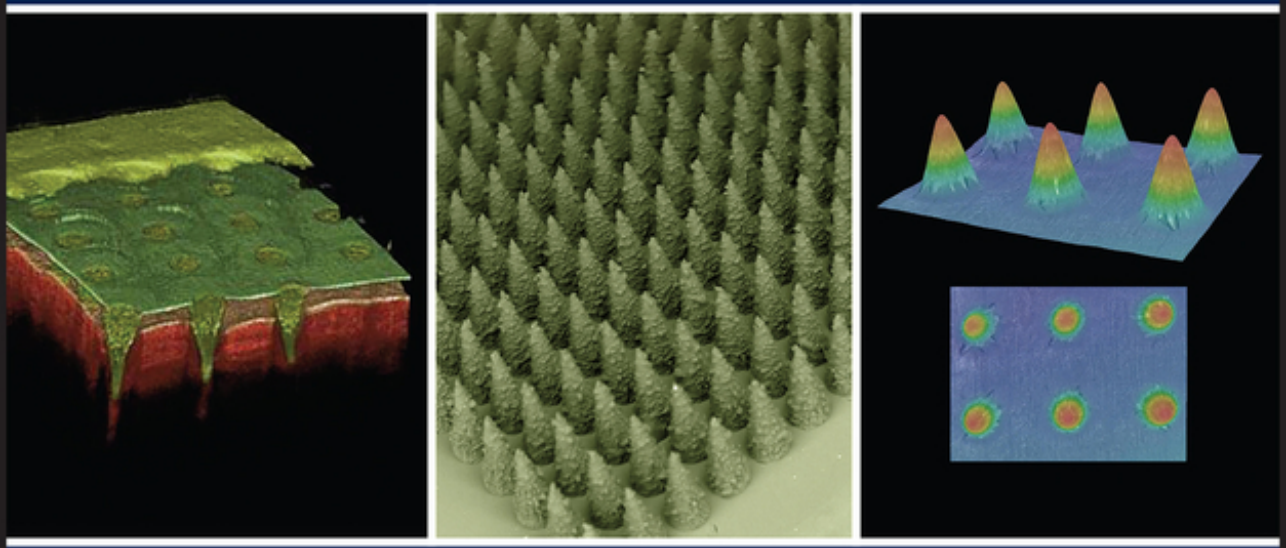

MICRONEEDLES FOR DRUG AND VACCINE DELIVERY AND PATIENT MONITORING

EDITED BY
RYAN F. DONNELLY | THAKUR RAGHU RAJ SINGH
ENEKO LARRAÑETA | MAELÍOSA T.C. McCRUDDEN

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Edited by
Ryan F. Donnelly, Thakur Raghu Raj Singh, Eneko Larrañeta, and Maelíosa T.C. McCrudden
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Professor Ryan F. Donnelly holds the Chair in Pharmaceutical Technology in the School of Pharmacy at Queen's University Belfast. A registered Pharmacist, his research is centred on design and physicochemical characterisation of advanced polymeric drug delivery systems for transdermal and topical drug delivery, with a strong emphasis on improving patient outcomes. He is currently developing a range of novel microneedle technologies through independent research, but also in collaboration with several major pharma partners. He has obtained substantial Research Councils UK, charity and industrial funding and authored over 500 peer-reviewed publications, including four patent applications, four textbooks, 23 book chapters and more than 150 full papers. He has been an invited speaker at numerous national and international conferences. Professor Donnelly is Editor-in-Chief of *Recent Patents on Drug Delivery & Formulation* and a member of the Editorial Advisory Boards of several leading pharmaceutical science journals. He won the Controlled Release Society's Young Investigator Award in 2016, BBSRC Innovator of the Year and the American Association of Pharmaceutical Scientists *Pharmaceutical Research* Meritorious Manuscript Award in 2013, the GSK Emerging Scientist Award in 2012 and the Royal Pharmaceutical Society's Science Award (2011).

Dr Thakur Raghu Raj Singh is Senior Lecturer in Pharmaceutics in the School of Pharmacy at Queen's University Belfast. He obtained his PhD in Drug Delivery from the School of Pharmacy at Queen's in 2009, his MSc in Pharmaceutical Sciences from University Science Malaysia in 2006 and his BPharm from Jawaharlal Nehru Technological University, India in 2002. Dr Thakur's research interests are in the design and physicochemical characterisation of advanced polymeric drug delivery systems for ocular, transdermal and topical applications. In particular, his current research involves fabrication and design of novel long-acting injectable and implantable drug delivery systems for treating back-of-the-eye disorders. Dr Thakur's ocular drug delivery research has led to formation of a university spinout company, Re-vana Therapeutics Ltd, for which he is the Co-founder and Chief Scientific Officer. He has authored over 140 scientific publications, including one patent, 46 peer-reviewed research papers, eight book chapters and three textbooks and has been an invited speaker at a number of national and international meetings. He is currently an Editorial Board member of the *Journal of Pharmacy & Pharmacology*, *Chronicles of Pharmacy* and *Science Domain International*, and acts as a scientific advisor to *The Journal of Pharmaceutical Sciences*, in addition to regularly acting as a reviewer for many other international scientific journals.

Dr Eneko Larrañeta works as Lecturer in Pharmaceutical Sciences in the School of Pharmacy at Queen's University Belfast. His main fields of expertise are drug delivery and pharmaceutical materials. During the last five years, he has been working on several projects to develop more efficient drug delivery systems for the oral and transdermal routes. For this purpose, he worked on the development of advanced systems, such as nanoparticles and microneedles. He worked on the development of nanoparticles for oral delivery of difficult-to-deliver drugs and nutraceuticals at the University of Navarra (Spain) under the supervision of Professor Juan Manuel Irache, an internationally renowned expert on mucosal drug delivery. In addition to oral drug delivery, he has done extensive work on microneedle-mediated transdermal drug delivery with Professor Donnelly's Group. He has worked on a range of projects funded by the European 7th Framework Programme and the UK's Biotechnology & Biological Sciences Research Council. Moreover, he has also worked on projects sponsored by global not-for-profit organisations, as well as the pharmaceutical and cosmeceutical industry. Using his previous research experience, the main objective of his current research is to develop materials that can be easily transferred to industry for ultimate patient benefit. He has published 20 scientific articles in indexed journals and has been co-author of two book chapters.

