Kurdistan Region-Iraq
Ministry of Higher Education
And Scientific Research
Salahaddin University-Erbil

Skin Tissue Engineering

A Thesis

Submitted to the Council of the Education College -University of Salahaddin-Erbil in Partial Fulfillment of the Requirements for the Degree of Bachelor Science in Tissue Engineering - Biochemistry

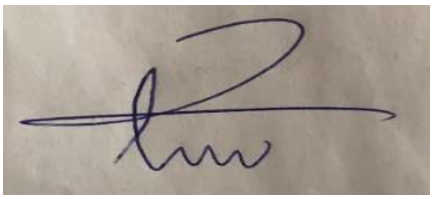
By
Noor Y. Othman

Supervised by
Lecturer: Hemn J. Majeed

April 2022 A.D. Ramadan 1442 Al-H. Gulan 2722 K.

Supervisor certification

I certify that this thesis was prepared under my supervision in the Department of chemistry, college of Education, University of Salahaddin-Erbil and hereby recommend it to be accepted in partial fulfillment of the requirements for the degree of Bachelor of Science in Tissue engineering/Clinical Biochemistry.

Signature: 

Supervisor: **Lecturer Hemn J. Majeed**

Date: / 4 /2022

Acknowledgements

*First of all, my thanks are addressed to **GOD** for his blessing me able to complete and perform this study with success.*

*Deepest gratitude with great respect to my supervisor, **Lecturer Hemn J. Majeed** for his continuous advice me and for taking the time to evaluate my work. I am also thankful for his encourage during my whole bachelor studies, which has always been supportive.*

Appreciation and gratefulness are dedicated to the presidency of Salahaddin University-Erbil, the deanery of College of Education and to the head of Chemistry Department for their valuable advice and guidance during the stages of the study.

Abstract

Tissue engineering, often known as bioengineering, is made up of three components: cells, scaffolds, and signaling molecules. These elements work together to promote the growth of new tissue. Tissue engineering also necessitates 3 dimensional cell cultures, which, unlike typical bidimensional cell cultures, have only lately been established.

This cell culture technique now has no doubt several advantages, including constant interchange of nutrients and oxygen, metabolite elimination, and mechanical support. as well as chemical stimulus All of these variables contribute to cell differentiation and facilitation proliferation. In the recent study, firstly we focused on the basic strategy of tissue engineering. Then, the skin and the anatomy of all parts of skin layers. In addition, the most suitable and available types of cells used in skin tissue engineering as well as the characteristic of scaffold and natural biomaterial used in this part such as Collagen and Elastin. Finally, we label application and conclusion.

CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

Symbol	*Description*
3D	Three Dimensional
SCs	Stem Cells
ECM	Extra Cellular Matrix
ESc	Embryonic Stem cell
iPSC	Induced Pluripotent Stem cell
HSC	hematopoietic stem cells
Gly-X-Y	Glycin – Prolin – 4. Hydroxyprolin
FDA	Food Drug Administration
UV	Ultra Violet

1. Introduction

Human tissue loss or end-stage organ failure resulting from an injury or a disease is a major health care problem in the world as the transplantation of tissues or organs in these patients is severely limited by availability of compatible donors. In this context, three-dimensional biofabrication techniques have emerged as powerful tools to produce functional homogeneous/heterogeneous substitutes (scaffolds), which have a huge potential to fulfill biological and mechanical requirements in order to achieve the full tissue regeneration. (Nuno Alves, et al, 2016)

Tissue engineering or Bioengineering, as defined by Langer and Vancanti, constitute an innovation in regenerative medicine and is based on three elements (i) cells; (ii) scaffold and (iii) growth factors. Scaffolds can be bi-dimensional and three-dimensional, bi-dimensional structures allow us to observe only cell behavior with reference to medium composition, cell-cell interaction, cell viability and cell differentiation. However, three-dimensional structures allow us more physiologically realistic factors including dynamic fluids rich in O_2, mechanical forces, and cell adhesion but this interaction is three-dimensional and can be modify cell behaviour (Breyner et al, 2011).

