DEVELOPMENT AND CHARACTERISATION OF BIOACTIVE GLASS BASED POLYMER COMPOSITE FILM FOR POTENTIAL APPLICATION IN WOUND HEALING

MAMUN KHAN SUJON

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DEVELOPMENT AND CHARACTERISATION OF BIOACTIVE GLASS BASED POLYMER COMPOSITE FILM FOR POTENTIAL APPLICATION IN WOUND HEALING

by

MAMUN KHAN SUJON

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DEDICATION

This thesis is dedicated to my wife and son

Dr. Shaila Zaman and Nabhan Ajwad Khan

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LIST OF ABBREVIATIONS

A/A	Antibiotic-Antimycotic
AB	Alamar blue
ADD	Agar disc diffusion
AFM	Atomic force microscopy
AgNPs	Silver nanoparticles
bFGF	Basic fibroblast growth factor
BG	Bioactive glass
BPWD	Biopad wound dressing
Ca/P	Calcium phosphate
CS	Chitosan
CaO	Calcium oxide
DDA	Degree of deacetylation
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco’s Phosphate Buffered Saline
ECM	Extracellular matrix
EDX	Energy dispersive X-ray spectroscopy
ERK	Extracellular signal-regulated kinase

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FBS	Foetal Bovine Serum
FDA	Food and drug administration
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared
HA	Hydroxycarbonate apatite
HE	Haematoxylin and Eosin
HIF	Hypoxia-induced factor
HIF-1α	Hypoxia-inducible factor-1 alpha
HPDF	Human primary dermal fibroblast
IGF-1	Insulin-like growth factor-1
LB	Luria-Bertani
MIC	Minimum inhibitory concentration
MT	Masson's Trichome
Na_2O	Sodium oxide
NHS	National Health Service
PCL	Poly-Ɛ-caprolactone
PDGF	Platelet derived growth factor
P_2O_5	Phosphorus pentoxide
PPh	Poly-phenols
PVA	Poly (vinyl alcohol)

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QCT	Quercetin
R_a	Root mean square roughness
R_{ku}	Kurtosis
R_{Sk}	Skewness
RMS	Root mean square
R_q	Mean roughness
SA	*Staphylococcus aureus*
SBF	Simulated body fluid
SE	Secondary electron
SEM	Scanning electron microscopy
SiO_2	Silicon oxide
SSD	Silver sulfadiazine
TEM	Transmission electron microscopy
TGF-α	Transforming growth factor alpha
TGF-β	Transforming growth factor beta
TSA	Tryptone soy agar
UV	Ultra violet
VEGF	Vascular endothelial growth factor
v/v	Volume per volume
w/v	Weight per volume

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XRD	X-ray diffraction

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LIST OF SYMBOLS

a.u	Arbitrary unit
at.%	Atomic percentage
cm^2	Centimetre square
°	Degree
°C	Degree Celsius
g	Gram
g/mol	Gram per mol
θ	Incidence Angle of X-Ray Beam
mol.%	Mole percentage
mg/mL	Milligram per millilitre
μm	Micrometre
%	Percentage
wt.%	Weight percentage

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LIST OF APPENDICES

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PEMBANGUNAN DAN PENCIRIAN FILEM KOMPOSIT POLIMER BERASASKAN KACA BIOAKTIF UNTUK POTENSI APLIKASI DALAM PENYEMBUHAN LUKA

ABSTRAK

Kejuruteraan tisu merupakan bidang penyelidikan yang berkembang pesat, memberikan tumpuan pada pembaikan, penjanaan semula, dan penggantian tisu serta organ yang rosak. Kajian ini memperkenalkan pembalut luka termaju yang boleh diserap, direka untuk mempercepatkan penyembuhan luka dan mengurangkan sisa klinikal, tempoh kemasukan ke hospital, dan beban kewangan. Kaca bioaktif (BG) yang secara tradisional dikaitkan dengan penjanaan semula tisu mineral telah menunjukkan potensi besar dalam pembaikan tisu lembut, terutama dalam penyembuhan luka melalui pembebasan ion terapeutik yang terkawal. Penggabungan polimer semula jadi kitosan (CS) dan polimer sintetik poli-ε-kaprolakton (PCL) ke dalam komposit meningkatkan biokeserasian, aktiviti antibakteria, morfologi permukaan, dan kekuatan mekanikal. Pelbagai komposisi BG (45S5 dan 50S8P) telah disintesis menggunakan kaedah sol-gel dan digabungkan dengan CS dan PCL untuk membentuk filem komposit BG/polimer melalui teknik pelarut tuangan yang menjimatkan kos. Analisis komprehensif telah dijalankan untuk pencirian struktur, kimia, morfologi, dan mekanikal filem komposit. Ujian bioaktiviti *in-vi*tro dalam cecair badan simulasi menilai potensi filem komposit untuk mendorong pembentukan hidroksiapatit (HA). Kesan biologi dinilai menggunakan ujian Alamar blue dan ujian migrasi calar dengan sel fibroblast dermal primer manusia. Keberkesanan antibakteria terhadap *Staphylococcus aureus* (*S. aureus*) diuji menggunakan ujian penyebaran

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cakera agar dan kepekatan penghambatan minimum. Selain itu, kajian *in-vi*vo melibatkan pemindahan kulit pada 20 arnab jantan baka New Zealand untuk menilai potensi penyembuhan luka dan disahkan melalui pemeriksaan histologi menggunakan pewarnaan Haematoxylin dan Eosin, dan Masson's Trichrome. Filem komposit BG/polimer menunjukkan kedua-dua fasa amorfus dan separa kristal, dengan kawasan amorfus disebabkan oleh campuran polimer dan puncak separa kristal berkaitan dengan fasa kombeite BG. Kehadiran kumpulan fungsi Si-O-Si mengesahkan bahawa rangkaian BG berasaskan silika, manakala kumpulan fungsi C-O-C, amida, O-H, dan N-H menunjukkan campuran polimer CS/PCL. Penilaian morfologi mencadangkan peningkatan kekasaran permukaan dan hidrofilik dengan penambahan zarah BG. Ujian bioaktiviti *in-vi*tro menunjukkan pemendapan HA pada permukaan filem komposit. Selain itu, penilaian biokeserasian menunjukkan peningkatan lekatan, percambahan, dan migrasi sel. Filem komposit menunjukkan aktiviti antibakteria yang dikaitkan dengan pelarutan ion BG, kesan pH, dan sifat antibakteria intrinsik CS dengan menghalang pertumbuhan bakteria *S. aureus*. Secara ketara, filem komposit 45S5 BG/CS/PCL menunjukkan tindak balas biologi, bioaktiviti, dan keberkesanan antibakteria yang luar biasa. Akhirnya, eksperimen *in-vi*vo mencadangkan bahawa filem komposit BG/polimer mempercepatkan penyembuhan luka dan mengurangkan keradangan di tapak luka. Potensi multifungsi filem komposit BG/polimer yang dibangunkan menjadikannya calon yang menjanjikan untuk digunakan dalam bahan pembalut luka termaju dan aplikasi kejuruteraan tisu.