Dr Maelíosa T.C. McCrudden is a Senior Research Fellow in the School of Pharmacy at Queen's University Belfast. Having received her PhD from Queen's in 2008, she first carried out postdoctoral research in the laboratory of Dr Fionnuala Lundy, determining the roles of specific innate host peptides in wound healing and defence mechanisms in the oral cavity. She then moved her research focus to the field of pharmaceutical sciences and has, since 2012, worked in the Microneedles Research Group of Professor Donnelly. Her research has centred on transdermal delivery of drugs and intradermal vaccine administration using a range of novel microneedle systems. She has worked on Research Council and pharmaceutical industry funded projects and has most recently worked with the international not-for-profit organisation, PATH, to deliver an anti-HIV drug in a sustained fashion. Over and above these research interests, she is passionately committed to science communication and was recognised nationally for her work by receipt of both the Mendel Gold Medal and the Eric Wharton Medal at the Westminster SET for Britain awards in 2016. Dr McCrudden has published 25 articles in peer-reviewed journals, in addition to seven review articles and one book chapter. She is a regular invited speaker at both national and international conferences.

Preface

The worldwide transdermal patch market approached $32 billion in 2015, despite limited innovation in this area over the previous five years. Indeed, the entire market is still based on only 20 drugs. This rather limited number of drug substances is attributed to the excellent barrier function of the skin, which is accomplished almost entirely by the outermost 10–15 μm (in the dry state) of tissue, the *stratum corneum* (*SC*). Before being taken up by blood vessels in the upper dermis and prior to entering the systemic circulation, substances permeating the skin must cross the *SC* and the viable epidermis. There are three possible pathways leading to the capillary network: through hair follicles with associated sebaceous glands, via sweat ducts, or across continuous *SC* between these appendages. As the fractional appendageal area available for transport is only about 0.1%, this route usually contributes negligibly to apparent steady state drug flux. The intact *SC* thus provides the main barrier to exogenous substances, including drugs. The corneocytes of hydrated keratin are analogous to "bricks," embedded in a "mortar" composed of highly organised, multiple lipid bilayers of ceramides, fatty acids, cholesterol and its esters. These bilayers form regions of semicrystalline gel and liquid crystal domains. Most molecules penetrate through skin via this intercellular micro-route. Facilitation of drug penetration through the *SC* may involve by-pass or reversible disruption of its elegant molecular architecture. The ideal properties of a molecule that can penetrate the intact *SC* well are:

- Molecular mass less than 600 Da.
- Adequate solubility in both oil and water so that the membrane concentration gradient, which is the driving force for passive drug diffusion along a concentration gradient, may be high.
- Partition coefficient, such that the drug can diffuse out of the vehicle, partition into, and move across, the *SC*, without becoming sequestered within it.
- Low melting point, correlating with good solubility, as predicted by ideal solubility theory.

Clearly, many drug molecules do not meet these criteria. This is especially true for biopharmaceutical drugs, which are becoming increasingly important in therapeutics and diagnostics of a wide range of illnesses. Drugs that suffer poor oral bioavailability or susceptibility to first-pass metabolism, and are thus often ideal candidates for transdermal delivery, may fail to realise their clinical application because they do not meet one or more of these conditions. Examples include peptides, proteins and vaccines, which, due to their large molecular size and susceptibility to acid destruction in the

stomach, cannot be given orally and, hence, must be dosed parenterally. Such agents are currently precluded from successful transdermal administration, not only by their large sizes, but also by their extreme hydrophilicities. Several approaches have been used to enhance the transport of drugs through the *SC*. However, in many cases, only moderate success has been achieved and each approach is associated with significant problems. Chemical penetration enhancers allow only a modest improvement in penetration. Chemical modification to increase lipophilicity is not always possible and, in any case, necessitates additional studies for regulatory approval, due to generation of new chemical entities. Significant enhancement in delivery of a large number of drugs has been reported using iontophoresis. However, specialised devices are required and the agents delivered tend to accumulate in the skin appendages. The method is presently best-suited to acute applications, with several commercialised products intended for regular at-home use by patients being withdrawn relatively quickly after market approval for a range of reasons. Electroporation and sonophoresis are known to increase transdermal delivery. However, they both cause pain and local skin reactions and sonophoresis can cause breakdown of the therapeutic entity. Techniques aimed at removing the *SC* barrier, such as tape-stripping and suction/laser/thermal ablation are impractical, while needle-free injections have so far failed to replace conventional needle-based insulin delivery. Clearly, a robust alternative strategy is required to enhance drug transport across the *SC* and thus widen the range of drug substances amenable to transdermal delivery.