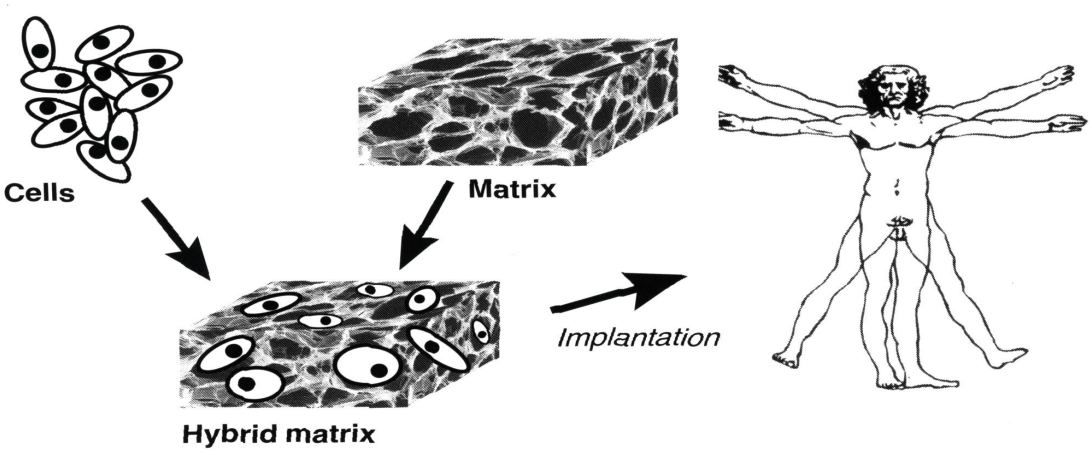


Figure 1. Schematic representations basic principle of tissue engineering

Tissue generation by autogenous cell transplantation is one of the most promising treatment concepts being developed as it eliminates problems of donor site scarcity, immune rejection and pathogen transfer. Cultured cells are seeded onto a three-dimensional biocompatible scaffold that will slowly degrade and resorb as the soft and hard structures grow and assimilate in vitro and/or in vivo. The 3D scaffold provides the necessary template for cells to proliferate and maintain their differentiated state. Ultimately, it defines the overall shape of the tissue-engineered transplant. (Hutmacher, D.W.et al, 2001).

Injury or damage to tissue and organs is a major health problem, resulting in about half of the world's annual healthcare expenditure every year. Advances in the fields of stem cells (SCs) and biomaterials processing have provided a tremendous leap for researchers to manipulate the dynamics between these two, and obtain a skin substitute that can completely heal the wounded areas. Although wound healing needs a coordinated interplay between cells, extracellular proteins and growth factors, the most important players in this process are the endogenous SCs, which activate the repair cascade by recruiting cells from different sites. Extra cellular matrix (ECM) proteins are activated by these SCs, which in turn aid in cellular migrations and finally secretion of growth factors that can seal and heal the wounds. The interaction between ECM proteins and SCs helps the skin to sustain the rigors of everyday activity, and in an attempt to attain this level of functionality in artificial three-dimensional (3D) constructs, tissue engineered biomaterials are fabricated using more advanced techniques such as bioprinting and laser assisted printing of the organs. (Singh, D., Singh, D. and Han, S.S., 2016)

2. Skin

The skin is the largest organ of the human body, representing approximately one-tenth of the body mass, and is necessary for animal survival. This organ serves several important functions, including physical barrier to the external environment, thermal regulation, and retention of normal hydration. When a large percentage of skin is lost, cultured epithelial sheets are routinely used to make autologous grafts, which can be lifesaving for patients with extensive burns (David J Wong and Howard Y Chang, 2009). Autologous keratinocytes can be isolated and cultured into cohesive sheets of epithelium that can be transplanted onto large skin defects on the patient. Clonogenic keratinocytes, termed holoclones, can be isolated from skin and serially propagated in culture for over 140 doublings, and have been shown to be bona fide multipotent stem cells based on their ability to renew multiple lineages in the skin (B Ter Horst, et al, 2018).

2.1 Skin anatomy and Functions

Skin is the largest and heaviest organ of the human body, with an area of 1.5–2.0 m^2 and a weight of 3.5–10 kg. This complex organ is the outermost barrier of the body that protects inner organs from microbial pathogens, mechanical and chemical insults, regulates the body temperature, gives support to blood vessels and nerves, and prevents dehydration. Furthermore, it is also involved in the immune surveillance and sensory detection process. Anatomically and functionally, the skin is formed by three connected layers, the epidermis, the dermis and the hypodermis (figure 2). The epidermis, the outermost layer, is primarily composed of stratified squamous epithelium of keratinocytes, which is derived from neurectoderm and comprises over ninety percent of epidermal cells. Keratinocytes are responsible for the cohesion of the epidermal structure and the barrier function. Pigment-containing melanocytes of neural crest origin, antigen-processing Langerhans cells of

mesoderm origin, and pressure-sensing Merkel cells of neural crest origin also reside within the epidermis.

The dermis is a connective tissue that is responsible for the mechanical properties of the skin. It is composed of fibroblasts of mesoderm origin, which lie within an extracellular specialized matrix. Collagens are interwoven with elastin, proteoglycans, fibronectin, and other components. The epidermis and dermis are connected by a basement membrane that is composed of various integrins, laminins, collagens, and other proteins that play important roles inregulating epithelial-mesenchymal cross-talk. The superficial papillary dermis is arranged in ridge-like structures called the dermal papillae, which contains microvascular and neural networks and extends the surface area for these epithelial mesenchymal interactions. Sebaceous glands, eccrine glands, apocrine glands and hair follicles are of neurectoderm origin and develop as down growths of the epidermis into the dermis. In addition, the dermis also contains blood vessels and lymphatic vessels of mesoderm origin, and sensory nerve endings of neural crest origin. The hypodermis, which is deep to the dermis, is composed primarily of adipose tissue of mesoderm origin, and separates the dermis from the underlying muscular fascia (David J Wong and Howard Y Chang, 2009).

