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Review

The structure and function of the stratum corneum

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ABSTRACT

Over the past 150 years the skin's structure and function has been the subject of much investigation by scientists. The stratum corneum (SC), the skin's outermost layer and interface with the outside world is now well recognized as the barrier that prevents unwanted materials from entering, and excessive loss of water from exiting the body. This review summarizes the major advances in our understanding of this formidable membrane. The structure of the SC is outlined as well as techniques to visualize the barrier. The lipid organization and ionic gradients, as well as the metabolic responses and underlying cellular signalling that lead to barrier repair and homeostasis are discussed. Finally, a brief overview of the molecular and genetic factors that determine the development of a competent permeability barrier is provided.

Contents

1. Introduction and historical background

The multilamellar structure of skin has been of interest to scientists since the 19th century. The early work of Homalle (1853) followed by Duriau (1856) recognized that skin layers, and specifically the epidermis and dermis, had different degrees of permeability. In a series of studies from 1924 to 1929, Hermann Rein was the first to demonstrate the presence of a barrier between the stratum corneum (SC) and viable epidermis based on the physiological behaviour of isolated human skin (Rein, 1924, 1925, 1926, 1929). The next 20 years flourished with studies that provided more definition to the barrier properties of skin, some of which were conflicting or even incorrect (Hediger, 1928; Miescher, 1931; Rothman, 1934; Wolf, 1939; Miescher, 1941). Wolf (1939), and Winsor and Burch (1944) confirmed that tape stripping, sand papering or chemical insult to the SC essentially removes skin barrier function and the later seminal work of Blank (1953) proved conclusively that the barrier function lay in the SC.

Over the recent decades, new methods of visualization (microscopy based on light, electrons, laser scanning confocal, scanning electrochemical micro laser and vibrating probe techniques) and physical characterization (infrared spectroscopy, thermal and X-ray diffraction techniques) became available to scientists. These found many applications in studying the barrier property of proteins, lipids and water found in various cellular biomembranes (Potts et al., 1985; Golden et al., 1986; Turner and Nonato, 1997). With reference to skin, these techniques allowed the detailed characterization of the membrane's structure and permeability with implications for the fields of drug delivery as well as toxicology. Importantly, candidate selection for passive transdermal and topical formulations, as well as an understanding of the various approaches to develop (trans)dermal delivery systems was

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also advanced by these investigations (Scheuplein, 1967; Katz and Poulsen, 1971; Michaels et al., 1975; Albery and Hadgraft, 1979; Machado et al., 2010).

In the past 20 years new paradigms on skin structure and its biochemistry, particularly the stratum corneum and viable epidermis have been elucidated. This has lead to a better understanding of skin permeability, barrier homeostasis, as well as the finer details of the two compartment model of the stratum corneum. Newer and diverse visualization tools ranging from devices to fluorescent dyes and tracers have helped reach the current state of knowledge of the stratum corneum. At the biochemical level, an enormous amount of data on the importance of lipids, especially the ceramides (Long et al., 1985; Wertz et al., 1985; Downing et al., 1987) has been generated. We now understand that these molecules are not just responsible for formation of the compact intercellular lamellae, the primary basis of the permeability barrier, but that they also display diverse signalling functions necessary for cell proliferation and programmed cell death (Uchida et al., 2000; Holleran et al., 2006). The molecular investigations have been complemented by the application of spectroscopic, X-ray diffraction and other related techniques. Interrogation of the lipids in relation to hydration or temperature dependent changes in phase behaviour (Thewalt et al., 1992; Fenske et al., 1994; Kitson et al., 1994; Bouwstra et al., 1996, 1997, 1999) has provided much insight into their critical functions. Although impossible to review all these facets of barrier research in one article, most of the above mentioned aspects will be discussed further; albeit briefly, in the next sections.

2. Structure of the stratum corneum

The stratum corneum has an elegantly simple two-compartment structural organization at the light microscopic level, with the corneocytes embedded in a lipid matrix, as visualized by frozen sections, swollen in alkaline buffer and stained with a dye (Christophers and Kligman, 1964), or when stained with Nile red, a fluorescent lipid stain (Simonetti et al., 1995). This prompted its comparison to a "brick and mortar system" (Fig. 1) originally described by Michaels et al. (1975). At the ultrastructural level, both the bricks and the mortar components of the paper-thin SC have incredible structural and functional complexity, metabolic adaptations and ability for autopoesis (self-maintenance by constant renewal), and several attributes of a smart material (Menon and Elias, 2001) which will be expanded on further in a later section.

