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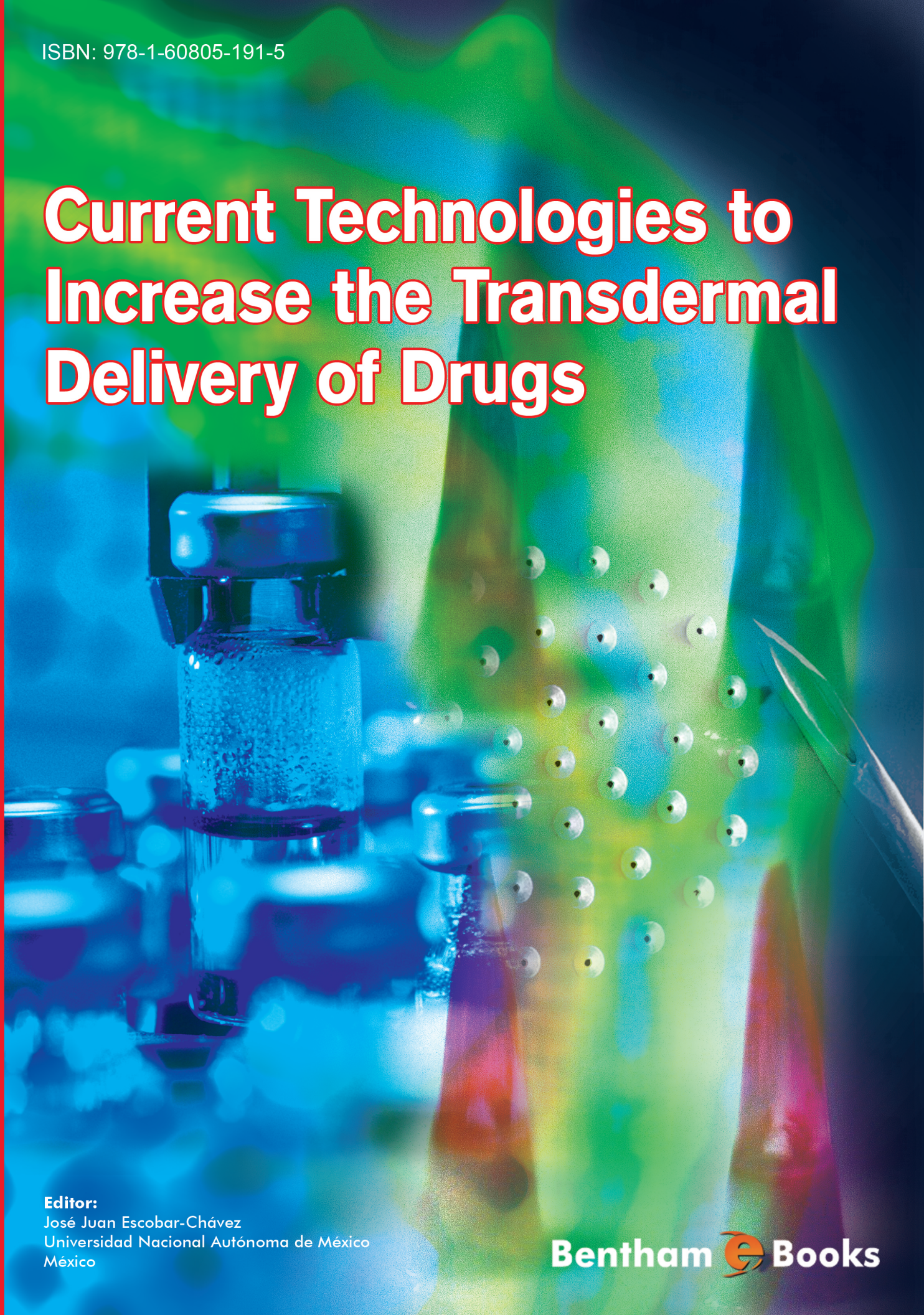
Current Technologies to Increase the Transdermal Delivery of Drugs



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"CURRENT TECHNOLOGIES TO INCREASE THE TRANSDERMAL DELIVERY OF DRUGS"

By

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DEDICATION

To my Family and Adalberto de la Fuente Chávez

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FOREWORD

Pharmaceutical knowledge has grown exponentially over the last 30 years. We now have a much clearer understanding of how drugs are absorbed into, distributed within, and cleared from the body.

The potency of agents with which we deal continues to increase, and our ability to unravel mechanisms of action proceeds. New drugs –in particular peptides, proteins and other biological response modifiers- are being developed and new challenges await pharmaceutical scientists. Controlled drug delivery represents a field that must keep pace with changing nature of chemotherapy. Tighter control of drug input into the body in both quantitative and temporal senses is crucial, and fabrication of new delivery systems must respond to this demand for increased sophistication.

Transdermal delivery has become an important means of drug administration. A number of scientists in this area has dramatically increased and multiple symposia have focused on the subject.

The objective of this book is to provide a general and an updated overview of the theoretical and practical aspects of iontophoresis, electroporation, sonophoresis, microneedles, chemical enhancers and transdermal nanocarriers systems on the delivery of transdermal drugs. Such a generalized approach would be helpful in drug discovery, drug delivery, drug design and toxicological research.

The contributors to this text have been directed to emphasize current above mentioned technologies involved in transdermal drug delivery. Authors were selected for their knowledge and reputation in their subject area, and for their ability to address objectively the topics of this book. I believe that they have performed this task effectively, producing a text that will facilitate and optimize future developmental programs in transdermal drug delivery.

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PREFACE

The proposed e-book provides an overview of current technologies to increase the topical/transdermal delivery of drugs, its protocols, advantages and limitations and an emphatic point in the uses and applications of these mechanisms. For this reason, this e-book provides exclusive chapters on Chemical Enhancers, Iontophoresis, Sonophoresis, Electroporation, Microneedles and more recently the use of micro/nanoparticles to deliver drugs throughout the skin.

Currently, there are no exclusive books available on techniques to increase the topical/transdermal drug delivery addressing each of the techniques mentioned above in a deep and detailed way**.** Brief chapters and books describing only one of the methodologies are available to readers only in some drug delivery or toxicology books. For these reasons, a more detailed discussion of current mechanisms to increase the penetration of drugs through skin is currently needed. This book presents a general overview of the theoretical and practical aspects of iontophoresis, electroporation, sonophoresis, microneedles, chemical enhancers and transdermal nanocarriers systems on the delivery of transdermal drugs. Such a generalized approach would be helpful in drug discovery, drug delivery and toxicological research.

A comprehensive book which provides the basis, the practical techniques and updated research information is necessary, for this reason this e-book will be an interesting option that could be used by students of the pharmacy area (biopharmacy, pharmaceutical technology, design and development of drugs, etc.), for the pharmacy and pharmaceutical technology departments of the different Universities all over the world, pharmaceutical technologists, dermatologists, scientists and pharmaceutical R & Ds.

Transdermal drug delivery has several potential advantages over other parenteral delivery methods. Apart from the convenience and noninvasiveness, the skin also provides a “reservoir” that sustains delivery over a period of days. Furthermore, it offers multiple sites to avoid local irritation and toxicity, yet it can also offer the option to concentrate drugs at local areas to avoid undesirable systemic effects. However, at present, the clinical use of transdermal delivery is limited by the fact that very few drugs can be delivered transdermally at a viable rate. This difficulty is because the skin forms an efficient barrier for most molecules, and few noninvasive methods are known to significantly enhance the penetration of this barrier.

In order to increase the range of drugs available for transdermal delivery the use of chemical and physical enhancement techniques have been developed in an attempt to compromise skin barrier function in a reversible manner without concomitant skin irritation. Recently, several alternative physical methods have emerged to transiently break the stratum corneum barrier and also the use of chemical enhancers continues expanding. The projectile methods use propelled microparticles and nanoparticles to penetrate the skin barrier. Microneedle arrays are inserted through the skin to create pores. “Microporation” creates arrays of pores in the skin by heat and RF ablation. Also, ultrasound has been employed to disrupt the skin barrier. All these methods have their own advantages and drawbacks, but a reality is that new developments are expected in the future to make these methods even more versatile.

This e-book reviews the use of chemical enhancers and physical methods as iontophoresis, sonophoresis, electroporation, microneedles and nanocarriers to increase the penetration of drugs throughout the skin. After an introduction, the protocol, advantages and limitations, the focus turns to the relevance of experimental studies. The available techniques are then reviewed in detail, with particular emphasis on topical/transdermal delivery.

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CHAPTER 1

The Skin: A Valuable Route for Administration of Drugs

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Abstract: The skin is the largest organ of the body and its main function is to protect the organism against undesirable effects of the environment. The skin is composed of three different layers: epidermis, dermis and hypodermis. The epidermis contains the stratum corneum, the uppermost layer of the epidermis, that acts as the barrier function of the skin due to its very high density and its low hydration. The dermis is an extensive vascular network providing skin nutrition, repair, thermal regulation and immune response. The hypodermis acts as a heat insulator, a shock absorber, and an energy storage region. There are also several appendages in the skin: hair follicles, sebaceous, sweat glands and nails. The skin properties play an important role to allow penetration of topically applied drugs or substances into the skin. Drug permeation through the skin include the diffusion through the intact epidermis and the skin appendages. In this chapter we reviewed structure, immunological and electrical properties, penetration routes of drugs throughout skin, types of skin and the most common skin disorders that affect humans.

SKIN STRUCTURE

The skin is the largest organ of the body with a surface area of about 2 m^2 and accounting for more than 10 % of body mass. Its main function is to protect the organism against undesirable effects of the environment. Essentially, the skin is composed of three different layers: epidermis, dermis and hypodermis (Fig. **1**). A basement membrane separates the epidermis and dermis, whereas the dermis remains continuous with the subcutaneous and adipose tissues [1]. It is well known that the stratum corneum, the uppermost layer of the epidermis, acts as the barrier function of the skin [2]. There are several appendages in the skin, which include hair follicles, sebaceous and sweat glands and nails, but these occupy only about 0.1 % of the total human skin surface [3, 4].

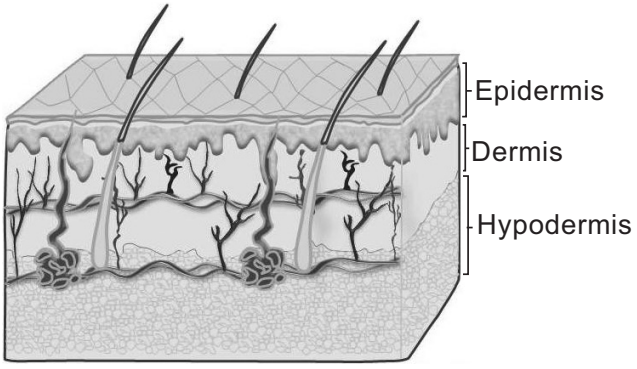

Figure 1: Schematic representation of the skin structure.

Epidermis

Stratum Corneum

The stratum corneum is the heterogeneous outermost layer of the epidermis and is approximately 10-20 μm thick. The stratum corneum consists of about 15 to 25 layers of flattened, stacked, hexagonal, and cornified cells embedded in an intercellular matrix of lipids (Fig. **2**). These lipid domains form a continuous structure so they are considered to play a crucial role in the maintenance of the skin barrier that helps avoid transepidermal water loss.

José Juan Escobar-Chávez (Ed)

Each cell is approximately 40 μm in diameter and 0.5 μm thick. The thickness varies according to areas such as the palms of the hand and soles of the feet as well as areas of the body associated with frequent direct and substantial physical interaction with the physical environment [5].

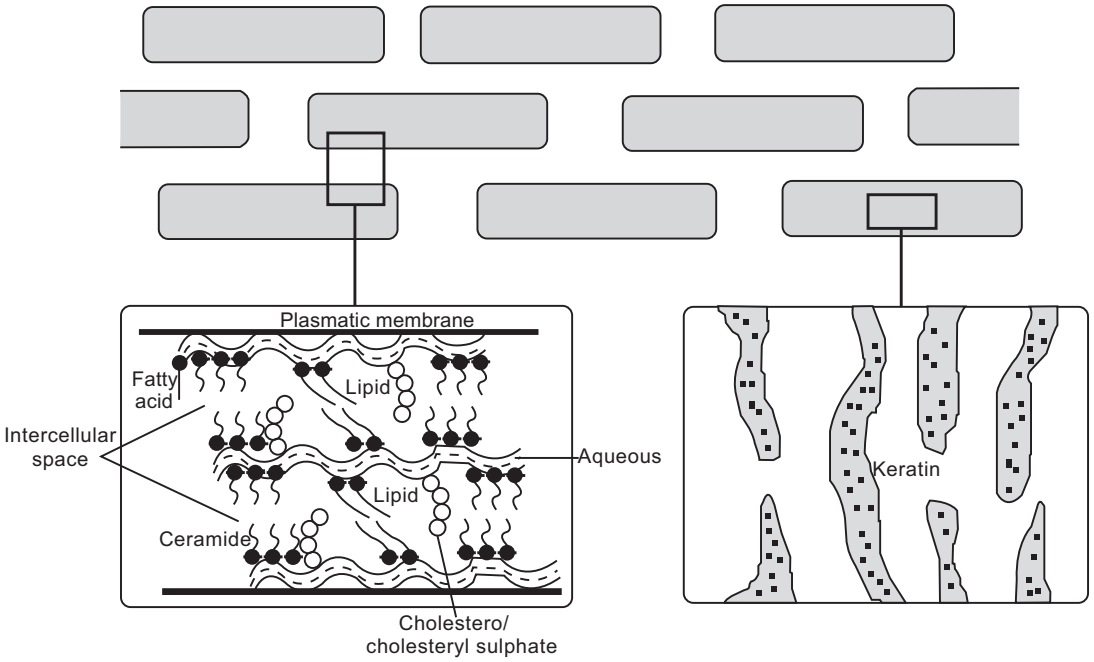

Figure 2: Simplified diagram of stratum corneum.

The stratum corneum barrier properties may be partly related to its very high density (1.4 g/cm^3 in the dry state) and its low hydration of 15–20 %, compared with the usual 70 % for the body. Each stratum corneum cell is composed mainly of insoluble bundled keratins (70 %) and lipid (20 %) encased in a cell envelope, accounting for about 5% of the stratum corneum weight. The permeability barrier is located within the lipid bilayers in the intercellular spaces of the stratum corneum [6-8] and consists of ceramides (40–50%), fatty acids (15–25%), cholesterol (20–25%) and cholesterol sulphate (5–10 %) [9-13].

The barrier function is further facilitated by the continuous desquamation of this horny layer with a total turnover of the stratum corneum occurring once every 2–3 weeks. The stratum corneum functions as a barrier are to prevent the loss of internal body components, particularly water, to the external environment. The cells of the stratum corneum originate in the viable epidermis and undergo many morphological changes before desquamation. Thus, the epidermis consists of several cell strata at varying levels of differentiation.

The origins of the cells of the epidermis lie in the basal lamina between the dermis and viable epidermis. In this layer there are melanocytes, Langerhans cells, Merkel cells, and two major keratinic cell types: the first functioning as stem cells having the capacity to divide and produce new cells; the second serving to anchor the epidermis to the basement membrane [14]. The basement membrane is 50–70 nm thick and consists of two layers, the lamina densa and lamina lucida, which comprise mainly proteins, such as type IV collagen, laminin, nidogen and fibronectin. Type IV collagen is responsible for the mechanical stability of the basement membrane, whereas laminin and fibronectin are involved with the attachment between the basement membrane and the basal keratinocytes. The cells of the basal lamina are attached to the basement membrane by hemidesmosomes, which are found on the ventral surface of basal keratinocytes [15]. Hemidesmosomes appear to comprise three distinct protein groups: two of which are bullous pemphigoid antigens (BPAG1 and BPAG2), and the other epithelial cell specific integrins [16, 17, 18]. BPAG1 is associated with the organization of the cytoskeletal structure and forms a link between the hemidesmosome structure and the keratin intermediate filaments. The integrins are transmembrane receptors that mediate attachment between the cell and the extracellular matrix. Human epidermal basal cells contain integrins $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_6\beta_4$. Integrin $\alpha_6\beta_4$ and BPAG2 appear to be the major hemidesmosomal protein contributors to the anchoring of the keratinocyte, spanning from the keratin intermediate filament, through the lamina lucida, to the

lamina densa of the basement membrane [19]. In the lamina densa, these membrane-spanning proteins interact with the protein laminin-5 which, in turn, is linked to collagen VII, the major constituent of the anchoring fibrils within the dermal matrix. It has also been suggested that both BPAG2 and integrin $\alpha_6\beta_4$ mediate in the signal transductions required for hemidesmosome formation and cell differentiation and proliferation. Integrin $\alpha_3\beta_1$ is associated with actin and may be linked with laminin-5. Epidermal wounding results in an up-regulation of these proteins that appears to be involved with cell motility and spreading. The importance of maintaining a secure link between the basal lamina cells and the basement membrane is obvious, and the absence of this connection results in chronic blistering diseases such as pemphigus and epidermolysis bullosa.

Dermis

The dermis is about 0.1–0.5 cm thick and consists of collagenous (70 %) and elastin fibres. In the dermis, glycosaminoglycans or acid mucopolysaccharides are covalently linked to peptide chains to form proteoglycans, the ground substance that promotes the elasticity of the skin. The main cells present are the fibroblasts, which produce the connective tissue components of collagen, laminin, fibronectin and vitronectin; mast cells, which are involved in the immune and inflammatory responses; and melanocytes involved in the production of the pigment melanin [19]. Nerves, blood vessels and lymphatic vessels are also present in the dermis.

Contained within the dermis is an extensive vascular network (Fig. **3**) providing for the skin nutrition, repair, and immune responses for the rest of the body, heat exchange, immune response, and thermal regulation. Skin blood vessels derive from those in the subcutaneous tissues (hypodermis), with an arterial network supplying the papillary layer, the hair follicles, the sweat and apocrine glands, the subcutaneous area, as well as the dermis itself. These arteries feed into arterioles, capillaries, venules, and, thence, into veins. Of particular importance in this vascular network is the presence of arteriovenous anastomoses at all levels in the skin. These arteriovenous anastomoses, which allow a direct shunting of up to 60% of the skin blood flow between the arteries and veins, thereby avoiding the fine capillary network, are critical to the skin's functions of heat regulation and blood vessel control. Blood flow changes are most evident in the skin in relation to various physiological responses and include psychological effects, such as shock (''draining of color from the skin'') and embarrassment (''blushing''), temperature effects, and physiological responses to exercise, hemorrhage, and alcohol consumption.

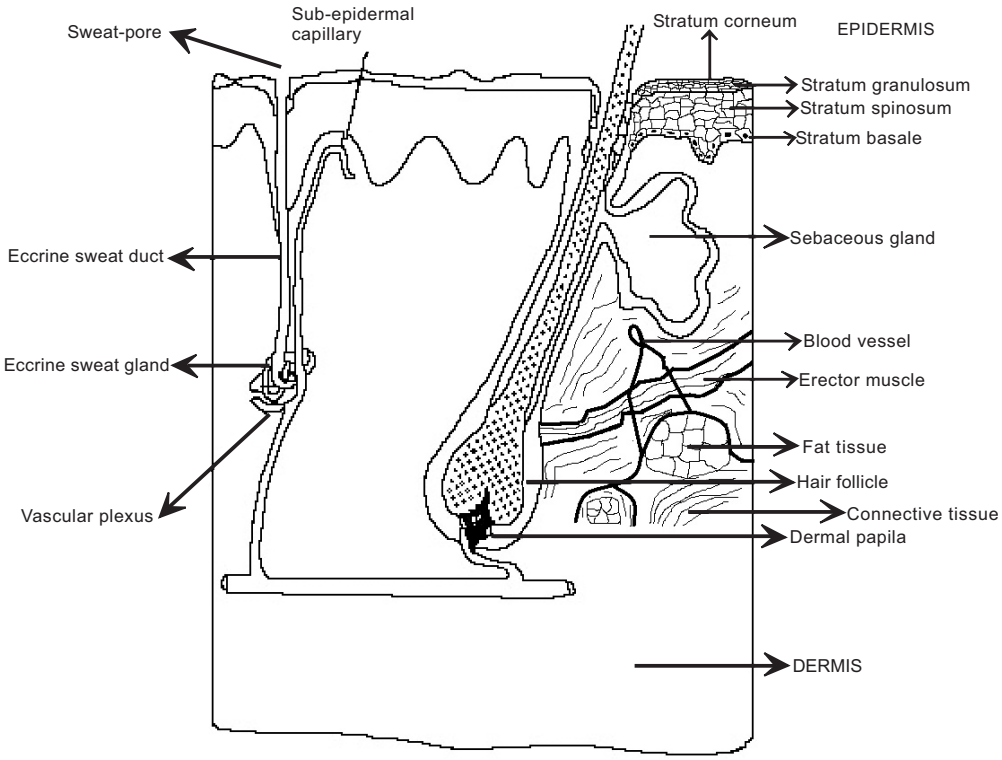

Figure 3: Components of the epidermis and dermis of human skin.

The lymphatic system is an important component of the skin in regulating its interstitial pressure, mobilization of defense mechanisms, and in waste removal. It exists as a dense, flat meshwork in the papillary layers of the dermis and extends into the deeper regions of the dermis. Also present in the dermis are a number of different types of nerve fibers supplying the skin, including those for pressure, pain, and temperature [20].

Epidermal appendages such as hair follicles and sweat glands are embedded in the dermis [21].

Hypodermis

The deepest layer of the skin is the subcutaneous tissue or hypodermis. The hypodermis acts as a heat insulator, a shock absorber, and an energy storage region. This layer is a network of fat cells arranged in lobules and linked to the dermis by interconnecting collagen and elastin fibers. As well as fat cells (possibly 50% of the body's fat); the other main cells in the hypodermis are fibroblasts and macrophages. One of the major roles of the hypodermis is to carry the vascular and neural systems for the skin. It also anchors the skin to underlying muscle. Fibroblasts and adipocytes can be stimulated by the accumulation of interstitial and lymphatic fluid within the skin and subcutaneous tissue [22].

The total thickness of skin is about 2–3 mm, but the thickness of the stratum corneum is only about 10–15 μm.

Skin Appendages

There are four skin appendages: the hair follicles with their associated sebaceous glands, eccrine and apocrine sweat glands, and the nails [4], but these occupy only about 0.1 % of the total human skin surface (Fig. **4).**

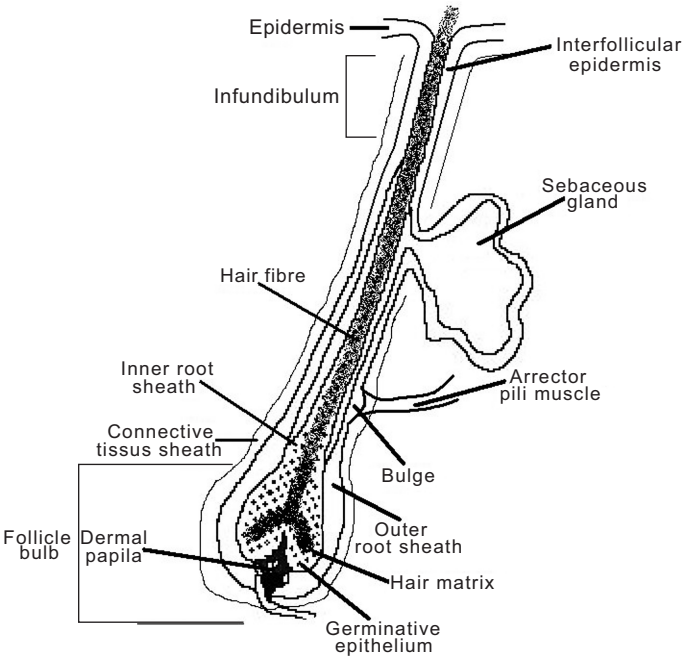

Figure 4: Schematic representation of the pilosebaceous unit showing both the hair follicle and sebaceous gland.

The pilosebaceous follicles have about 10 to 20 % of the resident flora and cannot be decontaminated by scrubbing. The hair follicles are distributed across the entire skin surface with the exception of the soles of the feet, the palms of the hand and the lips. A smooth muscle, the erector pilorum, attaches the follicle to the dermal tissue and enables hair to stand up in response to fear. Each follicle is associated with a sebaceous gland that varies in size from 200 to 2000 μm in diameter. The sebum secreted by this gland consisting of triglycerides, free fatty acids, and waxes, protects and lubricates the skin as well as maintaining a pH of about 5. Sebaceous glands are absent on the palms, soles and nail beds. Sweat glands or eccrine glands respond to temperature via parasympathetic nerves, except on palms, soles and axillae, where they respond to emotional stimuli via sympathetic nerves [19]. The eccrine glands are epidermal structures that are simple, coiled tubes arising from a coiled ball, of approximately 100 μm in

diameter, located in the lower dermis. It secretes a dilute salt solution with a pH of about 5, this secretion being stimulated by temperature-controlling determinants, such as exercise and high environmental temperature, as well as emotional stress through the autonomic (sympathetic) nervous system. These glands have a total surface area of about 1/10,000 of the total body surface. The apocrine glands are limited to specific body regions and are also coiled tubes. These glands are about ten times the size of the eccrine ducts, extend as low as the subcutaneous tissues and are paired with hair follicles.

Nail function is considered as protection. Nail plate consists of layers of flattened keratinized cells fused into a dense but elastic mass. The cells of the nail plate originate in the nail matrix and grow distally at a rate of about 0.1 mm/day. In the keratinization process the cells undergo shape and other changes, similar to those experienced by the epidermal cells forming the stratum corneum. This is not surprising because the nail matrix basement membrane shows many biochemical similarities to the epidermal basement membrane [23,24]. Thus, the major components are highly folded keratin proteins with small amounts of lipid (0.1–1.0%). The principal plasticizer of the nail plate is water, which is normally present at a concentration of 7–12 %.

SKIN FUNCTIONS

Many of the functions of the skin can be classified as essential to survival of the body bulk of mammals and humans in a relatively hostile environment. In a general context, these functions can be classified as a protective, maintaining homeostasis or sensing. The importance of the protective and homeostatic role allows the survival of humans in an environment of variable temperature; water content (humidity and bathing); and the presence of environmental dangers, such as chemicals, bacteria, allergens, fungi and radiation. In a second context, the skin is a major organ for maintaining the homeostasis of the body, especially in terms of its composition, heat regulation, blood pressure control, and excretory roles. It has been argued that the basal metabolic rate of animals differing in size should be scaled to the surface area of the body to maintain a constant temperature through the skin's thermoregulatory control [25]. Third, the skin is a major sensory organ in terms of sensing environmental influences, such as heat, pressure, pain, allergen, and microorganism entry. Finally, the skin is an organ that is in a continual state of regeneration and repair. To fulfill each of these functions, the skin must be tough, robust, and flexible, with effective communication between each of its intrinsic components mentioned above.

The stratum corneum also functions as a barrier to prevent the loss of internal body components, particularly water, to the external environment. The epidermis plays a role in temperature, pressure, and pain regulation.

Appendage functions are following: hair follicle and sebaceous gland fulfill with protect (hair) and lubricate (sebum), eccrine and apocrine glands have the functions of cooling and vestigial secondary sex gland, respectively; and nails has the function of to protect.

The hypodermis acts as a heat insulator, a shock absorber and an energy storage region. One of the major roles of the hypodermis is to carry the vascular and neural systems for the skin.

IMMUNOLOGICAL AND ELECTRICAL PROPERTIES

Contained within the dermis is an extensive vascular network providing for the skin nutrition, repair, and immune responses and, for the rest of the body, heat exchange, immune response, and thermal regulation.

It is known that Langerhans cells reside in the epidermis and express a high level of major histocompatibility complex class II molecules and strong stimulatory functions for the activation of T lymphocytes. The Langerhans cells comprise 2–4 % of the cells of the epidermis and are also found in lymph nodes. They act on antigens and present them to lymphocytes and thus provide immune surveillance for viruses, eoplasms and non-autologous grafts. The keratinocytes also play a role in immunity [19]. The Langerhans cells are dendritic-shaped cells which are located in the basal parts of the epidermis. In recent years, the concept of skin associated lymphoid tissue (SALT) has evolved in which Langerhans cells in the epidermis are believed to act as antigenic traps, and the antigen-laden cells then migrate into dermal lymphatic channels to present the information to T lymphocytes in lymph nodes. When allergens penetrate into the skin, they can in some cases lead to allergic contact dermatitis, which is

characterized by redness and vesicles, followed by scaling and dry skin. Relevant compounds in skin immunology are the eicosanoids. Eicosanoids, which are oxygenated metabolites of 20-carbon fatty acids, especially arachidonic acid, are a class of compounds which have a role in the pathophysiology of inflammatory and immunological skin disorders. For example, leukotrienes play a central role in the pathogenesis of psoriasis, a chronic, scaly and inflammatory skin disorder [26].

The main barrier of mammalian skin to the transport of ions and molecules, particularly charged molecules, is its outermost layer, the stratum corneum. This layer is a heterogeneous, dead layer about 10 to 15 /μm thick and consists of flattened remnants of cells (corneocytes) and about one hundred lipid bilayer membranes arranged in series, as it was discussed before [28]. One of the electrical properties more important of the skin is the impedance. Electrical impedance is defined as the opposition that show the skin when a current through itself.

It is widely accepted that the main electrical impedance resides in the stratum corneum while the impedance of the other layers is several orders of magnitudes lower [29]. This resistance is due to the water content of the stratum corneum is very low, not more than 20%, compared to 70% in the underlying tissue [30]. This means that the skin impedance is dominated by the passive electrical behaviour of the stratum corneum and significant differences in impedance values among different anatomical regions of normal skin have been found [31]. The low frequency pathway is dominated by the appendages such as hair follicles and sweat ducts. Lipid lamellae are borderlines between very low conductivity (lipids) and high conductivity (electrolyte) forming a capacitor [32]. There are two distinguishable pathways involving the lipid layers: a direct pathway through the corneocytes and a tortuous pathway using hydrated sites around the corneocytes. Technically, we can model this as a resistor for the appendages and a resistor-capacitor combination for each capacitive pathway in parallel. Since the parameters of the capacitive pathways are distributed, the number of resistor-capacitor combination should be enormous. This combination system showed by the stratum corneum is very reactive and it shows more impedance than resistance [29]. The skin capacitance is a measure of the charge storage capacity of the skin. Therefore, electroporation is known to dramatically change the electrical resistance of lipid-based barriers, and cell membranes. More recently electroporation has been suggested as being responsible for the rapid and large electrical changes that occur because of 'high-voltage' pulsing of tissues [33].

The complex electrical impedance of skin has been studied in some reports. It has used hairless mouse skin to measure the impedance of skin as a function of frequency, and resistance and capacitance. The results shown that the impedance became independent of frequency, suggesting that the capacitive properties of barrier had been lost. The results provide mechanistic insight into ion conduction through the skin and into the role of stratum corneum lipids in skin capacitance that increasing the ionic strength of the bathing medium, and increasing the magnitude of current, decreased resistance, whereas capacitance was, in general, unchanged. These changes occurred rapidly. The decrease in resistance with increasing the ionic strength of the bathing medium was consistent with elevated ion levels within the ion-conducting pathways of the membrane. The decrease in resistance by increasing the magnitude of current seems to be related to alteration of the current-conducting pathway. With increasing temperature, resistance also decreased while capacitance increased. The most marked changes occurred at the phase transition temperature (60°C) of the stratum corneum lipids; resistance fell dramatically and capacitance steadily increased [34].

The impact of physical and chemical perturbation of the stratum corneum on the barrier function of mammalian skin has been investigated in several reports. It has been studied, the application direct-current electrical in full-thickness hairless rat skin as a function of tape-stripping and delipidization. So samples subjected to tape-stripping or immersions in chloroform/methanol were highly conductive. Collectively, such findings would indicate that the stratum corneum serves as the principal barrier to the transport of ionic permeants into and through the skin, and that specific lipid components likely regulate the integrity of the intercellular lipid domain under the influence of electric current [35]. These results agree with those found in which the effects of current density on the temperature dependence of the electrical properties of human stratum corneum were investigated *in vitro* at two different current densities: 13 and 130 $\mu A/cm^2$. At both current densities three characteristic temperature intervals were distinguished: (1) A lower interval, from 20 to about 60°C at the lower current density and from 20 to about 50°C at the higher current density. In this interval a constant activation energy for ion transport and a gradual decrease of the resistances were found, whereas the capacitances were almost constant; all changes within this interval were thermo-reversible; (2) A middle interval, from 60 to about 75°C at the lower and from 50 to about 75°C at the higher current

density. Within these temperature ranges, a rapid and thermo-irreversible decrease of the resistances was observed, accompanied by an increase of the capacitances, these temperatures corresponded with the temperature interval of the gel-liquid phase transition of stratum corneum lipids; and (3) A higher interval, from 75 to 95°C, within which the resistance did not decrease any further, although the capacitance increase continued. Therefore the thermal analysis of electrical properties has shown that the resistances of human stratum corneum are closely associated with the intercellular lipid lamellae, whereas the capacitances are determined by both the intercellular lipid lamellae and protein-bound lipids. Furthermore, under influence of an electrical field the lipid phase transition temperature is shifted downward, indicating that the electrical field is capable of modifying the arrangements of stratum corneum lipids [36].

It has been studied in several investigations that a large electric field (high-voltage pulses) across the stratum corneum lipids leads to creation of aqueous pathways and simultaneously provides a local driving force, namely, an electrical potential gradient across the skin, for transport drugs through these pathways, then electroporation of the stratum corneum occurs [37-40]. Human skin has been observed using Cryo-scanning, transmission and freeze fracture electron microscopy. The *in vitro/in vivo* studies showed that iontophoresis (electric current) resulted in the formation of intercellular water pools (*in vitro* observation) and a weakening of the desmosomal structure (*in vivo* observation) only in the upper part of the stratum corneum, which can be observed on Figs. **5** and **6**. However, no changes in the lipid organization were observed *in vitro* and *in vivo* at the current densities of 0.5 and 0.25 mA/cm^2, respectively [41].

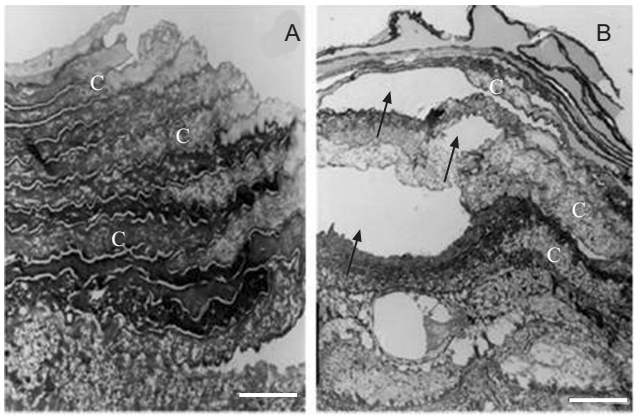

Figure 5: Transmission electron micrographs of human dermatomed skin. A) Anodal part, after 15 h of passive diffusion, overview of stratum corneum, (C) corneocytes. Scale bar represents 4500 nm. B) Anodal part, 6 h of passive diffusion and 9 h of iontophoresis at a current density of 0.5 mA/cm^2, black arrows indicates areas with cell detachment; Scale bar represents 1800 nm, [41].

An increase in stratum corneum hydration has been observed too after *in vivo* or *in vitro* application of various iontophoresis protocols by Fourier transformed infrared spectroscopy (FT-IR) it last provides information on the molecular level in the skin structure. Low current densities did not affect the structure of stratum corneum sheets; however, increased current densities, resulted in a number of changes to the lipid organization, suggesting that the electric field can perturb the intercellular lamellar ordering in the stratum corneum [42-44].

Another study analyzed the short high-voltage and long medium-voltage pulses to induce events within the multilamellar stratum corneum; Moreover, the results provided insight of the aqueous pathways created by the electric field. Most importantly, long medium-voltage pulses appeared to be more efficient in promoting transport of sulforhodamine across skin than short high-voltage pulses, and this might be especially for large compounds, such as heparin and therapeutic proteins [45].

Recently some attempts have been made to use chemical "enhancers" that result in chemical modification of the stratum corneum. Of all purely physical methods for enhancing transdermal drug delivery, iontophoresis is one of those very important for drugs and candidate drugs are too large, or are electrically charged in order to permeate the SC significantly. Therefore, a relatively low transdermal voltage (0.1-5V) is used to drive molecular transport [40].

In addition water is known as an effective penetration enhancer and could therefore play a role in the increased skin permeability observed after current termination [46].

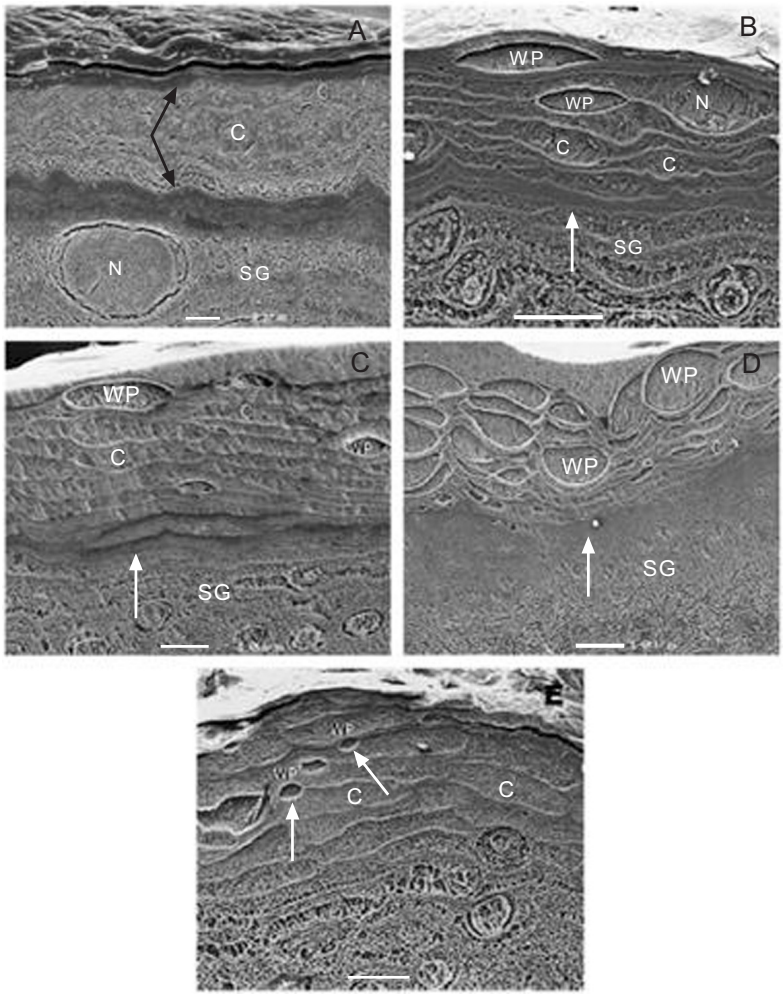

Figure 6: Cryo-scanning electron micrograph of human abdomen dermatomed skin after different treatments. A) A constant relative humidity controlled using a saturated solution of Na_2CO_3 (40%, w/v) at 25 °C for 15 h. Low hydration areas are indicated by black arrows (control). Nuclei (N) are also present in the graph. Scale bar represents 1 µm. B) Cryo-scanning electron micrograph of human abdomen dermatomed skin after 15 h of passive diffusion. Corneocytes (C) are shown to be strongly swollen with a few water pools (WP) in the intercellular regions. The circular regions in the stratum corneum represent remnants of cell nuclei (N). The non-swelling cells, indicated by a white arrow, are located in an interface between the stratum corneum and stratum granulosum (SG). Scale bar represents 10 µm. C) Cryo-scanning electron micrograph of human abdomen dermatomed skin after 15 h of passive diffusion. Corneocytes (C) are shown with a few water pools (WP) in the intercellular regions. The non-swelling cells, indicated by a white arrow, are located at an interface between the stratum corneum and stratum granulosum (SG). Scale bar represents 10 µm. D) Cryo-scanning electron micrograph of human abdomen dermatomed skin after 6h of passive diffusion and 9 h of iontophoresis with a current density of 0.5 mA/cm^2. Water pools (WP) are present in the intercellular regions. The non-swelling cells, indicated by a white arrow, are located in an interface between the stratum corneum and stratum granulosum (SG). Scale bar represents 10 µm. E) Cryo-scanning electron micrograph of human abdomen dermatomed skin after 6 h of passive diffusion and 9 h of iontophoresis with a current density of 0.5 mA/cm^2. Water pools (WP) are present in the intercellular regions (pointed by white arrows). Scale bar represents 10µm [41].

ROUTES OF PENETRATION OF DRUGS

The determination of penetration pathways of topically applied substances into the skin is the subject of several investigations. The permeation of drugs through the skin includes the diffusion through the intact epidermis y through the skin appendages. These skin appendages are hair follicles and sweat glands which form shunt pathways

through the intact epidermis, occupying only 0.1% of the total human skin [47]. It is known drug permeation through the skin is usually limited by the stratum corneum. Two pathways through the intact barrier may be identified, the intercellular and transcellular route, which are shown in the Fig. **7**:

a) The intercellular lipid route is between the corneocytes.

Interlamellar regions in the stratum corneum, including linker regions, contain less ordered lipids and more flexible hydrophobic chains. This is the reason of the non-planar spaces between crystalline lipid lamellae and their adjacent cells outer membrane. Fluid lipids in skin barrier are crucially important for transepidermal diffusion of the lipidic and amphiphilic molecules, occupying those spaces for the insertion and migration through intercellular lipid layers of such molecules [48, 49]. The hydrophilic molecules diffuse predominantly "laterally" along surfaces of the less abundant, water filled inter-lamellar spaces or through such volumes; polar molecules can also use the free space between a lamella and a corneocyte outer membrane to the same end [50].

b) The transcellular route contemplates the crossing through the corneocytes and the intervening lipids [51].

Intracellular macromolecular matrix within the stratum corneum abounds in keratin, which does not contribute directly to the skin diffusive barrier but supports mechanical stability and thus intactness of the stratum corneum. Transcellular diffusion is practically unimportant for transdermal drug transport [52].

The narrow aqueous transepidermal pathways have been observed using confocal laser scanning microscopy (CLSM). Here regions of poor cellular and intercellular lipid packing coincide with wrinkles on skin surface and are simultaneously the sites of lowest skin resistance to the transport of hydrophilic entities. This lowest resistance pathway leads between clusters of corneocytes at the locations where such cellular groups show no lateral overlap. The better sealed and more transport resistant is the intra-cluster/inter-corneocyte pathway [53]. Hydrophilic conduits have openings between ≥5 μm (skin appendages) and ≤10 nm (narrow inter-corneocyte pores). So sweat ducts (≥50 μm), pilosebaceous units (5–70 μm), and sebaceous glands (5–15 μm) represent the largest width/lowest resistance end of the range. Junctions of corneocytes-clusters and cluster boundaries fall within the range [54]. It was determined that the maximally open hydrophilic conduits across skin are approximately 20–30 nm wide, including pore penetrant/opener thickness [53]. Another studies revealed the width of the negatively charged hydrophilic transepidermal pores expanded by electroosmosis to be around of 22–48 nm [55]. Lipophilic cutaneous barrier is governed by molecular weight and distribution coefficient rather than molecular size [54]. The relative height of cutaneous lipophilic barrier consequently decreases with lipophily of permeant, but molecules heavier than 400–500 Da are so large permeants to find sufficiently wide defects in the intercellular lipidic matrix to start diffusing through the lipidic parts of cutaneous barrier [54,56,57].

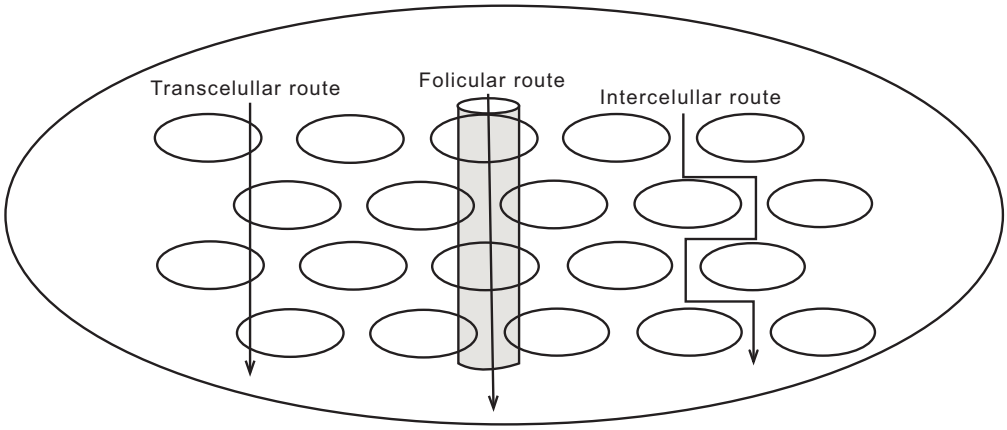

Figure 7: A schematic representation of penetration routes of drugs throughout the skin.

The contribution to transdermal drug transport can increases with the pathways widening or multiplication, for example such that is caused by exposing the stratum corneum to a strong electrical (electroporation/iontophoresis), mechanical (sonoporation/sonophoresis), thermal stimulus, or suitable skin penetrants [58].

Recently, follicular penetration has become a major focus of interest due to the drug targeting to the hair follicle is of great interest in the treatment of skin diseases. However due to follicular orifices only occupying ~0.1% of the total skin surface area, it was assumed as a non important route. But a variety of studies shown the hair follicles as could be a way to trough the skin [59-64].

The effect of ultrasound on the histological integrity and permeability properties of whole rat skin *in vitro* has been investigated [59]. The results showed high intensity ultrasound irradiation (1 to 2 W cm^{-2}) irreversibly damaged cutaneous structures and the increase percutaneous transport rate of permeants. In contrast, skin integrity was largely maintained with low intensity ultrasound (0.1 to 1 W cm^{-2}) which merely discharged sebum from the sebaceous glands so as to fill much of the hair follicle shafts and it was reduced the transport rate significantly for hydrophilic molecules that penetrate via this route.

Confocal laser scanning microscopy has been used to study the entry of drugs through the skin. It was visualized in the fresh human scalp skin on-line the diffusion processes of a model fluorophore into the hair follicle at different depths. Up to a depth of 500 μm in the skin, a fast increase of fluorescence is observed in the gap followed by accumulation of the dye in the hair cuticle. Penetration was also observed via the stratum corneum and the epidermis. Little label reached depths greater than 2000 μm. Therefore the gap and the cuticle play an important role in the initial diffusion period with the label in the cuticle originating from the gap [62]. Such follicular pathway also has been proposed for topical administration of nanoparticles and microparticles and it has been investigated in porcine skin, because in recent studies the results have confirmed the *in vitro* penetration into the porcine hair follicles might be considered similar to those on humans *in vivo*. After topical application of dye sodium fluorescein onto porcine skin mounted in Franz diffusion cells with the acceptor compartment beneath the dermis, the fluorescence was detected on the surface, within the horny layer, and in most of the follicles confirming the similarity in the penetration between porcine and human skin [63]. So nanoparticles have been studied in porcine skin revealing in the surface images that polystyrene nanoparticles accumulated preferentially in the follicular openings, this distribution was increased in a time-dependent manner, and the follicular localization was favored by the smaller particle size [65]. In other investigations, it has been shown by differential stripping the influence of size microparticles in the skin penetration. It can act as efficient drug carriers or can be utilized as follicle blockers to stop the penetration of topically applied substances [64].

In vitro drug penetration through human scalp skin has been compared with that via human abdominal skin to clarify the usefulness of intrafollicular delivery, these results showed the permeation of lipophilic melatonin and hydrophilic fluorouracil through the scalp skin was much higher than that via the abdominal skin, being 27 and 48 times respectively [66]. Therefore the drug delivery through the scalp skin will offer an available delivery preferably for drugs with hydrophilic characteristics [60]. It has been reported that above a critical log $K_{o/w}$ value, lipophilicity seems to be an important modulator of drug absorption into follicular orifices, and below of it lipophilicity does not apparently influence the follicular contribution in an obvious way. Here, aldosterone, cimetidine, deoxyadenosine and adenosine were investigated in order to know the influence of the lipophilicity above the penetration follicular. One hand, for the two most lipophilic drugs drug entry via follicular pores was very minor. In the other hand a small decrease in solute lipophilicity produced an appreciable increase in the contribution of the follicular orifices. Follicular contributions were 60, 58, 46 and 34% for aldosterone, cimetidine, deoxyadenosine and adenosine respectively [67].

It has already been postulated that certain molecules can hydrogen bond to groups present on the surfaces of follicular pores [68]. However, more studies have to be made in order to identify all the molecular properties that influence drug penetration into hair follicles.

Nowadays, there are currently a number of methods available for quantifying drugs localized within the skin or various layers of the skin. To date, a direct, non-invasive quantification of the amount of topically applied substance penetrated into the follicles had not been possible. Therefore, stripping techniques, tape stripping and cyanoacrylate skin surface biopsy have been used to remove the part of the stratum corneum containing dye topically applied. Thus, the "differential stripping" has been shown as a new method that can be used to study the penetration of topically applied substances into the follicular infundibula non-invasively and selectively [69]. However future research in this field should incorporate a greater number of validation studies.