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DEVELOPMENT AND CHARACTERISATION OF BIOACTIVE GLASS BASED POLYMER COMPOSITE FILM FOR POTENTIAL APPLICATION IN WOUND HEALING

ABSTRACT

Tissue engineering is a thriving research area of interest, focuses on the repair, regeneration, and replacement of damaged tissues and organs. This study introduces a novel resorbable advanced wound dressing designed to expedite wound healing and reduce clinical waste, hospitalisation durations, and financial burdens. Bioactive glasses (BGs), traditionally associated with mineralised tissue regeneration, have shown enormous promise in soft tissue repair, particularly in wound healing through the controlled release of therapeutic ions. The incorporation of natural polymer chitosan (CS) and synthetic polymer poly-ε-caprolactone (PCL) into the composite enhances biocompatibility, anti-bacterial activity, surface morphology, and mechanical strength. Different BG compositions (45S5 and 50S8P) were synthesised using sol-gel method and incorporated with CS and PCL to fabricate BG/polymer composite films via a cost-effective solvent-casting technique. A comprehensive analysis was conducted for structural, chemical, morphological, and mechanical characterisation of the composite film. *In-vitro* bioactivity assays in simulated body fluid assessed the composite film's potential to induce hydroxycarbonate apatite (HA) formation. Biological impact was evaluated using Alamar blue assay and scratch migration assays with human primary dermal fibroblast cells. Anti-bacterial efficacy against *Staphylococcus aureus* (*S. aureus*) was examined using agar disc diffusion and minimum inhibitory concentration assays. Furthermore, *in-vivo* studies involved skin

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transplantation on 20 male New-Zealand breed rabbits to assess wound healing potential and validated through histological examination using Haematoxylin and Eosin, and Masson's Trichrome staining. The BG/polymer composite films exhibited both amorphous and semi-crystalline phases, with amorphous regions attributed to the polymer blend and semi-crystalline peaks corresponding to the combeite phase of BG. The presence of Si-O-Si functional groups confirmed a silica network-based BG, while C-O-C, amide groups, O-H, and N-H functional groups indicated the CS/PCL polymer blend. Morphological assessment suggested increased surface roughness and hydrophilicity upon inclusion of BG particles. *In-vitro* bioactivity assay revealed HA deposition on the composite film surface. Additionally, proliferative activity assessment demonstrated enhanced cell adhesion, proliferation, and migration. The composite films showed anti-bacterial activity attributed to BG ion dissolution, pH effects, and CS's intrinsic anti-bacterial properties by inhibiting *S. aureus* bacterial growth. Notably, the 45S5 BG/CS/PCL composite film exhibited exceptional biological response, bioactivity, and anti-bacterial efficacy. Finally, *in-vivo* experiments suggested that BG/polymer composite film accelerated wound healing and reduced inflammation at the wound site. The multifunctional potentiality of the developed BG/polymer composite film makes it as a promising candidate for use in advanced wound dressing materials and tissue engineering applications.

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CHAPTER ONE

INTRODUCTION

1.1 Overview

The skin is the largest organ in human, encompassing an approximate area of 2 m^2 (Khalili et al., 2018). There are three distinct layers of the skin, including the epidermis, dermis, and hypodermis, each of which possesses inherent self-renewal capabilities and perform a diverse array of functions (Yildirimer et al., 2012). Primarily, the skin serves as a protective barrier, shielding internal organs from microbial invasion and radiation, while also controlling body temperature (Böttcher-Haberzeth et al., 2010). Aside from these functions, the skin plays a crucial role in maintaining the body's immune system and detecting sensory stimuli (Böttcher-Haberzeth et al., 2010).

Injuries to the skin are known as wounds (Dhivya et al., 2015). Generally, wounds can be divided into two main categories: acute and chronic wounds. Acute wound is a sudden injury to the skin. The healing process of an acute wound typically takes between 2 to 3 months, depending on the depth and size of the injury within the epidermis or dermis (Robson et al., 2001). On the other hand, a chronic wound, which does not heal as effectively or as quickly as they should, poses a significant risk, including burns, decubitus ulcers, infections, and leg ulcers (Dhivya et al., 2015). The nature and functional components of wound dressings have improved over the years in an effort to accelerate wound healing. Many wound care products are available in the market for the purpose of aiding the healing of wounds (Thu et al., 2012). Their

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primary function is to keep the wound moist, clean and prevent harmful bacteria from entering the wound (Boateng et al., 2008).

For decades, wound management has utilised natural and synthetic bandages, cotton wool, lint, and gauzes of various degrees of absorbency for each product. For example, a gauze dressing made of cotton, rayon, or polyester fibres is somewhat protective against bacterial infections. These dressings are use fibres and some sterile gauze pads are used to absorb fluid and exudates from open wounds. These dressings need to be changed frequently to prevent maceration of healthy tissues. The dressings become moistened when excessive wound drainage occurs, making it painful to remove them. Natural cotton wool bandages are made from cellulose, while synthetic bandages are made from polyamide materials. In case of venous ulcers, cotton bandages provide sustained compression while high compression bandages and short stretch compression bandages provide retaining light dressings (Boateng et al., 2008). It is important to note that, these conventional dry dressings served as passive wound protection methods. However, their limitations became apparent, particularly in their inability to provide a moist environment conducive to wound healing (Boateng et al., 2008). For instance, dry dressings are designed to absorb wound exudate, but they do not retain any of the moisture, resulting in the diversion of moisture away from the wound site and creating a dry environment. Wound exudate plays a vital role in the healing process as it provides necessary nutrients and white blood cells (Sweeney et al., 2012). Dry dressings do not manage this exudate effectively. Moreover, a dry environment can lead to tissue dehydration and cell death, which are not conducive to wound healing (Xiao and Miwa, 2022).