The main structural components of this composite system may be represented by a cartoon (Fig. 2).

1) *The corneocytes*: stacked up to 18–20 layers depending on the anatomic location in the body; these provide the physical barrier.
2) *Corneodesmosomes*: functioning as "spot weldings" or "rivets" to hold the corneocytes together. Desmosomes are programmed to go through a gradual degradation process so as to enable the orderly desquamation of outermost, worn-out corneocytes.
3) *The mortar lipids filling the tortuous pathway between the stacked corneocytes*: a highly complex mixture of about 13 species of ceramides, cholesterol, and free fatty acids in an equimolar ratio; these provide the permeability barrier.
4) *A battery of lipolytic and proteolytic enzymes*: involved in the processing of pro-barrier lipids and degradation of desmosomes, respectively, they contribute to ongoing biochemical activities in the stratum corneum, which was once thought to be inert and dead.
5) *The secreted contents of epidermal lamellar bodies at the interface of stratum corneum and the stratum granulosum*: these are the pro-barrier lipids which give rise to the multiple lipid lamellae of the SC and which are interspersed with the enzymes and antimicrobial peptides.

All these components are crucial to the stratum corneum barrier, which is viewed as a challenge to transdermal drug delivery. Interfering with, or altering the functional properties of any one of these components can weaken the barrier.

3. Visualization tools to study the SC

The major visualization techniques to study the SC to date may be classified as follows:

a) *Optical microscopy*. Conventional light and fluorescence microscopy confirmed the basic concept of lipids being sequestered into the extracellular spaces surrounding corneocytes, as well as the progressive change in the profile of lipids from the base to the top of the stratum corneum (Brody, 1989; Veiro and Cummins, 1994). Laser confocal and two photon microscopy used in conjunction with a vast array of newer fluorescent tags and dyes (such as marketed by Molecular Probes®) have revealed ion fluxes and pH changes with great accuracy (Prausnitz et al., 1996; Hanson et al., 2002).
b) *Chemical microscopy and physical imaging techniques*. Polarized light microscopy, acoustic microscopy, vibrational (infra red and Raman) microscopy and photon tunnelling (Dines et al., 1984; Groh et al., 1992; Lieberman et al., 1996; Zhang et al., 2007) have all contributed to the understanding of corneocyte structures, defining the biophysical and molecular properties of lipids and the effects of temperature and absorption promoters on barrier lipid behaviour. Stimulated Raman scattering (SRS) microscopy (Freudiger et al., 2008) is an exciting, label-free technique that is currently being fine-tuned for imaging sebaceous as well as SC

Fig. 1. Brick and mortar structure of the stratum corneum: corneocytes as bricks, and intercellular lipids (arrowheads) as mortar.

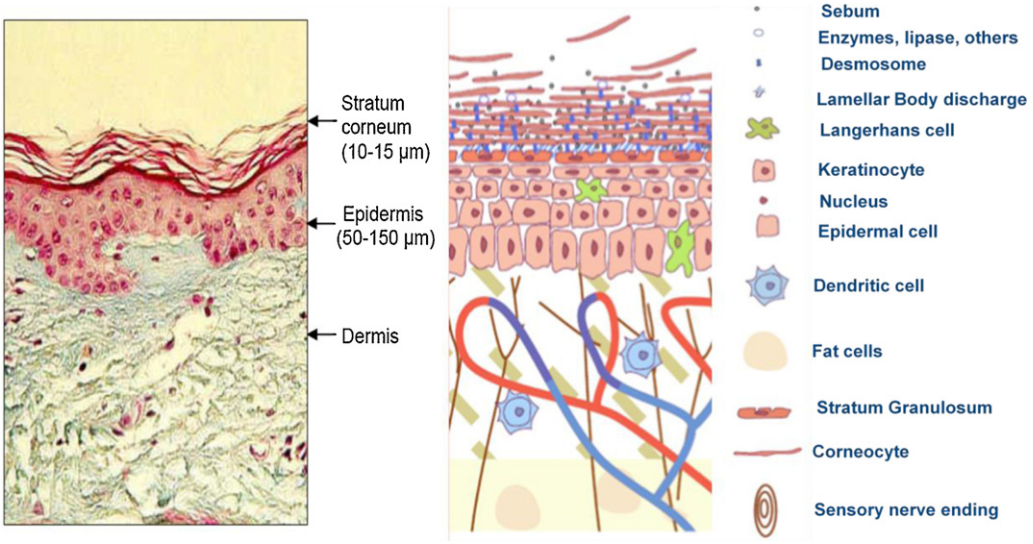

Fig. 2. Structure of the skin.