Microneedle arrays are minimally invasive devices that can be used to by-pass the *SC* barrier and thus achieve transdermal drug delivery. Microneedles (MN) (50–900 μm in height, up to 2000 MN/cm^2) in various geometries and materials (silicon, metal, polymer) have been produced using recently developed microfabrication techniques. Silicon MN arrays are prepared by modification of the dry- or wet-etching processes employed in microchip manufacture. Metal MN are produced by electrodeposition in defined polymeric moulds or photochemical etching of needle shapes into a flat metal sheet and then bending these down at right angles to the sheet. Polymeric MN have been manufactured by micromoulding of molten/dissolved polymers. MN are applied to the skin surface and pierce the epidermis (devoid of nociceptors), creating microscopic holes through which drugs diffuse to the dermal microcirculation. MN are long enough to penetrate to the dermis but are short and narrow enough to avoid stimulation of dermal nerves. Solid MN puncture the skin prior to application of a drug-loaded patch or are pre-coated with drug prior to insertion. Hollow bore microneedles allow diffusion or pressure-driven flow of drugs through a central lumen, while polymeric drug-containing microneedles release their payload as they biodegrade in the viable skin layers. *In vivo* studies using solid MN have demonstrated delivery of oligonucleotides, desmopressin and human growth hormone, reduction of blood glucose levels from insulin delivery, increase of skin transfection with DNA and enhanced elicitation of immune response from delivery of DNA and protein antigens. Hollow MN have also been shown to deliver insulin and reduce blood glucose levels. MN arrays do not cause pain on application and no reports of development of skin infection currently exist.

MN have been considered for a range of other applications, in addition to transdermal and intradermal drug/vaccine delivery. These include minimally invasive therapeutic drug monitoring, as a stimulus for collagen remodelling in anti-ageing strategies and for delivery of active cosmaceutical ingredients. MN technology is likely to find

ever-increasing utility in the healthcare field as further advancements are made. However, some significant barriers will need to be overcome before we see the first MN-based drug delivery or monitoring device on the market. Regulators, for example, will need to be convinced that MN puncture of skin does not lead to skin infections or any long-term skin problems. MN will also need to be capable of economic mass production.

In this book, we review the work that has been carried out on MN to date in both the academic and industrial sectors. We have looked in detail at both *in vitro* and *in vivo* studies and covered the important area of MN-based vaccines. We also consider safety and public perception aspects of MN and discuss newer applications of this exciting technology, such as delivery of gene therapies, photodynamic therapy, ocular delivery and enhanced administration of nanomedicines.

The MN field continues to expand, with ever-increasing numbers of publications that are increasingly being cited (Figure 1). However, the number of commercialised products on the market remains disappointing. Indeed, no true MN array drug delivery system is currently available to patients. Research work is largely confined to universities and specialised drug delivery companies, many of which have been spun-out from universities. It is hoped that the exciting data generated and published will encourage large pharmaceutical and medical device forms to make the considerable financial investment necessary for scaled-up manufacture and comprehensive clinical trials over the coming years so that MN technology will finally deliver the impact envisioned by research scientists.

We took a very different approach to the production of this book, as compared with how we wrote the first text on microneedles published by Wiley in 2012. The first book was written by four authors, with Desmond Morrow and David Woolfson coming on board with Raj and myself. Given the expansion of the field, Desy's move to New Zealand and David's well-deserved retirement, I asked Eneko Larrañeta and Maelíosa McCrudden to join Raj and I as Editors of this new book. Instead of writing the entire book ourselves, we asked members of my research Group and close collaborators to each co-author a chapter in an area of their specialisation. This approach worked extremely well, with our hectic schedules of research over the past year only slightly delaying delivery of the final text.

Editing this text took quite a considerable amount of time and I would like to thank my wife Johanne for her patience and support throughout the project. I am also grateful to Raj, Eneko and Maelíosa for agreeing to assist me in the editing of the book and for their work in co-authoring several chapters. This is now the fourth book I have worked on with Raj and it has been every bit as enjoyable collaborating with him again this time. This has been a new experience for Eneko and Maelíosa and I hope that this is the first of many books for them.

I am highly appreciative of the past and present members of the Microneedles Group at Queen's for their hard work and imagination in the lab. Special mention must go to the current Group members: Maelíosa, Aaron Courtenay, Ismaiel Tekko, Lalit Vora, Patricia Gonzalez-Vazquez, Helen Quinn, Mary-Carmel Kearney, Aoife Rodgers, Bridie Dutton, Sara Cordeiro, Emma McAlister, Eman Migdadi, Rehan Al-Kasasbeh, Iman Hamdan, Kurtis Moffatt, Dian Permana, Fabiana Volpe-Zanutto, Boonnada Pamornpathomkul, Michelle Barreto-Requena, Yadira Pastor-Garcia, Ke Peng, Delly Ramadon, Sarah Stewart, Inken Ramöller, Heba Abdelazim and Alvaro

