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International Materials Reviews

Recent concepts in biodegradable polymers for tissue engineering paradigms: A critical review --Manuscript Draft--

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Abstract:	<p>Tissue engineering and regenerative medicine are emerging as future approaches for the treatment of acute and chronic diseases. However, many challenging clinical conditions exist today and include congenital disorders, trauma, infection, inflammation and cancer, in which hard and soft tissue damage, organ failure and loss are still not treated effectively. Regenerative medicine has contributed to a number of innovations through artificial implants and biomedical materials, with advances are continually being made. Researchers are constantly developing new biomaterials and tissue engineered technologies to stimulate tissue regeneration in order to repair and replace damaged or malfunctioning organs. However, the challenge continues to lie in devising effective biomedical materials that can be implanted as scaffolds. Various approaches are emerging, according to the organ, tissue, disease and disorder. Scaffolds are implanted cell-free, or incorporated with stems cells, committed cells, or bioactive molecules. Irrespective, engineered biomaterials are required to regenerate and ultimately reproduce the original physiological, biological, chemical and mechanical properties over time. This is enabled by providing a three-dimensional architecture for cells to adhere, migrate, proliferate within, and differentiate appropriately for the growth of new tissues to provide a relevant structure, and in so doing, restore function. Biodegradable materials have been used extensively as regenerative therapies since their advent in early 20th century. One notable example is the development of surgical fixation devices. The selection, design and physicochemical properties of these materials are important and must consider biocompatibility, biodegradability and minimal cytotoxicity in the host to enable cell-proliferation, cell-matrix interactions and intercellular signalling for stimulating tissue growth. In this review, we critique the most studied and recently developed biodegradable</p>

polymers with the aim of highlighting recent trends and developments for targeting organ and tissue regeneration. Tissues and organs considered include the skin, nerves, blood vessels, heart, cornea, bone, dental and oral structure, trachea cavity. The limitations and future challenges of naturally occurring and bio mimetic tissue-engineered materials are also discussed.

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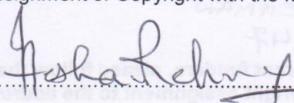
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Comments from the Editors and Reviewers:

Dear Professor Withers,

We are pleased to provide further revisions to this manuscript as requested by Reviwer#1

As you can see below from reviewers#1 comments that at this stage he had only two minor issues; first was grammar and second was mismatch between different paragraphs. Prof John W Haycock and we have extensively worked on these issues and we have also inserted new text and suitable references where these were needed.

Now, a high standard article has been produced which warrants publication in International Materials Review.

Please see enclosed marked and clean copies of this manuscript for your consideration please.

Reviewer #1: This is the third time that I review this paper, and I am very disappointed for two reasons:

1.some improvements have been made, however still conceptual mistakes and english grammar mistakes are present

Now we have corrected all the Conceptual and English mistakes, Prof John Haycock one of the co-author of this review article and a native English speaker has thoroughly corrected this revised draft.

2. the authors have done a huge work in reviewing the literature, and this work would deserve a publication.

Despite the second consideration, the manuscript cannot be published as it is, and new versions cannot be submitted "ad libitum".

There is also a huge mismatch between the quality of the paper and the level of the language of different paragraphs. Why?

Thanks to the reviewer that he admires our efforts.

These all mismatches have been corrected throughout the manuscript. We have added additional text now where it was needed (please follow these in track changes).

Yours sincerely

Dr Ihtesham Ur Rehman

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4 **Recent concepts in biodegradable polymers for tissue engineering**
5 **paradigms: A critical review**
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4 **Abstract**
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7 Tissue engineering and regenerative medicine are emerging as future approaches for the treatment
8 of acute and chronic diseases. However, many challenging clinical conditions exist today and
9 include congenital disorders, trauma, infection, inflammation and cancer, in which hard and soft
10 tissue damage, organ failure and loss are still not treated effectively. Regenerative medicine has
11 contributed to a number of innovations through artificial implants and biomedical materials, with
12 advances are continually being made. Researchers are constantly developing new biomaterials and
13 tissue engineered technologies to stimulate tissue regeneration in order to repair and replace
14 damaged or malfunctioning organs. However, the challenge continues to lie in devising effective
15 biomedical materials that can be implanted as scaffolds. Various approaches are emerging,
16 according to the organ, tissue, disease and disorder. Scaffolds are implanted cell-free, or
17 incorporated with stems cells, committed cells, or bioactive molecules. Irrespective, engineered
18 biomaterials are required to regenerate and ultimately reproduce the original physiological,
19 biological, chemical and mechanical properties over time. This is enabled by providing a three-
20 dimensional architecture for cells to adhere, migrate, proliferate within, and differentiate
21 appropriately for the growth of new tissues to provide a relevant structure, and in so doing, restore
22 function. Biodegradable materials have been used extensively as regenerative therapies since their
23 advent in early 20th century. One notable example is the development of surgical fixation devices.
24 The selection, design and physicochemical properties of these materials are important and must
25 consider biocompatibility, biodegradability and minimal cytotoxicity in the host to enable cell-
26 proliferation, cell-matrix interactions and intercellular signalling for stimulating tissue growth.
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44 In this review, we critique the most studied and recently developed biodegradable polymers with
45 the aim of highlighting recent trends and developments for targeting organ and tissue regeneration.
46 Tissues and organs considered include the skin, nerves, blood vessels, heart, cornea, bone, dental
47 and oral structure, trachea cavity. The limitations and future challenges of naturally occurring and
48 bio mimetic tissue-engineered materials are also discussed.
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54 **Key words:** Biodegradable Polymers; Skin; Heart; Vascular Arteries; Dental Regeneration; Bone,
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56 Cornea
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1. Introduction

Regenerative nanomedicine has been widely accepted which has an objective to develop the compatible biomaterials to interact with cells/tissues present in the site of implantation¹⁻³. Tissue engineering and regenerative medicine exploits properties taken from the life sciences, engineering and physical sciences⁴⁻¹⁰, and is therefore not only a highly interdisciplinary field of research, but in practice comprised of many novel and speciality areas¹¹. Humans in their life time, experience a number of acute diseases and traumas that affect cells, tissues and organs^{4, 6}, which leads to the degeneration of living cells and tissues, or the malfunctioning of an entire organ system⁷. Physicians traditionally prescribe drug therapies to induce tissue regeneration, but there are occasions when organ transplantation is the only option. Organ replacement is constrained by two reasons – firstly, artificial substitutes are not usually as effective or as long lasting when compared to the original tissue, and secondly organ donation and transplantation is highly reliant on a limited donor supply. The success of organ donation is also complicated by the potential of disease transmission or mismatching or rejection of the donor tissue. In this situation, there is utmost demand of developing biological substitutes via different chemical and biological strategies^{12, 13}. Within this review, we discuss recently developed innovative and well experimented biodegradable materials that address the aforementioned hindrances. Furthermore, we highlight recent trends and achievements accomplished in organ or tissue regeneration research. Tissues and organs considered for this purpose include the skin, cornea, nerves, blood vessels, heart, trachea, bone, dental and oral cavity. The limitations and future challenges of naturally occurring and biomimetic tissue-engineered materials are also discussed.

2. Tissue Engineering and regenerative medicine

In tissue engineering, biomedical substitutes as biomaterials are continually being developed that completely (or partly) replace damaged tissue. An essential role for bio mimetic materials is to provide a 3-dimensional matrix as a scaffold. The materials must also be permissive for ensuring the maintenance of cells and signals for regenerating the particular tissue or organ¹⁴⁻¹⁶. Regenerated tissues must reinstate, maintain and augment function thereafter. Numerous biomaterials have been used as alternative treatments for damaged tissues or dysfunctional organs,

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4 with the healing of fractured bones being a key example. The physical, chemical and biological
5 properties of biomaterials can be tuned depending upon the organ in question and biological
6 environment of the host cells or tissue. Tissue engineered materials may be functional at the time
7 of implantation, or have the ability to integrate and form the expected function after implantation
8 in the host. In either case the biomaterial must integrate favourably with transplanted or recipient
9 cells in order to play a key role for tissue regeneration through cell-cell signalling, production of
10 growth factors, proliferation, differentiation and the formation of an extracellular matrix (ECM)<sup>17-
11 22</sup>.