(Courtesy of Sean Cleary)

lipids, besides solvents (DMSO) or drugs (retinol) that permeate the SC.

c) *Electron microscopy (EM)*. Transmission electron microscopy (TEM) is possibly the singularly most important visualization tool that shaped the current understanding of skin barrier lipid organization, its alterations by chemical and physical penetration enhancers, and the structural basis of a 'pore-pathway' in the stratum corneum (Menon & Elias, 1997) which has been much debated in the past (Flynn, 1989).

Along with conventional TEM (with OsO_4 and RuO_4 post-fixation to visualize SC lipids), techniques which employ special ion capture, cytochemical imaging of ion gradients, freeze fracture, immuno EM for specific, subcellular localization of proteins, cryo EM for circumventing fixation artefacts in fine structure, cryoelectron diffraction for local variations in lipid organization, have been applied to the study of the SC (Pfeiffer et al., 2000; Vielhaber et al., 2001; Menon, 2002; Norlén et al., 2003). These have greatly advanced our understanding of, not only the SC, but also have allowed tracking of the tracers and drugs delivered via passive or active trans-dermal systems. Scanning electron microscopy (SEM), especially environmental SEM or variable pressure SEM which do not require dehydration or coating of the samples have been particularly instructive in this regard.

d) In vivo confocal reflectance microscopy and confocal Raman microscopy, as well as several non-invasive techniques for monitoring the pH, TEWL, hydration, elastic module, sebum secretion, and blood flow have enhanced our ability not only to monitor drug delivery, but also to monitor the local effects of such treatments, including the process of barrier recovery locally (Fluhr et al., 2000; Caspers et al., 2001; Sauermann et al., 2002). Thus we now understand not only the chemical and biophysical properties of SC, but also we may define where in the SC specific microdomains are located (pools of water, pH microdomains, permeated molecules, depots of drugs within the SC).

4. Current understanding of skin barrier function

Skin provides barriers to diverse physical and chemical stressors that it encounters in the environment, including xenobiotics originating from industrial, agricultural and recreational activities. As skin is not just the limiting boundary layer; but also our interface with the habitat, it functions as a dynamic feature rather than a fixed, inflexible barrier layer, as several recent reviews and books have emphasized (Elias and Feingold, 2006; Tobin, 2006; Menon and Kligman, 2009). Its fine structural organization as a composite material also has changed the view of SC as a uniform "membrane" once prevalent in the Pharma industry.

Corneocytes. Although it had been established earlier that the permeability barrier was located in the SC, in the 1970s the membrane was regarded as an inert "basket weave" of terminally differentiated flattened and enucleated cells filled with keratin and bounded by a cornified envelope. A series of investigations in the next decade, employing ultrastructural methods dispelled the notion of the SC as a passive occluding membrane of the epidermal machinery below. These studies, reviewed by Elias (1991), unravelled the true nature of the SC organization (Fig. 3) and the formation of the barrier by secretion of epidermal lamellar bodies, enriched in cholesterol, sphingolipids, fatty acids and a battery of enzymes at the stratum corneum–stratum granulosum interface. The significance of the, highly cross-linked cornified envelope, and the role of filaggrin protein inside the corneocyte in forming a proper "scaffold" for the mortar lipids has also come to light in more

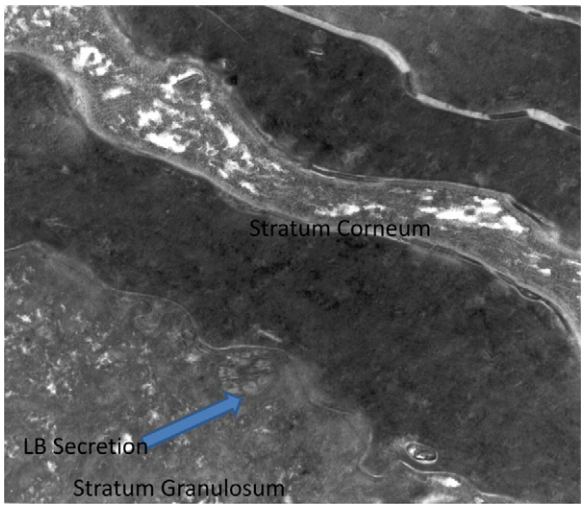

Fig. 3. Electron microscopy image of the stratum corneum–stratum granulosum interface.