SKIN TYPES

The history of classifying skin types has had a considerable progress made with continuing awareness. There are different ways in order to make a classification about the skin. Among the numerous skin classifications that are proposed, the one most closely connected with cosmetological requirements distinguishes four different types: normal, oily, dry, and mixed. Skin can have different appearances directly related to the water and fatty content of the hydrolipidic film, it depends on its state, activity, and defense capacity. Fatty deficiency, indispensable for retaining water in the teguments, favors its evaporation and therefore skin drying, whereas an excess of lipidic components favors a state defined as oily. This classification must be used cautiously, because the criteria of selection to define each category are difficult to standardize since they vary from one case to another, for example, severe changes in epidermal water content associated with superficial pH changes can modify the skin's appearance and lead one to establish a visual diagnosis of dry skin, whereas it may be actually an oily skin [70, 71].

Dry skin would mainly correspond to structural and functional modifications of the components of the epidermis. In skin normal, the corneal layer is made up of a regular assembly of corneocytes, forming a structure of modulated thickness with unique physical qualities. Each corneocyte contains dampening substances called natural moisturizing factors, resulting from the enzymatic degradation of the fillagrines, which fix a certain quantity of inter-corneocytar water and therefore exert a decreasing osmotic pressure as they migrate to the surface. Any decrease in the enzymatic function therefore plays an important part on the natural moisturizing factors content and consequently on the osmotic pressure and on the opening of corneosomes, consequently easing a disorganized desquamation as it is observed with xerosis [72]. This dysfunction actually depends on a qualitative and quantitative change of enzymes and/or on an inadequate change of the pH of the stratum corneum [73]. The cohesion of corneocytes also depends on a complex mixture of lipids that constitute the lamellar structure (made up of fatty acids, sterols, and ceramides coming from the keratinosomes) [72].

It has been shown the importance of four factors predisposing to dry skin:

a) The lack of water of corneocytes, directly depending on the presence of natural moisturizing factors.

b) The epidermal hyper-proliferation, resulting from a deficiency in the renewal process of the keratinocytes.

c) The change of lipidic synthesis at cell level.

d) The deterioration of the functionality of skin barrier, following a degradation of intercellular cohesion.

The factors mentioned above are interdependent. So, dry skin should be characterized by its rough appearance, without referring to its hydration level [74]. Recent investigations have tested the influence of the inflammatory process or of the content in calcium ions of the epithelial cells in skin drying, showing that the supply of nonsteroidal anti-inflammatory agents or of calcic regulators did not significantly modify the skin's state [75, 76]. On the other hand, the use of specific inhibitors of tryptic proteases, and particularly of "plasminogen activation system," showed a capacity for restoring the normal state of the skin and for simultaneously suppressing all the changes related to skin drying, notably against the mechanisms of cell regulation and differentiation [77]. These works suggest that skin drying does not correspond to an irreversible state but involve a dysfunction the traditional "balance moisture theory" and the "protease regulation theory" [77, 78]. Its reparation implies the restoration of the epidermal barrier, actually damaged by the loss of fat and dehydration of the superficial layers of the stratum corneum.

Oily skin would result from an excessive seborrheic production, invading skin surface and possibly hair. Oily skin and dry skin therefore correspond to two states that must not be opposed to each other, as some skins can be "dry" or "oily" and dehydrated at the same time. Whereas dry skin reflects a functional change of different skin components, the oily skin results from an overactivity of the sebaceous glands, leading to an overproduction of sebum overflowing on the skin, giving it a characteristic oily and shiny appearance. In fact, sebum results from the disintegration of specific cells, the sebocytes, and a short time before they are secreted from the sebaceous gland. Once again it results from a cell differentiation. Originally, sebum contains squalene, waxes, triglycerides, and sterols. Under the effect of resident bacteria, one part of the triglycerides is immediately hydrolyzed, and the main

part of the cholesterol is esterified, the sebum excreted containing a significant quantity of free fatty acids contributing to the acidity of the pH of the skin surface. Then this sebum blends with epidermal lipids produced from the destruction of the desquamated horny cells that also contain triglycerides and cholesterol to form the surface lipidic film covering the stratum corneum. Human beings have the particularity to have at their disposal sebaceous glands almost all over the body, but their activity is not the same on all the anatomical sites. The production of sebum is more important on head, face, neck, shoulders, and thorax, areas where a hyperseborrhea can be the conjunction of a high production of the glands and of a greater number of glands [79]. The change of its rate of production depends on genetic, endocrinic, and environmental factors [80]. The opposite of oily skin would not be dry skin since they can coexist [81]. Finally, at cosmetological level, it must be retained that oily skin is sometimes erythrosic, easily irritable, and particularly fragile.

There is no definition of normal skin; however it can be defined in comparison with the other skin types: a normal skin is not a dry skin, not an oily skin, not a mixed skin, and no more a pathological skin.

A normal skin according to its structure and its functions, should be a smooth skin, pleasant to touch, because of the cohesion of the cells of its more superficial layers; a firm and supple skin because of the existence of a dense supportive tissue and of the presence of numerous elastic fibers of good quality; a mat skin through its balanced seborrheic production; a clear and pinkish skin because of the perfect functionality of its microcirculatory network. In reality, a skin complying with all these characteristics would only exist in the healthy child before his/her puberty [82]. At cosmetological level, it can be considered normal skin as a young skin, structurally and functionally balanced and requiring no care apart from those necessary for its cleaning.

Mixed skin corresponds to a complex skin where the different types previously described coexist on different areas of body or face. The characteristic example is the face, where solid and oily skin with well-dilated pores on the medio-facial area can coexist with a fragile skin with fine grains on cheeks. Such a skin requires conjugating the particularities and sensitivities peculiar to normal, dry, and oily skins.

Sensitive skin is a special case that has been reported. Racial, individual, and intra-regional differences in the skin reactivity to a number of external stimuli have been widely documented during the last 20 years. This suggest that a specific reactivity, more frequent in the populations with light skin, corresponds to the conjunction of a different aspect of the skin barrier and vascular response and to a heightened neurosensory input, all related to a genetic component [83, 84].

The biophysical characteristics of skin also vary according to sex and age and can differ for the same subject according to the anatomical site considered. So, the distribution of these different types of skin widely varies according to the ethnical group we are referring to. Moreover the interindividual variations or those that can result from the methodological approach or from the material of measurement used, many authors have tried to identify the influence of the race, sex, and age of the populations observed and even the anatomical site on which the observations are made by the results obtained. The results of these investigations are sometimes contradictory, but there are some tendencies to be taken into consideration when conducting studies on the human being. The good previous knowledge of these differences is notably essential to know the efficacy, acceptability, and even tolerance of pharmaceutical or dermatological products applied topically.

Important functional differences exist between races and correspond to their necessary adaptation to the environment they are meant to live in. So, whereas the mean thickness of the horny layer is similar between the different races, the number of cell layers in the stratum corneum of the black skin is higher than that noted in Caucasian or Asian skins. Black skins therefore have a more compact stratum corneum with a greater cohesion between cells that makes them difficult to remove [85, 86]. However, the surface of corneocytes is identical for all the types of skin. In apparent contradiction to this greater cell cohesion, the spontaneous surface desquamation is significantly more important in blacks than in Caucasians or in Asians [87]. Interracial differences also exist concerning the melanocytic system. Basically each type of skin has the same number of melanocytes per unit of surface, but there is no similarity concerning their structure and their functionality [86]. Whereas the melanosomes are small and concentrated in the keratinocytes to be then degraded in the superficial layers of the epidermis of Caucasian skins, they are much bigger, widely scattered in all the layers of the keratinocytes and are not degraded

when they arrive in the horny layer of black skins, giving them a characteristic color [88]. Colorimetric and spectrophotometric studies have shown that the interindividual and intersexual differences of skin coloration in the different races are mainly related to the blood concentration in hemoglobin for the Caucasian subject, both to the hemoglobin and melatonic pigment content in the Asian subject, and only to the concentration in melanin in the black subject [89]. With respect to skin appendages, it even never has been possible to demonstrate a possible racial incidence on sebaceous secretion as some authors report a more important activity for black skins, whereas others report no substantial difference in sebaceous production between races in their comparative studies [90,91]. The advancement of knowledge enables today to retain the assumption that the genetic factors and the intrinsic differences between ethnical groups actually have less importance than their capacity for adaptation to the environment they live in [92]. Pigmentation favors a better protection against sun radiations and therefore actinic aging. This can explain why, from this point of view, aging is quicker for the Asian skin [93].

Morphological differences have been found in the skin according to the sexes. One of them is the skin thickness that is greater in men on most of the sites usually used for biophysical measurements than for women, the skin is thicker at dermal level [94-96]. Other authors reported no significant differences for the forearms [97, 98]. Observations made on male and female Asian subjects enabled to show no difference between sexes concerning the number of layers of coenocytes. The skin thickness would reduce more quickly with aging in women than in men [99]. There has been increasing interest in studying gender differences in skin to learn more about disease pathogenesis and to discover more effective treatments [100]. The physiology of body organs can be affected by gender. Skin and skin appendages are influenced by sex hormones. Skins of men and women differ in hormone metabolism, hair growth, sweat rate, sebum production, surface pH, fat accumulation, serum leptins, etc [101]. The knowledge of epidermal thickness is of great significance in many areas of medical and biological research and it could be influenced by several constitutional factors, such as age, gender, skin type, and anatomic site. It was assessed optical coherence tomography *in vivo* to investigate the factors mentioned before. The epidermal thickness was assessed in six different body sites of young (20–40 years old) and old (60–80 years old) caucasians, respectively. Comparison of young and old Caucasians demonstrated a significant decrease of epidermal thickness with age in all anatomic sites investigated. Epidermal thickness assessed in males and females did not significantly differ, except for forehead skin which is significantly thinner in old females than in males [102].

The influence of the aging of the skin on its structure and functionality has obtained relevant results. Age has a direct impact on the evolution of most of the biophysical parameters of the skin. In the adult person, epidermal proliferation rate decreases with age. It can be 10 times higher in younger (second decade) than in older (seventh decade) individuals, and for a given age, the decrease was demonstrated to be 10 times faster in sun-exposed areas than in unexposed ones. These constant reductions seem to be independent of the ethnic origin and season [103].

The differences that exist between anatomical sites are wide. The spontaneous changes of the skin's state over time according to intercurrent-factors that depend on physiological and hormonal variations and on its proper aging an approach can only be performed case by case. The skin's thickness is not the same between anatomical sites as established in the publications of many authors through numbered data and different instrumental measurements. So, the skin's thickness measured in the subject of Caucasian race is less on the forearm than on the forehead, of the order of 0.9 and 1.7 mm, respectively [94]. These values are slightly higher than those described by others but it can be taken into account as the approach by a more elaborated technique based on high-resolution scanning [94, 104–106]. Moreover there are great variations for the same area.

Measurements performed with a scanner on 22 anatomical sites of young male and female Caucasians enabled to note that the skin is all the more echogenic since it is thinner and that at acoustic level the response of the reticular dermis is denser than that of the papillary dermis. This acoustic density, also inversely proportional to the skin's thickness, is consequently variable according to the thickness of the anatomical sites measured [96]. It must be underlined that in spite of differences in the absolute values from site to site, the evolution of the response of a given site can be predictive for other sites in the same person. So, the volar forearm is considered as representative of the face for measuring the skin's hydration and biomechanical properties [107].

There are important natural variations in the skin color between anatomical sites induced by sun exposure. This is another classification of the skin according to its photosensitive. Skin phototype was firstly proposed by Fitzpatrick

based on skin response of the Caucasian, whereas the Japanese skin type was proposed for Japanese skin by Satoh and Kawada [108]. The Fitzpatrick Skin Phototype Classification remains the gold standard. Current clinical assessments of skin color and photosensitivity include the physician-diagnosed skin phototype scale, which relies on the visual assessment of pigmentation as an indicator of skin responses to sunlight.

The original version of the physician-diagnosed skin phototype scale developed by Fitzpatrick categorized skin response to UV exposure into 1 of 4 types (I-IV), and more pigmented skin types (V and VI) were included after subsequent revision [109]; however this system fails to accurately predict skin reactions. The Roberts Skin Type Classification System is a tool to predict the skin response to injury from cosmetic procedures and identify the propensity of sequel from inflammatory skin disorders. It can be a predictor of an impending complication, such as hyperpigmentation and scarring, which can then be avoided. In addition, it includes the skin phototype and photoage [110]. Objective measures of pigmentation fail to correlate well with race, whereas race correlates moderately with physician-diagnosed skin phototype. Including objective methods of analyzing skin color may reduce subjective influences of race in assessing photosensitivity and potential risk for skin cancer [111].

Another clinical measure used to predict photosensitivity is the minimal erythema dose, which is based on correlation to dose-response curves. The technique of spectrometry has been used to assess both the minimal erythema dose and the minimal melanonogenic dose as indicators of erythema induced by UV radiation [112]. The skin phototype concept is practicable and useful for predicting individual's sensitivity to UV, risk and preventive factors, and choosing sunscreens even with the limitation [108]. In a group of 190 white healthy subjects the skin type classification method was found valuable for differentiating subgroups with various degrees of sun sensitivity. Sun-sensitive skin types 1 and II were significantly more common among persons with light hair color or freckles, or both. In each skin type category the proportion of subjects with a minimal erythema dose decreased significantly with increasing skin type number. The contribution of freckles to % of the minimal erythema dose was skin type dependent. Age, sex, or eye color had no independent effect on % of the minimal erythema dose. The association of skin types I and II, red or blond hair, and freckles with decreased the minimal erythema dose may reflect genetically controlled predominance of pheomelanin (a photosensitizing molecule) in the skin of subjects with these phenotypes [113].

The understanding and quantification of racial differences in skin functions are important for the treatment and prevention of skin diseases and skin care. A key feature that characterizes race is skin colour: pigmented skin is different from fair skin in terms of responses to chemical and environmental insults and requires specific skin care. Different risk factors among racial groups for the development of skin disease after exposure to the same insults have been described. The interpretation of pathophysiological phenomena should consider not only anatomical and functional characteristics of ethnic groups but also socioeconomic, hygienic and nutritional factors. Sensitive skin is a complex problem with genetic, individual, environmental, occupational and ethnic implications [114]. Studies have been carried out to evaluate the influence of age and sun-exposure on the main clinical signs of Asian skin ageing [115]. One hundred and sixty Chinese and 160 French age-matched women (age range: 20–60 years old) were clinically examined and scored by the same dermatologist. Facial wrinkles and pigmented spots (on face and hands) were assessed in situ and standardized photographs of the face were taken. Results showed for each facial skin area, wrinkle onset is delayed by about 10 years in Chinese women as compared to French women. Facial wrinkling rate over the years is linear in French women and not linear in Chinese women who appear to experience a fast ageing process between age 40 and 50. Pigmented spot intensity is a much more important ageing sign in Chinese women (30% of women over 40) than in French women (severe for less than 8% of women, irrespective of age). The skin color of Asians ranges from light brown to dark brown, as is more pigmented, the acute and chronic cutaneous responses to UV irradiation seen in brown skin differ from those in white skin of Caucasians [116]. Although limited data are available, it is commonly considered that Europeans and Asians have different skin ageing features. These results require to be confirmed on broad studies [115].

SKIN DISORDERS

Since skin is the largest organ in the body, skin-based diseases are among the most common diseases in the human population, ranging from cancerous to noncancerous diseases caused by infection, inflammation, and autoimmune disorders. The occurrence of skin diseases varies between continents with people being exposed to different

elements. The most common skin diseases found around the world are acne, psoriasis, eczema, keloids, rosacea, alopecia areata, vitiligo (pigmentation disorder), warts, urticaria, pediculosis and leprosy [117].

Cutaneous growths that are found in the pediatric and adolescent population include acrochordons, dermatofibromas, keloids, milia, neurofibromas, and pyogenic granulomas. Treatment of these growths usually involves observation or curettage with electrodessication. Infectious etiologic agents of skin disease include bacteria, fungi, and viruses. Impetigo is a bacterial infection which may present as a bullous eruption or as erosion with a honey colored crust [118].

A disorder autoimmune is alopecia areata which is an inflammatory condition, often reversible hair loss affecting mainly children and young adults. Clinically, round hairless patches appear on the scalp while hair follicles remain intact. This skin disorder is related with the distal part of the human hair follicle immune system, especially with the interacting intraepithelial T cells. The cause of this condition is diverse and seems to involve T cell–mediated immunologic changes, neuropeptides, genetic disposition to autoimmunity, and distress [119].

Acne is another common disorder experienced by up to 80% of individuals between 11 and 30 years of age, and by up to 5% of older adults [120]. It is a common multifactorial disorder of the pilosebaceous follicles, involving sebaceous hyperplasia, follicular hyperkeratinization, hormone imbalance, bacterial infection, immune hypersensitivity and in some cases, there is evidence of genetic influence. Microbial colonization is a factor for the development of acne due to metabolism of the *Propionibacterium acnes* bacteria [121,122]. Therapeutic options include topical as well as oral antibiotics and retinoids. Extreme caution must be used when prescribing retinoids because these agents are teratogenic [118]. Subtypes of acne vulgaris are indicative of the particular cause of the disease, such as acne fulminans or cosmetica. Subclassifications as acne conglobata and acne fulminans are both forms of cystic acne characterized by the formation of deep inflammatory lesions that often cause scarring. Acne can also be further defined by the age at onset, as with neonatal or infantile acne [123]. This condition can be psychologically debilitating and, therefore, proper treatment is of paramount importance. It has been reported a psychiatric disturbance in approximately 30% of dermatology patients. Early recognition and treatment of depression associated with skin disorders can lead to improved therapeutic outcomes and may avert disastrous outcomes, including suicide [124].

Historically, acne-like diseases, such as rosacea, steroid acne, and Gram-negative folliculitis, were considered to be subcategories of acne. However, these diseases are now classified as acneiform eruptions because of the absence of a comedon stage in their pathogeneses. This reclassification may have ramifications on the clinical management of these disorders [123].

Rosacea is an inflammatory condition, predominantly on the face presenting papules and pustules. It is often associated with telangiectasia and a marked tendency to flushing. There may be associated conjunctivitis, keratitis and blepharitis. It is most common in women aged 30–50 years. Sun-screens are usually recommended because photodamage has been implicated in the pathogenesis of rosacea, and sunlight may aggravate the disorder [125].

Gram-negative folliculitis is a sudden eruption of pustular lesions that is often seen in patients taking long-term antibiotics and is commonly mistaken for a flare of acne. Relapse is common, however, and a course of oral isotretinoin has superseded these treatment options [125].

Dermal rashes may be localized or generalized. Treatment of generalized drug eruptions involves elimination of the inciting agent, topical antipruritics, and systemic corticosteroids for severe reactions. Vascular anomalies are most commonly exemplified as port wine stains and hemangiomas. Port wine stains may be treated with pulsed dye laser or may be observed if they are not of concern to the patient or physician. Hemangiomas typically spontaneously regress by age ten; however, there has been recent concern that certain cases may need to be treated [118].

Inflammatory skin diseases account for a large proportion of all skin disorders and constitute a major health problem worldwide. Psoriasis, atopic dermatitis, poison ivy, and eczema are another skin disorders. Contact dermatitis, atopic dermatitis, and psoriasis represent the most prevalent inflammatory skin disorders and share a common efferent T-lymphocyte mediated response. Oxidative stress and inflammation have recently been linked to cutaneous damage in

T-lymphocyte mediated skin diseases, particularly in contact dermatitis [126]. Poison ivy and atopic dermatitis may also present with bullous and vesicular changes. Therapy typically consists of topical emollients; phototherapy is reserved for refractory cases [118]. Perioral dermatitis is commonly seen in women aged 20–35 years. It presents as red papules that form superficial plaques around the perioral area, nasolabial folds and/or lower eyelids. It is minimally itchy. The cause is unknown, though many patients give a history of use of topical corticosteroids, which may provoke the disorder. Oral tetracyclines are the treatment of choice. Topical corticosteroids should be avoided; they may reduce inflammation, but their withdrawal results in a rebound flare [125,127].

Other bacterial infections include erythema chronicum migrans, and cellulitis. Fungal infections include the various forms of tinea and are usually treated with topical antifungals. Viral infections include warts, varicella, molluscum contagiosum, and herpes. Treatment varies from observation or antivirals for varicella to cryosurgery. Finally, scabies and lice are infectious agents that can be treated with permethrin and pyrethrin solutions [118].

In addition, it is known that factors inherent to individuals can affect the permeation of substances. Such factors include age, anatomical site, hydration and damage of the stratum corneum [128].

Recent advances on gender differences have been made in our understanding of these differences in skin histology, physiology, and immunology, and they have implications for diseases such as acne, eczema, alopecia, skin cancer, wound healing, and rheumatologic diseases with skin manifestations. It has been observed that sex steroids modulate epidermal and dermal thickness as well as immune system function, and changes in these hormonal levels with aging and/or disease processes alter skin surface pH, quality of wound healing, and propensity to develop autoimmune disease, thereby significantly influencing potential for infection and other disease states [100]. Other disorders in women's connective tissue mainly in the skin, bone and blood vessels are caused by oestrogen deficiency in the menopause. Numerous studies prove that collagen loss in the postmenopausal years is the cause of alterations such as a thinning of the skin and osteoporosis [129,130]. Immunohistochemical, transmission electron microscopy and computer-assisted image analysis methods have been used to determine the collagen IV content and the epithelial basement membrane in a total of 35 (from 35 to 60 years) women who had been admitted for skin biopsies taken from a site 6 cm above the pubic symphysis. The results shown that type IV collagen content decreased with age after 35 years although the epithelial basement membrane thickness increased, which suggests a reduction in tissue turnover. More research is needed to translate current findings to clinically significant diagnostic and therapeutic applications. These advances will enable us to learn more about disease pathogenesis, with the goal of offering better treatments [100].

It is important mention the skin of the child is more sensitive than that of the adult, so greater care is required in prescribing remedies in order to avoid injury. So, certain dermatoses which affect children exclusively or predominantly include infantile eczemas, papular urticaria, tinea capitis, pyoderma, scabies and angiomas. However, the correct diagnosis is very essential in order to get a successful treatment [129,130]. More than 111 million children are believed to have pyoderma, with many also co-infected with scabies, tinea, or both. These skin disorders cannot be differentiated by ethnicity or socioeconomic status but, in high-prevalence areas, poverty and overcrowded living conditions are important underlying social determinants. Each infection is transmitted primarily through direct skin-to-skin contact. For many Indigenous children, these skin conditions are part of everyday life and rarely directly resulting in hospitalization or death [131-134]. Nowadays, minocycline is a new therapeutic option for pyoderma gangrenosum and sarcoid [135].

Skin diseases commonly seen in the elderly are mainly due to effects of sun damage or vascular disease. Chronically sun-exposed skin becomes thin, loses collagen, and has disrupted elastin and decreased glycosaminoglycans. The result is skin that breaks easily, bruises, sags, irritates easily, and itches. The spots and bumps that patients associate with age are all sun-induced [136-138].

Internal diseases can manifest in a myriad of skin dermatoses ranging from single disorders such as calciphylaxis, cryoglobulinemia, amyopathic dermatomyositis, and Raynaud phenomenon, to spectrum disorders such as the neutrophilic dermatoses and morphea [132]. Factors such as the temperature can provoke disorders in the skin. These temperature-dependent skin disorders have been studied for a long time. Temperature plays a direct role in some of the physical urticarias and is one of several important pathogenic factors in conditions such as Raynaud's

syndrome, cold panniculitis, and cryoglobulinemia. One main role of the skin is in thermoregulation, where cutaneous blood flow, and hence skin temperature, vary widely in order to help preserve core body temperature. In some cases of skin disorders, under extreme conditions, frostbite may occur and prolonged exposure to moderate degrees of heat or cold can result in erythema abigne and chilblains [139,140].

Some common skin manifestations are found too in association with systemic diseases as lupus erythematosus, scleroderma, dermatomyositis, sarcoidosis and diabetes, with other conditions that it is important for the general physician to recognize [133,134]. It has been also evaluated the presence of skin diseases in diabetic children and adolescents. Thirty-six patients with type 1 diabetes mellitus showed skin problems: the most frequent disease was skin infection, followed by necrobiosis lipoidica; this last disorder is linked to the presence of microvascular complications. The skin problems were more frequent in children with long duration of disease than in patients with duration less than 7 years. All the patients who had limited joint mobility showed scleroderma [141]. Results of another study coincide with the results before, about skin microvascular functional alterations in both extremities characterized by an absence of capillary reserve. These results are clinically relevant, since in patients with type 1 diabetes, mortality rates for cardiovascular disease, and especially for ischemic heart disease, are raised in comparison with the general population at all adult ages and in both sexes. This severity of microvascular alterations in these patients could eventually predict the occurrence of cardiovascular disease and earlier mortality [142].

Recently the elucidation of hereditary skin disease genes, by genome analysis, including functional and positional cloning has been studio in many investigations. In more than fifty skin disorders, not only the chromosomal localizations, but also the abnormalities of the disease genes have been identified. Investigative tests such as testing for mutation in the haemochromatosis gene in patients with porphyria cutanea tarda have become important [98]. As a resource for candidate genes, the expressed gene catalogues generated by large scale cDNA sequencing analysis are available. The isolation of disease genes may not directly serve to provide any therapeutic aids; moreover it can help us to understand the pathogenesis and diagnosis of these skin disorders [143-145].

CONCLUSIONS

Skin is the largest organ of the body and protects us from microbes, helps regulate body temperature, and permits the sensations of touch, heat, cold and pain. It is very important to know composition and architecture of healthy and unhealthy skin in order to understand and explain the possibly routes of penetration of drugs throughout the permeability barrier of skin (stratum corneum) with the purpose of offering better treatments.

Transdermal drug delivery is hardly an old technology, and the technology no longer is just adhesive patches. Due to the recent advances in technology and the incorporation of the drug to the site of action without rupturing the skin membrane by using chemical and physical enhancers (iontophoresis, electroporation, sonophoresis, microneedles and nanocarriers), transdermal route is becoming the most widely accepted route of drug administration. It promises to eliminate hypodermic needles for administration of a wide variety of drugs in the future.

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CHAPTER 2

Chemical Enhancers

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Abstract: The transdermal administration of drugs is an effective alternative to conventional methods such as oral or subcutaneous injections, as it overcomes the difficulties associated with these routes. Several methodologies have been developed in order to enhance drug transdermal absorption. Chemical percutaneous enhancers have long been used to increase the range of drugs that can be effectively delivered through the skin. To date, a vast array of chemicals has been evaluated as enhancers. This chapter reviews the principal chemical percutaneous enhancers and their mechanisms of action. The techniques to determine permeation enhancement and their uses in topical/transdermal formulations are also discussed.

INTRODUCTION

The application of preparations to the skin for medical purposes is as old as the history of medicine itself, with references to the use of ointments and salves found in the records of Babylonian and Egyptian medicine. The historical development of permeation research is well described by Hadgraft and Lane [1]. Over time, the skin has become an important route for drug delivery in which topical, regional or systemic effects are desired. Nevertheless, skin constitutes an excellent barrier and presents difficulties for the transdermal delivery of therapeutic agents, since few drugs possess the characteristics required to permeate across the stratum corneum in sufficient quantities to reach a therapeutic concentration in the blood. In order to enhance drug transdermal absorption different methodologies have been investigated developed and patented [2,3]. Examples include the use of drug derivatives, drug-saturated systems, physical techniques such as iontophoresis and sonophoresis, micro needles, biochemical strategies such as liposomal vesicles and enzyme inhibition, and chemical strategies using percutaneous enhancers that facilitate the diffusion of drugs through the stratum corneum.

Chemical percutaneous enhancers have long been used to increase the range of drugs that can be effectively delivered through the skin. To date, a plethora of chemicals have been evaluated as enhancers, but their inclusion in topical or transdermal formulations is limited due to fact that the underlying mechanisms of action of these agents remain unclear. Although different chemicals are employed by the industry as percutaneous enhancers, some of which have several desirable properties, to date none has proved to be ideal. An ideal chemical penetration enhancer should have the following attributes [4]:

- It should be non-toxic, non-irritating and non-allergenic.
- It should work rapidly, and its activity and duration of effect should be both predictable and reproducible.
- It should exert no pharmacological activity within the body.
- It should work unidirectionally; i.e. it should allow therapeutic agents into the body whilst preventing the loss of endogenous material from the body.
- When removed, the skin's barrier properties should return both rapidly and fully.
- It should be compatible with both excipients and drugs.
- It should be cosmetically acceptable and, ideally, odourless and colourless.

MECHANISM OF ACTION OF CHEMICAL PERCUTANEOUS ENHANCERS

There are three major potential routes of percutaneous penetration: appendageal, transcellular (through the stratum corneum), and intercellular (through the stratum corneum). There is a weight of evidence that suggests that passage

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through the intact stratum corneum constititutes the predominant route by which most molecules penetrate the skin [5], as the appendageal route is characterized by a limited available fractional area of 0.1%. In this way, diffusion through the skin is controlled by the particular characteristics of the stratum corneum. In order to obtain a sufficient drug flux and, in turn, the therapeutical objectives in question, an alternative is to use chemical percutaneous enhancers. These substances alter some of the properties of the stratum corneum. The postulated mechanisms of action of enhancers have been reviewed by Williams and Barry [6]. The mechanism by which these molecules operate is not fully understood, but they are thought to act directly on the skin or by producing a modification of the formulation.

Direct Effects of Enhancers on the Skin

The lipid–protein-partititioning theory sets out the mechanisms by which enhancers alter skin lipids, proteins and/or partitioning behaviour [7]:

- They act on the stratum corneum intracellular keratin by denaturing it or modifying its conformation, causing subsequent swelling and increased hydration.
- They affect the desmosomes that maintain cohesion among corneocytes.
- They modify the intercellular lipid domains to reduce the barrier-like resistance of the bilayer lipids. Disruption to the lipid bilayers can be homogeneous when the enhancer is distributed evenly within the complex bilayer lipids, but the accelerant is more likely to be heterogeneously concentrated within the domains of the bilayer lipids.
- They alter the solvent nature of the stratum corneum, thus aiding the partitioning of the drug or a co-solvent into the tissue.

Indirect Effects of Enhancers on the Skin

Chemical enhancers can produce:

- Modification of the thermodynamic activity of the vehicle. The permeation of a good solvent from the formulation, such as ethanol, can increase the thermodynamic activity of a drug.
- It has been suggested that, by permeating through the membrane, a solvent can ‘drag’ the permeant with it, though this concept is somewhat controversial and requires confirmation.
- Solubilising the permeant within the donor (e.g. with surfactants), especially when solubility is very low, as in the case of aqueous donor solutions, can reduce depletion effects and prolong drug permeation.

CLASSIFICATION OF PERCUTANEOUS CHEMICAL ENHANCERS

Different authors have proposed a percutaneous enhancers classification method [8,9] but the diverse properties and various mechanisms of action of chemical enhancers make this a difficult task. For this reason, the classification of percutaneous enhancers is frequently based on the chemical class to which the compounds belong [10,11]. Table **1** shows the principal classes of percutaneous enhancers. The main compounds of each of these chemical classes and their characteristics are detailed in this chapter.

Table 1: Principal classes of percutaneous enhancers.

CHEMICAL CLASS	COMPOUNDS
Water	Water
Sulfoxides and similar chemicals	Dimethyl sulfoxide, Dodecyl methyl sulfoxide
Ureas	Urea
Alcohols	

Table 1: cont....

Alkanols	Ethanol
Fatty alcohols	Caprylic alcohol
Glycols	Propylene glycol
Pyrrolidones and derivatives	N-methyl-2-pyrrolidone, 2-pyrrolidone
Azone and derivatives	Azone® (1-dodecylazacycloheptan-2-one)
Dioxolane derivatives	SEPA®
Surfactants	
Anionic surfactants	Sodium lauryl sulfate
Cationic surfactants	Cetyltrimethyl ammonium bromide
Nonionic surfactants	Sorbitan monolaurate, Polisorbate 80
Zwitterionic surfactants	Dodecyl dimethyl ammoniopropane sulfate
Terpenes	Menthol, Limonene
Fatty acids	Oleic acid, Undecanoic acid

Water

Water is the most natural penetration enhancer [12]. The hydration of the stratum corneum is one of the most important factors in determining the successful penetration of a drug through the skin. For this reason, factors that modify skin hydration affect the permeability of the skin.

The water content of the human stratum corneum is typically around 15–20% of the dry tissue weight, although obviously this varies according to the external environment, in particular with respect to humidity [6]. Occlusion modulates the hydration of the stratum corneum and generally increases the transdermal absorption of drugs. Consequently, when a systemic effect is required from a dermatological formulation, lipophilic vehicles or occlusive patches are employed [13]. However, it has been reported that occlusion does not enhance the transdermal delivery of some hydrophilic compounds, and that occlusion may cause local skin irritation [14].

The mechanism of action by which water increases transdermal drug delivery is unclear, although there is an enormous amount of knowledge regarding the stratum corneum and the effect of water on this structure. The water within tissue can modify the solubility of a drug in the stratum corneum and alter the partitioning of the drug from the vehicle into the skin. This mechanism could partially explain elevated hydrophilic drug fluxes under occlusive conditions, but fails to provide an answer for the hydration-enhanced delivery of lipophilic permeants. Since the principal barrier against transdermal drug delivery is the lipids of the stratum corneum, high water content generated by occlusion or soaking would be expected to cause swelling of the polar head group regions of the bilayers and, consequently, to disrupt these domains. On the other hand, it has been shown that hydration does not alter the packing arrangements of the intercellular lipid bilayers [15]. Another theory holds that the swelling of corneocytes (due to the water absorbed by the cells) has an impact on the lipid structure between the corneocytes, thereby causing disruption to the bilayer packing. Nevertheless, experimental evidence obtained by electron microscopy shows no gross distortion of the lipid domains in a fully hydrated stratum corneum, even though the intercellular lipid bilayers may contain water pools with vesicle-like structures [16]. Moreover, it has been reported that, following one or two days of occlusion, the corneocytes swell as a result of the hydration, the intercellular spaces become distended, and the lacunar network becomes dilated. The distension of lacunae create a continuous “pore pathway” in the stratum corneum through which polar and non-polar substances can permeate easily [17].

Sulfoxides and Similar Chemicals

Dimethylsulphoxide (DMSO) enhances the transdermal permeation of a large number of drugs [18,19]. This compound was one of the first penetration enhancers and is among the most widely studied. It is often used in areas of pharmaceutical sciences as a “universal solvent”, and is employed as a cosolvent in the vehicle of a commercial preparation of idoxuridine used to treat severe herpetic infections of the skin, particularly those caused by herpes simplex. DMSO alone has also been applied topically to treat systemic inflammation, although currently it is used

only to treat animals. The mechanism of action of DMSO has been extensively studied. It has been argued that DMSO promotes permeation by reducing skin resistance to drug molecules or by promoting drug partitioning from the dosage form. Additionally, DMSO denatures the intercellular structural proteins of the stratum corneum [20], and may alter the physical structure of the skin by elution of lipid, lipoprotein and nucleoprotein structures of the stratum corneum [21].

However, some problems are attributed to the use of DMSO as a chemical penetration enhancer. Its effects are concentration-dependent, and a concentration above 60% is generally needed for optimum enhancement efficacy. At these relatively high concentrations, DMSO can cause erythema and wheals of the stratum corneum, and may denature some proteins. The adverse effects reported for twice daily treatment with 90% DMSO for 3 weeks are erythema, scaling, contact urticaria, stinging and burning sensations, and systemic symptoms [22]. A further inconvenient side effect is the foul odor on the breath and garlic-like taste that DMSO produces in patients, which is due to the metabolite dimethylsulphide produced by the solvent.

These problems in the use of DMSO as a chemical penetration enhancer have promoted the employment of compounds that are structurally similar to sulfoxides. Dimethylacetamide (DMAC) and dimethylformamide (DMF) are similar powerful aprotic solvents, but are less potent as penetration enhancers. DMF increases transdermal absorption by increasing both the diffusion and the partitioning of drugs [23,24]. Structural analogues, such as decylmethylsulphoxide (DCMS), have also been used. This compound has been shown to act reversibly on human skin and to exert a concentration-dependent effect. DCMS is a potent enhancer of hydrophilic permeants, but is less effective at promoting the transdermal delivery of lipophilic agents [6].

Urea

Urea is one of the components of the natural moisturising factor (NMF) of the skin. Topical preparations of synthetically manufactured urea can be effective in treating scaly and itchy dry skin conditions including atopic dermatitis, psoriasis and ichthyosis. The skin effect of urea has been attributed to the hydrating abilities of this compound. Urea at high concentrations also has keratolytic properties, which is the case when used in combination with salicylic acid for keratolysis [25]. The enhancer effect of urea is attributed to the increase it produces in the water content of the stratum corneum and its keratolytic properties, but urea itself exerts only a minor penetration enhancer effect. Urea-related compounds have been used to obtain a more potent enhancer effect than urea itself [26].

Alcohols: Alkanols, Fatty Alcohols and Glycols

Ethanol is the most commonly used alcohol for the purpose of enhancing the transdermal penetration of drugs. It frequently forms part of transdermal formulations and is the solvent of choice for use with many patches, as it increases the permeation of a large number of drugs. Ethanol acts as a penetration enhancer through various mechanisms. The permeation of ethanol into the skin can alter the solubility properties of the tissue, and consequently improves the partitioning of a drug into the membrane [27]. Ethanol can also increase the solubility of a drug in a vehicle when employed as the solvent. Furthermore, the rapid permeation of ethanol or its evaporation from the donor phase can modify the thermodynamic activity of the drug within the formulation. In addition, as a volatile solvent, ethanol may extract some of the lipid fraction from within the stratum corneum when used at high concentrations over prolonged periods, thus improving the drug flux through the skin [6]. Apart from ethanol, other alkanols have been studied as percutaneous enhancers. Alkanols act on the permeability of the skin through different mechanisms. The alkyl chain length of the alkanol is an important parameter in the enhancing effect of percutaneous penetration, which appears to increase as the number of carbon units increases, eventually reaching a maximum [28]. In addition, alkanols of a lower molecular weight are thought to act as solvents that enhance the solubility of drugs in the matrix of the stratum corneum. Disruption of the integrity of the stratum corneum by more hydrophobic alcohols almost certainly also contributes to the enhancing effect of these compounds [29].

Structure/activity relationships for fatty alcohol penetration enhancement have been established by analyzing melatonin permeation through porcine and human skin *in vitro* [30]. When the activity of the saturated fatty alcohols of octanol and myristyl was compared, a parabolic relationship was detected and a maximum enhancement effect was established for decanol. There was also a general increase of enhancement activity when one or two unsaturated

bonds were added to the alcohols, but activity dropped when three double bonds were introduced. Phenylalcohols also exhibit enhancement activity, with *in vitro* studies demonstrating that 2-phenyl ethanol, 3-phenyl propanol and cynamil alcohol are percutaneous penetration enhancers of 5-fluorouracil [31].

The molecular complexity of different glycol molecules is a determinant of their efficacy as permeation enhancers. Solubility of the drug in the delivery vehicle is markedly influenced by the number of ethylene oxide functional groups in the enhancer molecule; this modification of solubility may either enhance or retard transdermal flux, depending on the drug and delivery environment in question [32]. Propylene glycol is frequently used as a vehicle for penetration enhancers and exerts a synergistic action when used with other enhancers, such as Azone® and oleic acid. The efficacy of propylene glycol as a permeation enhancer has been questioned, as evidence suggests at best only a very mild enhancement effect for molecules such as estradiol and 5-fluorouracil. Propylene glycol permeates easily through the human stratum corneum, and its mechanisms of action seem to be similar to those suggested above for ethanol. Permeation of the solvent into the tissue can alter the thermodynamic activity of the drug in the vehicle, which would, in turn, modify the driving force of diffusion; the solvent may partition into the tissue and facilitate the uptake of the drug into skin, and there may be some minor disturbance to intercellular lipid packing within the stratum corneum bilayers [6].

Pyrrolidones and Their Derivatives

Pyrrolidones and their derivatives have been used as percutaneous enhancers of hydrophilic and lipophilic drugs. The most common, N-methyl-2-pyrrolidone (NMP), has been widely employed to enhance absorption of many drugs by the skin. 2-Pyrrolidone and NMP have been assessed with respect to enhancing the topical bioavailability of the steroid betamethasone-17-benzoate, using dimethylisosorbide (DMI) as the standard solvent [33]. Pyrrolidones produce greater stratum corneum reservoirs than DMI, but are less suitable for clinical use due to their irritation potential [34].On the plus side, pyrrolidones partition easily into the human stratum corneum, where they alter the solvent nature of the tissue.

The influence on skin permeation of several enhancers prepared with 2-pyrrolidone containing a short alkyl group at the 1 position and a dodecyl group at the 3 position of the pyrrolidone ring has been studied. Results showed that the length of the short alkyl group at the 1 position considerably influenced the enhancing activity, and 1-propyl and 1-butyl-3-dodecyl-2-pyrrolidone proved to be effective enhancers of the penetration of indomethacin [35]. An almost semilog linear relationship between enhancement potency and carbon number of the alkyl chain was observed. These results suggest that the enhancer action resides in the alkyl group and that the nature of the polar head group may not be intrinsically important for this type of permeation enhancers [36].

Azone® and Derivatives

Azone® is the registered trademark of the chemical compound laurocapram (1-dodecylazacycloheptan-2-one), which was specifically developed as a chemical penetration enhancer and was patented in 1976. Azone® is compatible with most organic solvents and is an excellent solubilizer of a wide variety of drugs. It is of a high chemical stability and excipient compatibility. Safety studies conducted with Azone® in human subjects have shown that this compound is not irritating or allergenic when applied to human skin [37]. Azone® is an effective percutaneous enhancer for hydrophilic and lipophilic drugs [38]. The enhancing effect produced by Azone® depends strongly on the concentration (it is effective only at relatively low concentrations of 1-10%), the vehicle used, the level of skin hydration (occluded/non occluded) and other factors. Furthermore, as explained previously, the enhancer effect of Azone® increases when it is used in combination with propylene glycol.

The effect of Azone® on the fluidity of the lipid fraction of the stratum corneum has been thoroughly studied [39, 40, 41, 42]. It has no direct effect on the stratum corneum proteins; rather, it increases the moisture content of the stratum corneum. It is directly partitioned into the lipid bilayer, whose fluidity it increases, hence promoting the penetration of the drug (Fig. **1**).

The chemical structure of Azone® is considered to be a hybrid of two potent permeation enhancers; pyrrolidone and decylmethylsulphoxide. The polar ring and the long alkyl chain present at position 1 contribute to its action. Nevertheless, the compounds obtained by replacing this long alkyl chain by different terpenes, which are analogues

to Azone®, exert an enhancing effect on the transdermal absorption of mitomycin C in hairless mouse and rat skin [43]. Derivatives with a C_{10} carbon terpene chain and an azacyclo ring with one carbonyl group also produce enhancing effects. An increase in the length of the terpene chain and the number of carbonyl groups in the compound reduces this activity. Derivatives with an alkyl chain induce more severe primary irritation than those with a terpene chain [44]. Furthermore, Azone® derivatives with varying azacyclo ring nuclei (maintaining a constant side alkyl chain) were investigated for their permeation enhancing properties with respect to six drugs with varying lipophilicities. N-Dodecyl-2-pyrrolidinone and N-dodecyl-2-piperdinone were shown to be the most effective for hydrophilic drugs [45].

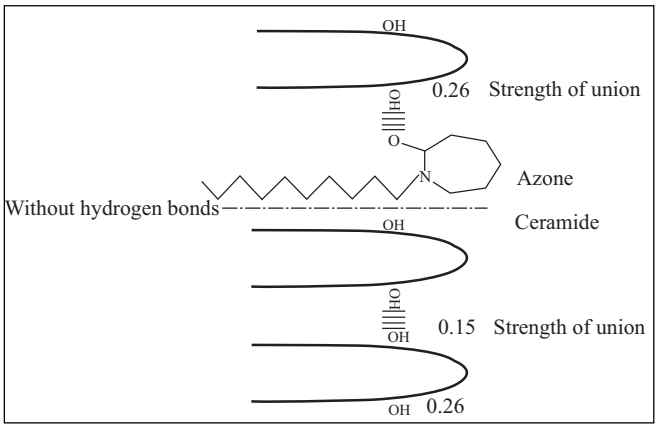

Figure 1: Schematic representation of the mechanism of action of Azone®.

Dioxolane Derivatives

A family of chemical enhancers using dioxolanes and dioxanes known as SEPA® have been specifically designed and synthesized to act as percutaneous penetration enhancers. SEPA is an acronym for "Soft Enhancement of Percutaneous Absorption," where "soft" refers to a temporary and reversible absorption enhancement due to the rapid breakdown of the enhancer. The SEPA family includes several compounds commonly used in the flavour and fragrance industries. All consist of only carbon, hydrogen and oxygen molecules (they do not contain nitrogen) in order to minimize the risk of being metabolized to potentially toxic compounds. One of these SEPA® molecules, 2-n-nonyl-1,3- dioxolane, was selected for development after *in vitro* diffusion studies demonstrated it to produce the greatest degree of enhancement. The enhancement effect of SEPA® is attributed to an increase in the mobility of the hydrocarbon chain of skin lipids or the disruption of the lipid layer, coupled with a modification of protein hydrophobic interactions [46]. SEPA® is used to enhance some drugs *in vivo* [47].

Fatty Acids

A large number of fatty acids have been reported to be effective enhancers of the percutaneous absorption of a wide range of drugs [48, 49, 50, 51]. The efficacy of these enhancers is related to their structure [52,53]. Table **2** shows the classification of fatty acids as a function of their chemical structure. A recent review has detailed the different fatty acids that have been studied as skin penetration enhancers and the factors governing their activity as such [54].

Table 2: Classification of fatty acids as a function of their chemical structure.

FATTY ACIDS	COMPOUNDS
Saturated	
	Caprylic acid
	Capric acid
	Lauric acid
	Myristic acid
	Palmitic acid

Table 2: cont....

Unsaturated		
	Monounsaturated	Oleic acid
		Elaidic acid
	Polyunsaturated	α-Linoleic acid
		γ-linolenic acid
		α-Linolenic acid
Branched		
		10- methyl palmitic acid
		7- methyl octanoic acid
		9- methyl decanoic acid
		8-ethyl decanoic acid
		10- ethyl dodecanoic acid

The skin perturbation effects of a number of fatty acids - namely, straight chain saturated, monounsaturated and polyunsaturated acids - have been demonstrated with the human stratum corneum. Aungst *et al.* studied the enhancing effect of the carbon chain length of fatty acids on naloxone penetration through human skin *in vitro* [51], and the enhancer effect of fatty acids on the percutaneous absorption of propanolol has also been evaluated [55]. Maximum skin penetration enhancement has been observed with fatty acids with a chain length of approximately 12 carbons [51]. Fatty acids with shorter chains are likely to have insufficient lipophilicity for skin permeation, whereas those with longer chains are sure to have a much higher affinity for the lipids in the stratum corneum, thereby delaying their own permeation and that of other permeants. Unsaturated fatty acids, particularly those of *cis* conformation and C18 chain lengths, have been shown to be more effective enhancers of the permeation of naloxone across human skin than their corresponding saturated fatty acids. As the number of double bonds increases from one (oleic acid) to two (linoleic acid), there is a substantial increase in the flux of naloxone. However, an increase in the number of double bonds to three (linolenic acid) does not produce a further increase in the flux [51]. The presence of double bonds in the structure is thought to cause the formation of kinks in the lipid structure of the stratum corneum, thereby altering the ordered lipid array [50] and forming separate fluid states that disrupt the endogenous lipids [56,57].

The ratio of the delta/omega chain length of the cis-unsaturated fatty acid determines the efficacy of these compounds as percutaneous penetration enhancers. This ratio suggests that skin distribution increases as the position of the double bond shifts towards the hydrophilic end [58].

Among unsaturated fatty acids, oleic acid is reported to be an effective skin penetration enhancer for polar and non-polar drugs [39]. Oleic acid is both GRAS-listed and included in the FDA Inactive Ingredients Guide [59]. Oleic acid, together with its methyl and ethyl esters, is the subject of a large proportion of the registered patents of fatty acid transdermal enhancers [60,3]. It has been shown to be a more potent penetration enhancer when combined with propylene glycol [61], inciting drastic alterations in the membrane structure and causing defects in the interface between solid and liquid domains that can reduce either the diffusional path length or the resistance of the stratum-corneum [62]. Furthermore, a synergist effect has been established for unsaturated fatty acids and benzyl alcohol [63].

The enhancing effects of the shorter chain non-terminal type of branching fatty acids have been shown not to differ significantly from those of linear fatty acids of the same carbon number [64]. Larger terminal-branched fatty acids produced a greater disruption of the lipid chain packing than the linear-chain, and exerted a greater influence on their permeation enhancer effect. Terminal ethyl branching has been shown to be more effective than methyl branching [65] with respect to permeation enhancement; the incorporation of such chains into the stratum corneum lipid lamellae demands more space and, thus, causes a more pronounced disturbance of the skin. Moreover, it has been reported that branching near the polar head decreases enhancing activity. Such branching can sterically hinder the polar group and decrease its hydrogen bonding ability [66].

Surfactants

Surfactants are used as emulsifiers and as physical stabilizing, wetting and suspending agents in many topical pharmaceutical and cosmetical formulations and agro-chemical preparations. It is well known that surfactants have effects on the permeability characteristics of several biological membranes, including the skin [67]. For this reason, they have been employed to enhance the permeation rates of several drugs, which make the extent to which human skin is exposed to these chemicals of relevance.[68,51]. Surfactants are typically composed of a lipophilic alkyl or aryl fatty chain together with a hydrophilic head group. Surfactants are often classified as anionic surfactants, cationic surfactants, non-ionic surfactants and zwitterionic surfactants according to their hydrophilic head group.