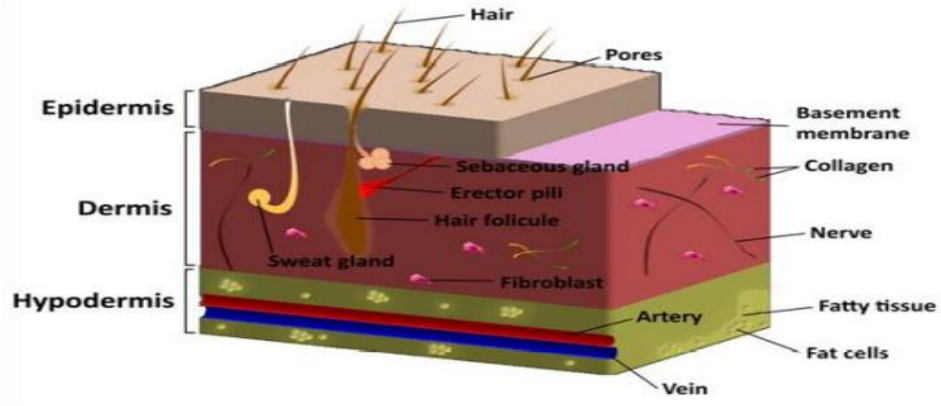


Fig 2. Schematic representation of the structure of normal skin tissue

Skin is the outermost tissue of the body and the largest organ in terms of both weight and surface area. It has an area of approxinatly 16,000 cm^2 for an adult and represents about 8% of the body weight. As seen in Figure 3 skin has a very complex structure that consists of many components. Cells, fibers and other components make up several different layers that give skin a multi-layered structure. Veins, capilaries and nerves form vast networks inside this structure. In addition, hairs stick out from the inside of skin. Numerous fine hair furrows are scattered over the surface of skin.

Skine performs a wide variety of functions resulting from chemical and physical reactions inside these components. The major function of skin is to act a barrier to the exterior environment. It protects the body from friction and impact wounds with its flexibility and toughness. Harmful chemicals, bacteria, viruses, and ultraviolet light are also prevented from entering the body by the skin. In also prevent water loss and regulates body temperature by blood flow and evaporation of sweat. These funcionalities are critical to our well-being.

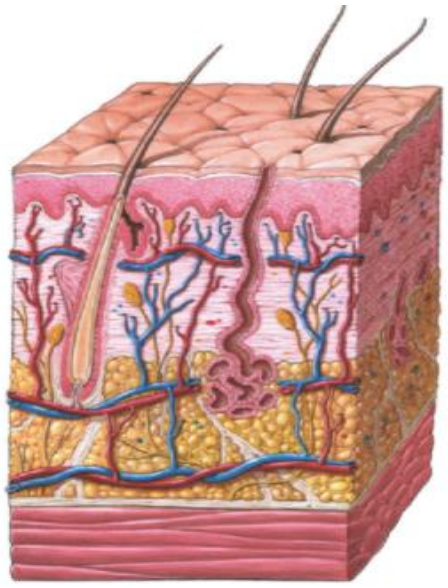


Fig 3. A cross-sectional schematic diagram of skin. Skin is a complex multi-layered tissue consisting of various types of components, including veins, capillaries, hairs, cells, fibers, etc. (image courtesy of A.D.A.M.)

2.2 skin layer

Cellular-level elements (level 1) form the three different skin layers; *epidermis, dermis, and subcutis*. These layers are composed of different types of cellular-level elements. Hence, they are very different in terms of structure and function. As a result, they exhibit different types of light propagation.

2.2.1 Epidermis

The epidermis is the outermost layer of skin. There are no veins and capillaries in this layer. Its thickness is about 0.2 mm on average and this thickness varies depending on the location on the body. Furthermore, the thickness also varies according the volume of water that epidermis holds.

The epidermis is further divided into five sublayers. From the bottom (innermost), these sublayers are *stratum basale, (basal cell layer), stratum spinosum (prickle cell layer), stratum granulosum (granular cell layer), stratum lucidum (clear layer), and stratum corneum (horny cell layer)* (see figure 4).

The epidermis is a metabolically active tissue. Keratinocytes produced in stratum basale move upward to the outer surface. This process is called *turn-over*. During this *turn-over*, keratinocytes change their structures and physiological functions. One cycle of this turn-over process take about 28 days.

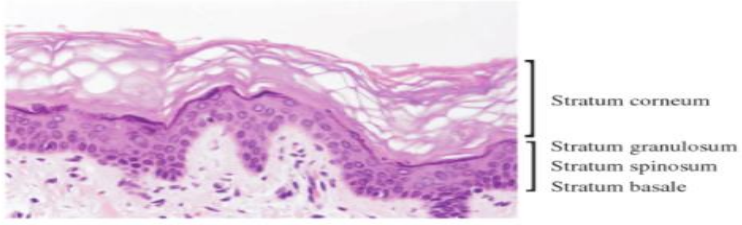


Fig 4. Microscopic image of the epidermis. The epidermis is composed of four sublayers: stratum corneum, stratum granulosum, stratum spinosum and stratum basale. In the sole and the palm, there is an additional layer called stratum lucidum underneath stratum corneum.

The main function of the epidermis is to protect the skin from potential threats and also to act as an efficient barrier at the top of the skin. As can be observed in figure 5A, the epidermis is composed of five distinct cell layers, according to the different stages of keratinocyte maturation. From the deepest to the most superficial, these layers are known as strata basale, spinosum, granulosum, lucidum, and corneum.