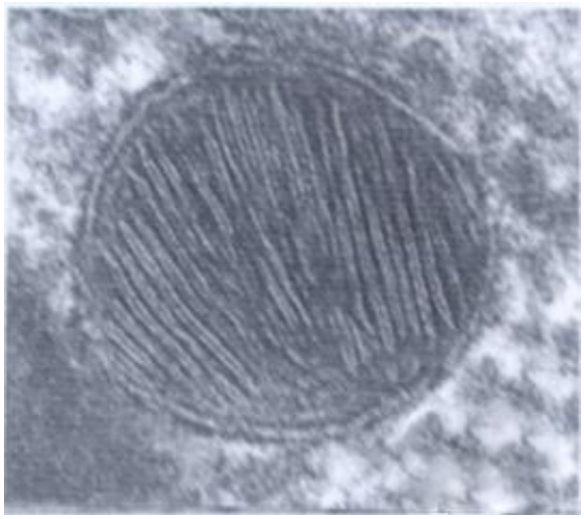

Fig. 4. Epidermal lamellar body from murine epidermis: OsO_4 post fixation.

recent investigations (Presland et al., 2001, 2004). Filaggrin mutations are now known to underlie several disorders of cornification and atopic dermatitis (Sandilands et al., 2009).

SC homeostasis. Stratum corneum maintains its homeostasis, that is, renewal and replacement of exfoliated cell layers by a series of well balanced events. These include epidermal proliferation, and progressive differentiation involving synthesis of lipid enriched lamellar bodies (LB), secretory organelles ranging from 0.2 to 0.5 μm in diameter (Fig. 4). Synthesis and sequestration of keratin protein within the corneocytes leads to the formation of the thickened corneocyte membrane. The secretion of LB before the cornification process allows the lipids to surround each corneocyte having been sequestered in the extracellular space of the stratum corneum. Thus, this tissue is characterized by the "brick-and-mortar" organization where the corneocytes forming the 'bricks' and extracellular lipids can be represented as the "mortar".

Lamellar bodies (LB). Synthesis of LBs begins at the suprabasal epidermal layer, and culminates in the uppermost cells of the stratum granulosum, where they occupy about 20% of the cell volume. They are polarized to the apical border of the uppermost SG cell (subjacent to the SC), and secreted contents of LBs occupy the interface between these two layers (Fig. 4). The use of water soluble tracers such as colloidal lanthanum showed that both the efflux and influx of water across the SC is blocked at the site where the LBs are secreted, i.e. the SG–SC junction (Elias and Brown, 1978; Landmann, 1988). Freeze fracture studies and ruthenium tetroxide post-fixation techniques (Madison et al., 1987; Hou et al., 1991) helped elucidate the sequence of events that follow the secretion of epidermal lamellar bodies involving the fusion of disk like contents of the LBs, and their progressive conversion to tight lamellar sheets occluding the space between the adjacent corneocytes (and the corneodesmosomes that hold them together). Cytochemical localization, as well as experimental studies with various enzyme inhibitors further delineated the role of enzymes (lipases, phospholipase, sphingomyelinase, B glucocerebrosidase, cholesterol sulphatase) in conversion of these disks into the compact lamellar structures that surround the corneocytes (Elias et al., 1984; Menon et al., 1986; Holleran et al., 1991, 1993; Man et al., 1993; Madison et al., 1998; Madison, 2003). A recent proteomics characterization of the LB identified over 980 proteins which is likely to be an incomplete characterization of the contents (Raymond et al., 2008).

Lipids. Investigations using animal models with specific gene defects or knock-outs further extended the knowledge of enzymes such as B glucocerebrosidase (Holleran et al., 1994), stearoyl CoA desaturase (Miyazaki et al., 2005), and Elovl4, which is involved in elongation of long chain fatty acids, necessary for ceramide formation (Cameron et al., 2007; Li et al., 2007). Ceramides are uniquely significant in the formation of the covalently bound lipid envelope of corneocytes (Behne et al., 2000; Zheng et al., 2011). Ceramide 1 consists of sphingosine and long chain unsaturated, mono-and di-unsaturated omega hydroxy acids. The other major lipid components of the SC are cholesterol and fatty acids. Cholesterol amounts to 25% by weight or 30 mol% of the SC lipids and is crucial for promoting the intermixing of different lipid species Free fatty acids account for about 10% of SC lipids or 15 mol%, and consist predominantly of long chain saturated fatty acids having more than 20 carbon atoms. Oleic acid (6%) and linoleic acid (2%) are the only unsaturated fatty acids detected unbound in the SC. Deficiencies in any one of these three lipid species result in barrier abnormalities characterized by increased trans-epidermal water loss (TEWL) as well as observable alterations in the ultrastructural features of the SC extracellular domains (Holleran et al., 2006).