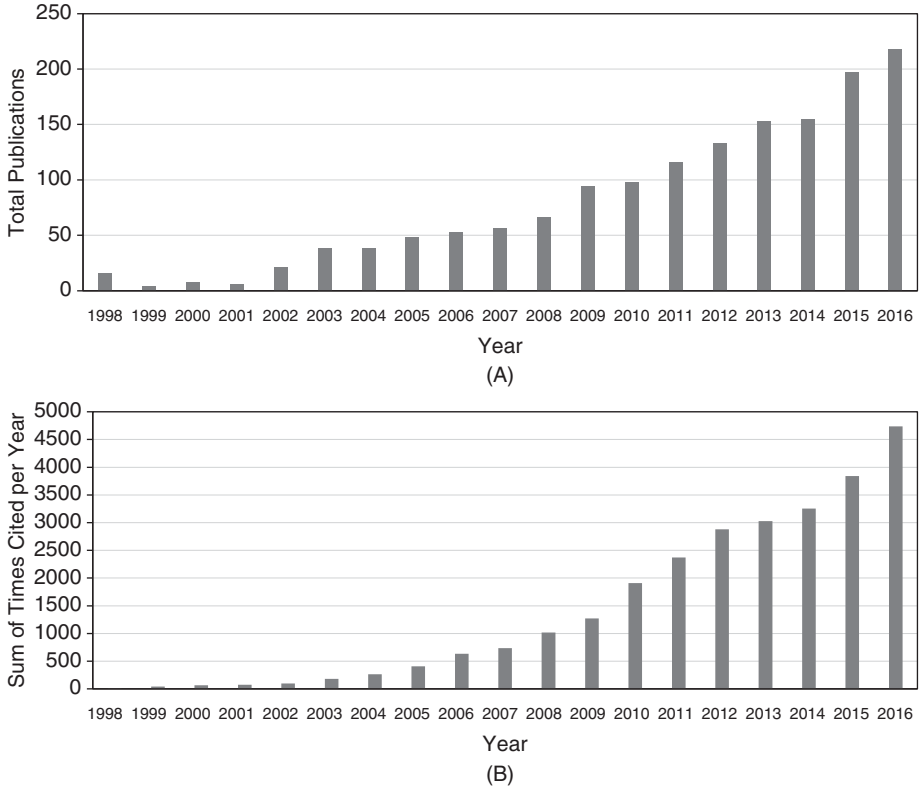

Figure 1 (A) Total number of journal articles published on microneedles, by year, since the first publication in 1998 and (B) total number of citations of microneedles articles, by year, since 1998.

Carcamo-Martinez. I would also like to acknowledge BBSRC, EPSRC, MRC, The Wellcome Trust, PATH, USAID, Invest Northern Ireland, Action Medical Research, Prostate Cancer UK, Arthritis Research UK and The Royal Society for funding my work in this area. I have been fortunate to have many excellent industrial collaborators who continue to support the translation of my research towards commercialisation and patient benefit, but confidentiality prevents direct acknowledgement. You know who you are – thank you! Emma Strickland and Elsie Merlin from Wiley provided considerable help and encouragement as we completed this project and their support and guidance are greatly appreciated. Judith Egan-Shuttler is also thanked for her work as the copyeditor. It is my hope that this book will serve as a comprehensive overview of the field and, hence, that it will be of use to those new to microneedles, as well as people already engaged in work in this area in both industry and academia.

Belfast, October 2017 *Ryan F. Donnelly*

1

Genesis of Transdermal Drug Delivery

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On the basis of the drug delivery systems, the market can be segmented into eight categories: oral, pulmonary, transdermal, injectable, ocular, nasal, implantable and transmucosal drug delivery. The most common routes of drug delivery are oral, which represent the largest market share (more than 50%), followed by transmucosal (26.2%) and transdermal delivery (12%) [1]. The conventional routes of oral drug delivery have many inherent limitations that could potentially be overcome by advanced drug delivery methodologies, such as transdermal drug delivery (TDD).

The administration of chemical agents to the skin surface has long been practiced, whether for healing, protective or cosmetic reasons. Historically, the skin was thought to be totally impervious to exogenous chemicals [2]. Thus, topical drug therapy typically involved the localized administration of medicinal formulations to the skin, generally when the skin surface was breached by disease or infection and a route of drug absorption into the deeper cutaneous layers was consequently open. However, once it was understood that the skin was a semi-permeable membrane rather than a totally impermeable barrier, new possibilities arose for the use of this route as a portal for systemic drug absorption.

In the early twentieth century, it was recognised that lipophilic agents had increased skin permeability, and in 1919 the barrier properties of the skin were attributed specifically to the outermost layers [3]. Scheuplein and Blank thoroughly investigated skin permeability to a wide range of substances *in vitro* [2]. They modelled skin as a three-layer laminate of *stratum corneum*, epidermis and dermis, with drug permeation driven by Fickian diffusion. By digesting the epidermal layer, the *stratum corneum* was separated from the lower layers of the skin and was determined to be the principal barrier to drug absorption.

Transdermal drug delivery refers to the delivery of the drug across intact, healthy skin into the systemic circulation [4]. The diffusive process by which this is achieved is known as percutaneous absorption. The drug initially penetrates through the *stratum corneum* and then passes through the deeper epidermis and dermis, without drug accumulation in the dermal layer [5]. When the drug reaches the dermal layer, it becomes available for systemic absorption via the dermal microcirculation. Thus, classical topical formulations can be distinguished from those intended for transdermal drug delivery in that,

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whilst the former are generally applied to a broken, diseased or damaged integument, the latter are used exclusively on healthy skin where the barrier function is intact.

It is, indeed, fortuitous for all of us that the skin is a self-repairing organ. This ability, together with the barrier protective properties associated with the integument, is a direct function of skin anatomy. Therefore, in order to develop an effective approach to transdermal drug delivery, it is necessary to be aware of how skin anatomy restricts the percutaneous absorption of exogenously applied chemicals.

1.1 Skin Anatomy

As the largest, and one of the most complex, organs in the human body, the skin is designed to carry out a wide range of functions [6]. Thus, the skin forms a complex membrane with a non-homogenous structure and a surface area of 1.7 m^2, compromising 16% of the total body mass of an average person (Figure 1.1). It contains and protects the internal body organs and fluids, and exercises environmental control over the body with respect to temperature and, to some extent, humidity. In addition, the skin is a communicating organ, relaying the sensations of heat, cold, touch, pressure and pain to the central nervous system.

1.1.1 The Epidermis

The multilayered nature of human skin can be resolved into three distinct layers. These consist of the outermost layer, the epidermis, beneath which lies the much larger dermis and, finally, the deepest layer, the subcutis. The epidermis, which is essentially a stratified epithelium, lies directly above the dermo–epidermal junction. The viable epidermis is often referred to as the epidermal layers below the *stratum corneum*. This provides mechanical support for the epidermis and anchors it to the underlying dermis. The junction itself is a complex glycoprotein structure about 50 nm thick [7].

