Novel Delivery Systems for Transdermal and Intradermal Drug Delivery
Novel Delivery Systems for Transdermal and Intradermal Drug Delivery
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Novel Delivery Systems for Transdermal and Intradermal Drug Delivery

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Unfortunately during the preparation of this book, one of the authors, Dr Sian Lim died in a tragic cycling accident and left behind a wife Evelyn, a daughter Caelyn and a son Elijah. Sian was a brilliant scientist and an expert formulator who was involved in the development of over 20 topical and transdermal medicines that are now on the market. We would like to dedicate this book to his memory.
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About the Editors

Ryan F. Donnelly
Ryan Donnelly graduated with a BSc (First Class) in Pharmacy from Queen’s University Belfast in 1999 and was awarded the Pharmaceutical Society of Northern Ireland’s Gold Medal. Following a year of pre-registration training spent in Community Pharmacy, he returned to the School of Pharmacy 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. He was promoted to Senior Lecturer in 2009, Reader in 2011 and, in 2013, to a Chair in Pharmaceutical Technology.

Professor Donnelly’s research is centered on design and physicochemical characterisation of advanced polymeric drug delivery systems for transdermal and topical drug delivery, with a strong emphasis on improving therapeutic outcomes for patients. His bioadhesive patch design was used in successful photodynamic therapy of over 100 patients and this technology has now been licensed to Swedish Pharma AB, for whom Professor Donnelly acts as a Technical Director. Currently, Professor Donnelly’s group is focused on novel polymeric microneedle arrays for transdermal administration of ‘difficult-to-deliver’ drugs and intradermal delivery of vaccines and photosensitisers. His work has attracted funding of approximately £4.5 million, from a wide range of sources, including BBSRC, EPSRC, MRC, the Wellcome Trust, Action Medical Research, the Royal Society and the pharmaceutical and medical devices industries.

Still at a relatively early stage of his career, he has authored over 350 peer-reviewed publications, including 4 patent applications, 3 textbooks and approximately 120 full papers. He has been an invited speaker at numerous national and international conferences. Professor Donnelly is the Associate Editor of Recent Patents on Drug Delivery & Formulation and a member of the Editorial Advisory Boards of The American Journal of Pharmacology and Toxicology, Pharmaceutical Technology Europe, Expert Review of Medical Devices and Journal of Pharmacy & Bioallied Sciences and is Visiting Scientist at the Norwegian Institute for Cancer Research, where he is Associate Member of the Radiation Biology Group.
His work has attracted numerous awards, including the BBSRC Innovator of the Year Award and the American Association of Pharmaceutical Scientists Pharmaceutical Research Meritorious Manuscript Award in 2013, the GSK Emerging Scientist Award in 2012; he is a previous winner of the Royal Pharmaceutical Society’s Science Award (2011), the Queen’s Improvement to Society Award (2011), an Innovation Leader Award from the NHS Research & Development Office (2009) and a Research Scholarship from the Research Council of Norway (2004). In 2013, he was listed in the 40 most influential business leaders in Northern Ireland under the age of 40 by Belfast Media Group. Professor Donnelly’s microneedles work has featured on the front cover of Journal of Controlled Release and BBSRC Business and he has represented BBSRC and the Royal Society of Chemistry at Parliamentary Receptions at Westminster and Stormont, respectively. He has been extensively involved in activities promoting public engagement with science through regular interviews on television and radio and online platforms, such as You Tube, Twitter and Tumblr. His Pharmacists in Schools Programme has made over 100 school visits and his work featured at the 2014 Great British Bioscience Festival.

**Thakur Raghu Raj Singh**
Thakur Raghu Raj Singh is Lecturer in Pharmaceutics at the School of Pharmacy, Queen’s University Belfast. Dr Singh’s research interests lie 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 chronic ocular diseases. Dr Singh has authored over 90 scientific publications, including 40 full papers and a textbook on microneedles. He has been an invited speaker at a number of national/international meetings.