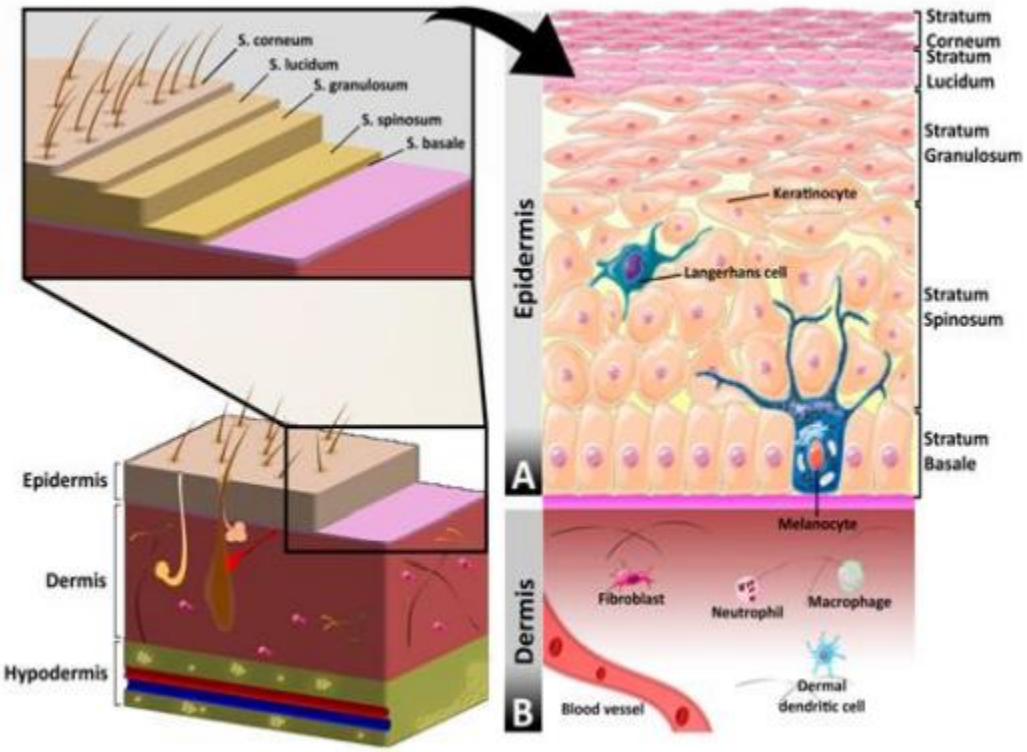


Figure 5. Representation of the structure of the skin. The outermost layer of the epidermis, the stratum corneum, is fundamental for avoiding microorganisms' penetration and also protect the skin from environmental insults. The other strata (lucidum, granulosum, spinosum and basale) are fundamental for the remodeling of the stratum corneum. The epidermis also contains Langerhans cells and melanocytes (A). The dermis has as mainly cellular components the fibroblasts but also contains cells of the immune system (neutrophils and macrophages), blood vessels, and nerve fibers (B).

2.2.2 Dermis

The dermis is the second layer of skin, beneath the epidermis layer. Tis layer is much thicker than the epidermis (usualy 1-4 mm) the main components of the dermis are collagen and elastin fibers. Compared to the epidermis, there are much fewer cells and much more fibers in the dermis.

This layer supplies energy and nutrition to the overlying epidermis and also provides mechanical integrity to the skin due to the arrangement of collagen fibers that are deposited by local fibroblasts. The dermis is composed of two distinct layers: the upper papillary dermis and the deeper reticular dermis. The papillary dermis is essentially composed of loose connective tissue, namely elastin fibers interspersed with collagen fibers. It also holds numerous blood vessels that assure nutrients delivery, remove waste products and also allow the regulation of the body temperature. In turn, the reticular dermis is denser and composed by larger collagen fibers that confer flexibility to the skin. Fibroblasts are the main cell type found in the dermis and they are responsible for the production and deposition of extracellular matrix (ECM) components. The dermal layer also contains cells of the immune system (neutrophils and macrophages), lymphatic vessels, nerve fibers, sweat and sebaceous glands, the deep portion of hair follicles and endothelial cells (figure 5B).

2.2.3 Subcutis or Hydrodermis

Subcutis, or hydrodermis, is the third layer beneath the dermis. It is important to note that it is not categorized as another skin layer. Subcutis is an elastic layer and includes a large amount of fat cells that work as a shock absorber for blood vessels and nerve endings. The thickness of this layer is reported to be 4-9 mm on average. However, the actual thickness differs from person to person and also depends on the body region.

Hypodermis is the deepest skin layer and it is mainly composed of adipose tissue. This layer insulates the body and provides mechanical protection against physical shock. Structurally, hypodermis is divided into lobules containing adipose cells separated by fibro-vascular septa. The septa are composed of collagen and reticulin fibers, blood and lymphatic vessels. In addition to adipose cells, hypodermis also contains fibroblasts and macrophages, that have an important role in the stimulation of thermogenesis during cold exposure or exercise.

((Igarashi, T., Nishino, K. and Nayar, S.K., 2007) (Figueira, D.S.R., 2016))

3. Cells

Tissue engineering strategy demands high numbers of cells, therefore, ideal cell sources for tissue engineering application must be easily isolated, expandable to higher passages, be non-immunogenic and have a protein expression pattern similar to the tissue regenerated.

Keratinocyte derived from autologous tissue constitutes the most obvious choice to be used in tissue engineering, for their absence of immunogenicity and possibility of limited expansion in vitro. However, this methodology suffers from many limitations, such as the generation of a second site of Skin lesion, as well as the limited amount of cells obtained at the end of the procedure. As an alternative, stem cells present a great therapeutic potential due to their capacity of differentiation to many cell lineages. These cells are able to self-renew and proliferate for long periods in vitro. Stem cells are divided into two great classes: adult and embryonic stem cells and also divided based on their differentiation potential. even though they may also be described based on their differentiation potential (Breyner et al, 2011).

