FUNCTIONAL BIO-BASED MATERIALS FOR REGENERATIVE MEDICINE:

FROM BENCH TO BEDSIDE - PART 1

Editors:
Mohd Fauzi Mh Busra
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Functional Bio-based Materials for Regenerative Medicine: From Bench to Bedside (Part 1)

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PREFACE I

I would like to congratulate all the authors who took their valuable time to contribute to the book titled "FUNCTIONAL BIO-BASED MATERIALS FOR REGENERATIVE MEDICINE from Bench to Bedside". This book is a dedication to the founder of the Centre for Tissue Engineering and Regenerative Medicine (CTERM), University Kebangsaan Malaysia, Prof. Dato' Dr Ruszymah Hj. Idrus. It has been an honour to have been under her supervision and now expanding on our own across the continents through collaborating with talented, hardworking scientists in the tissue engineering and regenerative medicine field on various state-of-the-art technologies and biobased materials used for the tissue-engineered skin, bone, heart, respiratory tract and other vital organs.

In this marvellously insightful book, the authors offer some valuable strategies, technologies and artificial intelligence in the established technology covering tissue engineering as an essential field for various health applications to help not only scientists, researchers and healthcare providers but also the general public to remain updated with the current work happening worldwide.

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PREFACE II

The regenerative medicine is currently an established technology, covering tissue engineering as a significant field in various health applications. Traditionally, tissue engineering is a combination of cells, biomaterials, and biomolecules to replace or repair damaged tissues. The current advanced technology in the tissue engineering field focuses on the functionalisation of invented bio-based materials. It includes the development of a hybrid or composite biomatrix together with cells-secreted products and related active compounds for better performance in enhancing the rapid therapeutic mechanism. The mode of action for the invented product depends on its formulation or a three-dimensional design of the biomatrix.

This book provides detailed information on the latest advancements in bio-based materials treatment strategy for various tissue applications, encompassing skin, bone, heart, respiratory tract and other vital organs. This is followed by future perspectives on the new emerging treatment in the field of tissue engineering and regenerative medicine.

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CHAPTER 1

Acellular Strategy of Functional Biomaterials for Tissue Wound Healing

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Abstract: The skin is known as the largest organ in the human body as it functions to regulate the temperature in the human body and acts as the first-line defence. The skin consists of two layers: the epidermis (the outer layer of skin) and dermis layers (the inner layer of the skin) occupied by specific skin cells. Whenever the skin barrier is compromised, the skin heals following four phases: haemostasis, inflammation, proliferation, and remodelling. Wound healing takes a few weeks for acute wounds, however it takes a longer period to heal chronic wounds. Chronic wound complication extends the inflammation phases during the wound healing process and becomes a significant problem in the healthcare field. Therefore, various treatments were produced to reduce the healing time in chronic wounds and produce less scarring. Acellular treatments have gained attention in wound healing research as these treatments have a lower risk of rejection and are easily obtained through nature or lab. Acellular treatments include growth factors, bioactive molecules, and peptides that are clinically proven to have faster healing time and reduce scarring as these treatments are readily available in the market. Biomaterials have become a novel study in wound healing research due to their vast potential as alternative treatments for skin wound healing. Therefore, the chapter discussed the acellular strategies for tissue wound healing.

Keywords: Functional biomaterials, Skin, Tissue engineering, Wound healing, Wound treatment.

INTRODUCTION

Skin is the largest organ in the human body. It can weigh up to 15% of the adult's total body weight with a surface area of up to 2 m^2 [1]. The skin acts as our body's

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initial barrier against potential insults from the exogenous environment, such as pathogens, UV light, hazardous chemicals, and mechanical injuries [2]. Besides that, humans can sense touch, temperature, and pain *via* skin sensations, which allows us to perceive signals from the outside world. The diffusion of substances like oxygen, carbon dioxide, and topical drugs into the human body is also made possible by the skin.

Generally, the human skin is constituted of the epidermis and dermis layer (Fig. **1**). The epidermis and dermis layer are separated by a layer of basement membrane formed by an interconnected network of extracellular matrix (ECM) macromolecules [3]. Besides that, the epidermal rete ridges and dermal papilla are both distinct microarchitectures that can be found between the epidermis and dermis layers. These structures combine together and exhibit an undulated wave-shaped pattern to form the dermal-epidermal junction (DEJ) [3]. Other than providing mechanical support, the basement membrane at the DEJ also allows cell-cell and cell-matrix interaction between the epidermis and dermis [4]. The epidermal cells recognise the basement membrane as an adherence site and utilise it as a reference layer to separate themselves from the dermis.

Epidermis Layer

The epidermis layer contains multiple layers of keratinocytes, which include the stratum basale, stratum spinosum, stratum granulosum, and the outermost stratum corneum. The stratum basale is accommodated with keratinocytes, continuously differentiating and proliferating from their stem cell progeny [5]. Next, keratinocytes in the stratum spinosum exhibit a polyhedral "spine" like morphology that extends and connects with the surrounding cells *via* desmosomes. The stratum granulosum possesses diamond-shaped keratinocytes that contain electron-dense keratohyalin granules [5]. The outermost stratum corneum layer comprises dead keratinocytes that travel toward the outer skin surface over time, forming a cornified layer of cells [6]. These cells then undergo a desquamation process and shed off from the skin. A complete cycle of keratinocytes progressing from the stratum basale to the stratum corneum takes approximately 28 days [7].

Dermis Layer

The dermis layer consists of two regionally distinct layers: the superficial papillary later and the deeper reticular layer. The papillary dermis is composed of loose connective tissue and is constantly interconnected to the epidermis *via* the DEJ [8]. The papillary dermis is mainly populated by fibroblasts, with the addition of immune cells like macrophages and neutrophils that are activated by pathogenic infections [8]. Thin collagen fibers along with elastic fibers can also

be found at this site [9]. On the other hand, the reticular dermis is thicker and deep within the skin. It comprises mainly dense connective tissues like thick collagen bundles and exhibits less cellular complexity [9]. The reticular dermis demonstrates a net-like structure contributed by the meshwork of fibers. Among the extracellular matrix found in the reticular dermis, collagen fibers provide tensile strength to the skin, while elastin fibers provide the skin with elasticity which enables movement. In addition, the collagen fibers can bind to the water molecules and retain the skin hydration.

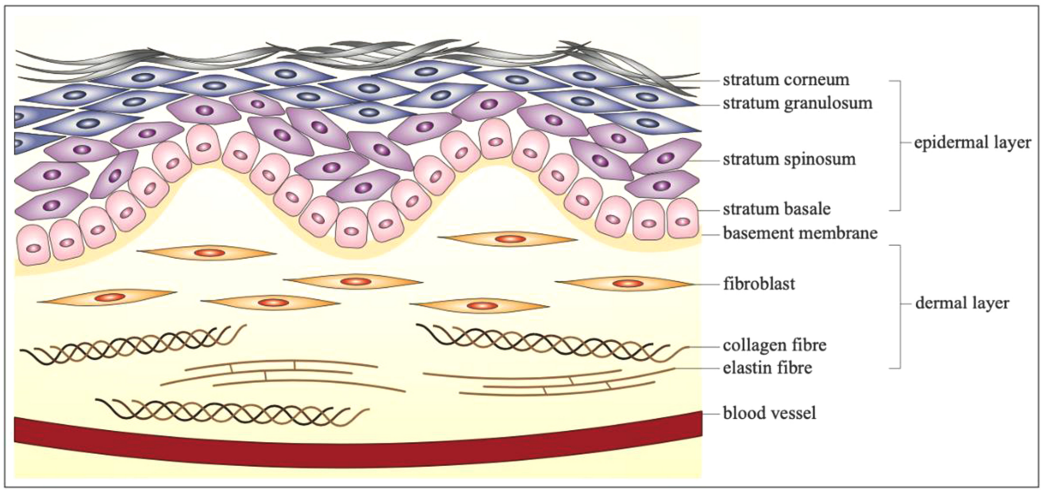

Fig. (1). Structure of the skin. The skin comprises the epidermal and dermal layers separated by the basement membrane. The epidermal layer is composed of four different layers of keratinocytes, the innermost layer is the stratum basale, followed by the stratum spinosum, stratum granulosum, and stratum corneum. The dermal layer contains fibroblasts as the primary cell type. Collagen fiber and elastin fiber can also be found in the dermal layer.

MECHANISMS OF WOUND HEALING

Generally, the wound healing process consists of four overlapping and continuous phases, which are haemostasis, inflammation, proliferation, and remodelling. The wound healing process involves the cellular components like macrophages, fibroblasts, keratinocytes, and the extracellular matrix produced by these cells.

Platelets are activated at the wound site upon skin injury to form aggregates [10]. These aggregates release clotting factors that promote fibrin deposition at the wound site [10]. The fibrin clot acts as a provisional matrix where the aggregated platelets are encapsulated within [11]. Growth factors are also released in this stage, which signals the cells to migrate to the wound site and perform their function [10].

CHAPTER 2

The Utilisation of Animal By-products for the Production of Potential Biomaterial in Tissue Engineering and Regenerative Medicine

Noor Amirrah Binti Ibrahim[1], Maheswary Thambirajoo[1], Nusaibah Sallehuddin[1], Mohd Fauzi Mh Busra[1] and **Manira Maarof[1,*]**

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Abstract: The development of biomaterials in tissue engineering has already started decades ago. A wide variety of biomaterials are being used as alternatives in clinical applications. Lately, animal by-products have increased in demand for natural substrates in various sectors. As in tissue engineering, animal-based biomaterials are from different resources or origins of animal species that are being studied and applied for disease treatments. In addition to this, novel biomaterials are being produced that could imitate the physiology of natural healing mechanisms or the regeneration of certain tissues. Thus, the efficiency in utilising animal by-products could alleviate the waste management cost and scarcity of materials, which could reduce environmental pollution. This book chapter discusses different classifications of animal byproducts, their unique characteristics, and the advantages of these products that could embark as new alternative approaches for treating diseases.

Keywords: Animal by-products, Biomaterials, Clinical applications, Regenerative medicine, Tissue engineering.

INTRODUCTION

Animal by-products are considered natural sources of biomaterials. They are advantageous for implants as they can be readily available, safe for the environment, and may have biomimetic properties in the cellular environment. In this regard, the field of regeneration through biomimetics, or mimicking nature, is expanding. However, there can be an issue of immunogenicity and instability, as natural products tend to denature or decompose easier, even when it has not reached their melting temperatures. Although this can provide limitations in mass

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production or fabrication in various sizes and shapes, it is important to emphasise that natural biomaterials have fewer toxicity issues than synthetic materials.

Additionally, beneficial functional groups, binding sites, and other biochemical signals might be integrated which can promote tissue regeneration. This chapter intends to highlight common sources of animal by-products such as fibrin, collagen, gelatin, elastin, chitosan, and other related animal by-products that were purified and manipulated. We discuss further animal by-products currently being used as biomaterials and summarise the areas of potential application in the tissue engineering and regenerative medicine field.

Utilisation of Fibrin, Fibrinogen, and Fibronectin

Fibrin is a scaffold formed after tissue injury from the action of thrombin on fibrinogen which initiates hemostasis and provides the initial matrix essential for cell adhesion, migration, proliferation, and differentiation [1 - 3]. Fibrinogen is a glycoprotein crucial to many biological processes, such as hemostasis, wound healing, inflammation, and angiogenesis [4]. Following tissue injury, proteins such as fibronectin and vitronectin, growth factors such as fibroblasts growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1, and enzymes such as plasminogen and tissue plasminogen activator (tPA) specifically bind to the fibrin matrix, initiating wound healing through specific receptor-mediated interactions with cells [3].

