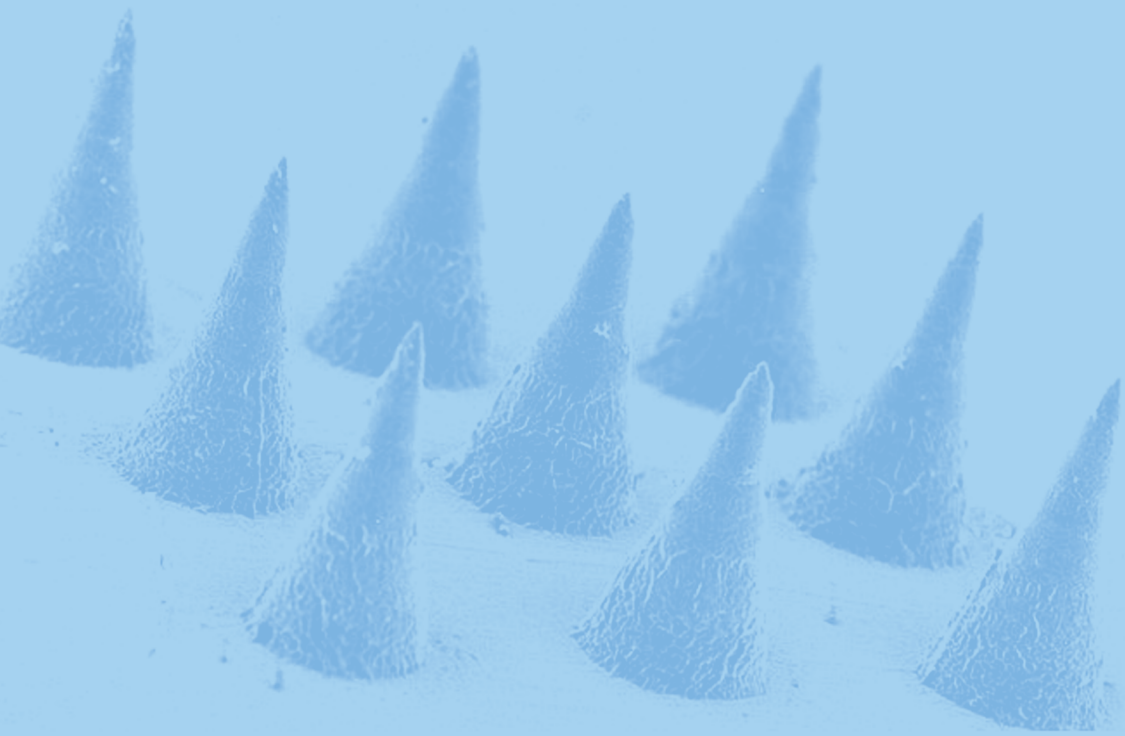
MICRONEEDLE-MEDIATED TRANSDERMAL AND INTRADERMAL DRUG DELIVERY

Ryan F. Donnelly | Thakur Raghu Raj Singh
Desmond I.J. Morrow | A. David Woolfson



WILEY-BLACKWELL

Microneedle-mediated Transdermal and Intradermal Drug Delivery

Microneedle-mediated Transdermal and Intradermal Drug Delivery

Ryan F. Donnelly, BSc, PhD, PGCHET, MRSC, MPSNI
Thakur Raghu Raj Singh, BSc, MSc, PhD
Desmond I.J. Morrow, MPharm, PhD, MPSNI
A. David Woolfson, BSc, PhD, CChem, FRSC, FPSNI

School of Pharmacy
Queen's University
Belfast, UK

WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Registered Office
John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Offices
9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell

Library of Congress Cataloging-in-Publication Data

Microneedle-mediated transdermal and intradermal drug delivery / Ryan F. Donnelly ... [et al.].
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-65489-7 (cloth)
1. Microinjections. 2. Transdermal medication. 3. Drug delivery systems. I. Donnelly, Ryan F.
[DNLM: 1. Microinjections–methods. 2. Administration, Cutaneous. 3. Drug Delivery Systems. 4. Injections, Intradermal. WB 354]
RM169.M53 2012
615.1'9–dc23

2011022675

A catalogue record for this book is available from the British Library.
Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Set in 10/13pt Palatino by SPi Publisher Services, Pondicherry, India

1 2012

Contents

Preface

Recently, the transdermal route has vied with oral treatment as the most successful innovative research area in API delivery. In the USA (the most important pharmaceutical market), out of 129 API delivery products under clinical evaluation, 51 are transdermal or dermal systems; 30% of 77 candidate products in preclinical development represent such API delivery. The worldwide transdermal patch market approaches $20 billion, yet is 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 organized, 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 microroute. 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 penetrating 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 realize their clinical application because they do not meet one or more of the above 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, specialized devices are required and the agents delivered tend to accumulate in the skin appendages. The method is presently best-suited to acute applications. 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 (MNs) (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 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.