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19 A number of studies have been conducted in the last few decades for developing new and improved
20 bio-mimetic materials for a wide range of biomedical applications²³⁻²⁶. Developing physiologically
21 functional artificial tissues and organs is a pre-requisite of tissue engineering, and technological
22 developments in tissue engineering continually underpin progress. One of the earliest approaches,
23 that of cell-seeded scaffolds has led to the current advances, which include new materials and
24 methods of fabrication resulting into well-engineered biocompatible systems¹⁵. In addition, nano-
25 structuring of biomaterial scaffolds from nanoparticles, nanocomposites and organic-inorganic
26 hybrid polymer materials have showed progress in organ regeneration. Various synthetic and
27 natural polymers and their composite materials have been used to fabricate scaffolds for bone
28 tissue engineering, nerve regeneration, controlled drug release, tooth structure regeneration,
29 guided tissue regeneration, reinforcement of dental composite, bone and cartilage regeneration²⁷.
30 Moreover, micro fabrication technologies, such as lithography²⁸, bio printing^{29, 30}, micro
31 moulding³¹ or photolithography³² are now becoming more routine and are emerging as powerful
32 tools for the manufacture of biomaterials and tissue engineered constructs. Use of these micro and
33 nanotechnologies not only replicates cell-scale complexities by providing the cells with a
34 microenvironment that mimics the native structure, but also allows obtaining 3D architectures<sup>15,
35 33-36</sup>. The advances in biomaterials science is also complimented with progresses in cell and
36 molecular biology, in particular induced pluripotent stem cells which makes tissue engineering a
37 highly multi-disciplinary discipline. The challenge however is whether this promise will fulfil the
38 long awaited desire of having readily available methods and facile approaches for regenerating
39 tissues and organs¹⁴.

2.1- Biomaterials in Tissue Engineering

During recent years, developments in tissue engineering, regenerative medicine, gene therapy, and controlled drug delivery have placed demand on the need for better biomaterials. This includes a detailed understanding of biodegradability, in particular polymers, and ‘tailoring ‘new materials where degradability is carefully controlled^{37, 38}. Native ECM has an ability to coordinate stromal cells for synthesising new tissue (e.g. if injured) with control over tissue structure through the regulation of cell phenotype. Biomaterials act as an artificial extracellular matrix (ECM) and in the context of a scaffold must have biological and mechanical properties that match the native body tissue, they must facilitate the localization and delivery of cells and/or transforming factors to desired sites in the body. This includes providing a two or three-dimensional space for the formation of new tissues with appropriate structure, and guiding the development of new tissues with appropriate function. The design and selection of the biomaterial is therefore an important factor in the development of engineered tissues, and preferably be capable of controlling the structure and function of the engineered tissue in a predesigned manner³⁹. Among these properties, the release of degradation products should not provoke inflammation and must be removed from the body via metabolic pathways. According to American Standard Testing Materials (ASTM D20-96) degradation is defined as, “*plastic designed to undergo a significant change in chemical structure under specific environment conditions resulting in a loss of some properties and its applications in a certain period*”. The degradation rate and the concentration of degradation products in the tissues must therefore be of an acceptable level^{40,41}. The more general definition of polymer degradation is; “*the chemical degradation of macro-molecules to achieve the perfect difference from the materials physical degradation*”⁴². However, degradation must be used instead of *biodegradation* when the mechanism of *chain scission* is not known or demonstrated as being cell-mediated⁴¹. Degradation mechanisms include hydrolytic, enzymatic and biodegradation²⁴. However, it is necessary to consider abiotic reactions (e.g. photo degradation, oxidation and hydrolysis) that may also alter the degradation of a polymer either before or during the reaction, (or not) due to environmental factors⁴³. Sometimes the definition of biodegradation is not accurately described⁴⁴, for example a material can undergo degradation by enzymes *in vitro*, however, this degradation may fail *in vivo* due to the absence of the required body enzymes. Therefore, biodegradation is caused by cell activity. Similarly, *in vivo*, degradation as a result of hydrolysis by water located in tissues and organs is not biodegradation; it should be described as

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4 hydrolysis or a hydrolytic degradation. Technically, biodegradation is the breakdown of a material
5 due to specific biological activity and the mechanism related to this activity is proved. Related to
6 this is where a cell-mediated chemical modification arises in which a main *chain scission* event is
7 strictly speaking bio-alteration (and not biodegradation).
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10 11 12 13 **2.2- Biodegradable Polymers**