Fibrin is superior to collagen in initiating the proliferation and differentiation of osteoblasts [5]. In bone tissue engineering, fibrin is used as fibrin glue, bead, scaffold, coated on scaffold, or hydrogel. Fibrin can be fabricated either from the autologous or allogeneic origin, from fibrin precursors, which are fibrinogen and thrombin [6]. Nevertheless, fibrin possesses fast degradation and weak mechanical property; hence, fibrin is prepared in combination with other materials to eliminate these disadvantages [7].

Glue

Fibrin glue can be injected directly into bone defects or mixed with other cells or materials prior to injection. Autologous fibrin glue injected into mandibular defects of humans revealed increases in the growth of both fibroblasts and osteoblasts as well as the rate of revascularization [8]. Injection of platelet-enriched fibrin glue to autogenous bone grafts effectively enhanced the vertical alveolar ridge and maxillary sinus floor augmentation in dogs [9].

Bead

Implantation of fibrin microbeads-mesenchymal stem cells (MSCs) after cultured in an osteogenic medium into mouse skull defect successfully revealed bone-like tissue with a calcium/phosphorus ratio similar to the native bone after 2 months [10]. In addition, implantation of 3 mm periosteal cells encapsulated fibrin beads into ulna defects of rabbits showed bone healing after 28 days [11].

Coating Agent

Biphasic calcium phosphate (BCP) coated fibrinogen implanted in a rabbit calvarial model revealed a significant enhancement in bone healing. This enhancement was attributed to the increase in the formation of native fibrin clots, which improve the migration of fibroblasts and vascular cells into the wound area as well as increase the initial stability of ceramic particles [12]. Besides, Abiraman *et al*. revealed that the implantation of calcium phosphate and silicate-coated fibrin glue into the muscles of mice showed an increase in bone regeneration [13].

Scaffold

Santos *et al*. reported that the incorporation of fibrinogen into chitosan scaffolds improved bone formation in a critical-size bone defect in rats [14]. Besides, the implantation of BMSCs or calvaria-derived osteoblasts seeded within a fibrin matrix into the polycaprolactone (PCL) scaffolds into critical-size calvarial defects in rabbits showed an increase in bone regeneration [15].

Utilisation of Collagen and Gelatin

Collagen

One of the nutrient classes available that are obtained from animal by-products is protein. The major protein constituents of animal bones, skin, and connective tissues are collagen, which accounts for 25-33% of the total protein content in animals [16]. The most abundant sources of collagen from animal by-products are from the bovine hide, pigskin, and bones from cattle and pork; however, many researchers in recent times have also explored collagen properties of other sources such as ovine, goat, poultry, shellfish, and different species of fishes [17, 18]. In recent years, collagen has been used vastly in the medical field mainly to treat heart diseases, osteoarthritis, enuresis (involuntary urination), in tissue engineering as a skin substitute or biomaterial grafting, and to promote the formation of blood flow for diseases related to inflammation of the joints (arthritis), obesity and diabetes [19]. Among the 28 major types of collagen, Type

CHAPTER 3

Chitosan-Based Nanoparticles in Tissue Regeneration

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Abstract: Chitosan is a unique polymer owing to its cationic property that allows interactions with various biological entities and is subsequently produced into novel functional products for biomedical applications, including tissue regeneration. Its cationic nature is conferred by amino groups present in its structure that are also responsible for various properties, including antibacterial activity. Chitosan is a biomaterial that has been extensively used in tissue engineering due to its ability to facilitate three-dimensional (3D) cell growth and proliferation as well as organize the deposition of collagen, the important processes in wound healing. Moreover, chitosan is a biocompatible and biodegradable polymer, making it an outstanding material for tissue engineering applications. Besides, chitosan possesses biological or pharmacological activities such as hemostatic, antioxidant, antimicrobial, and anti-inflammatory, further expanding its biomedical applications. In tissue engineering, chitosan has been developed as scaffolds in the form of membranes, sponges, nanofibers, and hydrogels for treating various tissue damages. They are used to provide a suitable environment for supporting the growth of cells. In combination with nanotechnology, chitosan is converted into nanoparticles that possess unique properties and hence, they have been utilised in wound healing, cartilage, and bone regeneration. This chapter highlights the roles of chitosan-based nanoparticles in tissue regeneration along with their recent developments.

Keywords: Biomaterials, Drug delivery systems, Nanotechnology, Nanoparticle, Nanocomposite.

INTRODUCTION

Chitosan entered the pharmaceutical arena in the early 1990s, and since then, it has been extensively researched to generate various products based on it. Chitosan is a polysaccharide consisting of two units in a linear conformation; N-acetyl-d-glucosamine and D-glucosamine. Chitosan is derived from chitin, which is

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mainly obtained from the shells of crustaceans or fungal mycelia *via* deacetylation [1]. Chitosan has been a material of choice for the preparation of nanoparticles for the last few decades for various applications due to its non-toxic property. Chitosan has been regarded as safe and obtained U.S. Food and Administration (FDA) approval for wound dressings and dietary applications [2]. The presence of primary amino groups that are protonated in an acidic environment contributes to the cationic character (positive charge) of chitosan. Hence, it provides an option to produce nanoparticles using polyanions such as tripolyphosphate (TPP), dextran sulphate (DS), genipin, acrylic acid, and many other cross-linking agents. In addition, side chain attachment can be made to the hydroxyl groups of chitosan without affecting its biophysical properties [3]. The reactive hydroxyl group can also react covalently or non-covalently with various cross-linker reagents.

Chitosan Nanoparticles

Nanoparticles have recently emerged as one of the important technologies owing to their unique properties, particularly their large surface area to volume ratio that carries high surface reactivity, leading to their exploitation in biomedical applications [4]. Chitosan is one of the most used natural polymers in the synthesis of nanoparticles due to its attractive characteristics, including non-toxic, biodegradable, and biochemical properties as well as medicinal benefits such as having antimicrobial, hemostasis, and antioxidant effects (Fig. **1**). In combination with other agents that promote cell or tissue growth, synergistic or multi-action effects could be obtained for accelerating tissue repair or regeneration. These are the reasons for a wide range of applications of chitosan nanoparticles in the biomedical field.

Polymeric nanoparticles were first described in the late 80s, while chitosan nanoparticles were reported for the first time by Ohya *et al*. in 1994 [5]. In the study, chitosan nanoparticles were developed as a carrier for an anti-cancer drug, 5-fluorouracil. The chitosan nanoparticles were obtained by cross-linking the emulsified polymer with glutaraldehyde, a chemical crosslinking agent [5]. Many other methods have been described since then, including ionotropic gelation [6], microemulsion [7], emulsification solvent diffusion [8], and polyelectrolyte complex [6]. Cross-linking process of chitosan increases its viscosity, which is suitable for applications in wound dressing and tissue regeneration. Chitosan nanoparticles are also being used as delivery systems for various therapeutic agents, including drugs, proteins, and peptides (*e.g*., cytokines, growth factors) and genetic materials (*e.g*., plasmid DNA [pDNA], oligonucleotides, and small interfering RNA [siRNA]) in treating diseases and illnesses including cancer, and wounds as well as combating infections (Fig. **2**).

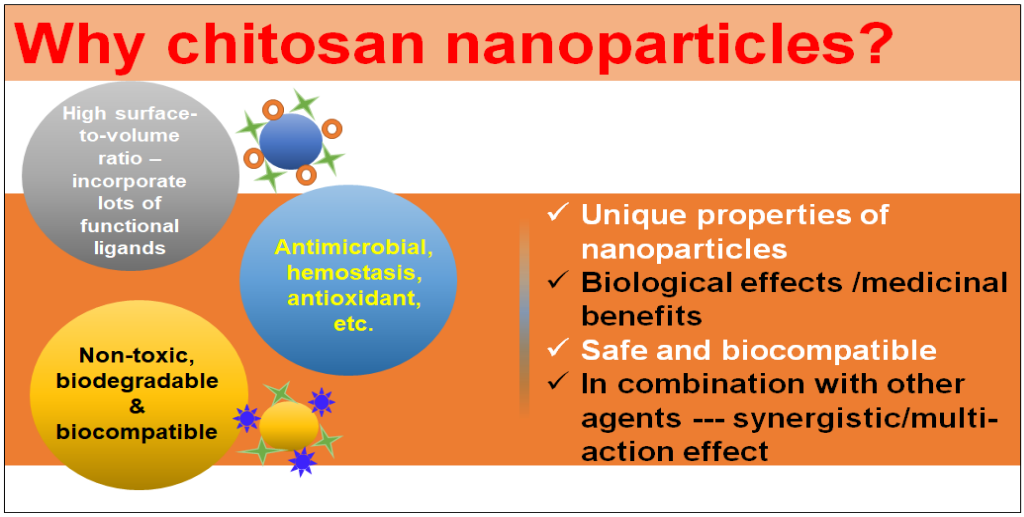

Fig. (1). The reasons for the wide range of applications of chitosan nanoparticles in the biomedical field.

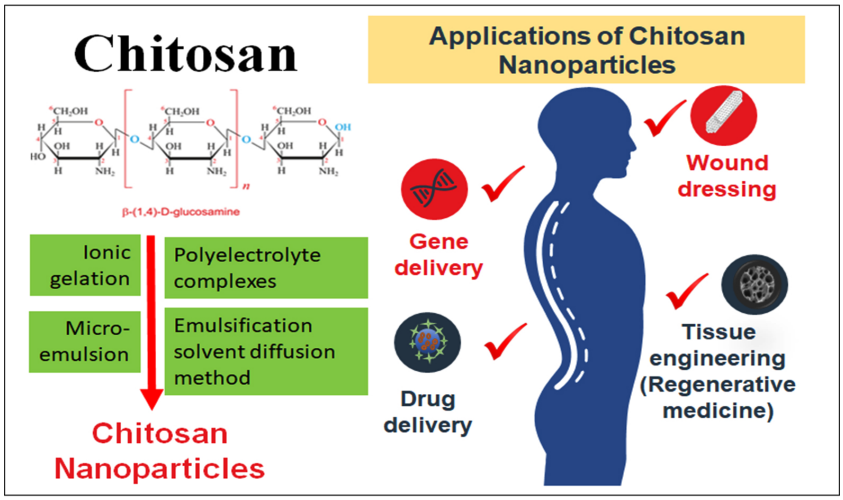

Fig. (2). Preparation methods and the applications of chitosan nanoparticles in the biomedical field.

The Roles

Chitosan has been developed primarily as the building component of scaffolds (*e.g.*, films, membranes, hydrogels, sponges, nanofibers) for tissue engineering applications. Chitosan in the form of nanoparticles has also been extensively used in tissue regeneration. Nanoparticles are defined as structures with a size ranging from 1 to 100 nm. Nevertheless, particles that are up to several hundred nanometers in size can also be categorised as nanoparticles [9]. Nanoparticles are generally used as drug delivery systems for achieving sufficient pharmacological

CHAPTER 4

Likes and Dislikes: Cell Preference in the Context of Biomaterials

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Abstract: Cell adhesion is a complex mechanism that involves a dynamic interaction between the cell surface protein and specific ligands. It has become a crucial part to be understood when it comes to cell adhesion to biomaterial, especially in the tissue engineering field. In this chapter, we narratively discussed the basic principle of cell adhesion and the factors that affect this process. The characterisation of cells on biomaterials has also been discussed, as well as their application in the tissue engineering context.

Keywords: Biomaterial, Cell adhesion, Cell signaling, Microenvironment, Tissue engineering.