Furthermore, semi-solid formulations such as creams and gels, commonly utilised in wound care, presented challenges regarding maintaining therapeutic drug

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concentrations on moist wound surfaces over extended periods of time, which often led to insufficient retention times and issues such as leakage and messiness in highly exuding wounds (Saghazadeh et al., 2018). Thus, modern wound dressings are designed to promote wound healing and prevent dehydration rather than just covering it. They keep the wound from getting dry and prevent it from getting worse. The benefits of moist wounds over dry wounds include preventing tissue dehydration and cell death, accelerating angiogenesis, and reducing dead tissue and fibrin buildup (Junker et al., 2013). It is important to provide adequate blood supply, tissues, and white blood cells for debridement and decontamination (Vivcharenko and Przekora, 2021). Oxygen perfusion increases collagen synthesis and angiogenesis, which improves wound healing (Yip, 2015). Wound healing is also influenced by age, nutrition status, systemic diseases such as diabetics, medication use, chemotherapy and most usual bacterial infection. Additionally, the size and depth of the wound play crucial roles in determining the rate and efficacy of healing. These factors collectively contribute to the complexity of wound management and crucial for effective wound management. The selection of wound dressing products is very difficult because of the wide range of dressing and materials available on the market depending in the cause and type of wound. As part of the modern wound dressing category, there are passive, interactive and bioactive products made of synthetic polymers. In passive products, the wound is covered with non-occlusive materials, such as gauze and tulle, which restore its function. As a barrier against bacterial penetration into the wound environment, interactive dressings are semi-occlusive or occlusive, for instance, films, foam, hydrogel and hydrocolloids (Dhivya et al., 2015).

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Nowadays, the biodegradability and biocompatibility characteristics of films fabricated using solvent cast method have advantageous for drug delivery to moist surface including wounds, nasal, vaginal, and buccal cavities (Ayensu et al., 2012; Kianfar et al., 2012). In order to promote healing in shallow wounds, an ideal film dressing must be flexible, homogeneous and smooth (Boateng et al., 2009). They provide a simple and effective way to create moist wound environment. The flexibility of films makes them easy to apply, especially around joints and other difficult areas. The dressings are made of transparent polyurethane that allows water vapour, oxygen, and carbon dioxide to pass through and provides autolytic debridement of the eschar, while being impermeable to bacteria (Dhivya et al., 2015). In the beginning, films were made from nylon derivatives that were supported by polyethylene frames, which made them occlusive. Nylon-derived film dressings have limited absorption capacities causing maceration of wounds and healthy tissues around the wound, hence, limited use for highly exudating wounds at first (Dhivya et al., 2015). However, transparent films permit inspection of wound closure without the need to remove the wound dressing. They are highly elastic and flexible, allowing conformity to any shape without the need for additional taping. Hence, the dressings are suitable for superficial, and shallow wounds with low exudates. Various film dressing differs in terms of their conformability, vapour permeability, adhesion, absorbability and extensibility among other characteristics (Ezzelarab et al., 2019). Biomaterials may provide a solution to the shortcomings of film dressings.

In modern wound dressings, bioactive dressings are made with biomaterials that play an important role in the healing process. In addition to being biocompatible, biodegradable, and nontoxic, these dressings are generally made up of natural tissues or synthetics such as collagen, hyaluronic, chitosan (CS), alginate, and elastin

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(Habibovic and Barralet, 2011; Ishihara et al., 2002). Depending on the wound nature and type, polymers of these materials may be used alone or combined. The healing process of wounds can be enhanced by incorporating growth factors and anti-bacterial agents in biological dressings. Researchers have noted that collagen is an important structural protein that plays a crucial role in healing natural wounds (Mathew-Steiner et al., 2021). Collagen stimulates fibroblast activity and endothelial migration when it comes into contact with wound tissue (Mian et al., 1992). CS, on the other hand, promotes the growth of granulation tissues during wound healing. It is derived from the N-deacetylation of chitin, a marine polysaccharide. Among its unique properties is its ability to be biocompatible, biodegradable, anti-bacterial, and highly adhesive. However, one of the characteristics of CS-pure scaffolds is usually a low strength property, limiting its clinical application (Xianzhen Dong et al., 2020). A hybrid system of bioactive glass and poly(vinyl alcohol) and CS scaffolds, designed by Mansur and Costa (Xianzhen Dong et al., 2020) showed improved physical, mechanical, and biological properties.

Bioactive glass (BG) is the first man-made inorganic material to be used in bone tissue engineering due to its excellent ability to promote osteogenesis properties (Jones, 2015; Yang et al., 2015). In recent years, BG has also been used in soft tissue engineering. BG is composed of silicon dioxide (SiO_2), sodium oxide (Na_2O), calcium oxide (CaO), phosphorus pentoxide (P_2O_5) in different ratio for different composition and the first synthesised composition was 45S5 Bioglass® by Prof. L. L. Hench (Hench, 2006). A reaction on the surface of BG releases soluble ions such as silicon (Si), calcium (Ca^{2+}), and phosphate (PO_3^{4-}) (Jones, 2015). The ionic products of BG have been shown to strongly affect cell behaviour, notably promoting osteogenic differentiation in stem cells as well as endothelial cell vascularisation, which

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ultimately stimulates osteogenesis and angiogenesis (Gorustovich et al., 2010). In recent years, it has been shown that BG stimulates angiogenesis and increases the secretion of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hypoxia-inducible factor-1 alpha (HIF-1α), from fibroblasts (Gorustovich et al., 2010). The importance of these angiogenic growth factors for wound healing has been well established.

1.2 Problem statement

Globally, skin disruptions and wounds, whether chronic or acute, pose a threat to patients' welfare present a substantial challenge to healthcare systems. The wound management alone is estimated to cost the National Health Service (NHS) 8.3 billion dollar annually, with approximately 3.8 million people in the UK suffering from chronic wounds (Hoang et al., 2022). In Malaysia, the cost of wound management is estimated to be 0.5127 dollar billion annually (Queen and Harding, 2023). The contributing factors include the rising prevalence of diabetes and obesity, and an aging population. In diabetes patients, ulcers can develop up to 25% of the time, further complicating wound management (Naves, 2016). The global market for advanced wound care is projected to exceed £14.8 billion by 2024, driven by advancements in technology and an increasing demand for effective wound management solutions (Guest et al., 2015).

