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Editors

Nanocosmetics and Nanomedicines

New Approaches for Skin Care

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Ruy Beck, Silvia Guterres,
and Adriana Pohlmann (Eds.)

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Forward

The interaction of particles with biological systems unravels a series of new mechanisms not found for molecules: altered bio-distribution, chemically reactive interfaces, and the combination of solid-state properties and mobility. The tremendous progress and recent advances of nanotechnology has not been accompanied by sufficient studies of nanomaterial toxicity even though they possess unique, completely new properties. One certainty: the toxicity of nanomaterials can neither be extrapolated from the toxicity of bulk materials nor from the toxicity of their constituents in molecular/ionic form. Therefore the research on nanotoxicity is of extremely high scientific, social, and economic value in particular for human applications. As nanomaterial-based products enter the market, there is an urgent need for related research in order to prevent dramatic consequences of any health-oriented issues caused by nanotechnology-driven products. Indeed, the results of research on nanotoxicity have profound significance because the design of nanomaterials used in industry and consumer products should be based on the outcome of such work. This is more urgent in view of the infancy of these studies and that many of the data available on the biological effects of nanomaterials do not always come from studies that can be considered reliable. The responsibilities are enormous not only in social terms but also this research has multi-billion dollar significance for industry and an even greater value for consumers and health care. Therefore one of key questions is to understand the behavior of nanoparticles in biological systems that will certainly opens up new directions for medical treatments and is essential for the development of safe nanotechnology. The widespread use of untested nanomaterials can cause an enormous cost of care for health problems for the word population. In particular, in Brazil the applications and uses of "bio" stands among the most productive and successful area of the Brazilian "nano" initiative in both academia and industry. Indeed, not only in terms of the number and impact of the papers (more than a half of the Brazilian scientific papers in "nano" are from the nano bio area) but also the numbers of commercial products that are already cases of success. With this in mind Pohlmann, Beck and Guterres have ingeniously collected a series of state of the art contributions on an important and hot area on the use of nanomaterials on the use of nanocosmetics and nanomedicines for skin care. The book NANOCOSMETICS AND NANOMEDICINES: New approaches for skin care will certainly stands as the landmark and part of the classical literature on nanomaterials and skin care.

Jairton Dupont, Tarragona, 30th January 2011.

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Part I

Fundamentals of Skin Delivery

Chapter 1
Transport of Substances and Nanoparticles across the Skin and *in Vitro* Models to Evaluate Skin Permeation and/or Penetration

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Abstract. Nanotechnology can be used to modify the drug permeation/penetration of encapsulated substances, through the manipulation of many different factors, including direct contact with the skin surface and controlled release. In general, nanoparticles cannot cross the skin barrier, which can be explained by the cell cohesion and lipids of the *stratum corneum*, the outermost skin layer. The device most commonly used to study the transport of substances and nanoparticles across the skin is the Franz vertical diffusion cell, followed by the substance quantification in the receptor fluid or determination of the amount retained in the skin. Microscopy techniques have also been applied in skin penetration or permeation experiments. This chapter will present the fundamental considerations regarding the transport of encapsulated substances and/or nanoparticles across the skin, the experimental models applied in these studies and a review of the main studies reported in the literature in order to allow the reader to gain insight into the current knowledge available in this area.

1.1 Introduction

The skin is the largest organ of the human body, presenting a total area of close to 2 m^2. It acts as a barrier between the organism and the external environment [1]. Important skin functions include protection against UV radiation, physical and chemical damage and microbiological attack, maintenance of the body temperature and sensorial functions such as pain and temperature [2].

The skin is mainly composed of two layers (epidermis and dermis) besides the subcutaneous tissue [3]. It is composed of a variety of different cells, being considered more complex than the brain regarding this aspect [1]. The epidermis is composed of several lipids including phospholipids, phosphatidylcholine, cholesterol and triglycerides [4]. The main cell types found in the epidermis are keratinocytes, melanocytes, Langerhans cells, and Merkel cells [3]. The epidermis is

divided into several layers and its outermost layer, the *stratum corneum*, is responsible for the barrier function of the skin due to its lipophilicity and high cohesion between cells [5, 6]. The *stratum corneum* is composed of keratinized corneocytes embedded in lipid bilayers [2]. Ceramides, cholesterol and free fatty acids comprise its extracellular lipid compartment [7]. The dermis is the layer next to the subcutaneous tissue and it is composed of collagen, elastin, glycosaminoglycans, and fibroblasts. This layer is highly vascularized besides containing the appendices (sweat glands and pilosebaceous units) and leucocytes, adipocytes and mast cells [3].