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15 Polymeric materials have been used in clinical applications for a number of years^{45,46,47}, however
16 the clinical and biological requirements vary according to the nature of the application. Numerous
17 techniques have been used to modify and fabricate different compositions to achieve exact
18 requirements for clinical use⁴⁸, typically based on control of molecular weight, polydispersity,
19 crystallinity, thermal transition and degradation rate. All of these factors can strongly affect the
20 polymer scaffold properties⁴⁹. There are three general types of biodegradable polymers: synthetic,
21 natural and hybrid materials, which have been gaining recent attention due to their superior
22 characteristics in regenerative therapies. These materials can be produced with high structural
23 precision employing assembly strategies to control properties such as stiffness, degradation and
24 porosity⁵⁰. A wide range of natural and synthetically derived polymers are capable of undergoing
25 degradation, however synthetic biodegradable polymers have found more versatile and diverse
26 biomedical applications, arguably due to a more facile ability to undertake tailorable designs and
27 chemical modifications⁵¹.
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31 The most commonly used synthetic polymers for tissue engineering and drug delivery are aliphatic
32 polymers, and include poly (lactic acid) (PLA) and poly (glycolic acid) (PGA), poly (lactic-co-
33 glycolide) (PLGA), poly (ϵ -caprolactone) (PCL), poly (p-dioxanone), plus copolymer soft
34 trimethylene carbonate and glycolide. These materials are approved by the U.S. Food and Drug
35 Administration (FDA) for human clinical applications⁵². PLA exists in three forms - D-PLA
36 PDLA, L-PLA (PLLA). Blends of D-PLA and L-PLA (PDLLA), PLA, PGA and PLGA⁵³⁻⁵⁵ have
37 been used clinically to treat patients suffering from damaged or lost organs or tissues and for drug
38 delivery systems⁵⁶⁻⁵⁹. These polymers have been demonstrated as being biocompatible and
39 degrading into non-toxic products, with a controllable degradation rate when implanted *in vivo*.
40 Other biodegradable synthetic polymers include poly anhydrides, polyphosphazenes,
41 polyurethanes, poly(glycerol sebacate), synthetic hydrogels and functional synthetic polymers,
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4 with a range of other tissue engineering applications including restorable sutures^{60,61}, drug delivery
5 systems^{62,63}, artificial skin⁶⁴⁻⁶⁶, wound healing^{62,67,68} and orthopaedic implants⁶⁹.
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9 These synthetic biodegradable polymer materials can be synthesized by controlling their
10 fundamental building block units to give various properties such as uniformity, which are free
11 from immunogenicity. High molecular weight aliphatic polyesters are mostly synthesized by
12 condensation or ring-opening polymerization. The basic and generic structure of all aliphatic
13 polyesters is very similar and the only difference is the pendant groups, where a change contributes
14 to differences in molecular weight and crystallinity. This directly affects the kinetics of
15 degradation⁴⁹. These synthetic polymers contain chemical bonds in their backbone that undergo
16 breakdown in the presence of water. Polymers having functional groups of esters, ortho-esters,
17 anhydrides, amides, urethane, lactones, and lactams are categorized as polymers that degrade
18 through hydrolysis. However, it is important to clarify that *in vivo*, degradation resulting solely
19 from hydrolysis through water present in tissues is not biodegradation, and should be referred to
20 as hydrolysis or hydrolytic degradation⁴¹.
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32 In addition, synthetic hydrolytically degradable polymers possess number of advantages compared
33 to natural polymers when used in biomedical applications, including tailored-made porosity,
34 degradation time and mechanical characteristics⁶⁸. They are often cheaper than biological scaffolds
35 and can be produced in large quantities under controlled conditions, and have a long shelf life⁷⁰.
36 Synthetic polymers are generally preferred for medical applications, due to manufacturing
37 reproducibility compared to natural polymers, which in contrast have little control over chemical
38 structure. Furthermore, synthetic polymers do not tend to display problems when used as
39 biomedical implants due to this minimal batch-to-batch variation, and are therefore more
40 reproducible³⁷. The degradation of synthetic polymers is very much dependent upon the chemical
41 structure of their functional groups. Different polymers show a variable degree of degradation, as
42 some are more water stable than others. Chemical structure therefore plays an important role in
43 materials selection and design for tissue engineering scaffolds. Figure 1 shows the order of
44 hydrolytic degradation of various chemical structures. Based on these observations one can design
45 and select a specific polymer for a required biomedical application. Chemical reactivity depends
46 upon the level of electrophilicity of e.g. a carbonyl moiety (C=O) and stability of the leaving group.
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4 Natural biopolymers include polysaccharides(e.g. starch, alginate, chitin/chitosan, hylauronic acid
5 derivatives) or proteins (e.g. soy, collagen, fibrin gels, silk)⁷¹. They serve as intrinsic templates for
6 cell attachment and growth because of their inherent biocompatibility. However, they also have an
7 ability to stimulate an immune response. The molecular structure of natural polymers is highly
8 organized containing extra cellular ligands that can bind to cell receptors. Although naturally
9 derived polymers are biocompatible, there are some disadvantages including not being available
10 in bulk quantities, being expensive, and difficulty in processing into a desired shape when used as
11 a scaffold for tissue engineering. The degradation rate of both natural and synthetic polymers can
12 vary from patient to patient, because the degradation of natural polymer materials is dependent
13 upon enzyme activity, which is a variable within patients.
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23 Generally, the majority of naturally occurring polymers are degraded under enzymatic
24 conditions³⁷. For example, chitin as enzymes are well known for the degradation of chitin
25 (chitinases originating from fungi, bacteria, and plants etc.^{72, 73}.) Degradative chitinases are divided
26 into two groups; endo- and exo-chitinases. Figure 2(A) shows the pattern of chitin degradation by
27 various catalysts including endo- and exo-chitinases (interestingly, lysozyme is also known to
28 break the β -1,4-linkage in the natural carbohydrate polymers^{74, 75}). Chitosanase leads the β -1,4-
29 linkage in the D-glucosamino moieties in the chitosan as shown in Figure 2(B). Hyaluronic acid
30 undergoes catalytic degradation in the presence of mammalian hyaluronidase, assisting in the
31 hydrolysis of 1,4-bonds between the D-glucuronic acid and N-acetyl-D-glucosamine, as shown in
32 Figure 2(C).
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43 Another very common naturally derived polymer is collagen, of which 28 different types have
44 been described. Collagen is found in mammalian connective tissues; consisting of up to 30% of
45 all proteins that are present in the human body that provides strength and flexibility to tissues. The
46 most common, representing about 90%, is type I collagen. Collagen I is abundantly found in
47 tissues, with higher levels found in tendon, skin, bone and fascia. It has been extensively
48 researched for developing biomaterials in tissue engineering⁷⁶. Because of its distinctive physical
49 strength, porosity and biological properties i.e., phylogenetical studies showed a primary sequence
50 and helical structure as well as mild immune-reactive recital⁷⁷⁻⁷⁹. Collagens undergo degradation
51 in the presence of collagenases and metalloproteases. Collagenases belong to the family of
52 endopeptidases, and metalloproteases are proteases that require a metallic catalyst for their
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4 activity. These enzymes attack the collagen in the triple helix region, which is composed of
5 polypeptide strands bearing tri-amino acid blocks of glycine-proline-hydroxyproline, and
6 responsible for the repeating helical structure⁸⁰ [Figure 2(D)].
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11 **(Insert Figure 02)**
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16 Hybrid or composite materials behave in such a way that better properties are achieved for scaffold
17 function and have been commonly used for clinical applications⁸¹⁻⁸³. Polymeric material blends
18 have been fabricated by the combination of synthetic and natural, natural plus natural and synthetic
19 plus synthetic polymers, to improve the mechanical properties, to improve processability, to lower
20 production costs, or to improve cell compatibility⁸⁴. Bioactive phases increase hydrophilicity and
21 water absorption of the polymer matrix, which can change the degradation behaviour of the
22 polymers by allowing rapid exchange of protons in water from ceramics⁸⁵. Biodegradable hybrid
23 materials may provide a number of benefits, e.g., an enhanced environment for cell seeding,
24 survival, growth, and differentiation due to the osteoconductive function imparted by bioceramics
25 (which increases mechanical properties essential for load bearing applications⁸⁶). The
26 composition, structural and functional versatility of hybrid materials accounts for a range of
27 tuneable physicochemical properties, which are highly suitable for designing organ specific tissue
28 engineering constructs.
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42 **3. Organ Specific Regeneration using Biodegradable Materials**
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44 **3.1 Skin**
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46 Skin is the most exposed and largest body organ with an approximate surface area of 1.5-2.0 m² in
47 the adult human body and 12-15% by weight. It is a multifaceted organ that is frequently subject
48 to burn and wound injuries. Skin anatomy reveals a three-layered structure: the stratified
49 epithelium or *epidermis*, separated from an underlying tissue stroma or *dermis* and a well-
50 characterized cellular layer of subcutaneous tissue or hypodermis⁸⁷. The functions of skin are to
51 maintain the integumentary system (that includes but is not limited to) protection against any
52 external physical, chemical and biological insults⁸⁸, preventing excess water loss from the body
53 and thermoregulation. Skin damage can have a number of causes e.g., traumatic injury, burns,
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4 surgery, non-healing ulcers and chemical injury. These can cause extensive skin loss and require
5 instant treatment to restore structure and function. For several decades, scientists and clinicians
6 have been designing and fabricating new tissue engineering scaffolds to develop artificial skin or
7 wound healing strategies⁸⁹.
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11 A noticeable breakthrough is in the development of artificial skin, which is being used in burn
12 patients⁹⁰⁻⁹³. The plus point of such a development is that artificially grown skin can be stored in
13 tissue banks and be used when required. The major drawback with existing burns treatments using
14 skin grafting is that patients need to wait for a number of weeks while the skin is grown
15 autonomously. The donor site for this is also a new wound and thus a potential site for infection
16 or scarring. In addition, the donor site is limited and so a major limitation in patients with extensive
17 burns. Figure 3 illustrates an example of a patient treated using tissue engineered skin.
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30 In 1997, the first tissue engineered skin product TransCyte (Shirepid, California, USA) was
31 launched. This was a non viable product that was comprised of silicone, a nylon membrane and
32 collagen containing neonatal fibroblasts grown for 17 days to produce a matrix. It was followed
33 by Apligraf[®] (Organogenesis, Canton, USA) in 1998. Apligraf was used in the chronic
34 wounds healing when such wounds were previously failed in healing by using other methods of
35 treatment. Fifty-six percent of patients treated using Apligraf had full wound healing in
36 comparison with 37% of patients who were treated using standard wound care protocols. Figure 4
37 shows the appearance of a wound before and after application of Apligraf⁹⁴.
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50 Dermagraft[®] (Advanced BioHealing, Westport, Conn) is a synthetic product which either uses
51 polygalactic or polyglycolic acid meshes combined with neonatal fibroblast to enhance wound
52 healing as temporary skin substitutes. It was followed by the development of OrCel[®] (Ortec
53 International US Inc., New York, USA) in 2001.
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4 According to a research report compiled by ECRI Institute/Evidence-based Practice Centre (EPC)
5 under contract to the Agency for Healthcare Research and Quality (AHRQ, USA)⁹⁵ its use is only
6 for chronic wounds such as diabetic foot ulcers, pressure ulcers, and vascular ulcers (including
7 venous ulcers and arterial ulcers)⁹⁶. A wealth of literature reviews elsewhere⁹⁷⁻⁹⁸ document a range
8 of skin substitutes and techniques investigated for *in vitro* testing and employed as model skin⁸⁸,
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13 95, 97-104.