For many years, traditional dressings such as cotton wool, lint, gauze have been commonly used for wound care, primarily to keep wounds clean and prevent bacterial infections. However, these dressings often stick to granulation tissue, causing pain during removal and failing to maintain a suitable moist environment for optimal

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healing. In contrast, modern wound dressings have emerged as the preferred choice for treating various wound types due to their exceptional biocompatibility and biodegradability (Nguyen et al., 2023). These advanced dressings are engineered to provide optimal healing conditions by regulating temperature and moisture levels, offering pain relief, enhancing hypoxic environments, protecting wounds from external bacteria, and preventing cross-infection. They are specifically formulated to facilitate and manage key phases of the wound healing process, including inflammation, proliferation, and remodelling. Currently, several types of modern dressings are utilised in clinical practice, including hydrocolloid, alginate, hydrogel, foam, and film dressings (Nguyen et al., 2023). For example, alginate and hydrogel dressings are non-adhesive, causing less pain during dressing changes and thus addressing the limitations of traditional dressings. In conjunction with their advantages, many studies on modern dressings such as foams, hydrogels, alginates, hydrocolloids, and films are carried out to solve clinical problems in treating wounds.

Given these advantages, extensive research is focused on modern dressings such as hydrogel, alginates, hydrocolloids, foams, patches, and films to solve clinical problems in wound treatment (Xin Dong et al., 2017; Kataria et al., 2014; Kuddushi et al., 2020; Labovitiadi et al., 2013; Rezvani Ghomi et al., 2019). These dressings not only ensure a conducive environment for wound healing but also significantly enhance patient comfort and recovery outcomes. Developing cost-effective and efficient wound dressings is crucial to meet the growing medical needs and reduce the financial burden on healthcare systems. Despite their numerous advantages, modern wound dressings face several limitations. The production process for these advanced materials is often complex, and there are significant challenges in ensuring the consistent quality of biological materials used in these dressings. Additionally, the effectiveness of the

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individual components in a clinical setting is not always well-established, hindering their widespread utilisation. Thus, material scientists are increasingly drawn to explore this field, undertaking extensive trials and experiments to rigorously evaluate the actual efficacy of modern wound dressings in promoting wound healing.

Therefore, in this present study, a BG/polymer composite film was developed using solvent casting technique. As BG and CS has anti-bacterial and cell proliferating properties, this composite is well suited for wound dressings, while Poly-Ɛ-caprolactone (PCL) provides mechanical stability. This fabricated composite film has a cell-proliferating component that can facilitate the transition from an inflammatory phase to a proliferative phase. The anti-bacterial component may facilitate this process. As well as helping the film in terms of tensile strength during application, the mechanical part may assist in degradation after application. Consequently, it is likely to have promising properties in terms of promoting wound healing, improving patient compliance, shortening the medical care, and reducing treatment costs. Shortening the medical care in wound healing can lead to significant cost savings for both patients and healthcare systems. Reduced hospital stays, fewer medical visits, and lower use of medical supplies, all contribute to cost-effectiveness.

1.3 Objective of the study

1.3.1 General objective

This study aimed to develop and characterise BG/polymer composite film for potential application in wound healing.

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1.3.2 Specific objectives

a) To synthesise 45S5 and 50S8P BG powder using sol-gel method and subsequently, characterise in terms of structural, chemical and morphological properties.

b) To fabricate BG/polymer and polymer blend composite films using solvent casting technique and subsequently, characterise the composite films in terms of structural, chemical, morphological and mechanical properties.

c) To investigate the *in-vitro* bioactivity by immersing the BG/polymer and polymer blend composite films into simulated body fluid (SBF) solution.

d) To investigate the *in-vitro* proliferative activity and wound healing potentiality the BG/polymer and polymer blend composite films towards human primary dermal fibroblast (HPDF) cells.

e) To investigate the *in-vitro* anti-bacterial activity of the BG/polymer and polymer blend composite films towards *Staphylococcus aureus* bacterial strain.

f) To investigate the *in-vivo* biocompatibility of the BG/polymer composite film for potential soft tissue regeneration by transplanting the composite film onto the rabbit skin.

g) To validate the soft tissue regeneration potential of the BG/polymer composite film through histological evaluation using Haematoxylin and Eosin (HE) staining and Masson's Trichome (MT) staining.

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1.4 Research questions of the study

a) Will the 45S5 and 50S8P BG powder in combination with CS and PCL components able to form composite film using solvent casting method?

b) Does BG/polymer and polymer blend composite films become bioactive while being immersed in SBF solution?

c) Does the BG/polymer and polymer blend composite films induce the proliferation of HPDF cells *in-vitro*?

d) Do BG/polymer and polymer blend composite films possess *in-vitro* wound healing potential towards HPDF cells?

e) Does BG/polymer and polymer blend composite films possess anti-bacterial activity against *S. aureus* bacterial strain?

f) In terms of physical, chemical, mechanical, and morphological characterisation as well as bioactivity, proliferative activity, anti-bacterial investigations, which of the fabricated BG/polymer and polymer blend composite films holds the greatest potential as wound dressing?

g) Would BG/polymer composite film be as biocompatible as the commercial wound dressing for the potential for soft tissue regeneration?

1.5 Hypothesis of the study

a) The 45S5 and 50S8P BG powder with CS, and PCL components, is able to form composite film using the solvent casting method.

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b) The BG/polymer and polymer blend composite films is able to exhibit bioactivity when immersed in SBF solution.

c) The BG/polymer and polymer blend composite films induce the proliferation of HPDF cells *in-vi*tro.

d) The BG/polymer and polymer blend composite films show *in-vitro* wound healing potential towards HPDF cells.

e) The BG/polymer and polymer blend composite films possess anti-bacterial characteristics against the *S. aureus* bacterial strain.

f) Based on the physical, chemical, mechanical, and morphological characterisations, as well as bioactivity, proliferative activity, and anti-bacterial investigations, it is hypothesised that a specific fabricated BG/polymer composite film will demonstrate the greatest potential as a wound dressing material.

g) The BG/polymer composite film will exhibit biocompatibility in rabbit skin comparable to that of a commercial wound dressing product, indicating potential for soft tissue regeneration.