Considering the skin anatomy and physiology, some active substances will not provide the desired activity after their cutaneous administration. Nanotechnology can be used to modify the drug permeation/penetration by controlling the release of active substances and increasing the period of permanence on the skin [8, 9] besides ensuring a direct contact with the *stratum corneum* [10] and skin appendices [11, 12] and protecting the drug against chemical or physical instability [13, 14, 15, 16]. Also, depending on their sizes and structures, some nanostructures can also penetrate across the skin [17]. The aim of this chapter is to discuss the permeation and penetration of nanoencapsulated substances and nanoparticles through the skin. The transport across the skin and the *in vitro* models and membranes used to evaluate the skin permeation and/or penetration are also reviewed.

1.2 Transport across the Skin

Despite the efficient barrier property of the skin, some substances can penetrate across its different layers [18]. The *stratum corneum*, known as a coherent and compact membrane, is the limiting layer for the penetration process, acting as a passive diffusion barrier [2, 19]. The permeability of some substances through the full-thickness of skin is at least 14 times lower than that of the same substances through the dermis [18]. The integrity of the *stratum corneum* and the concentration of the applied drug are important aspects that influence the drug penetration profile [19]. The lipids of the *stratum corneum*, especially ceramides, are important components in terms of its barrier function [2, 20].

There are some passive routes by which a molecule can cross the *stratum corneum* (Fig. 1.1): intercellular (through solubilization in the extracellular lipids arranged into structured bilayers), transcellular (through the corneocytes and the lipid bilayers) and appendageal (through either the sweat glands or hair follicles) [1]. However, the contribution of the latter route is considered small since the skin appendices occupy 0.1% of the skin surface [21]. The sweat glands are not a common pathway for drugs to pass through the skin due to the tortuous pathway and the ascendant sweat. The hair follicles, on the other hand, are common mechanism of transport for some ions, polyfunctional polar compounds and high molecular weight molecules [5, 22]. It has been verified that the hair follicles, despite their smaller superficial area, play an important role in the permeation of nanoencapsulated substances, since they serve as nanoparticle depots [11, 23].

The impermeability of skin can be a drawback when this route is desirable for the delivery of active substances. Only a small percentage of a substance reaches

its target when topically applied [1]. The *stratum corneum* is slightly permeable to both hydrophilic and lipophilic compounds. Thus, the transcellular and intercellular routes are the most important pathways across the skin [19]. The partition coefficient of a substance between the epidermis and its pharmaceutical form is a determining factor in its penetration into the epidermis, which is the determining step of its diffusion rate across the skin [18].