Recently, MNs 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 potentially novel applications of this exciting technology moving forwards. It is our 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 MN as well as people already engaged in work in this area.

Writing this text took considerable time and we would like to thank our families for their patience and support throughout the project. We are also

grateful to past and present members of the Microneedles Group at Queen's for their hard work and imagination in the lab; Dr Martin Garland, Dr Corona Cassidy, Dr Elizabeth Ryan, Dr Cian McCrudden, Dr Rita Majithiya, Sharifa Al-Zahrani, Ella Mahmood, Karen Mooney and Ester Caffarel. We would also like to acknowledge BBSRC, EPSRC, The Wellcome Trust and The Royal Society for funding our work in this area. Karen Moore from Wiley-Blackwell provided considerable help and encouragement as we completed this project and her support and guidance are greatly appreciated.

Ryan Donnelly
Raj Singh
Des Morrow
David Woolfson
(Belfast, UK)

About the Authors

Dr Ryan F. Donnelly is Senior Lecturer in Pharmaceutics at the School of Pharmacy, Queen's University Belfast. Dr Donnelly obtained a BSc (1st Class) in Pharmacy from Queen's University Belfast in 1999. Following a year of Pre-Registration training spent in community Pharmacy Practice, he registered with the Pharmaceutical Society of Northern Ireland. He then returned to the School of Pharmacy in 2000 to undertake a PhD in Pharmaceutics. He graduated in 2003 and, after a short period of post-doctoral research, was appointed to a Lectureship in Pharmaceutics in January 2004.

His research is centred on design and physicochemical characterization of advanced polymeric drug delivery systems for transdermal and topical drug delivery, with a strong emphasis on improving therapeutic outcomes for patients. Still at an early stage of his career, he has authored over 200 peer-reviewed publications, including three patent applications, two text-books and approximately 80 full papers. He has been an invited speaker at numerous national and international conferences. Dr Donnelly is the Associate Editor of *Recent Patents on Drug Delivery & Formulation* and a member of the Editorial Advisory Boards of *Pharmaceutical Technology Europe* and *Journal of Pharmacy and Bioallied Sciences* and is a Visiting Scientist at the Norwegian Institute for Cancer Research, where he is an Associate Member of the Radiation Biology Group. He leads the microneedles research programme in the School of Pharmacy and his work is currently funded by BBSRC, EPSRC, The Wellcome Trust, The Royal Society and the pharmaceutical and medical devices industries.

Dr Thakur Raghu Raj Singh is Lecturer in Pharmaceutics at the School of Pharmacy, Queen's University Belfast. Dr Singh holds a degree in Pharmacy from Jawaharlal Nehru Technological University, India and an MSc in Pharmaceutical Sciences from University Science Malaysia. He received his PhD in Pharmaceutics from Queen's University Belfast in

2009. Dr Singh's research interests are in the use of novel injectable polymeric systems for controlled drug delivery and transdermal delivery using microneedles. Dr Singh has published more than 20 international publications and has presented his work at both national and international conferences.

Dr Desmond I.J. Morrow is an Honorary Research Fellow at the School of Pharmacy, Queen's University Belfast. Dr Morrow obtained his MPharm (1st Class) in Pharmacy in 2002 and his PhD in drug delivery from Queen's University Belfast in 2008. During his career, he has previously worked in community pharmacy, hospital pharmacy and in academia and is currently employed in the pharmaceutical industry. He has authored over 30 publications in the field of topical and transdermal drug delivery and now has research interests in solid oral dosage form design and process scale-up.

Professor A. David Woolfson holds the Chair in Pharmaceutics and is Head of the School of Pharmacy at Queen's University Belfast. Professor Woolfson is Chair of the British Pharmacopoeia Commission, leader of the UK Delegation to the European Pharmacopoeia Commission and a former UK designated expert on formulated pharmaceutical products at the European Pharmacopoeia Commission. He is a member of the Council of the Pharmaceutical Society of Northern Ireland and holds Fellowships from that body and from the Royal Society of Chemistry. He founded the McClay Research Centre for Pharmaceutical Sciences at Queen's.