Directly above the undulating ridges of the dermo–epidermal junction lies the basal layer of the epidermis, the *stratum germinativum*. This layer is single cell in thickness with columnar-to-oval shaped cells, which are actively undergoing mitosis. As the name implies, the *stratum germinativum* generates replacement cells to counterbalance the constant shedding of dead cells from the skin surface. In certain disease states, such as psoriasis, the rate of mitosis in this layer is substantially raised in order to compensate for a diminished epidermal barrier, the epidermal turnover time being as fast as four days. As the cells of the basal layer gradually move upwards through the epidermis, they undergo rapid differentiation, becoming flattened and granular and the ability to divide by mitosis is lost. Directly above the *stratum germinativum* is a layer, several cells in thickness, in which the cells are irregular and polyhedral in shape. This layer is the *stratum spinosum*, and each cell has distinct spines or prickles protruding from the surface in all directions. Although they do not undergo mitosis, the cells of this layer are metabolically active. The prickles of adjacent cells interconnect via desmosomes or intercellular bridges. The increased structural rigidity produced by this arrangement increases the resistance of the skin to abrasion [7].

As the epidermal cells migrate upwards towards the skin surface they become flatter and more granular in appearance, forming the next epidermal layer, the *stratum*

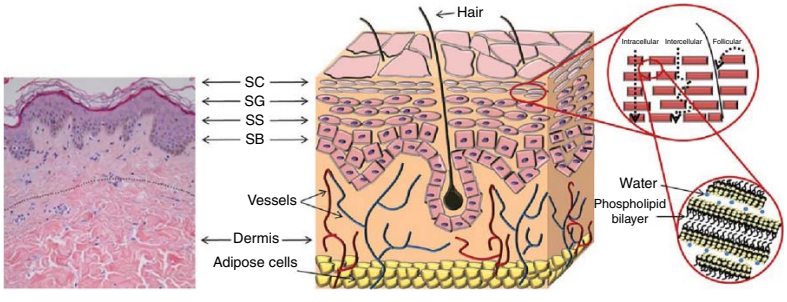

Figure 1.1 Schematic representation of the skin. Comparison with a histological section of mammary skin from a 19 year old patient (left). From inside to outside, the adipose cells, the dermis (the only vascular layer; thickness 0.3–4 mm; fibroblasts, sweat glands and hair follicles are present), the epidermis (thickness 100–150 μm) composed of *stratum basale* (*SB*), *stratum spinosum* (*SS*), *stratum granulosum* (*SG*), and *stratum corneum* (*SC*). Cells differentiate from *SB* to *SC* up to lose their nuclei. In the *SC* cells (corneocytes) are embedded in a matrix of lipid bilayers (brick and mortar model) (right). Penetration routes of molecules are reported with dotted arrows. The number of *SC* layers depends on the body site, age, skin condition and skin hydration (generally 6–20 but also 86 in the heel). Total thickness of *SC* is 10–30 μm. Reproduced with permission from [30] R.J. Scheuplein (1972) Properties of the skin as a membrane. *Adv. Biol. Skin*. 12: 125–152 .

granulosum, consisting of a few layers of granular cells. Their appearance is due to the actively metabolising cells producing granular protein aggregates of keratohyalin, a precursor of keratin [8]. As cells migrate through the *stratum granulosum*, cell organelles undergo intracellular digestion and disappear. The cells of the *stratum granulosum* die due to degeneration of the cell nuclei and metabolic activity ceases towards the top of this layer. A further differentiation of cells above the *stratum granulosum* can be seen in sections taken from thick skin, such as on the palm of the hand or the sole of the foot. This distinct layer of cells is termed the *stratum lucidum*. The cells of this layer are elongated, translucent and anuclear.

1.1.2 The *Stratum Corneum*

The *stratum corneum* is the most superficial layer of the epidermis, and thus the skin. It is in direct contact with the external environment and its barrier properties may be partly related to its very high density (1.4 g/cm^3 in the dry state) [9]. It is now well accepted that this layer constitutes the principal barrier for penetration of most drugs. The horny layer represents the final stage of epidermal cell differentiation. The thickness of this layer is typically 10 μm, but a number of factors, including the degree of hydration and skin location, influence this. The *stratum corneum* consists of 10–25 rows of dead keratinocytes, now called corneocytes, embedded in the secreted lipids from lamellar bodies [10]. The corneocytes are flattened, elongated, dead cells, lacking nuclei and other organelles [11]. The cells are joined together by desmosomes, maintaining the cohesiveness of this layer [12]. The heterogeneous structure of the *stratum corneum* is composed of approximately 75–80% protein, 5–15% lipid and 5–10% other substances on a dry weight basis [13, 14].

The majority of protein present in the *stratum corneum* is keratin and is located within the corneocytes [14]. The keratins are a family of α-helical polypeptides. Individual molecules aggregate to form filaments (7–10 nm diameter and many microns in length) that are stabilised by insoluble disulfide bridges. These filaments are thought to be responsible for the hexagonal shape of the corneocytes and provide mechanical strength for the *stratum corneum* [15]. Corneocytes possess a protein-rich envelope around the periphery of the cell, formed from precursors, such as involucrin, loricrin and cornifin. Transglutaminases catalyse the formation of γ-glutamyl crosslinks between the envelope proteins that render the envelope resistant and highly insoluble. The protein envelope links the corneocytes to the surrounding lipid enriched matrix [12].

The main lipids located in the *stratum corneum* are ceramides, fatty acids, cholesterol, cholesterol sulfate and sterol/wax esters [14]. These lipids are arranged in multiple bilayers called lamellae. Phospholipids are largely absent, a unique feature for a mammalian membrane. The ceramides are the largest group of lipids in the *stratum corneum*, accounting for approximately half of the total lipid mass [16], and are crucial to the lipid organisation of the *stratum corneum* [12].