Cationic surfactants are more destructive to skin tissue then other types, exerting a greater enhancing effect than, for example, anionic surfactants. In turn, the anionic type produces a greater enhancement than its non-ionic counterparts [69]. Anionic and cationic surfactants have the potential to damage human skin, as they swell the stratum corneum and interact with intercellular keratin. Non-ionic surfactants, on the other hand, are widely regarded as safe. Consequently, anionic and non-ionic surfactants have received the most attention as percutaneous enhancers. The former type seems to function by altering the barrier function of the stratum corneum. Sodium lauryl sulphate (SLS) is an anionic, amphiphilic surfactant extensively used in consumer products and for industrial purposes. However, its widespread topical use can cause irritation. The effect of SLS on the skin has been extensively studied, and has been attributed to the removal of intercellular lipids and subsequent increase in trans-epidermal water loss [70,71]. SLS also binds extensively to intracellular keratin, which explains some of its irritant effects, such as tightness and roughening of the skin [72]. Furthermore, SLS fluidizes the lipid bilayers in the stratum corneum and inserts itself between the lipids [73]. Borrás-Blasco *et al*. reported that SLS increased the penetration rates of compounds with a lower-than-optimum lipophilicity value ($\log P_{oct}<3$) but did not affect penetrants with a $\log P_{oct}$ above this value [74].

Of the major classes of surfactants, non-ionics have long been recognized as the least toxic and with the lowest irritant potential, which is why they are widely used in topical formulations. They also have an effect on the permeability characteristics of the skin [75,76]. These properties endow non-ionic surfactants with the potential to be effective penetration enhancers for use in transdermal delivery systems [77].

The hydrophobic portion of non-ionic surfactants usually consists of alkyl or acyl chains that are attached to a polar head group, which in many non-ionic surfactants is a polyoxyethylene chain. Many reports have focused on the importance of the length of the alkyl chain with respect to the potency of surfactants as penetration enhancers [78,79]. One can deduce from these reports that the strongest effects are observed with molecules with a C12 alkyl chain. However, it is not yet clear how the polar head group influences the activity of surfactants as enhancers. For instance, reports of surfactant-induced alterations in the permeability of biological membranes to alkyl ether ethoxylates (Brij®) and nonyl phenol ether ethoxylate surfactants indicate that the length of the ethoxy chain is important. In fact, a parabolic relation was observed and was relative to the degree of ethoxylation [78,79].

Polysorbates are another kind of surfactants that are widely employed in the preparation of pharmaceutical formulations, as they have been shown to enhance the permeation of some drugs [80,81]. Cappel and Kreuter compared the potential of several polysorbates as enhancers of the transdermal penetration of methanol and octanol. Positive effects were observed only for the permeation of methanol, with the more lipophilic polysorbates 21 and 81 altering the barrier properties of the skin to a greater extent than their hydrophilic analogs [81]. Polyoxyethylene alkyl ethers and esters have been shown to be more effective enhancers of permeation than polysorbates [77].

A study involving several series of chemical enhancers has demonstrated that their potency as enhancers is essentially independent of their polar functional group, except in the case of n-alkyl-azacycloheptanones [82]. In contrast, a study of non-ionic surfactants and Azone® revealed that the nature of the enhancer head group allowed it to exert an important influence on cutaneous barrier impairment [76].

In general, the effect of a surfactant on membrane permeability is the result of its interaction with the membrane and that of the permeant with the micelle. Therefore, the concentration of the surfactant is of key importance to enhancement activity [83,84].

Terpenes

Terpenes have been used for a number of therapeutic purposes including antispasmodics, carminatives, antiseptics, flavouring agents and perfumery. Terpenes are found in essential oils, many of which are employed in aromatherapy. These compounds have been extensively used as percutaneous chemical enhancers to enhance the permeation of both lipophilic and hydrophilic drugs [85].

Terpenes are classified by the FDA as generally safe (GRAS). They cause no skin toxicity and, if any, only mild irritation [86,87]. Moreover, though considered to be skin irritants, they do not cause lasting erythema [88].

The chemical structure of terpenes consists of repeated isoprene (C_5H_8) units. Table **3** provides a detailed classification of the different terpenes according to the number of isoprene units (i.e. monoterpenes have two isoprene units (C_{10}), sesquiterpenes have three (C_{15}), and diterpenes have four (C_{20})) and chemical groups (i.e. hydrocarbons, alcohols, esters, ketones, phenols, ethers and oxides). Additionally, terpenes can also be classified as linear, monocyclic or bicyclic.

Table 3: Classification of terpenes as a function of their chemical structure.

TERPENES	
Number of isoprene units	
C_{10}	Monoterpenes
C_{15}	Sesquiterpenes
C_{20}	Diterpenes
C_{25}	Sesterterpenes
C_{30}	Triterpenes
C_{40}	Tetraterpenes
Chemical groups	
Hydrocarbons	D-Limonene
Alcohols	Linalool, Geraniol
Aldehydes	Cinnamic aldehyde,
Ketones	Carvone, Thujone
Phenols	Eugenol, Thymol
Ethers	Cinelol, Anethol
Oxides	1,8-cineole

Aqil *et al.* have reviewed the status of terpenes as penetration enhancers, which is related to their chemical structure and the physico-chemical properties of the drug in question, such as lipophilicity, size and chirality, boiling point, energy of vaporization and degree of instauration [89]. In relation to their size, smaller terpenes tend to be more active permeation enhancers than their larger counterparts. Furthermore, hydrocarbon and non-polar terpenes such as limonene seem to be particularly good enhancers of lipophilic permeants such as indomethacin. Conversely, polar terpenes (such as menthol, 1,8-cineole) provide a better enhancement of hydrophilic permeants. Such a relationship tends to imply that one of the mechanisms of these agents is their modification of the solvent nature of the stratum corneum, by which drug partitioning into the tissue is improved. However, limonene (a non-polar terpene) produces a greater enhancement effect for the hydrophilic permeant sumatriptan succinate than for bisabolol and 1,8-cineole [90]. Many terpenes permeate easily through the human skin [91], with large amounts being found in the skin after its application via a patch [92]. This permeation can alter the thermodynamic activity of the permeant in the formulation, as terpenes are generally good solvents. Terpenes may also modify drug diffusivity through the membrane; studies have indicated that D-limonene and 1,8-cineole disrupt stratum corneum bilayer lipids, whereas nerolidol, a long chain sesquiterpene, reinforces the bilayers, possibly by positioning itself alongside the stratum corneum lipids [93].The effect of 1,8-cineole and menthol on stratum corneum lipids and the permeation of

zidovudine across human cadaver skin was studied by Narishetty and Panchagnula. They concluded that terpenes enhance the transdermal permeation of zidovudine and other drugs by disrupting the highly ordered subcellular packing of stratum corneum lipids [94]. A recent review of the potential mechanisms of action of terpenes as penetration enhancers has highlighted an increase in the solubility of drugs in skin lipids, disruption of lipid/protein organization and/or extraction of the skin micro constituents that are responsible for maintenance of barrier status [95].

TECHNIQUES TO DETERMINE PERMEATION ENHANCEMENT

The great majority of studies of the effects of enhancers on skin permeability have been carried out by means of *in vitro* diffusion experiments in which various kinds of diffusion cells have been used. The most well-known of these cells are the Franz diffusion systems. These cells have two receptor compartments - donor and receptor (donor positioned above receptor) – between which the skin is placed. In general, the skin is pretreated with a solution of the chemical enhancer to be evaluated. The transdermal flux (J) of drugs can be estimated from the slope of the linear region (steady-state portion) of the accumulated amount of drug in the receptor compartment versus time plot. Permeation enhancing activity, expressed as enhancement ratio of flux (ER_{flux}), is determined as the ratio between the flux value obtained with the chemical enhancer and that obtained with the control.

A number of variables can strongly influence the permeation enhancement of drugs. The most important are the skin used in the experiments, temperature, humidity, enhancer concentration, vehicle employed and degree of saturation of the drug in the donor and receptor compartments.

Human skin is usually considered the best choice for *in vitro* diffusion studies, but can be difficult to obtain. Therefore, the use of animal models is sometimes necessary. Different animal models, including rat, pig, mouse, monkey and snake, have been used in diffusion experiments. However, it is important to take into account the validity of the model in question, as permeability can vary depending on the species used. For example, if the literature regarding the effects of water on transdermal permeation is consulted, we can see how different species respond in different ways. Bond and Barry showed that hairless mouse skin is unsuitable as a model for imitating the human stratum corneum to examine hydration effects; rodent skin permeability rose over 50-fold with respect to human skin membranes when both models were hydrated for 24 h [96]. In this way, the literature reporting enhancing effects on skin permeability using animal models should be viewed with some caution.

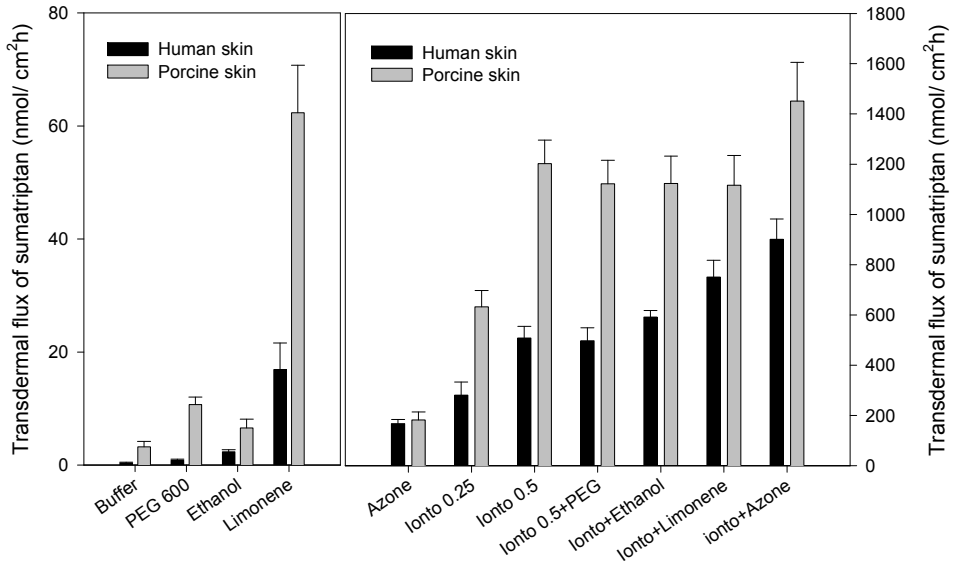

Figure 2: Comparison of the transdermal flux of sumatriptan across human and porcine skin.

The application of chemical enhancers and other physical methods such as iontophoresis have traditionally been evaluated separately, but more recently the combined application of different strategies has been evaluated with the aim of enhancing transdermal drug transport as much as possible [97,98,99]. In our laboratory, we have investigated the effect of the percutaneous enhancers ethanol, PEG 600, limonene, Azone® and iontophoresis (0.25 and 0.5 mA/cm^2) on the transdermal flux of sumatriptan [100]. We have used human and porcine skin in order to compare the results in two species. Our findings are shown in Fig. **2**, which shows that porcine skin was more permeable than human skin. Although the results obtained with porcine skin reproduce the sequence of those obtained with human skin, a more in-depth analysis reveals some differences. In relation to porcine skin, ethanol and PEG 600 did not produce any significant increment of the transdermal flux of sumatriptan with respect to the control. This shows that porcine skin is more permeable than human skin but less sensitive to slight variations in transdermal flux.

It should be stressed that, although porcine skin does not reproduce exactly the same results as those obtained with human skin, a linear correlation does exist between the transdermal fluxes observed in the two membrane models. Even though some of the values deviate from predicted values (see Fig. **3**), the correlation is statistically significant ($r > 0.964$). The value of the slope of the correlation is 1.79 ± 0.10. Therefore, the fluxes observed with porcine skin should generally be 1.8-fold higher than those observed with human skin.

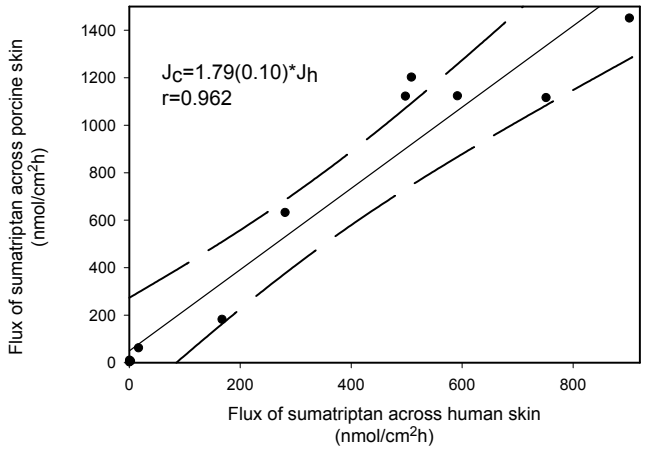

Figure 3: Lineal correlation between the sumatriptan transdermal flux values obtained with human skin and porcine skin. Dashed lines represent the 95% confidence interval of the regression.

It is important to point out that most studies of chemical percutaneous enhancers have been performed with the enhancer in solution and by applying a pretreatment of the skin with a chemical enhancer solution. For this reason, when evaluating the enhancing effect and irritation potential of a penetration enhancer with respect to a certain drug, the influence of said enhancer on the overall performance of the formulation must be properly assessed. The delivery of the formulation/patch or production method can cause the enhancer activity of the chemical compound to vary. The compatibility of a percutaneous enhancer with the drug and other excipients (release liner, backing membrane, etc.) should also be evaluated. The effect of the enhancer on the stability and performance of the transdermal product throughout its shelf life needs to be assured.

In addition, it should be kept in mind that an enhancer can increase the permeation of not only the drug but also the other excipients in a formulation. Ideally, an enhancer should selectively increase the permeation of the drug, as the permeation of other excipients tends to cause local and systemic toxic reactions.

USES IN TOPICAL/TRANSDERMAL FORMULATIONS

A remarkable amount of research concerning chemical enhancers is available [101,102] and it has been reviewed in this chapter, some examples of drugs delivered thorugh out the skin using chemical enhancer are shown in Table **4.**

In addition, a great deal of work has been carried out by pharmaceutical companies whose results tend to be converted into patents rather than scientific publications. Ahad *et al*. have recently published a comprehensive review of the efficacy of penetration enhancers in recent patents [103].

Table 4: Examples of drugs delivered through out the skin using chemical penetration enhancers.

Drug	**Chemical enhancer used**
Sodium salicylate [104,105] Sodium naproxen [106] Ibuprofen [45,107] Nonivamide acetate [108] Meloxicam [109] Flurbiprofen [110] Naloxone [111] Nortriptyline hydrochloride [112] Furosemide [113] Methotrexate [37] Sumatriptan succinate [114]	Azone®
Sodium naproxen [106] Sodium diclofenac [115] Lidocaine [116] Testosterone [117] Mometasone furoate [118] Ketorolac [119]	Transcutol ®
Haloperidol [120] Indomethacin [121] Leuprolide [122]	Urea
Tizanidine hydrochloride [123] Minoxidil [124] Metopimazine [125] Nortriptyline hydrochloride [112,126]	Alcohols
Lidocaine [127] Bupranolol [128] Propanolol [129] Acyclovir [130]	Pyrrolidones
Tizanidine hydrochloride [123] Daphnetin [131] Nitrendipin [132]	Fatty acids
Diclofenac [133] Nortiptyline hydrochloride [112] Verapamil hydrochloride [134] Minoxidil [124]	Terpenes
Retinol [135] Morphine [136] Arginine vasopressin [137] Insulin [99] Enoxacin [138]	Surfactants

CONCLUSIONS

The authors concluded that fatty acids are the most promising class of penetration enhancers because of their similarities to skin lipid architecture. Another promising group of chemical enhancers are terpenes, which are non-toxic, non-irritant and are generally regarded by the FDA to be safe. Terpenes have been applied to promote the percutaneous absorption of hydrophilic and lipophilic drugs, as in the case of sulfoxides such as DMSO and DMF. DMSO and DMF are some of the earliest chemical penetration enhancers, but there are many reports of their high irritation potential. Other compounds such as Azone®, surfactants and polyols have also been documented and

patented [3,103]. Williams and Barry remark that many of the chemicals described above are used in combination in topical and transdermal formulations for varying purposes. For instance, a topical preparation may contain propylene glycol as a vehicle, a surfactant to solubilize the drug and a terpene as a fragrance material. The efficacy of some commercially available topical formulations is a result of the penetration enhancement produced by these agents, though manufacturers do not tend to recognize that they employ excipients specifically for this purpose [6].

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CHAPTER 3

Transdermal Iontophoresis

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Abstract: Among the different methods of incrementing the transdermal absorption of drugs, iontophoresis has been the focus of considerable research. The fundamentals of iontophoresis are presented in this chapter along with the factors that need to be considered in order to apply this technique effectively (i.e. current density, ionic strength, and pH). The main applications of iontophoresis for the topical and systemic delivery of drugs are also discussed. Finally, the usefulness of reverse iontophoresis is addressed.

INTRODUCTION

Transdermal administration of drugs represents an interesting alternative to oral and parenteral routes. Nevertheless, the stratum corneum constitutes a significant barrier to the entrance of any drug into the body. Transport through the skin highly depends on the physicochemical properties of the molecule in question, with the most important factors being lipophilicity, molecular weight and solubility. The number of drugs whose characteristics allow them to permeate through skin is low, and, consequently, an enhancement approach may need to be employed to achieve the objective of topical or transdermal delivery. Among the different techniques that can be used to enhance transdermal permeation, that of iontophoresis has attracted the attention of many researchers.

Iontophoresis facilitates drug passage through the skin by means of the application of a low density electrical current. Compared with other techniques used to increase transdermal absorption, iontophoresis is advantageous in certain circumstances:

1. The application of an electric field across the skin mainly enhances the transport of hydrophilic molecules in the ionic state, and accordingly represents an aid to peptide and oligonucleotide drug administration [1,2].
2. The drug can be administered using an aqueous solution; a specific formulation is not strictly required.
3. There are different approaches that can be employed to apply a current. Iontophoresis has been put forward as a means for the controllable and reliable administration of drugs through the skin.
4. Iontophoresis considerably reduces inter and intra-individual variability, since the rate of drug delivery is more dependent on the current applied than on the characteristics of the stratum corneum [3].

THEORETICAL BASIS

Iontophoresis consists of the application of a low density current and low voltage via an electrical circuit constituted by two drug reservoirs (anode and cathode) deposited on skin surface. During application of the current, the drug is repelled by the corresponding electrode and pushed through the stratum corneum. During application of an electrical current, a substance can pass through the skin by electromigration, electroosmosis or passive diffusion (Fig. **1**). The latter of the three mechanisms is a result of changes caused by the electric field to the permeability of the skin, and its effects are negligible compared with those of the other two mechanisms.

Skin is a complex membrane and controls the movement of molecules across it in the presence of an electric field. Skin has an isoelectric point (pI) of 4–4.5. Above this pH, the carboxylic acid groups are ionized. Therefore, at higher pH values, the skin behaves as a permselective membrane which especially attracts cations that have been repelled by the anode, thus favouring the passage of molecules by electromigration [4,5]. The movement of small-sized cations (mainly Na^+) generates a solvent flow that promotes the passage of non-charged molecules through the

José Juan Escobar-Chávez (Ed)

skin. This process is identified as electroosmosis [6,7]. Electrical mobility decreases with molecular weight, and, as a consequence, the electroosmotic contribution becomes increasingly important for larger molecules [8].

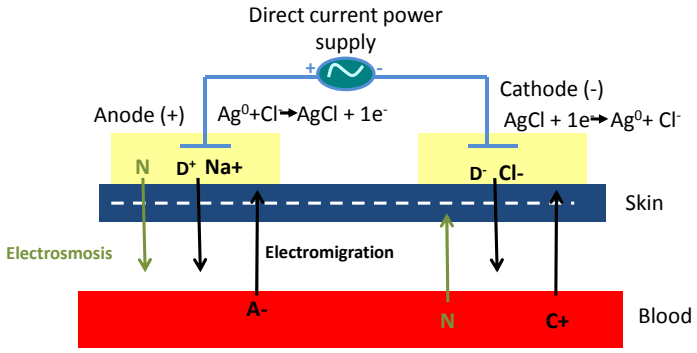

Figure 1: Diagram of the direction of movement of substances during application of iontophoresis. Ions are repelled by the electrode of the same charge and attracted by the electrode of the opposite charge (electromigration). Neutral substances are transported with the solvent flow (electroosmosis), which at physiological pH favours the movement from the anode to the skin.

The dependence of iontophoretic flux on the intensity of the current applied has been clearly demonstrated by Faraday's law [9-11]: $J_a = (t_a I)/(Z_a F)$ where J_a is the flux (in moles per unit time), t_a is the transport number, Z_a is the valence of ion a, I is the current applied (Amperes), and F is Faraday's constant (Coulombs/mol). The transport number, t_a, is the fraction of the total current transported by a specific ion, and is a measure of its efficiency as a charge carrier: $t_a=I_a / I$. It follows that knowledge of a compound's transport number allows the feasibility of its iontophoretic delivery or extraction to be predicted. The sum of the transport numbers of all the ions present during iontophoresis equals 1 ($\Sigma ti=1$), illustrating the competitive nature of electrotransport. Since small ions are more mobile than the drug to be administrated, they compete to drive the current; consequently, the drug's flux through the skin is highly dependent on the concentration of the different ions in the solution [12].

The optimal situation for iontophoresis is the so-called "single-ion" case, in which competing co-ions are absent [13]. Under these circumstances, the ion of interest competes only with the endogenous counter-ions, and its transport number (flux) attains a maximum value that is independent of the ionic concentrations [14-16]. However, in practice, this ideal situation is difficult to establish, because pharmaceutical formulations frequently include charged additives (buffering agents, viscosity modifiers and preservatives) which decrease the efficiency of the transport of the ion in question [17]. To reduce this competing effect as much as possible, additives (buffers) should be of a large molecular size (i.e. HEPES (N-[2-Hydroxiethyl] piperazine-N-[2-ethanesulfonic acid]) instead of phosphates as buffers).

It follows that the transport efficiency of an ion depends on its physicochemical properties, which determine its mobility, and the corresponding characteristics of the co-ions and counter-ions present, whereas the concentration of the drug solution does not seem to be an important factor.

Considering the above equation, when the concentration of the competing ions is constant, if the current is doubled, the number of ions transferred across the skin is increased by a factor of two. Similarly, if the current is turned off, ion flow through the membrane should return to the passive level. Even though the drug may carry only a fraction of the total charge (or may even be primarily transported by electroosmosis), its flux will be directly proportional to the density of the applied current.

Although increasing the current produces an increase in iontophoretic transport, the response can plateau at higher current levels, suggesting the presence of a saturation phenomenon. Once a maximum transport number is achieved, further increases in current have no effect [13].

It has been demonstrated that a reduction of the concentration of competing ions in the solution has a greater impact on drug flux than an increment of current density (Fig. **2**) [18].

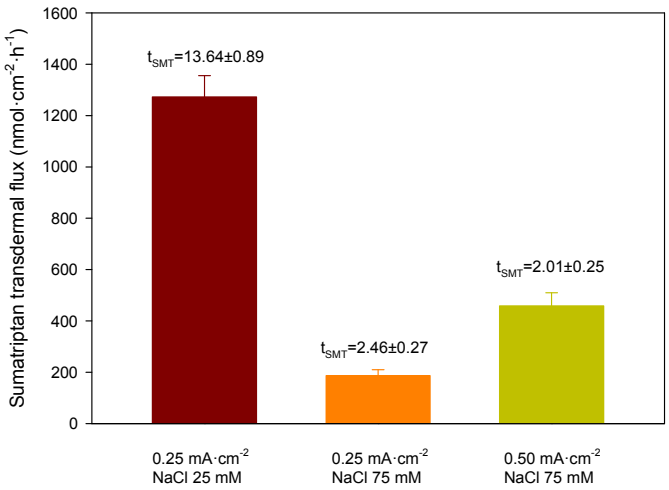

Figure 2: Sumatriptan transdermal flux and transport number (t_{SMT}) obtained at different current densities and NaCl concentrations in the donor.

Another parameter that requires attention is pH value. The pH of the drug-containing solution in contact with the skin has an impact on iontophoretic transport through two distinct mechanisms. It has an effect on the degree of ionization of the drug; an increase of pH will reduce the ionized fraction of weak bases and result in a decreased electromigratory contribution to the iontophoretic transport. However, the electroosmotic solvent flow is increased when the skin pI is surpassed. Therefore, increasing the pH can affect the iontophoretic delivery of a weakly basic molecule in two opposing ways. In this way, as we approach the pKa of the drug, the contribution of electromigration to the transport of the drug will be reduced; however, at a higher pH, (above skin pI), electroosmosis begins to contribute as the membrane becomes completely ionized. The relative impact and net effect of these opposing trends depend on the physicochemical properties of the molecule in question [4]. On the other hand, low and high pHs will lead to substantial concentrations of hydroxonium and hydroxide ions, respectively. These ions have high electrical mobilities and, at elevated concentrations, can cause significant reductions in the iontophoretic delivery of larger drug molecules with lower mobilities that may be present at low concentrations, as detailed before.

The density of current usually employed in iontophoresis is below 0.5 mA/cm^2. Such densities are well tolerated [19] and generate voltages in the range of 1-10 V. Acceptable levels of current density and total current are dependent on the treatment area and duration of current passage [20, 21]. Iontophoresis is associated with minor local effects that disappear after the application of a current, such as erythema or tickling [22].

Research in the field of iontophoresis has evaluated both constant current and constant voltage, and the first of the two has been shown to be preferable. During current application skin resistance (R) decreases; considering Ohm's law (V=I*R), if the voltage (V) remains constant, the intensity (I) must increase. Since the magnitude of I determines the drug flux, this situation implies a loss of control in the process. In order to control drug delivery, either a constant or pulsed direct current can be applied between the two electrodes placed on the skin surface.

An iontophoretic dose is usually expressed in milliampere*minutes (mA*min), which is the total dose delivered and corresponds to the product Current × Treatment time. A typical iontophoretic dose is 40 mA*min (4 mA*10 min application), but can vary from almost 0 to 80 mA*min [23]. The application time never usually exceeds 20 minutes because, otherwise, the skin may be damaged.

After iontophoresis, the time required for restoring skin resistance to basal levels depends on the duration of application of the current and its density [20, 21, 24].

During the passage of a drug through the skin under the influence of electric current, a penetrant takes the pathways of lowest electrical resistance - in other words, an appendageal pathway (i.e. a skin appendage, such as a hair follicle) or can pass through a non-appendageal pathway.

The contribution of follicular versus non-follicular transport depends primarily on the physico-chemical properties of the penetrant and the nature and properties of the membrane in question. Normally, the former predominates in accordance with the established fact that ions tend to take the path of least resistance. This has been proven by laser scanning confocal microscopy and the vibrating probe electrode technique [25]. The non-appendageal route has been identified as the intercellular pathway consisting of polar regions in the lipid lamella, which has been confirmed using electron and fluorescence spectroscopy [26].

IONTOPHORETIC DEVICES

The components of an iontophoretic installation include a direct current (DC) power supply, a milliamperimeter, a timer, a rheostat and 2 electrodes. In the design, production, combination and validation of these components, safety, convenience, reliability and reproducibility are essential.

The DC sources of commercially available iontophoretic units are based either on line power or on one-way or rechargeable batteries. Currently available systems include an operator-controlled current, dosage and time of application delivery (by means of a keypad) and a fuse for protection against system malfunction if the current exceeds 4 mA.

Apart from the power source, the electrodes are the most critical parts of the device. The electrodes conventionally used in iontophoresis can be classified as inert electrodes (metals such as stainless steel, platinum, carbon or aluminium) or reversible electrodes (Ag/AgCl). Although the so-called 'inert' electrodes are indeed inert with respect to taking part in the electrochemical reactions, they are known to cause electrolysis of water, which lead to pH shifts [27] that can interfere with the drug passage.

One electrode is connected to an adhesive drug reservoir patch, constituted by a sponge that is filled with the solution at the time of application, and the other electrode is linked to a gel return patch (placed a few centimeters apart) that closes the circuit. To properly administrate the drug by means of iontophoresis it is important to avoid leakage of the solution. In this sense, there are different patch sizes, each of which corresponds with a volume capacity. There are also patches that combine anode and cathode in a unique adhesive system (Fig. **3**), such as the disposable 24-hour iontophoresis patch (Companion 80®, Iomed Inc, USA), which delivers a 80 mA*min dose over a 24 hour period. This system has an area of 6.45 cm^2, and a textile reservoir with of capacity of 1.1 mL. Depending on the characteristics of a drug, the reservoir must be connected to the anode or cathode. Both kinds of systems are versatile and allow the administration of a great variety of drugs.

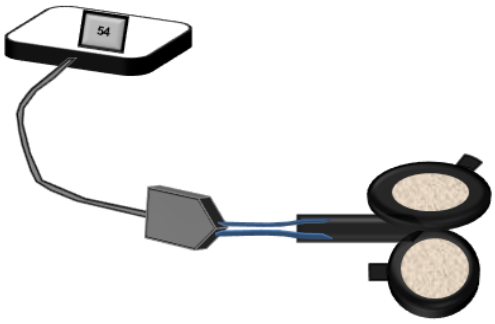

Figure 3: Iontophoretic device: The electrodes are a patch system.

Also available are disposable systems loaded with a particular drug, such as LidoSite® (Vyteris Inc, USA), which includes a 2% lidocaine and 0.01 mg/mL epinephrine solution used to achieve local anaesthesia, and the fentanyl iontophoretic transdermal system Ionsys® (Ortho-McNeil/Alza Corp/JNJ, Mountain View, CA) for the control of acute pain.

DIRECT IONTOPHORESIS

Iontophoresis can be employed either to assist the passage of the substances of a formulation across the skin or to extract from the body analytes representative of physiological or pathological circumstances (see Fig. **1**). The first is

known as direct iontophoresis and the later as reverse iontophoresis. Direct iontophoresis can be anodal if the drug is neutral or positively charged and cathodal if the drug is negatively charged. Although cations have better properties for iontophoresis, anions can also increase their transdermal drug flux with respect to passive diffusion [28].

Iontophoresis has been used to deliver drugs to skin structures or tissues immediately beneath the skin, as well as for achieving systemic effects. In the following sections, some of the uses of iontophoresis and the most recent research in this field are discussed.

Iontophoresis for Topical Delivery

The most extended uses of iontophoresis are the treatment of palmoplantar hyperhidrosis and the diagnosis of cystic fibrosis. However, iontophoresis is also used for the topical delivery of others drugs such as lidocaine, acyclovir and dexamethasone.

One of the oldest applications of iontophoresis is the treatment of palmoplantar hyperhidrosis [29]. Hyperhidrosis, an excessive rate of sweat secretion from the eccrine glands, is a disabling condition that affects both children and adults. Iontophoresis is one of the most effective, safest and inexpensive treatment options available. Although it has been applied for a long time in the treatment of this pathology, the mechanism of action of iontophoresis is unknown. Different options have been considered in the treatment of hyperhidrosis with iontophoresis, including the application of tap water, saline, botulinum toxin or anticholinergics. Anodal iontophoresis is more efficient than cathodal iontophoresis, and tap water is more effective than saline [30]. Iontophoresis with anticholinergics is more effective than tap water iontophoresis, but may induce systemic side effects [31]. Recently the administration of botulinum toxin by iontophoresis (the dose was administered in one 30 min session with a current intensity of 3 mA) has been shown to reduce sweating more quickly and for a longer period than saline, without side-effects [32].

Cystic fibrosis is a result of a mutated gene encoding the cystic fibrosis transmembrane conductance regulator. Among other functional problems, mutations in this gene cause extensive dysfunction of the exocrine glands. Based on this, the diagnosis of cystic fibrosis is a sweat-test. It consists of the application of pilocarpine to a small area of an arm or leg for 5 min by means of a constant current of about 2.5–3.0 mA in order to stimulate sweating. The sweat is then collected and analyzed for volume and chloride content [33].

Topical delivery of anaesthetics during dermal surgery remains one of the most common topical applications of iontophoresis. Hydrochloride salts of anaesthetics of the amide type, such as lidocaine [34], bupivacaine, etidocaine, mepivacaine, prilocaine and ropivacaine, have been widely studied [35]. Lidocaine has been successfully formulated in an iontophoretic patch as a means of dermal anaesthesia (Vyteris Inc, USA) and was approved by the FDA in 2007. There are different studies that demonstrate that lidocaine iontophoresis and the eutectic mixture EMLA are of a similar efficacy for providing analgesia, which is necessary in situations such as cannulation or CO2 laser surgery of superficial skin lesions [36, 37].

The efficacy of topical creams and ointments in the treatment of Herpes-labialis is controversial, showing very little improvement with respect to spontaneous healing. This poor outcome is attributed to the inadequate penetration by the drug of the intact skin and insufficient delivery to the basal epidermis, the targeted site of infection. The possibility of enhancing the skin permeability of acyclovir via an electrical current has been investigated in several research projects. Morel *et al.* have demonstrated that a single dose of a conventional formulation of acyclovir via topical iontophoresis is a convenient and effective treatment for cold sores [38]. Acyclovir is an ampholyte drug with two pKa values (2.27 and 9.25). Below pH 2, acyclovir is for the most part positively charged, while between pH 3 and 8.5 it is mainly neutral, and at pH values greater than 9.5 it is predominantly negatively charged. This means that acyclovir can be delivered ionized by anodal or cathodal iontophoresis or by electro-osmotic flow as a neutral species depending on the pH selected for the iontophoresis [39]. Recently, Shukla *et al.* [40] have tested the iontophoretic delivery of acyclovir from different formulations, and their results show that only acyclovir in the water phase of the cream was available for transport and that pH-11 gels produced a statistically significant increase in acyclovir dermis exposure when compared to the neutral cream formulations, without any sign of skin irritation. These results highlight the relevance of the characteristics of a formulation for the efficacy of iontophoresis.

Administration of dexamethasone sodium phosphate (DXP) is a major application of iontophoresis in the treatment of local pain and soft-tissue inflammation and in sports medicine. DXP easily penetrates the skin and provokes blanching, but the challenge is to ensure that a sufficient amount of DXP reaches the target tissue beneath the skin despite the clearance by systemic circulation. Iontophoretic treatment of various musculoskeletal problems with DXP has been the subject of clinical studies employing a great variety of protocols, many of which have reported beneficial effects [41, 42], though improvement in the patient's condition has not been always observed [43, 44]. The disodium salt DXP is water soluble and, at a physiological pH, is present mainly in its dianionic form (pKas: 1.9 and 6.4). Again, it has recently been demonstrated that, to achieve optimal iontophoretic delivery, DXP must be delivered from the cathode and formulated rationally, excluding mobile co-anions [45].

There are many drugs whose iontophoretic delivery is under different stages of investigation *in vitro*, *in vivo* in animals, or in clinical assays in human volunteers. One interesting application of iontophoresis could be the administration of antineoplasic agents for the treatment of certain skin cancers, since it avoids the scarring associated with surgery and the long-term complications of radiation therapy, as well as the adverse effects derived from the systemic administration of antineoplasics. In this sense, Chang *et al.* [46] investigated the iontophoresis of cisplatin in the therapy of basal and squamous cell carcinomas in the skin in patients that refused surgery. Cisplatin was applied in the anode together with the vasoconstrictor epinephrine hydrochloride, and the dose of drug and current applied depended on the size of the lesion. Of the 15 patients studied, 11 showed a partial reduction in lesion area or complete response. There were no incidences of systemic side effects (e.g. nausea or vomiting), although there were reports of a minor burning sensation at the cathode. The authors concluded that small lesions responded best and suggested a treatment schedule involving a daily iontophoretic therapy of 20–30 min for 5 days followed by a 2-week recuperation period.

Smith *et al.* [47] investigated the iontophoresis of vinblastine sulfate to treat cutaneous lesions associated with Kaposi sarcoma in human immunodeficiency virus-positive patients. They applied a vinblastine solution by iontophoresis after application of a lidocaine epinephrine solution in the same way. The results in a non-HIV infected group showed signs of local erythema, which cleared up within 2 weeks. In the HIV-1 patients, who were treated over a period of 6 months, there was less inflammation, though this may have been due to immunosuppression, since the patients were at advanced stages of the illness. All of these patients showed significant clearing of the lesions.

The effect of iontophoresis in the transdermal flux of the antineoplasic drug methotrexate has also been investigated [48]. The transdermal flux of methotrexate is higher when applied by means of cathodal iontophoresis than when delivered passively. These results point to the possibility of developing a topical dosage form of this drug, as yet unavailable, for the treatment of psoriasis. This topical form is of utmost interest, since the systemic use of this drug can provoke numerous side effects including nausea, vomiting, fatigue, headache, dyspnea, leucopenia, thrombocytopenia, anemia and hepatic toxicity.

Among other examples of the possible use of iontophoresis for the treatment of topical lesions is that evaluated in studies related with the administration of small interfering RNA (siRNA), a double-stranded molecule that can be designed to hybridize with a specific mRNA sequence. siRNA inhibits the translation of numerous genes both *in vitro* and *in vivo*. Therefore, the topical introduction of siRNA targeted against genes involved in various cutaneous disorders represents a novel therapeutic approach to the treatment of inherited skin diseases, viral infections, skin cancer and atopic dermatitis. Kigasawa *et al.* [49] reported successful iontophoretic delivery of naked siRNA into the epidermis of the rat, which suppressed the expression of an endogenous immunoregulatory cytokine interleukin-10 that is found in elevated levels in atopic dermatitis skin lesions.

The topical therapy of nail diseases, especially onychomycosis, and, to a lesser extent, nail psoriasis, is desirable to avoid the side effects associated with systemic therapy, to increase patient compliance and to reduce the cost of treatment. Systemic therapy, however, continues to be the mainstay of treatment due to the poor permeability of the nail plate to topically applied drugs. For effective topical therapy, ungual drug permeation must be enhanced, and, among other possible strategies, iontophoresis has recently been assayed. In this context, the iontophoretic trans-ungual flux of terbinafine hydrochloride (the most potent antifungal agent against dermatophytes) has been studied *in vitro*, and the results suggest that iontophoresis significantly increases the permeation of terbinafine through the

nail and forms a drug depot in the nail. Nair *et al.* [50] have observed a correlation between electrical dose and the amount of drug permeating through and loading into the nail, which suggests the advantage of individualization of treatment by manipulating the applied coulombic dose.

Hao *et al.* [51, 52] used the model compounds tetraethyl ammonium ion, mannitol and urea to demonstrate that the main mechanism of transport during ungual iontophoresis is electrorrepulsion and that the contribution of electroosmotic flow to the overall iontophoretic flux is low. Their results are consistent with the existence of both proton- donating and -accepting functional groups in the nail plates and a pI ~ 5. They observed that the net surface charge density of nail plates increased when the solution's pH varied from the pI of the keratins in the nail plates. Their results also showed that transungual electroosmosis was affected by the ionic strength of the solution used and that a decrease in solution ionic strength enhanced electroosmotic transport. Although electroosmotic flow is minor during iontophoresis, the results obtained to date are encouraging for positively charged molecules.

Furthermore, a device has been designed specifically for ophthalmic applications; namely the Visulex® Iontophoretic System (Aciont Inc.). This system consists of a user applicator, a dosing controller and connecting wires, and includes software and an algorithmic control along with a proprietary multi-electrode monitoring system. The applicator slips into the lower cul-de-sac and the return electrode can be positioned anywhere on the body to complete the electrical circuit. Hastings MS *et al.* [53] tested the device *in vitro* and *in vivo* (in New Zealand white rabbits) with DXP, and the results obtained were promising. Although at the early stages, this is an interesting new field of research for the treatment of back-of-the-eye diseases.

Iontophoresis for Systemic Delivery

Systemic administration of drugs is also possible by means of iontophoresis. The only system commercially available at present is the fentanyl iontophoretic transdermal system mentioned above. It is indicated for the short-term management of acute postoperative pain in adult patients requiring opioid analgesia during hospitalization. It is designed to operate for 24 hours after the first activation and allows 6 doses per hour up to a maximum of 80 doses, after which the system shuts off. Subsequently, a new transdermal system is required if the fentanyl administration is to be continued. The dose is controlled by the amount of electrical current applied and is designed not to exceed 40 mcg per activation. The transdermal system uses a 10-minute transdermal infusion for each 40 μg dose.

Currently, the iontophoretic delivery of apomorphine for the treatment of idiopathic Parkinson's disease is being evaluated in human subjects. Although the results obtained require further confirmation, they are so far encouraging [54], especially when the skin is previously treated with surfactants [55]. *In vivo* studies in rats point to the possibility of achieving therapeutic levels of the anti-Parkinsonian ropinirole hydrochloride by means of iontophoresis [56]. The administration of drugs for the treatment of migraine also represents an interesting alternative for iontophoresis. Different *in vitro* [18] and *in vivo* studies have been developed with sumatriptan [57], and the results support the possibility of using this technique to reach adequate plasma levels for migraine treatment. Similar results have been obtained with zolmitriptan [58].

Peptide drugs including various series of amino acid derivatives and tripeptides, thyrotropin release hormones, LHRH and analogues, vasopressin and calcitonin can also be administered by means of this technique. Many *in vitro* and *in vivo* studies have been performed with these molecules [59-61]. Nevertheless, it is difficult to predict the blood levels than can be reached after iontophoretic administration of any of these drugs. Computational studies of 3D quantitative structure-permeation relationships suggest that iontophoresis is favoured by peptide hydrophilicity and hindered by voluminous, localized hydrophobicity [62, 63]. This is particularly the case when the bulky lipophilic moiety is directly adjacent to a positively charged residue, as occurs in nafarelin and leuprolide [62]. It has been suggested that this structure inhibits electroosmosis, thereby reducing iontophoretic transport [64].

One peptide that has focused the attention of researchers in the field of iontophoresis is insulin. Although hexameric insulin is not transported through skin when iontophoresis is applied, the flux of monomeric human insulin (Mean molecular weight ~6000Da, negatively charged) increases significantly with respect to passive diffusion. The administration of insulin by means of iontophoresis is hindered by many obstacles, such as the degradation caused by contact with the anode, which can be solved by placing the insulin solution in the cathodal chamber. A

considerable number of *in vitro* studies have investigated the effect of iontophoretic parameters on insulin delivery and have demonstrated the physiological effect of iontophoretically-delivered insulin on blood glucose levels in different small animal models, particularly mice, rats and rabbits [65-73]. Although these studies prove that iontophoretic delivery of insulin is feasible in these animals (frequently after compromising the stratum corneum), their results need to be extrapolated to humans, where significantly greater quantities of the hormone are required for pharmacologic effect.

Calcitonin is a 32 amino acid peptide with a molecular weight of ~3500 Da which, under physiological conditions, is positively charged. Different iontophoretical studies have been developed with this peptide. The possibility of delivering calcitonin topically to the dentin for the treatment of invasive cervical resorption [74] and systemically for the treatment of osteoporosis and Paget's disease has been reported. No studies have yet been developed in humans, though salmon calcitonin, which is more potent than the human form, has been applied by means of iontophoresis in animal skin models, reaching blood levels comparable to those produced by intravenous infusion [61, 75].

REVERSE IONTOPHORESIS

Reverse iontophoresis across the skin is a potentially useful alternative for non-invasive clinical and therapeutic drug monitoring. During current application, reverse iontophoresis allows the movement of neutral and positively charged entities into the cathode while negatively charged entities move into the anode. The main problem with this is that skin, per se, contains some of the entities to be analyzed, which implies that there is a period of time within which it is necessary to withdraw skin reserves and after which it is possible to correlate extracted levels of the analytes with levels in the blood [76-79]. Another limiting factor of reverse iontophoresis is the low levels extracted, which make it difficult to quantify the extracted molecules. Nevertheless, findings in this field are interesting.

The first approved use of reverse iontophoresis was the extraction of glucose from beneath the skin. The GlucoWatch G2® Biographer (Cygnus Inc., Redwood City, CA, USA) is a device that permits the non-invasive extraction of glucose across the skin, allowing the glycemia to be evaluated every 10 min over several hours. In this system, glucose is extracted by the cathode due to the electroosmotic flow generated by the application of a low level current and is then quantified; that is, glucose is oxidized by glucose oxidase to release the hydrogen peroxide detected in the system by the biosensor [80, 81].

Although the detection of glucose is currently the only application on the market, a great deal of research is being carried out in this field, and the results obtained with some analytes are encouraging. In this sense, aminoacids, urea, lactate, lithium, valproate, phenytoin and amikacine are the focus of most of the attention.

Amino acids are biological markers, the plasma levels of which may be used to detect inherited metabolic diseases. They are excellent candidates for non-invasive monitoring via reverse iontophoresis due to their small molecular weight and polar nature. Charged amino acids are primarily extracted by the electrode of opposite polarity, while zwitterionic species are extracted, more or less equally, by both the anode and cathode. Different authors have shown how the transport of amino acids across the skin can be significantly enhanced by iontophoresis [82], and pilot investigations (*in vitro* and *in vivo*) of the reverse iontophoretic extraction of phenylalanine for the diagnosis of phenylketonuria have been reported [83, 84]. Recently, *in vivo* studies of the simultaneous extraction of 17 aminoacids by reverse iontophoresis have suggested the feasibility of extracting some of them at physiologically relevant levels *in vivo* [77, 85].

Other analytes that can be extracted by reverse iontophoresis and whose potential utility in the field of diagnosis have been explored are; urea, used for the non-invasive diagnosis of individuals with chronic kidney disease [86]; lactate, a widely used marker of tissue distress in critically ill patients and of sports performance [87]; and drugs such as lithium [79, 88], phenytoin [79, 88, 89], valproate [90] and amikacine [91], which require routine drug monitoring to assess adequate plasma levels.

CONCLUDING REMARKS

As discussed in this chapter, iontophoresis has attracted the attention of much research in recent years. Knowledge of this technique has increased and the influence of formulation factors on its efficacy has been demonstrated. The

electrode chosen to place the drug solution or formulation, the pH of the formulation and the presence of counter ions can radically affect the efficacy of the technique. These findings demonstrate that, although iontophoresis has been employed for some time now, the optimum conditions for its effective application have not always been respected. Iontophoresis cannot be considered a technique for the general application of drugs in local therapy; nevertheless, it may sometimes be indicated, such as in the case of local anaesthesia with lidocaine or the treatment of local pain, since onset is faster than that of topical forms and is better tolerated than subcutaneous injections and infiltrations. The main inconvenience of this mode of application at the present time is its cost in comparison with that of more traditional therapies.

The systemic administration of peptidic drugs represents an important challenge, since they are sensitive to the gastrointestinal environment and must be administered either subcutaneously or intravenously in most cases. In this sense, iontophoresis represents a promising alternative and may soon make the systemic administration of some small peptides possible. Of course this technique does not constitute an alternative for the administration of all kinds of drugs, as only molecules of a moderate molecular weight, which tend to be positively ionized or neutral, allow its advantages to be availed of. An important limiting factor that should be considered is that, although iontophoresis increases transdermal flux with respect to passive diffusion, only molecules that are not required in high levels in the body can benefit from this technique. In order to overcome this problem, the possible synergistic effect between chemical enhancers, sonophoresis, electroporation or microneedles on the one hand and iontophoresis on the other has been studied for some drugs [3]. As an example, the results obtained combining chemical enhancers and iontophoresis with sumatriptan [92], atenolol [93] and metopimazine [94] are encouraging, since they demonstrate that the combination of the two strategies increases the transdermal flux of the tested compounds.

One application of iontophoresis that is especially promising is reverse iontophoresis. After the recent commercialization of the GlucoWatch® for glucose monitoring it is likely that other substances will be monitored by means of iontophoresis in the near future. Without doubt this will represent a step forward in the field of diagnosis, especially for children, since this technique avoids the stress associated with injections.

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CHAPTER 4

Sonophoresis: A Valuable Physical Enhancer to Increase Transdermal Drug Delivery

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Abstract: Transdermal drug delivery offers an attractive alternative to the conventional drug delivery methods of oral administration and injection. However, the stratum corneum acts as a barrier that limits the penetration of substances through the skin. Application of ultrasound to the skin increases its permeability (sonophoresis) and enables the delivery of various substances into and through the skin.

Ultrasound has been used extensively for medical diagnostics and to a certain extent in medical therapy (physiotherapy, ultrasonic surgery, and hyperthermia). Nevertheless, it has only recently become popular as a technique to enhance drug release from drug delivery systems. A number of studies suggest the use of ultrasound as an external mean of delivering drugs at increased rates and at desired times.

This chapter presents the main findings in the field of sonophoresis, namely transdermal drug delivery and transdermal monitoring. Particular attention is paid to proposed enhancement mechanisms and future trends in the field of cutaneous vaccination and gene delivery.

Key words: Sonophoresis, phonophoresis, ultrasound, skin, physical enhancers, transdermal drug delivery.

INTRODUCTION

Therapeutic applications of ultrasound pre-date its use as an imaging technique. It was recognized in 1927 that ultrasound (ULTS) could produce lasting changes in biological systems, and this was the start of both safety studies, and of ultrasound therapy [1].

Absorption of ultrasonic energy leads to tissue heating, and this has been used with therapeutic intent in many conditions. More recently it has been realized that benefit may also be obtained from the non-thermal effects that occur as ULTS travels through tissue.