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1.6 Flow chart of the study

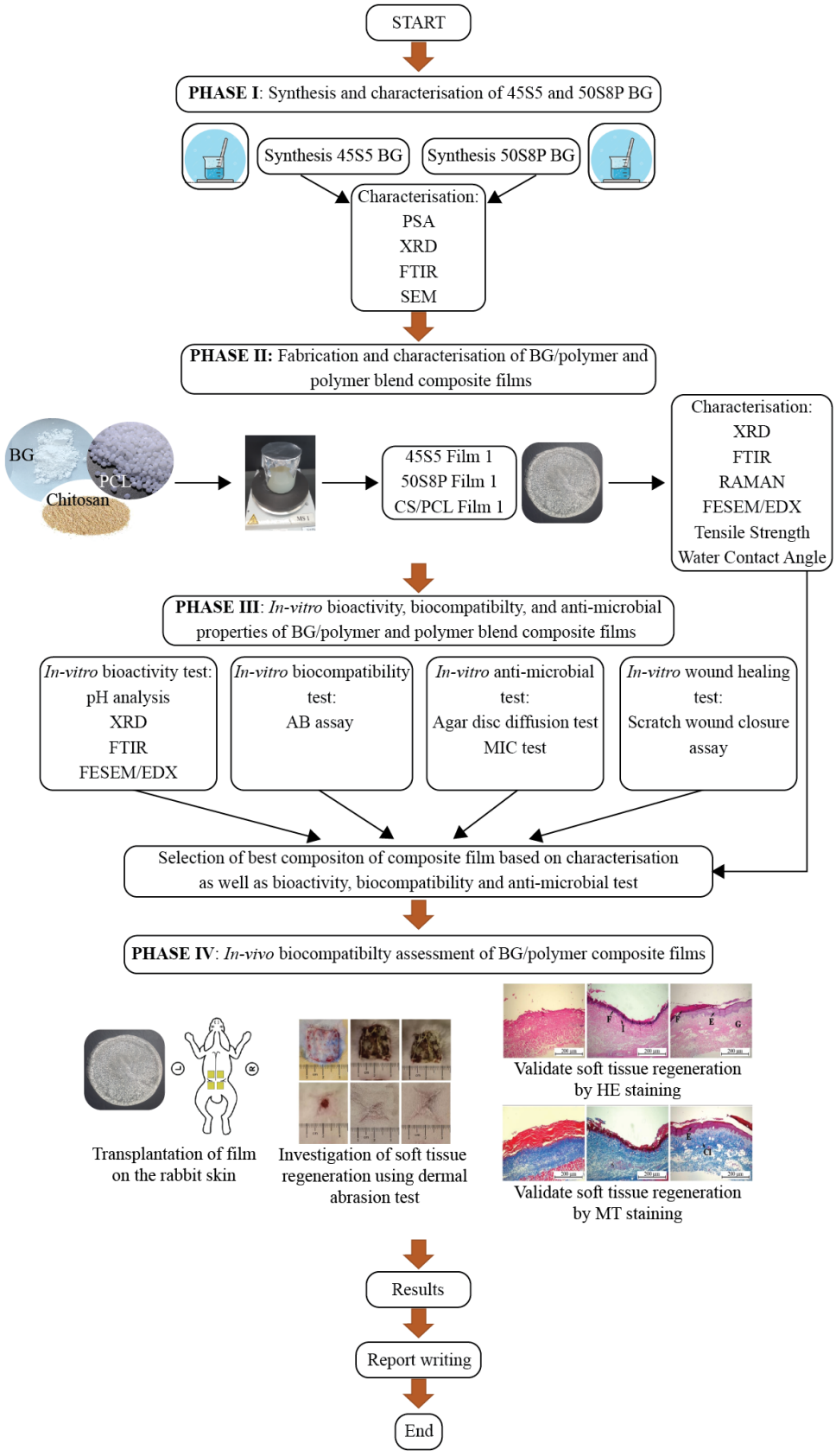

Figure 1.1: Flow chart of the study

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1.7 Scope of the study

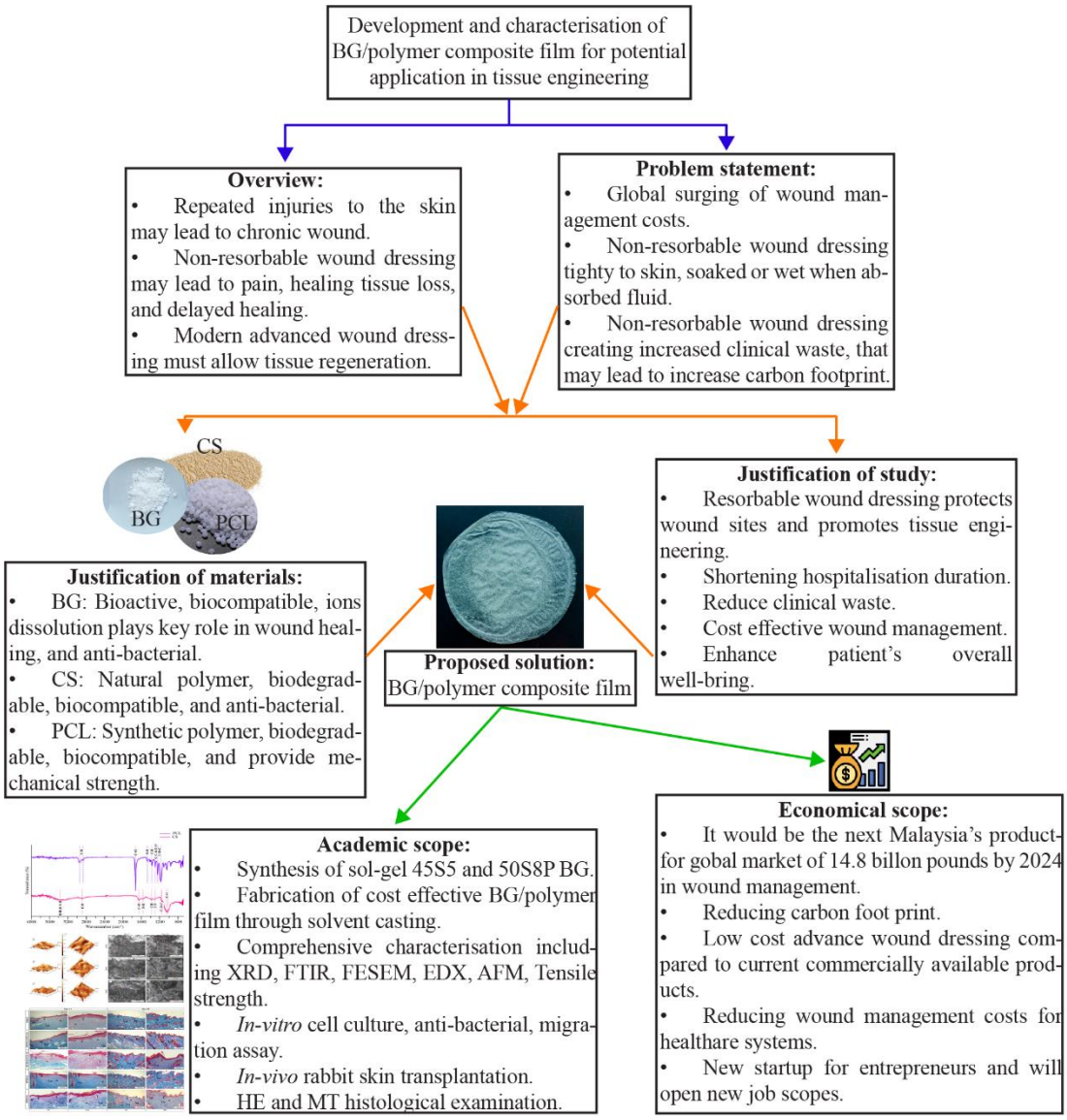

Figure 1.2: Scope of the study

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CHAPTER 2

LITERATURE REVIEW

2.1 Tissue engineering

Tissue engineering constructs have emerged as a promising approach for the functional restoration of tissues, addressing the human body's limited ability to regenerate organs (Han et al., 2020). Strategies for tissue regeneration, aimed at replacing or repairing damaged tissues, encompass cell therapy, growth factors, and biomaterial scaffolds, either independently or in combination. The field of tissue engineering, which began in the early 1990s, is defined as “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function” (Han et al., 2020). This discipline is fundamentally based on the "Tissue Engineering Triad," consisting of three essential components: cells, biomaterial scaffolds, and stimuli. Each of these elements is crucial for the successful application of tissue engineering principles.

The matrix or scaffold plays a crucial role in supporting living cells and directing the development and regeneration of new tissue. These scaffolds can originate from natural or synthetic materials, or a combination of both. The cells can be seeded onto the scaffold prior to implantation, or they may migrate into an acellular scaffold post-implantation. In both instances, the scaffold induces microenvironmental stimuli that influence cell behaviour. In the field of tissue engineering, biomaterials are employed to provide micro and nanostructural characteristics, morphology, and surface properties that support cellular activity. Additionally, these biomaterials can be loaded with specific growth factors. When used as matrices or scaffolds, biomaterials can be