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16 Extra Cellular Matrix (ECM) plays a vital role in tissue engineering. It supports the growth of
17 proliferating cells (in particular, fibroblasts) and serves as a scaffold post injury, and hence is a
18 major component in the process of tissue regeneration. Fibroblasts produce ECM constituents and
19 through it communicate with each other. The ECM can signal to the fibroblasts and control cell
20 phenotype, genetic expression, development, protein expression and the function of these cells.
21 Such interactions are influenced by the microenvironment, which provides a niche for homeostatic
22 modulation of ECM. When considering skin substitutes, development of biomedical materials
23 should ideally aim at mimicking the ECM by incorporating appropriate factors, or pharmacological
24 agents, at physiological quantities and durations⁹⁷. Numerous biomaterials are employed as skin
25 implants, ranging from naturally occurring collagen gels/sponges, alginates, polypeptides, glycol
26 saminoglycans, hyaluronan and fibronectin to synthetic materials e.g., polyvinyl chloride, poly
27 lactate/glycolate fabrics (PLGA) etc. ^{88, 97, 105}. Researchers are constantly trying to find an ideal
28 skin graft¹⁰⁶. Huss et al¹⁰⁷ reported on the development of biodegradable polyurethane-urea
29 (PUUR) scaffold for dermis regeneration. After *in vitro* and *in vivo* assessments, the fibrous and
30 porous forms of PUUR scaffold showed biocompatibility with human dermal fibroblasts. Thus,
31 the cells could attach, proliferate and migrate around the biodegradable scaffolds. Porous scaffold
32 discs of dimensions 4 mm diameter, 2 mm-thick) with a polymer solution (of 12% w/w or 9%
33 w/w) were inserted intra-dermally into four volunteer healthy patients. Increased growth of
34 fibroblasts was observed on all materials and after eight weeks, the scaffolds were fully occupied
35 with fibroblasts. Production of procollagen was observed that signified the existence of functional
36 and active cells. The fibroblasts stained immune histochemically for procollagen and von
37 Willebrand factor, demonstrating neocollagenesis and angiogenesis contained within the scaffolds.
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4 These biodegradable materials have shown potential applications in dermal regeneration ^{91, 107, 108}.
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6 Among various biodegradable polymers for skin regeneration, polyurethanes have attracted
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8 interest due to their tuneable mechanical properties, biocompatibility and structural adaptability
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10 ¹⁰⁹. In a number of other studies ¹¹⁰⁻¹¹², polyurethane-based dressings are reported which are
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12 considered as superior to hydrogels. Furthermore, successful culturing of keratinocytes has also
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14 been reported on polyurethane membranes ¹¹³. Nonetheless, biodegradable polyurethanes as
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16 dermal scaffolds have not been fully explored. Greenwood *et al.* ¹⁰⁹ reported on a study of a dermal
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18 skin substitute for restoration of major skin loss caused by burn injury. The authors carried out *in*
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20 *vitro* studies on three derivatives of NovoSorb™ Polynovo Ltd. Australia), a class of
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22 biodegradable polyurethane used as a dermal scaffold. Results showed biocompatibility, nominal
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24 cytotoxicity on skin cells and facilitation of cell development i.e., growth of human keratinocytes,
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26 dermal fibroblasts and microvascular endothelial cells in co-culture. Furthermore, one of the skin
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28 substitutes (BTM-2, Biodegradable Temporising Matrix, PolyNovo Ltd. Australia) exhibited a
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30 desired degradation profile for a dermal scaffold and was developed into a 3-dimensional porous
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32 matrix for further studies. *In-vivo* studies ¹¹⁴ were carried out in both rats and sheep, with
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34 subcutaneous implantation of three NovoSorb™ derivatives which revealed no toxic effects. The
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36 authors demonstrated an inflammatory response and granulomatous reactions that were
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38 comparable to clinically used materials, e.g. sutures and Integra™ (Integra Life Sciences
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40 Corporation, NJ, USA) dermal substitutes.

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42 These dermal scaffolds, both the non-optimised skin substitutes and Integra™, restrict wound
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44 contraction and allow re-epithelialisation over the dermal granulation tissue with the growth of
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46 normal basement membrane. Both *in vitro* and *in vivo* studies show that a basic bi-layered
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48 composite skin was created, which may eliminate a dependence on skin auto grafts via non-
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50 optimized BTM-2 matrix. The authors suggest further the generation of vascular structures by
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52 culturing MVECs in the BTM-2 matrix, with the potential for developing a ‘pre-vascularized’
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54 composite skin substitute (and is of relevance to other tissue engineered organs ^{109, 114}).

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56 Wang et al ¹¹⁵ reported an interesting study for skin restoration and wound healing. They introduced
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58 a novel collagen/hyaluronic acid (HA)/gelatine based sponge-like scaffold for human skin
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60 regeneration. The scaffold offered an optimal pore size with an average pore diameter of
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62 132.5±8.4µm observed under SEM. The swelling ratio was examined by water absorption and
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4 showed a value of over 20g water/g of dried scaffold. Enzymatic degradation was demonstrated
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6 by lysozyme, hyaluronidase and collagenase I assays in a time- and dose-dependent fashion and
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8 observed by measuring a reduction in weight. The scaffold degraded gradually to $38.1 \pm 2.6\%$ and
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10 $36.4 \pm 5.1\%$ of original weight after one week using 10,000 and 30,000 U/mL of lysozyme
11
12 respectively. Similarly, when using 30 U/mL of hyaluronidase, the scaffold maintained about 10%
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14 weight after a 5-day examination. With 50 U/mL of hyaluronidase, the scaffold was degraded after
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16 7 days. Furthermore, in 20 U/mL collagenase I, the scaffold degraded almost completely in
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18 approximately 3 hours. In contrast, 10 U/mL reported 45% of remaining scaffold in comparison
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20 to its starting weight. It was further investigated that with human skin cells growing for 7 days,
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22 SEM studies indicated surface degradation of the scaffolds. This was attributed to enzymatic
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24 digestion, signifying the biodegradable properties of the scaffolds. Human epidermal
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26 keratinocytes, melanocytes and dermal fibroblasts were cultured on the porous scaffold and
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28 immunofluorescence microscopy confirmed a normal human skin layer distribution i.e., the
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30 scaffold was able to mimic the human epidermis and dermis structures. Furthermore, the authors
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32 reported that the amount of collagen was quantified to 50% higher after skin cell seeding, as
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34 compared to cells seeded on culture wells. The *in vivo* histological outcomes showed that the
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36 scaffold wound healing was faster, with no further inflammation or side effects¹¹⁵.

37 Recently, Lagus *et al*¹¹⁶ in a clinical/histological study compared three different strategies to heal
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39 excised burn wounds by using Integra® (Integra LifeSciences Corporation, USA), Split Thickness
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41 Skin Graft (STSG) from a donor, and a viscose cellulose sponge Cellonex™ (Vivoxid Ltd,
42
43 Finland), respectively. Integra®, is a biodegradable porous skin substitute consisting of bovine type
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45 I collagen and chondroitin-6-sulphate from shark's cartilage with a temporary epidermal substitute
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47 layer made of 0.1mm synthetic polysiloxane matrix. The silicone layer regulates the moisture
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49 content from the wound to a permeability value of 0.5 mL/cm^3 , reported for the epidermis of
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51 human skin¹¹⁷. This layer also provides a protective barrier to the host body undergoing a thin split
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53 thickness skin graft substitution from infectious microorganisms. It facilitates the formation of a
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55 neodermis, autologous extracellular matrix (ECM) and wound bed for a thin STSG. In contrast,
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57 Cellonex™ viscose sponge, can be obtained from cellulose. It has shown granular tissue growth
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59 on wound beds and is known for its optimal pore-size, open cell-to-cell structures, homogeneity
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61 and purity. The sponge contains a viscose cellulose matrix as a main component, which is
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63 supported by cotton fibres¹¹⁸. The flexible architecture allows the free passage of cells into the
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4 inner parts of the sponge¹¹⁹. However, *in vivo* cellulose sponge degradation is believed to be result
5 of chemical, biological, and mechanical interactions¹²⁰. These materials were tested and compared
6 in ten adult patients¹¹⁶ and results showed that STSGs performed well in muscle fascia, on
7 vascularized Integra[®] and on wound surfaces possessing a cellulose sponge. Minimal
8 inflammation was observed in Cellonex[™] treated areas, in contrast to other materials. Most
9 neutrophils, histiocytes, and lymphocytes were observed with significant differences on days 7
10 and 14. Entire vascularization of Integra[®] occurred later, as compared to the other materials (STSG
11 showed most myofibroblasts on day 14). However, it was noted that fibroblasts and myofibroblast
12 number may show a slow increase in Integra[®], in contrast to wound beds treated with other
13 materials. Furthermore, it was also revealed that both the maturation of scar tissue and the fibres
14 of Integra[®] may persist for a year or longer, respectively. From the results, it was concluded that
15 Integra[®] is a better skin substitute as compared to other materials, but that from the 12 month
16 investigations of histological and immune histochemical outcomes, proposed that three strategies
17 could be clinically adopted¹¹⁶.

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31 Hypopigmentation is the common problem while using tissue engineered skin grafts to treat burn
32 wounds¹²¹. A study has been published to investigate the difference between the normal and
33 vitiligo melanocytes in artificial skin grafts. It was observed that skin fibroblasts regulate the
34 pigmentation in tissue engineered skin grafts. Figure 5 shows melanocytes in the epidermis in
35 tissue engineered skin. Melanocyte function depends upon fibroblast presence. In the absence of
36 melanocytes the authors observed no pigmentation. In the presence of fibroblasts and melanocytes
37 (isolated from pale skinned patients) unpigmented skin was observed [Figure 5(A)], whereas in
38 the absence of fibroblasts under same conditions pigmentation arose [Figure 5(B)]¹²².