Dr Singh is currently an Editorial Board Member of the International Journal of Pharmacy and Chronicles of Pharmacy and Scientific Advisor to the editors of the Journal of Pharmaceutical Sciences. He is a reviewer for at least 18 other international scientific journals. Following his appointment as Lecturer in August 2010, he has secured funding of approximately £560 000 from Invest NI, WHO and industry. Dr Singh’s group is currently working on design and development of injectable in situ implant-forming systems for ocular drug delivery, funded by Invest Northern Ireland, and on industrial development of novel non-aqueous-based protein eye drops for the treatment of age-related macular degeneration and diabetic retinopathy.
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The series *Advances in Pharmaceutical Technology* covers the principles, methods and technologies that the pharmaceutical industry uses to turn a candidate molecule or new chemical entity into a final drug form and hence a new medicine. The series will explore means of optimizing the therapeutic performance of a drug molecule by designing and manufacturing the best and most innovative of new formulations. The processes associated with the testing of new drugs, the key steps involved in the clinical trials process and the most recent approaches utilized in the manufacture of new medicinal products will all be reported. The focus of the series will very much be on new and emerging technologies and the latest methods used in the drug development process.

The topics covered by the series include the following:

**Formulation:** The manufacture of tablets in all forms (caplets, dispersible, fast-melting) will be described, as will capsules, suppositories, solutions, suspensions and emulsions, aerosols and sprays, injections, powders, ointments and creams, sustained release and the latest transdermal products. The developments in engineering associated with fluid, powder and solids handling, solubility enhancement, colloidal systems including the stability of emulsions and suspensions will also be reported within the series. The influence of formulation design on the bioavailability of a drug will be discussed and the importance of formulation with respect to the development of an optimal final new medicinal product will be clearly illustrated.

**Drug Delivery:** The use of various excipients and their role in drug delivery will be reviewed. Amongst the topics to be reported and discussed will be a critical appraisal of the current range of modified-release dosage forms currently in use and also those under development. The design and mechanism(s) of controlled release systems including macromolecular drug delivery, microparticulate controlled drug delivery, the delivery of biopharmaceuticals, delivery vehicles created for gastrointestinal tract targeted delivery, transdermal delivery and systems designed specifically for drug delivery to the lung will
all be reviewed and critically appraised. Further site-specific systems used for the delivery of drugs across the blood–brain barrier including dendrimers, hydrogels and new innovative biomaterials will be reported.

**Manufacturing:** The key elements of the manufacturing steps involved in the production of new medicines will be explored in this series. The importance of crystallisation; batch and continuous processing, seeding; and mixing including a description of the key engineering principles relevant to the manufacture of new medicines will all be reviewed and reported. The fundamental processes of quality control including good laboratory practice, good manufacturing practice, Quality by Design, the Deming Cycle, Regulatory requirements and the design of appropriate robust statistical sampling procedures for the control of raw materials will all be an integral part of this book series.

An evaluation of the current analytical methods used to determine drug stability, the quantitative identification of impurities, contaminants and adulterants in pharmaceutical materials will be described as will the production of therapeutic bio-macromolecules, bacteria, viruses, yeasts, moulds, prions and toxins through chemical synthesis and emerging synthetic/molecular biology techniques. The importance of packaging including the compatibility of materials in contact with drug products and their barrier properties will also be explored.

*Advances in Pharmaceutical Technology* is intended as a comprehensive one-stop shop for those interested in the development and manufacture of new medicines. The series will appeal to those working in the pharmaceutical and related industries, both large and small, and will also be valuable to those who are studying and learning about the drug development process and the translation of those drugs into new life saving and life enriching medicines.