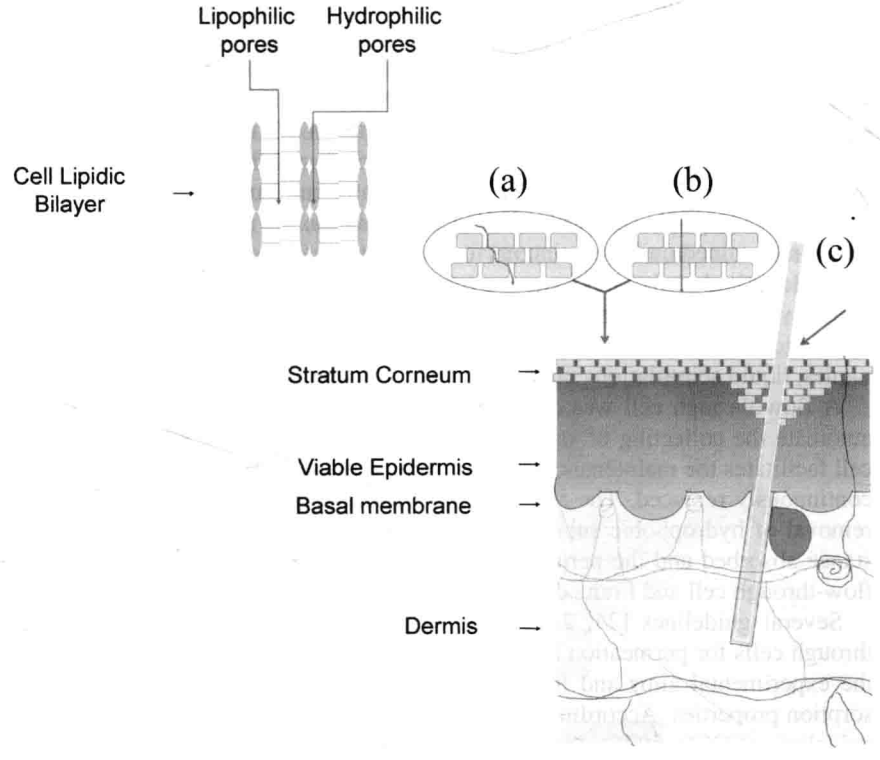

Fig. 1.1 Mechanisms of transport across the skin. (a) intercellular, (b) transcellular and (c) hair-follicles.

1.3 *In Vitro* Models and Membranes Used to Evaluate Skin Permeation and/or Penetration

1.3.1 In Vitro Diffusion Models

The guidelines for dermal absorption of the World Health Organization (WHO) [24] differentiate between the processes of skin permeation and penetration. Penetration is the entry of a substance into a particular layer or structure, whereas permeation is the transport from one layer into a second layer, these being associated with different functions and structures.

The diffusion cells are the systems normally used to evaluate the drug permeation through the skin [21]. There are vertical or horizontal cells, associated with the drug diffusion, and static or flow-through cells, considering the receptor fluid [24]. Thus, it is important to select a model where the transport is limited only by the skin and not by a stagnated diffusion, which can occur particularly in the case of highly lipophilic substances [5].

Franz (1975) [25] developed a static vertical diffusion cell and verified a good relation between the *in vivo* and *in vitro* data for organic substances. The Franz diffusion cell is the most common type of diffusion cell, mainly because of its low cost [2, 5, 26]. Furthermore, it is useful for studying semisolid formulations and is ideal for simulating *in vivo* performance [24]. In the Franz technique, a membrane (skin) is placed between the diffusion compartments of the cell. The dermis, when present, is turned toward the receptor compartment, which is usually filled with an isotonic saline solution or phosphate buffer containing surfactants or cosolvents in the case of lipophilic substances in order to maintain the sink conditions. The system is maintained under continuous magnetic stirring (Fig. 1.2) [21, 26]. The sample is deposited in the donor compartment on the epidermis. The diffusion rate of the active substance to the receptor compartment is determined by an appropriate and validated analytical method, such as chromatographic or spectroscopic techniques, liquid scintillation counting or other suitable methods [27, 28].

A flow-through cell was developed by Bronaugh and Steward (1985) [29] to automate the collecting of samples from a two-compartment cell. Moreover, this cell facilitates the maintenance of the system viability because the receptor fluid is continuously replaced. The flow-through cell mimics the blood flow through the removal of hydrophobic substances from the skin. However, the amount of substance absorbed and the period necessary for its removal are similar for both the flow-through cell and Franz diffusion cell [26].

Several guidelines [24, 27] recommend static Franz diffusion cells or flow-through cells for permeation studies. The choice of the cell type must be based on the experimental aims and the substance characteristics, such as theoretical absorption properties. According to the Scientific Committee on Consumer Products guidelines (SCCP, 2006), the static diffusion cell presents the advantage of easy quantification, since the receptor fluid is not replaced continuously, only when the sampling takes place. Recently, the development of equipment based on static permeation cells has presented the possibility of automatic sampling from the receptor fluid, which facilitates the collection and replacement of the medium without the formation of air bubbles.