ULTS therapies can broadly be divided into ‘‘high’’ power and ‘‘low’’ power therapies where high power applications include high intensity focused ULTS and lithotripsy, and low power encompasses sonophoresis, sonoporation, gene therapy and bone healing. Apart from physiotherapy uses, ULTS therapies are currently not widespread.

ULTS has been used in the medical field for several decades. There are three distinct sets of ULTS conditions based on frequency range and applications. These are shown in Table **1.**

Table 1: Classification of ULTS as a function of frequency used

1)	High frequency (3–10 MHz) or diagnostic ULTS,
2)	Medium frequency (0.7–3 MHz) or therapeutic ULTS, and
3)	Low frequency (18 to 100 KHz) or power ULTS.[2]

The principal medical applications of ULTS are shown in Table **2:**

Table 2: Medical applications of ULTS

(a) As a diagnostic tool at various anatomical sites (eyes and orbit, head, neck, chest, breast, abdomen, and pelvis),
(b) For physical therapy,
(c) In surgery, and
(d) In dentistry.
(e) For the treatment of tumors

ULTS was initially investigated for treating localized skin conditions [3] and joint inflammation.[4] More recently, there has been considerable interest in developing ULTS as a technique to enhance transdermal drug delivery [5] and to enable the relatively noninvasive extraction of analytes from the blood [6] The scope of this article is, first, to detail the relationship between ULTS frequency and improved molecular transport across the skin; second, to describe the mechanisms by which ULTS enhances transdermal transport; and third, to review the increasing sonophoretic enhancement literature to assess the potential for practical transdermal applications in drug delivery and noninvasive clinical chemistry.

A number of excellent reviews that have been published contain detailed discussions concerning many aspects of sonophoresis [7-13]. The present chapter shows an updated overview of the use of sonophoresis in the pharmaceutical field, specifically in the area of topical and transdermal drugs. This focus is justified due to the magnitude of the experimental data available with the use of this technique. The use of sonophoresis in experimental medicine and pharmaceutical sciences has a long history.

THE ULTRASOUND

Skin is the largest and most accessible organ of the body, is advantageous portal for drug delivery [14-21]. In addition to offering improved patient compliance over needle-based delivery and avoiding first-pass drug metabolism common in oral drug delivery, the transdermal route provides a unique platform for sustained and controlled delivery of therapeutics.

Many agents are applied to the skin either deliberately or accidentally, with either beneficial or deleterious outcomes. The main interest in dermal absorption assessment is related to: a) Local effects in dermatology (e.g., corticosteroids for dermatitis); b) transport through the skin seeking a systemic effect (e.g., nicotine patches, hormonal drug patches, etc.); c) surface effects (e.g., sunscreens, cosmetics, and antiinfectives) [22]; d) targeting of deeper tissues (e.g., nonsteroidal anti-inflammatory agents) [23-35]; and e) unwanted absorption (e.g., solvents in the workplace, pesticides or allergens) [36,37]. Fig. **1** summarizes the process of percutaneous absorption.

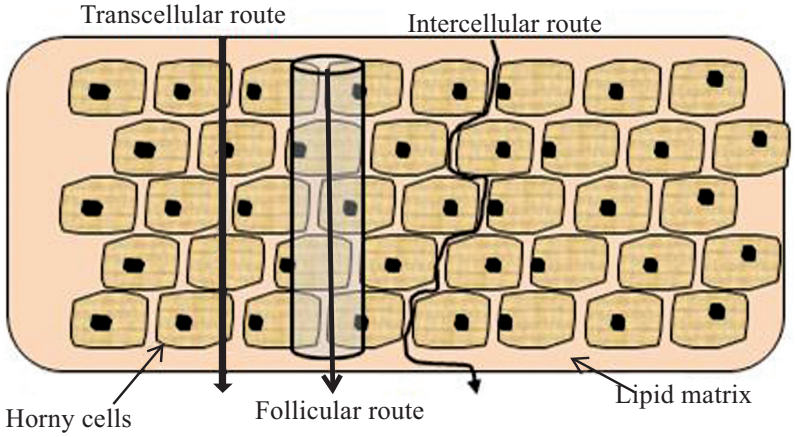

Figure 1: Processes of percutaneous absorption

The skin became popular as a potential site for systemic drug delivery, on the one hand, because of the possibility of avoiding the problems of stomach emptying, pH effects, enzyme deactivation associated with gastrointestinal passage, and hepatic first-pass metabolism; and on the other hand, due to its capability to enable input control.

Sound is a form of mechanical energy that is propagated from one point to another by the interaction between neighboring oscillating particles [38]. The direction of propagation is parallel to the direction of oscillation and, hence, sound is defined as a longitudinal wave. Because its propagation depends entirely on the creation of alternating regions of molecular compression and rarefaction, sound cannot exist in a vacuum. The pressure variation has the same propagation speed and frequency as the oscillations of the molecules about their equilibrium positions. Acoustic waves with frequencies between 20 Hz and ~20 KHz fall in the audible range. The term ultrasonic refers to sound waves whose frequency is >20 KHz (Fig. **2**). The intensity (I, expressed in W/cm^2), or concentration of power within a specific area in an ULTS beam, is proportional to the square of the amplitude, p, which is the maximum increase or decrease in the pressure relative to ambient conditions in the absence of the sound wave. The complete relationship is:

$$I = p^2/2\rho c$$

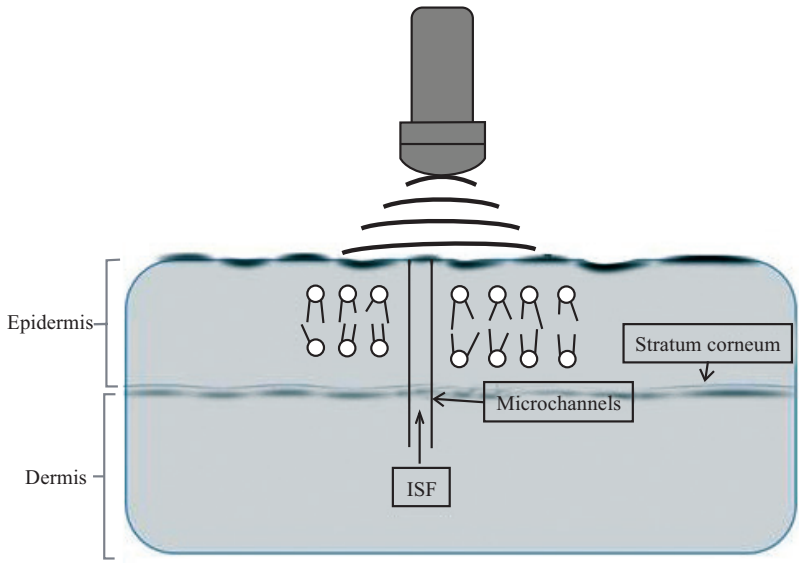

Figure 2: Picture of how ultrasound disrupts stratum corneum: Permeability barrier of skin.

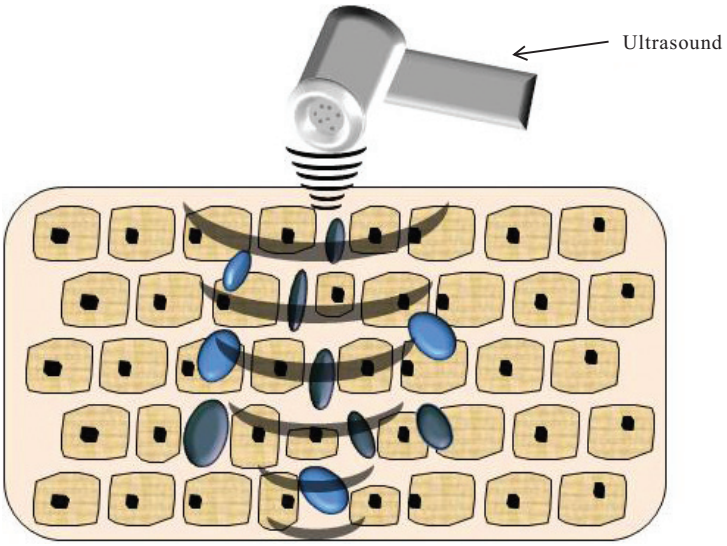

Figure 3: Enhanced permeation by disruption of lipid barrier and cavitation by use of ULTS.

Where ρ is the density of the medium and c is the speed of the sound (in human soft tissue, this velocity is 1540 m/s). The intensity is progressively lost when a sound wave passes through the body or is deviated from its initial direction, a phenomenon referred to as attenuation. In homogeneous tissue, the attenuation occurs as a result of absorption, in which case the sound energy is transformed into heat and scattered [39]. The source of sound waves in a biomedical ultrasound device is a piezoelectric crystal transducer. The crystal material may be quartz or another polycrystalline material, such as lead–zirconate–titanium or barium titanate [39]. The sound waves are produced in response to an electrical impulse in the piezoelectric crystal, allowing the conversion of electrical into mechanical or vibrational energy; this transformation requires a molecular medium (solid, liquid, or gas) to be effective. Following the external perturbation, groups of molecules oscillate in phase and transmit their kinetic energy to nearby molecules [2]. The ULTS beam is composed of two fields, the ‘‘near field,’’ in the region closest to the transducer face, and the ‘‘far field,’’ corresponding to the conical diverging portion of the beam (see Fig. **3**). The parameters controlling this configuration of the ULTS beam are principally the frequency and the size of transducer [39].

MECHANISMS OF ACTION

Although considerable attention has been given to the investigation of sonophoresis in the past years, its mechanisms were not clearly understood, reflecting the fact that several phenomena may occur in the skin upon ULTS exposure. These include:

i) Cavitation.

ii) Thermal effects.

iii) Induction of convective transport.

iv) Mechanical effects.

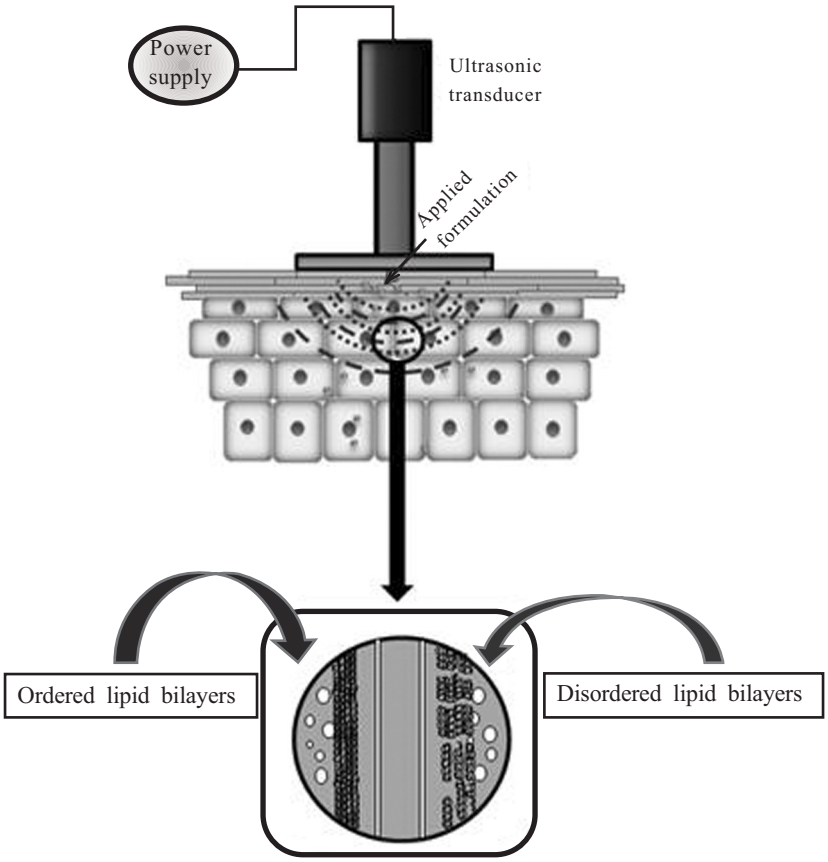

Figure 4: Basic principle of sonophoresis. ULTS pulses are passed through the probe into the skin fluidizing the lipid bilayer by the formation of bubbles caused by cavitation.

Cavitation Effects

Cavitation is the formation of gaseous cavities in a medium upon ULTS exposure (Fig. **4**). The primary cause of cavitation is ULTS-induced pressure variation in the medium. Cavitation involves both the rapid growth and collapse of a bubble (inertial cavitation), or the slow oscillatory motion of a bubble in an ULTS field (stable cavitation). Collapse of cavitation bubbles releases a shock wave that can cause structural alteration in the surrounding tissue [40] ULTS can generate violent microstreams, which increase the bioavailability of the drugs [41]. Tissues contain air pockets that are trapped in the fibrous structures that act as nuclei for cavitation upon ultrasound exposure. The cavitational effects vary inversely with ULTS frequency and directly with ULTS intensity. Cavitation might be important when low-frequency ULTS is used, gassy fluids are exposed or when small gas-filled spaces are exposed.

Cavitation occurs due to the nucleation of small gaseous cavities during the negative pressure cycles of ULTS, followed by the growth of these bubbles throughout subsequent pressure cycles. Whenever small gaseous nuclei already exist in a medium, cavitation takes place preferentially at those nuclei [42-44]. This cavitation leads to the disordering of the lipid bilayers and formation of aqueous channels in the skin through which drugs can permeate [45-47].

Thermal Effects

Absorption of ULTS increases temperature of the medium. Materials that possess higher ULTS absorption coefficients, such as bone, experience severe thermal effects compared with muscle tissue, which has a lower absorption coefficient [48]. The increase in the temperature of the medium upon ULTS exposure at a given frequency varies directly with the ULTS intensity and exposure time. The absorption coefficient of a medium increases directly with ULTS frequency resulting in temperature increase.

A study [49] suggested the use of a new safety parameter, time to threshold (TT). TT indicates the time after which a threshold temperature rise is exceeded, and how long a piece of tissue can be safely exposed to ULTS, provided the safe threshold is known.

Convective Transport

Fluid velocities are generated in porous medium exposed to ultrasound due to interference of the incident and reflected ULTS waves in the diffusion cell and oscillations of the cavitation bubbles. Fluid velocities generated in this way may affect transdermal transport by inducing convective transport of the permeant across the skin, especially through hair follicles and sweat ducts. Experimental findings suggest that convective transport does not play an important role in the observed transdermal enhancement [42].

Mechanical Effects

ULTS is a longitudinal pressure wave inducing sinusoidal pressure variations in the skin, which, in turn, induce sinusoidal density variation. At frequencies greater than 1 MHz, the density variations occur so rapidly that a small gaseous nucleus cannot grow and cavitational effects cease. But other effects due to density variations, such as generation of cyclic stresses because of density changes that ultimately lead to fatigue of the medium, may continue to occur. Lipid bilayers, being self-assembled structures, can easily be disordered by these stresses, which result in an increase in the bilayer permeability. This increase is, however, non-significant and hence mechanical effects do not play an important role in therapeutic sonophoresis. Thus cavitation induced lipid bilayer disordering is found to be the most important cause for ultrasonic enhancement of transdermal transport [42].

ADVANTAGES AND DISADVANTAGES OF SONOPHORESIS

Sonophoresis is capable of expanding the range of compounds that can be delivered transdermally. In addition to the benefits of avoiding the hepatic first-pass effect, and higher patient compliance, the additional advantages [50] and disadvantages that the sonophoretic technique offers can be summarized as follows in Table **3**.

Table 3: Advantages and disadvantages of using sonophoresis as a physical penetration enhancer

Advantages	Disadvantages
Enhanced drug penetration (of selected drugs) over passive transport	Can be time-consuming to administer
Allows strict control of transdermal penetration rates	Minor tingling, irritation, and burning have been reported (these effects can often be minimized or eradicated with proper ULTS adjustment [51])
Permits rapid termination of drug delivery through termination of ULTS	SC must be intact for effective drug penetration
Skin remains intact, therefore low risk of introducing infection	
Less anxiety provoking or painful than injection	
In many cases, greater patient satisfaction	
Not immunologically sensitizing	
Less risk of systemic absorption than injection	

APPLICATIONS OF ULTRASOUND

The unique and promising release of drugs by sonophoresis renders it an attractive candidate as a physical enhancer to administer drugs throughout the skin [52-89]. This is emphasized in Tables **4** and **5**, which summarizes the research on sonophoresis uses and many experimental conditions used in the transdermal administration of drugs.

Table 4: Research on uses of sonophoresis to administer different drugs through the skin

Anesthetics		
Research	**Outcome**	**Author (Ref.)**
Topical skin penetration of lidocaine	Increase in the concentration of lidocaine transmitted into rabbit subdermal tissues when topical application was followed by use of ULTS	Wells *et al.* [52]
Double blind, vehicle-controlled, crossover trial in healthy volunteers for lidocaine cream	No increase in absorption of lidocaine cream by using ULTS	McEnlay *et al.* [53]
Trial in healthy volunteers for lidocaine oil	Other variables include differences in ULTS frequencies and drug concentrations.	Novak *et al.* [54]
Skin lidocaine penetration	250 kHz induced the highest penetration of lidocaine.	Griffin and Touchstone [55]
Anesthetic effect of lidocaine in legs of hairless mice	ULTS in conjunction with a topical aqueous lidocaine solution was rapidly effective in inducing an anesthetic effect in the legs of hairless mice	Tachibana *et al.* [56]
Sonophoresis of topical benzocaine and dibucaine	No detectable increase in the rate of anesthetic penetration	Williams *et al.* [57]
Administration of lidocaine hydrochloride transdermally on healthy volunteers applying 0.5 MHz ULTS.	0.5 MHz ULTS in sonophoresis for conduction anesthesia using lidocaine hydrochloride for a nerve block, it is more effective than the 1 MHz that is widely used in clinical situations	Kim *et al.*[58]
Permeation of procaine hydrochloride through cell monolayers applying therapeutical ULTS.	Extent and velocity of the permeation of procaine hydrochloride through MDCK monolayer can be controlled by sonophoresis	Hehn *et al.* [59]
Analgesic and anti-inflammatory drugs		
Effect of intensity, mode, and duration of ULTS application on the transport of three non steroidal anti-inflammatory drugs (NSAIDs) across cellulose membrane and hairless rabbit-skin	Demonstrated the synergistic effect of temperature and ULTS operation parameters on drug transport of NSAIDs	Meshali *et al.* [60]
Effect of an ULTS (1 MHz) on transdermal absorption of indomethacin from an ointment in rats	Intensity and duration of application play an important role in the transdermal sonophoretic delivery; intensity of 0.75 W/cm^2 for 10 min was most effective for delivering indomethacin	Miyazaki *et al* [61]

Table 4: cont....

Study of the influence of ultrasound on percutaneous absorption of ketorolac tromethamine *in vitro* across hairless rat skin	A significant increase in permeation of ketorolac through rat skin was observed with the applied sonication at 3 W/cm^2 when compared with permeation at 1 and 2 W/cm^2.	Tiwari *et al.* [62]
To determine if a ketorolac tromethamine (KT) gel solution could be administered *in vivo* via phonophoretic transdermal delivery using pulsed ULTS by examining its anti-inflammatory effects in a rat carrageenan inflammation model.	The transdermal application of KT gel using sonophoresis had significant anti-hyperalgesic and anti-inflammatory effects. These findings suggest that the transdermal administration of a KT gel using sonophoresis with pulsed ULTS might be useful for treating acute inflammation and pain.	Yang *et al.* [63]
Application of ultraphonophoresis of 5% ibuprofen nurofen gel to affected joints of 20 patients.	Analgesic efficacy of transcutaneous 5% gel nurofen in osteoarthrosis.	Serikov *et al.* [64]
Examination of therapeutic effects of sonophoresis with ketoprofen in gel form in patients with enthesopathy of the elbow.	Positive effects of sonophoresis using a pharmacologically active gel with ketoprofen were shown to be highly significant in both assessments, objective (clinical examination) and subjective (interview). The pain symptoms in the elbow resolved in most of the patients.	Cabak *et al.* [65]
Quantitative study of sodium diclofenac (Voltaren Emulgel, Novartis) phonophoresis in humans	Previously applied therapeutic ULTS irradiation enhances the percutaneous penetration of the topical diclofenac gel, although the mechanism remains unclear	Rosim *et al.* [66]
Investigation of *in vitro* penetration and the *in vivo* transport of flufenamic acid in dependence of ULTS.	Using this *in vitro* model it is possible to compare the transdermal delivery of commercial flufenamic ointment in volunteers.	Hippius *et al.* [67]
Antibiotics		
Effect of ULTS on the delivery of topically applied amphotericin B ointment in guinea pigs.	Amphotericin B content in the skin and subcutaneous fatty tissues was much higher when the drug was delivered in the presence of ULTS.	Rornanenko and Araviiskii [68]
Administration of tetracycline in healthy rabbits using electrophoresis and sonophoresis	It was found that the tissue levels of tetracycline administered with the modified methods of electro and sonophoresis increased with an increase in the current density or ULTS intensity, the procedure time and antibiotic concentration.	Ragelis *et al.* [69]
Immunosuppressives		
Investigated the topical transport of Cyclosporin A using low-frequency US throughout rat skin	The enhanced skin accumulation of Cyclosporin A by the combination of low-frequency ULTS and chemical enhancers could help significantly to optimize the targeting of the drug without of a concomitant increase of the systemic side effects.	Liu *et al*.[70]
Evaluation of the efficacy of low frequency sonophoresis (LFS) at 25KHz produced by a sonicator apparatus for treatment of alopecia areata, melasma and solar lentigo.	The study showed that LFS, a not aggressive technique, enhanced penetration of topic agents obtaining effects at the level of the epidermis, dermis and appendages (intradermal delivery), giving better results in the treatment of some cosmetic skin disorders.	Santoianni *et al*.[71]
Anticancer drugs		
Application of a method using ULTS and nano/microbubbles to cancer gene therapy using prodrug activation therapy.	Dramatic reductions of the tumor size by a factor of four.	Aoi *et al*.[72]
Investigation of competitive transport across skin of 5-fluorouracil into coupling gel under the influence of ULTS, heat-alone and Azone® enhancement.	Ultrasonication produced a decrease in percutaneous drug penetration. This effect was due to the diffusive loss of the hydrophilic substance 5-fluorouracil from the skin surface.	Meidan *et al.* [73]
Insulin		
To determine if the 3x1 rectangular cymbal array perform significantly better than the 3x3 circular array for glucose reduction in hyperglycemic rabbits.	Using the rectangular cymbal array, the glucose decreased faster and to a level of -200.8±5.9 mg/dL after 90 min.	Luis *et al.* [74]
To demonstrate ultrasonic transdermal delivery of insulin *in vivo* using rabbits with a novel, low-profile two-by-two ULTS array.	For the ULTS-insulin group, the glucose level was found to decrease to -132.6 ± 35.7 mg/dL from the initial baseline in 60 min	Lee *et al*.[75]
The purpose of this study was to demonstrate the feasibility of ULTS-mediated transdermal delivery of insulin *in vivo* using rats with a novel, low profile two-by-two US array based on the "cymbal" transducer.	For the 60-min ULTS exposure group, the glucose level was found to decrease from the baseline to -267.5 ± 61.9 mg/dL in 1 h. Moreover, to study the effects of ULTS exposure time on insulin delivery, the 20-min group had essentially the same result as the 60-min exposure at a similar intensity.	Smith *et al.* [76]
Corticosteroids		
Determination of the effect of ULTS on the transcutaneous absorption of dexamethasone.	A sonophoretic effect occurred with dexamethasone when its application saturated the skin.	Saliba *et al.* [77]
To determine if ULTS enhances the diffusion of transdermally applied corticosteroids.	The effects of sonophoresed dexamethasone can be measureed in terms of reduced collagen deposition as far down as the subcutaneous tissue but not in the submuscular or subtendinous tissue	Byl *et al.* [78]

Table 4: cont....

Comparison of effectiveness of 0.4% Dexamethasone sodium phosphate (DEX-P) sonophoresis (PH) with 0.4% DEX-P iontophoresis (ION) therapy in the management of patients with knee joint osteoarthritis	Significant improvement in total WOMAC scores was observed in 15 (60%) and 16 (64%) patients in the PH and ION groups respectively, indicating no significant difference in the improvement rate.	Akinbo *et al*.[79]
Designing a sonophoretic drug delivery system to enhance the triamcinolone acetonide (TA) permeability.	The highest permeation of TA was observed under the ULTS treatment conditions of low frequency, high intensity, and in continuous mode.	Yang *et al.* [80]
Cardiotonics		
The sonophoresis of digoxin *in vitro* through human and hairless mouse skin.	There was no enhancement of digoxin absorption across human skin by ULTS.	Machet *et al.* [81]
Vasodilators		
Skin penetration enhancement effect of ULTS on methyl nicotinate in 10 healthy human volunteers.	ULTS treatment applied prior to methyl nicotinate led to enhanced percutaneous absorption of the drug	McEnlay *et al*.[82]
Hormones		
Effect of permeation enhancers and application of low frequency (LUS) and high frequency ultrasound (HUS) on testosterone (TS) transdermal permeation after application of testosterone solid lipid microparticles (SLM).	Skin exposure to HUS or LUS before application of 1% dodecylamine for 30 min had no superior enhancement effect over application of either LUS or HUS alone. Application of drug loaded SLM offered skin protection against the irritation effect produced by TS and 1% DA.	El-Kamel *et al.* [83]
Cicatrizants		
The effectiveness of sonophoresis on the delivery of high molecular weight (MW) hyaluronan (HA) into synovial membrane using an animal model of osteoarthritis (OA).	Synovial fluid analysis revealed increased absorption and fluorescence microscopy showed deeper penetration of both HA1000 and HA3000.	Park *et al.* [84]
Calcein		
The skin permeation clearance of model hydrophilic solutes, calcein (MW 623) and-labeled dextrans [MW 4400 (FD-4) and MW 38000 (FD-40)], across the skin under the influence of ULTS.	Good correlations were observed between the $3H_2O$ flux and solute clearances and, unexpectedly, the slope values obtained from linear regression of the plots were consistent for all solutes examined.	Morimoto *et al.* [85]
Oligonucleotids		
Assessment of the potential of low frequency ULTS (20 kHz, 2.4 W/cm^2) in delivering therapeutically significant quantities of anti-sense oligonucleotides into skin.	Microscopic evaluations using revealed heterogeneous penetration into the skin. Heterogeneous penetration led to the formation of localized transport pathways, which occupied about 5% of the total exposed skin area.	Tezel *et al.* [86]
Stimulants		
The effect of low-frequency sonophoresis on fentanyl and caffeine permeation through human and hairless rat skin.	Discontinuous ULTS mode was found to be more effective in increasing transdermal penetration of fentanyl while transdermal transport of caffeine was enhanced by both continuous and pulsed mode.	Boucaud *et al.* [87]
Calcium		
Manipulation of the Ca^{2+} content of the upper epidermis by sonophoresis across hairless mouse SC.	Sonophoresis at 15 MHz did not alter barrier function.	Menon *et al.* [88]
Panax notoginseng		
Effect of a therapeutic US coupled with a Panax notoginseng gel for medial collateral ligament repair in rats.	This study reveals a positive ultrasonic effect of Panax notoginseng extract for improving the strength of ligament repair.	Ng *et al.* [89]
Other applications ***i)To study the mechanisms of penetration due to US throughout the skin***		
To demonstrate the calcein permeability through the localized transport regions (LTRs) from the exposure to the ULTS/ Sodium lauryl sulphate (SLS) system.	LTRs and the non-LTRs exhibit significant decreases in skin electrical resistivity relative to untreated skin, suggesting the existence of two levels of significant skin structural perturbation due to ULTS exposure in the presence of SLS.	Kushner IV *et al.* [90]
To shed light on the mechanism(s) by which low-frequency ULTS (20 KHz) enhances the permeability of the skin.	Significant fractions (30%) of the intercellular lipids of SC were removed during the application of low frequency sonophoresis.	Alvarez-Roman *et al.* [91]
Investigation of short time sonication effects of human skin at variable intensities and on the dynamics of fluorescein transport across the skin.	A short application of ULTS enhanced the transport of fluorescein across human skin by a factor in the range of 2–9 for full thickness skin samples and by a factor in the range of 2–28 000 for heat-stripped SC samples	Cancel *et al.* [92]
Use of quantum dots as a tracer and confocal microscopy and transmission electron microscopy (TEM) as visualization methods, on low frequency sonophoresis.	ULTS significantly increased the frequency of occurrence of the otherwise scattered and separated lacunar spaces in the SC. A significant increase in lacunar dimensions was observed when 1% w/v sodium lauryl sulfate was added to the coupling medium.	Paliwal *et al.* [93]

Table 4: cont....

ii)Kelloids		
ULTS therapy with a water-based gel alone	"Complete flattening" of keloids in two young men when 1 MHz at 0.8 W/cm 2 was applied for approximately 4 minutes.	Walker [94]
ii) Tumours		
Optimization of ULTS parameters for *in vivo* bleomycin delivery	An effective antitumor effect was demonstrated in solid tumors of both murine and human cell lines.	Larkin *et al.* [95]
Investigation of high-intensity focused ULTS (HIFU) exposure of (111) In-MX-B3.	The HIFU exposure shortened the peak tumor uptake time (24 vs. 48 h for the control) and increased the peak tumor uptake value (38 vs. 25 %ID/g for the control). The HIFU effect on enhancing tumor uptake was greater at earlier times up to 24 h.	Khaibullina *et al.* [96]
Suppurative wounds		
Treatment of suppurative wounds with ULTS.	sonophoresis of ethylenediaminetetra acetic acid with the quinoxaline antibiotic dioxidine was effective in accelerating wound purification an delimination of necrotic issues	Levenets [97]. Shuvalov, and Poliakov [98]
Treatment of suppurative wounds with ULTS.	Sonophoresis of a 1% papain solution together with dimethyl sulfoxide was an effective method for treating purulent wounds and inflammatory infiltrates.	Matinian *et al.* [99]

Table 5: Experimental conditions for study the pass of drugs throughout the skin by sonophoresis.

Drug	Experimental conditions used by different researchers
Lidocaine [52-58]	▪ Healthy volunteers ▪ Rabbit skin ▪ Different drug concentrations and frequencies (250-100kHz)
Procaine hydrochloride [59]	▪ Cultured MDCK (Madin Darby Canine Kidney) epithelial cell a model ▪ Continuous irradiation of 1.0 W/cm^2
Piroxicam [60]	▪ Cellulose membrane and rabbit skin ▪ 0.5 to 3.0 W/cm^2
Ibuprofen [60]	▪ Cellulose membrane and rabbit skin ▪ 0.5 to 3.0 W/cm^2
Sodium diclofenac [60,66]	▪ Cellulose membrane and rabbit skin ▪ 0.5 to 3.0 W/cm^2 ▪ Humans
Indomethacin [61]	▪ Rat skin ▪ Range of intensities (0.25, 0.5, 0.75, and 1 W/cm^2), Time 5-20min.
Ketorolac tromethamine [62.63]	▪ Rat skin ▪ Rat carrageenan inflammation model. ▪ Continuous mode, at an intensity of 1-3 W/cm^2 and a frequency of 1 MHz for 30 min.
Ketoprofen [65]	▪ Humans ▪ Pulse mode of ULTS and an intensity of 0.8 W/cm^2
Flufenamic acid [67]	▪ Human skin ▪ ULTS energy was supplied for between 5 and 30 min at a range of intensities up to 1.5 W/cm^2
Amphotericin B [68]	▪ Guinea pigs ▪ Use of dimethyl sulfoxide as a chemical enhancer
Tetracycline [69]	▪ Healthy rabbits ▪ Galvanization apparatus "Potok-1"
Ciclosporin A [70]	▪ Rat skin ▪ Pretreatment of skin with chemical enhancers, such as Azone® and sodium lauryl sulfate ▪ Trimodality treatment comprising of pretreatment with Azone®+ULTS in combination followed by electroporation
Methyl prednisolone [71]	▪ Human patients affected with alopecia ▪ Frequency 25kHz

Table 5: cont....

Ganciclovir [72]	▪ Mice bearing subcutaneous tumors ▪ ULTS and nano/microbubbles ▪ Low-intensity pulsed ULTS (1 MHz; 3 W/cm^2)
5- Fluorouracil [73]	▪ Whole rat skin ▪ heat-alone and Azone® enhancement
Insulin [74-76]	▪ Hyperglycemic rabbits and New Zealand white rabbits ▪ 3x1 rectangular cymbal array ▪ 50 mWcm^{-2} ▪ Rats ▪ Low profile two-by-two ULTS array based on the "cymbal" transducer ▪ ULTS exposure for 60 min (100 mW/cm^2)
Hydrocortisone acetate and Dexamethasone [78]	▪ Yucatan pigs ▪ 1.5 W/cm^2, 1 MHz, 5 minutes
Dexamethasone sodium phosphate [79]	▪ Patients with knee joint osteoarthritis ▪ ULTS waves of 1 MHz frequency was applied for 5 minutes
Triamcinolone acetonide [80]	▪ Mouse skin ▪ Frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm^2), and duty cycle (continuous, pulse mode)
Digoxin [81]	▪ Human and hairless mouse skin ▪ Continuous mode at an intensity of 1 and 3 W/cm^2 and a frequency of 3.3 MHz for 10 min
Methyl nicotinate [82]	▪ Healthy human volunteers ▪ 3.0 MHz, 1.0 W/cm^2 continuous output for 5 min
Testosterone [83]	▪ Abdominal rat skin ▪ Permeation enhancers were 1% oleic acid or 1 % dodecylamine were used as penetration enhancers
Hyaluronan [84]	▪ An animal model of osteoarthritis (knee rabbits) ▪ 1 MHz, 400 mW/cm^2 was applied to the knees for 10 min treatment bid
Calcein [85]	▪ Hairless rat skin ▪ 41 kHz, 60-300 mW/cm^2
Oligonucleotids [86]	▪ Human skin ▪ 20 kHz, 2.4 W/cm^2
Fentanyl and caffeine [87]	▪ Human and hairless rat skin ▪ 20 kHz ULTS applied at either continuous or discontinuous mode and with an average intensity of 2.5 W/cm^2
Calcium [88]	▪ Hairless mouse SC ▪ 15 MHz
Panax notoginseng [89]	▪ Mature male Sprague-Dawley rats receiving surgical transection to the left medial collateral ligament ▪ Treatments started on day 3 after surgery for six days per week over a two-week period ▪ 4 min of pulse ULTS (1 MHz) at the intensity of 0.5W/cm^2

Anesthetics

Most local anesthetics have poor topical skin penetration. Novak observed an increase in the concentration of lidocaine transmitted into rabbit subdermal tissues when topical application was followed by use of ULTS [52]. In a double blind, vehicle-controlled, crossover trial in healthy volunteers, McElnay *et al.* [53] reported no increase in absorption of lidocaine cream. There are several explanations for these contrasting results. McElnay *et al.* used lidocaine in a cream vehicle, whereas Novak [54] used lidocaine in oil: Other variables include differences in ULTS frequencies and drug concentrations. Griffin and Touchstone [55] found that 250 kHz induced the highest penetration of drug, whereas 1000 kHz consistently resulted in the least amount of recovered cortisol. McElnay *et al.* did not state why they chose 870 kHz, a frequency closer to 1000 than 250 kHz. In addition, the method that McElnay *et al.* used to test absorption (pin prick) may not have been sensitive enough to detect differences in absorption. Tachibana and Tachibana [56] showed that ULTS in conjunction with a topical aqueous lidocaine solution was rapidly effective in inducing an anesthetic effect in the legs of hairless mice. Immersion in lidocaine without ULTS did not produce analgesia. Similarly, use of ULTS without lidocaine had no anesthetic effects. These findings differed from those of Williams [57] who used an electrical sensory perception threshold technique to study

sonophoresis of topical benzocaine and dibucaine. With low-intensity ULTS (0.25 W/cm^2) and a high frequency (1.1 MHz), Williams found no detectable increase in the rate of anesthetic penetration. In light of the differences in experimental variables and methods in these studies, more research is necessary to elucidate the factors that affect sonophoresis of anesthetics.

Low-frequency ULTS has a significant effect on the transdermal permeation of high molecular weight drugs. However, the rate of permeation in pulsed mode is quite low necessitating considerable time to apply the ULTS. 0.5 MHz, which is a relatively higher frequency in the low-frequency range, can be applied in high intensity in continuous mode. Kim *et al.* [58] used a transducer to administer an anesthetic drug transdermally on healthy volunteers. The anesthetic effect was measured following administration on placebo, lidocaine HCl alone and lidocaine HCl with 0.5 and 1.0 MHz ULTS. In surface anesthesia, the sonophoresis group showed a significantly higher pain threshold than the other groups but there was no significant difference between the sonophoresis groups according to the ULTS frequency. In conduction anesthesia, the 0.5 MHz sonophoresis group showed a significant change in their pain threshold and amplitude of sensory nerve action potential compared with the other groups. Although there are limitations in applying 0.5 MHz ULTS in sonophoresis for conduction anesthesia using lidocaine hydrochloride for a nerve block, it is more effective than the 1 MHz that is widely used in clinical situations.

With the aid of permanent cultured MDCK (Madin Darby Canine Kidney) epithelial cell a model for investigations dealing with sonophoresis effects was developed by Hehn *et al.* [59]. The permeation of procaine hydrochloride through cell monolayers was examined while applying therapeutical ULTS simultaneously. It could be shown that this permeation follows Higuchi kinetics. A comparison of the velocity factors, using a continuous irradiation of 1.0 W/cm^2, shows about a 4.8 fold increase. Single ULTS pulses, however, result in a short-time enhancement of the permeation. The conclusion can be drawn that extent and velocity of the permeation of procaine hydrochloride through MDCK monolayer can be controlled by sonophoresis

Analgesic and Anti-Inflammatory Drugs

Meshali *et al.* [60] evaluated the effect of intensity, mode, and duration of ULTS application on the transport of three nonsteroidal anti-inflammatory drugs (NSAIDs) across cellulose membrane and rabbit-skin. Ibuprofen, piroxicam and diclofenac sodium were used as the model drugs. ULTS had a significant and positive effect on the transport of the model NSAIDs across cellulose and rabbit skin membranes. Increasing ULTS intensity from 0.5 to 3.0 W/cm^2 led to a proportional increase in drug transport. Continuous ULTS mode was more effective in enhancing drug transport than the pulsed mode. This study demonstrated the therapeutic potential of ULTS in transdermal delivery of NSAIDs and the synergistic effect of temperature and ULTS operational parameters on drug transport.

The effect of ULTS (1 MHz) on transdermal absorption of indomethacin from an ointment was studied in rats by Miyazaki *et al.* [61]. ULTS energy was supplied for between 5 and 20 min at a range of intensities (0.25, 0.5, 0.75, and 1 W cm^{-2}). The pronounced effect of ULTS on the transdermal absorption of indomethacin was observed at all ULTS energy levels studied. The intensity and the time of application were found to play an important role in the transdermal sonophoretic delivery system of indomethacin; 0.75 W/cm^2 appeared to be the most effective intensity in improving the transdermal absorption of indomethacin, while the 10 min ULTS treatment was the most effective. Although the highest penetration was observed at an intensity of 0.75 W/cm^2, 0.5 W/cm^2 was preferred because intensities of less than 0.5 W/cm^2 of ULTS for 10 min did not result in any significant skin temperature rise nor did it have any destructive effect on rat skin.

Tiwari *et al.* [62] studied the influence of ULTS on percutaneous absorption of ketorolac tromethamine *in vitro* across rat skin. Sonication was carried out with a continuous mode, at an intensity of 1-3 W/cm^2 and a frequency of 1 MHz for 30 min. A significant increase in permeation of ketorolac through rat skin was observed with the applied sonication at 3 W/cm^2 when compared with permeation at 1 and 2 W/cm^2. Enhanced ketorolac penetration at 3 W/cm^2 can be explained by the mechanical and/or thermal action of ULTS waves. Pretreatment of skin by 5% d-limonene in ethanol for 2 hr followed by sonication at 3 W/cm^2 (30 min) significantly enhanced the permeation of ketorolac when compared with passive flux with or without enhancer pretreatment.

The aim of Yang *et al.* [63] was to determine if a ketorolac tromethamine (KT) gel solution could be administered *in vivo* via sonophoresis using pulsed ULTS by examining its antihyperalgesic and antiinflammatory effects in a rat

carrageenan inflammation model. The changes in the mechanical and thermal hyperalgesia, nociceptive flexor reflex (NFR), as well as the swelling changes were determined. According to the antihyperalgesia and antiinflammation tests, which were used to determine the change in the pain threshold, NFR and swelling showed that the group given the sonophoretic transdermal delivery of KT exhibited significantly more noticeable anti-hyperalgesic and anti-inflammatory effects than those treated with the simple application of a KT gel. These findings suggest that the transdermal administration of a KT gel using sonophoresis using pulsed ULTS might be useful for treating acute inflammation and pain.

To compare efficacy of pain syndrome relief in osteoarthrosis at conventional administration and sonophoresis of ibuprofen (nurofen gel), Serikov *et al.* [64] applied sonophoresis of 5% gel nurofen to affected joints of 20 patients of the study group. The control group received only local treatment with 5% gel nurofen on the affected joints three times a day. The course of pain syndrome was assessed by the visual analogue scale (VAS). Monitoring of side effects, total blood count, blood biochemistry, and urine analysis were made. They recorded significant attenuation of pain by VAS at rest, in palpation, in walking in both groups. In spite of initial pain intensification in sonophoresis, positive dynamics of pain decline in the study group was more significant. Thus, their study supports anesthetic efficacy of transcutaneous 5% gel nurofen in osteoarthrosis and a rise of this efficacy in combined use of ULTS and this medicine in sonophoresis.

Cabak *et al.* [65] examined the therapeutic effects of sonophoresis with ketoprofen in gel form in patients with enthesopathy of the elbow. Ultrasonic therapy and sonophoresis have their primary application in the physical therapy of this disorder. The main aim of Cabak *et al.* was to assess the effectiveness of sonophoresis. The research group consisted of 19 patients diagnosed with enthesopathy of the lateral and medial epicondyle. In the statistical analysis they included 28 elbow joints treated with sonophoresis. The effects of therapy were compared with a control group of 20 patients who were treated with only ULTS therapy. The therapeutic series consisted of 10 treatments, using the pulse mode of ULTS and an intensity of 0.8 W/cm^2 in both groups. The clinical examination and interview consisted of specific tests, and were separately collected. The positive effects of sonophoresis using a pharmacologically active gel with ketoprofen were shown to be highly significant in both assessments, objective and subjective. The pain symptoms in the elbow resolved in most of the patients. There were statistically significant differences between sonophoresis and ULTS therapy. Their results support the application of sonophoresis with ketoprofen in the treatment of epicondylitis.

A quantitative study of sodium diclofenac (Voltaren Emulgel, Novartis) sonophoresis was undertaken in humans by Rosim *et al.* [66] Fourteen healthy human volunteers were submitted to ULTS irradiation on two 225 cm^2 areas on the dorsum (group A), followed by the application of the medication gel, and the plasma diclofenac mass was measured at 1, 2 and 3 h later by HPLC. The same procedure was repeated one month later with the same volunteers but with the ULTS equipment switched off for the control group (group B). The plasma diclofenac mass was significantly higher in group A than in group B at 1 h (0.0987 μg/mL as opposed to 0.0389 μg/mL) and 2 h (0.0724 μg/mL as opposed to 0.0529 μg/mL), but not at 3 h (0.0864 μg/mL as opposed to 0.0683 μg/mL). The authors conclude that previously applied therapeutic ULTS irradiation enhances the percutaneous penetration of the topical diclofenac gel, although the mechanism remains unclear.

Although topical drugs are usually applied at a convenient site, the target for the drug interaction may be systemic. The purposes of the study of Hippius *et al.* [67] were to investigate the *in vitro* penetration and the *in vivo* transport of flufenamic acid in dependence of ULTS. They designed a shonophoretic drug delivery system to investigate the influence of ULTS on transmembrane transport of different drugs. They investigated the absorption of flufenamic acid in a buffer medium in dependence of ULTS energy and application time. ULTS energy was supplied for between 5 and 30 min at a range of intensities up to 1.5 W/cm^2, energy levels commonly used for therapeutic purpose. The pronounced effect of ULTS on the transmembrane absorption of the drug was observed at all ULTS energy levels studied. The time of application was found to play an important role in delivery and transport of drug. Dependent on time, they observed a rise of temperature up to 4.5°C. The highest penetration was observed at an intensity of 1.0 W/cm^2 after 30 min. These results were not significantly different from concentration measurements after 30 min and 0.5 and 1.5 W/cm^2. It seems that arise of drug concentration is caused by effects of temperature and by variation of membrane delivery in dependence of temperature. Using this *in vitro* model they noted it is possible to compare the transdermal penetration and absorption of commercial flufenamic ointment in volunteers.

Antibiotics

Romanenko and Araviiskii [68] studied the effect of ULTS on the delivery of topically applied amphotericin B ointment in guinea pigs. They found that amphotericin B content in the skin and subcutaneous fatty tissues was much higher when the drug was delivered in the presence of ULTS. The highest levels of drug delivery produced involved preliminary treatment with dimethyl sulfoxide in combination with ULTS.

Ragelis *et al.* [69] performed a total of 451 experiments (130 with the method of electrophoresis and 321 with the method of sonophoresis) on healthy rabbits of the same species, age, weight and sex with the use of the galvanization apparatus "Potok-1" and ULTS therapeutic apparatus "VTP-1". The penetration levels of tetracycline into the tissues after its administration with the modified methods of electrophoresis and sonophoresis were determined. The dependence of the process on the current density, ULTS intensity, time and antibiotic concentration was studied. The efficacy of the routine electro and sonophoresis methods was compared with that of the modified ones. It was found that the tissue levels of tetracycline administered with the modified methods of electro- and sonophoresis increased with an increase in the current density or ULTS intensity, the procedure time and antibiotic concentration. When tetracycline was administered with the modified method of electrophoresis its levels were highest in the skin, lower in the muscles and minimum in the bones. With the use of the modified method of sonophoresis the highest levels were in the skin, the lowest in the bones and the minimum in the muscles.

Immunosuppressive Drugs

Liu *et al.* [70] investigated the topical transport of Cyclosporin A using low-frequency ULTS throughout rat skin. Studies of intensity and exposure time acting on the deposition of Cyclosporin A into deeper skin of *in vitro* sonophoresis were performed. Low-frequency ULTS increased the amount of Cyclosporin A retained in the skin only seven times than the passive diffusion. Furthermore, they also tested the synergistic effect of ULTS and other approaches such as chemical enhancers and electroporation on topical drug delivery of Cyclosporin A. They found that the efficacy of low-frequency ULTS in enhancing topical delivery could be further increased by pretreatment of skin with chemical enhancers, such as Azone® and sodium lauryl sulfate (SLS). Meanwhile only a small amount was seen to across the full skin into the receiver compartment. Trimodality treatment comprising of pretreatment with Azone®+ULTS in combination followed by electroporation was not effective in enhancing the topical delivery of Cyclosporin A. However, this combination strategy increased the penetration of Cyclosporin through rat skin by order of 15. In general, the enhanced skin accumulation of Cyclosporin A by the combination of low-frequency ULTS and chemical enhancers could help significantly to optimize the targeting of the drug without of a concomitant increase of the systemic side effects.

Santoianni *et al.* [71] evaluated the efficacy of low frequency sonophoresis (LFS) at 25KHz produced by a sonicator apparatus for treatment of alopecia areata, melasma and solar lentigo. Thirty patients affected by alopecia areata were treated by application of methylprednisolone or cyclosporine solution followed by LFS. In a case-control study 48 women with melasma and 48 with solar lentigo were also treated by depigmenting emulsion and LFS application. For alopecia areata after 36 applications with LFS and 3-month treatment the results were: 57 percent partial regrowth and 29 percent total with methylprednisolone; and 33 percent partial regrowth and 34 percent total when cyclosporine was used. For melasma and solar lentigo the results when the drug application was followed by LFS, were after 3-month and twice a week application: 75 percent complete depigmentation and 25 percent partial for melasma, 43 percent total regression and 57 percent partial for solar lentigo. This was the first report of sonophoresis at a frequency of 25 KHz in dermatocosmetology. The study showed that LFS, a not aggressive technique, enhanced penetration of topic agents obtaining effects at the level of the epidermis, dermis and appendages (intradermal delivery), giving better results in the treatment of some cosmetic skin disorders.

Anticancer Agents

Aoi *et al.* [72] evaluated the application of a method using ULTS and nano/microbubbles to cancer gene therapy using prodrug activation therapy. Low-intensity pulsed ULTS (1 MHz; 3 W/cm^2) and NBs were used to transduce the herpes simplex thymidine kinase (HSVtk) gene *in vitro*, leading to gene transfer. The addition of ganciclovir (GCV) to the transduced cells led to HSVtk/GCV-dependent cell death mediated by apoptosis. This technology was then assessed *in vivo*, using mice bearing subcutaneous tumors (1 MHz; 3.0 W/cm^2). Gene transfer to the tumor,

measured by luciferase activity, was transient, with a peak of expression 24 h after transduction, and decreased at 48 h, demonstrating the transient nature of ULTS/NB-mediated gene transfer. The therapeutic potential of this approach was evaluated through repeated intratumoral gene delivery using ULTS/NB-mediated transfer of the HSVtk gene, followed by recurrent administration of GCV, using two different experimental treatment protocols. In both cases, dramatic reductions of the tumor size by a factor of four were observed. Altogether, these data demonstrate the potential of ULTS/NB as a new physical gene delivery method for cancer gene therapy.