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engineered with precise designs to direct cell growth effectively (Chan and Leong, 2008).

Current strategies for tissue regeneration often fall short of achieving satisfactory results, prompting the development of novel approaches to address these challenges. Animal studies have shown that biomimetic scaffolds can effectively support the regeneration of damaged tissues (Paltanea et al., 2023). These engineered scaffolds must not only perform the necessary functions for the specific tissue but also restore it with accurate cellular phenotypic expression. In addition to genetic factors, the cellular microenvironment within the body plays a crucial role in determining cell function. Understanding the role of the microenvironment is vital for designing and fabricating scaffolds that mimic the native extracellular matrix (ECM). The primary function of a scaffold is to facilitate cell growth in three dimensions (3D), which is essential for all tissues and organs. To engineer a functional scaffold that mimics the characteristics of native tissue, it is necessary to incorporate key physical and biological factors into the scaffold to create an optimal healing environment (Norouzi et al., 2024). Figure 1 provides a schematic illustration of the essential factors in the design and development of tissue engineering substitutes. Moreover, Bioreactors are instrumental in this process, offering a controlled chemical, electrical, or mechanical environment for cell-seeded scaffolds or standalone cells. This controlled environment facilitates the growth and remodelling phase of the constructs during in vitro studies or prior to implantation. By enhancing the constructs' characteristics in this manner, bioreactors significantly improve their success rates following implantation (Norouzi et al., 2024).

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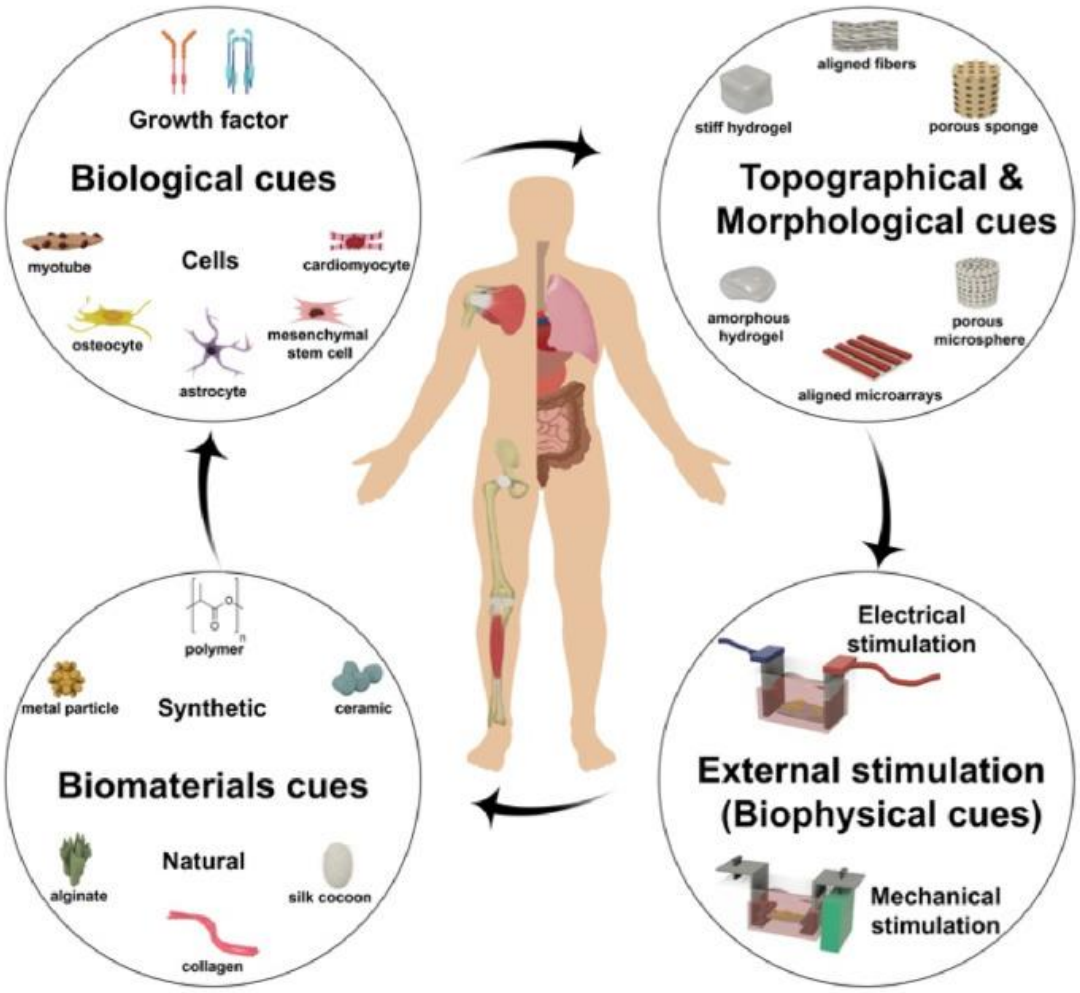