Dennis Douroumis
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Preface

Medicines have been delivered across the skin since ancient times. However, the first rigorous scientific studies involving transdermal delivery seeking to determine what caused skin to have barrier properties that prevent molecular permeation were not carried out until the 1920s. Rein proposed that a layer of cells joining the skin’s stratum corneum (SC) to the epidermis posed the major resistance to transdermal transport. Blank modified this hypothesis after removing sequential layers of SC from the surface of skin and showing that the rate of water loss from skin increased dramatically once the SC was removed. Finally, Scheuplein and colleagues showed that transdermal permeation was limited by the SC by a passive process. Despite the significant barrier properties of skin, Michaels and coworkers measured apparent diffusion coefficients of model drugs in the SC and showed that some drugs had significant permeability. This led to the active development of transdermal patches in the 1970s, which yielded the first patch approved by the United States Food and Drug Administration in 1979. It was a 3-day patch that delivered scopolamine to treat motion sickness. In 1981, patches for nitroglycerin were approved. Understanding of the barrier properties of skin and how they can be chemically manipulated was greatly enhanced in the 1980s and early 1990s through the work of Maibach, Barry, Guy, Potts and Hadgraft. Today there are a number of transdermal patches marketed for delivery of drugs such as clonidine, fentanyl, lidocaine, nicotine, nitroglycerin, oestradiol, oxybutynin, scopolamine and testosterone. There are also combination patches for contraception, as well as hormone replacement.

Recently, the transdermal route has vied with oral treatment as the most successful innovative research area in drug delivery. In the United States (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 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 microroute. Facilitation of drug penetration through the SC may involve bypass or reversible disruption of its elegant molecular architecture. The ideal properties of a molecule penetrating intact SC well are as follows:

- 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 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, robust alternative strategies are
required to enhance drug transport across the SC and thus widen the range of drug substances amenable to transdermal delivery.

Recently, nanoparticulate and super-saturated delivery systems have been extensively investigated. Nanoparticles of various designs and compositions have been studied and, while successful transdermal delivery is often claimed, therapeutically useful plasma concentrations are rarely achieved. This is understandable, given the size of solid nanoparticles. So called ultra-deformable particles may act more as penetration enhancers, due to their lipid content, while solid nanoparticles may find use in controlling the rate or extending the duration of topical delivery. Super-saturated delivery systems, such as ‘spray-on’ patches may prove useful in enhancing delivery efficiency and reducing lag times.

Amongst the more promising transdermal delivery systems to emerge in the past few decades are microneedle (MN) arrays. MN arrays are minimally invasive devices that can be used to bypass the SC barrier and thus achieve transdermal drug delivery. 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 MNs arrays are prepared by modification of the dry- or wet-etching processes employed in microchip manufacture. Metal MNs 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 MNs have been manufactured by micromoulding of molten/dissolved polymers. MNs are applied to the skin surface and pierce the epidermis (devoid of nociceptors), creating microscopic holes through which drugs diffuse to the dermal microcirculation. MNs are long enough to penetrate to the dermis but are short and narrow enough to avoid stimulation of dermal nerves. Solid MNs puncture skin prior to application of a drug-loaded patch or are pre-coated with drug prior to insertion. Hollow bore MNs allow diffusion or pressure-driven flow of drugs through a central lumen, while polymeric drug-containing MNs release their payload as they biodegrade in the viable skin layers. In vivo studies using solid MNs have demonstrated delivery of oligonucleotides, desmopressin and human growth hormone, reduction of blood glucose levels from insulin delivery, increase in skin transfection with DNA and enhanced elicitation of immune response from delivery of DNA and protein antigens. Hollow MNs 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 cosmeceutical 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 recently on innovative transdermal delivery systems 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 skin characterisation, since thorough understanding of this is vital when designing delivery systems to overcome its barrier function. We also consider safety and public perception aspects of new
delivery systems and discuss potentially-novel applications of these exciting technologies moving forwards. Since scientists in the cosmetics field have borrowed techniques and formulation designs from the transdermal field, we also look at the recent innovations in this area. Importantly, the final chapter discusses the process of commercialisation of skin delivery systems. 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 transdermal delivery, as well as people already engaged in work in this area.