Meidan *et al.* [73] investigated the competitive transport across skin and back-diffusion of 5-fluorouracil into coupling gel under the influence of ULTS, heat-alone and Azone® enhancement. The ULTS effect on 5-fluorouracil penetration through whole rat skin was investigated in modified diffusion cells using a commercial ULTS generator which was calibrated with a bilaminar membrane hydrophone. Ultrasonic dosimetry measurements demonstrated that the skin membrane was subjected to a complex and unpredictable standing wave field which induced physiologically acceptable heating of the tissue. Surprisingly, ultrasonication produced a decrease in percutaneous drug penetration. Quantification studies indicated that this effect was due to the diffusive loss of the hydrophilic substance 5-fluorouracil from the skin surface into the overlying volume of coupling gel. This phenomenon could be duplicated by the application of conductive heating, indicating that the thermal effects of ULTS were probably responsible for accelerated 5-fluorouracil diffusion through the gel. This study acutely demonstrates how formulation design of the donor vehicle/coupling gel may radically affect therapeutic efficacy in sonophoretic systems.

Insulin

Circular cymbal ULTS arrays have been shown to be effective in delivering therapeutic levels of insulin in rats, rabbits, and pigs. To improve delivery efficiency, a rectangular cymbal design was desired in order to achieve a broader spatial intensity field without increasing the size of the device or the spatial-peak temporal-peak intensity. Luis *et al.* [74] with a similar intensity (50 mW/cm^2), determined if the 3x1 rectangular cymbal array could perform significantly better than the 3x3 circular array for glucose reduction in hyperglycemic rabbits. Rabbit experiments were performed using three groups: nonsonicated control, ULTS exposure using a circular cymbal array, and ULTS exposure using a rectangular cymbal array. Rabbits were anesthetized and a water tight reservoir that held the insulin was fastened on the rabbit's thigh. At the beginning of the experiment and every 15 min for 90 min, the blood glucose level was determined. For comparison between individual rabbits, the absolute level is normalized by subtracting out the baseline in order to arrive at the change in glucose level. For the control group, the normalized glucose level increased to +80.0±28.8 mg/dL. Using the circular array, the glucose level decreased to -146.7±17.8 mg/dl at 90 min. However, using the rectangular cymbal array, the glucose decreased faster and to a level of -200.8±5.9 mg/dl after 90 min. These results indicated the feasibility of the rectangular cymbal array as an improved device for drug delivery.

Recent studies have shown that ULTS-mediated transdermal drug delivery offers a promising potential for noninvasive drug administration. The purpose of Lee *et al.* [75] was to demonstrate ultrasonic transdermal delivery of insulin *in vivo* using rabbits with a novel, low-profile two-by-two ULTS array based on the cymbal transducer. As a practical device, the cymbal array (f = 20 kHz) was 37 x 37 x 7 mm^3 in size and weighed less than 22 g. Using the same array on hyperglycemic rats, their previous experiments demonstrated that blood glucose would decrease 233.3±22.2 mg/dL in 90 min from 5 min of pulsed ULTS exposure. With a similar intensity (Isptp = 100 mW/cm^2, 20% duty cycle), their goal was to determine if the same effect could be achieved with rabbits. Experiments were performed in New Zealand white rabbits divided into three groups: two controls and one ULTS with insulin exposure. At the beginning of the experiment and every 15 min for 90 min, 0.3 mL of blood was collected from the ear vein to determine the blood glucose level using a glucose monitoring system. For both controls, insulin-no ULTS and saline-ULTS, the blood glucose level varied from the initial baseline by approximately 75 mg/dL. However, for the ULTS-insulin group, the glucose level was found to decrease to -132.6 ± 35.7 mg/dL from the initial baseline in 60 min. Even after the array and insulin reservoir were removed, the blood glucose level of ULTS-insulin group continued to decrease to -208.1±29 mg/dL from the initial baseline. These results indicate the feasibility of using a low-cost, lightweight cymbal array for enhanced transdermal insulin delivery using ULTS.

Smith *et al.* [76] demonstrated the feasibility of ULTS-mediated transdermal delivery of insulin *in vivo* using rats with a novel, low profile two-by-two ULTS array based on the "cymbal" transducer. As a practical device, the

cymbal array (f = 20 kHz) was 37 x 37 x 7 mm in size, and weighed less than 22 g. A total of 20 Sprague-Dawley rats (350 to 450 g) were divided into four groups, two controls and two ULTS exposures, with five rats in each group. The rats were anesthetized and shaved; a water-tight standoff reservoir, which held the insulin or saline, was sealed against the rat's abdomen and the ULTS array. At the beginning of the experiment and every 30 min for 90 min, 0.3 mL of blood was collected from the jugular vein to determine the blood glucose level. For comparison between the rats, the change in the glucose level for each rat was normalized to a baseline (i.e., 0 mg/dL). The first control group used insulin in the reservoir with no ULTS and the second control group had saline in the reservoir with ULTS operating at I(SPTP) = 100 mW/cm^2 for 60 min. For the experiments, the third group employed insulin with ULTS exposure for 60 min (I(SPTP) = 100 mW/cm^2), whereas the last group used insulin with ULTS operating with a 20-min exposure (I(SPTP) = 100 mW/cm^2) to examine the effects of time on delivery. For the 60-min ULTS exposure group, the glucose level was found to decrease from the baseline to -267.5 ± 61.9 mg/dL in 1 h. Moreover, to study the effects of ULTS exposure time on insulin delivery, the 20-min group had essentially the same result as the 60-min exposure at a similar intensity, which indicates that the expose time does not need to be as long for delivery.

Corticosteroids

Saliba *et al.* [77] found that a sonophoretic effect occurred with dexamethasone when its application saturated the skin. 2-way repeated-measures analysis of variance revealed a significant main effect for ULTS treatment. The rate of appearance and the total concentration of dexamethasone in the serum were greater in subjects after sonophoresis than after sham ULTS. The sham group had only trace amounts of dexamethasone in the serum, indicating that drug absorption was negligible without the ULTS energy. The effect size of the sonophoresis conditions fell within a 95% confidence interval after the baseline measurement.

Although physical therapists and physicians often treat patients with local musculoskeletal inflammation using topically applied steroids enhanced with ULTS, there is a paucity of research confirming that sonophoresis significantly enhances drug diffusion. The purpose Byl *et al.* [78] was to determine if ULTS enhances the diffusion of transdermally applied corticosteroids. Diffusion was measured secondarily in terms of collagen deposition [estimated by levels of hydroxyproline in polytetrafluroethylene (ePTFE) tubing] and cellular activity (measured by levels of DNA). Sixteen pieces of ePTFE tubing were subcutaneously implanted on the dorsum of five mini Yucatan pigs. Pairs of tubing were randomly assigned to sham control or treatment groups. Over the paired ePTFE tubes in the treatment groups, a single transdermal application of hydrocortisone acetate (HC) or dexamethasone (DX) was applied to the skin by rubbing, sonating with the drug mixed in the acoustic gel (1.5 W/cm^2, 1 MHz, 5 minutes), or injecting the drug into the tubing. Four additional ePTFE tubes were threaded in the extremities, two submuscularly and two subtendinously, with random assignment to a sham control or a DX sonation treatment group. At the end of a week, the mean hydroxyproline levels in the swine were lower than expected (mean = 9.3 μg/cm compared to an expected mean = 22.2 μg/cm). Comparing the control and skin-applied groups with the injected and sonated treatment groups, the hydroxyproline was found to be 50% lower in the DX-injected, DX-sonated, and HC-injected sites. However, statistically there were no significant differences in DNA or hydroxyproline levels between the HC subcutaneous control and treatment groups or the DX submuscular and subtendinous groups. There was a significant main effect of group on hydroxyproline levels in the group of DX-treated, subcutaneously implanted ePTFE tubes ($p = 0.001$). Post hoc testing revealed a significant difference between the skin-rubbed and control groups together compared to the DX-injected and DX-sonated groups together ($p = 0.001$). These findings indicate that the effects of sonophoresed DX can be measured in terms of reduced collagen deposition as far down as the subcutaneous tissue but not in the submuscular or subtendinous tissue. However, a single application may not have a measurable effect on cellular activity after 7 days of healing. The unusually low level of hydroxyproline across all groups suggests that sonophoresis with steroids may have had a systemic as well as a local effect.

Many treatment options, including non-pharmacological and pharmacological measures, have been recommended in the management of osteoarthritis (OA). Among the non-pharmacological approach is physiotherapy, which involves the use of physical modalities like, heat therapy, exercise therapy, electrical stimulation, therapeutic ULTS, iontophoresis, and sonophoresis. The study of Akinbo *et al.* [79] was designed to compare the effectiveness of 0.4% Dexamethasone sodium phosphate (DEX-P) sonophoresis (SPH) with 0.4% DEX-P iontophoresis (ION) therapy in the management of patients with knee joint OA. Fifty patients with a mean age of 53.6±8.9 years were randomly

assigned to PH or ION groups with 25 patients in each group. ULTS waves of 1 MHz frequency was applied for 5 minutes to the target knee, so also was the direct current for 10 minutes for 10 sessions treatment period. Western Ontario and McMaster University Osteoarthritis Index (WOMAC) scores, 20 meters ambulatory time, and knee range of motion (ROM) were evaluated before and after therapy as the outcome measures.

At the end of two weeks, significant improvement in total WOMAC scores was observed in 15 (60%) and 16 (64%) patients in the SPH and ION groups respectively, indicating no significant difference in the improvement rate. Twenty meters ambulatory time and knee range of motion also improved significantly in both groups, yet these variables showed no significant difference between the two groups. Both therapeutic modalities were found to be effective and generally well tolerated after 10 treatment sessions. DEX-P sonophoresis was not superior to DEX-P iontophoresis in the treatment of patients with OA of the knee.

Triamcinolone acetonide (TA) is a corticosteroid that is used in the systemic and topical treatment of many inflammatory diseases. In this study, Yang *et al.* [80] designed a sonophoretic drug delivery system to enhance the TA permeability and the influence of ULTS was examined. In order to establish the transdermal delivery system for TA, a hydrophilic carbopol gel containing TA was prepared after adopting sonophoresis. A permeation study through mouse skin was performed at 37 °C using a Franz diffusion cell, and the ULTS treatment was carried out for 10 h. The level of TA permeation through the skin was evaluated under various ULTS conditions including the frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm^2), and duty cycle (continuous, pulse mode) using a 0.5% TA gel. The highest permeation was observed under the ULTS treatment conditions of low frequency, high intensity, and in continuous mode.

Cardiotonics

Machet *et al.* [81] studied the sonophoresis of digoxin *in vitro* through human and hairless mouse skin. Sonication was carried out with continuous mode at an intensity of 1 and 3 W/cm^2 and a frequency of 3.3 MHz for 10 min. Sonication at 3 W/cm^2 significantly increased the absorption of digoxin through mouse skin. Percutaneous penetration was not increased using an intensity of 1 W/cm ^{2} under the same experimental conditions. Enhanced digoxin penetration at 3W/cm^2 can be explained by the mechanical and/or thermal action of ULTS waves. Thermal simulation from electrical resistance increased digoxin flux in comparable amounts to those obtained by sonication at 3 W/cm^2. There was no enhancement of digoxin absorption across human skin by ULTS, probably due to dermal retention of this lipophilic drug.

Vasodilators

McElnay *et al.* [82] investigated the skin penetration enhancement effect of ULTS on methyl nicotinate in 10 healthy volunteers in a double-blind, placebo-controlled, crossover clinical trial. Each treatment consisted of the application of ULTS massage (3.0 MHz, 1.0 W/cm^2 continuous output) or placebo massage (0 MHz) for 5 min to the forearms of the volunteers, followed by a standardized application of methyl nicotinate at intervals of 15 sec, 1 min, and 2 min post-massage. Percutaneous absorption of methyl nicotinate was monitored using laser Doppler velocimetry. ULTS treatment applied prior to methyl nicotinate led to enhanced percutaneous absorption of the drug, for example, ULTS treatment data versus control data at 2 min showed significant increases in the peak blood flow (125.8 ±12.0 vs 75.3±10.4% flux) and in the AUC for blood flow (2630.3 ± 387.5 vs 1567.6 ±183.5% flux.min). The results of this study suggest that ULTS affects the skin structure to provide skin penetration enhancement. This finding was consistent with the proposed hypothesis that sonophoresis acts by disordering the structured lipids in the SC.

Hormones

El-Kamel *et al.* [83] investigated the effect of permeation enhancers and application of low frequency (LULTS) and high frequency ULTS (HULTS) on testosterone (TS) transdermal permeation after application of testosterone solid lipid microparticles (SLM). SLM formulations contained 10% compritol and 5 mg TS /g of SLM. The permeation experiments were performed using Franz diffusion cells and abdominal rat skin. The examined permeation enhancers were 1% oleic acid (OAc) or 1 % dodecylamine (DA). HULTS (1 MHz) was applied in a continuous mode for 1h at intensity 0.5 W/cm^2. Different intensities and application time of pulsed LULTS (20 kHz) were also

examined. Additionally, the effect of combination of ULTS and OAc or DA was investigated. Skin irritation and histological changes were also evaluated. The results revealed that SLMs have an occlusive effect on the skin. Statistical analysis revealed the following order for the permeation of TS: 1% DA for 30 min>HUS +1% DA for 30 min= HULTS=HULTS + SLM containing 1% OA> SLM containing 1% OA=control. At total application time of LULTS 6, 12, and 15 min the flux increased by 1.86, 4.63, and 4.77 fold, respectively. The enhancement effect of different intensities of LULTS was not directly proportional to the magnitude of intensity. Skin exposure to HULTS or LULTS before application of 1% DA for 30 min had no superior enhancement effect over application of either LUS or HUS alone. Application of drug loaded SLM offered skin protection against the irritation effect produced by TS and 1% DA. Histological characteristics of the skin were affected to various extents by application of enhancers or ULTS. In general, application of LULTS gave higher TS permeation than HULTS. However, safe application of LULTS should be practiced by careful selection of exposure parameters.

Cicatrizants

Park *et al.* [84] determined the effectiveness of sonophoresis on the delivery of high molecular weight (MW) hyaluronan (HA) into synovial membrane using an animal model of osteoarthritis (OA). A total of 1000 kDa (HA1000) and 3000 kDa (HA3000) HA were labeled with fluorescein and injected into the knees of rabbits. Low-intensity continuous ULTS at 1 MHz, 400 mW/cm^2 was applied to the knees for 10 min treatment bid. Synovial fluid analysis revealed increased absorption and fluorescence microscopy showed deeper penetration of both HA1000 and HA3000, more so with the latter. Histological examination indicated that ULTS treatment resulted in no apparent damage to the synovial membrane. These results suggested that simultaneous sonication with HA injection might compensate for the short half-life of HA. Consequently, this dual treatment would render HA a far more effective tool in the management of OA.

Calcein

Morimoto *et al.* [85] examined a relationship between hydrophilic solute and water (vehicle) transports in the excised hairless rat skin in the presence of ULTS (41 kHz, 60-300 mW/cm^2) irradiation and also conducted skin surface observation using confocal microscopy. When the applied intensity was increased stepwise over the rage of 60–300 mW/cm^2, the transport of tritiated water ($3H_2O$) was increased 140-fold in an intensity-dependent manner and this returned to normal on stopping the ULTS application. The skin permeation clearance (Al/h) of model hydrophilic solutes, calcein (MW 623) and-labeled dextrans [MW 4400 (FD-4) and MW 38000 (FD-40)], across the skin under the influence of ULTS was plotted against the corresponding $3H_2O$ flux (Al/h) to estimate the potential contribution of convective solvent flow, induced by the ULTS application, to the solute transport. Good correlations were observed between the $3H_2O$ flux and solute clearances and, unexpectedly, the slope values obtained from linear regression of the plots were consistent for all solutes examined (1.04F0.29 for calcein, 1.07F0.17 for FD-4, and 1.08F0.23 for FD-40, respectively). Transport of intact FD-4 and FD-40 was confirmed by gel permeation chromatography. When the skin surface and deeper regions of the skin after sonophoresis of FD-40 were observed using a confocal microscope, the fluorescence of FD-40 was uniformly distributed in the area under the ULTS horn and also evident in crack-like structures in the boundary of the horn. On the other hand, a hexagonal structure of horny cells in the SC observed by post-staining with rhodamine B was fully conserved in the area under the horn. These findings suggest that 41 kHz ULTS can increase the transdermal transport of hydrophilic solutes by inducing convective solvent flow probably via both corneocytes and SC lipids as well as newly developed routes. Their observation also suggests that 41 kHz (low frequency) ULTS has the potential to deliver hydrophilic large molecules transdermally.

Oligonucleotids

Topical delivery of oligonucleotides, though attractive for the treatment of skin disorders, is limited by the low permeability of the SC. Tezel *et al* [86] assessed the potential of low frequency ULTS (20 kHz, 2.4 W/cm^2) in delivering therapeutically significant quantities of anti-sense oligonucleotides into skin. Estimated concentrations of oligonucleotides (ODNs) in the superficial layers of the skin ranged from 0.5% to 5% of the donor concentration after a 10-min application of ULTS and ODN. Microscopic evaluations using fluorescently labeled oligonucleotides and sulforhodamine B revealed heterogeneous penetration into the skin. Heterogeneous penetration led to the formation of localized transport pathways (LTPs), which occupied about 5% of the total exposed skin area.

Immunohistochemical studies using an oligonucleotide that reacts specifically with an antibody also confirmed penetration of ODNs into LTPs. Histological studies revealed that no gross structural changes were induced in the skin due to ULTS application. These results show successful delivery of anti-sense oligonucleotides using low-frequency ULTS.

Stimulants

The effect of low-frequency sonophoresis on fentanyl and caffeine permeation through human and hairless rat skin was studied *in vitro* by Boucaud *et al.* [87]. Experiments were performed using 20 kHz ULTS applied at either continuous or discontinuous mode and with an average intensity of 2.5 W/cm^2. The results showed that low-frequency ULTS enhanced the transdermal transport of both fentanyl and caffeine across human and hairless rat skin. This was explained by both increasing flux during sonication and shortening the lag time. Discontinuous mode was found to be more effective in increasing transdermal penetration of fentanyl while transdermal transport of caffeine was enhanced by both continuous and pulsed mode. Histological and electron microscopy studies showed that human and hairless rat skin was unaffected by ULTS exposure. Further studies will be necessary to determine the relative contribution of ULTS parameters in low-frequency ULTS induced percutaneous enhancement of drug transport.

Calcium

Menon *et al.* [88] manipulated the Ca^{2+} content of the upper epidermis by sonophoresis of aqueous solutions containing physiologic Ca^{2+} (and K^{2+}) versus ion-free solution across hairless mouse SC. Sonophoresis at 15 MHz did not alter barrier function, but in the absence of Ca^{2+} the extracellular calcium content of the outer epidermis, as revealed by ion capture cytochemistry, was displaced downward the basal layer and dermis. In contrast, following sonophoresis of Ca^{2+}-containing solutions, the extracellular Ca^{2+} gradient became obscured by excess Ca^{2+} in the cytosol at all levels of the epidermis. These results demonstrated that the epidermal extracellular calcium content in the upper epidermis can be manipulated by sonophoresis without prior barrier disruption, and that changes in the Ca^{2+} gradient induce lamellar body secretion, independent of barrier disruption

Panax Notoginseng

Ng, *et al.* [89] examined the phonophoretic effect of a therapeutic ULTS coupled with a Panax notoginseng (PN) gel and compared it with a therapeutic ULTS alone for medial collateral ligament repair in rats. Twenty mature male Sprague-Dawley rats receiving surgical transection to the left medial collateral ligament (MCL) were divided randomly into three groups: ULTS (n = 7), ULTS with PN coupling gel (PNULTS, n = 7) and control (n = 6). The treatments started on day 3 after surgery for six days per week over a two-week period. The ULTS group received 4 min of pulse ULTS (1 MHz) at the intensity of $0.5W/cm^2$ with a normal ultrasonic coupling gel. The PNULTS group received the same ultrasound treatment, but with a coupling gel that contained PN extract. The control group received a placebo ULTS treatment similar to the other two groups. On day 17, the ligaments were mechanically tested for load-relaxation, stiffness and ultimate tensile strength (UTS). Values of the left side were normalized against that of the right side of each animal for analysis. Results revealed significantly higher normalized stiffness and UTS in the PNUS group than the other two groups, but insignificant difference in load-relaxation among all groups. This study reveals a positive sonophoretic effect of Panax notoginseng extract for improving the strength of ligament repair than ULTS therapy alone.

Other Uses

To Study the Mechanisms of Penetration Due to Ultrasound Throughout the Skin

Kushner IV *et al.* [90] Recent advances in low-frequency ULTS have focused on the existence of hypothesized localized transport regions (LTRs). However, there has been no actual experimental demonstration that the hypothesized LTRs are, in fact, localized regions of high permeability. Through a series of low-frequency sonophoresis experiments conducted with full-thickness pig skin, in the presence of the surfactant sodium lauryl sulfate (SLS), in which they have separately measured the transport of calcein through the LTRs, which have areas ranging from 10 to 40 mm^2, and the surrounding regions of the skin (the non-LTRs) by means of a novel masking technique, they demonstrate that the calcein permeability through the LTRs is approximately 80-fold higher than the

calcein permeability through the non-LTRs, suggesting that the LTRs are structurally perturbed to a greater extent than the non-LTRs from the exposure to the ULTS/SLS system. In addition, they propose basic models to predict the total skin transdermal permeability from the transdermal permeabilities of the LTRs and the non- LTRs, and then compare the predictions to the experimental data obtained from the masking experiments. They also demonstrate that both the LTRs and the non-LTRs exhibit significant decreases in skin electrical resistivity relative to untreated skin (5000-fold and 170-fold, respectively), suggesting the existence of two levels of significant skin structural perturbation due to ULTS exposure in the presence of SLS. Finally, an analysis of the porosity/tortuosity ratio (e/t) values suggests that trans-cellular transdermal transport pathways are present within the highly permeable, and highly structurally perturbed, LTRs.

Alvarez-Román *et al.* [91] sheded light on the mechanism(s) by which low-frequency ULTS (20 KHz) enhances the permeability of the skin. The physical effects on the barrier and the transport pathway, in particular, were examined. The amount of lipid removed from the intercellular domains of the SC following sonophoresis was determined by infrared spectroscopy. Transport of the fluorescent probes nile red and calcein, under the influence of ULTS, was evaluated by laser scanning confocal microscopy. The results were compared with the appropriate passive control data and with data obtained from experiments in which the skin was exposed simply to the thermal effects induced by ULTS treatment. Significant fractions (30%) of the intercellular lipids of the SC, which are principally responsible for skin barrier function, were removed during the application of low frequency sonophoresis. Although the confocal images from the nile red experiments were not particularly informative, ULTS clearly and significantly (again, relative to the corresponding controls) facilitated transport of the hydrophilic calcein via discrete permeabilized regions, whereas other areas of the barrier were apparently unaffected.

Lipid removal from the SC is implicated as a factor contributing the observed permeation enhancement effects of low-frequency ULTS. However, microscopic observations imply that sonophoresis induces localized permeation pathways at discrete sites.

Cancel *et al.* [92] investigated the effects of short time sonication of human skin at 20 kHz and at variable intensities and duty cycles on the dynamics of fluorescein transport across the skin as well as the changes in the skin's structural integrity. They found that a short application of ULTS enhanced the transport of fluorescein across human skin by a factor in the range of 2–9 for full thickness skin samples and by a factor in the range of 2–28 000 for heat-stripped SC samples The electrical resistance of the skin decreased by an average of 20% for full thickness samples and 58% for SC samples. Increasing the duty cycle from 10 to 60% caused a significant increase in permeability enhancement from 2.3 to 9.1, and an increase in intensity from 8 to 23 mW/cm^2 induced a significant increase in permeability enhancement from 2 to 7.4, indicating a clear dependence of the permeability on both duty cycle and intensity. The increase in solute flux upon ULTS exposure was immediate, demonstrating for the first time the fast response dynamics of sonophoretic enhancement.

In addition, a quantitative analysis of the thermal and convective dispersion effects associated with ULTS application showed that each contributes significantly to the overall permeability enhancement observed.

Low-frequency sonophoresis (LFS) has been well documented to enhance the permeability of skin to macromolecular drugs via induction of localized transport regions. However, the organizational details of epidermis, specifically SC, during sonophoresis are beyond the resolution limit of common histo-optical microscopy tools, which fail to reveal any notable structural alterations in these regions at a submicroscopic scale. Paliwal *et al.* [93] used quantum dots (QDs) as a tracer and confocal microscopy and transmission electron microscopy (TEM) (with OsO_4 and RuO_4 post-fixation) as visualization methods, on LFS induced permeation pathways in the SC. QDs (20 nm diameter) penetrated well beyond the SC. TEM revealed that ultrasound significantly increased the frequency of occurrence of the otherwise scattered and separated lacunar spaces in the SC. A significant increase in lacunar dimensions was observed when 1% w/v sodium lauryl sulfate was added to the coupling medium. These studies show that LFS induces dilatation and higher connectivity of voids in the SC, possibly leading to formation of a three-dimensional porous network, which is capable of transporting QDs as well as macromolecules across the SC. This contention is consistent with previously conceived theoretical mechanistic understanding of LFS-induced enhanced transport across the skin.

Kelloids

ULTS therapy with a water-based gel alone was reported to result in "complete flattening" of keloids in two young men when 1 MHz at 0.8 W/cm^2 was applied for approximately 4 minutes. 74 Long-term follow-up and controlled studies, however, must be done to further evaluate the efficacy of such treatment [94].

Tumors

Bleomycin is a nonpermeant, hydrophilic macromolecule with a high intrinsic anticancer cytotoxicity. However, the cytotoxic potential of the drug is restricted by its low membrane permeability. Application of low-intensity ULTS to growing tumors enhances intracellular delivery of bleomycin after IP or intratumoral administration, thereby potentiating its cytotoxicity. Optimization of ULTS parameters for *in vivo* bleomycin delivery was undertaken by Larkin *et al.* [95] and an effective antitumor effect was demonstrated in solid tumors of both murine and human cell lines. Cell death after treatment was shown to occur by an apoptotic mechanism. The results achieved in these experiments were equivalent to those achieved using electroporation to mediate delivery of bleomycin-electrochemotherapy. They found that, although temperature rises of up to 5 °C occur using the optimized ULTS conditions, this effect is not responsible for the potentiated drug cytotoxicity. This technique could be used with focused ULTS or with endoscopic ULTS probes to develop a localized and effective anticancer treatment with little or no systemic toxicity.

Khaibullina *et al.* [96] determined if pulsed high-intensity focused ULTS (HIFU) exposures could enhance tumor uptake of (111)In-MX-B3, a murine IgG1kappa monoclonal antibody directed against the Le(y) antigen. MX-B3 was labeled with (111)In, purified, and confirmed for its binding to the antigen-positive A431 cell line. Groups of nude mice were inoculated subcutaneously with A431 tumor cells on both hind flanks. A tumor on one flank was treated with pulsed-HIFU; the other tumor was used as an untreated control. Within 10 min after the HIFU exposure, the mice received intravenous (111)In-MX-B3 for imaging and biodistribution studies. Mice were euthanized at 1, 24, 48, and 120 h after injection for biodistribution studies. The HIFU exposure shortened the peak tumor uptake time (24 vs. 48 h for the control) and increased the peak tumor uptake value (38 vs. 25 %ID/g [percentage injected dose per gram] for the control). The HIFU effect on enhancing tumor uptake was greater at earlier times up to 24 h, but the effect was gradually diminished thereafter. The HIFU effect on enhancing tumor uptake was substantiated by nuclear imaging studies. HIFU also increased the uptake of the antibody in surrounding tissues, but the net increase was marginal compared with the increase in tumor uptake. This study demonstrates that pulsed-HIFU significantly enhances the delivery of (111)In-MX-B3 in human epidermoid tumors xenografted in nude mice. The results of this pilot study warranted further evaluation of other treatment regimens, such as repeated HIFU exposures for greater delivery enhancement of antibodies labeled with cytotoxic radioisotopes or pulsed-HIFU exposure in addition to a combined therapy of (90)Y-B3 and taxol to enhance the synergistic effect.

Suppurative Wounds

Sonophoresis has also been studied in the treatment of suppurative wounds. Levenets [97], Shuvalov, and Poliakov [98] found that the sonophoresis of ethylenediaminetetlaacetic acid with the quinoxaline antibiotic dioxidine was effective in accelerating wound purification and elimination of necrotic issues. Matinian *et al.* [99] similarly reported that the sonophoresis of a 1% papain solution together with dimethyl sulfoxide was an effective method for treating purulent wounds and inflammatory infiltrates. They found that sonophoresis of the aforementioned solutions almost halved the healing time.

CONCLUSIONS

The use of Sonophoresis in skin treatment therapy is to provide enhanced permeability of the skin. This permeabilisation of the skin allows a far higher absorption of the active ingredients the therapist is attempting to infuse. The result will be both a more economical use of the therapeutic agents applied, and a greater response to the agent due to better penetration.

In summary, even though today only nine drugs are administered transdermally in general practice, with the advent and development of ULTS mediated transdermal transport; patches may soon become the name of the game. Besides,

taking into account the varied possible applications of sonophoretic transdermal drug transport in fields of biotechnology and genetic engineering, we can envision a whole gamut of newer technologies and products in the foreseeable future for all transdermal drug delivery including the use of physical enhancers like sonophoresis (Fig. **5**).

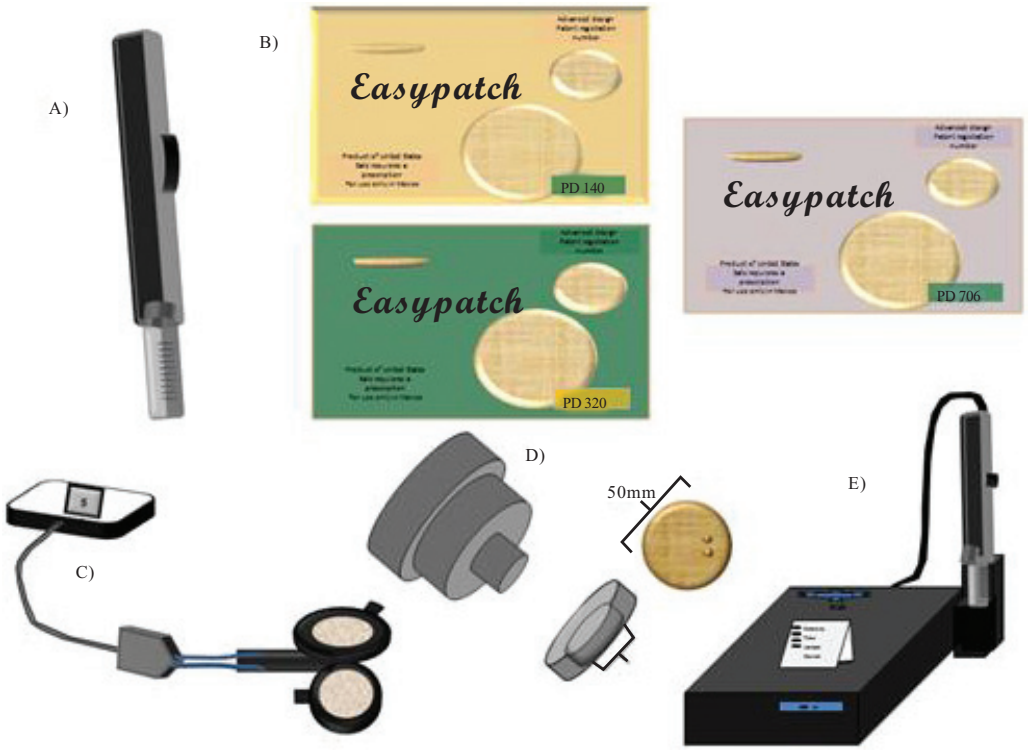

Figure 5: Images of transdermal products: a) Jet injection device, b) Transdermal patches, c) Iontophoresis device, d) Microneedles device, e) Sonophoresis device.

Current evidence [9,13] suggests that sonophoresis are promising methods of enhancing topical delivery of both dermatologic and nondermatologic drugs (Table 4 and 5). These methods may enable precise control of transdermal drug delivery rates by varying ULTS frequency. Further controlled studies of this modality are necessary to determine optimal technique and conditions for safe and efficacious utilization. Future interest may also focus on systemic sonophoretic delivery of peptide and protein drugs. ULTS has also been shown to enhance transdermal transport synergistically with other penetration enhancers, such as chemical enhancers and electrical methods (iontophoresis and electroporation). These studies suggest that sonophoresis may provide a powerful new approach to transdermal drug delivery.

Studies on the physics of ULTS as an enhancer of transcutaneous drug delivery must also be continued. This research should be closely paralleled with research on the variables of ULTS delivery, the length of a treatment session, and the duration of sonophoresis treatments in terms of effectiveness of diffusion at the local as well as systemic level. This same research should be come out with asymptomatic subjects as well as those with pathology.

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CHAPTER 5

Electroporation of the Skin

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Abstract: Transdermal drug delivery offers an attractive alternative to the conventional drug delivery methods of oral administration and injection. However, the stratum corneum acts as a barrier that limits the penetration of substances through the skin. Application of high voltage pulses to the skin increases its permeability (electroporation) and enables the delivery of various substances into and through the skin.

The application of electroporation to the skin has been shown to increase transdermal drug delivery. Moreover, electroporation, used alone or in combination with other enhancement methods, expands the range of drugs (small to macromolecules, lipophilic or hydrophilic, charged or neutral molecules) which can be delivered transdermally.

The efficacy of transport depends on the electrical parameters and the physicochemical properties of drugs. The *in vivo* application of high voltage pulses is well tolerated but muscle contractions are usually induced. The electrode and patch design is an important issue to reduce the discomfort of the electrical treatment in humans.

This chapter presents the main findings in the field of electroporation. Particular attention is paid to proposed enhancement mechanisms and trends in the field of topical and transdermal delivery.

Key words: Skin electroporation, Transdermal electroporation, physical enhancers, skin, transdermal drug delivery.

HISTORICAL PERSPECTIVE ON ELECTROPORATION

In 1802 study, J. W. Ritter was the first to notice an electrophysiological phenomenon that may be related to what we now call "reversible electroporation". He reports the observation of a contraction that occasionally occurs when a strong electric current passing through a stretch of muscle-nerve preparation is interrupted [1]. This phenomenon, which became known as *Ritter's opening tetanus,* was not understood at the time. However, later research performed in the mid 20th century attributed the phenomenon to an electric field induced "breakdown" of the cell membrane.

Perhaps a first reference to what is now called "irreversible electroporation" can be found in a late 1800's book by A.D. Rockwell [2]. In experiments with red blood cells and Leyden jars it is reported that "Under the discharges of the Leyden jar the red corpuscles (of the blood, i.e. red blood cells) change their shape and lose their color". This is probably a description of hemolysis induced by irreversible electroporation [3]. Leyden jars were capacitors used to accumulate the charge generated by the electrostatic generators so that it was possible to produce high-voltage and high-current short pulses as it is required for electroporation of cells in suspension.

A 1936 report of G.M. McKinley [4] is relevant to this review in the sense that, from his own observations and those of others he concludes that damage caused to living tissues by high frequency electric fields (10 to 100 MHz) cannot be only from thermal origin, particularly in the case of nervous tissue. He even brought forward the ideas that this special "agent" (of damage) is associated with the electric field and that it can be used as a minimally invasive ablative method, which will be selective to some specific tissues.

Electroporation, in the conventional sense, is the phenomenon in which cell membrane permeability to ions and macromolecules is increased by exposing the cell to short high electric field pulses (Fig. **1**). The increase in permeability is attributed to the electric field induced "breakdown" of the cell membrane and the formation of nano-

scale defects or "pores" in the membrane – and hence electro-"poration". Although relevant scientific observations were made since the 18th century, the electroporation phenomenon was not identified as an increase of membrane permeability until the mid 20th century. Electroporation can be of two types - reversible and irreversible. In irreversible electroporation (IRE) the electric field is such that the membrane permeabilization leads to cell death. This may be caused by either permanent permeabilization of the membrane and cell lysis (necrosis) or by temporary permeabilization of a magnitude which can cause a severe disruption of the cell homeostasis that can finally results in cell death, either necrotic or apoptotic. In reversible electroporation the electric pulse causes only a temporary increase in permeability and the cell survives. The reversible electroporation mode has numerous applications in biotechnology and medicine both, *in vitro* (DNA electrotransfer) and *in vivo* (electrogenetherapy and electrochemotherapy). Irreversible electroporation has applications in the food industry (where it is known as a pulsed electric field (PEF)), for sterilization and in medicine for tissue ablation [5].

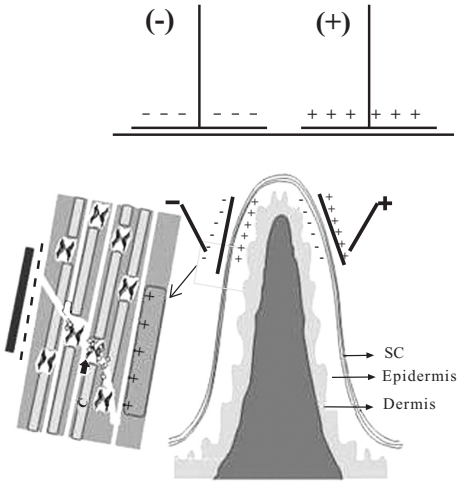

Figure 1: Schematic drawing showing skin and subcutaneous tissue being clamped between a pair of plate electrodes. During the pulse application, charges build up across the stratum corneum.

ELECTROPORATION OF CELLS

Perhaps the first publication that suggests the possibility of cell membrane breakdown due to an electric field is an 1951 paper by A.L. Hodgkin [6]. In discussing the Ritter's opening tetanus phenomenon, described earlier, he states that it is associated with "… (the breakdown)… of the insulating properties of the membrane … under the influence of the abnormally high potential difference". The wording is suggestive of the concept of breakdown of a cell membrane viewed as a dielectric layer. Modeling the cell membrane as a dielectric was already introduced earlier. In 1925, H. Fricke [7] was able to hypothesize a reasonable value for the membrane thickness (30 nm instead of the actual 5 nm) by analyzing the passive electrical properties of red blood cells under the assumption that the cell membrane acts electrically as a thin dielectric layer. Viewing the cell membrane as a thin dielectric it was reasonable to expect that some sort of dielectric rupture phenomenon could exist in the case of living cells as it happens in most dielectrics. However, the idea that electroporation is some sort of dielectric rupture by electron avalanche is now discredited [8].

The first fundamental study on cell electroporation is a 1960's series of three papers by Sale and Hamilton. They show that the electric field strength for inducing the lysis of various organism by what is now called "irreversible electroporation' ranges from 3.1 kV/cm to 17 kV/cm (fields for 50 % population lysis with a protocol of 10 pulses of 20 μs). The equivalent induced transmembrane voltages was calculated in the range from 0.7 V to 1.15V [9]. In order to compute these transmembrane voltages, Sale and Hamilton employed a model in which the cell was considered to be a conductive sphere isolated from the external conductive medium by a thin dielectric layer. Then they employed equations derived from those of J.C. Maxwell for calculating the conduction through a suspension of spheres [10, 11]. The transmembrane voltage (V_m) has a maximum at the poles facing the electrodes (i.e. direction of the electric field, **E**) and its value at those two points is $V_m = (3/2)\times a\times|\mathbf{E}|$ where a is the radius of the cell. At any point of the cell the transmembrane voltage is $V_m=(3/2)\times a\times|\mathbf{E}|\times\cos(\theta)$ where θ is the polar angle measured from the

center of the cell with respect to the direction of the field. This expression is usually referred to as the *Schwan's equation* [12]. In their papers Sale and Hamilton also suggested a mechanism for electroporation stating that the transmembrane potential induced by the external field may cause "conformational changes in the membrane structure resulting in the observed loss of its semipermeable properties"

Some of the major advances in the field of reversible electroporation occurred in the 1970's and 1980's. In their 1972 paper, E. Neumann and K. Rosenheck, show that electric impulses of about 18 to 24 kV/cm and about 150 μsec long produce reversible permeabilization of the cell membrane of chromaffin granules of bovine-medullary cells used as vesicles for epinedrine, norepinephrine, ATP and proteins [13]. Experiments done at 0 °C show that the largest increase in temperature is 6 °C and that the observed effect of reversible permeabilization is therefore not thermal. However, while their experiments are the first to involve reversible electroporation, Neumann and Rosenbeck try to relate their observations to the physiological release of hormones and neurotransmitters in neurons, rather than what was later called electroporation. In 1977, K. Kinosita and T. Tsong, [14] proposed that the permeabilization of the cell membrane due to the application of electric pulses is related to the formation of several pores with radii in the range of a few angstroms. In classic osmotic mass transfer experiments with red blood cells they showed that the size of these pores can be varied and that these pores eventually reseal. The sealing process was found to be strongly temperature dependent. In the early eighties, the use of reversible electroporation to produce fusion between cells is described in a paper by Zimmermann [15]. In a now classical paper Neumann and his collaborators coined the term *electroporation* to describe the cell membrane breakdown discussed in this review and introduce the use of reversible electroporation for the insertion of genes into cells [16]. They also present a classical thermodynamic analysis of the formation of pores during electroporation.

In 1984, H. Potter *et al.* [17] designed an electroporation cuvette suitable for cells in suspensions and microbiology researchers started to employ electrophoresis power supplies in order to perform gene transfection by electroporation. Soon after, multiple commercial generators specifically intended for electroporation were developed and now this transfection technique is very common in microbiology laboratories. Summaries on the technique and its applications can be found in several edited books, e.g. [18, 19].

In 1997 K.H. Schoenbach *et al.* [20, 21] reported the first *in vitro* study on the use of very high voltage pulses of "submicrosecond" duration. Numerous papers have been published since then on the use of pulses with a duration of some nanoseconds or tens of nanosecond [20-25]. The main motivation for this line of research line came from the believe that those ultra-short pulses, known as *nanosecond Pulsed Electric Field* (nsPEF), could be able to induce electroporation of intracellular membranous structures (e.g. mitochondria) without disturbing the cell membrane. However, recent computer models [26] and experimental results [27] indicate that cell membrane electroporation also occurs when nanosecond pulses are applied. That is, with nsPEF all the membranous structures of the cell are electroporated (some authors refer to this phenomenon as *supraelectroporation*). It is interesting to point out that researchers in this field found that nsPEF can induce apoptosis [28] and that they succeeded in inhibiting tumor growth after nanosecond pulses were applied *in vivo* [29, 30].

ELECTROPORATION OF TISSUE

The use of reversible electroporation in tissue (Fig. **2**) can be traced to two mid 1980's papers by M. Okino and H. Mohri [31] and S. Orlowski *et al*., [32] who proposed, independently, the use of electroporation to reversible permeabilize cells in tissue and thereby introduce more effectively cytotoxic agents into malignant cells, for treatment of cancer.

The first report on the use of reversible electroporation to introduce plasmid DNA into a living tissue was published in 1991 by A.V. Titomirov *et al.* [33]. Gene delivery to cells in tissue has now become an area of major importance to biotechnology and medicine in which reversible electroporation plays a central role. It has also found applications in treatment of cancer, e.g. [34, 35]. Some of the reviews and edited books written on this topic include [36-38].

In 1991 the group of L.M. Mir published two breakthrough papers on the use of reversible electroporation to treat cancer by facilitating the penetration of anticancer drugs, such as bleomycin, in the malignant cells. They coined the term *electrochemotherapy* to describe this procedure [39] and reported the first clinical trial in the field of

electroporation [40]. Electrochemotherapy is now one of the most important applications of reversible electroporation and is being used clinically to treat cancer patients. Probably the most updated review information on the topic can be found in [41-44].

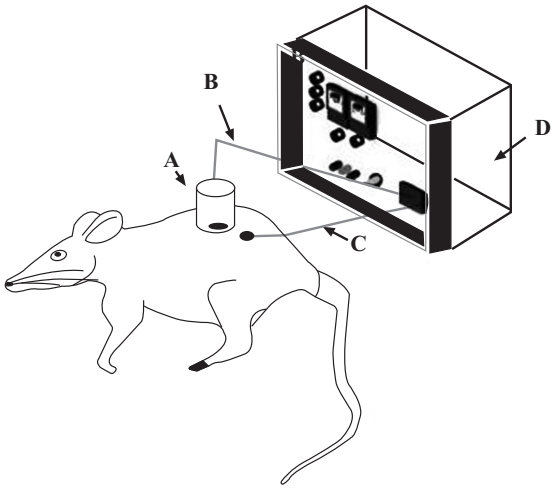

Figure 2: Diagram representing the *in vivo* experimental electroporation. A sampling chamber glues on the skin surface of a rat (A). Ag/AgCl electrodes place in the sampling chamber and secure on the skin surface (B and C), they are connected to porator (D).

The latest advance in the field of electroporation in tissue is the use of irreversible electroporation in a non-thermal mode for tissue ablation [45, 46]. The basic concept in this application is that when irreversible electroporation is delivered to targeted tissues in such a way that the Joule heating induced temperature elevation is restricted to levels that do not substantially affect biological molecules, the damage to cells is restricted to the cell membrane and the extracellular scaffold remains intact. This facilitates selective ablation of cells while retaining mechanical and structural integrity of the tissue scaffold. A recent review of advances in this field can be found in [47].

ELECTROPORATION OF THE SKIN

Historical Background

In a 1913 set of lectures, A.J. Jex-Blake reviews knowledge on the lethal effects of human made electricity and lighting [48]. He notes that burns observed in industrial accidents with electricity are related to thermal effects whereas electrical injuries from lighting do not seem to be always from thermal origin. His observations can, in hindsight, be related to electroporation of the skin. A non-lethal effect of lighting on humans is the emergence of red Lichtenberg figures on the skin that disappear in a few days. It is now thought that they are caused by "red blood cells extravasated into the superficial layers of the skin from capillaries secondary to the dielectric breakdown of the skin and subsequent massive electron shower" [49]).

The field of skin electroporation is made of two aspects. The first deal with electroporation in a conventional sense in relation to the cells of the skin and the second is unique and relates to transdermal effects.

Studies on electroporation of epidermal cells were reported soon after electroporation became an acceptable tool in biotechnology, in the early 1980's. For instance an 1986 paper reports the use of electroporation of murine epidermal cells for transfection of cells, which expressed the foreign DNA [50]. Primary human skin fibroblasts, a source of phenotypicall and karyotipical normal human cells were reported in an 1988 report to be easily transfected with exogeneous DNA plasmids [51]. The process of reversible and irreversible electroporation in cells from the skin is similar to that of any other cell, and therefore it lends itself to all the applications used with electroporation in any type of cell.

The concept of transdermal electroporation may be traced to fundamental research on the breakdown of flat lipid bilayer membranes reported in the late 1970's and early 1980's. For instance, the group of Zimmerman performed pulsed electric field experiment with lipid bilayers from oxidized cholesterol/n decane and showed that charging the

membrane to voltages on the order of 300 mV causes irreversible membrane rupture. In a variety of experiments it was found that there are also other conditions in which the membrane breakdown is reversible and the potentials across the membrane which cause the effect are in the range also reported by Sale and Hamilton with cells, i.e. on the order of 1V [52]. Further studies on lipid bilayers calculated electric field induced formation of pores with a radius of 4 nm, that can reseal [53]. Other studies on planar lipid bilayers exposed to rectangular voltage pulses of 150 – 700 mV showed evidence of reversible electric breakdown of the lipid layer. The resealing occurred in two time scales, a rapid one in less than ms and a slower one which lasted seconds and even minutes [54].

The first true skin electroporation experiment was done in the frog (Rana pipens) skin and the results were compared to electroporation results from flar lipid bilayers [55]. Exposing the viable skin to electroporation type pulses and measuring the transtissue potential it was observed that the potential decayed with two distinct time constants of 0.3 ms and 2 ms, which is indicative of the temporal dimension of the permeabilization of the skin. Resealing occurred in the time frame of 2-3 minutes after the pulse stopped, demonstrating repeatability and providing an indication of the time scale of skin permeabilization during reversible electroporation. The 1991 work of Titomirov *et al*., [33] which was discussed earlier and established DNA transfection in tissue was also done with the skin cells of newborn mice. Electroporation was used to transfect skin cells of newborn mice, *in vivo*. The plasmid DNA was introduced subcutaneously and followed by high-voltage pulses applied to the skin pleat.

The seminal paper which established electroporation as a mechanism to enhance transdermal drug delivery was published in 1993 [56]. The paper addresses the fact that mammalian skin functions as a barrier to mass transfer through the outermost dead layer of the skin, the stratum corneum. Transdermal transport normally occurs primarily through the intracellular lipids organized in bilayers. Small molecular weight lipophilic drugs can be effectively delivered by passive transdermal delivery. However, the stratum corneum does not permit passage of polar/hydrophilic molecules and macromolecules. The paper suggests that microsecond to millisecond electroporation type pulsed electric fields applied across the skin produce, in a manner similar to that found in studies on flat lipid bilayers, trans bilayer aqueous pores. It reports that electroporation produces transient structural changes in the skin resulting in an up to four orders of magnitude increase in transdermal mass transfer flux of polar molecules in human skin *in vitro* and animal skin *in vivo*. Subsequent to this paper researchers began to compare the established technique of transdermal iontophoresis (e.g.[57]), with the new technique of transdermal electroporation (e.g. [58]). The technical difference between these methods is that iontophoresis employs much lower voltages for longer periods of time than electroporation.