Horizontal diffusion cells, which are also called side-by-side cells, are less common. Here, the cell also comprises two compartments, and the donor and the receptor (usually containing equal volumes) are separated by a membrane [30]. This kind of system is useful for studying mechanisms of diffusion through the skin, such as permeation from one stirred solution into another stirred solution through a membrane [24].

Besides the assay of the active substance in the receptor compartment, the amount of the substance retained on the skin or penetrated into different skin layers is also commonly evaluated using diffusion cells. In order to determine this

profile, the skin is cleaned after the permeation experiment to remove the excess of formulation. The *stratum corneum* layers are usually removed by the tape stripping technique where adhesive tapes are placed on the skin surface and the layers are taken by applying constant pressure [21, 31]. In addition, the epidermis and dermis can be separated by heat and force [32, 33]. The amount of substance remaining in each layer is extracted using an appropriate solvent and assayed by a validated analytical method.

Studies on the penetration of active substances into different skin layers can also be performed using the alternative Saarbrücken diffusion model [9, 34, 35]. In this system, the skin is placed onto a filter paper wetted with Ringer solution, which is placed into the hole of a Teflon block. The Teflon block with the sample is fitted into the cavity of a Teflon punch and applied to the skin surface. A standard weight is placed on the top of the punch for 2 min to increase the contact between the skin and the formulation. The gap between the two Teflon pieces is then sealed to avoid the loss of water from the skin and the system is placed into a plastic box at 32 ± 1°C. The skin is removed at different time intervals and tape stripping is carried out. After this procedure, the skin is separated into parallel sections using a cryomicrotome and extraction techniques are performed for the quantification of the drug [36].

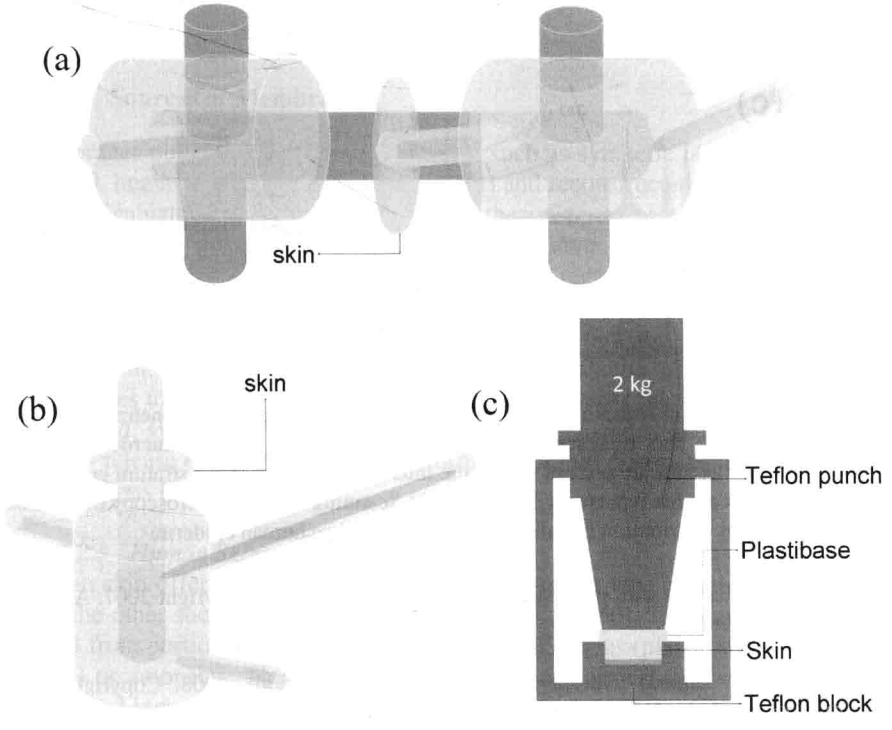

Fig. 1.2 (a) Horizontal diffusion cell, (b) Vertical diffusion cell and (c) Saarbrucken model.