WHAT IS CELL ADHESION?

Generally, cells need to be adhered to surfaces in order to survive. This adhesion mechanism has been taken over by cell membranes which consist of various proteins that are responsible for adhesion regulation. The dynamic process of adhesion between cell surface molecules and the appropriate ligands determines the strength of adhesion. Adhesion can be found between adjacent cells (cell-cell adhesion) as well as between cells and the extracellular matrix (ECM) (cell-matrix adhesion) [1]. Cell adhesion receptor molecules are also influenced by their microenvironment that consists of extracellular matrices and also neighboring cells. Those receptors are connected to specific transduction pathways when activ-

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ated by specific proteins, for instance, the integrin signalling pathway, which is regulated by ILK. Integrin can be activated *via* two different mechanisms, whether from the outside cell by multivalent binding of integrin with an extensive molecule network (*i.e.*, glycosaminoglycan and glycoconjugate) or from inside of the cells, which are regulated by ILK that consequently activates their downstream proteins such as GSK3 and Akt pathways.

Cell adhesion is an interaction mechanism between cell surface molecules with any surface biologically. Generally, adhesion molecules include integrins, cadherins, lectins, selectins, and Ig superfamily Cell Adhesion Molecules (CAMS). These proteins are typically transmembrane receptors consisting of three domains which are: an intracellular domain, a transmembrane domain, and an extracellular domain. Those molecules can connect the cells to each other in many different ways, for instance, *via* junctional complexes. Tight junctions build a seal between adjacent cells and are connected to actin filaments. Adherens junctions are plaques of classical cadherins linked to the actin cytoskeleton, while gap junctions connect the cytoplasm of two adjacent cells and are linked to microfilaments. In addition, cells also can connect to each other through desmosomal cadherins that are linked to intermediate filaments. Selectins and Ig-superfamily CAMs can promote homophilic adhesion outside of junctions, while the integrins can bind in a heterophilic manner (Fig. **1**).

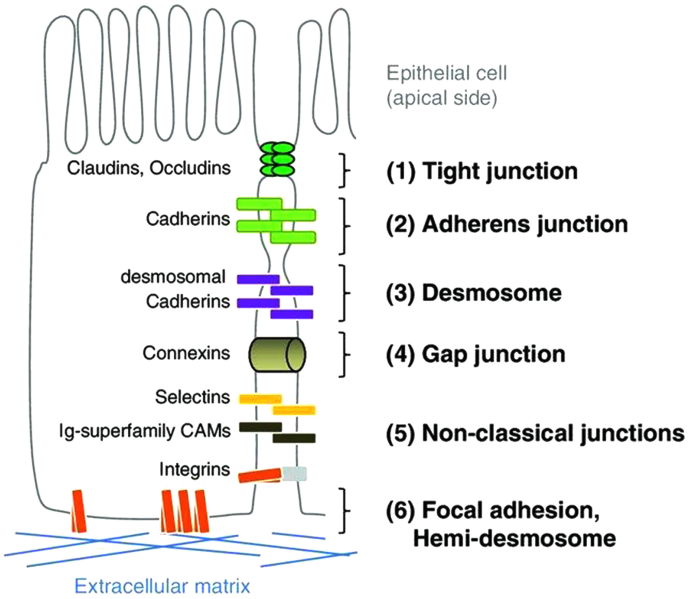

Fig. (1). Cell adhesion molecules (CAMs) and junctional complexes are abundant in epithelial tissues [2].

FACTORS AFFECTING CELL ADHESION

Cell adhesion is essential for regulating proliferation, differentiation, and phenotypic behavior, which affects tissue development and function. The interaction of cells with materials is a highly complex process that is influenced by a variety of factors [3]. Cell adhesion and proliferation on materials are affected by surface properties such as wettability (hydrophilicity/hydrophobicity or surface free energy), chemistry, charge, topography, and rigidity. According to a researcher Anselme [4], these surface properties specify how biological molecules adsorb to the surface and, more specifically, the orientation of adsorbed molecules.

Biomaterial surfaces with amino-, hydroxyl-, carboxyl-, sulfonic-, and acylamino groups promote cell adhesion and development. The cell adhesion, proliferation, and growth performance in the aqueous cell culture medium of the polymer surface grafted with the amine group are better than the hydroxyl group and the amide group because of their positively charged characteristics. Positively charged surfaces often promote cell adhesion, spread, and growth more effectively than negatively charged or neutral surfaces [5]. The degree of cell adhesion and proliferation can be affected by the hydrophilicity of a material surface.

The hydrophilicity of a substance was believed to influence the surface energy (surface tension), which regulated serum proteins that adhered to the material andin turn regulated biological responses, including cell adhesion and proliferation [6]. Despite the fact that hydrophobic surfaces bind more proteins, several cell studies have shown that cells bind and spread more efficiently on hydrophilic surfaces than on hydrophobic surfaces [7].

Surface topography and its chemical composition are essential factors in cell adhesion. Indeed, cell adhesion and proliferation are influenced by surface roughness. Surface roughness is classified into four grades based on the irregularities of the material surface; macroscopic roughness, microscopic roughness, submicron surface roughness, and nanometer roughness [8]. Different surface roughness has different effects on the cells. Since cells have enough space to spread and expand between macroscopic irregularities, macroscopic roughness has a little impact on cell adhesion behaviour. Micron and sub-micron surface roughness has a dual effect on cell growth and adhesion but has a positive effect. According to a researcher Zhao (2006) [9], the number of MG63 cells in a titanium disc with submicron surface roughness is less than the number of MG63 cells in a flat nanostructure. Nano-roughness is known to be the most similar to natural tissue morphology, and it is an ideal factor that promotes cell adhesion, development, and maturation. For example, in human venous endothelial cells,

CHAPTER 5

Injectable *In Situ* Hydrogels for Regenerative Medicine Applications

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Abstract: Regenerative medicine (RM) is a field of study that helps repair or restore native tissue function which has lost its functionality due to chronic diseases and trauma. The regeneration process can be promoted by constructing biomimetic systems, which can support cellular growth and proliferation. In this regard, the development of injectable hydrogels has gained enormous attention in recent times. An arrangement of cells and bioactive molecules in the three-dimensional extracellular matrix created by injectable gels is favorable for the regeneration of damaged tissues. Ideally, the injectable hydrogel remains in the solution form before injection and rapidly undergoes gelation at the physiological condition. A high water content, mechanical strength, scope of improved functionalization, injectability, and ease of implantation make the injectable hydrogel an ideal candidate for tissue-specific repair. This chapter aims to concisely summarize the mechanism and recent fabrication advancement of the injectable hydrogel that is being used in RM applications. A vast number of injectable hydrogels have been discovered for bone, cartilage, skin, and cardiovascular tissue regeneration, which are discussed in detail in the chapter. In gist, it is expected that injectable hydrogels will become a promising tool for a variety of tissue repair applications shortly.

Keywords: Cross-linking, Gelation mechanism, Injectable hydrogels, Regenerative medicine, Tissue engineering.

INTRODUCTION

In the last couple of decades, the use of hydrogels for the treatment, restoration, and replacement of tissues and organs has increased multi-fold. This has opened up a new field of medical research called Tissue Engineering (TE) and Regenerative Medicine (RM), which deals with regenerating damaged or diseased tissues and organs. The TE and RM technology usually employ biologics like

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cells, growth factors, and scaffolds that help in the regeneration process. Hydrogels, a type of scaffold, have come up as an effective treatment modality under regenerative medicine. Hydrogels are polymeric materials with the ability to swell and retain a substantial portion of water within their structure. They serve as artificial extracellular matrices (ECM) during the regeneration process. However, the use of hydrogels is not only meant for providing structural support but also to supply growth factors and nutrients to the growing cells. These polymeric architectures are also reported to control the spatiotemporal activities of the cells and, hence, help tune the properties of the regenerated tissues and organs [1]. The ECMs are the polymeric blends that form an interlocking mesh and are responsible for keeping the cells together in a tissue or organ.

The naturally-occurring ECMs are mainly composed of fibrous proteins and glycosaminoglycans (GAGs) [2]. Chemically, GAGs can be categorized as carbohydrates. Some of the common GAGs include chondroitin sulfate and heparin sulfate. The conjugation of the GAGs and ECM proteins is known as proteoglycans. Another carbohydrate-based polymer includes hyaluronic acid (HA). HA is a non-proteoglycan polysaccharide, which is the primary constituent of ECM in the load-bearing joints. The different proteins that are present in the ECMs include collagens, elastin, laminin, and fibronectin. Among these proteins, the collagen group of proteins is present in abundance in the ECMs. The adhesion of the cells to the ECM is controlled by the fibronectin and laminin proteins. These protein molecules bind with the integrin proteins of the cells through the cellular adhesion molecules (CAMs). In short, the cellular proliferation within the ECM is not only greatly reliant on growth factors and cell-to-cell interactions but also on the chemical structure and physical properties of ECM.

The polymeric architecture of hydrogels serves as artificial ECM (aECM). These porous polymeric structures allow the spatiotemporal growth of the cells. The diffusion of cellular wastes away from the cells significantly minimizes the cellular damage. This is possible as the aECMs allow easy diffusion of the nutrients and wastes within their architecture. The optimization of the diffusion process plays a significant role in designing aECMs. Accordingly, different researchers have proposed to develop aECMs with unique architectures for RM applications. Some of these architectures include hydrogels, scaffolds, nanofibers, and nanostructures. Many a time, the defect site has an irregular shape, and inserting an implant/scaffold requires a complicated surgical procedure.

However, the injectable hydrogel can be placed at the deeply located injured site in a minimally invasive manner [3]. The injectable hydrogels generally form *in*

situ hydrogels when injected into the human body. Injectable hydrogels are prepared as pre-formed extrudable hydrogels. *In situ* hydrogels are formed when precursor molecules are placed together at the site with required physiological conditions. Since all the *in situ* hydrogels are injectable, they can be placed under the broad category of injectable hydrogels. An injectable hydrogel can properly align to the margins of the damaged area and hence provide a better and faster recovery. The creation of aECM, having properties similar to the native tissue, further assists in the induction of cell growth and vascularization from neighboring tissue. The injectable hydrogels can evade the first-pass metabolism [4]. First-pass metabolism is a common terminology that is linked with drug metabolism, taking place in the liver. Due to this, there is a rapid decrease in the drug concentration before reaching the target site. As the injectable hydrogels are administered through subcutaneous or transdermal routes, the chances of them bypassing the first-pass metabolism are higher. The use of this type of formulation for the treatment of disorders has shown greater patient compliance. Besides the above-said advantages, injectable hydrogels provide an easy tool to efficiently fill up even complex voids. In the current chapter, advances in the field of injectable hydrogels toward different RM applications will be discussed.