Professor Woolfson's research interests are in polymeric drug delivery systems for transdermal and intravaginal applications, bioadhesion and biomaterials. He has published several books on drug delivery and more than 300 research papers and articles, and has an extensive portfolio of research grants. Professor Woolfson is on the Editorial Advisory Board of CRC Books Inc. He has contributed to the development of a number of marketed pharmaceutical products, notably the percutaneous anaesthetic product, Ametop™, and has authored a book on *Percutaneous Local Anaesthesia*. More recently, he has focused on intravaginal drug delivery, notably in the development of elastomeric intravaginal rings for HIV microbicides and mucosal vaccines. He has extensive experience of technology transfer and intellectual property in the pharmaceutical sector.

CHAPTER 1

Transdermal Drug Delivery

1.1 Genesis of transdermal drug delivery

The administration of chemical agents to the skin surface has long been practised, whether for healing, protective or cosmetic reasons. Historically, the skin was thought to be totally impervious to exogenous chemicals [1]. 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 semipermeable 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 recognized that more lipophilic agents had increased skin permeability. The barrier properties of the skin were attributed specifically to the outermost layers in 1919 [2]. Scheuplein and co-workers thoroughly investigated skin permeability to a wide range of substances *in vitro* [3]. 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, 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 and into the systemic circulation. The diffusive process by which this is achieved is known as percutaneous absorption. Thus, classical topical formulations can be distinguished from those intended for transdermal drug delivery in that, whilst the former are generally

Microneedle-mediated Transdermal and Intradermal Drug Delivery, First Edition. Ryan F. Donnelly, Thakur Raghu Raj Singh, Desmond I.J. Morrow and A. David Woolfson.

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. So effective is the skin as a barrier to the external environment that, even now, the majority of licensed preparations applied to the skin are aimed at delivering the drug for a local, rather than a systemic, action.

1.2 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 [4]. Thus, the skin forms a complex membrane with a nonhomogenous structure (Figure 1.1). It contains and protects the internal body organs and fluids, and exercises environmental control over the body in respect of 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.

1.2.1 The epidermis

The multilayered nature of human skin can be resolved into three distinct layers. These are 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. 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 [5].

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,

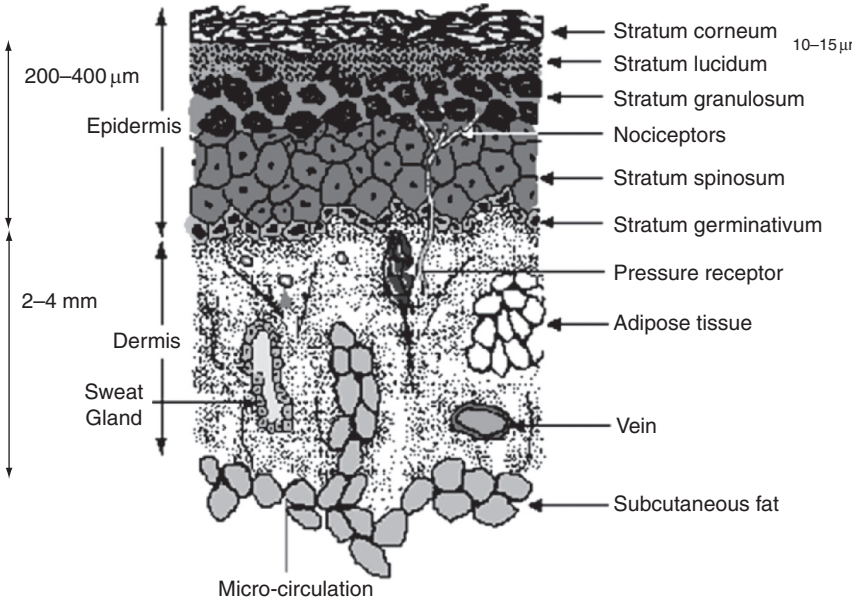


Figure 1.1 Diagrammatic representation of the major features of skin anatomy.

becoming flattened and granular. 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.

As the epidermal cells migrate upwards towards the skin surface they become flatter and more granular in appearance, forming the next epidermal layer, which is the stratum granulosum, consisting of a few layers of granular cells. Their appearance is due to the actively metabolizing cells producing granular protein aggregates of keratohyalin, a precursor of keratin [6]. 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, which is now substantially removed from nutrients supplied via the dermal circulation, is the stratum lucidum. The cells of this layer are elongated, translucent and anuclear.