Transdermal Electroporation Protocols

A number of papers were published over the years which investigate what are the optimal electroporation parameters for transdermal transport of various substances. For instance a study of transdermal delivery of metoprolol, which employed several orders of magnitude longer pulses than conventional electroporation in cells, found that when 5 single pulses (each separated by 1 min) were applied at varying voltages from 24 to 450 V (pulse time 620 ms) a linear correlation occurs between pulse voltage and cumulative metoprolol transported after 4 h. Varying pulse duration of 5 single 100 V pulses from 80 to 710 ms (each pulse also separated by 1 min) showed that cumulative metoprolol transported after 4 h increased linearly with the pulse time. [59]. Transdermal transport of another highly-charged macromolecule (heparin), using high-voltage pulses believed to cause electroporation was reported in [60]. The transdermal heparin transport across human skin *in vitro* occurred at therapeutic rates (100-500 micrograms/cm^2h). In contrast, fluxes caused by low-voltage iontophoresis having the same time-averaged current were an order of magnitude lower. However, while heparin transported across the skin was biologically active, it had only one eighth the anticoagulant activity of heparin in the donor compartment due to preferential transport of small (less active) heparin molecules. This dependence of mass transfer on molecular weight is an important consideration that needs to be made when delivering transdermally cocktails of compounds. We believe that this is not unique to electroporation and it is relevant to any method that employs natural or forced diffusion across the skin. Another early application of transdermal electroporation was for fentanyl [61]. Fentanyl is a synthetic opioid with short-acting analgesic activity after intravenous or subcutaneous administration. The low molecular weight, high potency and lipid solubility of fentanyl make it suitable for delivery via the transdermal therapeutic system (TTS) [62]. However, experiments with hairless rat skin have shown that the application of electric pulses can strongly promote transdermal delivery of fentanyl compared to passive diffusion through untreated skin. It was also

found that the choice of the waveform of the electric pulses is important: "at the same applied energy, a few exponentially-decaying (ED) pulses increased fentanyl permeation more than a few square-wave pulses and to the same extent as the repeated application of higher voltage-shorter duration ED pulses". However a central issue in the paper is that transdermal electroporation is effective not only for polar molecules but also for molecules that exhibit lipid solubility. Further studies on transdermal delivery of fentanyl were performed *in vitro* with full-thickness hairless rat skin [63]. Skin electroporation was carried out with five exponentially-decaying pulses of 100 V applied voltage and around 600 ms pulse duration. Results have shown that rapid transport occurred during pulsing due to electrophoresis and diffusion through highly permeabilized skin. Slow post-pulse passive transport was observed and explained by lasting changes in skin permeability.

Transdermal delivery of peptides, such as insulin, by electroporation was reported in [64]. Compared to passive diffusion, use of multiple pulses (25 pulses, 10 ms each) with an electrode voltage of 200 V resulted in a sixty-fold increase in the delivery of cyclosporin-A to the skin. In contrast, attempts to deliver from a solution of cyclosporin-A prepared in 40% ethanol (EtOH) in phosphate buffered saline (PBS) using iontophoresis did not result in any significant increase in drug delivery. Transdermally delivered cyclosporine-A was mostly bound to the skin and only a small amount was seen to cross the full skin into the receiver compartment [65].

Transdermal transport of methylene blue, a water soluble cationic dye, was studied with two electrodes places on the outer surface of excised full thickness porcine skin [66]. Methylene blue was applied to the skin beneath the positive electrode; 1 ms pulses of up to 240 V were delivered at frequencies of 20-100 Hz for up to 30 min. The amount of dye in a skin sample was determined from absorbance spectra of dissolved punch biopsy sections. Transport induced by electric pulses was more than an order of magnitude greater than that seen following iontophoresis. The paper suggests that the enhanced cutaneous delivery of methylene blue is due to a combination of *de novo* permeabilization of the stratum corneum by electric pulses, passive diffusion through the permeabilization sites, and electrophoretic and electroosmotic transport by the electric pulses.

A comparison was made between transdermal fluxes of tetracaine achieved with passive diffusion and either electroporative pulse and iontophoresis [67]. The results show that electroporation is effective in enhancing transdermal delivery of tetracaine and it functions better than iontophoresis. Electroporation was performed using (square-wave pulse, voltage 130 V, pulse time 0.4 s, pulse frequency 40 pulses per minute and iontophoresis using (0.2.mA.cm^{-2}, lasting for 4 h). The flux of tetracaine at 0.25 h after electroporation (number of pulses - 400) was 54.6+/-6.0 microg.cm^{-2}.h^{-1} while that after iontophoresis was 17.4+/-5.8 microg.cm^{-2}.h^{-1} and that after passive diffusion was 8.2+/-0.5 microg.cm^{-2}.h^{-1}. [67].

Transdermal delivery of methotrexate (MTX) with relevance to the treatment of psoriasis and neoplasic diseases was studied by placing electrodes either side-by-side on the surface of excised full thickness pig skin, or on a piece of skin clamped between compartments of a vertical diffusion chamber. Sixty rectangular electric pulses at 120 V, 1 ms and 1 Hz were applied across the skin and the solution kept on the skin for 10 minutes. Cumulative drug transport was measured by radioactive tracing, using [3H]-methotrexate, from punch biopsy samples taken from under the cathode. Results show that using side-by-side electrodes, treatment with the pulses alone resulted in a 2.5-fold increase in MTX transport; adding anionic lipid enhancers to the pulses resulted in a 4.4-fold enhancement compared with passive diffusion. Concurrent iontophoresis made a nonsignificant contribution. MTX penetration profiles indicated that more than half of the MTX was confined to the epidermis and papillary dermis. In conclusion electroporation of MTX with an anion lipid enhancer under a mild hyperthermic environment provided a significant transdermal delivery within a short application time [68].

Combined Electroporation Modalities

It is natural to consider the combination of the two electric based methods for enhancing transdermal transport, electroporation and iontophoration. It is useful to bring here and at this stage the difference between electroporation and iontophoresis as they emerge from an important study by Chen, Langer and Weaver, in their own words, [69] "High voltage pulsing of human skin (approximately 100 V across the skin, 1 ms pulses) has been hypothesized to cause electroporation of the stratum corneum, and to cause large fluxes of drugs and other molecules across the skin, through newly created aqueous pathways. In contrast, iontophoresis (<0.5 mA per cm^2, <1 V across the skin) has

long been used in transdermal drug delivery, and is believed to involve pre-existing pathways associated with hair follicles and sweat ducts. Either high voltage pulsing or iontophoresis was applied to human, hairless rat, or black rat snake skin. Hairless rat skin contains more hair follicles than human skin, and snake skin does not contain any hair follicles. All three types of skin had comparable electrical resistances at low voltages; however, the iontophoretic transport of charged fluorescent molecules was significant for human and hairless rat skin, but no transport occurred across snake skin, indicating that hair follicles and sweat ducts play a major role in iontophoresis. Electroporation caused large molecular transport for all three types of skin, and involved spontaneously forming localized transport regions, not associated with appendages. These experiments thus provide further support for the hypothesis that high voltage pulsing causes electroporation in the stratum corneum, and that this transport mechanism is fundamentally different from iontophoresis." Another review study that compares iontophoresis and electroporation can be found in [70]. Combining electroporation with iontophoresis was proposed shortly after the introduction of the concept of transdermal electroporation [71]. The authors have delivered the luteinizing hormone releasing hormone trough human skin using a combination of first electroporation followed by iontophoration. The electroporation parameters are typical, a single square pulse of 1 kV delivered for five ms. This is followed by iontophoresis in a range from 0 to 4 mA/cm^2. As expected the single electroporation pulse increased the flux by five to ten times as compared to the transdermal transport without electroporation. The effect of electroporation was reversible. Considering that electroporation, presumable, causes the formation of aqueous pores and the reduction of the skin electric resistance, the potential applied across the skin after electroporation is higher than when the same iontophoretic current is used without electroporation.

Combination of iontophoresis and/or electroporation was used *in vitro* to deliver the calcium regulating hormones, salmon calcitonin (sCT) and parathyroid hormone (1-34) (PTH) through human cadaver epidermis. A sCT (50 microg/ml) or PTH (1-34) (100 microg/ml) formulation was prepared in citrate buffer (pH 4.0 or 5.0, respectively). Iontophoresis was applied using a constant current power source and electroporation with an exponential pulse generator. Silver/silver chloride electrodes were used. A combination of electroporation and iontophoresis resulted in higher transdermal permeation than either one technique alone. The transdermal transport of salmon calcitonin by pulsing with 15 pulses (1 ppm) of 500 V (200 ms) followed by iontophoresis led to a quick input and high flux. The average transdermal voltage was only about 50 V [72].

Ultrasound and sonophoresis are also used to enhance transdermal transport, e.g. [73, 74]. Similarly to the synergistic effect of iontophoresis and electroporation, the MIT group also reports a synergistic effect of electric fields and ultrasound. [75].

The idea to use macromolecules as additivites to chemically enhance electroporation induced transdermal transport was reported in [76]. Enhancement of transdermal mannitol transport *in vitro* by heparin, dextran-sulfate, neutral dextran, and poly-lysine was examined. While skin electroporation increased transdermal mannitol delivery by approximately two orders of magnitude, the addition of macromolecules further increased transport up to five-fold. Although unable to enhance passive or iontophoretic transport, the tested macromolecules enhance electroporation-assisted delivery, tentatively, by stabilizing the increased permeability caused by high-voltage pulses. The transport enhancing macromolecules were effective for hours after pulsing, presumable by a macromolecule-skin interaction. "No enhancement was observed during passive diffusion or low-voltage iontophoresis, suggesting that macromolecules interact specifically with transport pathways created at high voltage. Although all macromolecules studied enhanced transport, those with greater charge and size were more effective." At the same time as the paper discussed above another study was published to report that heparin, a linear, highly charged macromolecule, significantly alters the molecular transport capacity and lifetime of aqueous pathways across human stratum corneum created by short, high-voltage (approximately 100 V) pulses. It has been shown that these pulses increase rates of transdermal transport by several orders of magnitude, via a mechanism hypothesized to involve electroporation. [77]. The observed increase in post-pulse skin permeability and persistent lower skin resistance with heparin was explained by heparin molecules being long enough to span the five to six lipid bilayer membranes that separate corneocytes within the SC. The hypothesis is that heparin molecules were trapped within the skin, holding open pathway segments connecting adjacent corneocytes.

A combined high voltage pulsed electric field and topical sodium thiosulfate intervention has shown that significant macromolecule transdermal fluxes occurred only if a pathway enlarging molecule (sodium thiosulfate) was present

during the electroporation. *In vitro* experiments with electroporation and sodium thiosulfate demonstrate that transdermal macromolecule fluxes of 10^{-9} to 10^{-8} mol h^{-1} cm^{-2} (10 to 100 microg h^{-1} cm^{-2}) or greater are possible for lactalbumin and an antibody (IgG), which are potentially therapeutic values for peptides, proteins and nucleic acids. In the absence of sodium thiosulfate, only the flux of a small molecule (sulforhodamine) increased significantly, consistent with many previous studies. The results provide support for the hypothesis that high voltage pulses leading to transdermal voltages U(skin) > 50 V create straight-through aqueous pathways that penetrate multilamellar bilayer membranes, corneocyte envelopes and corneocyte interiors within the stratum corneum and that chemical additives such as sodium thiosulfate create enlarged and more stable aqueous pathways [78].

A very thorough study has shown that anionic phospholipids, but not cationic or neutral phospholipids, enhance the transdermal transport of molecules by electroporation. When added as liposomes to the milieus of water-soluble molecules to be delivered through the epidermis of porcine skin by electroporation, these phospholipids enhance, by one to two orders of magnitude, the transdermal flux. Encapsulation of molecules in liposomes is not necessary. In the presence of 1 mg/ml Dimyristoylphosphatidylserine (DMPS) in the transport milieu, the flux of FITC-Dextran-4k was enhanced by 80-fold and reached 175 $\mu g/cm^2$/min. The authors suggest that after being driven into the epidermis by negative electric pulses, saturated anionic phospholipids mix and are retained better by the SC lipids. Anionic lipids prefer loose layers or vesicular rather than multilamellar forms, thereby prolonging the time for structural recovery of SC lipids to the native multilamellar form [79]. The same group has demonstrated enhanced transdermal insulin delivery using lipid enhanced electroporation. Fluorescence microscopy revealed that the insulin transport was mainly through the lipid multilayer regions that surround the corneocytes [80].

Electroporation application in the presence of electrolytes, particularly $CaCl_2$, was found effective in increasing transdermal delivery of water-soluble macromolecules. The effects of electroporation (300 V, 10 msx10 times) and 150 mM NaCl or $CaCl_2$ on skin permeation of higher molecular weight compounds, fluorescein isothiocyanate (FITC)-dextrans (FD-4, FD-10 and FD-40; average molecular weight, 4.4, 9.6 and 35.6 kDa, respectively) was studied using excised hairless rat skin. The observed steady state flux of FD-4 was 1.3 $pmol/cm^2/h$ after electroporation without NaCl or $CaCl_2$. The flux did not differ greatly from that without electroporation. In contrast, a much higher steady state flux was observed after electroporation with NaCl or $CaCl_2$ (2.5 and 8.2 $pmol/cm^2/h$, respectively). For FD-10 and FD-40, no flux was detected with electroporation in water (without electrolytes) or without electroporation. On the other hand, high skin permeation was observed after electroporation in NaCl or $CaCl_2$ solution (FD-10: 7.5 and 18.2 $pmol/cm^2/h$, FD-40: 4.5 and 9.3 $pmol/cm^2/h$ in NaCl and $CaCl_2$, respectively). The effects of $CaCl_2$ on FD permeation were greater than those of NaCl [81].

A recent combination of methods employs lasers and electroporation for delivery of methotrexate (MTX) via the skin route [82]. The technique combined an erbium:yttrium-aluminum-garnet (Er:YAG) laser and electroporation using a nude mice animal model. The combined application of the laser and electroporation significantly enhanced the permeation of MTX. The enhancing effect was more pronounced after applying the laser. Er:YAG laser pretreatment on the skin produced a 3- to 80-fold enhancement dependent upon the magnitude of the laser fluency. Using electroporation, treatment with 10 pulses resulted in a twofold increase in MTX flux. A combination of laser pretreatment and subsequent electroporation for 10 minutes resulted in a higher drug permeation than either technique alone.

Experimental Methods to Study Transdermal Electroporation

The use of fluorescence measurements to evaluate transdermal transport processes for studying transdermal electroporation was introduced in [83]. Fluorescence measurements continue to be an important method for studying mass transfer across the cell membrane. In addition to fluorescence measurements, using electrical measurements and tissue electric parameters as a means to evaluate the effects of electroporation was employed already in fundamental studies on the effect of electroporation on the lipid bilayer. A study that tried to characterize the transdermal electroporation effects of various pulses through the electrical impedance method was described in [84]. The study finds that exponential pulses of 1 ms results in what may be called reversible transdermal electroporation when the skin voltages are between 40 and 80 V and begins to be irreversible from voltages of 90 V and completely reversible after 130V. Since the study was done at 25 C, it is quite likely that at body temperatures the required voltages for these conditions will be lower with an increase in temperature. (Higher temperatures favor electroporation).

The group of Chizmadzhev has made important contributions to the understanding of the mechanism of skin electroporation through fundamental research on the kinetics of skin electric properties in relation to pulsed electric field parameters. [85]. They report a study that resembles their earlier work on plane lipid bilayers, and which produced conceptually similar findings. That study investigates the electric current I(t) passing through human skin samples of full thickness *in vitro* during the application of rectangular voltage pulses (amplitude, 10-60 V; duration, 5-8 ms). During electroporation, the current was found to rapidly decrease, to pass through its minimum, and then to increase slowly. "With the increase in voltage, the minimal current grew; the dropping branch became less pronounced (up to its complete disappearance at 40 V); and the position of the minimum shifted to short times." These features were explained by "the assumption that the electrical properties of the skin at a voltage less than 30 V are determined by macropores of skin appendages (hair follicles, sweat glands, etc.). The dropping branch of the current was a superposition of the charging current of the macropore wall capacity and the conductive current through the electroporated walls. At voltages over 30 V, increases in current and conductivity are determined by electroporation of the lipid-corneocyte matrix of the skin outermost layer (stratum corneum)." The post - electroporation skin resistance shows that the electrical properties of the skin become restored to their original values after less than one minute for pulses of 10 V. The reversal lasted up to dozens of minutes at voltages above 30 V.

Electric properties measurements across full thickness porcine skin by two electrodes attached to the stratum corneum of the excised skin was also reported in [86]. Qualitatively similar to other studies, skin resistance dropped to about 20% of its prepulsing value when pulsed with a dosage higher than 0.4 V-s (with 20-40 V across each skin path), but recovered rapidly within seconds after the pulse. Long-term permeabilization of the skin required repeated pulsing with a minimum potential of 160 V. The maximum long-term resistance drop, to 35% of the initial value, required a dose greater than 200 V-s, recovering slowly and seldom completely in tens of minutes to hours.

A freeze fracture electron microscopy and differential thermal analysis study were performed to study experimentally the effects of high voltage pulses on the skin [87]. Because the time involved between the application of the electric field and freeze embedding does not allow observation of events occurring during electroporation only "secondary" phenomena could be observed. Nevertheless, "The freeze-fracture electron microscopy study revealed a dramatic perturbation of the lamellar ordering of the intercellular lipid after application of HVP. Most of the planes displayed rough surfaces. The lipid lamellae exhibited rounded off steps or a vanished stepwise order. There was no evidence for perturbation of the corneocytes content."

To compare the effects of short high-voltage and long-medium voltage pulses on skin electroporation the group of Weaver have measured skin electric properties as well as transdermal transport of sulforhodamine, a fluorescent polar molecule of 607 g/mol and a charge of -1. Comparing the outcome from the intermittent application of short (approximately 1 ms) high-voltage (approximately 100 V across skin) pulses with a few applications of long (=100 ms) medium-voltage (>30 V across skin) pulses shows that whereas both protocols induced similar alterations and recovery processes of skin electrical resistance, long pulses of medium-voltage appeared to be more efficient in transporting molecules across skin. Transport was concentrated in localized transport regions for both protocols but transport regions created by long pulses were an order of magnitude larger than those formed by short pulses, while the short pulses created an order of magnitude more transport regions [88].

Magnetic resonance microscopy of skin from hairless rats was conducted for conditions of the low voltage and low current iontophoresis, i.e., 0 to 20 V, and 0 to 0.5 mA/cm^2, and high voltage electrical fields, i.e., 220 V, 1 ms pulses repeated once per second. It was found that for low voltage pulses the skin structure, as observed by magnetic resonance microscopy, did not significantly change until 20 Volts were applied across the 0.1 cm thick skin. After 20 V, the viable epidermis appeared to swell this result is consistent to observations from scanning electron microscopy and other research from the literature. In the case of iontophoresis, water self-diffusion coefficients in the epidermis and hair follicle regions at all voltages were affected by the electric field. The high voltage appeared to hydrate the stratum corneum in agreement with published literature on electroporation [89].

Mechanisms of Transdermal Electroporation

Mathematical models and theoretical explanations for the electroporation driven transdermal drug delivery process were introduced first in 1995 by the groups of Weaver and Chizmadzhev [90]. Additional elaboration on the theory,

which postulates two paths for electroporation induced transdermal transport, through pores formed in the multiple lipid bilayers connecting corneocytes and through appendage cells, were published over the years, e.g. [91].

The essence of the theory as well as the most updated literature on this work can be found on the MIT web site of Weaver [92]. Following is a quotation from that site: "Rapid, controlled molecular transport across human skin is of great interest for transdermal drug delivery and non-invasive chemical sensing. The main barrier is the stratum corneum (SC), which can be described by a ``brick wall'' model in which the dead, hydrated corneocytes are the bricks, and the surrounding multilamellar lipid bilayer membranes are the mortar. Small lipid-soluble molecules can partition into the SC, and then diffuse across the lipid bilayer membranes, but water soluble molecules, particularly charged molecules, cannot penetrate significantly by this route. Our general hypothesis is that high voltage (HV) pulsing (Uskin > 50V) creates aqueous pathways (``pores'') through stratum corneum (SC) lipid bilayer membranes, a more specific hypothesis is that short pathway segments are formed across 5--6 lipid bilayer membranes which connect adjacent corneocyte interiors forming transcellular straight-through pathways. Moderate voltage (MV) (Uskin = 5 to 50V) pulses appear to electroporate cell linings of the appendages. Our overall aim continues to be understanding of the mechanism of electrical creation of pathways, and the associated ionic and molecular transport. Previous work shows that pulsing causes large and rapid increases in the flux of charged molecules across human skin. The basic idea is to permeabilize the SC (reversibly or irreversibly, under control), to provide major improvements in transdermal drug delivery and the possibility of better minimally-invasive sampling of subcutaneous fluid analytes (e.g. glucose)." Several publications review these theoretical models, e.g. [93].

Temperature is also considered to play a role in the permeabilization. Studies have found highly localized Joule heating in the electroporation induced transdermal channels. It was found that there was "a small rise (about 17°C) for short, large pulses (1 ms, 100 V across the SC), but was increased (about 54°C) for long, large pulses (300 ms, 60 V across the SC). The latter case appears to result in irreversible structural changes like vesicularization of the lipid lattice." [94]. A different study shows that electroporation at mild hyperthermia temperatures resulted in delivering much higher quantities of macromolecules [95]. The results of these two studies may be related.

A flow through sampling system was used to measure the response of human skin *in vitro* to a series of exponential pulses (time constant of 1 ms; peak transdermal voltages of one pulse every 5.6 s). Four negatively charged hydrophilic fluorescent tracer molecules were employed: sulforhodamine, lucifer yellow, cascade blue, and calcein (molecular weights of 450 to 625 Da). The study has shown that all four molecules exhibited a transition from small to large fluxes at Uskin of approximately 50 V. The authors suggest that this behavior may reflect a transition from electroporation of the skin's appendages to electroporation of the multilamellar bilayer membranes within the stratum corneum [96].

The effects of transdermal electroporation temperature on the transport processes through the skin were studied extensively in fundamental studies by the group of Pliquett. The findings were reported in several studies, e.g. [97]. The research recognizes that electroporation of skin is accompanied by local heating. Of particular interest are studies which show that the transport of medium-sized, ionic molecules occurs through localized transport regions (LTR). "The size of a LTR increases with the pulse length, whereas the density of the LTRs increases with increasing voltage, for instance at U(SC=)80 V, the LTR cover approximately 0.02--1% of the surface area. The state of low resistance within the LTR is long-lived. During high voltage application, the center of the LTR is heated above the phase transition temperature of the SC lipids (70°C) and the heat front propagates outwards. Inside the SC, the pulse causes aggregates of small-sized vesicles. At a higher temperature, the aggregate formation and their disappearance are delayed. Multiple pulses with the applied voltage of U(appl)=80 V induce the formation of long-lasting vesicle aggregates with a diameter of 1--30 micrometers, covering 0.05--0.5% of the total sample area. The electric energy dissipated within the LTR during high voltage application is apparently sufficient to raise the temperature well above the phase transition temperature of the lipids of the SC, accounting for the conformational changes from the multi-lamella to the vesicular structures." Another, convincing model by Pliquett suggests the following sequence of thermal events during pore formation between adjacent corneocytes: "(1) the PEF (pulsed electric filed) rapidly charges the stratum corneum near the electrode until the transepidermal potential difference is large enough to drive water into a small region of the stratum corneum, creating new aqueous pathways. (2) PEFs then drive a high current density through this newly created electropore to generate Joule heating that warms the pore perimeter. (3) This temperature rise at the perimeter increases the probability of further electroporation there as

the local sphingolipids reach their phase transition temperature. (4) This heat-generated wave of further electroporation propagates outward until the surface area of the pore becomes so large that the reduced current density no longer generates sufficient heat to reach the phase transition temperature of the sphingolipids. (5) Cooling and partial recovery occurs after the field pulse. This process yields large, high permeability regions in the stratum corneum at which molecules can more readily cross this skin barrier. We present a model for this process that predicts that the initial radius of the first aqueous pathway is approximately 5nm for a transdermal voltage of 60V at room temperature." [98].

Thermal models of skin electroporation were also reported in [99]. This paper reports the use of a composite model in calculating the electric and thermal effects associated with skin electroporation. A three-dimensional transient finite-volume model of *in vivo* skin electroporation was developed to emphasize the importance of representing the skin's composite layers and to illustrate the underlying relationships between the physical parameters of the composite makeup of the skin and resulting thermal damage potential.

The group of Miklaccic has a developed thorough numerical models of skin electropermeabilization based on experiments in which plasmids were delivered to a rat skin using external plate electrodes. [100]. The experiments showed that skin layers below the stratum corneum can be permeabilized in this way. A numerical model of the transdermal electroporation process was made, using the finite element method. The model is based on the tissue-electrode geometry used in the *in vivo* experiments and takes into account the layered structure of skin and changes of its bulk electrical properties during electroporation. The results obtained with the model were found to be in good agreement with the *in vivo* results of gene transfection in rat skin. Such a model can be used in the future to optimize and develop electrodes and pulse parameters for transdermal electroporation. A more advanced model which incorporates also the local transport regions discussed earlier can be found in [101].

Delivery of Transdermal DNA

Delivery of transdermal DNA is the basis for successful transdermal DNA vaccination. While the fact that electroporation can deliver DNA into cells was established since the early 1980's, to the best of our knowledge the first report on successful transfer of DNA through the dermis by electroporation was given in 1995 [102]. The electroporation parameters with which fluorescein-labeled antisense oligodeoxynucleotides (ODNs) corresponding to the promoters of the protooncogene c-myb (24-mer) and the oncogene c-myc (15-mer) were transported through the human skin *in vitro* by electroporation were a skin voltage of 80V delivered for 1.1. ms every five seconds. Fluorescent imaging has shown that there was no transport below 70 V and the transport was localized in regions of 30 microns in diameter.

The group of Teissie in France, has shown that electroporation can be used to effectively transfect beta-galactosidase injected in a murine tumor with surface electrodes in contact with the skin. [103].

In a study on electroporation it was found that long duration (100-500 ms)--medium voltage (100-200 V)--exponentially decaying pulses appeared to be the best for delivering oligonucleotides by transdermal electroporation to the viable hairless rat skin as an alternative to passive topical delivery of oligonucleotides. Phosphorothioate derivatives were preferred to the phosphodiester congeners as the former were found to be much less degraded when extracted from the tissues. After delivery by electroporation, therapeutic levels of oligonucleotides were reached in the viable tissues of the skin (above 1 microM or 10 microM in intact or stripped skin respectively) [104].

A very interesting set of two studies were reported by [105, 106]. The first study is comprised of *in vivo* rodent, porcine, and primate experiments and is aimed at enhancing nonviral transgene delivery to skin. The study has identified a compound (aurintricarboxylic acid or ATA) that enhances transfection activity of "naked" plasmid and pulsed electrical fields (electroporation or EP). It boosts transgene expression to an average of 115-fold more than that observed with free DNA ($P < 0.00009$). When plasmid is intradermally injected with or without ATA, the transfected cells are typically restricted to the epidermis. However, when electroporation is added after the same injection, larger numbers of adipocytes and fibroblasts and numerous dendritic-like cells within the dermis and subdermal tissues are transfected. In the second study, a HBV sAg-coding plasmid was used to test skin electroporation mediated nucleic acid vaccination in a murine model. The analysis of humoral immune responses

including immunoglobulin subclass profiles revealed strong enhancement of electroporation-mediated nucleic acid vaccination relative to naked DNA injection.

In a study intended to examine the use of electroporation to deliver plasmid DNA to cells of the skin, intradermal injection with a plasmid encoding interleukin-12, the induced serum concentrations of gamma-interferon, were as much as 10 fold higher when electroporation was used. The results demonstrate that transdermal electroporation can be used to augment the efficiency of direct injection of plasmid DNA to skin [107]. A clinical implementation of the concept was reported in [108]: The reports states that "A phase I dose escalation trial of plasmid interleukin (IL)-12 electroporation was carried out in patients with metastatic melanoma. Patients received electroporation on days 1, 5, and 8 during a single 39-day cycle, into metastatic melanoma lesions with six 100-mus pulses at a 1,300-V/cm electric field through a penetrating six-electrode array immediately after DNA injection. Pre-and post-treatment biopsies were obtained at defined time points for detailed histological evaluation and determination of IL-12 protein levels. RESULTS: Twenty-four patients were treated at seven dose levels, with minimal systemic toxicity. Transient pain after electroporation was the major adverse effect. Post-treatment biopsies showed plasmid dose proportional increases in IL-12 protein levels as well as marked tumor necrosis and lymphocytic infiltrate. Two (10%) of 19 patients with nonelectroporated distant lesions and no other systemic therapy showed complete regression of all metastases, whereas eight additional patients (42%) showed disease stabilization or partial response. CONCLUSION: This report describes the first human trial, to our knowledge, of gene transfer utilizing *in vivo* DNA electroporation. The results indicated this modality to be safe, effective, reproducible, and titratable."

Recently a comprehensive report was presented that recommends the use of a combination high voltage low voltage pulse sequences for gene delivery in several tissues including the skin [109]. Because of its importance the abstract of the papers is quoted here in its entirety: "Gene electrotransfer is gaining momentum as an efficient methodology for nonviral gene transfer. In skeletal muscle, data suggest that electric pulses play two roles: structurally permeabilizing the muscle fibers and electrophoretically supporting the migration of DNA toward or across the permeabilized membrane. To investigate this further, combinations of permeabilizing short high-voltage pulses (HV; hundreds of V/cm) and mainly electrophoretic long low-voltage pulses (LV; tens of V/cm) were investigated in muscle, liver, tumor, and skin in rodent models. The following observations were made: (1) Striking differences between the various tissues were found, likely related to cell size and tissue organization; (2) gene expression is increased, if there was a time interval between the HV pulse and the LV pulse; (3) the HV pulse was required for high electrotransfer to muscle, tumor, and skin, but not to liver; and (4) efficient gene electrotransfer was achieved with HV field strengths below the detectability thresholds for permeabilization; and (5) the lag time interval between the HV and LV pulses decreased sensitivity to the HV pulses, enabling a wider HV amplitude range. In conclusion, HV plus LV pulses represent an efficient and safe option for future clinical trials and we suggest recommendations for gene transfer to various types of tissues." It is interesting to notice the qualitative similarity between the proposed new method and the combination of transdermal electroporation and iontophoresis.

Electrochemotherapy of the Skin

Electrochemotherapy - which employs a combination of injected drugs and electroporation has been also used for the skin. A first clinical report of electrochemotherapy for the skin can be found in [110]. An application for basal cell carcinoma can be found in [111]. The study reports partial response in tumors after electrical pulses were delivered to tumor nodules by means of caliper electrodes following a administration of systemic doses of bleomycin. A follow up clinical study, which contains similar data and is probably a follow up to the 1985 paper of Heller, is reported. The study goal is to determine if electrochemotherapy is effective against primary and metastatic cutaneous malignancies. Six patients, three with malignant melanoma, two with basal cell carcinoma, and one with metastatic adenocarcinoma, were enrolled in the study. [112]. The patients received a 10 unit/m^2 dose of bleomycin administered intravenously at 1 to 1.5 units/minute. This was followed by eight 99 microsecond pulses at an amplitude of 1.3 kV/cm administered directly to the tumors 5 to 15 minutes after the bleomycin was completely infused. Pulses were administered after the injection of 1% lidocaine solution around the treatment site. The authors report that "Two of three melanoma patients had objective responses. In these two patients, five of six treated tumors decreased in size, and three completely responded. Untreated tumors displayed continued growth. Objective responses were observed in both basal cell carcinoma (BCC) patients. One patient had partial responses in both treated tumors. The other patient had one of four primary BCCs respond completely, and the remaining three

respond partially. Patients with metastatic breast adenocarcinoma showed complete responses in both treated nodules after ECT. All patients tolerated the treatment well with no residual effects from the electric pulses." [112].

Clinical studies on treatment of melanoma with electrochemotherapy was reported in [113]. The report states that: "Nineteen patients with metastatic melanoma were enrolled in a phase two, randomized, open-label study comparing intralesional bleomycin+EPT with intralesional bleomycin alone. Of 18 study lesions, 13 (72%) showed a complete response, one (5%) showed a partial response, three (18%) showed no change and one (5%) showed disease progression over a period of greater than 12 weeks. This represents a 78% objective response rate, which was significantly greater than the 32% response rate observed in the 19 patients with tumours treated with intralesional bleomycin alone (chi=7.94, 1 df, P=0.005). An additional 36 lesions, not enrolled in the study, were also treated with bleomycin+EPT. Of the total of 54 lesions treated with bleomycin+EPT, there was a 72% objective response rate. EPT treatment was well tolerated and was performed on an outpatient basis."

A more recent review of the use of electrochemotherapy for cutaneous and subcutaneous tumours is found in [114]. The study presents an overview of preclinical and clinical studies. In clinical studies electrochemotherapy has proved to be a highly efficient and safe approach for treating cutaneous and subcutaneous tumour nodules. The treatment response for various tumours (predominantly melanoma) was approximately 75% complete and 10% partial response of the treated nodules. The advantages of electrochemotherapy a are high effectiveness on tumours with different histologies, simple application, minimal side effects and the possibility of effective repetitive

Reviews

Several reviews have been published over the years on skin electroporation and they have contributed to better understanding the use of this physical enhancer on transdermal drug delivery [115-126].

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CHAPTER 6

Transdermal Drug Delivery Using Microneedles

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Abstract: Microneedles have been introduced as a measure to solve the shortcoming of normal transdermal patches and hypodermic injections as they are able to pierce through the stratum corneum, the top sub-layer of skin, in a simple and almost painless manner. Previous studies have shown microneedles to enhance drug delivery through the skin by significant amount as compared to the conventional drug delivery methods using transdermal patches. A number of designs have been proposed using both solid and hollow microneedles of different types of materials. These studies show the effects of a number of factors that affect the drug delivery efficiency using these microneedles. Mathematical models have also been proposed to represent transport of drug through the skin and blood concentration in the blood using microneedles. These models provide the relationships between parameters such as needle length and skin thickness, and factors such as permeability of the drug through the skin and the force to insert the microneedle into the skin. Different applications of microneedles in transdermal drug delivery have been proposed in different publications, which may include, e.g., delivery of vaccines for immunization, insulin delivery for controlling glucose level in diabetic patients, and others. The results from the existing studies have shown microneedles to have promising prospects in transdermal drug delivery applications. This chapter aims to review various aspects of transdermal drug delivery by microneedle arrays.

Key words: Microneedles, Microneedle arrays, Skin, Transdermal drug delivery.

INTRODUCTION

Transdermal drug delivery (TDD) has been used for decades to deliver various drugs. The drug delivery method is however limited by the protective layer of the skin, the stratum corneum (SC), which protects the body by preventing the entry of foreign substances [1]. Therefore, one of the challenges in TDD so far has been to devise methods to improve the permeability of this barrier, thereby increasing the range of drugs that can be delivered transdermally. Over the past few years, different methods have been proposed as means to overcome the barrier of the stratum corneum in order to aid transdermal drug delivery, e.g., chemical penetration enhancers, iontophoresis, sonophoresis, and others. However, the high cost, complexity and the difficulty to deal with these methods at home pose problems for the users [2-4]. Micron sized needles to penetrate the upper layer of the skin without reaching the dermis which are commonly known as microneedles have recently been introduced as an efficient method to deliver drugs transdermally in an almost painless method [5].

The first microneedles for transdermal drug delivery were fabricated by Henry *et al* [5]. The authors performed a set of experiments which showed that inserting microneedles into the skin enhanced the permeation of substances through the skin. Over the years, different types of microneedles have been designed by other researchers as well, varying in their materials of fabrication, shapes, dimensions, modes of application, etc. These microneedles have also been shown experimentally to increase the drug permeability across skin by orders of magnitude *in vitro* for a range of drugs varying in molecular size [6-9]. The current applications of microneedles mainly include delivery of vaccine, insulin delivery and acne treatment, as discussed again later.

This chapter is aimed at reviewing microneedles for transdermal drug delivery. The technique and different methods of its applications as well as types of microneedles are discussed. This chapter also reviews the methods involved in the fabrication of microneedles. Subsequently, the mathematical models governing drug delivery using microneedles are discussed. The chapter is composed of nine sections with additional sections devoted to conclusions and references. The first section is an introduction which gives a review of the basic concepts and technique of various transdermal drug delivery methods including microneedle. The second section discusses the conventional transdermal drug delivery devices and the disadvantages of this method while the use of microneedles for

José Juan Escobar-Chávez (Ed)

transdermal drug delivery is explained in the third section. Fabrication of microneedles is briefly discussed in the fourth section followed by the uses and applications in the fifth section. Furthermore, the advantages and disadvantages of microneedles in drug delivery are discussed in the seventh section. Finally the mathematical models for drug delivery using microneedles are presented in the eighth section and a conclusion is given in the final section.

CONVENTIONAL DRUG DELIVERY METHODS

The need to deliver drugs transdermally arises from the need to bypass the alimentary canal as some drugs tend to be digested by enzymes in this region or are metabolized in the liver [10]. For example, oral delivery has a disadvantage associated with the degradation, first pass metabolism and may be susceptible to poor absorption [11-12], and hence have a low bioavailability [13-14]. A common alternative to the oral route is through the skin using hypodermic needle injection which has been the gold standard for drug delivery for over a century [9, 15]. This method is rather painful, requires medical expertise, reduces patience compliance due to the inconvenience, increases chance of infection, need avoidance of the same puncture site (i.e., different points along a vein), etc. [16-21]. Another alternative that has been available for the past two decades is the transdermal patch for delivering drugs such as nicotine, scopolamine and nitroglycerin in a non-invasive manner. Transdermal drug delivery has a number of advantages over both oral delivery and hypodermic needles. Drugs delivered transdermally avoid the harsh conditions in the gastro-intestinal tract implying that the drugs have higher bioavailability, provide a controlled release rate, maintain therapeutic concentrations of the drugs in the blood over long periods [22], etc. These are important for drugs such as insulin where the patient might require a basal rate throughout the day [23] for a long period of time.

Transdermal drug delivery is seen as a good form of drug delivery because it is easy to use, safe and painless [20]. However, this method is limited to low molecular weight, lipophilic drugs with high potency [20]. For high molecular weight drugs such as insulin the normal transdermal patches could not be applicable since the drugs are unable to diffuse through the skin at a therapeutically relevant rate.

MICRONEEDLES - THE NOVEL TRANSDERMAL DRUG DELIVERY SYSTEM

Another method for bypassing the stratum corneum barrier is through the use of microneedles, which have been introduced as a form of transdermal drug delivery. They can be seen as a hybrid between the conventional hypodermic needle and the normal transdermal patches but without the demerits of both methods [5]. These micron sized needles pierce the stratum corneum and the needles should extend into the epidermis but are not expected to touch the nerves in the dermis [5]. The drug diffuses across the rest of the epidermis into the dermis where it is absorbed into the blood circulation.

In order to fully understand the application of microneedles it is important to understand the skin structure. The stratum corneum acts as the major barrier in transdermal drug delivery. In the conventional transdermal therapeutic system (TTS) patch approach across the skin, the drug is in contact with the stratum corneum through which it diffuses into the lower layers of the skin in order to reach the capillaries in the dermis [24] where it is absorbed into the blood. The diffusion of drug through the stratum corneum is very slow and this affects the rate of drug delivery. Various attempts have been made to increase the permeability of the stratum corneum to substances so that the range of drugs delivered through the transdermal route can be increased. As mentioned previously, these may include iontophoresis, electroporation [25] ultrasound [26] chemical penetration enhancers (e.g., sodium lauryl sulphate) [27]. Penetration enhancers, for example, can be used to increase the permeability of substances in skin by dissolving the lipids joining two cells together thereby creating a route for penetration [28]. Although these methods have proven to be effective in increasing the permeability of the stratum corneum, they incur disadvantages such as skin irritation and some are not so practical.

Microneedles are a relatively novel innovation that brings together the fields of engineering, pharmaceutics and biology. The idea of microneedles for use in drug delivery were first patented in 1976 but the first microneedles were fabricated by Hashmi *et al* [29] where the group used “micromechanical piercing structures” (microneedles) to deliver DNA to nematodes. Later in the 90’s the first studies on microneedles for transdermal drug delivery

application was introduced by Henry *et al* [5]. The group used the poke with patch (see section 6.4) method to demonstrate that with the use of microneedles the permeability of drugs in skin can be increased by up to three orders of magnitude, while limited insertion tests on human subjects showed microneedles to be painless.

Further studies were done by McAllister *et al* [9], where microfabrication techniques were introduced for microneedles made out of silicon, metal, polymer materials, etc. These microneedles improved the skin permeability of macromolecules such as calcein, insulin and bovine serum albumin. In the same study, microneedles were shown to inject insulin into diabetic rats at a pharmacologically relevant rate enough to significantly reduce blood glucose level. Microneedles had the ability to enhance cellular uptake of calcein, where microneedles were placed into a cell culture medium and analysis showed improved uptake of calcein after treatment with microneedles.

Insertion of microneedles in combination with iontophoresis has been demonstrated to increase transdermal flux by 100-fold in comparison to iontophoresis alone [30]. High levels of oligonucleotides were also detected in the skin after delivery with microneedles without iontophoresis. Protein antigens were successfully coated into microneedles [31] and the antigens were delivered into the skin and caused an antibody immune response. The delivery of naked plasmid into skin using microneedles has also been studied [32]. Blunt tipped microneedles were used to scrape the skin in order to enhance delivery. Microneedle delivery was seen to induce stronger and less varied immune response relative to when hypodermic needles were used. In another study Stoeber and Liepmenn [12] injected insulin into chicken thighs using microneedle arrays. The pair modeled the flow of the fluid through the microneedles using the modified Bernoulli equation which they verified during their experiments.

From these studies it is apparent that microneedles have quite a variety of possible applications. This method of drug delivery appears to be a promising venture especially in transdermal drug delivery.

MICRONEEDLE TYPES AND FABRICATION TECHNIQUES

Microneedles are available as both solid and hollow microneedles made of various materials as discussed in the following sections. Fig. **1** is a two dimensional schematic view of a solid and a hollow microneedle. Microneedles can vary according to their tip shape, e.g., volcano like, micro-hypodermis and snake-fang design [33] or the overall shape, e.g., pyramidal, spiked, candle-like and spear-shaped structure [34, 35]. Till date, five methods of transdermal delivery mediated by microneedles have been attempted [19]:

- Poke with patch approach
- Coat and poke approach
- Dip and scrape
- Dissolving microneedles
- Injection through hollow microneedles

The poke with patch approach was proposed by Henry *et al* [5]. Using this technique Henry *et al* [5] fabricated microneedles out of silicon which were then inserted into the skin to pierce the stratum corneum and create micro conduits through which drug can enter into the lower layers of the epidermis. In general, the microneedles create holes in the skin through which the drugs can diffuse, bypassing the stratum corneum to reach the epidermis where they can diffuse faster.

The coat and poke approach involves coating the drug to be delivered around the surface of the microneedle. By inserting the microneedles through the skin, the drug coating dissolves off in the skin fluid and the dissolved drug diffuses through the skin [36] into the blood microcirculation. The coating methods generally used are roll coating, spray coating and dip coating. Gill and Prausnitz [36] proposed a dip coating method suitable for efficient coating at the micron scale. This coating method is discussed later in section 6.5.3.

Microneedles with the drugs encapsulated within the microneedle structure are referred to as dissolving microneedles [37]. In this method, the coated microneedle is made from a biodegradable polymeric material. The drug is released in a controlled manner as the microneedle dissolves off when inserted into the skin.

The dip and scrape method involves placing the array in contact with the drug solution and then scraping multiple times across the skin to create microabbrassions. This method has been used to deliver DNA vaccines into mice skin [32]. The results indicated an improved delivery as compared to topical application of the naked DNA plasmid unto untreated skin.

Fluid injection through hollow microneedles occurs where the microneedles are designed with holes at the centre through which drugs are microinjected into the lower layers of the skin and then diffuses across the viable skin until it reaches the blood vessels in the dermis [12]. Hollow microneedles have also been designed with side openings as an attempt to improve drug flow [38]. However, the hollow microneedles have generally received less attention to date as they are inherently weak structurally and have practical problems such as the insertion of microneedle into skin [39].

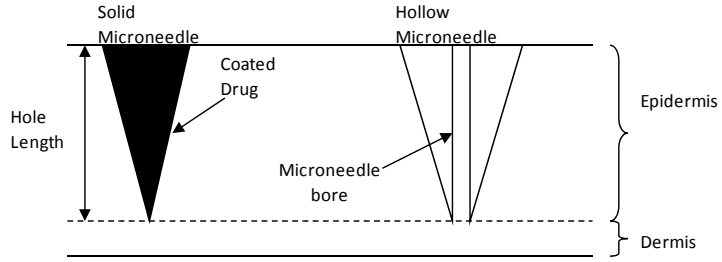

Figure 1: Two dimensional view of hollow and solid microneedle inserted into the skin.

Solid Microneedles

The solid microneedle was first fabricated to increase gene transfection [29]. After this work was published, the concept of applying the solid microneedle for the use of transdermal drug delivery has also appeared. For example, solid microneedle has been successfully used for vaccine injection to prevent influenza [2]. Many solid microneedles have been developed for a variety of different drugs both *in vitro* and *in vivo*. These solid microneedles are easier to fabricate, have better mechanical strength and sharper tips as compared to hollow microneedles [40-41] which might result in higher drug transport [42]. Fig. **2** is an illustration of drug diffusing from the surface of solid coated microneedles into the skin. The red arrows indicate the direction of flux.

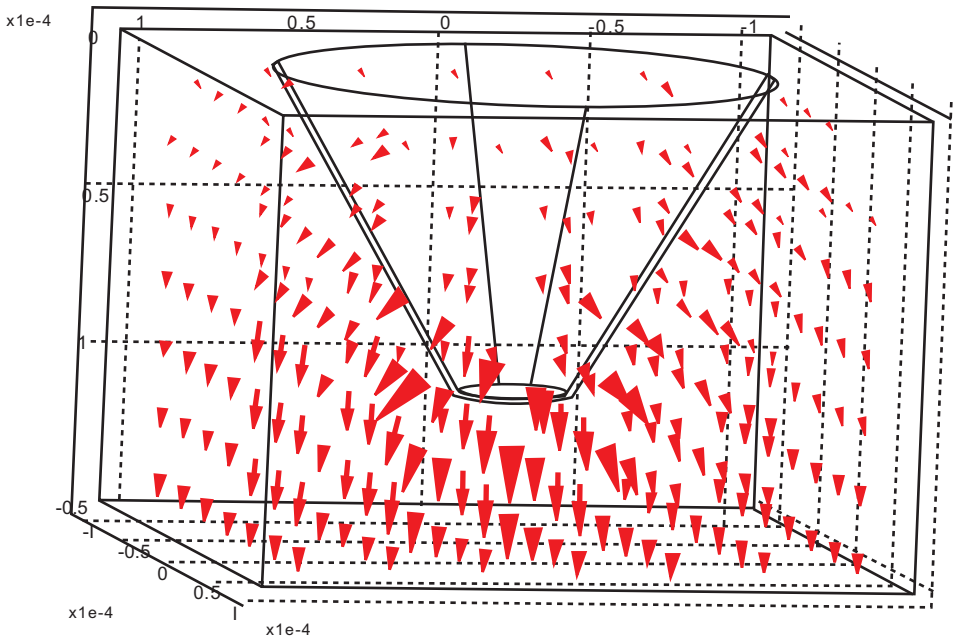

Figure 2: Three dimensional view showing drugs diffusing from the surface of a solid microneedle into the skin.

Solid Microneedles Made of Silicon

When microneedles first began to be used in transdermal drug delivery [5], they were made out of silicon. Therefore, solid silicon microneedles have been widely used for the transdermal drug delivery studies [16, 43, 44]. However, silicon is expensive and brittle, it therefore breaks easily during the penetration across skin [45], Henry *et al* [5] used silicon to fabricate the microneedles and realized that calcein can be delivered and that permeability across skin was increased when using this technique.

Another study using the same microneedles concluded that permeability across skin was also increased when using these microneedles to deliver insulin and bovine serum albumin [9]. The microneedles fabricated were in a 20 by 20 array and each microneedle has a base of 80 μm and a height of 150 μm with a tip radius of 1 μm. Silicon microneedles having a length of 50-200μm with a square tip have also been made and inserted into mice skin for gene delivery [32]. In another gene delivery, solid silicon microneedles have been used across human skin *in vitro* [46].

Solid Microneedles Made of Polymer

As mentioned previously, silicon is brittle and this led to the use of other materials. Polymer has been used as an alternative material because it is a cheaper and stronger material which could reduce tissue damage [47]. In spite of these advantages, polymer increases the bluntness of the microneedle tip due to the low modulus and yield strength of polymer [48]. On the other hand, polymer microneedles have a main limitation with its mechanical properties which could cause needle failure during the penetration across skin [49]. This is because microneedle failure force depends on the mechanical properties of the polymer [20,49]. Different kinds of polymer microneedles have been designed to increase permeability across skin as discussed below.

Bevelled Solid Microneedles Made of Polymer

Bevelled tip microneedles have been fabricated using biodegradable polymers [50]. Poly-glycolic acid (PGA) was chosen as the main material of this microneedle because it is relatively inexpensive and believed to be mechanically strong [51]. For example these types of microneedles have been fabricated by Park [50] as shown in Fig. **3**. These microneedles were arranged in an array of 20 needles in each row with a centre-to-centre spacing of 1400 μm by 6 columns with a centre-to-centre spacing of 400 μm. In total, there were 120 needles in this array which has an area of 9 × 9 mm.