The bricks and mortar model of the *stratum corneum* (Figure 1.1) is a common representation of this layer [17]. The bricks correspond to parallel plates of dead keratinised corneocytes, and the mortar represents the continuous interstitial lipid matrix. It is important to note that the corneocytes are not actually brick shaped, but rather are polygonal, elongated and flat (0.2–1.5 μm thick and 34.0–46.0 μm in diameter) [11]. The "mortar" is not a homogenous matrix. Rather, lipids are arranged in the lamellar phase (alternating layers of water and lipid bilayers), with some of the lipid bilayers in the gel or

crystalline state [18]. The extracellular matrix is further complicated by the presence of intrinsic and extrinsic proteins, such as enzymes. The barrier properties of the *stratum corneum* have been assigned to the multiple lipid bilayers residing in the intercellular space. These bilayers prevent desiccation of the underlying tissues by inhibiting water loss and limit the penetration of substances from the external environment [18].

1.1.3 The Dermis

This region, also known as the corium, underlies the dermo–epidermal junction and varies in thickness from 2 to 4 mm. Collagen, a fibrous protein, is the main component of the dermis and is responsible for the tensile strength of this layer. Elastin, also a fibrous protein, forms a network between the collagen bundles and is responsible for the elasticity of the skin and its resistance to external deforming forces. These protein components are embedded in a gel composed largely of mucopolysaccharides. The skin appendages, such as the sebaceous and sweat glands, together with hair follicles, penetrate this region. Since these open to the external environment they present a possible entry point into the skin [19, 20].

The dermis has a rich blood supply extending to within 0.2 mm of the skin surface and derived from the arterial and venous systems in the subcutaneous tissue. This blood supply consists of microscopic vessels and does not extend into the epidermis. Thus, a drug reaching the dermis through the epidermal barrier will be rapidly absorbed into the systemic circulation, a key advantage of the use of microneedles to by-pass the barrier to drug penetration offered by the *stratum corneum* [6, 11].

1.1.4 Skin Appendages

The skin appendages comprise the hair follicles and associated sebaceous glands, together with the eccrine and apocrine glands. Hairs are formed from compacted plates of keratinocytes, with the hair shaft housed in a hair follicle formed as an epidermal invagination. Associated flask-like sebaceous glands are formed as epidermal outgrowths. The sebaceous gland secretes an oily material (sebum), which plays a role in lubricating the skin surface and maintaining skin pH at around 5 [21]. Hairs can be pigmented or non-pigmented and can extend more than 3 mm into the hypodermis [22]. In humans, the skin density of these units varies with body region. For example, on the face, follicular openings can account for up to 10% of the surface area, whilst on other parts of the body, these orifices make up only 0.1% of the surface area [22]. Thus, a transfollicular route may be important for certain veterinary transdermal drug delivery applications, where the hair follicle density is much higher, but not in humans.

The eccrine glands respond to increased temperature and stress by exuding a dilute salt solution (sweat), where its evaporation plays an important thermoregulatory role. The coiled and tubular eccrine glands are located in the dermal tissue, and are connected to a duct that ascends towards the surface. They are distributed throughout the body surface, being particularly concentrated in the hands and feet [21]. Humans have approximately 3–4 million eccrine glands on their skin, which produce as much as 3 litres of sweat per hour [23]. The apocrine glands are found closer to the epidermal–dermal boundary and are associated with the axillae and ano-genital regions [21]. Apocrine ducts exit to the skin surface via the hair follicle [23].

1.2 Routes to Percutaneous Drug Absorption

It is now well established that the *stratum corneum* is the principal barrier to the percutaneous absorption of exogenous substances, including drugs seeking to use the skin as a portal via transdermal drug delivery. There are three routes by which a drug can, in theory, breach the *stratum corneum* barrier, thus reaching viable tissue and, ultimately, the skin microcirculation (Figure 1.1). From here, entry is made into the systemic circulation to complete the drug absorption process. The available routes are: trans-appendageal, via the hair follicles and sweat glands (sometimes referred to as the shunt route); transcellular, by diffusion through and across the corneocytes; and intercellular, by diffusion through the ordered domains of intercellular skin lipids. The relative contributions of the pathways to the overall drug flux are governed by the physicochemical properties of the permeating molecule, the fractional area of the route and whether drug permeation is facilitated in any way by disruption of the skin barrier.

An elegant model for the percutaneous absorption of a topically applied drug has been proposed [24] based on an analogy between the flow of electrons in an electrical circuit through series and parallel resistors, and the passive diffusional flow of a drug through the resistances offered by the various skin components. The current flow is driven by an electrical potential gradient whereas the diffusional drug flow, in contrast, is driven by a concentration gradient across the skin (Figure 1.2).

Skin diffusional resistances can be thought of as the transepidermal and transappendageal routes, in parallel. The transepidermal resistance is essentially that offered by the *stratum corneum*. As with the ohmic magnitude of an electrical resistance, the chemical magnitude (R) of a membrane resistor with respect to drug diffusion through

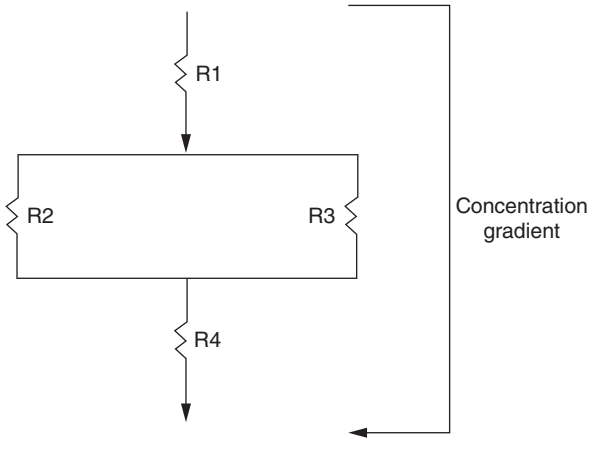

Figure 1.2 Series and parallel resistances to percutaneous drug penetration.