1.2.2 The stratum corneum

The stratum corneum, or horny layer, is the outermost layer of the epidermis, and thus the skin. It is now well accepted that this layer constitutes the principal barrier for penetration of most drugs [7]. 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. For example, the stratum corneum on the palms and soles can be, on average, 400–600 µm thick [7] whilst hydration can result in a 4-fold increase in thickness [8].

The stratum corneum consists of 10–25 rows of dead keratinocytes, now called corneocytes, embedded in the secreted lipids from lamellar bodies [7]. The corneocytes are flattened, elongated, dead cells, lacking nuclei and other organelles [9]. The cells are joined together by desmosomes, maintaining the cohesiveness of this layer [10]. 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 [11].

The majority of protein present in the stratum corneum is keratin and is located within the corneocytes [11]. The keratins are a family of

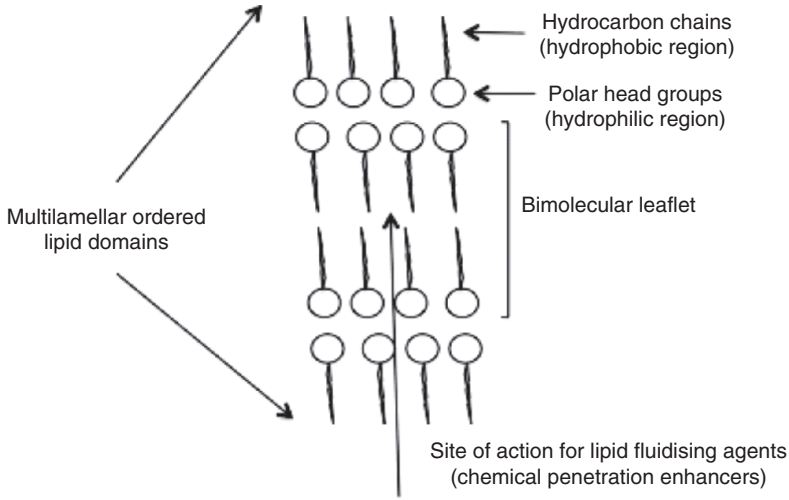


Figure 1.2 Arrangement of lipids in the stratum corneum.

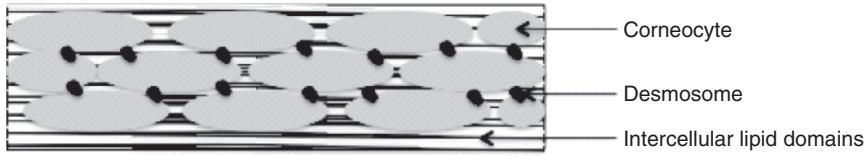


Figure 1.3 'Bricks and mortar' model of stratum corneum.

α-helical polypeptides. Individual molecules aggregate to form filaments (7–10 nm diameter and many microns in length) that are stabilized by insoluble disulphide bridges. These filaments are thought to be responsible for the hexagonal shape of the corneocyte and provide mechanical strength for the stratum corneum [12]. Corneocytes possess a protein rich envelope around the periphery of the cell, formed from precursors, such as involucrin, loricrin and cornifin. Transglutaminases catalyze the formation of γ-glutamyl cross-links between the envelope proteins that render the envelope resistant and highly insoluble. The protein envelope links the corneocyte to the surrounding lipid enriched matrix [10].

The main lipids located in the stratum corneum are ceramides, fatty acids, cholesterol, cholesterol sulphate and sterol/wax esters [11,12]. These lipids are arranged in multiple bilayers called lamellae (Figure 1.2). 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 [13], and are crucial to the lipid organization of the stratum corneum [10].

The bricks and mortar model of the stratum corneum (Figure 1.3) is a common representation of this layer [8]. The bricks correspond to parallel plates of dead keratinized 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) [9]. 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 [14]. 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 [14].

1.2.3 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.

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 in the use of microneedles to by-pass the barrier to drug penetration offered by the stratum corneum.

1.2.4 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 flasklike 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 around 5 [15]. This mixture of lipids acts as a plasticizer for the stratum corneum and maintains an acid mantle of about pH 5 on the skin surface. Hairs can be pigmented or nonpigmented and can extend more than 3 mm into the hypodermis [16]. 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 [16]. 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 gland is located in the dermal tissue, and is connected to a duct that ascends towards the surface.