3.1 Stem cells

Tissue-resident stem cells are surrounded by a microenvironment known as ‘stem cell niche’ which is specific for each stem cell type. This niche comprises of cell-intrinsic and -extrinsic factors like biochemical and biophysical signals, which regulate stem cell characteristics and differentiation. Biochemical signals have been thoroughly studied however, the effect of biophysical signals on stem cell regulation is yet to be completely understood. Biomaterials have aided in addressing this issue since they can provide a defined and tuneable microenvironment resembling in vivo conditions.

3.1.1 Embryonic stem cell or pluripotent cells

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocyst-stage embryos. Their importance to modern biology and medicine derives from two unique characteristics that distinguish them from all other organ-specific stem cells identified to date. First, they can be maintained and expanded as pure populations of undifferentiated cells for extended periods of time, possibly indefinitely, in culture. Unlike transformed tumor cell lines, ES cells can retain normal karyotypes following extensive passaging in culture. Second, they are pluripotent, possessing the capacity to generate every cell type in the body (Gordon Keller 2005).

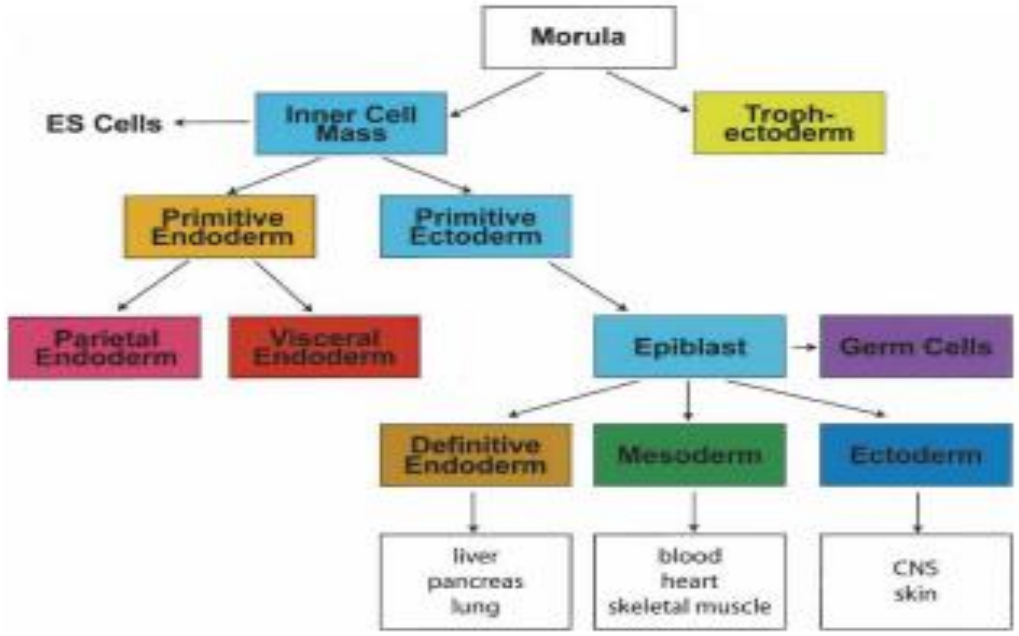


Figure 6. Scheme of early mouse development depicting the relationship of early cell populations to the primary germ layers.

3.1.2 Adult stem cell or Multipotent stem cells

Stem cells are self-renewing cells that can differentiate into specialized cell type(s). Pluripotent stem cells, i.e. embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC) differentiate into cells of all three embryonic lineages. Multipotent stem cells, like hematopoietic stem cells (HSC), can develop into multiple specialized cells in a specific tissue. Unipotent cells differentiate only into one cell type, like e.g. satellite cells of skeletal muscle. There are many examples of successful clinical applications of stem cells. Over million patients worldwide have benefited from bone marrow transplantations performed for treatment of leukemias, anemias or immune deficiencies. Skin stem cells are used to heal severe burns, while limbal stem cells can regenerate the damaged cornea. Pluripotent stem cells, especially the patient–specific iPSC, have a tremendous therapeutic potential, but their clinical application will require overcoming numerous drawbacks. Therefore, the use of adult stem cells, which are multipotent or unipotent, can be at present a more achievable strategy. Noteworthy, some studies ascribed particular adult stem cells as pluripotent. However, despite efforts, the postulated pluripotency of such events like “spore–like cells”, “very small embryonic-like stem cells” or “multipotent adult progenitor cells” have not been confirmed in stringent independent studies. Also plasticity of the bone marrow–derived cells which were suggested to differentiate e.g. into cardiomyocytes, has not been positively verified, and their therapeutic effect, if observed, results rather from the paracrine activity (Józef Dulak et al, 2015).

4. Characters of Scaffold and its Biomaterial

Tissue engineering is a new emerging biotechnology that focuses on the synthesis of new 3-D biofunctional materials to serve as porous scaffolds for cell attachment. These constructs, built from synthetic or natural polymers, can be used to produce neo-tissue with mature extracellular matrix and to guide the proliferation and spread of seeded cells in vitro and in vivo. The main requirements for skin biomaterials are biocompatibility, degradability and structural integrity.