Mechanism of Formation of Injectable Hydrogel

Very recently, researchers moved their focus to the hydrogel systems that are sensitive to physiological conditions. An injectable hydrogel is a category of hydrogel in which the precursor components are present in the form of a solution. Such systems are meant to be injected at the damaged tissue site using a syringe. The precursor undergoes a transition from solution to gel based on the physiological environments, thus forming a hydrogel. The transition can occur either due to physical gelation or chemical cross-linking. The gelation depends on the extent to which polymeric chain binds among themselves. In the physical gelation methods, two or more polymeric chains associate through weak physical bonds like hydrophobic, ionic, or simply self-assembly. However, the chemical cross-linking mechanism to form hydrogel occurs through the covalent bonding of polymeric chains. The mechanism for *in situ* gelation can comprise gel formation due to alteration in temperature or pH, cross-linking due to released ions in the system, or simply crystallization [5]. One common example of *in situ* hydrogel formation is chitosan and polyphosphate systems [6]. Chitosan is found to be soluble in an acidic solution, which is later neutralized by the addition of polyphosphates. The system remains in the liquid state at a lower temperature and converts into a gel at physiological temperature when injected subcutaneously. While injecting, the elastic modulus (G') of the solutions should be lower than the storage modulus (G”) to assist fluidity. However, once injected, the G'>G” will support gel formation [7]. Further, the functionality of the injectable hydrogel can

CHAPTER 6

Wound Healing Utilizing Electrical Stimulation Technique: Towards Application of Dielectrophoresis

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Abstract: Diabetes is a metabolic disorder characterised by long-term hyperglycemia caused by insulin resistance [1]. According to scientific studies, the number of people with diabetes climbed from 30 million in 1985 to 177 million in 2000 and is expected to increase to 552 million by 2030 [2, 3]. Diabetic patients suffer from impaired or delayed wound healing due to a few factors leading to the development of chronic wounds. Chronic wound management is becoming an economic burden on the healthcare system. With the rising prevalence of chronic diabetic wounds, finding effective treatment strategies and advancing therapy are critically important. Although the exploration of electrostimulation therapy for wounds is only very recent, it is a promising approach to expedite wound healing. With recent advancements in wearable device technology, a new treatment strategy that integrates electrical stimulation and biomaterial dressing has been adopted.

Keywords: Dielectrophoresis, Electrical stimulation, Electric field, Stimulation therapy, Wound healing.

ECONOMIC BURDEN OF CHRONIC WOUND

Diabetes is a metabolic disorder that causes hyperglycemia due to insulin resistance over an extended amount of time [1]. According to scientific studies, the number of people with diabetes raised from 30 million in 1985 to 366 million in 2011. The number is estimated to grow to 552 million by 2030 [2, 3].

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Diabetic patients have impaired or delayed wound healing that may lead to the development of chronic wounds. Diabetes slows wound healing by affecting each stage of wound healing, including haemostasis, inflammation, proliferation, and the modeling-modeling phase [4]. A diabetic wound is classified as a chronic wound as it often does not heal within 4 weeks. Diabetes leads to major complications such as poor wound healing, diabetic ulcerations, and limb amputation [5]. In addition, diabetes also includes a wide range of co-morbidities such as cardiovascular disease, stroke, chronic renal failure, and peripheral neuropathy. In diabetic individuals, foot ulcers have been identified as a clinical marker for limb amputation and fatality [6, 7]. Delays in the healing of chronic wounds may also lead to psychiatric stress and depression [8].

Chronic diabetic wounds are becoming a challenging clinical problem and lead to mortality if not treated effectively. Chronic wound management is becoming an economic burden on the healthcare system. This cost is associated with patient care, hospitalisation, and wound infections, all of which have medical, financial, and social implications for patients, their families, and society as a whole. With a rising prevalence of chronic diabetic wounds, finding effective treatment strategies and advanced therapy is critically important. Solving this issue will make a significant clinical impact on healthcare.

Understanding the factors of wound healing impairment and wound healing mechanisms is vital to improving the wound management method. Significant progress has been achieved in the treatment of severe wounds over the previous few decades. Previous methods for wound management include wound dressings, negative wound pressure therapy [9], ultrasound, debridement, and skin substitutes [10]. These methods can be costly and time-consuming in demonstrating a positive outcome. Despite the numerous treatment approaches, current strategies are insufficient, as chronic diabetic wounds continue to be a major financial and clinical issue.

Numerous studies have supported the use of endogenous electric fields (EF) in conjunction with standard wound management. Electrical stimulation (ES) is the technique of employing a device to send electrical pulses through the skin. The electrode is usually placed directly on the skin and the wound in most research [11, 12]. ES has been investigated *in vitro* on various types of wound healing cells, including macrophages [13], fibroblasts [14, 15], endothelial cells [15, 16], keratinocytes [17, 18], and a few other cells have shown changes at different phases of wound healing.

ENDOGENOUS ELECTRIC FIELD

In 1843, Emil Du-Bois Reymond deduced that wounded skin is electrically positive compared to healthy skin. He employs a galvanometer from 2 miles of wire to measure about 1 μA flowing from his wounded finger [14]. Since then, it has been suggested that the electric current might be beneficial in enhancing wound healing. Research shows that the skin generates an endogenous electrical field (EF) ranging from 30 to 100 mV during wound healing [19]. The current flow is produced by the short-circuiting of the transepithelial potential (TEP) difference at a wound site, as shown in Fig. (**1**) [20, 21]. The TEP of 25 - 40 mV throughout all parts of our skin will rapidly force a positive current out of any wound [22]. When a wound occurred, a lateral voltage gradient of 100 - 200 mV/mm was generated across the wound. This number was the highest in open wounds and subsequently dropped as it healed [22].

Moreover, it was reported that, near the wounded area, cells migrate in response to the endogenous EF to heal the wound. Ions passing through cells and tissues cause tissue polarisation and a quantifiable EF gradient over the skin wound [20, 23]. The discovery and understanding of endogenous EF at the wound site aid in gaining knowledge of exogenous EF stimulation for rapid wound closure.

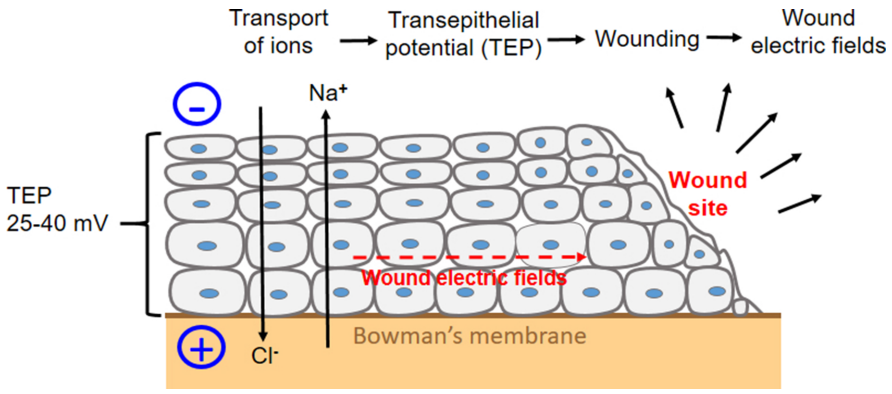

Fig. (1). The generation of an endogenous electric field (EF) at the wound site [21].

ELECTRICAL STIMULATION DEVICE: EXOGENOUS ELECTRIC FIELD

The electrical stimulation (ES) device is a biomimicry device in wound healing applications that mimics bioelectrical signals in the body [24]. Exogenous ES mimics the effects of the endogenous electric field (EF) to stimulate and guide cell migration, improving wound healing [25]. The directional cell migration towards the cathode or anode of an applied EF is known as electrotaxis or

CHAPTER 7

Modification and Treatment of Wound Dressing Material

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Abstract: The utilisation of bio-based materials in constructing an advanced wound dressing for regenerative medicine is not new. Due to the fundamental principle of tissue engineering in regenerative medicine, bio-based wound dressing formulated from by-products or animal or plant wastes could contribute significantly to healing processes. Bio-based materials help to regenerate and replace damaged tissues and shorten the recovery period without frequent dressing changes. The bio-based dressing development begins with a small-scale benchwork that focuses on modifying and treating the bio-based material before it is clinically applied for wound treatment procedures. The modified bio-based materials then turn into functional wound dressing in various forms such as hydrogel, membrane, sponges, film, or fibres. The dressing's therapeutic healing properties can be enhanced by subjecting it to physical, chemical, and biological treatments. The dressing principle must abide by tissue engineering needs and offer a broader range of alternatives that can clinically treat different types of wounds based on different etiology. Therefore, this book chapter highlights the advantages of a bio-based material and its modification for wound dressing for tissue engineering purposes in regenerative medicine.

Keywords: Bio-based material, Chemical treatment, Green product, Physical treatment, Tissue engineering, Wound healing.

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INTRODUCTION

The wound dressing application has become an essential step in the wound recovery management system for ages. Wound dressing development has evolved from indigenous to ingenious strategies with various material and technological interventions. The expanding growth of knowledge about wound healing and its recovery mechanism precedes the establishment of novel therapeutic modalities with new and thrilling notions, such as advanced wound dressing. The utilisation of bio-based materials in an advanced wound dressing with healing properties began as early as the Egyptian civilisation [1]. The Egyptians use “plaster” a mixture of lint (vegetable fibres), honey, and grease (animal fat) to cover the wound and protect it from infection. Currently, wound dressing technology is already connected to tissue engineering treatment, extending its advantages in regenerative medicine. Tissue engineering entirely relies on materials, methods, and tissue physiological aspects for repairing, development, and cell restoration after the treatment [2].

In terms of tissue engineering, wound dressing has various therapeutic abilities, such as antimicrobial, anti-oxidant, haemostasis, bioactive, and biomimetic properties, that contribute to an ideal micro-environment for rapid wound healing [3]. Utilising bio-based materials from natural sources (animal, plants, or bacteria) as an alternative for advanced wound dressing is captivating and has proven to work well in accelerating wound healing without any significant side effects. Therefore, natural-based materials such as cellulose, collagen, chitosan, and alginate are commonly used to produce advanced wound-dressing products for regenerative medicine [4]. They also offer many advantages over artificial materials due to their abundance, biocompatibility, biodegradability, non-toxicity, and durability. Moreover, their chemical and structural properties permit them to undergo physicochemical modification fashioned into various dressing forms and blended with other materials extending their medicinal properties. For example, collagen-chitosan hydrogel as a wound dressing moisturises the injury sites and promotes connective tissues near the wounded area to synthesise more extracellular matrix (ECM) components [5]. They are also efficiently proven to help in blood clotting and minimise bacterial infection. Additionally, collagen on the plasma membrane promotes cell growth, supports the tissue's structure, and anchors cells to one another [6]. Post-modification and treatment are also done to boost its mechanical strength, compatibility, and durability and control its solubility to match tissue engineering treatment requirements for regenerative medicine. To date, wound dressing technology from bio-based materials is not only exemplified with numerous healing properties, but its development has extended its abilities as a scaffold [7], 3D artificial skin, matrix for immobilisation of bioactive molecules, and biosensor [8] used for clinical treatment purposes.

However, healing abilities of bio-based material wound dressings are still bound to the four phases of the wound healing mechanism: i) Haemostasis, ii) Inflammation, iii) Proliferation, and iv) Remodelling [9]. In the haemostasis phase, platelets and fibrins in the blood are stimulated and form a clot as a stopper to avoid excessive bleeding. Then, the inflammatory phase is activated, where histamine from the mast cell near the injury area promotes leukocytes and macrophages, thus minimising the risk of bacterial infection at the injury site. Also, the injured area becomes red and warm due to more blood, fluid, and metabolic heat at the injury site. Then the accumulation of the phagocytes (macrophages, leukocytes), blood clotting elements, and exudate cause oedema (tissue swelling) and pain. The third and fourth phases, proliferemodeling remodelling/maturation involve angiogenesis, epithelisation, synthesis of ECM, and cell granulation, thus restoring the cosmetic, anatomy and physiology of the affected skin.

This chapter discusses the development and formulation of the bio-based wound dressing starting from a small-scale bench work preceding its therapeutic use for wound treatment in the hospital, especially on a chronic wound. Then, physical, chemical, and biological treatments to enhance its healing properties are outlined and reviewed. The modified bio-based wound dressing significantly promotes healing mechanisms, accelerates wound recovery, and replaces and repairs damaged tissues, thus restoring the skin's architecture and function. Furthermore, the regenerative medicine approach for bio-based wound dressing is expected to perform safer and better than the synthetic-based material, specifically in treating a chronic wound.