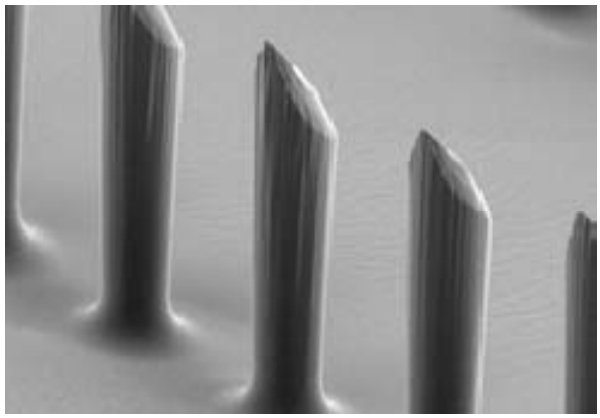

Figure 3: Bevelled solid microneedles made of polymer (with permission Park J-H *et al.* Copyright 2004, IEEE) [48].

Tapered Solid Microneedles Made of Polymer

Tapered microneedles have been fabricated with Poly-glycolic acid (PGA). For example, Park *et al* [19] proposed an array consisted of 200 needles with a height of 1500 μm, base diameter of 200 μm and tip diameter of 20 μm. In addition, tapered microneedles have been fabricated with biocompatible polycarbonate with various microneedle densities [52]. In another study, Park *et al* [53] demonstrated that calcein as a model drug could be delivered successfully when using both bevelled and tapered microneedles. On the other hand, in a different study Park *et al*

[49] concluded that tapered microneedles could solve the problem that arises with the polymer microneedles. In contrast, Kolli and Banga [54] demonstrated that solid maltose microneedle could be a promising technique to replace the biodegradable polymer. This is because maltose microneedles dissolve within minutes as compared to the biodegradable polymer which remains in the skin for period of time [54].

Solid Microneedles Made of Metal

Metal is the third material which is used to manufacture microneedles. It is mechanically strong and relatively cheap to produce. The main components of this type of microneedle patches are either stainless steel [55, 56] or titanium [47, 57].

Hollow Microneedles

Less attention has been given to hollow microneedles for drug delivery. This is because hollow microneedles are harder to use since they are weaker than solid microneedles requiring care in terms of needle design and insertion method [58]. The main purpose of this type of microneedles is to deliver drugs through the bore at the needle tip. This reduces the sharpness of needle tip which affect the penetration of this needle into skin. These issues have been resolved recently through development of a new microneedle design and insertion method [38-40, 59]. These include microneedles designed with the openings at the side rather than at the bottom as presented by Griss and Stemme [38]. Having the tip at the side allows for the design of sharper tips. Also designs for hollow microneedles with temporarily sealed tips have been proposed by Roxhed *et al* [40]. These microneedles have their tip closed initially; however they can be opened on insertion into the skin where the tip dissolves in the high saline solution in the interstitial fluid. The tips can also be opened as a result of applied pressure. Wang *et al* [39] proposed the use of rotary drilling and mechanical vibration as methods to enhance insertion of hollow microneedles. The group also carried out experiments that showed that retracting the microneedles halfway or vibrating the microneedles after insertion dramatically increased the fluid infusion flow rate. Nevertheless, one of the main advantages of hollow microneedle is that it offers continuous infusion through the skin [40].

Hollow Microneedles Made of Silicon

Hollow microneedles made of silicon [60, 61] have been studied and designed to enhance the transdermal delivery, by increasing permeability across skin. Silicon has been generally used in microelectronic industry with wide manufacturing experience [20]. Despite this advantage, it is a high-priced, not biocompatible and breakable material [20, 62-63]. The first design of this was a tapered needle in which there were 8 needles in each array and with a height of 200 μm and a lumen diameter of 40 μm [12]. Another type was the bevelled needle in which the length was 350 μm, the diameter of the tip was 70 μm, while the base was 250μm and the distance from the needle tip to the centre of the hole was 40 μm [64]. Examples of tapered and bevelled hollow microneedles are shown in Fig. **4**.

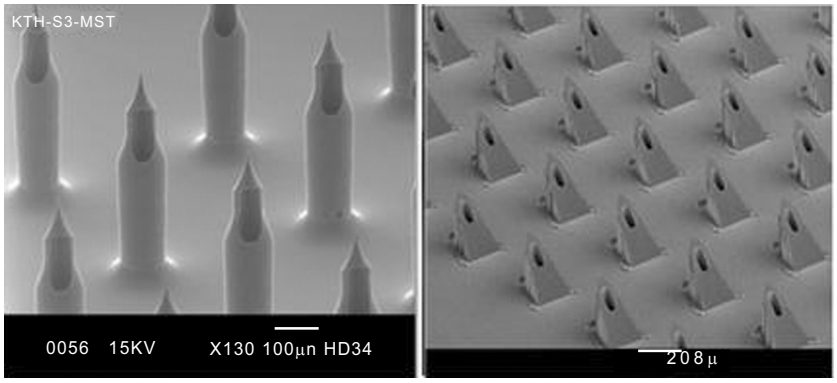

Figure 4: Left: Tapered microneedles (with permission Roxhed *et al.* Copyright 2008, IEEE) [102], Right: bevelled microneedles (with permission Gardeniers HJ *et al.* Copyright 2003, IEEE) [64].

Hollow Microneedles Made of Polymer

Polymer has been less widely used for fabricating hollow microneedles [65]. Nevertheless, it is a cheap and biocompatible material [20] with a lower melting temperature than silicon [66] which makes it an attractive option

sometimes as a possible material for microneedle. Furthermore, it may improve the deformation of a material substance because of the polymer viscoelasticity [20, 67].

Hollow Microneedles Made of Metal

As mentioned previously, metal is a cheap, strong and biocompatible material [62, 68]. In particular, it has been commonly used in hollow microneedles which require structural strength [20, 58]. However, there has been a concern about its safety in cases where the needles may break inside the skin during the insertion [49]. The hollow metal microneedles have been fabricated by using various metals such as nickel [69] and nickel-iron metal [70]. Fig. **5** shows examples of hollow metal microneedles.

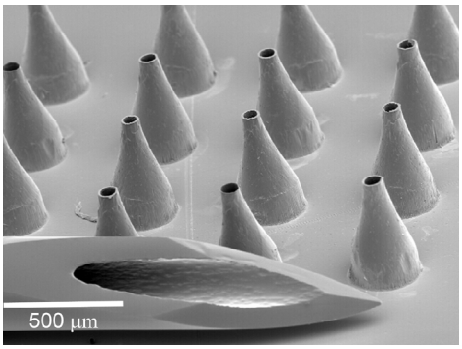

Figure 5: Tapered hollow metal microneedles (with permission Davis SP, *et al.* Copyright 2005, IEEE) [79].

MICRONEEDLE FABRICATION

Microneedles can be classified as BioMEMS, i.e., MEMS (microelctromechanical systems) devices for biological application. The techniques developed by the MEMS industry have made it possible to fabricate microneedles of different sizes and shapes with precise dimensions. Microfabrication (also known as micromachining or micro-manufacturing) can simply be explained as a technique that makes use of a set of manufacturing tools based on batch thin and thick film fabrication techniques, techniques which are common to the electronics industry [71]. Microfabrication is one of the many precision engineering disciplines which combine the use of serial direct write technologies with traditional precision machining methods which have been enhanced or modified for creating small three-dimensional structures with dimensions ranging from sub-centimeters to sub-micrometers [71].

The methods that have been adopted for microneedle fabrication include wet etching, deep reactive ion etching (DRIE) [72], microinjection moulding [73], isotropic etching, isotropic etching in combination with deep etching and wet etching respectively [74], dry etching, isotropic and anisotropic, photolithography, thin film deposition [74], laser cutting [56], and inclined LIGA process [58, 74, 75].

Since there is a great variety of MEMS fabrication process, it is difficult to define a general process for all the methods [76]. However it is quite apparent that all microfabrication processes start with lithography, followed by a number of other stages [71, 76-77]. Lithography is a process by which the master pattern of the desired material is transferred unto a solid material such as a silicon wafer; the most common type is photolithography. Photolithography involves creating a mask (a stencil used to repeatedly generate a desired pattern in resist-coated wafers) [71]. Photolithography is based on the fact that metal is opaque to ultraviolet light while glass is transparent. The metal acts as an absorber while the glass acts as the photoresist layer. A light field or dark field image is thereby produced which can be transferred unto the silicon wafer. Other types of lithography are x-ray lithography and charged particle beam lithography. Other techniques for lithography are also emerging and these include; proximal probe lithography, holographic lithography, stereolithography and lithography on non planar substrates [71].

The next stages of microfabrication involve the selective addition or removal of substrates to or from a material chemically or physically [71]. The most important removal process is etching; pattern transfer by chemical or physical removal of a material from a substrate, often involving the use of a mask. Etching can be in the dry or

liquid phase. There are also subtractive processes which do not make use of a mask such as focused ion beam, laser machining, ultrasonic drilling, electrochemical discharge machining and traditional precision machining [71].

MEMS technology makes it possible to fabricate a wide range of needle sizes and shapes with precision. This makes it feasible to experiment with different geometries in order to optimize drug delivery using microneedles.

Microneedle Designs and Geometry

In this subsection the various designs of microneedles are discussed as different designs have been adopted to meet the requirements for ensuring adequate insertion into the skin without failure of the microneedle. Over the past few years, microneedles have been designed for research purposes in different shapes and sizes. For instance Henry *et al* [5] fabricated conical microneedles with very sharp tips (radius of curvature <1μm), which were 150μm long. Martanto *et al* [56] fabricated solid metal microneedles with base cross sectional area of 50μm × 200μm tapering to a sharp tip with an angle of 20 degree, 1000μm in an array containing 105 microneedles. Ito *et al* [78] studied the feasibility of microneedles for insulin delivery using insulin loaded microneedles 3.24 ± 0.16mm in length with a base diameter of 0.55 ± 0.03mm. Davis *et al* [79] designed hollow microneedles, straight-walled, 180μm long with 125μm base diameter as well as tapered microneedles with base radius of 250μm, tip radius of 50 μm and a length of 500μm. This group also fabricated 4 × 4 arrays of hollow microneedles with tapered wall microneedles 500μm long with base and tip radius 300μm and 75μm, respectively. Gill and Prausnitz [19] fabricated solid microneedles with rectangular base and tapered tips with 700μm long, a width of 160μm and 50μm thickness.

The general idea behind this design is that they are long and strong enough to pierce the stratum corneum and short enough to avoid the nerves in the dermis.

General Requirements for Microneedles Design

Microneedles are meant to be disposable and hence, a cheap fabrication method should be employed in their manufacturing [37]. They can also be designed to be biodegradable (as mentioned earlier) so that their disposal has little environmental impact [37]. Microneedles must also be robust in design in order to withstand the forces that tend to bend or break them on insertion [58]. To ensure that both the insertion and delivery occur at the right location, they should be sharp enough and at least 100μm in length [12]. For example Stoeber and Liepmann [12] designed hollow microneedles with a wide base and sharp tip to give the microneedle strength and ease of penetration. It is also important that the material of fabrication is biocompatible with skin.

Studies have shown that factors such as microneedle geometry, coating depth on solid microneedle and skin thickness affect the drug delivery efficiency using microneedles [34, 80-82]. It is also important that microneedles insert effectively into the skin without breaking and that the patch stays on keeping the needle inserted without falling off [83]. The coatings must adhere well to the surface of solid microneedles without wiping off onto the skin surface [19]. All these design parameters have been taken into consideration in studies on microneedles and are in the process of being optimized.

Optimizing Microneedle Design

Wilke and Morrissey [84] optimized mask shape of microneedles for three different shapes. They concluded that the square mask shape is the optimum shape instead of diamond and circular shape. Khumpuang *et al* [85] determined the optimum location for various microneedle holes locations. The microneedle tip radius has also been optimized to improve the tip sharpness [86]. With drug delivery methods, it is therefore logical to expect the optimum values of drug permeability depending on the dimensions of the microneedles. In general, the main aim of drug delivery optimization is to deliver a small quantity of a given drug in an effective manner (i.e., microneedles) to avoid any problems such as the possibility of damaging the liver or low drug absorption [87]. However, other questions have appeared recently while using these microneedles, e.g., how to reduce the sizes of holes produced by microneedles so that transport of bacteria and other foreign particles can be minimized [88].

In addition, Lv *et al.* [89] illustrated the insulin distribution profiles across skin by proposing a theoretical model. They studied both the transient and spatial distribution in the skin tissue as well as in the drug solution. The geometry of microneedle models also has an important effect. For example, the transdermal drug delivery is

constrained by the surface area of the microneedles [19]. Teo *et al* [72] concluded that the radius of microneedles is a critical parameter when designing microneedles. Furthermore, the performance of the transdermal drug delivery process has been improved by increasing the number of microneedles [90]. A theoretical *in vitro* approach appeared in the literature that describes the permeability of the skin when microneedles are inserted and removed [9]. The calculations of this theoretical model agreed well with their experimental data with an accuracy of 95% [9]. From the first investigation, one can conclude that the permeability across skin increases by decreasing the surface area of the microneedles and increasing both the number of microneedles and the microneedle radius. This leads to the inclusion of another physical parameter which is the centre-to-centre spacing in order to increase the permeability across skin and, hence, obtain the optimum microneedle design for a given range of microneedle dimensions. Another mathematical model has been presented to determine the dosing rate needed for a given transdermal patch [91]. This has been done by computing the surface area of the patch, permeability across skin and the solubility of the drug [91]. However, this model has some limitation such as studying the influence of microneedle geometries. For example, the thickness of microneedles has been considered as an important parameter since it is related to the length of microneedle and hence its strength [92]. In addition, controlling the depth of penetration has a significant limitation when using the microneedles of different geometries [93]. Frameworks have been proposed for the optimization of microneedle geometry and distributions in terms of permeability [18, 82]. These frameworks were based on finite element simulations of microneedles inserted into the skin.

It is envisaged that designing microneedles adequately meeting the necessary requirements with respect to factors such as geometry and material of fabrication as discussed above, will lead to efficient drug delivery as the reported studies have shown.

Coating Microneedles

Gill and Prausnitz [36] developed a simple, versatile and controlled microneedle coating process. The microneedles were fabricated using the laser cutting technique and coated using the developed novel dip coating method, respectively. They were able to coat uniformly a range of substances such as calcein, insulin, vitamin B and plasmid DNA around microneedles by performing this coating method. The same group, later in 2007 used the same method to develop a rational basis for designing coating solution formulations for uniform and thick coatings [19]. The factors that affected the coating thickness, coating concentration and uniformity were analyzed. These factors include concentration of surfactant, type of surfactant, viscosity enhancer and number of dips.

To counter the effect of the surface tension and low viscosity which might prevent drug molecules and particles from adhering to the surface of the microneedles the coating solution contained a viscosity enhancer and a surfactant. 1% w/v carboxymethylcellulose sodium salt was used as a surfactant and 0.5% w/v lutrol as viscosity enhancer together with the required concentration of the drug to be coated on.

Single microneedles were coated by dipping the microneedle horizontally into a coating solution held as a droplet on the tip of a 200μm large orifice pipette tip. The orifice is mounted on a clamp while the microneedle is mounted on a manual micropositioner. The microneedle was immersed and withdrawn from the coating solution while viewing under a stereo microscope.

Coating arrays of microneedles required the use of the micro-dip coating device designed by the pair [36]. The device consists of a reservoir containing the coating solution and a micropositioning dip coater as shown in Fig. **6**. The reservoir is made up of a back plate and a cover plate which is designed to restrict coating only to the microneedle shaft while preventing the coating solution from getting into contact with the array base. The drug is only absorbed from the microneedle shaft and any residue on the patch base will be wastage.

The bottom plate of the reservoir is designed through-hole to allow the entry of the coating solution into the feeding chamber. The cover plate is drilled with holes at the same interval from each other as the spacing on the microneedle array. These holes are to be in alignment with the microneedle array mounted on the micropositioner which allows the microneedle to be dipped and withdrawn from the coating solution in the chamber.

In this design, three linear micropositioning were assembled on a 6.35mm thick flat acrylic plate. The x- positioner was used to move the microneedle array into and out of the coating solution while the y- and z- positioner positioned

the coating reservoir. The coating was carried out manually while viewing from a stereo microscope. This method has the ability to coat a wide range of molecules around microneedles including calcein, bovine serum albumin and latex beads [36].

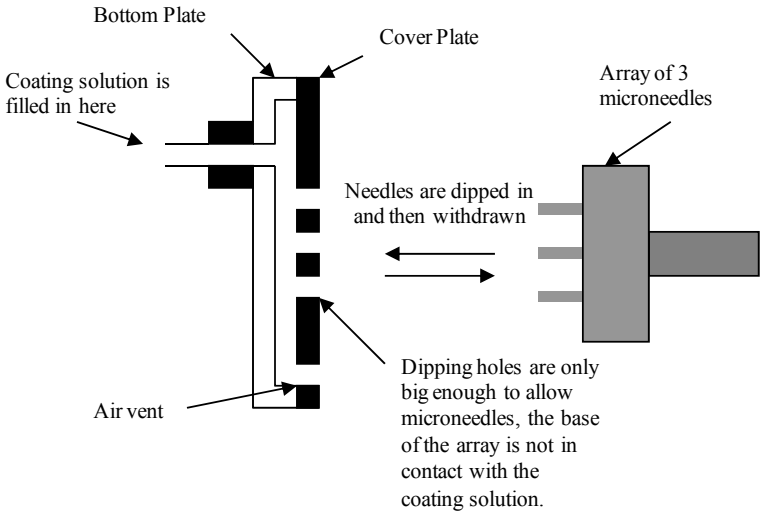

Figure 6: Schematic to illustrate the technique of the dip coating device.

All coatings were uniform and did not wipe off the surface of the skin on insertion. The pair was able to coat up to a milligram of drug on an array containing a few hundreds of microneedles. The drugs coated included calcein, vitamins, proteins and DNA. Coated microneedles are especially attractive as it is thought that storing a drug in solid phase as a coating on microneedles enhances its long-term stability even at room temperature [36].

USES AND APPLICATIONS OF MICRONEEDLE

Since their first fabrication, microneedles have been proposed and experimented for various medical applications as shown below:

- Vaccination against flu virus
- Cutaneous fluid extraction and glucose monitoring
- Acne treatment
- Delivery of nano-particles
- Insulin delivery

Microneedles have also been proposed for use in combination with other processes such as iontophoresis [22] to improve drug delivery. These applications are still under research as microneedles poses many revolutionary advantages to drug delivery. Although microneedles are commercially available in the form of Dermaroller™ (Arstheticare®) for cosmetics applications [94], experimental studies with the Dermaroller have shown their applicability in drug delivery as well as cosmetics [94].

Microneedles for Transdermal Immunization

Over the past few years, there has been a large amount of interest in transdermal cutaneous immunization. This area is particularly of interest due to the presence of a large number of antigen presenting cells (APC) in the skin. APCs such as the langarhans (LC) cells in the epidermis and the dendritic cells (DC) of the dermis, macrophages and monocytes, recognize and take up foreign antigens from this region and present them to the T and B cells in the draining lymph nodes initiating an immune response by the body [95-97]. This gives transcutaneous immunization

the advantage of dose sparring which is particularly important where vaccine is in short supply. Studies have shown that transcutaneous delivery requires lower doses and results in stronger immune response than the conventional intramuscular (IM) delivery method especially in people aged 18 to 60 [98, 99].

Despite the prospect of transcutaneous immunization, a convenient and reliable method is yet to be developed. Nevertheless, in some cases (e.g., smallpox immunization), scarification method is used. In this case, the skin is scrapped with needle and the live virus is delivered to the area. This leads to swelling and scaring of the immunization site and the doses delivered are variable and often inefficient. In particular, the administration of IM and SC creates inconvenience and discomfort to the patience.

Intradermal injection has been given to patients in trials to compare this method with intramuscular and subcutaneous vaccine delivery. Intradermal injection of a lower dose induced vigorous antibody response similar to when a full dose is given subcutaneously or intramuscularly. Intradermal injection was done with a 30 gauge needle which requires a high level of skill [98]. This is similar to subcutaneous injection which often results in local inflammation [98].

Researchers have recently presented microneedle patches as a better alternative for immunization. The vaccine can be coated unto microneedle array and presented as a simple patch which can allow patients to immunize themselves without the necessity for intense medical training [90]. Human trials are under way however experiments on mice [95-97] and guinea pigs [100] indicated microneedle immunization to be at least as effective as intramuscular immunization.

In the experiment carried out by Koutsanamos *et al* [95], arrays of metal microneedles were coated with influenza vaccine with an array contained 5 microneedles, each 700 μm long, 170 μm by 55 μm in cross section with a sharp tip having a radius of curvature of 5 μm. The microneedles were spaced out 1575 μm on the array, the dip coating method as discussed previously was used to coat inactivated H_3N_2 influenza virus unto them. Approximately 3 μm of the vaccine was successfully loaded onto each array and the coatings were uniform and adhered well to the surface of the microneedles. On insertion into the skin, the vaccine coating dissolved off within minutes. In another experiment, an electric applicator was used to enable insertion of shorter microneedles (i.e. 300μm) into mice skin [96]. The mice were anaesthetized with a ketamine and xylazine cocktail and the hair was removed from the dorsal caudal surface before the microneedles were inserted for 2 minutes and then removed.

Furthermore, another group of microneedles were administered with the same amount of vaccine via intra muscular injection, whereas another group was unimmunized and used as the control group [95]. Consequently, tests were carried out to compare the immune response shown by all three groups [95], and then 2 months after immunization, the mice were slowly administered 50 μl of the live virus intranasally.

Blood samples were taken from the mice 14 days after immunization and heamaglutinition inhibition (HAI) titers were determined for both groups. The microneedle immunized group was reported to have shown increased HAI titers at low dose (3μg) and HAI titers remained high for another 28 days. The intramuscularly immunized mice also showed increase in HAI titers after immunization. However the microneedle immunized group showed higher HAI titers at a higher dose (10μg) than the intramuscularly immunized group of mice at day 28. This showed that microneedles are capable of achieving high antibody titers at least comparable to intramuscular injection even at low doses of vaccine.

Circulating levels of influenza specific IgG (antibodies) were measured in the serum after 14 and 28 days. All immunized group showed an increase in antibody titre at day 14 compared to the unimmunized mice. After 28 days all mice immunized using microneedles showed increased antibody titers at both high and low does. The intramuscularly immunized group on the other hand showed increase in antibody titers after 28 days only at high doses (10μg). These results suggest that immunization by microneedles could perform better than intramuscular immunization.

The route of vaccine delivery was also shown to have an effect on the antibody isotype profile [95]. The IgG1/IgG2a ratio served as an indicator of whether the response is T helper type 2 biased or T helper type 1 based. The

intramuscularly immunized mice showed an IgG1/IgG2a ratio of 2 and 0.3 at 3μg and 10μg doses, respectively, after 14 days indicating a Th1 type response. The microneedle immunized group showed IgG1/IgG2a ratios of 24 and 4, respectively, indicating a Th2 type immune response. Moreover microneedle immunization prevented the replication of virus as well as intramuscular immunization when the viral titres in the lungs were compared to unimmunized group after 4 days of challenge. At 10 μg no virus was detected in the lungs and only minimal amounts were detected at 3μg dosage.

Analysis of antibody secreting cells in the spleen showed no difference between microneedle and intramuscularly immunized mice. On the other hand, the lungs ASC was high at both 3μg and 10μg doses for microneedle immunized mice whereas, it was only high at 10μg dose for intramuscularly immunized mice. Furthermore, memory B cell numbers obtained *ex vivo* was also the same for microneedle immunized and intramuscularly immunized mice [95].

The dip and scrape mode of delivery have also been used to successfully deliver DNA plasmid to mice [101]. A better immune response was observed while using microneedles to create microabrassions prior to topical application of the naked DNA plasmid as compared to injection based delivery using lancets [101].

So far, the results from the studies carried out on immunization using microneedle suggest microneedles to be at least as efficient as intramuscular injection and even better in some cases [95-100]. In addition to the added advantages of convenience, low risk method of microneedle application, it seems to have a good future prospect in immunization against influenza virus and immunization.

Cutaneous Sampling and Glucose Monitoring Using Microneedles

It seems there are two continuous glucose monitoring systems to monitor blood glucose level painlessly [102]. These are the Cygnus GlucoWacth® and the Miinimed CGMS™. Problems associated with these devices render them inadequate for regular glucose monitoring and, in addition to these disadvantages, regular fingerstick tests are still required to recalibrate the system [102]. Integrating a normal sized hypodermic needle into a silicon chip creates a challenging problem of scale [104]; hence a continuous glucose monitoring system based on normal hypodermic needles would not be feasible. On the contrary, microfabricated microneedles can be fabricated with great precision which allows the penetration depth to be controlled accurately [104]. This makes it possible to reach the upper part of the skin (i.e., epidermis) where the interstitial fluid can be extracted without causing pain, since the nerves are avoided.

Interstitial fluid (ISF) is in close proximity to the capillary blood flow for constituents of the blood responsible for maintaining homeostasis such as blood glucose level [104]. Experiments on the ear lobe of a human subject have been used to demonstrate the capability of microneedles to extract interstitial fluid from the epidermis. Microneedles with snake fang shaped tips were inserted into the skin and after 15-20 minutes, test strips were used to show that interstitial fluid has been extracted. The hollow microneedles draw ISF from the skin by capillary action. Filling the hollow microneedle is governed by surface tension, which is reliant on the imbalance of forces (such as Van Der Waals dipole-dipole interaction and hydrogen bonds) at an interface. This is essential to minimize the contact angle between the needle tip and the interstitial fluid in order to maximize the capillary force drawing the fluid into the hollow microneedles [104].

Studies have also been carried out on anaesthetized rats and conscious normal adult human subjects using glass microneedles with tip radius 15 – 40μm penetrating to depth of 700 – 1500μm across the skin [105]. By applying a vacuum of 200-500mm Hg for 2 – 10 minutes, ISF was successfully extracted and glucose level was measured. The glucose level in the ISF was compared to glucose level in the blood samples obtained from either the tail vein of rats or the finger stick on humans. The ISF glucose levels correlated well with that of blood [104-105] and a linear correlation factor was used to calibrate both glucose levels against each other. ISF extraction using microneedles is tested to be a painless method for glucose monitoring.

A prototype of a disposable microneedle based glucose monitoring devices has been designed by Zimmermann *et al* [106]. The fluid extraction chamber attached to the microneedle can be connected to a sensing device which measures and indicates the glucose concentration in the body (ISF). The commercially available glucose monitoring device incorporating silicon microneedles is the Kumetrix® [107]. This device can simply be placed on the skin

surface (e.g., on the top of the palm), and automatically draws blood painlessly within a second after which the device can be removed from the skin surface. It has been shown that less than 200 nanolitres of blood is withdrawn from the body and is drawn to the micro chamber where an automatic assay on the blood sample is performed [107]. The device calculates and displays the glucose concentration and results can be kept in memory for recall. Unlike its predecessors this device requires minimal amount of blood sample and is a less messy process [107].

In terms of fluid extraction, microneedles have been used in microdiyalysis to help counter the issue of large protein molecules blocking the device thereby reducing their shelf life and effectiveness. Microneedles serve a selectively permeable membrane that chooses the permeant by size restriction [104].

To sum up, microneedle aided fluid extraction from the body shows a promising potential for application in analysis of other bodily fluid components as well as in a continuous medical monitoring.

Acne Treatment

The effectiveness of the commonly available topically applied drugs for acne treatment is limited by the low rate of penetration through the stratum corneum whereas, the main cause of acne scarring occurs deeper into the skin. Therefore, in order to enhance the effectiveness of the topically applied treatments, the penetration of the drug needs to be deeper into the skin [108].

The idea to treat skin with microneedles before applying the skin treatment was recently presented by Wu *et al* [109]. An array of microneedles is expected to be used to pierce the stratum corneum after the skin treatment ointment is applied to the skin. Piercing of the skin with microneedles initially will aid the penetration of the compound in order to improve the treatment.

In addition, experiments have been carried out by applying the TheraJectMAT^{TM} dissolving microneedles containing API in a GRAS matrix to the surface of human skin with acne. The images of the skin surface before and after microneedle treatment showed an improvement within hours [108].

Acne affects up to 67.5% of the teenage population in the UK and studies show the disorder to have an effect on the quality of life of the sufferers [110]. According to these studies mentioned above, using microneedles could improve the effectiveness of acne therapeutics. As a result, this would have a significant impact if commercialized.

Delivery of Microparticles

Microneedles have been used to deliver microparticles varying in size from 1μm to 20μm [36]. Gill and Prausnitz [36] delivered barium sulphate particles with 1μm in diameter and latex beads with both 10 and 20μm in diameters into porcine cadaver skin. The barium sulphate particles were coated on solid microneedles and delivered into the skin without wiping off unto the skin surface even at low insertion speed of 0.5 to 1mm/s. However, to insert 20μm particles an insertion speed of 1 to 2cm/s was required and the microneedles had to be designed with holes or "pockets". These pockets where the microparticles were secluded had the ability to facilitate the delivery of microparticles without most of them ending up as residue on the skin surface. The delivery of 10μm particles was successful without pockets at high insertion speed of 1 to 2 cm/s.

In another study, McAllister *et al* [9] were able to show that the delivery of particles of 1μm in diameter is enhanced when the skin is pre-treated with microneedles by adopting the poke with patch approach. Therefore, it seems to us that the delivery of microparticles is important in order to facilitate controlled/ delayed delivery after the drug is inserted into the skin.

Microneedles for Insulin Delivery

There have been a number of researches into using microneedles for insulin delivery in diabetic patients. For example Martanto *et al* [56] used the poke with patch approach to deliver insulin to diabetic rats. Davis *et al* [79] developed a simple reproducible technique for fabrication hollow metal microneedles that were tested on diabetic rats for insulin delivery. In another study, McAllister *et al* [9] used microneedles to deliver insulin to hairless rats. Microneedles have been shown to deliver insulin with a significant biological effect as the blood glucose

concentration was reduced by substantial amount using microneedles. For example, arrays of 105 needles made of stainless steel have been inserted into diabetic rats by adopting the poke with patch approach [55]. Blood glucose level was reduced by up to 80% using hollow microneedles, similar to subcutaneous injection of about 0.05-0.5 units of insulin. This study showed insulin to be a good candidate for transdermal delivery using microneedles. Dissolving microneedles have also been shown to deliver insulin and cause a significant drop in blood glucose in hairless rats [78].

Clinical trials are ongoing into the application of microneedles for continuous insulin infusion in diabetic patients (www.Clinicaltrials.gov). The idea is to replace indwelling catheter used in the CSII with array of about 5 hollow microneedles, although meal-time subcutaneous injection of insulin might still be necessary. The extent of interest in the application of microneedles to insulin delivery is due to the current increase in the number of cases worldwide. According the WHO report [111], as of 2000, 171 million people in the world were suffering from diabetes and this is estimated to rise to 366 million by 2030 [111].

ADVANTAGES AND LIMITATIONS

Researches on microneedles have pointed out a number of advantages and limitations regarding microneedles for transdermal drug delivery. As more research is done, more of their advantages and limitations will become apparent. We discuss the general advantages and limitations of the microneedles briefly in this section.

Advantages

First of all, the microneedles possess all the advantages of transdermal drug delivery method. They are almost painless, require no medical expertise and have little risk of infection at injection sites [112]. In addition, they have the ability to overcome the barrier function of the stratum corneum without causing pain [113,114].

As stated previously, the nerve receptors are presented in the dermis below the epidermis and the barrier layer of the skin is the stratum corneum, the top layer of the skin. Microneedles only pierce the upper layer of the epidermis and hence, they cause no pain [113,114].

The simple process of placing the patch on the skin and allowing the drug to be delivered carries little or no risk and hence, no medical expertise is required [112]. This is unlike intramuscular injection or intravenous injection were trained practitioners must be employed [112]. Consequently, providing microneedles to patients for home use instead of frequent hospital visits for jabs may reduce the risk of contracting diseases.

The presence of antigen producing cells such as the langerhans cells and the dendritic cells in the skin makes microneedles as an alternative method for vaccine delivery. Researchers suggest the possibility of lower dose requirement and better immune response when using microneedles as opposed to intramuscular or subcutaneous injection [95-97, 99].

Storing a drug in a dried form as a coating could improve their shelve life even at room temperature [19]. The obvious advantage of this is the ease of storage of drugs and fewer drugs are wasted due to exceeding expiry date in storage.

Limitations

In spite of considering the microneedles as promising technology for transdermal drug delivery, they have some limitations. The small size of the needles limits the therapeutics delivery rates which are required in relatively low doses such as proteins and vaccines.

Skin thickness can vary according to age, anatomical region, race and sex [115]. Therefore, it is very important to consider the variation of skin thickness while designing microneedles since this could affect the natural behaviour of skin barrier and hence influence the drug delivery [18, 34, 80-81, 116]. To our best knowledge there is no detailed cost data to date, that gives comparison between the overall cost of microneedles and that of hypodermic injection. Despite the positive prospects of microneedles it is important that they can be affordable. As a result current studies

have presented fabrication methods that allow cheap mass production of microneedles [117]. Moreover it seems the cost of production also depends on the material from which the microneedles are being fabricated. For example fabrication of polymeric microneedles seems to be less expensive than silicon microneedle [1]. Some studies have also reported that the insertion of microneedles proves rather difficult *in vivo* [3, 39]. This is due to the cushion provided by the underlying fat and muscles giving the skin a viscoelastic property that makes insertion difficult [1]. For this reason in some experiments an electric applicator [3] or mechanical vibration [39] has been used to enhance insertion of microneedles.

MATHEMATICAL MODELS OF TRANSDERMAL DELIVERY BY MICRONEEDLES

Throughout the literature, several works have been conducted to assess the safety and efficacy of the microneedles for use in drug delivery. For example, the purposes of some of these studies have been to evaluate the microneedle strength and to predict the concentration of the drug in the blood following microneedle insertion.

Mathematical Models to Determine the Mechanical Properties of Microneedles

Aggarwal and Johnston [118] investigated the influence of various hollow microneedles shapes (i.e., square, rectangular and circular) on bending force and buckling force to obtain the optimal design. The results show that the circular microneedle has the maximum bending force as compared to both square and rectangular microneedles. Moreover, the bucking force reaches its highest value for both circular and rectangular microneedles. Therefore, the circular microneedle can withstand more force as compared to both square and rectangular microneedle. The effect of the microneedle length has also been studied. This may change the insertion force needed to penetrate the skin with a large number of microneedles [119]. An optimization approach has been proposed by Vasquez and Pelesko [120] to maximize the buckling force. The result indicates that the buckling force is higher as compared with the previous work done by Aggarwal and Johnston [118]. However, the permeability across skin has not been linked with the insertion force. Once the microneedles are inserted into skin, this will lead to a huge deformation of the skin [119]. As a result, this will damage the skin and hence influence or even reduce the permeability across skin [119]. In another study, a theoretical model has been presented by measuring experimentally, both the fracture force and penetration (insertion) force for solid and hollow microneedles [58]. The result suggests that there is a linear relationship between the penetration force and the full cross sectional area of the microneedles, whereas no relationship has been observed between the penetration force and the wall thickness. According to Davis *et al* [58], the full cross sectional area of microneedles is only a function of the microneedle tip radius. On the other hand, the fracture force decreases by decreasing wall thickness, wall angle and tip radius. Consequently, the highest safety margin (i.e., the ratio of fracture force to penetration force) occurs when microneedle has small tip radius and large wall thickness. However, the experiments were performed on human skin for various ages of Caucasian males. As mentioned previously, the skin thickness could vary according to different factors such as sex, anatomical sites, etc. and consequently this could influence the prediction of the insertion force [119]. The critical buckling load has been theoretically studied and numerically simulated for various microneedles geometries [121-123, 49, 57].

Mathematical Models to Determine the Microneedle Flow Rate

The volumetric flow rate of hollow microneedles has been measured by adopting the Hagen-Poiseuille equation [124]. A relationship has been observed between the pressure drop, microneedle radius, number of microneedles and flow rate, whereas the flow rate does not depend on the microneedle centre-to-centre spacing [115]. In another work, the liquid flow rate of hollow microneedle has been determined by applying Bernoulli equation [125]. The experimental results agreed well with the theoretical model which shows that Bernoulli equation is a useful model to describe the liquid flow through the microneedle lumen [125]. It has also been shown for a larger number of microneedles in an array; the delivery rate is lower as compared to smaller number of microneedles [119]. As a consequence the flow rate may affect the flow resistance in the bores of microneedles.

Mathematical Models to Determine the Permeability Across Skin

It is important to know the factors that affect permeability across skin when applying microneedle arrays as they could have significant impact on transdermal drug delivery. To date, different methods have been proposed to predict permeability across the stratum corneum which are mostly applicable for low molecular weight compounds. Wilschut *et al* [127] reviewed these models for predicting permeability across skin of transdermal delivery of low

molecular weight drugs. In these approaches, the stratum corneum is assumed to be the rate limiting layer and the resistance of the drug transport provided by the viable skin is ignored [128]. In the present context, the resistance of the stratum corneum is overcome by insertion of the microneedle and the rate limiting barrier of skin is the viable skin. Consequently, most of these approaches are not directly applicable. A number of attempts have been already made to evaluate permeability across skin when using microneedle arrays. Wu *et al* [129] obtained a relationship between the number of bores and permeability across skin using microneedles. They also found a relationship between molecular weight of macromolecules and permeability across skin for hairless rat's skin. Another relationship between permeability across skin and high molecular weight compounds was observed for hairless rat skin [130].

The transport of drug through the skin has been defined as a diffusion process represented by Fick's second law equation [22]:

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} \tag{1}$$

The flux term is related to the drug permeability in skin such that the steady state flux is simply a product of permeability (K) and concentration (C) [131]

$$J_{ss} = KC \tag{2}$$

The concept of effective skin thickness has been introduced and calculated by rearranging the Fick's first law equation for steady state diffusion [22].

$$J_{ss} = -D\frac{dC}{dx} \tag{3}$$

Where J_{ss} is the steady state flux of a drug across skin, D is the diffusion coefficient of the drug, C is the drug concentration and dC/dx is the concentration gradient. It has been defined that at steady state

$$\frac{dC}{dx} = \text{constant} = \frac{C_0 - C_1}{h_{eff}} \tag{4}$$

Where C_0 is drug concentration at the coated area of microneedles, C_1 is drug concentration at the epidermal/dermal junction and h_{eff} is the skin thickness after microneedle has been inserted.

By combining equation (3) with equation (4), the following equation for steady state diffusive flux J_{ss} can be obtained:

$$J_{ss} = \frac{-D(C_0 - C_1)}{h_{eff}} \tag{5}$$

When C_0 is defined to be zero (i.e., sink condition), the effective skin thickness is determined as follows [34, 80]:

$$h_{eff} = \frac{DC_1}{J_{ss}} \tag{6}$$

We illustrate the use of this system of equations as follows. The imposed boundary conditions for this purpose are as follows. We define that the full penetrated length (L) of the microneedle is coated. The initial concentration at time t=0 in the microneedle coating is 0, i.e.,

$$C = 0 \text{ at } 0 \le x \le L \text{ for } t = 0 \tag{7}$$

The dissolved drug concentration on the microneedle coating, C_s, remains constant.

$$C = C_s \text{ at } 0 \le x \le L \text{ for } t > 0 \quad (8)$$

At the epidermal/dermal junction (x = h) the concentration is kept as 0 at all times. This is based on the assumption that as drug diffuses through the epidermis it is immediately absorbed into the microcirculation in the dermis [80].

$$C = 0 \text{ at } x = h \text{ for } t > 0 \quad (9)$$

With the imposed boundary conditions (7-6.9) the software (SKIN-CAD^{TM} [p134]) was used to solve for values of diffusive flux at different times till the steady state diffusive flux was obtained.

Here, as an example, using computer simulations that incorporated the finite element method [132], the effects of two parameters, namely, microneedle tip radius and center-to-center spacing on permeability are demonstrated. Insulin (MW=5734, [133]) is used as the model drug having a diffusion coefficient of 1×10^{-10} m^2/s. This method has been used by Davidson *et al* [34] to calculate the effects of microneedle geometry on permeability of a model drug, insulin, in the skin.

In the first example the center-to-center spacing was varied while keeping other dimensions constant and the permeability was then calculated numerically. Figs. 6 and 7 shows the effect of varying the center-to-center spacing of microneedles in an array on the permeability of insulin across the skin. This is shown for a particular microneedle shape also shown in Fig. **7**. From the graph it can be seen that reducing the center-to-center spacing between the microneedles in an array will cause a noticeable increase in permeability.

Likewise Fig. **8** shows the effect of the microneedle tip radius on the permeability. Using the same microneedle domain, the tip radius was varied while keeping the base radius and the length constant. The permeability was then calculated at the different values of tip radius and the results compared in Fig. **8**. The results suggest that increasing the microneedle tip radius would cause an increase in permeability.

Studies have also been done to predict the concentration of the drug in the blood following drug delivery using microneedles [34, 80]. In this case the effect of the stratum corneum has been eliminated and the drug permeates through the viable epidermis and is absorbed by the blood vessels in the dermis [34, 80]. The effect of the microneedle geometry on the concentration profile has been investigated using mathematical simulations [80]. The concentration profile of insulin in the blood is predicted using equation 6.10 [22].

$$V_p \frac{dC_p}{dt} = \frac{dQ}{dt} S_a - K_E C_p V_p \quad (10)$$

Where V_p is the volume of distribution in the blood (m^3), C_p is the concentration of the drug in the blood (g/m^3), t is time (s), K_e is the elimination rate constant, dQ/dt is the rate of skin penetration(g/m^{2}s), S_a is the area of the drug releasing surface of the delivery system (m^2) [22].

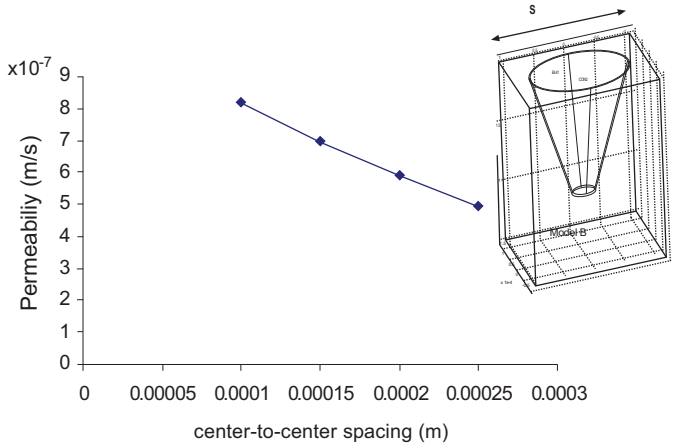

Figure 7: Influence of microneedle diameter on permeability. The shape of model B is shown on the side.

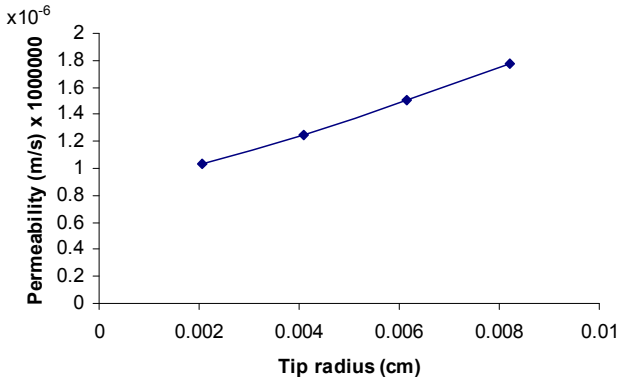

Figure 8. Effect of tip radius on permeability for model B.

An example of the effect of spacing and tip radius of microneedle on drug concentration in the blood after transdermal delivery using microneedles are analyzed. These results are shown in Figs. **9** and **10.** Fig. **9** suggests that the farther the microneedles are spaced from one another, the lower the peak blood concentration would be. A maximum blood concentration of about 0.0025 μg/ml was obtained with a center-to-center spacing of 0.01cm while a lower concentration of around 0.00150 μg/ml was reached with a spacing of 0.025cm.

Figure 10 shows the results of varying the tip radius of microneedles on the concentration of the drug in the blood. From the graph it can be seen that a higher concentration is reached for a wider tip radius.

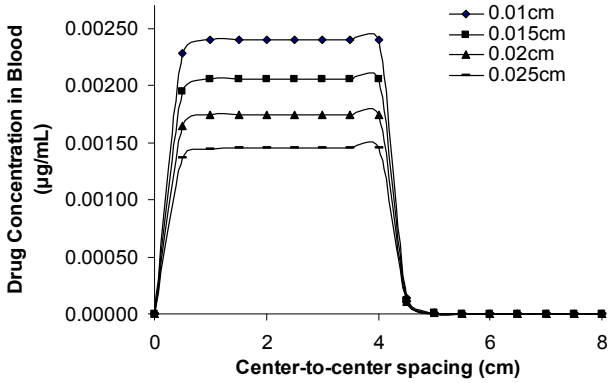

Figure 9: Effect of center-to-center spacing (S) on insulin concentration in the blood

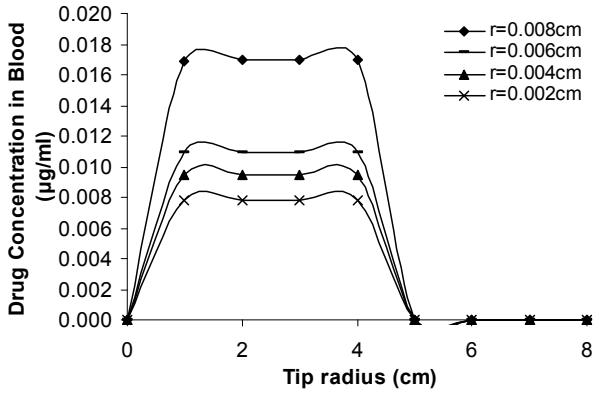

Figure 10: Effect of microneedle tip radius (r) on drug concentration in blood.

CONCLUSIONS

Transdermal patches are efficient in delivering lipophilic drugs with relatively small molecular size across the skin. For more potent drugs with larger molecular weight, alternative methods are required. This has led to the invention of microneedles. Sizes of microneedles are within the micron scale in order to facilitate painless drug delivery at rates that would otherwise not be achieved using ordinary transdermal patches. In this chapter we have given an overview of the concept and technique of microneedles used for transdermal drug delivery applications. We reviewed the different types of microneedles and the fabrication methods. It is important to design microneedles to meet certain requirements that ensure adequate drug delivery. For instance the microneedles must be strong enough to insert into the skin and the material from which they are fabricated must be biocompatible with the skin. The applications of microneedles in transdermal drug delivery have been discussed and these include insulin delivery, acne treatment, interstitial fluid extraction and delivery of nano-particles. Furthermore, different *in vitro* and *in vivo* studies that suggested possible improvements in areas such as insulin delivery and immunization with the use of microneedles have been discussed. Microneedles possess a number of advantages over other drug delivery methods such as ease of application and little risk of infection of the injection site. On the other hand microneedles are limited by their small size to drug required in small amounts such as proteins. The mathematical theories behind transdermal drug delivery are also briefly discussed. Different microneedle types and geometry have been mentioned to give an idea of the range of sizes and shapes of microneedles that can be fabricated through the microfabrication process. In addition to these, the governing equations of drug transport through the skin and blood concentration profile in the blood have been presented. Furthermore some sample results of computer simulations incorporating these equations to analyze the effect of parameters such as penetration depth of microneedle in skin, diameter of microneedle and number of microneedles in an array have been discussed. These mathematical models allow systematic prediction and optimization of the performance of microneedles for drug delivery. This chapter gives a good idea of the concept of microneedles and their relevance as medical tools especially for transdermal drug delivery.

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CHAPTER 7

Transdermal Nanocarriers

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Abstract: This chapter aims to show state-of-the-art transdermal drug-delivery nanocarriers. The advances in this field are very important at present because the skin offers many advantages over other organs due to its size. Depending on the location, we can deliver drugs very close to the target area. The skin provides protection against microorganisms, temperature, mechanical factors, etc. [1-3]. The development of submicron particles and other nanostructures in the pharmaceutical and cosmetic fields has been employed for the design of formulations for application through the skin [4-7]. The mechanism by which submicron particles traverse the skin is interesting and important as well as size and composition. The nature of drugs that can be applied through the skin offers many possibilities for future therapies. Both lipophilic and hydrophilic compounds can be used for this purpose, depending on the chosen nanocarrier. Finally, regarding the specific applications of nanocarriers, it is worth mentioning that scientists have used nanocarriers for several applications, including delivery of local and systemic therapies.

Key words: Nanoparticle, transdermal nanocarriers, nanotechnology, dendrimers, liposomes, nanoemulsions.

INTRODUCTION

The nanomedicine which is the application of technologies on the scale of 1 to 500 nm to diagnose and treat diseases, it has become in a very relevant topic nowadays. During the last century, there has been a lot of new research and patents regarding nanomedicine in health sciences. Also, by 2007, U.S. Government has launched at least eight centers of Cancer Nanotechnology Excellence and twelve Cancer Nanotechnology Platform Partnerships [8]. The objective of nanomedicine is to diagnose and preserve the health without side effects with noninvasive treatments. To reach these goals, nanomedicine offers a lot of new tools and capabilities. The manipulation that nanomedicine provides to the drugs and other materials in the nanometer scale can change the basic properties and bioactivity of materials. The solubility, increment in surface area, control release and site-targeted delivery are some characteristics that nanotechnology can manipulate on drug delivery systems.