We are indebted to the contributors for their hard work, openness to suggestions for directions of their chapters and prompt delivery of the chapters. Editing 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 the members of the Microneedles Group at Queen’s for their hard work and imagination in the lab: Dr Maeliosa McCrudden, Dr Ester Caffarel-Salvador, Dr Rebecca Lutton, Dr Eneko Larraneta, Dr Aaron Brady, Patricia Gonzalez-Vazquez, Eva Vicente-Perez, Joakim Kennedy, Helen Quinn, Aaron Courtenay, Mary-Carmel Kearney and Steven Fallows. Gratitude is also due to the members of the Ocular Delivery Group: Dr Chirag Gujral, Dr Hannah McMillan, Dr Ismaeil Tekko, Samer Adwan and Katie McAvoy. We would like to acknowledge BBSRC, EPSRC, MRC, the Wellcome Trust, PATH, Action Medical Research and the Royal Society for funding our work in this area. Sarah Tilley Keegan and Rebecca Stubbs from John Wiley & Sons provided considerable help and encouragement as we completed this project and their support and guidance are greatly appreciated.

Ryan Donnelly and Raj Singh
Belfast, 2014
1

Introduction

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The skin is the most physiologically complex and diverse organ of the human body. It has many roles, including the regulation of temperature, mechanical and protective functions. This latter function includes the regulation of water ingress and egress, as well as the prevention of entry into the body of exogenous chemical and biological entities.

The skin is the largest organ of the body, accounting on average for approximately 10% of body mass. It receives approximately one-third of the blood circulating throughout the body and has a surface area of approximately 2–3 m² [1]. It provides a robust, flexible and self-repairing barrier to the external environment and protects internal body organs and fluids from external influences, harmful molecules and micro-organisms. Its permeability limits excessive water loss and exercises temperature regulation over the body. The skin forms an extensive sensory surface, transmitting sensations such as heat, cold, touch, pressure and pain to the central nervous system. The skin is a multi-layered organ consisting of three main histological layers: the epidermis, the dermis and the subcutis. Mammalian skin is a stratified epithelium, and each layer will be considered individually, below, progressing from the innermost tissues to the outermost.

1.1 The Subcutis (Subcutaneous Fat Layer)

Immediately beneath the epidermis and dermis lies the subcutaneous fatty tissue layer (or subcutis or hypodermis). This layer provides support and cushioning for the overlying skin, as well as attachment to deeper tissues. It acts as a depository for fat and contains
blood vessels that supply the skin. It also acts as a heat insulator and a shock absorber. The subcutis is variable in thickness, ranging from a few centimetres thick in some regions, such as the abdominal wall, to areas where there is little or no fat, and the subcutis may be difficult to distinguish, such as the eyelid or scrotum. It is often difficult to distinguish the subcutis from the dermis, as both are irregular connective tissues, but the subcutis is generally looser and contains a higher proportion of adipose cells. The deeper layers of the subcutis are fully continuous, with layers of deep fascia surrounding muscles and periosteum.

1.2 The Dermis

The dermis, or corium, lies immediately below the dermo-epidermal junction. It is 10–20 times thicker than the epidermis and ranges from 0.1 to 0.5 cm in thickness, depending on its location in the body. It is a robust and durable tissue that provides flexibility and tensile strength. It protects the body from injury and infection and provides nutrition for the epidermis and acts as a water storage organ. The main feature of the dermis is a matrix of mechanically strong fibrous proteins, consisting mainly of collagen, but with elastin embedded in a gel-like mix of mucopolysaccharides [2]. Embedded within this matrix are various structures, including nerve tissues, vascular and lymphatic systems and the base of various skin appendages. The upper section of the dermis consists of loose connective tissue and a superficial, finely structured papillary layer which progresses upwards into the epidermis. The lower dermis is a coarse, fibrous layer which is the main supporting structural layer of the skin. The transition between epidermal and dermal structures occurs at the dermo-epidermal junction. Both the epidermis and dermis vary greatly in structure, with the former being mostly cellular in construction, whereas the latter contains few cells, other than mast cells. The dermis is the locus of the blood vessels in the skin, extending to within 0.2 mm of the skin surface and derived from the arterial and venous systems in the subcutaneous tissue. The blood vessels supply the hair follicles, glandular skin appendages and subcutaneous fat, as well as the dermis itself [1].