Bio-Based Material for Wound Dressing

Among bio-materials, organic biopolymers have attracted huge attention and interest to be tailored into advanced wound dressing with natural therapeutic healing properties. They are made up of a repeating unit of monomers, which are amino acids and monosaccharides, to form polypeptides (collagen, gelatine) and polysaccharides (cellulose, chitosan, alginate), respectively [10]. Due to their proven biocompatibility, non-toxicity, and biodegradability, natural biopolymers such as collagen, gelatine, chitosan, and alginate recapitulate the biological and physiochemical features of the ECM components [11]. In addition, they can also stimulate a cellular signaling pathway by triggering the key players in wound healing mechanisms, such as macrophages, fibroblasts, fibrinogen, and endothelial cells, thus facilitating the wound healing processes.

Wound healing is considered a natural immune system initiated as a response to breakage or injuries on the skin tissue [12]. Under normal circumstances, the

CHAPTER 8

Nanobio-Inspired Materials for Tissue Engineering

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Abstract: The utilization of nanoscale biomaterials in various fields of modern science has shown great change and benefits in this present era. Due to their unlimited potential, many researchers have focused on further studies, and today, they play a good role in human health improvement programmes. Accordingly, it is true that the use of nanomaterials in tissue engineering is one of the most advanced technologies in medical care. The technology which integrates materials science and engineering with biology in order to improve the induction of tissue regeneration is called tissue engineering. This technology helps in controlling the cellular combined with synthetically engineered materials and is used for various treatments. One of its main functions is the treatment of structurally degenerated organs in the human body. Tissue engineering techniques can be upgraded with distinct properties of nanomaterials like better adaptability, good response, delivery potential, and better controllability. Moreover, unlike other materials, they are highly efficient, reliable, and easily decompose. Depending on the type of application, different kinds of nanomaterials are used, such as polymers, metals, ceramics, and their various compositions. Consequently, it can be assumed that the approaches of incorporating nanomaterials in tissue engineering will enhance the disciplines of tissue regeneration.

Keywords: Tissue engineering, Nanomaterials, Nanoparticles, Biomaterials, Biomedical, Tissue regeneration.

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INTRODUCTION

Modern society has considered "Nanotechnology" as one of the best methods to explore many fields due to its unique properties. Particles of size smaller than 1 micrometre are synthesized using nanotechnology and they are known as nanoparticles. Likewise, any material structured using a micro or nanometer with at least one dimension is called nanomaterial [1]. There are different forms of nanomaterials, including nano metallic, nanosheets, nanofiber, and nanotubes. Their application has expanded in many products starting from small to big and has become a strategy to overcome difficult tasks in this advanced world. It is exploited in various important activities of agriculture, the food industry, the sports industry, health equipment, cosmetics, biomedical, *etc*. One of the crucial roles in biomedical is the use of engineered nanomaterials from both sources for the regeneration of tissues. There is a need to apply nanomaterials for tissue engineering due to the presence of a smaller number of donors for tissue and organ transplantation. New materials can be developed and manufactured with nanomaterial exposure whose function resembles the very tissue or organ [2]. A few years back, there was a report on incorporating nanometric materials for their regenerative properties and biocompatible mechanisms [3]. Initiating from that time, it was observed that different types of nanomaterials manufactured partially or fully using nanoparticles are being utilised in medicine [4]. The scaffolding of a cell with three-dimensional properties is required to detect cellular functions and deliver bioactive agents. These activities can only be a success with the approach of using nanoscale products [5]. The 3D scaffold structure with nanoparticles helps increase the mechanical properties of the template [8] and immediate reactive scaffolds, including pH, ionic strength, *etc*. [9]. In addition to this, it makes more upholds of electrical conductivity [10] and protects from the formation of bacterial colonies [11]. Moreover, various aspects like poor solubility, labile biomotion, and poor movement of bioactive agents make nanoparticles more advanced for application in bioactive agent delivery and monitoring [6]. There are also other reports of nanoscale products such as nanofibers and nano surface patterned for monitoring cell manner in tissue engineering. Nanomaterials like ceramics, metals, and artificial and natural sources of polymers used in preparing nanoparticles with more penetration ability, and high surface area, act as bio-ink supplements, and have anti-microbial properties [12]. Currently, nano-engineered materials are popularized more in cardiac tissue regeneration, vascular repair, skin wound healing, skin regeneration, eyes regeneration, skeleton muscular repair, peripheral and central nervous system regeneration, *etc*. [7]. Another use of nanobiomaterials is the development of worthy and agreeable materials that can manage the biological, chemical, structural, and mechanical microenvironment to achieve cell delivery and tissue regeneration. Furthermore, they fasten cell adhesion, growth and

development, proliferation, and division even by excluding the use of drugs. Hence, the use of material surfaces and structures with nanoscale features, eventually known as nanobiomaterials, has become a necessary tool for better treatment, diagnosis, and organ transplantation [14]. Their addition are more advantageous than conventional methods and results in fewer side effects when compared to older therapies. Also, the conventional method of treatment consumes a lot of time and is low in efficiency. The activity of nanobiomaterials for tissue engineering amplifies tissue regeneration with minimized immune protection from infection. For instance, the use of nano biomaterials in tissue engineering brings more positive results and can be developed by employing nano-engineered components.

Bioinspired Nanomaterials as Biotemplates

Biotemplates are a biological template that is structured by architectures as containers in the form of viral capsids. Their main function is utilizing them as the carrier of DNA assays and immunoassays. These containers are also used as drugs, catalysts, and in the synthesis of new distinctive materials [13]. Biological templates have unique physicochemical features which play a role in the delivery of drugs and in proteomics. They are also the role model of bio nanostructures, also known as bio-inspired nanomaterials [18]. Meanwhile, the report for biological macromolecular use, which resembles a template for the production of new nanomaterials, is also revealed [15]. Many researchers have focused their studies on cages of proteins that are non-natural, greatly symmetric, and protein systems with multiple functions. They have different boundaries, *viz.*, internal, external, and mid-phase units [16, 17]. Protein cages are used to produce inorganic nanoparticles, and Ferritins were the first protein cages to be used. Ferritins have the capacity to store and sequester iron [19]. Additionally, a report on producing Fe_3O_4 nanoparticles using empty ferritin cages known as apoferritins was found [20]. Some common uses of protein cages include the storage of nucleic acid, sequestration, and biomineralization. It is also applied to transport and deliver nucleic acids among dissimilar inorganic surroundings to progress negative charges from Li batteries [16, 21]. Moreover, protein cage template nanoparticles are the major components for catalysis. Here, ferritin-encapsulated silver nanoparticles are produced genetically in the presence of Ag-bound peptides inside the cage of ferritin. After this process, they are again synthesized chemically to reduce the binding of silver ions and peptides to silver nanoparticles [22]. Advances in modern science have made a gateway for the synthesis of 3D molecules and suitable physicochemical and mechanical activities. A dendrimer is a macromolecule that has many branches and is symmetric. Dendrimers have distinct properties as they are monodispersed with a well-defined figure of end sets [23]. Some of their unique characteristics include high physicality, good

CHAPTER 9

The Potential of Plant-based Composite Material for Regenerative Medicine

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Abstract: Regenerative medicine focuses on replacing injured tissues and organs by utilising biophysical and biochemical cues to develop bioscaffolds suitable for regenerating damaged or injured tissues. The scaffold has an important role in guiding the development of the regenerative process that allows the migration and attachment of cells and, to a certain extent, becomes the source of nutrients influencing the cells or tissue's biological mechanism. Hence, the selection of biomaterials is important to ensure biocompatibility and suitable mechanical properties for tissue engineering. Different sources and types of biomaterials can be used in the fabrication of scaffolds, including composites. Composite biomaterials consist of more than one material with different morphologies and compositions, causing it to become multiphased, which can ameliorate the scaffold's mechanical properties, flexibility, and structural properties to ensure a suitable microenvironment for cell growth and viability. Nevertheless, biocompatibility issues need to be addressed, particularly with synthetic materials, which led investigators to explore other sources and types, such as plant-based biomaterials, to fabricate suitable and safer composites. Aside from being more sustainable and perhaps more eco-friendly, plant-based composite materials fulfill the criteria required for biomaterials and exhibit many advantages that can be adapted in their fabrication techniques. Hence, in this chapter, the advantages and development of plant-based materials will be discussed, focusing on the potential of oil palm and konjac plants as sources of biomaterials.

Keywords: Bioscaffold, Biomaterials, Composites, Plant-based, Regenerative medicine.

INTRODUCTION

Regenerative Medicine

Regenerative medicine is the current rising field of medicine, focusing on regeneration and replacing incapacitated human cells, organs, and tissues caused

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by external injuries and the development of diseases due to age and/or hereditary factors. The fundamental aim of regenerative medicine is to engineer damaged organs and tissues by restoring them to their normal function. The restoration processes use engineered external biological materials, which are usually incorporated with the self-healing ability of the human body to improve the damaged organs and tissues. Hence, the term 'tissue engineering' has been regularly used in regenerative medicine. Regenerative medicine has vast potential in many health spectra and can be equally important to vaccines, antibiotics, and even monoclonal antibodies [1]. Chronic diseases, such as cardiovascular disease, diabetes mellitus, and cancer, have long been the focus of regenerative medicine and tissue engineering, potentially being newly used or as an alternative to current treatment standards.

Decades of research have seen regenerative medicine as the vortex of other related fields assimilating together, such as cell biology, chemistry, material science, engineering, and life sciences. Despite the complexity, regenerative and tissue engineering use the fundamentals of cells as the basic building blocks of tissues, and with the capability of biomaterials to develop a suitable matrix or scaffold for supporting, receiving, and transmitting signalling molecules leading to responses and interactions. This process is the determinant that can affect the fate of the cell. By understanding this aspect, researchers can manipulate the communication activities between cells to regenerate cells and tissues of choice. Consequently, scaffolds in regenerative medicine have a central role in guiding cells or tissue development into a mature and functional one for clinical application. This is also due to the biochemical factors that allow the migration and attachment of cells, the source of nutrients, and the ability to impose adjustment to the cell's behavior [2].

The basic 'ecosystem' of studying scaffold production and engineered tissues involves biofabrication techniques, types of biomaterials for scaffold production, and cell sources. Biofabrication techniques include electrospinning, bioprinting using 3D technology, and nanotechnology [3 - 6]. The selection of biomaterials is crucial in controlling the scaffold's physical, mechanical, and chemical properties. These are important in ensuring effective methods in tissue engineering and the availability of complaisant biomaterials. The American National Institute of Health defines biomaterials as 'Any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period; augmenting or replacing parts of any tissue, organ, or function of the body, to maintain or improve the quality of life of the individual' [7]. Biomaterial is synonymous with a 'remote controller' due to its ability to react when subjected to external cues, such as chemical, physical, temperature, light, sound, humidity, redox potential, enzyme activity, and pH, thus affecting the cell's fate [8 - 10].

Consequently, biomaterial designs must emphasise the proper chemical, physical, mechanical, and structural properties for the scaffold to be biodegradable, cost-effective, non-antigenic, and biocompatible. Depending on the intended use, the material's selection for making biomaterials must be based on the molecular weight, shape, solubility, structure, hydrophilicity, hydrophobicity, and surface energy. Different categories of biomaterials used in the fabrication of scaffolds can be grouped as metals, ceramics, polymers, and composites used individually or in combination. The closest resemblance of structure between synthetic polymers and polymers that exist in natural tissues has been reckoned for its use as a biomaterial [11]. These substances are attractive due to their low elastic modulus and high strength properties [12]. In the following sections of this chapter, the current development of biomaterials and the potential application of selected plant-based composite material in regenerative medicine will be reviewed.