A scaffold used for tissue engineering requires a porous structure with a porosity not less than 70% and interconnected pores which allow cell growth and proliferation, in the form of three-dimensional porous structure with heterogeneous pore size. The pore structure of the scaffolds formed of the freeze-drying technique used in their fabrication. (Gaspar, A., Moldovan, et al, 2011)

The purpose of engineering new biological material is that it can restore or replace a damaged or diseased tissue and organs. The two most important components that decide the fate of tissue engineered construct are cells and artificial extra cellular matrices (ECMs), also known as scaffold or biomaterials that support cellular growth, differentiation, and migration. Creating a construct by blending the principles of life sciences, developmental biology, and engineering that can address clinical problems, has been the focus of all researchers working in the area of regenerative medicine. The foundation of the current research efforts in the field of tissue engineering is to recapitulate development processes that occur in vivo in clinical scenarios. This could be achieved with increased understanding of the roles of scaffold, stem cells, and signaling interaction of cells with artificial ECM. However, before aiming to repair any tissue or organ, understanding of its anatomical structure and biogenesis is critical as it allows the users to control the conditions that could affect the neo-tissue formation. In the case of skin, a

fundamental understanding of structure and functional relationship between normal and pathological tissue is required (Singh, D., Singh, D. and Han, S.S., 2016).

4.1 Collagen

Collagen is a natural polymer abundant in all vertebrates, which provides the major mechanical support for cell attachment. It is a biomaterial of interest to the medical community due to its advantageous properties that recommend it for tissue engineering. These properties are conferred by the molecule's native structure and chemical composition. Many types of have been discovered, which differ in their three-dimensional structure and their amino acid sequence, in order to meet the functional needs of different tissues. In recent years, special attention was paid to, due to its excellent biocompatibility and its ability to degrade into well-tolerated compounds. The favorable influence of on cell infiltration and wound healing. (Gaspar, A., Moldovan, et al, 2011). This natural biomaterial is distinct from other proteins in that the molecule comprises three polypeptide α-chains that assemble together which form a unique triple-helical structure (Figure 7). This tight wrapping into a triple-helix and covalently crosslinked to each other stabilize the fibrils collagen and provides great tensile strength. Each of the α chain is composed of more than a thousand amino acids with the repeating structure –Gly-X-Y- in which glycine essential at every third residue, while X and Y can be any amino acid but mostly frequent proline at X position and 4-hydroxyproline at Y position (Van der Rest and Garrone 1991).

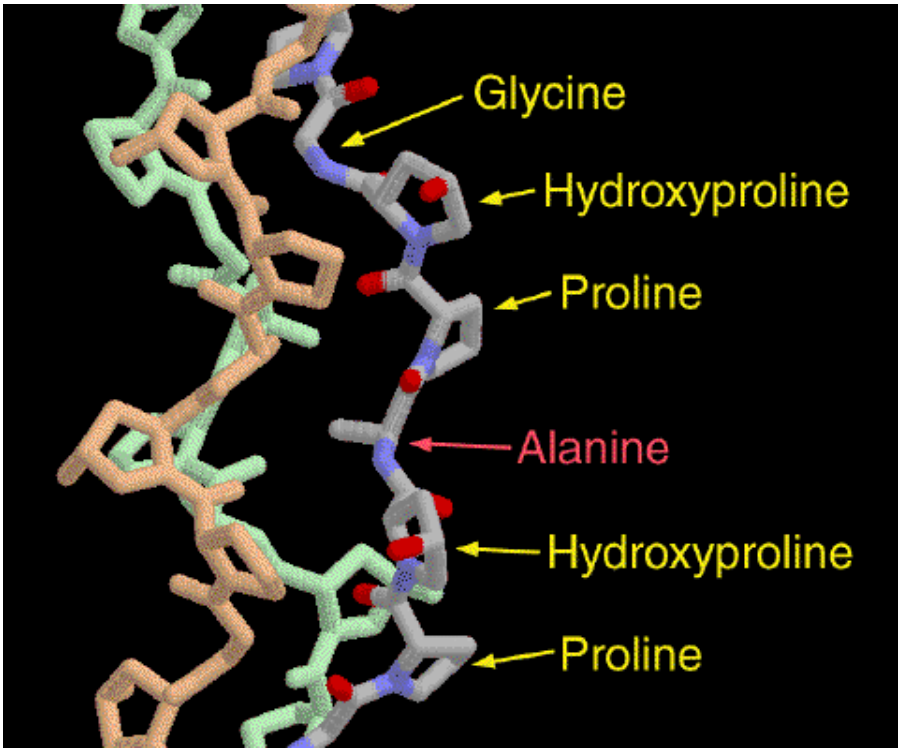


Figure 7: Schematic representation of self-assemble of triple-helical collagen fibril molecules (http://www.rcsb.org/pdb/101/motm.do?momID=4).

The most widely used tissue equivalent scaffolds clinically, is for replacement dermal equivalents. The majority of products available rely upon a fabricated collagen mesh, mainly without a cellular component. Generally, collagen is isolated from tissue samples, and in the process the collagen is greatly disaggregated and denatured, often resulting in complete loss of the original architecture and packing, and comprising mainly of fragments of the original protein. The majority of these scaffold equivalents are composed solely of collagen, without the addition of other extracellular matrix components.

Integra is the first FDA-approved skin substitute consisting of a suturable, semi-permeable silicone elastomer (polysiloxane, Dow Corning Liquid Silastic Medical Adhesive Type A) cured to an underlying dermal component made of a degradable

crosslinked (vacuum dehydration and gluteraldehyde) coprecipitation of bovine collagen and (8%) chondroitin 6-sulfate (a shark cartilage derived glycosaminoglycan). Integra is used to reconstruct the skin in a two stage procedure in surgically excised burn injuries or in excised benign or malignant lesions. After it has integrated, generally 2-3 weeks after implantation the silicone membrane is removed and the neodermis is grafted with a split thickness skin graft (Cheema, U. et al 2011).