The vasculature of the skin is responsible for regulating the skin temperature, supplying nutrients and oxygen to the skin, removing toxins and waste products and for assisting in wound repair. Clearly, the vasculature also plays an important role in the removal of locally absorbed chemicals, carrying them into the systemic circulation. The blood supply to the skin can sit relatively close to the skin surface, meaning that exogenous penetrants are removed into the circulation from around the dermo-epidermal junction. Thus, for percutaneous absorption into the systemic circulation, including transdermal drug delivery, the blood supply to the skin facilitates the maintenance of a concentration gradient between the material applied to the external skin surface and the vasculature, across the skin barrier. Such clearance may also be facilitated by the lymphatic system, which is similarly located at a comparable distance from the exterior of the skin to the blood supply [3, 4].

1.3 Skin Appendages

Associated with the skin are several types of appendages, including hair follicles and their associated sebaceous glands (Figure 1.1) and eccrine and apocrine sweat glands.
On average, human skin contains 40–70 hair follicles and 200–250 sweat ducts/cm² of skin. The skin appendages occupy approximately 0.1% of the total human skin surface [4, 6], although this varies from region to region. Hairs are formed from compacted plates of keratinocytes and reside in hair follicles formed as an epidermal invagination. The associated sebaceous glands (Figure 1.1) are formed as outgrowths of the follicle and secrete an oily material – sebum – onto the skin surface. Sebum is a combination of various lipids and acts as a plasticiser for the stratum corneum, maintaining an acidic mantle of approximately pH 5 [6]. The eccrine glands are principally concerned with temperature control and are responsible for secretion and evaporation of sweat when stimulated by an increase in external temperature or emotional factors. These glands commonly occupy only $10^{-4}$ of the total skin area, and extend well into the dermis. Whereas eccrine glands are found throughout the body, apocrine glands are located in specific regions, such as the axillae and anogenital regions. Similar to eccrine glands, they descend into the dermis.

1.4 The Subcutaneous Sensory Mechanism

The extensive size of the skin lends itself to act as a major source of sensory input for the sensory nervous system. It provides information about the environment from both direct contact and from more remote sources, such as the effect of radiation on skin temperature. Cutaneous fibres within the dermis form a plexus lying parallel to the surface of the skin. This plexus is composed of unmyelinated and myelinated fibres, organised in the same manner as the parent nerve trunks. The dermal networks send twisted extensions into the
papillae, where they form loops which return to the superficial part of the plexus. From the plexus some individual fibres extend to supply particular locations. The terminal branches of each fibre interconnect with and superimpose themselves on each other [7] in such a way that every area in the skin is supplied by several different fibres. Each of these fibres ends in at least one particular receptor. Most of the cutaneous receptors can be excited by various stimuli, but it is the different thresholds of the stimuli required to provoke responses that yields specifically to these receptors [8].