Enzymes. Proteolytic enzymes secreted via LBs lead to progressive dissolution of the corneodesmosomes thus allowing the orderly desquamation of corneocytes. The degradation of corneodesmosomes leaves 'gaps' or 'lacunae' within the multiple lamellar barrier structures, which play an important role in transdermal permeation, which will be discussed further by the authors in a future review. More recently, anti-microbial peptides such as beta defensins and cathelicidins (Oren et al., 2003) were co-localized to the lamellar bodies and found within the extracellular matrix of SC thus, linking the formation of a permeability barrier with an anti-microbial barrier (Braff et al., 2005). This innate immunity, which further underlies the skin's ability for repairing itself, is crucial for survival. The application of simple emollient formulations has been shown to alter desquamatory and inflammatory enzyme activity in the SC (Mohammed et al., 2011).

It is also important to note that the SC is intimately linked with the stratum granulosum both structurally and functionally. Structurally, the two are held together by transitional desmosomes; functionally, the paracellular barrier formed by tight junctional complexes located to the SG, also supplements the permeability barrier.

5. Genes that regulate skin barrier formation

In this era of molecular biology, there has been a huge interest in identifying the genes that control development in general, and skin differentiation in particular, with a view to understanding the skin diseases and dysfunctions that are a consequence of a defective permeability barrier. Knock-out mouse models are one of the most convenient ways to study the effects of single gene deletions, loss of function or gain of function mutations on the skin barrier. The newborn animals are easily tested for the skin's ability to exclude the penetration of a dye (the dye exclusion test) that can show if there is a complete absence of barrier, or if there are site specific barrier defects. The latter is also valuable to evaluate the progressive pattern of skin maturation in normal controls. The list of such genes and publications is rather extensive, and it is not the intent of the present authors to provide a review in its entirety. For the sake of convenience and brevity, we have grouped these into: upstream genes that direct development through transcription factors; genes specific for structural proteins of skin; genes for functional enzymes such as proteases (leading to desquamation of corneocytes) and their inhibitors, enzymes involved in lipid metabolism (synthesis and processing of SC lipids); genes for various ion channels (Aqp, TRVP) and receptors (RARs, ARNt, etc.).

Upstream genes. The Grainy head-like 3 (Grhl3) gene encodes a transcription factor which has crucial roles in epidermal morphogenesis during embryonic development, with deficient mice exhibiting failed skin barrier formation (Boglev et al., 2011). A complex of three amino acid loop extension (TALE) transcription factors PBX1, PBX2 and Pknox preferentially regulate Late Cornified Envelope (LCE) protein genes, and thereby the barrier development (Jackson et al., 2011). *Taf10* is a transcription factor required for the establishment of skin barrier function in embryonic, but not in adult mouse epidermis (Indra et al., 2005).

Genes for structural proteins. The most well known or well characterized is the loss-of-function mutation in the FLG gene (coding for profilaggrin), leading to ichthyosis vulgaris and atopic dermatitis. Decreased levels of natural moisturising factor (derived from filaggrin) lead to reduced water holding properties of SC, and a defective corneocyte envelope (CE) as well as an increased pH of SC (Sandilands et al., 2009). Keratin 5 knockout mice show neonatal lethality, owing to the fundamental role of Keratin 5 in skin integrity (Peters et al., 2001). On the other hand, knock-out of the loricrin gene does not have such a major impact (Jarnik et al., 2002).

Knockout mice models for desmoglein 3, desmocollin 1, desmoplakin, or DSG-1 (a component of corneodesmosomes) affect the structural integrity of SC, and hence the barrier functions of skin. In the nucleated layers of epidermis, the loss or down-regulation of tight junction proteins claudin 1 and claudin 23 also affect the permeability barrier adversely (De Benedetto et al., 2011).

Genes for enzymes. Knockout mice models for steroyl-coA desaturase2 (Miyazaki et al., 2005) and ELOV 14 (Cameron et al., 2007) have barrier deficiencies because of defects in synthesis of lipids and very long chain fatty acids respectively. In addition, loss or mutation in the beta glucocerebrosidase gene interferes with the processing of the secreted contents of lamellar bodies (Holleran et al., 2006), leading to a dysfunctional barrier, as exemplified by Gaucher's disease.