Figure 2.1: Schematic illustration of the essential factors in the design and development of tissue engineering substitutes. (Adapted from Norouzi et al., 2024)

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2.2 The anatomy of human skin

The skin is a complex and vital organ that plays multiple roles in protecting the underlying tissues from environmental stresses and facilitating sensory perception. Skin serves as a physical barrier, excluding potential pathogens and external stressors while also ensuring homeostasis at a fundamental level. Skin regulates body temperature through various mechanisms such as sweating, vasoconstriction, and vasodilation. Additionally, when exposed to ultraviolet radiation from the sun, the skin synthesises vitamin D, which serves as a semipermeable barrier to fluid entry and loss. Sensory receptors on the skin enable the perception of temperature, pressure, vibration, and injury. Immune cells are also present within the skin, which contributes to immunity development.

The adult human skin is divided into three distinct layers (Figure 2.2). The stratified squamous epidermis is the outermost layer and is largely composed of keratinocytes, melanocytes, Langerhans cells, sensory Merkel cells, as well as pigment-producing melanocytes. On the lower side of the epidermis is a basement membrane that separates it from the underlying dermis. There is an extracellular matrix (ECM) rich dermis that provides mechanical support for the epidermis as well as nerve fibres, blood vessels, and sebaceous units. The hypodermis is an area of loose connective tissue and fat, known as subcutaneous adipose tissue, beneath the dermis (Hofmann et al., 2023).

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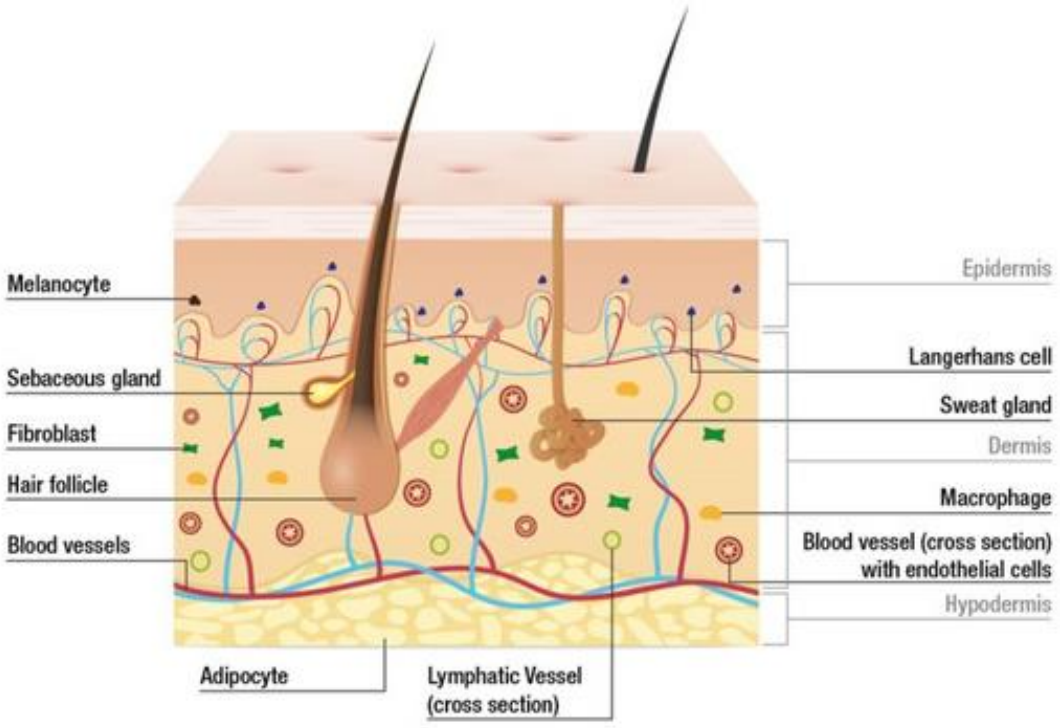

Figure 2.2: Schematic illustration of structural details of human skin. The skin is composed of three distinct layers: the epidermis, dermis and hypodermis. (Adapted from: Hofmann et al., 2023**)**

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2.2.1 Epidermis

Epidermis is the outermost layer of the skin and protects the body from external factors such as ultraviolet radiation, pathogens, chemicals, and water loss. As a stratified squamous epithelium, it is composed of several distinct layers with different functions and characteristics. The outermost layer is the stratum corneum, which is composed of several layers of dead, flat cells called corneocytes. It is believed that the stratum corneum acts as a barrier to water loss and prevents harmful substances from entering the body by providing keratin, a fibrous protein rich in strength and waterproofing properties (Srivastava et al., 2018). In addition to maintaining skin hydration and regulating body temperature, it also protects the skin from harmful substances. The stratum corneum is accompanied by the stratum lucidum, which is found only on thick skin areas such as palms and soles of the feet. There are translucent cells in this layer that do not have nuclei and organelles (Wertz, 2013). The stratum granulosum is the next layer, where keratinocytes undergo a significant change in their composition and structure (Houben et al., 2007). These cells contribute to the overall thickness and toughness of the skin in these regions. Keratohyalin granules are produced and accumulated by these cells, and they aid in the growth of keratin fibres. In addition to its role in the production of lipid-rich products, the stratum granulosum also contributes to the formation of lamellar bodies (Wertz, 2013). The lipids in the stratum corneum serve to strengthen the skin's barrier function by preventing the loss of water and maintaining skin hydration. There is stratum spinosum below the stratum granulosum, which is characterised by its spiny appearance under a microscope. There are several layers of keratinocytes that are connected to each other through desmosomes, specialised cell junctions that provide the epidermis with mechanical strength (Figure 2.3). In addition to being metabolically active, stratum spinosum cells

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contribute to the synthesis of structural proteins such as keratin. The stratum basale is also known as the basal layer or germinativum and is the deepest layer of the epidermis (Lee and Hwang, 2002) During active cell division, cuboidal or columnar cells replenish the upper layers of the epidermis in this layer. It contains basal cells that produce keratinocytes, as well as melanocytes that produce melanin to protect the skin from ultraviolet rays and to give it its colour (Proksch et al., 2008).