Composite Biomaterials for Biocompatibility Improvement

The production of biomaterials or biopolymers has adopted the application of synthetic- or inorganic-based substances or solvents. Polymers, such as polylactic acid (PLA), polyglycolide (PGA), and poly (lactic-co-glycolic acid) (PLGA) copolymers, have been extensively used in many bodies of literature as synthetic polymers for scaffolding in regenerative medicine [4, 13, 14]. Furthermore, issues of biocompatibility in using synthetic sources for biomaterials have compelled the use of naturally-derived polymers as biomaterials. Instances of naturally-derived polymers include chitosan, collagen, hyaluronic acid, and silk fibroin, which have been known to facilitate cellular processes of adhesion, migration, proliferation, and differentiation [11].

Unfortunately, the disadvantages of these natural polymers are limited in terms of their malleability to be processed into distinctive sizes and shapes. Therefore, the physical-chemical properties of the natural polymers are challenging to modify, leading to partial control of the scaffold's mechanical behavior. Despite the plasticity of synthetic polymers, they turn out to be too flexible, hence composite materials have attracted much interest as an alternative biomaterial in producing scaffolds for tissue engineering. Composite materials involve combining more than one material with different morphologies and compositions to achieve a scaffold with the required chemical, physical, and mechanical characteristics. Normally, composite materials consist of two components known as the matrix and inclusion or dispersed phase. The matrix is characterised by its high ductility and structure strength, which usually involve the inclusion of constituents that determine the finishing properties of the composites. In addition, composites can

CHAPTER 10

Current Trends and Future Perspective of Skin-Based Tissue Engineering

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Abstract: The skin regulates several important physiological processes which have a significant clinical influence on wound healing. Tissue-engineered substitutes may be used to help patients with skin damage to regenerate their epidermis and dermis. Skin replacements are also gaining popularity in the cosmetics and pharmaceutical sectors as a viable alternative to animal models for product testing. Recent biomedical advances, ranging from cellular-level therapies like mesenchymal stem cell or growth factor delivery to large-scale biofabrication techniques like 3D printing, have enabled the use of novel strategies and biomaterials to mimic the biological, architectural, and functional complexity of native skin. This chapter elaborates on some of the most recent methods of skin regeneration and biofabrication that use tissue engineering techniques. Current problems in manufacturing multilayered skin are discussed, as well as opinions on attempts and methods to overcome such constraints. Commercially accessible skin substitute technologies are also investigated, as an effort to mimic native physiology, the function of regulatory authorities in facilitating translation, and current clinical requirements. Tissue engineering may be used to develop better skin replacements for *in vitro* testing and clinical applications by addressing each of these viewpoints.

Keywords: Biomaterial, Current trend, Future perspective, Skin, Tissue engineering, Wound healing.

INTRODUCTION

Skin, being the largest organ, covers the body and accounts for around 8% of total body mass. The skin is also exposed directly to microorganisms and thermal, mechanical, and chemical elements, which prompt skin problems like trauma, and

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acute and chronic wounds [1]. Skin tissue engineering aims at substituting or replicating the tissue of the skin, which resembles the original physiological structure or function of the tissue.

A number of biomedical practices, such as disease modelling, novel clinical therapies, 3D bioprinting, *etc*., are used in skin tissue engineering. Skin substitute products may be divided into three types based on their anatomical structures: cellular epithelial autografts, engineered dermal substitutes and engineered dermo-epidermal substitutes [2].

Skin Structure and Function

The epidermis, the uppermost thin layer, is largely built of melanocytes and keratinocytes and is noticeably multi-layered and cellular. Together with skin components, including sebaceous glands, sweat glands, and hair follicles, the mammalian basal epidermal cells maintain homeostasis. A layer of keratinized dead cells, which serves as the primary barrier to the skin, is then formed by the multiple layers of the epidermis as a result of the keratinocytes' terminal maturation and differentiation [3].

Dermis, which is located directly underneath the epidermis, is the connective tissue that makes up most of the skin. It is made up of fibroblasts, extracellular matrices including collagen, glycosaminoglycans (GAGs), elastin, and skin components like hair follicles and sweat glands. The most common category of cells in the dermis secreting remodelling enzymes (collagenases and proteases) and accounting for the majority of the resilience and biomechanics of the skin is the fibroblast [4].

The last layer under the dermis is the hypodermis, which is highly vascularized and primarily made of adipose tissue [5]. Hypodermis is instrumental in the thermoregulatory and mechanical characteristics of the skin as well as acting as the protection between the skin and other skeletal elements.

Current Treatment Methods for Acute and Chronic Wounds

Determined by their etiology, skin wounds are often categorized as either acute or chronic. Mechanical trauma, burn wounds, and surgical excision of skin tumours are among the most frequent forms of acute skin wounds [6].

Many commercially available treatments and products are presently used for managing wounds based on the cellular and molecular basis of skin injury. There are many types of wound care dressings available depending on the nature and characteristics of the wound during healing. Besides promoting wound healing and preventing further infections, dressings are also important to stop bleeding

and activate haemostasis, and clotting, absorb exudates, protect the wound and surrounding tissues, and promote moist wound healing [7]. A moist wound environment must maintain important wound healing characteristics like enhanced epithelial migration (epithelisation), fibroblast functions, and collagen production. A quality wound dressing should have non-adherence to the wounds, it can be applied and removed without inducing pain, allow gaseous exchange and maintain temperature. The different types of dressings used for acute or chronic wounds are briefly described in Table **1**.

Table 1. Different types of wound dressings

Traditional wound dressings	**• Gauze dressings** **• Bandages** **• Non-adherent Moist (Tulle Gras Dressing)**
Modern wound dressings	• Semi-permeable film dressings (Opsite, Tegadarm) • Semi-Permeable foam dressing (Lyofoam, Alevyn, Tielle, PolyMem) • Alginate Dressings (Kaltostat, Sorbsan, Algisite) • Hydrocolloid dressings (Duoderm, Granuflex, Tegasorb) • Hydrofiber dressings (Hydrogel, Nu-gel, Intrasite)

Skin grafting is the superior method for curing this type of skin wound. By utilising the autologous skin of the patient, this method protects the deformed tissue and restores the barrier duty of the skin. This, however, comes with certain disadvantages like creating a new and painful wound site and the limitation of patient's supply of suitable harvest sites. According to research, hypertrophic healing and keloid formations are also common in patients treated with autologous skin grafts, especially in people who already have a genetic predisposition [8].

Chronic wounds, as opposed to acute skin injuries, occur as a result of improper wound healing mechanisms. Some examples are diabetic ulcers, venous leg ulcers, pressure ulcers, *etc*. An underlying comorbidity in these conditions prolongs inflammation and slows the wound-healing process of an open wound. This also comes with a high risk of infection [9]. Healing difficulties are exacerbated by tissue ischemia or constant pressure on the sites. Chronic wound treatments typically include treating the underlying infection and debriding contaminated areas. Amputation may be needed in serious situations. To avoid this situation, a variety of wound dressings have been created to protect the wound from infection as well as to aid in wound healing. By providing a hypoxic and wet wound environment, occlusive dressing stimulates angiogenesis and re-epithelization [10].

CHAPTER 11

3D-bioprinting for Tissue Engineering and Regenerative Medicine: Hype to Hope

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Abstract: Tissue replacement using engrafting biomaterials or artificial organs to restore lost functions post-injury is one of the leading regenerative medicine practices. The last two decades witnessed the emergence of many promising biofabrication approaches such as bioprinting. However, bioprinting allows the placement of complex structures that are multi-layer (using hydrogel biomaterials), multicellular, vascularized, and multifunctional. Different bioprinting approaches are being developed and used to print hundreds of promising bioinks combinations into tissue-specific niches to grow living organs for translation, disease modelling, and drug delivery. This book chapter reviews the three primary bioprinting techniques with their advantages and limitations. Moreover, this chapter discusses the natural and synthetic biomaterials and the additives and crosslinking methods used to fabricate functional bioinks that boost cell growth, proliferation, migration, differentiation, and homeostasis.

Keywords: Collagen, Crosslinking agents, Extrusion bioprinting, Natural-based bioinks.

INTRODUCTION

Bioprinting versatility and applications hold the bright future of enabling multidimensionality and biopatterning to develop multicellular systems in biomimetic architectures [1, 2]. Precise deposition of multiple lineage-specific cellular populations integrating biomaterials and growth factors in millions of points and curves on multiaxial dimensions is unachievable using methods other than bioprinting. Moreover, bioprinting is the leading edge of transitional research, contributing to building light-transmissive cornea [3], multi-layered vascularized skin [4], beating heart tissue [5], contracting skeletal muscle [6], and many other applications.

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The main aim of the 3D-bioprinting technique is to build reproducible and complex mimicry scaffolds that can support vascular evolvement, cellular activity, and tissue physiological functionality. Conventional scaffolding methods are time-consuming, inaccurate, unable to build tissue-specific architectures, and lack control over cell density and scaffolds' pore size essential to promoting cell migration activity [7]. Besides the high throughput and accuracy, 3D-bioprinting provides rapid fabrication of tissue-specific niches that enhance cell growth with minimal or no effect on cells viability (>90%) post-printing with low printing costs [8].

Generally, the bioprinting process in tissue engineering and regenerative medicine involves selecting suitable biomaterials (*i.e.*, natural and synthetic polymers), crosslinkers (to implement covalent bonding), and tissue-related cells. After that, CAD models imported from CT scans or magnetic resonance imaging (MRI) are used for designating patient-specific organs for bioprinting (Fig. **1**). Then, slicing software such as SIMPLIFY3D® converts the CAD design into defined layers with specific patterns and printing speeds/feed rates.

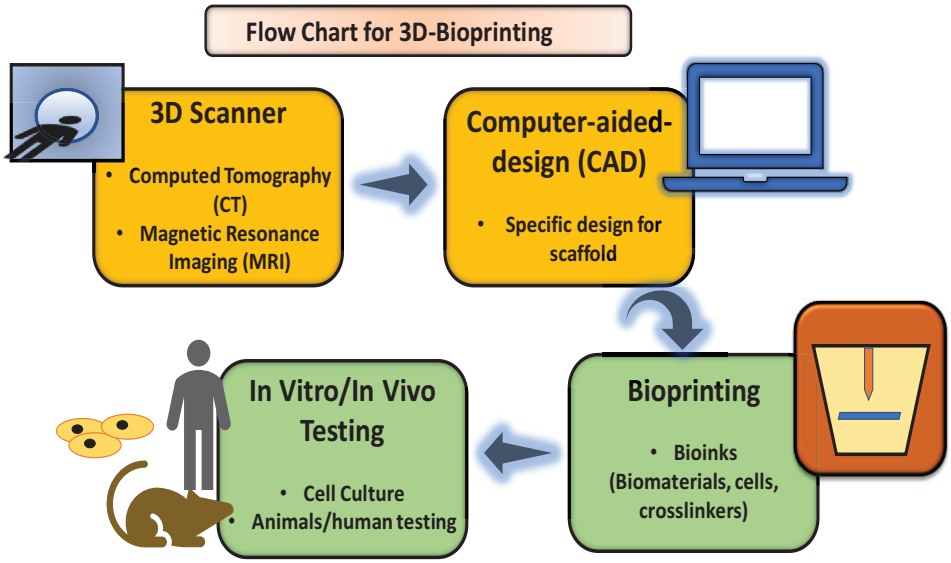

Fig. (1). Flow chart for 3D-bioprinting process starting from the 3D scanner, designing, fabrication by using a bioprinter, and product testing towards *in vivo* and *in vitro*.