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51 Biobrane[®] (Bertek Pharmaceuticals Inc., USA) due to its lower cost, ease of storage, application
52 and fix, and reliable when used according to guidelines and being efficacious in treating partial
53 thickness burns are the main reasons of its popularity in usage. By comparison of Biobrane[®] and
54 cadaveric allograft for temporizing the acute burn wound, Austin et al. founded that Biobrane[®] is
55 superior in terms of lower procedural time and associated cost because of mainly the relative ease
56 of its application. Currently Biobrane[®] is used as an alternative to cadaver allografts as temporizing
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4 dressings after excision of major burn injuries. However, the limitation of this technique is that
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6 wound bed must be meticulously prepared to prevent any infection and there is still a lack of
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8 existing literature and published clinical protocols proving that it could be a suitable replacement
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10 of the human skin allografts, especially in the treatment of full thickness burn wounds. Despite
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12 that, Biobrane® is still widely used as a synthetic skin substitute as well known for its success in
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14 the definitive management of partial thickness burns in many centres (Fig. 6)¹⁰⁸
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24 Advanced wound healing for diabetic foot ulcers (DFUs) has now started to focus on stem cell
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26 therapy for improved healing. Much of the research is still in the starting phases with a paucity of
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28 robust clinical trials, but it could prove to be an important method for advance wound healing in
29
30 difficult patient populations^{123, 124}.
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32 Adipose stem cells (ASCs) being a type of adult stem cells have been proven to be a useful cell
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34 resource for tissue regeneration. Cell therapy plays a major role in regenerative medicine of this
35
36 century where ASCs holds a key position. These cells have many clinical applications, including
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38 fat grafting, overcoming wound healing difficulties, recovery from local tissue ischemia and scar
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40 remodeling. Diabetic ulcers and chronic radiation ulcers are notorious for their recurrence. These
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42 lesions do not improve over time and tend to become worse. Recently, cell therapy using ASCs
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44 has been shown to be a good potential alternative technique because it is less invasive than re-
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46 constructive surgery and the cells can be directly placed onto target areas in cutaneous lesions.
47
48 Sufficient numbers of ASCs can easily be harvested by liposuction and fat tissue digestion. The
49
50 addition of cells to the defect may reinforce local regeneration capabilities that have been
51
52 exhausted during the course of prolonged disease processes. The ease of repeating the procedure
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54 during the course of regeneration is the main advantage of this type of tissue engineering. This
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56 type of cell-based therapy may be a good treatment option for small traumatic defects or skin
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58 cancers to avoid more substantial reconstructive surgeries using local flaps¹²⁵. In order to obtain
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60 recovery from ischemia, using ASCs is very effective until 4-5 days after the onset of
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62 complications and can reduce the area of necrosis in 7 days after the onset as shown in Fig 7¹²⁶.
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(Insert Figure 07)

3.2 Nerves

Compared to other types of trauma, nerve injuries are particularly complicated as mature neurons do not replicate. However, under the right conditions axon extensions of peripheral nerve injuries can regenerate, if reconnection with the distal stump arises eventually restoring function. Injuries to the central nervous system differ significantly from peripheral nerve injuries, in regards to outcomes following traumatic injury (in that permanent paralysis of organs distal to the injury site is usual). Major differences for this include the inability of spinal neurons to re-grow, predominantly due to biochemical inhibitory molecules secreted at the injury site and the formation of a glial scar. Current treatments for injuries to peripheral nerve defects typically rely on donor tissue obtained following second surgery, typically autologous nerve, vein, or arterial graft sutured to the two ends of the severed nerve¹²⁷. However, this method has raised the issue of functional loss at the donor site, formation of potential painful neuromas, structural differences between donor and recipient grafts, and shortage of graft material for extensive repair¹²⁸. Artificial nerve guidance conduits have been in development for many years, which bridge the gap between the nerve stumps and aid nerve regeneration. The guide may be implanted empty, or it may be filled with growth factors, cells, or fibres. Micro-braiding is a novel technique for the fabrication of polymeric nerve guide conduits composed of biodegradable PLGA fibres. The micro-braided nerve guide conduit with a fibre architecture has shown promotion of axonal regeneration, with no inflammatory response or swelling. It degraded from the implantation site after serving its purpose. An *in vivo* study was conducted on the sciatic nerve in rats and showed a 90% success rate¹²⁹. The results showed that the fibrous tubular structure did not collapse and had the necessary strength to withstand adjacent muscular forces surrounding the conduit. The micro-braided conduit had the required permeability to allow for the passage of nutrients from the external environment into the conduit lumen to promote nerve regeneration. In a separate study¹³⁰, the fabrication of a fibrous, porous, flexible and biodegradable tubular scaffold using PLGA and chitosan was proposed. Here, a PLGA conduit exhibited negligible or minimal swelling and thus maintained dimensional integrity. However, the chitosan conduit showed a 60% swelling, which had to be taken into consideration before designing the scaffold for practical applications. Both PLGA and chitosan

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4 scaffolds showed good biocompatibility. Cell morphology was not altered but remained similar in
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6 both polymers.

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8 An emerging area of nerve guide manufacture using synthetic materials includes the use of additive
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10 layer manufacturing. The very first 3D printed nerve guide was produced from poly (ethylene
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12 glycol; PEG) by UV light induced photo curing using stereo lithography. This enable a very precise
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14 shape and structure of guide to be manufactured via computer aided design and therefore has a
15
16 number of advantages over tradition manufacturing methods such as moulding and extrusion. A
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18 small common fibular nerve injury model (3mm) was studied in a mouse, which showed equivalent
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20 axon number and distance regeneration after 3 weeks compared to a nerve graft. Notwithstanding,
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22 the development of more suitable materials for nerve repair beyond PEG is currently ongoing.

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24 The idea that biomaterials might have electrically conductive properties for nerve repair has been
25
26 explored largely without success. However, composite materials from the blending of conductive
27
28 (CPs) and biocompatible polymers are fast emerging as successful biomaterials for the
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30 regeneration of the myocardium due to their unique conductive and biological recognition
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32 properties and can assure a more efficient electroactive stimulation of cells. Recently, research has
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34 been focused on the synthesis of conductive polymers to fulfil basic biocompatibility and
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36 biodegradability properties by combining conducting and degradable units¹³¹. A series of
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38 electroactive and biodegradable polymeric materials were prepared by blending PLLA and poly
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40 (glycol tetra-aniline) (PGTA). The blended polymers showed good solubility and thermal stability,
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42 the cytotoxicity and biocompatibility of the materials were evaluated with positive results
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44 obtained. Cell culture results showed that PLLA/PGTA blended materials could accelerate the
45
46 differentiation of rat C6 glioma cells compared with pure PLLA. They recommended that the 80/20
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48 wt.% PLLA/PGTA blend material showed the best effect and these biodegradable PLLA/PGTA
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50 polymer blends are shown to be electroactive¹³². A novel electrically conductive biodegradable
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52 poly phosphazene polymer containing aniline pentamer (AP) and glycine ethyl ester (GEE) as side
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54 chains was obtained by a nucleophilic substitution reaction. The electrical conductivity of the
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56 polymer was $\sim 2 \times 10^{-5}$ S/cm (i.e. in the semiconducting region) upon protonic-doped experiments.
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58 Furthermore, the polymer proved to promote cell adhesion and proliferation *in vitro* using
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60 Schwann cells. These polymers also showed good solubility in common organic solvents and good
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62 film-forming properties, and consequently potential applications as scaffolds for neuronal and
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64 cardiovascular tissue engineering applications¹³³.
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4 In another study, hyper-branched degradable conducting copolymers were blended with poly
5 caprolactone to construct electroactive tubular porous nerve conduits by a solution-
6 casting/particle-leaching method. Thermal and mechanical properties, hydrophilicity,
7 morphology, toxicity and conductivity (values between 3.4×10^{-6} and 3.1×10^{-7} S/cm were found,
8 depending on the composition) and were determined for blends doped with or without 10 camphor
9 sulfonic acid. The results obtained supported their potential for neural tissue engineering
10 applications¹³⁴. McKeon and group studied several polyaniline and poly(D,L-lactide)
11 (PANi/PDLA) mixtures at different weight percentages and were successfully electrospun from
12 1,1,1,3,3,3-hexafluoroisopropanol solutions and their conductivity and biocompatibility evaluated.
13 It was claimed that the successful results were only attained when the PANi content reached 25%.
14 Specifically, this scaffold could conduct a current of 5mA and had an electrical conductivity of
15 0.0437 S/cm. Primary rat muscle cells were able to attach and proliferate over all the new scaffolds,
16 which degraded during the process. The polymer degradation and shrinkage may prevent the blend
17 from being used as the primary component of a biomedical device, but its usefulness as a
18 biocompatible coating on devices such as sensors was proposed¹³⁵. Biodegradable semiconducting
19 melanin films have also been studied for nerve regeneration. Melanins are naturally occurring
20 pigments and exhibit unique electrical/biological properties and were used as melanin thin films
21 to enhanced Schwann cell growth and neurite extension, compared to collagen films *in vitro*.
22 Furthermore, melanin implants were significantly resorbed after 8 weeks¹³⁶.