R1 vehicle resistance
R2 appendageal resistance
R3 stratum corneum resistance
R4 viable tissue resistance

that membrane can be expressed as:

$$R = \frac{h}{FDK} \tag{1.1}$$

where h is the thickness of the resistor membrane, F is the fractional area of the route (where there is more than one pathway involved), D is the diffusion coefficient of the drug through that resistor (the ease of movement of the drug through the tissue) and K represents the capacity of a particular tissue for the drug (in effect, the partition coefficient of the drug between one tissue phase and that immediately preceding it). It follows that the rate of skin penetration of a given drug is inversely proportional to the total diffusional resistance due to the various skin layers and components.

The transepidermal route has a fractional area approaching unity. In the percutaneous absorption process the total diffusional resistance offered by this route would consist of the sum of resistances due to the *stratum corneum*, viable epidermis and dermis. However, any diffusional resistance due to the dermis is minimal compared with that of the *stratum corneum* and can be neglected.

The *stratum corneum* is a narrow layer; hence the value of h in Equation (1.1) is small, thus tending to reduce the diffusional resistance of this layer. However, the main factor to consider is the densely packed, organised anatomical characteristics of this layer, ensuring that its overall resistance to chemical penetration is substantial, notwithstanding the reduced thickness of the horny layer compared with that of the viable epidermis.

The transappendageal route has a very low fractional area [25]. Shunt diffusion of penetrants through the skin appendages appears to be of significance only during the initial phase following application of the drug. The higher diffusion coefficients through the appendages compared with the *stratum corneum* leads to an excess initial penetration via this route, with an exponential relationship with time compared with the linear time dependency of drug penetration that characterises the establishment of steady-state diffusion [2, 25, 26]. Thus, although the transappendageal route may be important initially, its small fractional area suggests that it is of no great significance in the overall percutaneous penetration of most topically applied drugs [27]. Given the tortuous nature of the skin ducts and glands, and the upwards flow of material towards the skin surface opposing the downwards diffusion of an applied drug, it is not surprising that the shunt route is unimportant in steady state drug diffusion through the skin [28]. However, the initial build-up of drug achieved by rapid diffusion along the appendageal route, probably the hair follicles, prior to the establishment of steady state transepidermal diffusion, may explain the appearance of vasoactive phenomena associated with nicotinates (erythema) and steroids (skin blanching), both effects rapidly seen following topical administration of these agents [29].

Since the transappendageal route can be neglected as a major contributor to the overall penetration of non-electrolytes, the overall resistance to the drug reaching its target site of action can be seen as analogous to the flow of current through electrical resistors in series. Thus, the total resistance (R) of the skin to the percutaneous absorption of a diffusing molecule can be described by:

$$R = \frac{h}{F_{sc}D_{sc}K_{sc}} + \frac{h}{F_{\mathrm{c}}D_{\mathrm{c}}K_{\mathrm{c}}} \tag{1.2}$$

where the denominator subscripts *sc* and c refer to the *stratum corneum* and viable epidermis, respectively.

The *stratum corneum* has been shown to have approximately 10^3 times greater resistance to water penetration than the dermis, and is thus even more resistant to the passage of polar solutes [30]. For non-polar lipophilic solutes the *stratum corneum* has a lower resistance than to the passage of water. Although the viable epidermis and the dermis are more resistant to the passage of non-polar compared with polar materials, as might reasonably be expected, this effect is relative and minimal, with only 4% of the total skin resistance being ascribed to these viable layers [30]. It is clear, therefore, that the passage of the drug through the *stratum corneum* is the rate-limiting step for the percutaneous absorption of both polar and non-polar molecules. The decreased resistance of the horny layer to lipophilic drugs dictates the use of lipophilic molecules for conventional transdermal delivery, that is, where diffusion is driven by the drug concentration gradient across the barrier.

Although numerous mathematical models are available to describe the process of percutaneous absorption, that proposed by Flynn and coworkers [31] provides a good description of the overall process involved in the percutaneous absorption of a drug. Where that drug is a relatively low molecular weight, lipophilic molecule, the model can be considerably simplified. Thus, the resistance to drug penetration of the dermis can be neglected since it is minimal compared with that of the *stratum corneum*. The transappendageal route is largely insignificant, and the resistance due to the viable epidermis is so small compared with that due to the *stratum corneum* that it approaches zero. Thus, the *stratum corneum* fractional area can, in this case, be taken as unity. When steady-state diffusion of the drug across the *stratum corneum* barrier has been established, the amount of material passing through the barrier per unit area of vehicle coverage per unit time, that is, the drug flux, J, is given by

$$J = \left(\frac{D_{sc}.K_{sc/\mathrm{w}}}{h_{sc}} \right) \Delta C \tag{1.3}$$

where $K_{sc/\mathrm{w}}$ represents the partition coefficient between the *stratum corneum* and the formulation vehicle and ΔC is the drug concentration gradient across the *stratum corneum*, which, assuming sink conditions is the effective drug concentration in the vehicle. This equation, which is essentially Fick's first law for a steady state [11, 32] can be simplified to:

$$J = P(\Delta C) \tag{1.4}$$

where P is the permeability coefficient of the drug through the skin; P is described by the term in parentheses in Equation (1.3).

Equation (1.3) provides a guide to those factors that can be acted upon to maximise the efficiency of the percutaneous absorption of a drug through the *stratum corneum* barrier. Clearly, little can be done to reduce the value of h, the barrier thickness, unless an adhesive tape stripping technique is employed [28]. The barrier thickness may be reduced in the event of an existing clinical disease state but otherwise it can be regarded as a constant.

The drug diffusivity in the *stratum corneum*, as measured by D_{sc}, is a physicochemical parameter of the chosen drug or drug combination. Although the barrier characteristics may be altered by the use of a chemical penetration enhancer [11, 14], the relative values of D_{sc} for different drug molecules will retain their same comparative ranking. An increase in the value of $K_{sc/\mathrm{w}}$, the vehicle/*stratum corneum* partition coefficient,

therefore represents the best available means to ensure that an adequate concentration of drug can penetrate through the *stratum corneum* barrier. In practice, therefore, a conventional approach to transdermal drug delivery via drug diffusion through the *stratum corneum* along a concentration gradient is highly dependent on the physicochemical properties of the drug, with some limited influence exerted by formulation factors. Hence, for water-soluble or large, particularly macromolecular, actives, other approaches are needed if the transdermal route is to be used to its full potential.