Protease inhibitors also have a role in regulating the orderly desquamation of the corneocytes, by preventing premature activation of proteases within the SC. Studies on SPINK 5(−/−) mice showed that LEKTI deficiency causes increased breakdown of DSG-1 and corneodesmosin, due to elevated activity of Kallikreins 5 and 7 (Descargues et al., 2005).

Genes for various ion channels and receptors. Aquaporin 3 (aquaglyceroporin) deficient mice showed reduced hydration of SC, as well as delayed barrier recovery, features which are corrected by topical application of glycerol (Hara and Verkman, 2003). Epithelial sodium channel (ENaC) while not required for the generation of the epidermal barrier, is needed in the maintenance of skin barrier function after birth, as shown by knockout mice studies (Charles et al., 2008). Genes encoding for the TRPV 3 channel may be working through transglutaminase or calcium in affecting the permeability barrier (Cheng et al., 2010).

The above account is by no means a complete list, but is a representative profile of the diverse set of genes that impact on the skin barrier formation or its maintenance.

6. Barrier repair—skin as an actively smart tissue

As the skin barrier is constantly exposed to changing environmental stressors, the SC and underlying epidermis must constantly be sensing, and responding to diverse stimuli, a fact not readily apparent from the static images one sees in a histological slide. The wound healing response of the epidermis, is perhaps one of the most visible manifestations of this adaptation, along with changes in pigmentation that accompany UV exposure. The invisible adaptations in the barrier are measured by non-invasive techniques (measurements of TEWL and hydration level, pH measurements, etc.), which have been used in conjunction with several microscopic visualization techniques and biochemical and biophysical measurements of enzymes that regulate lipogenesis, crucial for maintaining the permeability barrier, or more specifically; repairing the barrier following experimental disruption. Such sensing as well as repair and maintenance of the barrier is mediated via several signalling pathways in the epidermis.

SC and epidermal responses and signals. This has been one of the most intensely studied aspects of the permeability barrier in the last 20 years. Experimental studies using animal models showed that the uppermost cells of SG respond to barrier disruption by (i) an immediate secretion of nascent LBs, (ii) synthesis of new LBs and (iii) further secretion of the newly synthesized LBs, before their terminal differentiation (Menon et al., 1992a, b; Menon, 2002). An elaborate and responsive network in place within the epidermis, including ions (calcium, potassium, sodium), ionic channels, cytokines (IL 1, TNF, TGF beta), structural (caveolae, nuclear receptors) initiates and accelerates the multi-faceted, cellular and metabolic aspects of barrier repair which has been reviewed in detail by Elias and Feingold (2006).

Signal repair interference and inhibition. Treatments (including occlusive coverings), that interfere or inhibit some of these signals delay the barrier repair process. Besides, inhibitors of enzymes such as HMG-CO A reductase, serine-palmitoyl transferase (SPT), fatty acid synthase that block the synthesis of any of the three important barrier lipid species as well as exposure to adverse environmental conditions can also delay the barrier repair. This has led to the suggestion of the possibility of developing metabolic inhibitors which would allow a "window" for (trans)dermal delivery (Tsai et al., 1996). The major challenge for the industry is extrapolating from various skin models used for drug permeation for human applications (SC membranes, cadaver skin, skin equivalents) as well as from the animal data; the former may have inherent flaws or lack the repair response altogether and in the case of the latter the SC may differ significantly from human tissue (Menon, 2002).

7. Conclusions and outlook

Advances in our understanding of the skin and primary barrier component, the SC, have been reviewed. The major visualization tools which have lead to our current knowledge are highlighted and the essential role of lipids has also been considered. Regulation of skin barrier formation and response to experimental perturbation underlines the dynamic nature of the SC. The major challenge for the industry is extrapolating from various skin models used for human applications (SC membranes, cadaver skin, skin equivalents) as well as from animal data; the former may have inherent flaws or lack the repair response altogether and in the case of the latter the SC may differ significantly from human tissue. Aside from passive and active skin devices such as transdermal patches, lasers, iontophoresis and microneedles, we now see many new skin related applications that heretofore were not possible in earlier times. Looking to the future, stem cells and genes will provide new methods to develop skin substitutes for management of diabetic skin ulcers, wound healing, facial transplants, scar reduction, skin cell regeneration and therapy, induced pluripotent stem cells as well as gene based skin therapies.

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