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40 Among natural polymers, collagen^{137, 138}, chitosan¹³⁹ and alginate¹⁴⁰ have been used for
41 constructing nerve guidance channels. Addition of collagen gels to the lumina of nerve conduits
42 speeds the rate of nerve regeneration. A number of collagen based nerve tubes have shown to
43 support regeneration of nerve defects *in vivo*. However, repair was limited to gaps less than 30mm
44 long¹⁴¹. Alginate was employed in tubular and non-tubular repair of a long peripheral nerve defect
45 injury. *In vivo* studies showed the recovery of 50mm gap of the sciatic nerve of cats, treated by
46 tubular repair or non-tubular repair. In the tabulation group, a nerve conduit consisting of
47 polyglycolic acid mesh tube filled with an alginate sponge was implanted into the gap and the tube
48 was sutured to both nerve stumps. In the non-tabulation group, the nerve defect was repaired by a
49 simple interpolation of two pieces of alginate sponge without any suture. The animals in both
50 groups exhibited similar recovery of locomotor function. After three months, axonal elongation
51 and re-innervation in both the afferent and efferent systems were detected by electrophysiological

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4 examination. Intracellular electrical activity was also recorded, which is directly indicative of
5 continuity of the regenerated nerve and restoration of the spinal reflex circuit. Eight months after
6 surgery, many regenerated myelinated axons with fascicular organization of peri neural
7 (fibroblast) cells were observed within the gap, peroneal and tibial branches were found in both
8 groups, while no alginate residue was found within the regenerated nerves. Morphometric analysis
9 of the axon density and diameter revealed no significant differences between the two groups¹⁴².
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16 17 **3.3 Blood Vessels**

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19 There is a substantial patient demand for vascular bypass grafts due to atherosclerosis and related
20 cardiovascular diseases. Vascular disorders are the leading cause of mortality in Western countries.
21 Several studies have been focused on the development of biodegradable vascular grafts able to
22 temporarily substitute the blood vessel and allow for complete regeneration over a predetermined
23 time period. Several biodegradable synthetic polymers¹⁴³, and natural polymeric materials like
24 collagen¹⁴⁴ have also been evaluated for developing a successful vascular graft. However, due to
25 the lack of suitable mechanical properties, unsuitable rates of degradation and the poor capacity to
26 create an optimal microenvironment for cell adhesion and differentiation, none of these materials
27 has displayed the required properties for further application in the human body.
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35 However, different methods exist to prepare polymeric vascular grafts, which should allow greater
36 control on both the mechanical properties and the micro- and nanostructures of the product. An
37 ideal artificial graft should be mechanically compatible with the natural arteries and surrounding
38 tissue and should also mimic the extracellular matrix morphology; it should have a nano scale
39 topography (5 to 500nm) with high porosity and adequate pore sizes (5–500µm) to enhance cell
40 attachment and proliferation for the regeneration of the natural tissues. The first tissue-engineered
41 blood vessel substitute was created by Weinberg and Bell in 1986¹⁴⁵. They generated cultures of
42 bovine endothelial cells; smooth muscle cells (SMCs) and fibroblasts in layers of collagen gel
43 supported by a Dacron mesh. Although physiological pressures were sustained for only 3–6 weeks,
44 they did demonstrate the feasibility of a tissue-engineered graft with human cells. Since then,
45 strategies to create a suitable material for a vascular graft have focused on three areas of research:
46 1) coatings and surface chemical modifications of synthetic materials; 2) biodegradable scaffolds
47 and 3) biopolymers. Niklason and colleagues have developed a pulsatile bioreactor to remodel
48 PGA scaffolds seeded with bovine smooth muscle and endothelial cells¹⁴⁶. After a 10-week culture
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4 period, the resulting tissue-engineered vessel displayed a burst pressure of up to 2300mmHg. After
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6 5 weeks, the PGA scaffold had degraded to 15% of its initial mass¹⁴⁷. Shin'oka *et al* reported the
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8 use of PCL-based scaffolds to engineer venous blood vessels. The PCL/PLA copolymer was
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10 reinforced with woven PGA and seeded with autologous smooth muscle and endothelial cells
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12 harvested from a peripheral vein. After 10 days, the construct was implanted as a pulmonary
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14 bypass graft into a 4-year-old child¹⁴⁸. An alternative strategy to synthetic and degradable scaffold-
15
16 based vascular grafts is the manipulation of proteins that constitute the architecture of native ECM.
17
18 Weinberg and Bell first reported the use of collagen gels as substrates for cells in vascular tissue
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20 engineering. Since then, Habermehl and colleagues have developed a process to obtain large
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22 quantities of collagen from rat tail tendons to allow the scale-up of production¹⁴⁹. The
23
24 shortcomings of a relatively stiff collagen-based scaffold have motivated researchers to explore
25
26 the potential of more elastic fibrin gels in vascular tissue engineering¹⁵⁰. One such example is the
27
28 fibrin-based vascular graft developed by Swartz and colleagues, who incorporated bovine SMCs
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30 and endothelial cells into the gel¹⁵¹. The grafts were implanted in the jugular veins of lambs and
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32 remained patent for 15 weeks. Upon histological examination, the constructs were found to contain
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34 both collagen and elastin, with the mechanical integrity comparable to that of native coronary
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36 arteries. Recent developments in the field of nanotechnology have facilitated vascular tissue-
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38 engineering efforts in mimicking the nanostructure of native vasculature, thereby directing
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40 mechanical and biologic performance of the bulk material. One such application is electro spinning
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42 of synthetic polymers and naturally occurring materials into nanofibres^{152,153, 154}. In these studies,
43
44 use of electro spinning to create nano-fibrous scaffolds composed of collagen-blended degradable
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46 PLLA-co-PCL was demonstrated. Results indicated that the blended nano-fibres supported
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48 endothelial cell attachment and spreading, and preserved the endothelial cell phenotype¹⁵⁵.

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48 Poly amino acid-graft-polyester copolymers have been functionalized with heparin, for a potential
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50 use in tubular structures for vascular regenerative medicine¹⁵⁶. The fabricated scaffold had
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52 morphological characteristics like those of natural extracellular matrix, a suitable rate of
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54 degradation in simulated physiological medium (after 60 days approximately 50% of the scaffold
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56 degraded), the ability to be easily functionalize and allow endothelial cell adhesion and
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58 proliferation. Bio functional vascular grafts were synthesized by electrospinning PCL solutions¹⁵⁷.
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60 The obtained fibres showed tensile stresses above 2MPa and up to 7.4MPa and tensile strain at
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62 failure values in the range of 200–1200% after γ -sterilization. These values are above those for

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4 natural human blood vessels (1.4MPa and 100%). These PCL-based vascular grafts were
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6 implanted into rat's arterial circulation as an abdominal aortic substitute. All implanted grafts were
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8 fully potent up to 12 weeks after implantation, and none of the vascular grafts at the three different
9
10 time points (3, 6 and 12 weeks) demonstrated thrombosis or aneurismal dilatation. Histological
11
12 analyses revealed a homogeneous cellular infiltration associated with polymer degradation and
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14 extracellular matrix deposition, and a complete endolisation with little intimal hyperplasia.
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16 17 **3.4- Heart**

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19 Cardiovascular related deaths surpass cancer in general as the leading cause of death worldwide¹⁵⁸.
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21 The report, "Global Atlas on Cardiovascular Disease Prevention and Control" by the WHO has
22
23 identified cardiac related deaths will continue to increase in future¹⁵⁹. Increased interdisciplinary
24
25 research is therefore exploring multidimensional therapeutic aspects of cardiovascular diseases
26
27 and new materials are continuously being explored. However, current therapies dealing with
28
29 multifaceted cardiovascular damage lack the potential of intrinsic cardiac tissue regeneration¹⁶⁰.
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31 To date, the exact relationship between the components of engineered biomaterials, the immune
32
33 system and tissue regeneration has yet to be fully understood. The ultimate goal of tissue
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35 engineering is to develop therapeutic strategies that will stabilize, amend and improve
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37 cardiovascular anatomy and physiology¹⁶¹. Nowadays, cardiac tissue engineering and regenerative
38
39 medicine (TERM) has become the focal point for the repair of damaged heart tissue¹⁶². TERM
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41 related approaches have shown to minimize the need for ventricular remodelling. Different
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43 strategies have been adapted to design and fabricate polymeric scaffolds for heart tissue
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45 engineering¹⁶³. One potential application of polymeric scaffolds is the development of efficient
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47 degradable heart patches. These heart patches can provide an optimal platform for cellular growth
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49 over a period of time¹⁶⁴. A recent review focuses on the engineering of functional three-
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51 dimensional cardiac patches composed of various composite biomaterial including biodegradable
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53 materials¹⁶⁵.