4.2 Elastin

Biomaterials based upon elastin and elastin-derived molecules are increasingly investigated for their application in tissue engineering. This interest is fueled by the remarkable properties of this structural protein, such as elasticity, self-assembly, long-term stability, and biological activity. Elastin can be applied in biomaterials in various forms, including insoluble elastin fibers, hydrolysed soluble elastin, recombinant tropoelastin (fragments), repeats of synthetic peptide sequences and as block copolymers of elastin, possibly in combination with other biopolymers.

Elastin-based materials are becoming more and more popular as biomaterials for tissue engineering. This interest is established by its remarkable properties. Elastin is an extracellular matrix protein that provides elasticity to tissues and organs. Therefore, elastin is most abundant in organs where elasticity is of major importance, like in blood vessels, which stretch and relax more than a billion times during life, in elastic ligaments, in lung and in skin. (Daamen, W.F., et al, 2007)

5. Clinical Application

Two main groups of patients would profit most from a tissue engineered skin substitute. The first group includes burn patients, suffering from an acute life-threatening situation (Fig. 8A). Large and deep burn wounds leave little remaining healthy skin to be used for split-thickness skin grafts. The challenge here is to rapidly produce large quantities of autologous, dermo-epidermal substitutes. The second group denotes the elective or chronic situation. Disabling scars, giant nevi (Fig. 8B) or chronic ulcers are ideally replaced by a skin graft of matching size, texture, and colour. Full- and split-thickness skin grafts may not always be available in a sufficient quantity. The challenge in this respect is the engineering of functionally and cosmetically adjusted skin substitutes, ready for transplantation at a previously scheduled point in time. There exist several ‘‘commercial’’ treatment modalities next to skin grafting. These may or may not help to improve the structure and function of the grafted skin, such as some dermal substitutes or keratinocyte sprays. In any case, various basic problems are still encountered. For a given skin substitute to attach promptly after transplantation, a well prepared and vascularized wound bed is required. This is not always easy to achieve in deep burn wounds or with chronic wounds. If a dermal substitute reaches a threshold thickness, vascularization is too slow to assure nutrition of the overlying epidermis resulting in epidermal necrosis or graft loss. Therefore, most dermal substitutes thicker than 1 mm (Integra1, Matriderm1) are applied using a two-step approach. This avoids epidermal necrosis, as the dermal substitute is given sufficient time to vascularize. However, an additional operation is needed for transplantation of an epidermal component. This procedure is lengthy, gives no guarantee of success and is an additional stress factor for the patient. The transplanted epidermal component produces skin of varying quality and exhibits properties that are distinct from the original. Features of the

transplant may be missing elasticity, contraction of the graft, lack of pigmentation, and thereby lack of protection against UV radiation. All these factors let us conclude that there is still

a high potential for the development of novel, significantly improved skin substitutes. (Böttcher-Haberzeth, et al, 2010)

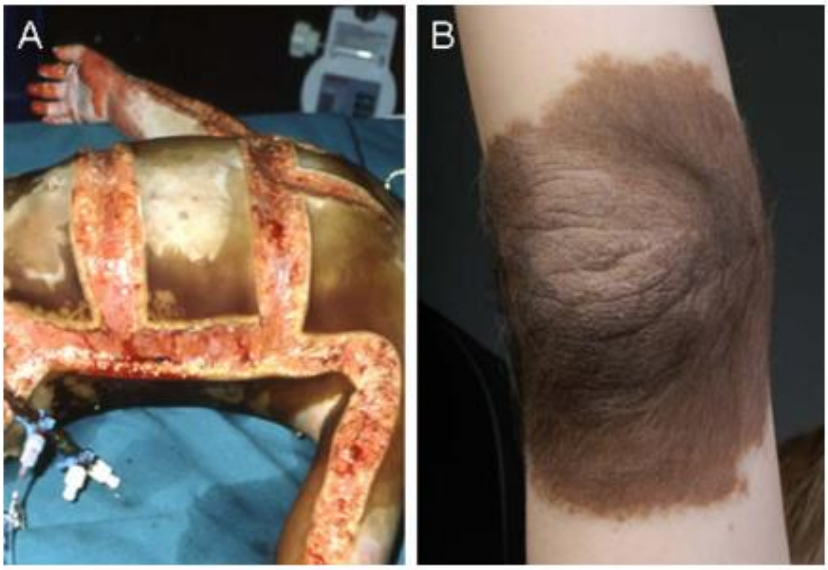


Fig. 8 – The need of tissue-engineered skin. (A) The acute situation. Patient, suffering from large and deep burn injury after escharatomy, necessitating necrectomy and skin coverage. Due to the large total body surface area affected, little healthy skin remains as donor site for a split-thickness skin graft. (B) The elective situation. Patient with a giant congenital melanocytic nevus, expanding over the elbow. After resection, the patient would benefit of the application of a tissue engineered skin substitute of matching color and texture to the adjacent skin.