1.3.2 Microscopy Techniques

Microscopy techniques are interesting tools to visualize the location of the particles or of the active ingredient in the skin tissue after *in vitro* or *in vivo* skin permeation or penetration studies. These studies provide information on the penetration pathway or permeation route of substances or particles across the skin [17, 37, 38]. Scanning Electron Microscopy (SEM), Confocal Laser Scanning Microscopy (CLSM), Transmission Electron Microscopy (TEM) and Scanning Transmission Ion Microscopy (STIM) are the microscopic techniques most commonly employed in this type of study [38, 39, 40, 41]. Figure 1.3 shows microscopy images obtained using these different techniques.

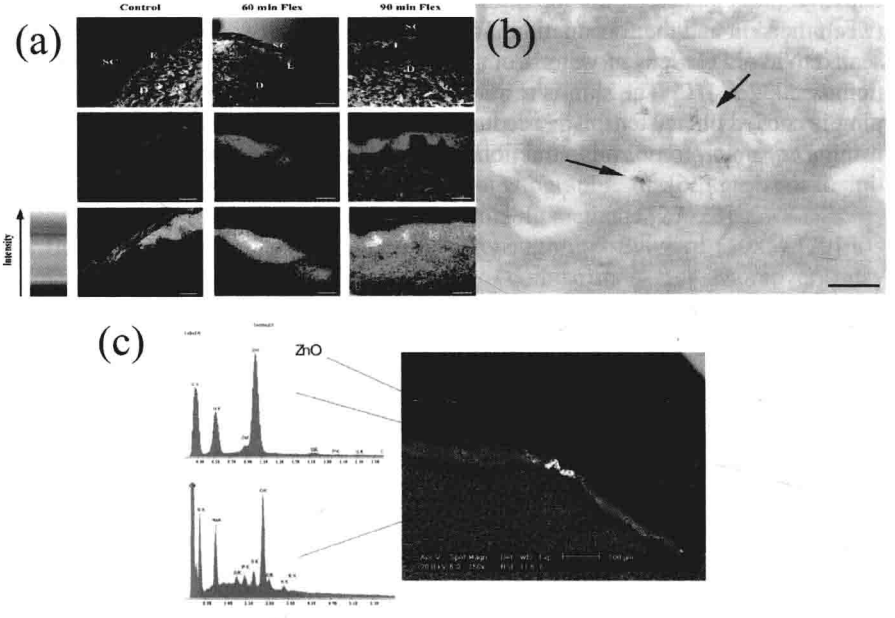

Fig. 1.3 (a)* Confocal scanning microscopy images showing fullerene penetration through the skin. All scale bars represent 50 μm. (b)* Transmission electron microscopy image showing that fullerenes are present within the intercellular space of the stratum granulosum cell layer. The scale bar represents 300 nm. (c)** Scanning electron microscopy of Zinc oxide nanoparticles distribution into different areas of *in vitro* human epidermis.

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1.3.3 Membranes

1.3.3.1 Types of Membranes

Since the skin is an organ with multiple layers, it can be used during *in vitro* skin permeation/penetration studies with its two basic layers, epidermis and dermis (*full-thickness skin*) or it can be submitted to treatments that separate its layers supplying different types of membranes (*split-thickness skin*) to be used for *in vitro* studies [26, 42, 43]. The choice of the membrane is related to the focus of the study (permeation or penetration). In the *full-thickness skin* membranes only the subcutaneous tissue is removed [26, 42, 43]. On the other hand, *split-thickness skin* can be obtained in several ways [26, 42, 43] and the isolation of one specific layer of a skin sample with a controlled thickness can be made using heat and force (*heat-separated epidermis*), dermatome (*dermatomed skin*) or enzymatic processes (*trypsin-isolated stratum corneum*) [44, 45]. Epidermis and *stratum corneum* membranes are more fragile and some mass balance techniques, such as tape stripping, cannot be applied [27]. Divergences can be found in the literature concerning the effect that these processes can have on the skin characteristics. Some studies have demonstrated that the skin barrier is reestablished with the hydration of these membranes [46, 47]. On the other hand, some reports have claimed that the viability of the skin and the flow through the epidermis can be disturbed by these processes [26, 48, 49].