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55 A three dimensional fibrin gel construct was reported by Ye *et al*¹⁶⁶, where different concentrations
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57 of apportioning (a protease inhibitor) promoted controlled degradation of the autologous scaffold
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59 seeded with fibroblasts. Microscopic studies of the developed tissue showed homogenous cell
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61 growth with no signs of toxic degradation or inflammatory reaction. However, the feasibility of
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4 forming a cardiovascular graft on the arterial side by the 1mm thick developed tissue appears
5 unlikely. A promising approach in cardiovascular tissue engineering was reported in which fibrin
6 gel was prepared by a non-woven poly glycolic acid (PGA) fibre mesh coated with
7 Polycaprolactone (PCL)¹⁶⁷. Human saphenous vein cells were seeded onto the fibrin gel and a
8 more mature extracellular matrix was produced in a short time span (days) with a decrease in the
9 loss of soluble collagen. Flanagan *et al* have reported an interesting study where fibrin-based heart
10 valves have been developed in a custom-designed bioreactor. The dynamic conditions were
11 optimized to accelerate the maturation of engineered valves¹⁶⁸. The experimental findings
12 demonstrated the potential repair and regenerative role of an injectable fibrin glue after a
13 myocardial infarction. This injectable fibrin glue could preserve infarct wall thinning and cardiac
14 function after myocardial infarction in MI-induced rat models. The decisive regenerative features
15 include; increased cell transplant survival, decreased infarct size and an increased blood flow to
16 the ischemic myocardium^{169,170}.

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29 Another study investigated the use of chitosan to increase the compression modulus of collagen
30 based injectable hydrogel matrices. It has been reported that endothelial cells formed significantly
31 more vascular-like structures on the collagen–chitosan matrix-hydrogels improved the ventricular
32 wall stability and showed an ability to reduce heart dilatation upon myocardial infarction (MI)¹⁷¹.
33 Silk protein fibroin of the Indian tropical tasar silkworm *A. mylitta* (AM) has been used by Patra
34 *et al* to develop 3D scaffolds for the *in vitro* engineering of a cardiac patch. The resulting
35 contractile patches were stable and demonstrated spontaneous beating for 20 days¹⁷². Biosynthetic
36 hydrogels of poly vinyl alcohol– alginate have also been prepared by Thankam *et al.*, these
37 consisted of a semi- and full-interpenetrating polymeric network (IPN hydrogel, PAHG)
38 harbouring it suitable for cardiac tissue engineering applications. Its amphiphilic nature and
39 moderate water content favoured cellular migration, growth and long term viability of L929
40 fibroblasts and H9C2 cardio myoblasts¹⁷³. Another methodology based on the blending of natural
41 polymers i.e. alginate and gelatine, were prepared in the form of films to be used as scaffold for
42 myocardial tissue engineering. Cell culture tests with C2C12 myoblasts, degradation in simulating
43 body fluids, showed best response for alginate/gelatine 20:80 blends¹⁷⁴. Gelatine and fibrin based
44 tissue engineered heart valve were designed and operated in a bioreactor with enhanced cell
45 attachment and alignment by Kim *et al.*¹⁷⁵.

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4 In 2007, Balguid *et al.* explored the role of collagen content and its cross-links in biomechanical
5 behaviour of human aortic heart valve leaflets and in tissue-engineered constructs. Collagen cross-
6 linked concentration showed a positive linear correlation with the modulus of elasticity, which can
7 enhance biomechanical function¹⁷⁶. It has been reported that collagen-glycosaminoglycan gels
8 matrices were used for mitral valve tissue engineering. Moreover, addition of chondroitin sulphate
9 (CS) resulted in a more porous model, which enhanced the bioactivity of seeded valve cells and
10 facilitating tissue remodelling¹⁷⁷. Gelatine/PCL hybrid fibrous scaffolds were synthesized by
11 electro spinning to obtain optimal fibre diameter, pore size and strength, promoting cell seeding
12 and finally development of constructs for cardiovascular tissue regeneration¹⁷⁸.
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21 In another study, Landa *et al.* investigated the use of bioresorbable alginate hydrogel to provide
22 mechanical and physical support to the damaged cardiac tissue after MI¹⁷⁹. Several recent reports
23 have shown the use of alginate hydrogels in delivery of sequential growth factor VEGF-A(165)
24 and PDGF-BB in a myocardial infarction model¹⁸⁰. These hydrogels were also able to controls
25 delivery of heat shock protein¹⁸¹ and serve as a carrier for dual delivery of insulin-like growth
26 factor-1 (IGF-1) and hepatocyte growth factor (HGF)¹⁸². Multi-layered cardiac grafts were
27 fabricated in vitro using biodegradable electrospun nano fibrous PCL meshes with a unique
28 extracellular matrix-like topography by Ishii and his co-workers¹⁸³. In another study, PLLA-co-
29 PCL (PLCL) nano-fibres were encapsulated with vascular endothelial growth factor (VEGF) using
30 two types of protective agents (BSA and dextran) through emulsion electrospinning. In vitro
31 release study demonstrated that the core-shell PLCL-VEGF-DEX nanofibers had potential as
32 sustained-release scaffold for cardiovascular tissue regeneration¹⁸⁴. Rat smooth muscle cells
33 (SMC) were seeded on biodegradable poly (ϵ -caprolactone-co-lactide) (PCLA) patches and were
34 checked for cellular penetration in vitro and in vivo. This work permitted the construction of an
35 autologous patch to repair congenital heart defects¹⁸⁵.
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52 To date several studies have focused on elastomeric biodegradable poly (glycerol sebacate) (PGS):
53 gelatine nano fibrous scaffolds and poly (glycerol sebacate) PGS/fibrinogen core/shell fibres.
54 These biomaterials exhibited well-defined anisotropy, mimicking the left ventricular myocardium
55 architecture that can be used as constructs for myocardial regeneration and repair^{186, 187}. The
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4 structural properties of the scaffolds had significant effect on cytoskeletal organization of the cells
5 as shown in Fig 8. Prabhakaran and his research group used a blend of synthetic (PLGA) and
6 natural (gelatine) polymer to obtain PLGA/Gel nano-fibres via electro spinning. Culturing of
7 cardiomyocyte cells on the scaffolds highlighted their potential as biomimetic cardiac patches¹⁸⁸.
8 In another study, a research group fabricated nano-fibrous scaffolds of electrospun random and
9 aligned PCL/gelatine to mimic structurally the oriented extracellular matrix (ECM), which provide
10 anisotropic wetting and mechanical properties compatible for cardiac regeneration¹⁸⁹. Composite
11 scaffolds of poly (1, 8-octanediol-*co*-citrate) and PLCL were evaluated for their mechanical and
12 biocompatibility properties. Electrospun scaffolds were elastic and hence provided the necessary
13 mechanical cues required for cardiac tissue repair¹⁹⁰. An electrospun poly(ethylene glycol)
14 dimethacrylate/poly (L-lactide) PEGDMA/PLA scaffold with biomechanical properties nearly
15 equal to native valve leaflets has also been reported¹⁹¹. Sant *et al* synthesized nano fibrous scaffolds
16 made up of blends of poly(glycerol sebacate) PGS prepolymer with PCL to address the mechanical
17 properties relevant to the human aortic valve leaflet¹⁹². For the regeneration of infarcted
18 myocardium, PGS short fibres were fabricated by co-axial electro spinning, with poly(glycerol
19 sebacate) (PGS) as core material and poly-L-lactic acid (PLLA) as shell material¹⁹³. A
20 mechanically compatible multi-layered scaffold of PCL sandwiched in a gelatine–chitosan
21 hydrogel was developed¹⁹⁴. This could be used as cardiac patch in tissue engineering applications
22 owing to its ability to sustain cardio-myocyte viability. Conductive nanofibrous scaffolds of
23 melanin, poly(L-lactide-*co*- ϵ -caprolactone) and gelatine can electrically stimulate cardio myocytes
24 to enhance cell proliferation and therefore are a potential candidate for cardiac patches as
25 demonstrated by Kai *et al*¹⁹⁵.