Nanotechnology applied to health sciences contains new devices used in surgery, new chips for better diagnostics, new materials for substituting body structures and some structures capable to carry drugs through the body for treatment of a lot of diseases. These structures can be made of a lot of different materials and they are very different in structure and chemical nature. All these nanostructures are called nanocarriers and they can be administrated into the organisms by all the routes.

Nanocarriers are a powerful weapon against a lot of illnesses since they are so small to be detected by immune system and they can deliver the drug in the target organ. For that reason, drug doses using nanocarriers decrease a lot and side effects decrease too.

The idea for using these tiny systems is not as new as we think but the use of nanocarriers in pharmaceutical products is not frequent, since the technology is expensive for certain types of nanoparticles and because nanocarriers need to be evaluated for demonstrating they do not have toxic effects. Nowadays the controversy of biological effects due to nanostructures is an open discussion, in one hand, the nanotechnologist continue making new and more sophisticated nanocarriers and in the other hand, toxicologist continue evaluating possible damaging effects.

Whatever it happens, nanotechnology is the new era and nanomedicine cannot be taking off. New nanocarriers will be created and the entire scientist working in nanomedicine bet for it to be the cure of diseases that in this moment are difficult to deal with.

José Juan Escobar-Chávez (Ed)

TRANSDERMAL NANOCARRIERS

There is a large variety of nanocarriers used in the pharmaceutical sciences as drug delivery systems. All of them accomplish the substantial task of carrying drugs to the target in a controlled manner. It is difficult to choose which of these carriers is better because ongoing research continually highlights new advantages and disadvantages for each particular carrier.

These carriers can be applied using different routes, and some of their characteristics make them better for one route than another. Materials used for their elaboration are also important, above all those relating to toxicity and metabolism side products.

Nanocarriers have demonstrated increased drug absorption, penetration, half-life, bioavailability, stability, etc. One of the less-used routes—but one of the most important—is the dermal route. Skin is the largest organ in the body, and it is connected with numerous systems, organs, glands, etc. This route is underutilized. In the past, this route was considered difficult to work with due to the fact that it allowed penetration by only a few selected molecules (i.e., small and lipophilic drugs). The use of permeation enhancers and nanocarriers allowed more drugs to be administered through the dermal route—not only small, lipophilic drugs but also larger, hydrophilic drugs. A large variety of nanocarriers are used for transdermal delivery (Table **1**). In this chapter, we focus on the most used and investigated in the pharmaceutical field: liposomes, dendrimers, nanoparticles and nanoemulsions.

Table 1: Examples of Nanocarriers used for drug delivery.

Type of transdermal Nanocarrier	Size range	Preparation Methods	Characteristics	References
Nanoparticles	10-1000 nm	-In situ polimerization. -Emulsification- evaporation. -Emulsificaction-diffusion.	Solid or hollow particles wich have entraped, binded or encapsulated drugs.	10
Solid lipid nanoparticles.	50-1000 nm	-High-pressure homogenization.	Similar to polymeric nanoparticles but made of solid lipids.	11
Inorganic nanoparticles.	<50nm	-Sol-gel technique	Nanometric particles, made up of inorganic compounds such as silica, titania and alumina.	12
Liposomes	25 nm-100 μm	-Sonication -Extrusion -Mozafari method	Vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments.	13
Dendrimers	3–10 nm	-Polymerization	Macromolecular high branched structures.	14
Quantum dots	2-10nm	-Colloidal assembly -Viral assembly. -Electrochemical assembly.	Made up of organic surfactants, precursors and solvents.	15
Lipid globules	1-100 nm	-Emulsification espontaneous systems.	Multicomponent fluid made of water, a hydrophobic liquid, and one or several surfactants resulting in a stable system.	16
Lipid microcylinders	<1 μm	-Self emulsification	Self organizing system in which surfactants crystallize into tightly packed bilayers that spontaneously form cylinders	17
Lipid microbubbles	<2 μm	-Sonication	Gas filled microspheres stabilized by phospholipids, polymers or low density proteins.	18
Lipospheres	0.2-100μm	-Melt method -Multiple microemulsion -Cosolvent method	Solid lipid core stabilized by a monolayer of phospholipids molecules embedded in the particle surface.	19
Ethosomes	<400 nm	-Cold method -Hot method	Non invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation.	20
Aquasomes	60-300 nm	-Self-assembling of hydroxyapatite by co-precipitation method	The particle core is composed of noncrystalline calcium phosphate or ceramic diamond, and it is covered by a polyhydroxyl oligomeric film.	21
Pharmacosomes	<200 nm	-Hand-shaking method -Ether-injection method	Pure drug vesicles formed by amphiphilic drugs	22
Colloidosomes	200nm – 1.5 □m	-Self-assembly of colloidal particles at the interface of emulsion droplets	Hollow capsules with elastic shells.	23
Niosomes	10-1000 nm	-Self-assembly of nonionic surfactant.	Bilayered structures made of non-ionic surfactant vesicles.	24

Table 1: cont....

Nanoemulsions	20-200nm	-High-pressure homogenization. -Microfluidization. -Phase Inversion Temperature.	Submicron emulsions o/w or w/o	25

All of these nanocarriers have been used for certain purposes but scientists still do not use them in a frequent basis. Most of them have a lot of potential as drug carriers.

The aim of this chapter is to provide a general scope of pharmaceutical nanocarriers. Special attention is placed on nanoparticles, liposomes, dendrimers and nanoemulsions since they are the most used carriers for topic/transdermal drug delivery (Fig. **1**). Some others nanocarriers are based on these three main nanocarriers mentioned above. For example, transformable liposomes, they are a kind of liposomes containing surface active ingredients in their membrane to let them decrease interfacial tension and enlarge/modify their structure.

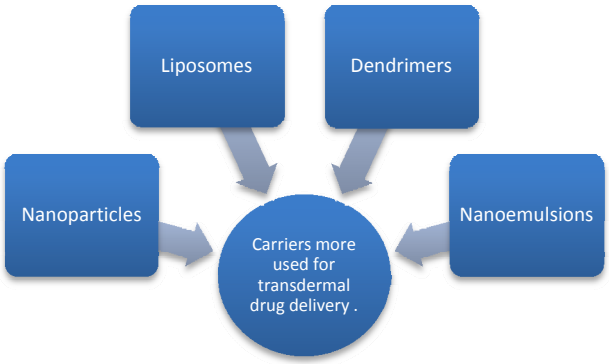

Figure 1: Carriers more used in transdermal drug delivery.

Liposomes

Liposomes (Fig. **2**) are hollow lipid bilayer structures that can transport hydrophilic drugs inside the core and hydrophobic drugs between the bilayer. They were created by Alec Bangham. Forty years ago he realized that phospholipids in aqueous systems can form closed, bilayered structures [26]. Liposomes have since become one of the pharmaceutical nanocarriers of choice for many applications. During recent years, many liposome-based drugs and biomedical products have been approved for use in the clinic.

Liposomes were used to study membrane processes and membrane-bound proteins. They were also proposed as drug carriers that reduce toxicity and increase efficacy. Early research did not produce sufficient *in vivo* results for liposome transport to be considered a valid option. Furthermore, liposomes did not show suitable extrapolation *in vitro*, nor did they demonstrate good stability or circulating time *in vivo*.

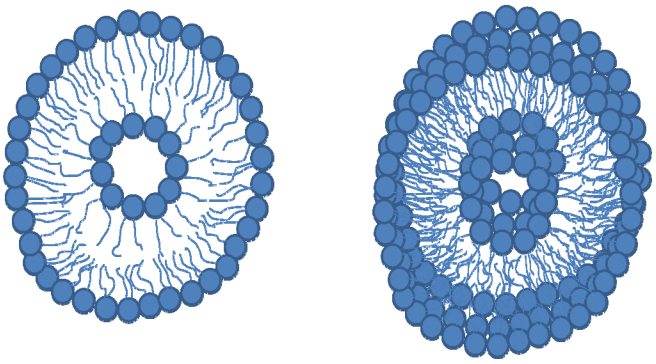

Figure 2: Liposomes contain a lipid bilayer.

Furthermore, the knowledge acquired in research on lipid polymorphism, the physiological mechanisms of *in vivo* liposome disposition, lipid-drug and lipid-protein interactions, changed early conceptions related to liposomes. The consequences of these findings were more stable liposomes, increased half-life, and better distribution. Fifteen years ago, liposomes were first used for drug delivery—thirty years after their creation by Bangham. Liposomes are currently being used for many anti-fungal and anti-cancer applications.

Liposomes have some interesting properties that make them very unique. The modification of some properties can give liposomes additional properties that make them better delivery systems.

First, liposomes can be surface-charged as neutral, negative or positive, depending on the functional groups and medium pH. This important characteristic can help liposomes with regard to distribution, stability, interaction with membranes, etc. Neutrally charged liposomes tend to form aggregates, and they can interact strongly with cells of the reticuloendothelial system; some authors have suggested the addition of small amounts of negatively charged lipids to stabilize them and diminish aggregation. Negatively charged liposomes tend to have better physical stability and less aggregation issues due to the repulsion forces. Such liposomes are composed of phosphatidylserine or phosphatidylglycerol, but they do not exhibit specific uptake *in vivo*; nonetheless, these liposomes penetrate cells faster than neutral liposomes. The inclusion of some glycolipids, such as ganglioside or phosphotidylinositol, results in longer circulation times because these glycolipids inhibit uptake by macrophages and reticuloendothelial cells. These liposomes are recognized by the receptors of certain cells, including macrophages. Positively charged liposomes are used for DNA delivery in gene therapy; they interact easily with serum proteins. Cationic liposomes interact with reticuloendothelial cells and they are excreted by lung, liver or spleen. The use of hydrophilic polymers in the membrane to avoid reticuloendothelial recognition is a good strategy in liposome fabrication, serving to also decrease aggregation through steric modification and surface hydration. This surface modification is performed using polyethyleneglycol (PEG) or gangliosides. The addition of hydrophilic polymers to the liposome membrane makes it unrecognizable to the macrophages and reticuloendothelial system [27].

Liposomes can encapsulate both lipophilic and hydrophilic drugs in a stable manner, depending on the polymer added to the surface (e.g., PEG). PEG does not significantly alter membrane charge. In addition, PEG is widely used in parenteral pharmaceutical applications. There are other polymers with similar properties such as poly (acrylamide) (PAA), poly (vinylpyrrolidone) (PVP) and poly (acryloyl morpholine) (PacM). These polymers have been conjugated with phospholipids for steric protection, but their safety in humans is not well understood [28].

Activation of the liposome surface is performed via three primary mechanisms: reaction between activated carboxyl groups and amino groups, yielding an amide bound; reaction between pyridyldithiols and thiols, yielding disulphide bonds; and reaction between maleimide derivatives and thiols, yielding thioether bonds. Liposomes can be surface modified by conjugation with proteins, peptides, polymers and other molecules.

Liposomes Classification

We can classify liposomes based on size and their number of lamellae (Fig. **3**). There are Small unilamellar visicles (SUV), Medium-sized unilamellar vesicles (MUV), large unilamellar vesicles (LUV), Giant unilamellar vesicles (GUV), Oligolamellar vesicles (OLV), Large multilamellar vesicles (LMV) and multivesicular vesicles (MVV). The range diameter for SUVs is from 25 nm to 100nm, MUVs diameter is between 100 nm and 500nm. LUVs, GUVs, OLVs, LMVs and MVVs have a diameter of few hundred nanometers to several microns. The thickness of the membrane measures approximately 5 to 6 nm.

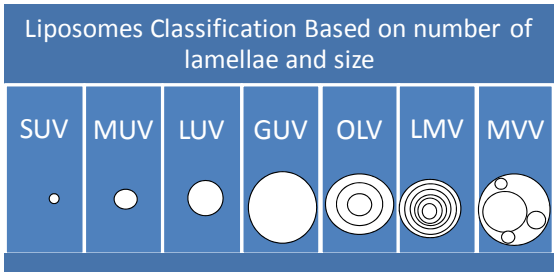

Figure 3: Liposomes Classification based on size and number of lamellae.

Liposomes could have different sizes, number of liposomes formed and position of the lamella These will depend on lipids used for their preparation, preparation technique and process variables.

Depending on these parameters, the behavior both *in vivo* and *in vitro* can change and opsonization processes, leakage profiles, disposition in the body and shelf life are different due to the type of liposome. Liposomes can also be classified regarding to the type of membrane as shown below in Table **2**

Table 2: Types of liposomes regarding to the membrane components.

Type of liposomes	Characteristics
Conventional	This type of liposomes basically have phospholipids in the bilayer. They can be negative or neutral charged.
Stealth	This type of liposomes are polymer covered, for prolonged circulation times.
Targeted	These have antibodies or antibodies fragments on their surface.
Cationic	These are cationic surfactant made to neutralize and transport negatively charged DNA.

Liposomes Preparation (Techniques)

Liposomes preparation techniques have been widely reported in a lot of research. All these techniques follow three basic steps with particular features depending on safety, potential scale up and simplicity:

1. Lipid must be hydrated
2. Liposomes have to be sized
3. Nonencapsulated drug has to be removed.

These three stages can be acomplish by different methods. Sometimes, it is used an especific hydration method, for example a mechanical method, after that, a sizing technique is applied for get a better size distribution and finally the nonencapsulated material is removed by Dialysis for instance. Table **3** shows lipid hydration methods, sizing liposomes techniques and tecniques for removal of nonencapsulated material.

Table 3: The three basic steps for liposomes preparation.

Lipid hydration	Liposomes Sizing	Removal of nonencapsulated Material.
a. Mechanical methods. -Vortex and hand shake. -Microfluidizer. -Bubbling inert gas. b. Methods based on replacement of organic solvents by aqueous media. -Removal of organic solvent before hydration. -Reverse phase evaporation. -Use of water immiscible solvents. -Use of water miscible solvents. c. Methods based on detergent removal. -Gel exclusion chromatography. -Slow dialisis. -Fast dilution. -Other related techniques d. Methods based on size transformation and fusion. -Spontaneous fusion. -Freeze thawing. -Freeze drying. -Dehydration. -Calcium ion induced fusion. -Detergent induced growth. e. Methods based on pH adjustement.	a. High Presure extrusion. b. Low presure extrusion. c. Ultrasonic treatment.	a. Dialysis. b. Ultracentrifugation. c. Gel Permeation Chromatography. d. Ion exchange resins.

Some LMVs are produced when a lipid film deposited by an organic solvent is hydrated and shaked at temperature above the phase transition temperature of the phospholipid. It is obtained a wide size distribution of liposomes dispersion but it is narrowed down by pressure extrusion or ultrasonication.

The three steps of producing liposomes can be applied depending on nature of phospholipids, production process, drug nature and features desired liposome.

Liposomes in Transdermal Delivery.

Liposomes were one of the first strategies for transdermal delivery. They are structures made of cholesterol and phospholipids. They can have different properties depending on the excipients with which they are synthesized and the process of their elaboration. The nature of liposomes makes them one of the best alternatives for drug delivery because they are non-toxic and remain inside the bloodstream for a long time. They are being successfully used in cancer therapy [29-32].

Nanoparticle size and formulation, as well as the presence of penetration enhancers and the physical state of the stratum corneum, affect the degree of transdermal drug penetration. Other important factors include lamellarity, lipid composition, charge on the liposomal surface, mode of application and the total lipid concentrations [33, 34]. Liposomes have been used successfully to transport drugs across the skin. Table **4** shows some examples of drugs delivered throughout the skin by using liposomes.

Table 4: Examples of transdermal drug delivery using liposomes.

Transdermal drug delivery using liposomes	References
Melatonin	35
Indinavir	36
Amphotericin B	37
Methotrexate	38
Ketoprofen	39
Estradiol	40
Clindamicyn Hydrochloride	41
Lignocaine	42

Some authors report the use of flexible vesicles in comparison with rigid vesicles to enhance penetration [43-50]. The interaction of liposomes with biological membranes occurs easily due to their lipid content [51]. The lipids present in the liposome bilayer can interact with lipids present in the stratum corneum [52], changing the structure of the upper skin. This change is beneficial for the penetration of lipophilic drugs into the stratum corneum. Some liposomes may have a deformable structure and pass through the stratum [53] or may accumulate in the channel-like regions in the stratum corneum [54], depending upon their composition. The driving force is nothing more than osmotic pressure; these liposomes are called transfersomes or transformable liposomes.

Transformable liposomes.

Transformable liposome is an idea that arose due to the need to reach narrow conducts to deliver drugs. The idea of using liposomes for drug delivering is very good since they have lipids very similar to biological membranes, but to going into some narrow zones it is a difficult task for these carriers. The idea of making less rigid the liposome membrane is a very interesting strategy to make liposomes more versatile. The incorporation of elements in the lipid bilayer to make it flexible has been the success of these carriers. Traditional transformable liposomes are made using surfactants in the lipid bilayer, but the idea of make flexible liposomes has been the goal of a lot of scientists, for that reason we can find another structures like these, for example, ethosomes that contain alcohol in the lipid bilayer for making them more flexible and be able to be deformed when a pressure is applied. In transdermal drug delivery the paracellular and intercellular pathways are very important but appendages routes have been of increasing interest lately. The use of flexible liposomes (transformable liposomes) is an invaluable strategy for reach the objective of drug deliver via transdermal route. Table **5** shows some examples of drugs delivered through the skin in flexible liposomes.

Table 5: Examples of transdermal drug delivery using transformable liposomes.

Transdermal drug delivery using liposomes	References
Diclofenac	55
Insulin	56, 57
Tetanus toxoid	58
Corticosteroids	59
Superoxide dismutase	60, 62
DNA	62
Triamcinolone-Acetonide	63
Ketoprofen	64
Interleukin-2	65

Dendrimers

Dendrimers are non-peptidic fractal 3D structures made of numerous small molecules (Fig. **4**). The term "dendrimer" is Greek: "dendra" means tree and "meros" means part. This name arose in the late 1970s by a research group formed by Vogtle Denkwalter, Tomalia, and Newkome [66]. The structure of these molecules results in relatively uniform shapes, sizes and molecular weights. They are a very good alternative to drug delivery systems; dendrimers can be used in anti-viral and anti-cancer pharmaceutical therapies, including vaccines [67, 68].

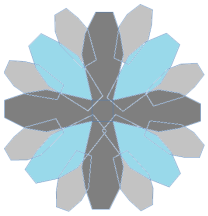

Figure 4: Dendrimer: Three-dimensional fractal structure.

The first material and most commonly used for dendrimer fabrication is poly (amidoamine) (PAMAM), which was initially synthesized by Dow Laboratories between 1979 and 1985. Dendrimers have been used in numerous applications: as light-harvesting agents [69,70], catalysts [71], chemical sensors [72] and cross-linking agents. In the context of controlled chemical delivery, dendrimers have been explored for drug delivery [73], gene therapy [74], and the delivery of contrast agents [75]. Additionally, they have been used in biomedical applications; including gene delivery, contrast agents, and oral drug delivery systems. Finally, dendrimers have been studied to assess biocompatibility and toxicity [76].

Dendrimers Classification

Dendrimers are monodisperse populations that are structurally and chemically uniform. They allow conjugation with numerous functional groups due to the nature of their branches. The amount of branches increases exponentially and dendrimers growth is typically about 1 nm per generation [77].

The dendrimers classification is based on the number of generations [78]. After the creation of a core, the stepwise synthesis is called first generation; after that, every stepwise addition of monomers creates the next generation. This approach allows an iterative synthesis, providing the ability to control both molecular weight and architecture. Table **6** shows molecular weight, diameters and surface groups by generation.

Table 6: Types of dendrimers depending on generation number.

Generation	Molecular Weight	Diameter by SEC ($\mathring{A}$)	Number of Surface Groups
0.5	924	27.9	6
1.5	2173	36.2	12
2.5	4671	48.3	24

Table 6: cont....

3.5	9668	66.1	48
4.5	19661	87.9	96
5.5	39648	103.9	192
6.5	79621	126.8	384
7.5	159568	147.3	768
8.5	319461	174.2	1536
9.5	639247	210.3	3072

Dendrimers Preparation

The main factor affecting dendrimer architecture and design is the selection of material for synthesis. These highly branched macromolecules are synthesized utilizing a step-wise approach with either linear or branched building blocks. The materials used for dendrimer synthesis allow the precise structure, molecular weight and chemical and physical properties to be manipulated.

These fractal-like structures have very good solubility in comparison with other non-fractal like polymers. This feature helps dendrimers to be easily characterized and purified.

Due to their 3D architecture, dendrimers possess low intrinsic viscosities in comparison with their linear counterparts. The kind of polymer chosen to construct the dendrimer is very important with regard to the final architecture and features. In addition, the use of branched monomers has the peculiarity of providing tailored loci for site-specific molecular recognition and encapsulation.

Notably, 3D and fractal architecture, as well as the peripheral functional groups, provide dendrimers with characteristic physical and chemical properties.

As previously noted, the discovery of dendrimers can be attributed to Vogtle *et al.*, who reported the first synthesis and characterization of a cascade molecule. Since this finding was published, many studies have documented the impressive versatility of dendrimers; they increase the solubility and decrease the toxicity of pharmaceutical molecules.

The trapping of molecules in a "carrier" is a concept used as far back as 1982 [79]. In comparison with linear polymers, dendritic structures have "dendritic voids" that give these molecules important and useful features. These spaces inside dendrimers can mimic the molecular recognition performed by natural proteins. The molecules have inside and outside receptors for binding molecules [80]. Furthermore, dendrimers have a high surface-charge density due to ionizable groups that help them to attach drugs by electrostatic forces, regardless of the stoichimetry. This dendrimer-drug association provides drugs with better solubility, increasing their transport through biological membranes and sometimes increasing drug stability.

By designing dendrimer architecture, surface charge, etc., the drug can be attached in a predictable way and the release can be controlled. The interaction between dendrimers and drugs may involve simple entrapment and/or nonbonding interactions into or out of the structure.

The number of molecules that can be incorporated into dendrimers is related to the number of surface functional groups; therefore, later-generation dendrimers are more easily incorporated into dendritic structure. However, not all the functional groups are available for interaction due to steric volume, molecule rotation or stereochemistry effects. When generation number increases, so do chain folding and the degree of sphericity [81]. Dendrimers can have positive and negative charges, which allows them to complex different types of drugs [82]. For example, positively charged dendrimers interact with flexible linear polyanions.

Dendrimers in Transdermal Delivery

In some researches, dendrimers are used for transdermal drug delivery. They show promising results in the delivery of drugs such as tamsulosin [83], indomethacin [84], ketoprofen and diflunisal [85] and 5-fluorouracil [86] (Table **7**). The main problems with this kind of transdermal carrier are poor biodegradation and inherent cytotoxicity [87].

Table 7: Examples of transdermal drug delivery using dendrimers.

Transdermal drug delivery using dendrimers	References
Tamsulosin	83
Indomethacin	84
Ketoprofeno	85
Diflunisal	85
5-fluorouracil	86
Peptides	89

Transdermal drug delivery has many advantages over the other routes: fewer side effects, increased patient compliance, controlled release, and the lack of a hepatic first pass. In addition, the treatment can be interrupted if necessary [88]. Dendrimers are rarely linked to peptides. These macromolecules are formed from amino acids linked via peptide-amide bonds to the branches of dendrimers in the core or on the surface [89, 90]. Dendrimer-peptide systems have inherent advantages such as low toxicity. When they are bio-transformed, dendrimer-peptide systems produce amino-acid derivatives. Finally, the synthesis of these structures is less expensive and purification does not present any difficulty.

The study of permeation/deposition of dendrimer-peptides is not so common since analytical methods to quantify levels in human skin have not been developed for all kinds of dendrimers. There is a validated HPLC method for quantifying levels of simple PAMAM dendrimers in human skin, but there were no reports about analytical methods for cationic poly-amino acid dendrimers [91].

The main advantage of dendrimers is that they have multivalency [92], and it is possible to precisely control the functional groups on the surface [93]. Due to their form and size, these molecules can carry drugs, imaging agents, etc. Another advantage is their small particle size (1–10 nm) [94, 95]. Dendrimers interact with lipids present in membranes, and they show better permeation in cell cultures and intestinal membranes. Dendrimers also act like solubility enhancers, increasing the permeation of lipophilic drugs. On the other hand, they are not good carriers for hydrophilic drugs and the mechanisms underlying permeation enhancement and the interaction of dendrimers with skin are not known.

5-fluorouracil (5FU) (log $P = -0.89$) is one hydrophilic model drug, which has very poor penetration in skin [96]. This drug is used to treat skin diseases [97, 98]. Many strategies to increase skin permeation of this drug have been tested: prodrugs, terpenes, fatty acids, iontophoresis, sonophoresis and laser ablation [99-104]. Dendrimers have been proven to increase 5FU permeation across the skin by altering the skin structure.

Nanoparticles

The prevailing goal for pharmacists is direct delivery of the drug to the organ of interest. Advances in Nanotechnology have brought scientists closer to this goal. The tools created by new advances in biotechnology, knowledge related to immunity, genetics, virology, etc., and Nanotechnology has helped in the fight against pathogenic agents and difficult syndromes. The use of Nanotechnology in pharmaceutical applications is not new but has recently come to represent the best option for therapeutic treatment of many diseases. The definition of nanoparticles in the field of pharmacy differs slightly from that used in other fields, where the maximum size for nanoparticles is in the neighborhood of 100 nm. When discussing therapeutics, nanoparticles can be smaller than 1,000 nm [105].

Today, it is possible to insert many types of materials such as drugs, proteins, peptides, DNA, etc. into the nanoparticles. Using conventional therapeutic systems, it would be difficult to have these kinds of molecules available for therapeutics; furthermore, using these molecules in conventional therapeutic systems could be toxic and ineffective [106]. With oral administration, the drug can be destroyed and/or poorly absorbed. Moreover, degradation side-products could be toxic to the organism. Nanoparticles are constructed from materials designed to resist pH, temperature, enzymatic attack, or other problems [107, 108].

The nanostructures used in the pharmaceutical industry are able penetrate barriers and survive in the blood [109, 110]. The spatial positioning and the temporal delivery of these systems are their main advantages. Because nanoparticles reach their target and deliver the drug directly where it is needed, the toxicity of the drug decreases, and the therapeutic effect is increased. The size of a nanoparticle is incredibly small; therefore, nanoparticles are not detected or rarely detected by the immune system. This characteristic has greatly helped in developing therapeutic strategies to fight human immunodeficiency virus (HIV)-infected cells [111].

The advancements in pharmaceutical nanoparticle technology can be divided into three stages: first generation, second generation and third generation. The first generation involves those nanoparticles that had only one component in their structure, like albumin nanoparticles. These delivery systems are able to transport drugs in the blood until they reach the target. Second generation implies nanoparticles made of one main component (polymer, lipid, etc.) and additional molecules, such as polymeric nanoparticles in combination with PEG. These complexes are able to cross barriers and reach difficult targets such as the brain. The third generation is represented by nanoparticles that can be made of nanoparticles with one main component combined with a second component to allow them to cross difficult barriers or antibodies, antigens, vitamins, etc. to reach a specific target, like those nanoparticles made of a polymer in combination with PEG and antibodies used to reach certain tumors.

Nanoparticles are very effective in the treatment of diseases such as cancer and diabetes [112], which are serious health problems that lead to substantial economic losses; the estimated incidence for 2030 is shown in Fig. **5**.

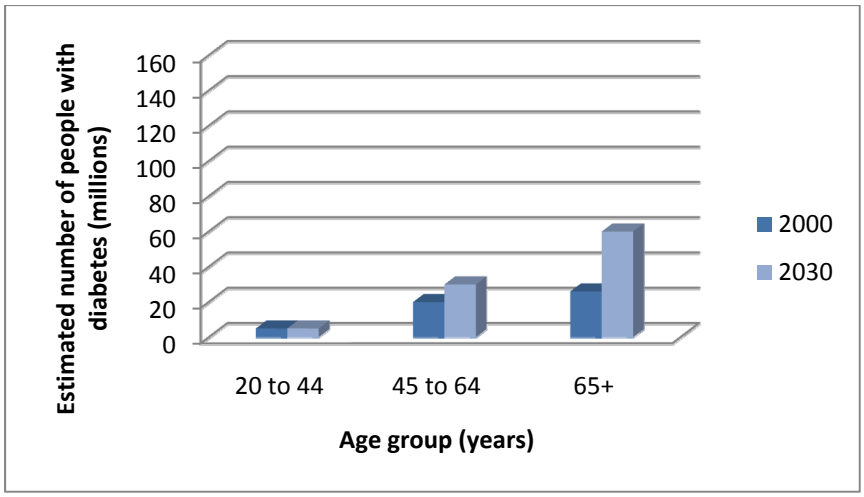

Figure 5: Diabetes in highly developed countries.

Nanoparticles have been used successfully in the therapy and diagnosis of cancer; this disease causes many deaths in developed countries every year [113]. While many cancer drugs destroy both cancer and healthy cells, nanoparticles represent a more targeted solution to drug delivery.

Nanoparticles have been also used for vaccine development because they offer features not found in other carriers: they are chemically stable and reproducible. As peptide vectors, nanoparticles have shown much better performance than traditional adjuvants in vaccine development [114]. Nanoparticles are important because many recently developed drugs are insoluble. One of the most helpful recent developments involves pH-sensitive nanoparticles [115]. Polymeric nanoparticles are used to deliver therapeutic agents for various types of tumors, diabetes, bone healing, and vaccination.

Nanoparticles Classification

They form colloids due to their size and can be classified as nanospheres or nanocapsules. Nanospheres are solid-core structures and nanocapsules are hollow-core structures (Fig. **6**). Drugs can be loaded into nanoparticles via encapsulation [116], surface attachment [117], or entrapping [118].

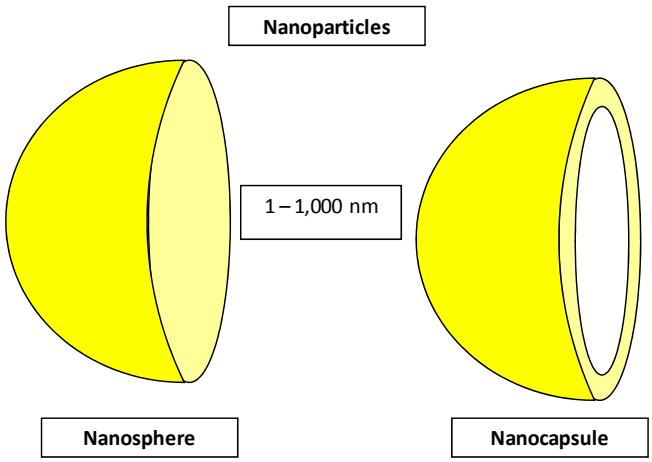

Figure 6: Nanoparticles: Nanospheres and Nanocapsules.

Nanoparticles can also be classified depending on the material they were made (Table **8**), for example, if they are made of lipids, the name is lipidic nanoparticles. Nanoparticles can be composed of polymers [119], lipids [120], polysaccharides [121] and proteins [122].

Table 8: Types of nanoparticles depending on material of preparation.

Material for producing nanoparticles	Nanoparticles name	References
Polymers	Polymeric Nanoparticles Nanospheres or Nanocapsules.	123
Lipids	SLN: Solid Lipid Nanoparticles LNS: Lipid Nanospheres NLC: Nanostructured Lipid Carriers	124 125 126
Polysaccharides	Chitosan nanoparticles Alginate nanoparticles	127 128
Proteins	Gelatin Nanoparticles Albumin Nanoparticles	129 130

Nanoparticles Preparation

Nanoparticles preparation techniques are based on their physicochemical properties and techniques derived from microparticles preparation methods (Table **9**).

They are made by emulsification-polymerization [131], in situ-polymerization (interfacial polymerization [132], gelation (crosslinking) [133], nanoprecipitation (solvent displacement) [134], solvent evaporation/extraction [135], inverse salting out, dispersion polymerization and other derived from these one.

Table 9: Preparation methods regarding on material used to produce nanoparticles.

Type of nanoparticle	Preparation technique	Important information	Reference
Albumin and Gelatin	Crosslinking agent	First type of nanoparticles	136
Acrylamide	Emulsion polymerization	Non biodegradable polymer	137
Polyalkylcyanoacrylate Nanospheres	Emulsion Polymerization	Biodegradable polymer	138
Methyl Methacrylate	Dispersion Polymerization	Non biodegradable polymer	139
Oil-containing nanocapsules	Nano precipitation	Used for liposoluble drugs	140
Polyalkylcyanoacrylate Nanocapsules	Interfacial polymerization	Use of negatively charged compounds to begin the polymerization	141
Polymeric Nanospheres	Solvent evaporation, salting out	Initially an emulsion o/w is done.	142,143
Nanocapsules	Solvent displacement, solvent extraction	Initially an emulsion o/w or w/o/w is done.	144
Chitosan nanospheres	Gelation	Tripolyphosphate	145
Alginate nanospheres	Gelation	Calcium	146

Nanoparticles in Transdermal Delivery

Two of the main options for transdermal delivery are the solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) [147]. Aside from lipid nanoparticles, polymeric nanoparticles are very good options for transdermal delivery because they can be tailor-made in different sizes and it is possible to modify surface polarity [148, 149] in order to improve skin penetration [150-156]. From the upper skin, nanoparticles can reach deeper skin regions because they exhibit mechanical flexion [157]. Nanoparticles can even travel from the skin to lymph nodes, representing a promising tool for immunomodulation [158]. Table **10** presents examples of drugs delivered throughout the skin by using nanoparticles.

Table 10: Use of nanoparticles in transdermal drug delivery.

Active Pharmaceutical Ingredient (API)	Type of nanoparticle	Reference
Mixnoxidil	Block copolymer Nanoparticle. poly(□-caprolactone)-block-poly(ethyleneglycol)	159
Triptolide	Solid Lipid Nanoparticle	160
DNA	Polysaccharide Nanoparticle. Chitosan/poly-γ-glutamic acid	161
DNA	multifunctional core-shell polymeric nanoparticle (PLGA core and a positively-charged glycol chitosan (GC) shell)	
Triamcinolone acetonide acetate	Solid Lipid Nanoparticle	162
Dexamethasone Phosphate	Polymeric Nanoparticle. Poly (Lactide-co-glycolide)	163
Cyclosporin A	Solid Lipid Nanoparticle	164
Flufenamic acid, Testosterone, Caffeine	Polysaccharide Nanoparticle. Propyl-starch derivatives	165
5-Fluorouracil	Polymeric Nanoparticle Poly (Lactide-co-glycolide)	166
Arthemeter	Lipid Nanoparticle.	167
Chlorhexidine	Polymeric nanoparticle.	168
Econazole nitrate	Lipid Nanoparticle.	169
Insulin	Chitosan Nanoparticle	170
Celecoxib	Nanostructured Lipid Carrier (NLC)	171
Coenzyme Q10	Nanostructured Lipid Carrier (NLC)	172

Nanoemulsions

Nanoemulsion are isotropic dispersed systems of two non miscible liquids, normally consisting of an oily system dispersed in an aqueous system (o/w nanoemulsion), or an aqueous system dispersed in an oily system but forming droplets or other oily phases of nanometric sizes. In pharmaceutical sciences, the main application of nanoemulsions corresponds to the oil in water (o/w) emulsions. These are thermodynamically non stable systems on the contrary with microemulsions, because nanoemulsions need high energy to produce them. They are susceptible to Oswald ripening and as a consequence susceptible to creaming, flocculation and other physical instability problems associated to emulsion. Even though, the instability of nanoemulsions, they can be stable (methastable) for long times due to the extremely small sizes and the use of adequate surfactants. Nanoemulsions can be used to formulate both hydrophobic and hydrophilic drugs because it is possible to make w/o nanoemulsions or o/w nanoemulsions. They are non-toxic and non-irritant systems for that reason they can be used for skin or mucous membranes, parenteral and non parenteral administration in general.

Nanoemulsions are sized between 20 and 200nm and they have had a lot of success in cosmetic and pharmaceutical fields due to the size and physicochemical properties. Their performance depends on surface charges and materials of fabrication. They can be associated to polymers and other macromolecules and they are used as nano reactors for the manufacture of other nanostructures.

Nanoemulsions Preparation

Nanoemulsions can be prepared by three methods mainly; all of them need energy to be produced. The main processes to produce nanoemulsions are shown in Fig. **7**.

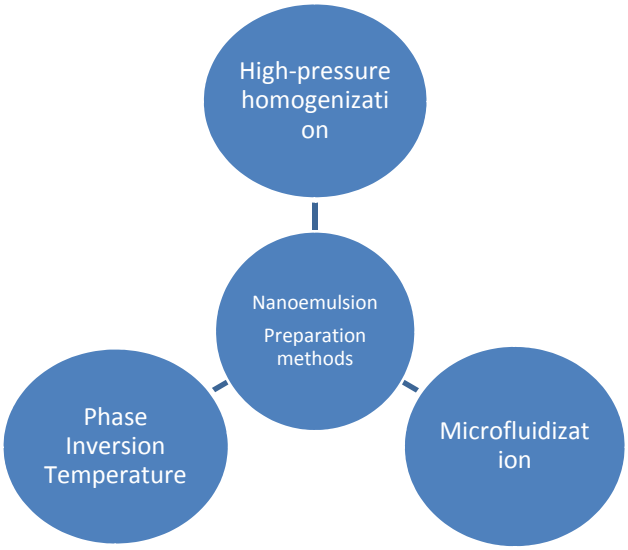

Figure 7: Nanoemulsions: Preparation methods.

The use of nanoemulsions is growing but due to the fact that in some cases they need to be prepared short time before use, complicates the storage for future dosage. Recently, some scientists are trying to produce self-nanoemulsifying systems for decreasing the instability problems [173].

Nanoemulsions in Transdermal Delivery

One promising field for nanoemulsions is in transdermal drug delivery, because due to the droplet sizes and materials nature; make this dosage form ideal for drug transport through the skin, both systemic and local. Transdermal delivery using nanoemulsions has been increasing in the past years, but nowadays it is not as used as nanoparticles delivery or liposomes delivery due to the stability problems inherent to this dosage form. Table **11** is showing some examples of transdermal drug delivery using nanoemulsions.

Table 11: Use of nanoemulsions in transdermal drug delivery.

Transdermal drug delivery using nanoemulsions	References
Gamma Tocopherol	174
Caffeine	175
Plasmid DNA	176
Aspirin	177
Methyl Salicylate	178
Insulin	179
Nimesulide	180

The use of nanoemulsions to deliver analgesics, corticosteroids, anti cancer agents, etc. is very important since these drugs are able to act immediately because they do not need to cross extra barriers; for example, nanoparticles barriers or dendrimers barriers. The drug is bio available easily and faster.

ADVANTAGES AND LIMITATIONS OF USING NANOCARRIERS FOR TRANSDERMAL DRUG DELIVERY

The advantages and limitations of using nanocarriers arise from their peculiar features: their tiny size, their high surface energy, their composition, their architecture, their attached molecules, etc. Comprehensive characterization, analytical evaluation, toxicological and pharmacological assessment will be necessary to determine the efficacy of using these nanostructures in disease therapy and diagnosis. Table **12** shows a summary of advantages and disadvantages for transdermal drug nanocarriers.

Table 12: Advantages and disadvatages of using nanocarriers.

Nanocarrier	Advantages	Disadvantages
Liposomes	Control release based on natural lipids.	When high-pressure homogenization is used decrease stability of high weight molecules.
	High biocompatibility.	Lipid crystallization leads to a lot of polymorphic issues.
	Simple manufacture.	Variable kinetics of distribution processes.
	Protein carriers increase stability of them.	They are susceptible of physical instability. Creaming, flocculation etc.
	High drug loads.	
Dendrimers	Increase stability of therapeutic agents	They have shown cellular toxicity.
	They are easily prepared and functionalized.	Elimination and metabolism could be a problem depending on the generation and materials.
	Increase bioavailability of drugs.	They synthesis costs are higher that other nanocarriers.
	They covalently associate drugs.	Hemolytic effects can be found.
Nanoparticles	They can be made of a lot of biodegradable materials.	Not enough toxicological assessment has been done.
	There are a lot of processes to prepare them.	It is difficult to develop an analytical method for drug delivery.
	They can include antibodies in their surface to reach target organs.	Some processes are difficult to scale up.
	Both hydrophilic and hydrophobic drugs can be loaded in a nanoparticle.	Sometimes, the size they reach is not enough to avoid immune system.
	They are able to avoid immune system due to their size.	
Nanoemulsions	They can be formulated as foams, liquids, creams and sprays.	They are susceptible to Oswald ripening.
	They are non-toxic and non irritant.	Surface charge has a marked effect on stability.
	Easily applied to skin and mucous membranes.	Variable kinetics of distribution processes and clearance.

The advantages of using nanocarriers are evident; first of all, they are able to reach target organs because they can be attached to antibodies, antigens, vitamins and other molecules to be more specific. Nanoparticles also deliver drugs in a controlled manner. Controlled release will be of increasing importance because, using a traditional system, drug delivery is sometimes rapid and plasmatic concentrations can result in toxic effects. Furthermore, a smaller amount of the drug is necessary when using nanoparticles due to the targeted nature of delivery; there are also fewer side effects. Nanoparticles travel largely undetected by the immune system. The immune response depends on both the size of the antigen as well as its composition. By hiding functional groups or protecting these groups with other molecules, immunogenic drugs can be released specifically in the target organ. Nanocarriers can penetrate biological membranes to deliver drugs for specific diseases.

Not all the attempts to use nanocarriers in pharmaceutical fields have been successful. The lack of basic knowledge about nanocarrier behavior makes this approach unpredictable. Future research will be necessary to elucidate the interactions between nanocarriers and other molecules as well as interactions between nanocarriers and biological entities. The toxicology of nanostructures is also a current concern. Materials behave very differently when they are diminished to nanosizes. Traditional laws do not work at this “meso-scale” in the same way as they function at the macro-scale. On the macro scale, bulk properties in a material predominate over surface properties. At the micro-scale, surface properties tend to dominate. At the meso-scale, both types of properties play significant roles [181,182]. It will be interesting to discover how nanoparticles interact with biological entities.

As this field transitions from academia to industry, concerns have surfaced regarding the toxicity of nanostructures [183-185]. Furthermore, the effects of metabolized/altered nanostructures on the biological system are difficult to predict. Nanostructures in wind, water and soil could be dangerous for many species. Regulatory agencies are taking action to assess new Nanotechnology-based products. In addition, the fabrication of nanocarriers at the industrial scale is difficult because scaling up from the lab scale could be difficult. The materials used to make nanocarriers are also very expensive. When drugs go off patent, drug prices drop and do not cover the costs of nanoparticle production.

CONCLUSIONS

In recent years, the main goal of pharmacists has been to target drugs effectively, thereby reducing side effects. Current strategies to accomplish these goals involve the use of novel drug delivery systems based on tiny structures

with long half-lives that deliver their cargo effectively. These structures are called nanocarriers and they were made to avoid immune system rejection and to reach target sites. The routes these nanocarriers follow are very different. Nanocarriers are particularly helpful in penetrating the skin. These delivery systems can deliver both hydrophilic and lipophilic molecules. Advances with regard to materials, fabrication methods and techniques facilitate the development of new and better nanocarriers. Nanocarriers with new features to reach specific organs, tumors, and cells are capable of crossing specific barriers and loading many different types of molecules, viruses, bacteria, etc. The fight against diseases is challenging, but now we have a better arsenal. However, the power of this methodology requires great responsibility and toxicological assessment needs to be done in order to avoid potentially disastrous consequences.

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Glossary

Arteriovenous anastomoses: connection between two blood vessels, resulting in multitude of arteries and veins serving the same volume of tissue.

Capacitor: passive electronic component consisting of a pair of conductors separated by *a* dielectric (insulator).

Cavitation: formation of gaseous cavities in a medium upon ultrasound exposure.

Chemical penetration enhancer: chemical substance that increases transdermal passage of a drug.

Corneocytes: cells located in the epidermis that are packed with fibrous protein called keratin.

Cryo-scanning, transmission and freeze fracture electron microscopy: A combination of three techniques, namely cryo-scanning electron microscopy in combination with cryoplaning, transmission electron microscopy and freeze fracture transmission electron microscopy.

Cuvette: is a small tube of circular or square cross section, sealed at one end, made of plastic, glass, or fused quartz (for UV light) and designed to hold samples for spectroscopic experiments.

Dendrimers: an artificially manufactured or synthesized large molecule comprised of many smaller ones linked together - built up from branched units called monomers**.**

Direct iontophoresis: application of an electrical current of low intensity to favour the passage of drugs from a formulation to the skin.

Electric field*:* is a field of force with field strength equal to the force per unit charge at that point. Basically, it is a field in which a charge experiences a force.

Electromigration: movement of ions caused by the application of an electrical field.

Electroporation: a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field.

Electroporators: appliances which create an electro-magnetic field in the cell solution. The cell suspension is pipetted into a glass or plastic cuvette which has two aluminum electrodes on its sides.

Electroosmosis: transport of neutral species during iontophoresis application due to the solvent movement produced by the small ions electromigration.

Epidermolysis bullosa: rare genetic disorder caused by a mutation in the keratin gene.

Fibroblasts: type of cell that synthesizes the extracellular matrix and collagen.

Hair follicle: narrow tubular cavity from which a hair grows and into which the sebaceous glands open. The follicle is lined by cells derived from the epidermal layer of the skin.

Hemidesmosomes: very small structures on the inner basal surface of keratinocytes in the epidermis of skin. They are similar in form to desmosomes when visualized by electron microscopy. Hemidesmosomes are asymmetrical and are found in epithelial cells connecting the basal face to other cells.

High frequency ultrasound: high intensity focused ultrasound or diagnostic ultrasound (3–10 MHz).

Hollow microneedles: micron sized needles with internal spaces used to store and deliver the drug or vaccine, in a design that is fairly similar to rows of tiny hypodermic needles.

Hypodermic needle: hollow needle commonly used with a syringe to inject substances into the body or extract liquids from the body.

Isoelectric point: is the pH at which a particular molecule or surface carries no net electrical charge**.**

Keratinocytes: the most common type of skin cells. They make keratin, a protein that provides strength to skin, hair, and nails.

Lamina densa: a component of the basement membrane zone between the epidermis and dermis of the skin, and is an electron-dense zone between the amina lucida and dermis, synthesized by the basal cells of the epidermis, and composed of type IV collagen, anchoring fibrils made of type VII collagen, and dermal microfibrils.

Lamina lucida: a component of the basement membrane which is founds between the epithelium and underlying connective tissue. It is a roughly 40 nanometer wide electron-lucent zone between the plasma membrane of the basal cells and the (electron-dense) lamina densa of the basement membrane.

Lipid bilayer mechanics: is the study of the physical material properties of lipid bilayers, classifying bilayer behavior with stress and strain rather than biochemical interactions.

Liposomes: is a tiny bubble (vesicle), made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases.

Low frequency ultrasound: power ultrasound (18-100 KHz).

Medium frequency ultrasound: therapeutic ultrasound (0.7–3 MHz).

Melanocytes: cells located in the epidermis that are responsible for producing melanin, a brown pigment that helps screen against the harmful effects of UV light.

Microneedles: Micron sized needles to penetrate the upper layer of the skin without reaching the dermis.

Milia: small subepidermal keratin cysts most commonly seen around the eyes. They present as 1–2 mm lesions resembling whiteheads.

Nanocapsule: is any nanoparticle that consists of a shell and a space, in which desired substances may be placed.

Nanomedicine: the medical application of nanotechnology and related research.

Nanoparticles: A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm.

Nanotechnology: refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the molecular level in scales smaller than 1 micrometer, normally 1 to 100 nanometers, and the fabrication of devices within that size range.

Pemphigus bullosa: a disease characterized by tense blisters on the skin. The condition is caused by antibodies that accumulate abnormally in a layer of the skin called the basement membrane.

Physical enhancers: Technologies to overcome the skin barrier to facilitate drug transport across the skin.

Proteoglycans: glycoproteins that are heavily glycosylated. They have a core protein with one or more covalently attached glycosaminoglycan chain.

Psoriasis: a genetically determined, autoinflammatory and chronic relapsing skin disorder without involvement of known infectious agents or antigens.

Pyoderma faciale: an inflammatory condition in women between 20–30 age years in the context of emotional stress and not necessarily in association with coexisting acne.

Reactance: Reflects impedance associated with passage of currents through the cell membranes.

Resistance: Measures impedance associated with the passage of current through intra- and extra-cellular fluids.

Reverse iontophoresis: application of an electrical current of low intensity to force the passage of substances from beneath the skin to the skin surface.

Solid microneedles: solid micron sized needles that penetrate transdermal or transmucosal tissues and thus overcome their barrier function.

Sonophoresis: process that exponentially increases the absorption of topical compounds (transdermal delivery) into the epidermis, dermis and skin appendages.

Sound: form of mechanical energy that is propagated from one point to another by the interaction between neighboring oscillating particles.

Ultrasonic: ultrasonic refers to sound waves whose frequency is >20 KHz.

Ultrasound: cyclic sound pressure with *a* frequency greater than the upper limit of human hearing.

Vitronectin: an abundant glycoprotein found in serum the extracellular matrix and promotes cell adhesion and spreading, inhibits the membrane damaging effect of the terminal cytolytic complement pathway, and binds to several serpin serine protease inhibitors.

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