1.3.3.2 Sources of Membranes

Membranes obtained from diverse sources, such as synthetic [43, 50, 51], vegetable [52], human [53, 33, 43], animal [43, 54] and reconstructed tissues [43, 45, 47] can be evaluated as candidates to predict the transport of substances across the skin and used during *in vitro* experiments. The advantages and disadvantages of these membrane models need to be investigated regarding their effectiveness to determine the transport of substances across the skin [26]. There is no consensus regarding the standardization of these sources in this type of study. In general, only human, animal or reconstituted skin models should be used as sources of membranes to evaluate the transport of substances across the skin [26, 27]. On the other hand, synthetic membranes are usually recommended and used to study the *in vitro* release of substances from semisolid or liquid pharmaceutical or cosmetic formulations [43, 55, 56, 57].

1.3.3.2.1 Human Skin

Human skin offers a greater possibility for *in vitro-in vivo* correlations compared with the other sources of membranes [33, 43, 58, 59, 60]. The samples can be obtained from portions remaining from surgical procedures (plastic surgery or amputation) or autopsies. The most commonly used anatomical regions are abdomen, breast and legs. In the case of skin membranes from a human source, *dermatomed skin* (200-500 μm) is recommended [27]. To minimize the variability in the results due to differences in the permeation properties of different anatomical regions and volunteers, the anatomical region as well as the sex, race and age of the donors

should be standardized [90, 92]. However, the availability of human skin sources is limited due to the need to acquire the consent of the volunteer [26].

1.3.3.2.2 Animal Skin

Skin samples obtained from animals allow easily obtainment and standardization of the anatomical region, sex and age of the animal. Thus, the skin of several animal species is frequently used as an alternative to human skin to evaluate the *in vitro* transport of substances [43]. The use of monkey, rodent, pig and snake skin is reported in the literature.

In the evolutionary chain, monkeys represent the species closest to humans. Comparative *in vitro-in vivo* studies demonstrate that the transport of substances across monkey skin is similar to that across human skin [62, 63, 64]. However, few studies have been carried out using monkey skin for ethical reasons.

Studies using rodent skin as a membrane model are more common for the evaluation of the *in vitro* transport of substances. As sources of membranes, the use of rat, mouse, guinea pig and rabbit skin has been reported. Although it is known that rat skin is around 10 times more permeable than the human skin [27, 64, 65] its use for *in vitro* experiments is still common in toxicity studies, making *in vitro-in vivo* correlations possible [64, 66]. Furthermore, a correlation between the skin diffusion coefficients for nortriptyline was observed on comparing Wistar rat skin and human *heat-separated epidermis* [67]. Considering mouse skin, only *full-thickness skin* membranes should be used since this type of skin presents a very low thickness [26].

Snake skin has been studied and recommended as a model for the *stratum corneum* membrane to evaluate *in vitro* penetration [68, 69]. The *stratum corneum* membrane is commonly employed to verify the barrier function of the skin, considering it as the main obstacle in the skin penetration of substances [45, 47].

Anatomically, pig skin presents the greatest similarity to human skin [43]. Thus, it is frequently used as a membrane to evaluate the transport of substances through the skin in *in vi*tro studies [43]. Generally, the samples come from the ears or from the abdominal or dorsal regions. Some particularities, such as size and density of hair follicles and the *stratum corneum* thickness, are indicated as reason for divergences in the results obtained from pig and human skin [34]. The evaluation of the relative permeability of some drugs through membranes obtained from different sources suggests differences in the following order: human < pig < rat < rabbit < mouse [70].

1.3.3.2.3 Regenerated Skin

The extrapolation of results obtained from skin permeation/penetration experiments carried out with animal membranes to the human skin is always questionable given the influence of the inter-species variability. Moreover, there are ethical questions regarding the use of animal skin [71].

The Organization for Economic Cooperation and Development has affirmed that the regenerated human skin models can be used to evaluate the *in vitro* transport of substances across the skin if there is equivalence [72]. However, this use requires validation of its applicability, which is still under evaluation [73]. The models of regenerated human skin commercially available are: Apligraf®, Epiderm®, SkinEthic® and EpiSkin®. Apligraf®/Graftskin® (Organogénese Incorporation) [74, 75]