3D-BIOPRINTING FOR SCAFFOLDING

Extrusion-Based Bioprinting

Extrusion-based bioprinting is considered the most convenient and popular method in bioprinting applications [9]. It involves injecting the hydrogel

throughout the printer nozzle (different nozzle diameters can also be used) and continuously stacking the hydrogel layer-by-layer [10]. Extrusion-based bioprinter relies on the simple principle of filling the syringe with the selected bioinks composed of biomaterials, cells, and needed crosslinkers (Fig. **2**). Despite the fact that extrusion-based bioprinting has been widely used in the tissue engineering field, printed scaffolds tend to have low cell viability post-printing, mainly due to the applied shear stresses on the bioink during printing [11]. This technique, however, showed better potential than some methods, such as solvent casting and phase separation, that usually involved more complex mechanisms [12]. For now, this technique is being rapidly improved and equipped with many printing heads and physical crosslinking accessories (*e.g.*, UV light) to improve cell viability and printing precision [13].

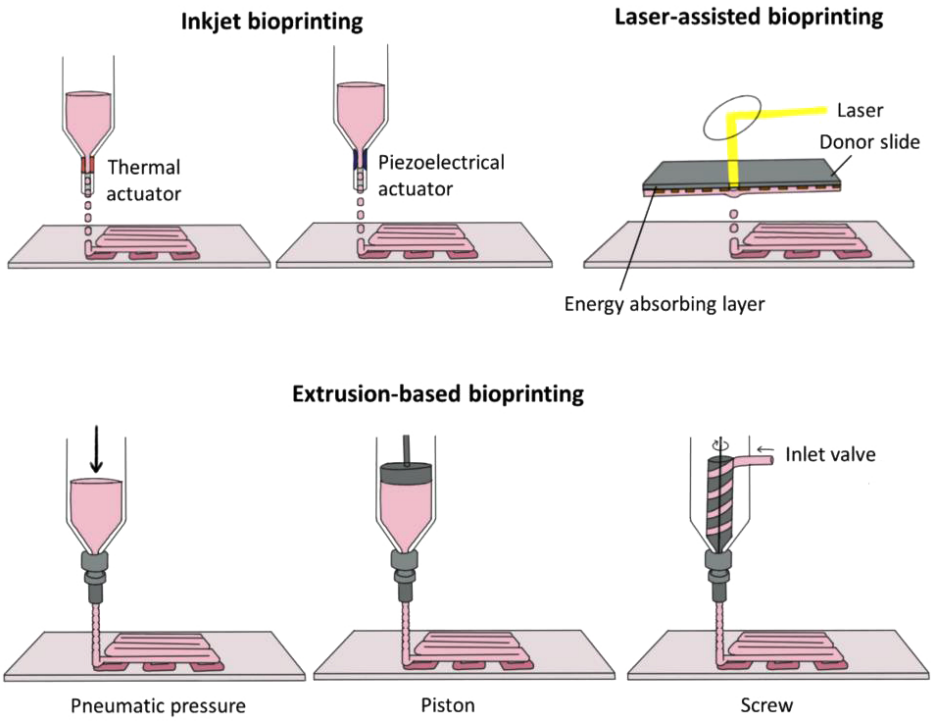

Fig. (2). The major most used 3D-bioprinting methods. (1) inkjet bioprinting (2) laser-assisted bioprinting (3) extrusion-based bioprinting [19].

Inkjet-Based Bioprinting

Inkjet-based bioprinting is the first existing bioprinting technique that has been innovated in the field (Fig. **2**). This technique involves the deposition of hydrogels encapsulated with cells in the form of droplets [14]. Two standard ejection methods are used (*i.e.*, thermal and piezoelectric actuators). The thermal inkjet

CHAPTER 12

Platelet-Rich Plasma and its Derivatives for Tissue Engineering

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Abstract: Platelet-rich plasma (PRP) is a well-established biological product used in the tissue engineering field to promote wound healing and tissue regeneration. PRP can form platelet gel with the addition of thrombin and/or calcium salts. Nonetheless, PRP is more commonly combined with biomaterial and cells for various tissue engineering applications. Over the years, PRP has been used in the dermatology field for hair follicle regeneration and wound healing, in the orthopaedic field for bone, muscle, tendon, and ligament repair, and in dentistry for many dental procedures, including dental implants. Despite the long historical use of PRP in the clinic, the PRP isolation technique is still continuously changing, evolving, and improving to increase the therapeutic effect of PRP. Nowadays, PRP is not only used as a biomaterial but it also can be used to replace foetal bovine serum and human serum in primary cell culture, especially for cell therapy purposes. PRP derivatives such as platelet lysate, platelet-derived growth factors, and platelet-derived extracellular vesicles also are precious functional materials used clinically in the tissue engineering field. In this book chapter, we review the different subclasses of PRP, including its derivatives, its research, and clinical applications, and underline the challenges of PRP in clinical translations.

Keywords: Biomaterial, Platelet-derived extracellular vesicles, Platelet-derived growth factor, Platelet-rich plasma, Tissue engineering.

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INTRODUCTION

Platelet-rich plasma (PRP) is a fraction of blood with a platelet concentration above the baseline [1]. PRP exists in various forms and is also known by other names such as platelet-rich concentrate, platelet-enriched plasma, platelet-rich growth factors, platelet releasate, and platelet gel [1 - 3]. These differences are attributed to different preparation methods that result in PRP with varying platelet concentration, fibrin concentration, the presence of leukocytes, and activation status [4]. PRP can form a platelet gel with the addition of thrombin and/or calcium chloride to activate the coagulation cascade, as it contains physiological levels of fibrinogen and coagulation factors [5]. The platelet gel can act as a scaffold for tissue engineering applications. A more recent variation of PRP is the collection of platelet-derived extracellular vesicles for therapeutic purposes in regenerative medicine [6].

PRP has been used in the field of tissue engineering and regenerative medicine as biological support to heal and regenerate tissues [7]. It is widely used as a biomaterial by dermatologists to promote wound healing, by orthopaedists to promote cartilage, tendon, ligament, and bone repair and regeneration, and by the dental surgeon as an adjuvant in a number of dental procedures such as alveolar wound healing, periodontal surgery, and dental implants. Additionally, PRP has also been used in other surgical fields, such as cardiovascular surgery, maxillofacial surgery, otolaryngology, and head and neck surgery [8].

Platelet contains alpha, delta, and lambda granules, which are known to be rich in biological factors that modulate wound healing and tissue regeneration [9]. PRP has been reported to be rich in growth and angiogenic factors such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), connective tissue growth factor (CTGF), insulin-like growth factor (IGF) and fibroblast growth factor (FGF) [10]. It also contains chemokines and cytokines such as RANTES (regulated upon activation, normal T cell expressed and presumably secreted), MIP-1a (macrophage inflammatory protein-1 alpha), MIP-2, MCP-1 (monocyte chemo-attractant protein-1), MCP-3, IL-8 (interleukin-8), LIX (lipopolysaccharide-induced CXC chemokine), GRO-α (growth-regulated oncogene-alpha), ENA-78 (epithelial cell-derived neutrophil-activating peptide-78), SDF-1α (stromal cell-derived factor-1 alpha), and PF4 (platelet factor 4), which modulate inflammation and tissue repair [9].

Generally, the growth factors, cytokines, and chemokines in PRP promote wound healing and tissue regeneration by enhancing native cell proliferation, regulating

cell differentiation, stimulating angiogenesis and vasculogenesis, chemotaxis, and modulation of inflammation [11]. Additionally, the fibrin clot serves as a provisional matrix to support cell migration and proliferation as well as permit the sustained release of bioactive factors.

In this chapter, we discuss various forms of PRP and their application in clinical settings. Additionally, we review the challenges of PRP therapy and its perspectives.

Platelet-Rich Plasma

PRP is a fraction of blood rich in platelet, which are minute non-nucleated bodies rich in biological factors [7]. A broad definition of PRP is the plasma fraction of blood with a platelet concentration above the baseline [1]. However, some researchers opt for more stringent criteria by defining PRP as blood plasma with platelet concentration two to five times higher than the baseline.

PRP can be prepared from autologous blood, eliminating the risk of pathogen transmission and immune rejection. Thus, the use of PRP is considered safe because of its autologous nature. Additionally, the preparation of PRP is relatively simple and can be done at the point of care. PRP can be prepared using a two-step centrifugation method [12]. The first step involves spinning the citrated blood at low gravity to separate the PRP and red cells. In the second step, the collected PRP can be concentrated by the second centrifugation at high gravity to discard the excessive plasma. Nowadays, the preparation of PRP has been made faster and simpler using the closed systems commercially available. Some of the commercial systems available include Biomet GPS, Cytomedix Angel, Magellan, Plateltex, and Arthrex ACP systems [13]. The final volume and concentration of PRP vary according to the systems employed. Isolated PRP can be stored at -80°C for an extended period without significant loss of growth factors [14]. Furthermore, PRP can be freeze-dried for easier and longer-term storage without affecting quality and clinical efficacy [15].

PRP is an inexpensive and readily available source of high concentrations of growth factors that can be produced using a minimally invasive method. It is rich in paracrine factors, which modulate tissue repair and regeneration. The most prominent paracrine factors include PDGF, TGF-β, PDAF, IGF-1, VEGF, EGF, HGF, FGF, and PF4 [9, 16]. PRP also contains extracellular matrix proteins such as fibronectin, fibrinogen, and thrombospondin-1. These bioactive factors have been reported to play a crucial role in the modulation of inflammation, cell bioactivities, angiogenesis, granulation tissue formation, and tissue fibrosis. Thus, PRP is extensively used for clinical purposes.

CHAPTER 13

Nanocollagen-graphene-antibiotic for Wound Healing

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Abstract: Nanotechnology is a greatly advancing field of scientific research due to its largely untapped potential, which may apply to various clinical uses. This book chapter focuses on the potential use of nanocollagen, graphene, and antibiotic components in biomaterial fabrication for wound healing. Nanocollagen is simply regular collagen broken down to the nanometer scale. Its nanocollagen-based biomaterials also conform to the ideals of tissue engineering, which are excellent biocompatibility with a high bioabsorption rate and little to no antigenicity while having an extensively cross-linked structure suitable for cellular growth and metabolism. Nanocollagen can be fabricated through electrospinning, nanolithography, self-assembly, and others. The physiology of wound healing follows specific proceedings, which are haemostasis, inflammation, and remodelling stages. The wound healing process may be improved through the use of nanocollagen biomaterials, together with the addition of graphene and antibiotics. Nanocollagen biomaterials aid in acting as a barrier for the wound against infections while providing collagen in the nanoscale to accelerate healing. The addition of antibiotics into the nanocollagen biomaterial aids in preventing bacterial infection by the inhibition of biofilm formation. Graphene, specifically in its oxide form, also acts as an antibacterial agent while potentially providing mechanical durability to the biomaterial scaffold. Along with the benefits of graphene oxide application in wound healing, its challenges are discussed in this book chapter. With that, this book chapter suggests the beneficial combinatorial factors of nanocollagen, graphene, and antibiotics that can potentially produce biomaterials with strong antibacterial properties while accelerating wound healing.

Keywords: Antibiotic, Biomaterial, Graphene, Nanocollagen, Wound healing.