There are three main categories of cutaneous receptor which are distinguished by their different sensitivities to stimuli: mechanoreceptors, thermoreceptors and nociceptors. Mechanoreceptors have high sensitivities to indentation or pressure on the skin, or to movement of the hairs. This group may be further subdivided into the rapidly adapting (RA) and slowly adapting (SA) receptor types. The RA mechanoreceptors include Pacinian corpuscles, found in both hairy and glabrous skin, and Meissner’s corpuscles, located in the glabrous skin of primates and hair follicle afferent units found only in hairy skin. Pacinian corpuscles are small pearl-shaped structures found in the deeper layers of the skin. They are 0.5–2 mm long and are composed of an ‘onion-like’ lamellar structure which is formed from non-nervous tissue. They contain an elongated nerve ending at its core which is not derived from the dermal plexus. The most important characteristic of the Pacinian corpuscle is its ability to detect mechanical vibrations at high frequencies, which may be relayed at greater than 100Hz/s. Such frequencies are often sensed in traumatised or unanaesthetised skin [9, 10]. The Meissner’s corpuscle is an encapsulated myelinated receptor which resides in the dermis of the human glabrous skin. It is tucked into the dermal papillae that fill the grooves formed by epidermal ridges. The entire corpuscle is surrounded by connective tissue, continuous with the perineurium, which is attached to the basal projections of the epidermal cells by elastin fibrils. The Meissner’s corpuscle discriminates highly localised sensations of touch, especially in the palmar regions where they are found in their highest density [11].

Hair follicle receptors are myelinated fibres, circumferentially arranged around the hair root sheath below the sebaceous gland which innervate hair follicles. Large hair follicles can be supplied by up to 28 fibres. The hair is well placed in its follicle to stimulate the nerve collar and is primarily associated with tactile sensations [12]. SA mechanoreceptors respond during skin displacement. They also maintain a discharge of impulses when the skin is held in a new position [8]. These receptors include the Ruffini endings and the C-mechanoreceptors. The Ruffini endings are encapsulated receptors which are found in the dermis of both hairy and glabrous skin and provide a continuous indication of the intensity of the steady pressure or tension within the skin [9]. C-mechanoreceptors have small receptive fields (about 6 mm²) in hairy skin and may emit a slowly adapting discharge when the skin is indented or when hairs are moved. Repetitive stimulation will, however, produce a rapid fall in excitability, and the receptors will fail to respond after 20–30s because the receptor terminals have become inexcitable [8].

Thermoreceptors are characterised by a continuous discharge of impulses at a given constant skin temperature which increases or decreases when temperature is raised or lowered. The receptive fields of the thermoreceptor are spot-like and cover an area of no more than 1 mm². Thermoreceptors are classed as either ‘cold’ or ‘warm’ receptors, with ‘cold’ receptors lying more superficially in the skin than ‘warm’ receptors. The depth of ‘cold’ and ‘warm’ receptors was estimated at about 0.15 and 0.6 mm, respectively, below the surface. The firing frequency accelerates in ‘cold’ receptors when the temperature is falling – and vice versa for the warm receptors. Such dynamic sensitivity
is high and permits the receptors’ response to relatively slow (<1°C in 30 s) and small changes in skin temperature [8].

Damaging or potentially damaging excitation of thermo- and mechanoreceptors is not necessary for such receptors to reach maximum activation, indicating their inability to control pain. They do, however, contribute to the sensory quality of perceived pain. The receptor systems that detect and signal high intensities of stimulation form a distinct class of sense peripheral organs called ‘nociceptors’. They have unencapsulated nerve endings and exhibit the smallest identified structures [9–15]. Nociceptors generally reside at the dermo-epidermal junction, and are either mechanical nociceptors, which respond to pin-pricks, squeezing and to crushing of the skin, or thermal (or mechanothermal) nociceptors which respond to severe mechanical stimuli and to a wide range of skin temperatures.

1.5 The Epidermis

The epidermis is the outermost layer of the skin. It is the thinnest part of the skin, with its thickness varying around the body – for example, the thickest skin is commonly found on the weight-bearing planter surfaces (feet and hands, ~0.8 mm) and the thinnest skin is normally found on the eyelids and scrotum (0.06 mm) [5]. Despite the extensive vasculature present in deeper tissues such as the dermis, the epidermis has no blood supply and passage of materials into or out of it is usually by a process of diffusion across the dermo-epidermal layer. It is essentially a stratified epithelium, consisting of four, or often five, distinct layers.