6. Conclusion and future Outlook

The goal of our review was to study molecularly specified bio scaffolds that supply cells with the necessary cues and instructions to produce the desired tissue/organ. Using highly purified biomaterials, including fibrillar scaffold molecules (collagen, elastin), modifying molecules (glycosaminoglycans), and effector molecules, several basic tailor-made scaffolds with a suitable porosity shape. These scaffolds have previously training physicochemical, biomechanical, and morphological properties, they have a lot of potential for tissue engineering in areas like the bladder, cartilage, and skin. Organ-specific scaffolds that imitate the desired microenvironment can now be created. This enables for the design of a specific scaffold on the drawing board, then its assembly in the lab. the design of a scaffold or tissue engineering of skin is given here (Fig. 9)

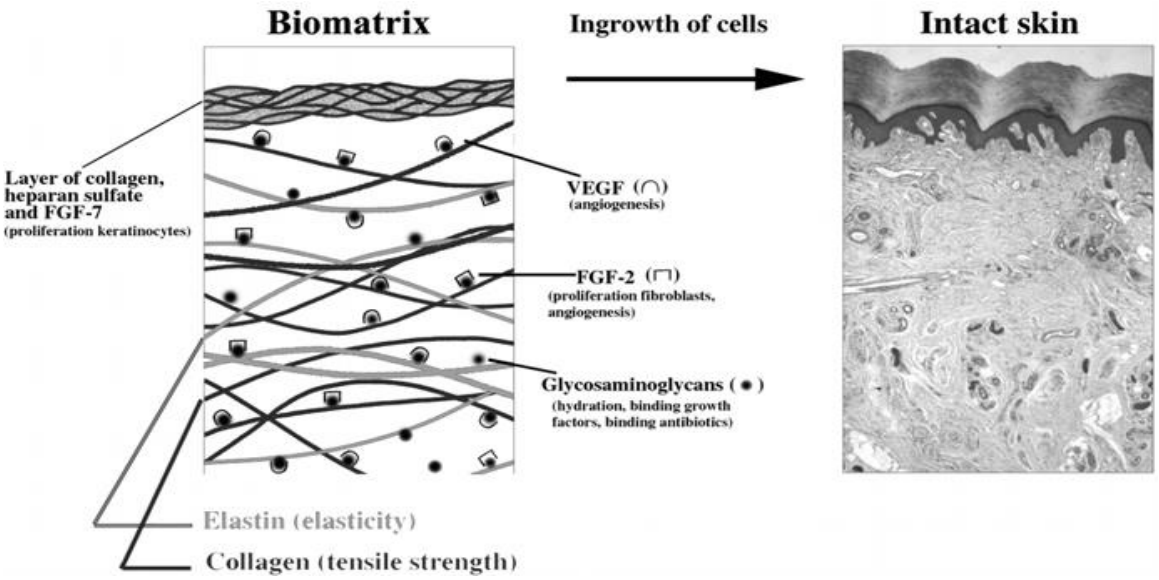


Fig. 9 A new acellular bilayered bioscaffold design for tissue-engineered skin.

7. References

Nuno Alves, Paula Faria, Artur Mateus and Pedro Morouço., 2016. Three.dimensional biofabrication tecnhiques for tissue regeneration. Journal of Biomedical MaterialsResearch (Applied Biomaterials), 53:6, 617–620, 2000

Breyner, N. M., Zonari, A. A., Góes, A. M., Gomes, D., Carvalho, J. L., & Gomide, V. S. (2011). Cartilage Tissue Engineering Using Mesenchymal Stem Cells and 3D Chitosan Scaffolds-In Vitro and in Vivo Assays. INTECH Open Access Publisher, (pp. 211-226).

Hutmacher, D.W., Goh, J.C.H. and Teoh, S.H., 2001. An introduction to biodegradable materials for tissue engineering applications. *Annals-academy of medicine singapore*, *30*(2), pp.183-191.

Singh, D., Singh, D. and Han, S.S., 2016. 3D printing of scaffold for cells delivery: Advances in skin tissue engineering. *Polymers*, *8*(1), p.19.

Wong, D.J. and Chang, H.Y., 2009. Skin tissue engineering. *StemBook [Internet]*. (2)

Ter Horst, B., Chouhan, G., Moiemen, N.S. and Grover, L.M., 2018. Advances in keratinocyte delivery in burn wound care. *Advanced drug delivery reviews*, *123*, pp.18-32.

Igarashi, T., Nishino, K. and Nayar, S.K., 2007. *The appearance of human skin: A survey*. Now Publishers Inc.

Figueira, D.S.R., 2016. *Desenvolvimento de novas abordagens terapêuticas para a regeneração da pele* (Doctoral dissertation, Universidade da Beira Interior (Portugal)).

Keller, G., 2005. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes & development, 19(10), pp.1129-1155.

Dulak, J., Szade, K., Szade, A., Nowak, W. and Józkowicz, A., 2015. Adult stem cells: hopes and hypes of regenerative medicine. *Acta Biochimica Polonica*, *62*(3).

Gaspar, A., Moldovan, L., Constantin, D., Stanciuc, A.M., Boeti, P.S. and Efrimescu, I.C., 2011. Collagen–based scaffolds for skin tissue engineering. *Journal of medicine and life*, *4*(2), p.172.

Van der Rest, M., & Garrone, R. (1991). Collagen family of proteins. The FASEB Journal, 5(13), 2814-2823.

Cheema, U., Ananta, M. and Mudera, V., 2011. Collagen: applications of a natural polymer in regenerative medicine. *Regenerative Medicine and Tissue Engineering-Cells and Biomaterials*, pp.287-300.

Daamen, W.F., Veerkamp, J.H., Van Hest, J.C.M. and Van Kuppevelt, T.H., 2007. Elastin as a biomaterial for tissue engineering. *Biomaterials*, *28*(30), pp.4378-4398.

Böttcher-Haberzeth, S., Biedermann, T. and Reichmann, E., 2010. Tissue engineering of skin. *Burns*, *36*(4), pp.450-460.

.