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INTRODUCTION

Recently, nanotechnology has been a highly sought-after topic in the scientific research field of regenerative medicine. Nanotechnology is a relatively new concept that has been advancing greatly throughout the years. The materials/ polymers involved in this field must be present in the nanometer (nm) size, usually between 1-100 nm, where its origins can be synthetic or natural. Synthetic materials, for example, include polylactic acid (PLA), polyethylene glycol (PEG), and bioactive glasses; natural materials would include collagen, cellulose, gelatin, silk fibroin, and elastin, to name a few [1, 2]. Nanocollagen fabricated into biomaterials is theorised to be absorbed more efficiently in comparison to bioscaffolds of regular collagen. For this book chapter, we will be focusing on the use of collagen in the nanometer size, as well as its effect on wound healing abilities, together with graphene and antibiotics integration.

Collagen

Prior to the fabrication of nanocollagen, collagen is initially sourced as a raw material. Collagen in its natural state is sized at 300 nm lengthwise with a diameter of 1.5 nm [3], comprising a four-level complex structure, from primary to quaternary structure. Its primary structure consists of a triplet amino acid sequence Gly-X-Y, which includes glycyl (GLY), proline (PRO), and 4-hydrocyproline (HYP), respectively. This amino acid sequence forms the secondary structure of collagen molecules through continuous repetition, which extends to the tertiary structure known as α-chain, forming a triple helix tropocollagen through three chains of parallel α polypeptide chains roped around each other. The final quaternary structure forms collagen fibers through the self-assembly of collagen fibrils Fig. (**1**)[4].

Collagen is a type of protein, naturally found abundantly in the extracellular matrix (ECM) of the human body, with some examples such as skin, tendon, and blood vessels [4]. There are at least 30 types of collagen identified to date, with collagen type I (Col I) being the most extensively studied form of collagen due to it being largely present throughout the body [5, 6]. Col I largely supports tendons and facias through having tensile strength and elasticity, successfully preventing morphological deformation from stress load [4]. It assembles and crosslinks independently to provide cellular support and mechanical strength for connective tissues [7]. Collagen extracted from the ECM provides an external mimicked environment of the body, further aiding in cellular responses such as migration, adhesion and proliferation.

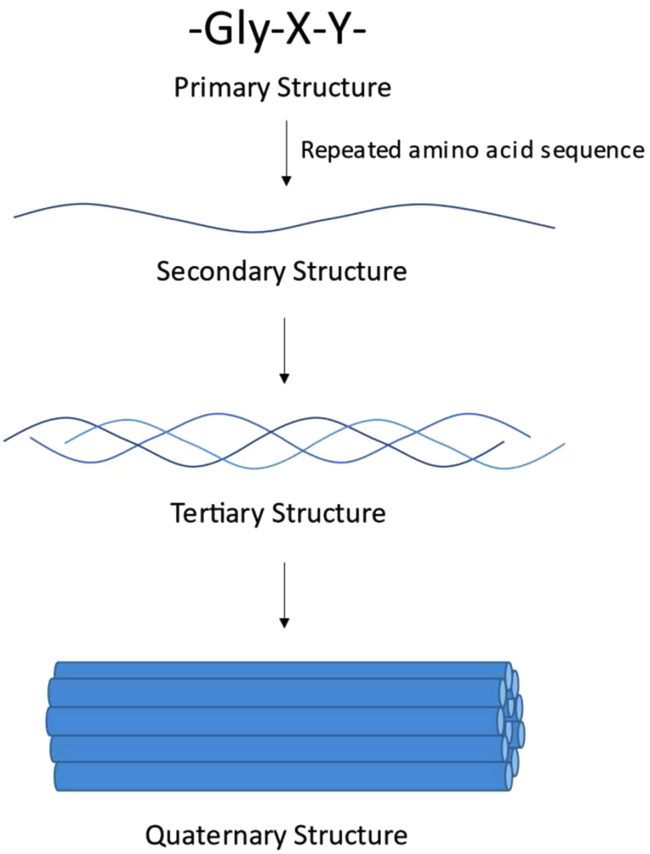

Fig. (1). Collagen fibril formation sequence.

Collagen is an ideal material for fabricating tissue-engineered biomaterials in regenerative medicine due to its biocompatibility, biodegradability, and versatility [4]. Collagen has little to no biocompatibility issues as it is widely found in the body, hence its external application on the human body would not trigger an immune response. Collagen as a biomaterial is biodegradable due to it being naturally sourced. It is also a versatile material through having the ability to conform to various three-dimensional (3D) scaffold shapes and sizes. While collagen as a biomaterial has its benefits, its most compounding disadvantages are poor mechanical properties and thermostability. Hence, modifications to collagen-based biomaterials may be the key to overcoming these limitations [6]. There is a myriad of methods for collagen modifications, however for this book chapter, we will focus on reducing collagen to nanocollagen.

CHAPTER 14

Engineering Skin for Wound Repair and Regeneration

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Abstract: Skin tissue engineering requires a multidisciplinary effort, reflecting the complexity of the organ that it attempts to replace after loss due to injury, trauma or as a consequence of diseases. Skin substitution aims to achieve complete closure of the existing defect and restoration of the normal function of the tissue. The major challenge faced whilst attempting to achieve such outcomes is successfully recapitulating the complex biology, chemistry and mechanical environments within native skin. Although major advances have been made to include biological entities (cells, biomolecules, small molecules) into engineered substrates to promote biological responses, the role that the substrate itself provides is often overlooked. In this chapter, we consider what might be required so that successful skin substitution post-trauma can be routinely achieved. This will require that researchers from different disciplines collaborate to ensure that not only should the cell types and matrices be carefully chosen, but in parallel, the resultant mechanical parameters need to be considered in the design process. We postulate that an engineering approach is required to recapitulate the skin by driving native healing pathways, ultimately creating a system where the synergistic effects are greater than simply the sum of its parts; where each partial component reflects various aspects of the human biology (cells, annexal structures, *etc*.), chemistry (materials, gradients, *etc*.) and physics (mechanics, *etc*.).

Keywords: Regeneration, Skin, Skin substitutes, Tissue engineering, Wound repair.

INTRODUCTION TO SKIN

The skin is the largest organ in the body and serves a complex purpose, protecting the inner organs from the physical and the biochemical factors of the environment, whilst still allowing the organism to interact with it and sense it. Despite its functional complexity, the skin has the ability to repair when superficially damaged. Deeper, more expansive defects, present a challenge for

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the organism to regenerate completely; with such defects often resulting in scarring, or entering a state of healing arrest known as a chronic wound [1]. 2.2 million people are affected by chronic wounds in the UK alone, accumulating a £5.3 billion annual expenditure for the UK National Health Service (NHS) [2]. Such prevalence is not isolated to the UK, with *ca.* 8.2 million patients suffering from wound healing complications within the US, incurring a financial burden of at least $28.1 billion [3]. Following the repair of such wounds, scarring becomes a huge challenge. It has been estimated that approximately 100 million new scars are formed annually, in the developed world alone, most arising from operations, however, at least a quarter result from trauma [4]. Statistically, wounds which do not heal within the first year of the injury translate to a 135% increase in expenditure, to the ones that do heal, which emphasises on the potential economic relief that skin engineering could offer in the field of wound healing [5].

Skin engineering is concerned with developing replacement therapies that guide the healing process in two main directions: towards closure of the existing defect, and recovery of the normal function of the tissue. The major challenge faced whilst attempting to achieve such outcomes, is successfully recapitulating the complex biology, chemistry and mechanical environments within native skin. Historically, much research has focused heavily on understanding the epidermis, examining the biology, structures and architectures [6, 7]. However, more recently, researchers have started to take a much closer look at the roles of other dermal layers having demonstrated the significance of the hypodermis when it comes to healing in much deeper wounds [8]. To add to the challenges posed in engineering replacement skin constructs, its thickness can vary from micro- to milli- metre length scales depending on where it is found on the body (eye lids to hands, stomach and back) [9 - 11]. Furthermore, skin mechanics are also location specific; where the state of tension and potential for retraction is dependent upon the body site. This is a characteristic originally discovered over 150 years ago by Langer who “mapped” skin contours in humans, which are known as Langer’s lines [12]. To this end, it becomes clear that engineering replacement tissue requires a much more bespoke approach incorporating both an understanding of the local biology (including how it becomes compromised throughout trauma) and the localised physico-chemical/mechanical components.

Skin Structure

As previously alluded to, such complexity as a direct function of the multi-layered/cellular/component tissue, requires a multi-disciplinary approach to skin repair, spanning numerous fields [16, 17]. Indeed, typical scientific approaches, systematically probing various components of the skin, in order to understand the overall picture, often require simplification of the system, isolating key variables.

For this reason, it is often easier to comprehend the structure of skin from two angles; 1) The physical and, 2) The biological structures of the tissue.

Physical Structure (Material Components)

Skin can be typically divided into 3 distinct layers: epidermis, dermis and subcutaneous/hypodermis (Fig **1**). The epidermis is the outer most layer of the skin, whose primary function provides a physical barrier to the surrounding environment as well as maintaining water balance in the tissue. Its role in protection is provided through its structure, where unlike the other two layers, it is mostly comprised of tightly packed cells. These cell layers can be sub-divided into 4 sections, stratum corneum, stratum granulosum, stratum spinosum and stratum basale (germinativum) with a fifth layer (Stratum lucidum) found only in thick skin (palms and soles) [18]. The cellular entities inter-dispersed between a complex lipid-protein matrix give rise to a "brick and mortar" microstructure that not only provides a highly, tortuous path for mass transport, but is inherently lipophilic [19].

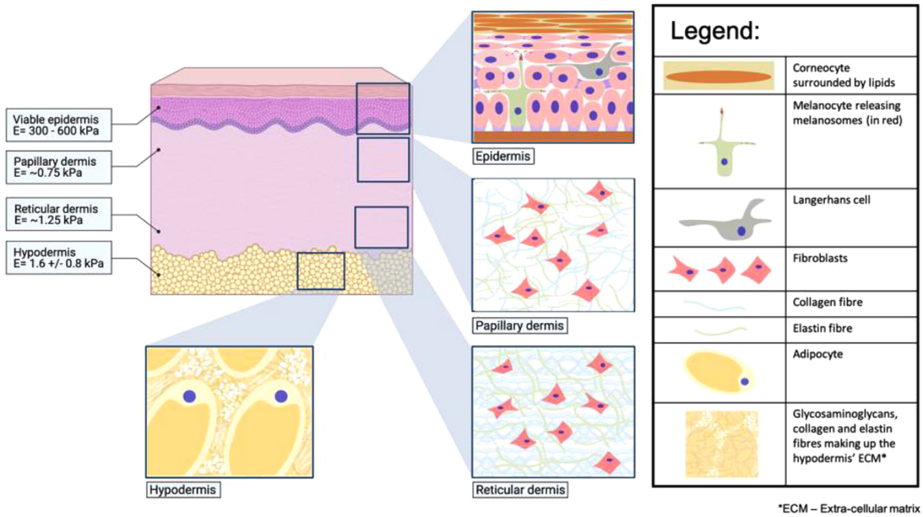

Fig. (1). Schematic demonstrating the complexity of the skin's structure and highlighting relevant mechanical stiffnesses [13 - 15].

In contrast, moving down through the skin layering, much of the dermis comprises a proteinaceous extracellular matrix (ECM). Again, the microstructure plays a key role in its function. The dermis is composed of two layers: papillary and reticular [1]. Importantly, the papillary dermis contains rete ridges ("finger-like" projections connecting the epidermis to the dermis) at the dermal-epidermal junction which prevents delamination upon shearing [11]. Surrounding these

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