1.6 The stratum germinativum

The deepest layer of the epidermis is the stratum germinativum, or basal layer. This metabolically active layer contains cells that are similar to those found in other tissues in the body, as they contain organelles such as mitochondria and ribosomes. It is often single celled in thickness and contains cuboid or columnar-to-oval-shaped cells which rest upon the basal lamina. The basal cells are continually undergoing mitosis, as they provide replacement cells for the higher (outer) epidermis. Basal keratinocytes are connected to the dermo-epidermal membrane by hemidesmosomes, which connect the basal cells to the basement membrane. Throughout the basal layer and higher layers of the epidermis, such as the stratum spinosum, keratinocyte cells are connected together by desmosomes. The basal layer is also the location of other cells, including melanocytes, Langerhans cells and Merkel cells. The basal cells become flatter and more granular as they move up through the epidermis.

1.7 The stratum spinosum

Immediately above the stratum germinativum is the stratum spinosum, or prickle cell layer. It is often described, in conjunction with the basal layer, as the Malpighian layer. It is several (usually between two and six) layers thick and forged from cells of irregular morphology, varying from columnar to polyhedral in structure as this layer progresses outward. Each cell possesses distinct tonofilamental desmosomes, characterised as prickles or spines,
which extend from the surface of the cell in all directions and which help to maintain a
distance of approximately 20 nm between cells. The prickles of adjacent cells link via inter­
cellular bridges, providing improved structural rigidity and increasing the resistance of the
skin to abrasion. Though lacking in mitosis, the prickle cell layer is metabolically active.

1.8 The stratum granulosum

The next epidermal tier is the stratum granulosum, or granular layer. It usually one to three
layers deep and consists of several layers of flattened, granular cells whose cytoplasm
contains characteristic granules of keratohyalin, which is responsible for their appearance.
It is produced by the actively metabolising cells and is believed to be a precursor of
keratin. The stratum granulosum is the skin layer where degradation of cell components
becomes significant, resulting in a decrease in metabolic activity which eventually ceases
towards the top of this layer due to the degeneration of cell nuclei, leaving them unable to
carry out important metabolic reactions.

1.9 The stratum lucidum

The layer above the stratum granulosum, the stratum lucidum, is easily observed on thick
skin, but may be missing from thinner skin, hence the often differing descriptions of the
epidermis as having four or five layers. It is often considered that the stratum lucidum is
functionally indistinct from the stratum corneum and that it may be an artefact of tissue
preparation and cell differentiation, rather than a morphologically distinct layer. The cells
are elongated, translucent and mostly lack either nuclei or cytoplasmic organelles. The
stratum lucidum exhibits an increase in keratinisation consistent with the progression of
cell flattening from the bottom to the top of the epidermis.

1.10 The stratum corneum

The outermost layer of the skin is the stratum corneum, often called the horny layer. It is
the final result of cell differentiation and compaction prior to desquamation and removal
from the body. While it is an epidermal layer it is often considered a separate layer of the
skin and is often described as such. It consists of a compacted, dehydrated and keratinised
multilayer, which is, on average, 15–20 cells thick; that is, around 10 µm in thickness when
dry, although it can swell to many times its thickness when wet. The formation of keratin
and the resultant death of these cells are part of the process of keratinisation, or cornification.
The stratum corneum is, in effect, the outer envelope of the body. In areas where the
stratum lucidum is apparent, the stratum corneum is much thicker, being designed to cope
with the effects of weight support and pressure. Its thickness also mirrors that of the viable
epidermis around the body. Thus, the epidermis in those regions, such as the palms and
soles, can be up to 800 µm in thickness, compared to 75–150 µm in other areas. Cells of the
stratum corneum are physiologically inactive, continually shedding and replenishing
themselves from the upward migration of cells from the underlying epidermal layers [1].