1.3 Facilitated Transdermal Drug Delivery

Transdermal delivery has potential advantages over other conventional routes of drug delivery. It can provide a non-invasive and painless alternative to parenteral routes [19, 32]. Furthermore, the pharmacokinetic profiles of drugs are more uniform with lower variability, resulting in compliance and adherence with drug use [19, 33, 34]. Transdermal administration can be stopped by removal of the patch, which puts the patient in control in the event of an adverse reaction [35]. The skin is the largest organ in the body, which allows many placement options for transdermal absorption and ease of access. TDD is used when there is a significant first-pass effect of the liver, since it avoids pre-systemic metabolism, thus improving bioavailability [20, 34]. In addition, it is also the best route for paediatric patients, and a suitable route for unconscious or vomiting patients or those who rely on self-administration. The skin is known to be a highly immunogenic site for vaccination, because this organ is known to be crowded with dendritic cells in both the epidermal and dermal layers, which play a central role in immune responses [36]. Therefore, TDD is an attractive and novel vaccination route for therapeutic proteins and peptides.

A three-day patch that delivers scopolamine to treat motion sickness was the first transdermal patch to be approved, in 1979 [34]. The biggest challenge for transdermal delivery is that only a limited number of drugs are suitable to be administered transdermally. The number of commercially available transdermal patches that are approved by the US Food and Drug Administration (FDA) is less than 20.

The use of this route is severely limited by the restrictions imposed by the lipophilic *stratum corneum* barrier, which allows a limited number of drug molecules with certain physicochemical properties to be delivered transdermally [20, 34]. These approved molecules are only up to a few hundred Daltons, with octanol–water partition coefficients that heavily favour lipids (log P = 1–3). Moreover, reasonable potency with doses of milligrams per day or less is required for candidates to become suitable for conventional TDD [15, 34]. Therefore, the transdermal delivery of hydrophilic drugs, peptides and macromolecules, for example, DNA or small-interfering RNA, has posed challenges [34, 36]. Penetration enhancement technology would broaden the range of drugs available for transdermal administration [20]. Technologies used to modify the barrier properties of the *stratum corneum* are classified into passive methods (chemical penetration enhancement) and physical methods.

Passive methods include modulation of formulation excipients and addition of chemical enhancers, in order to temporarily alter the barrier properties of the *stratum corneum* [13, 16]. Passive methods are inexpensive, available and easily incorporated into transdermal patches, such as chemical penetration enhancers

[14, 37]. Ideal penetration enhancers should be non-toxic, non-allergic, inert and work unidirectionally [14]. Chemical penetration enhancers facilitate drug permeation across the skin by various mechanisms without long-term damage to the skin [14]. The mechanisms of action of penetration enhancers are complex, so it is difficult to classify them accordingly. They have several mechanisms of action such as: enhancing solubility, improving partitioning between the formulation and the *stratum corneum*, fluidising the *stratum corneum* lipid bilayers, interaction with intercellular proteins, causing dissolution and disruption of *stratum corneum* lipids [11, 14, 26, 32]. In addition, they may enhance diffusion across the skin by increasing the diffusion coefficient of the drug in the *stratum corneum* through disruption of the barrier properties of the *stratum corneum* and increasing the drug's thermodynamic activity [14, 25, 32]. The most widely studied penetration enhancers are water, polyols, sulfoxides, azone, pyrrolidones, essential oils, surfactants, terpenes, fatty acids and urea [14]. Nonetheless, the major drawbacks of passive methods are that only modest degrees of increased flux can be achieved in practice and there is a time lag in drug release. Moreover, unacceptable skin irritation could occur, especially when using more than one enhancer in the formulation.

The recent trend in TDD is to use a novel vehicle. Recently, researchers have designed suitable drug delivery vehicles for transdermal patches, such as emugel, proniosomes, microemulsions and nanoemulsions, into the field of penetration enhancers [38]. Ammar and coworkers [39] designed a new transdermal formulation for tenoxicam and showed that proniosomal gels act as penetration enhancers that enhance the drug permeation from the skin barrier. Proniosomal gel formulations showed a significantly higher therapeutic compared with the oral tenoxicam tablets of the same dose on the market, thus revealing a more promising tenoxicam dosage form [39]. Barakat and coworkers [40] showed that nanoemulsions can be used as potential vehicles for improved transdermal delivery of indomethacin as an approach to eliminate the side effects of the oral dose.

Modulation of formulation excipients and addition of chemical enhancers can increase drug flux, but not sufficiently to ensure delivery of a pharmacologically effective concentration of the drug. Therefore, several new active rate-controlled transdermal drug delivery technologies (electrically-based, structure-based, velocity-based, etc.) have been developed for the transdermal delivery of wide ranges of drugs [41]. This is particularly of interest given the high economic value of the transdermal delivery market, despite the relatively small number of actives that can be delivered by this route [42]. Broadly, facilitated delivery falls into two categories: technological [42], of which microneedles, the subject of this text, is a good example; and formulation approaches, most notably the focus on nanoscale delivery systems [43]. The following are some of the technologies presently being considered as aids to transdermal drug delivery.

1.3.1 Electrical-based Devices

1.3.1.1 Iontophoresis

Perhaps the oldest method in use for facilitated transdermal delivery, this technique employs the application of physiologically acceptable electrical currents ($0.1–1.0\ mA/cm^2$) to drive charged drugs into the skin through electrostatic effects and make ionic drugs pass through the skin into the body by its potential gradient [20, 33,