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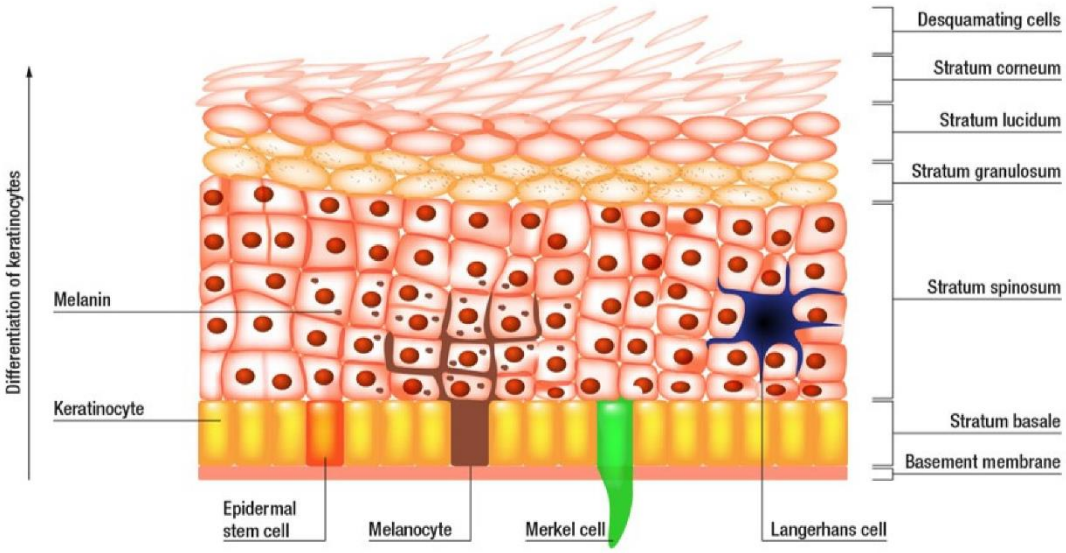

Figure 2.3: Schematic illustration of epidermal structure layers in details. (Adapted from: Hofmann et al., 2023)

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2.2.2 Dermis

Dermis, the middle layer of the skin, which is located between the epidermis and hypodermis. Dermis, which is primarily composed of connective tissue, plays a critical role in supporting the skin structurally, ensuring elasticity, and ensuring integrity of the skin (Reihsner et al., 1995). Papillary dermis and reticular dermis are the two distinct layers of the dermis. Papillary dermis consists of collagen and elastic fibres arranged loosely in the epidermis adjacent to it. This region contains dermal papillae which protrude into the epidermis, resulting in ridges that interlock with those of the epidermis, assisting in forming the fingerprints as well as enhance the gripping ability of the skin. Papillary dermis contains numerous blood vessels, lymphatic vessels, and sensory nerve endings that provide nutrients, remove waste, and perceive the environment (Zeng et al., 2017). An irregular layer of dense connective tissue lies beneath the papillary dermis known as the reticular dermis, which is thicker and denser than the papillary dermis. This layer is vital for providing strength, support, and elasticity to the skin. Among its primary constituents are collagen and elastin fibres, which are synthesised and maintained by dermis fibroblasts, comprising of larger blood vessels, nerves, hair follicles, sebaceous glands, sweat glands, and mechanoreceptors (Zeng et al., 2017). Inflammation responses, tissue repair, and collagen synthesis occur in the dermis, which is crucial to wound healing (Barbieri et al., 2014). Since collagen provides tensile strength and elastin provides elasticity, the dermis plays an important role in maintaining the integrity and functionality of the skin due to its complex structure and functions (Barbieri et al., 2014). It regulates body temperature by dilation and constriction of blood vessels.

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2.2.3 Hypodermis

Hypodermis is a deep layer of skin located below the dermis, often referred to as the superficial fascia or subcutaneous layer. The hypodermis is composed primarily of adipose tissue and serves a variety of important functions in the body. It serves as a cushioning layer, providing insulation, protection, and energy storage (R. Wong et al., 2016). The primary components of the hypodermis are adipocytes, or fat cells. Adipocytes are able to store triglycerides, which provide energy to the body when required. Adipose tissue is located in the hypodermis and plays role as a thermal insulation system (Ramakrishnan and Boyd, 2018). Aside from adipose tissue, the hypodermis also contains blood vessels, lymphatic vessels, and nerves which supply the skin and underlying tissues. It is believed that blood vessels in the hypodermis are essential for nutrient supply and waste removal, while lymphatic vessels assist with drainage of excess fluids and immune surveillance (Klar et al., 2017). In addition to providing stability and allowing movement and flexibility, the hypodermis also functions as a protective layer, cushioning the body against mechanical impacts and reducing injury risk (Diegel et al., 2018). It also connects the skin to underlying structures such as muscles and bones.

2.3 Types of wounds

In accordance with the Wound Healing Society, wounds are caused by disruptions of normal anatomy and function (Boateng et al., 2008). They are usually caused by physical or thermal damage or underlying physiological or medical conditions that lead to a rupture or defect of skin or body tissue (Boateng et al., 2008). It is also possible to define wounds as conditions in which homeostasis is quickly restored, as

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well as a discontinuity within the body (Enoch and Leaper, 2008). These wounds are classified into different categories according to their nature of repair and the underlying skin layers that have been affected (Boateng et al., 2008).

2.3.1 Nature of repair process

Wound healing is a complex process that involves multiple phases that will be discussed in the subsequent section. Wounds can be classified as acute or chronic depending on the type of repair process (Boateng et al., 2008).

2.3.1(a) Acute wounds

Acute wounds are usually caused by tissue injury, such as trauma or inflammation, and generally heal within eight to 12 weeks with minimal scarring (Boateng et al., 2008). Acute wounds are mechanical injuries caused by external influences, including an abrasion, an incision, a laceration, a degloving injury, or an ulcer. Additionally, surgical cuts, chemical injuries (burns), and thermal burns are included along with penetrating wounds (Kumar and Leaper, 2005).

2.3.1(b) Chronic wounds

A chronic wound is generally the result of tissue injury and usually takes beyond 12 weeks to heal. They often reoccur as a result of repeated tissue damage or underlying physiological conditions (Boateng et al., 2008). Chronic wounds are more difficult to treat than acute wounds due to the many complicating factors that accompany them. A number of factors contribute to this condition, including diabetes, malignancies,