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Scaffolds fabricated from PEG and modified electrospun PCL(ePCL) can serve as a foundation
for engineered heart scaffolds¹⁹⁶. Composite scaffolds consisting of polyglycolic acid coated with
a thin layer of poly-4-hydroxybutyrate can be used as tri leaflet heart valve scaffold^{197,198}. Other
biomaterials and tissue engineering research avenues for enhancing cardiac function focus on
matrices with appropriate mechanical strength for weak cardiac tissue and intrinsic regeneration
by incorporation of local drug delivery^{199, 200}. In a study by Elamparithi, a novel collagen type I
scaffold developed by electro spinning in the absence of copolymers showed higher levels of
desmin. This scaffold was seeded with primary neonatal rat ventricular cardio-myocytes
(NRVCM) and exhibited sustained cardiac contractile function over duration of 17 days²⁰¹. The

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4 prospect of limiting myocardial damage and facilitating repair and regeneration was addressed in
5 this study²⁰².
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10 **3.5- Cornea**

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13 Pathological conditions associated with cornea are reported as the major cause of vision
14 impairment. Corneal pathology accounts for 4.9 million blind cases worldwide²⁰³. Anatomically,
15 the transparent corneal layer serves to focus light as it enters the eye. Blindness related to corneal
16 disease include many conditions, e.g. keratoconus, Fuch's dystrophy and Stephen-Johnson
17 syndrome^{204 205}. The extracellular matrix (EMC) of the cornea is a highly compact and organized
18 architecture consisting primarily of collagen (types I to V). This EMC is currently under
19 investigation as a prospect therapeutic research area. As estimated by the World Health
20 Organization, corneal diseases are a major cause of vision impairment and blindness, second only
21 to cataracts as the leading cause of blindness²⁰⁶. Tissue engineering has been widely explored for
22 its role in regenerative medicine. Recently, significant progress in corneal tissue engineering has
23 been achieved, where researchers have reported on the development of a corneal construct either
24 by employing cellular or acellular based techniques that are biocompatible, with physiological
25 functional for long term endurance²⁰⁷. Tissue engineering has focused on developing corneal tissue
26 that can potentially mimic the native cornea. Synthesis of a corneal construct, epithelial and
27 endothelial layers in parallel with a network of nerves have been explored. Reports have identified
28 natural and synthetic polymers as the preferred choice of investigation²⁰⁵. In the last few years,
29 intensive research efforts have been focused to determine whether key properties of ECM
30 macromolecules can be replicated within tissue-engineered biosynthetic matrices to influence
31 cellular properties. Tissue engineering of the cornea could overcome shortages of donor corneas
32 for transplantation and improve quality. Hydrogels based corneal implants from concentrated
33 recombinant human type I and type III collagen have promoted stable regeneration of corneal
34 tissue²⁰⁸. For example, Madden *et al* reported on one of first successful demonstrations of primary
35 human corneal endothelial cells on fibroin coated with collagen. This step allowed the evaluation
36 of fibroin as a substratum for the transplantation of tissue-constructs for endothelial keratoplasty
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4 A simple corneal substitute was developed from carbodiimides and N-hydroxysuccinimide cross-
5 linked collagen and was found to be suitable for transplantation. This was employed in centres
6 having a shortage of corneas available for implants²¹⁰. An artificial cornea of collagen–chondroitin
7 sulphate foam approximately equal to human cornea thickness seeded with human endothelial cells
8 proved that these collagen-chondroitin sulphate scaffolds are good substrates for artificial cornea
9 construction²¹¹. Collagen hydrogel matrices were developed by Li *et al* from collagen I cross-
10 linked with a copolymer based on N-isopropyl acryl amide, acrylic acid and acryloxysuccinimide.
11 These hydrogels were found to be non-toxic and allowed epithelial cell overgrowth and optical
12 clarity superior to the human cornea²¹². Fibrillar collagen sponges were used as a substrate for
13 culturing human kerocyte, epithelial and endothelial cells. This synergy promoted a wound
14 healing in the eye²¹³. Liang *et al.* reported on the formation of an *in situ* biodegradable and non-
15 toxic composite hydrogel for corneal endothelium reconstruction²¹⁴. The hydrogel was made by
16 self-cross-linking of water-soluble chitosan and oxidized sodium alginate.
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29 Biodegradable chitosan–PEG hydrogel films (CPHF) and chitosan; PCL blends with excellent
30 biocompatibility are enviable candidates as substrates for the regeneration and transplantation of
31 CECs (corneal endothelial cell)^{215,216}. The transplantation of fibroblast precursors on gelatine
32 hydrogel into the corneal stroma may be a possible treatment for corneal stromal regeneration²¹⁷.
33 Some recent studies revealed fabrication of cross-linked porous gelatine scaffolds for *in vitro*
34 cultivation of corneal endothelial cells (CECs)²¹⁸. These were modified with chondroitin sulphate,
35 which enhanced proliferative and biosynthetic capacity of cultured cells²¹⁹. Cross-linked porous
36 gelatine hydrogel discs were inspected for their potential as cell sheet carriers for corneal
37 endothelial cell therapy. These could efficiently deliver the cell sheet transplants at the site of
38 injury²²⁰. Lawrence *et al.* reported that silk protein films could support corneal cell functions and
39 were used to reproduce corneal stromal tissue building²²¹. Dual layer scaffolds were prepared from
40 Silkworm (*Bombyxmori*) silk fibroin for corneolimb reconstruction of diseased or damaged
41 ocular surface. These fibroin membranes showed potential as a substrate for human limbal
42 epithelial (L-EC) and limbal mesenchymal stromal cell (L-MS) cultivation²²². Porous silk fibroin
43 film were synthesized by mixing of silk fibroin and poly(ethylene glycol) (PEG) followed by the
44 removal of PEG from porous films. These films were used as biocompatible carriers to deliver
45 corneal epithelial cells to ocular surface²²³.
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4 Klenkler *et al.* modified polydimethylsiloxane (PDMS) surfaces with epidermal growth factor
5 (EGF) to improve the growth of corneal epithelial cells²²⁴. The design of artificial limbal stem cell
6 niches for cell delivery to cornea was explored by the fabrication of biodegradable poly(lactic-co-
7 glycolic acid) 50:50 electrospun membranes²²⁵, which have the potential to support the growth of
8 limbal epithelial cells for periods of at least of 2-3 weeks in culture^{226, 227}. Biodegradable PLGA
9 membranes containing micro-pockets mimicking an ex vivo 3D cornea model can potentially
10 contribute to the migration of limbal cell from limbal explants^{228, 229, 230}. Fig 9 reveals degradation
11 of PLGA membranes when placed in media with and without cells and showed that fibres lost
12 integrity over 6 weeks' time. Poly (2-hydroxyethyl methacrylate) (PHEMA) hydrogels were used
13 for functional polymeric artificial corneas (keratoprosthesis)²³¹. Optically transparent,
14 biocompatible and biodegradable poly(ethylene glycol) (PEG)-based hydrogel films (PHFs)
15 appeared good candidates for regeneration and transplantation of corneal endothelial cells (CECs)
16 by Ozcelik *et al*²³².

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32 Recently, much attention has been dedicated to the use of blends of biodegradable polymers to
33 get interpenetrating polymer networks (IPNs). These hydrogels showed better mechanical
34 properties and harnessed greater multi-functionality appropriate for keratoprosthesis²³³. A number
35 of researchers have investigated the synthesis of poly(ethylene glycol)/poly(2-
36 hydroxyethylmethacrylate) (PHEMA)-based IPN hydrogels and chitosan-PCL-blended
37 membranes as carrier for corneal endothelial cell (CEC) transplantation^{234, 235}. A number of studies
38 have been reported on the pros and cons of using polymers such as collagen and fibrin based carrier
39 materials in limbal stem cell deficiency (LSCD) remedy²⁰⁴. In 2010, recombinant human collagen
40 type III (RHCIII) hydrogels that facilitated nerve regeneration and stromal cell were developed as
41 corneal constructs. Although promising, these implants presented some limitations, including
42 sufficient cell division speed to evade infection and fibrosis in some cases.²³⁶ This data identifies
43 the need to develop corneal implant that can be accurately grafted and in parallel allow rapid
44 healing process. Recent studies have highlighted the importance of post fabrication remodelling in
45 achieving positive clinical outcome²³⁷.

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4 RHCIII–MPC hydrogels were fabricated using 2-methacryloyloxyethyl phosphorylcholine (MPC)
5 that promoted fibronectin printing. The micro-patterns of 30µm size generated in these RHCIII–
6 MPC hydrogels showed optimal mitotic division and cell attachment. *In vivo* studies have yet to
7 replicate these properties exhibited by RHCIII–MPC hydrogels²³⁸. Biomaterials owing to their
8 therapeutic properties have shown promising avenues in corneal repair and regeneration. At
9 present, research is being focused on corneal regeneration *in vitro* and *in vivo* using polymer
10 (gelatine, alginate and chitosan) based hydrogels, and constructs²³⁹. A recent study has shown that
11 primary human corneal keratocytes were more compatible with silk fibroin films fabricated by
12 centrifugal force. Bombyx mori cocoons were used to retrieve silk fibroin (SF). SF has been used
13 in corneal tissue engineering and approved by FDA for soft tissue repair. SF films prepared by
14 centrifugal force had smooth surfaces, transparency and elasticity, rendering favourable
15 environment for cell growth²⁴⁰.
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27 **3.6- Dental and Oral Structure**

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29 The basic knowledge about the biology of the oral and tooth structure as well as the information
30 about fundamentals of materials and techniques applied to tissues, constitute the basis for
31 restorative dentistry and help in creating biological approaches to tissue regeneration. A suitable
32 inductive carrier is essential for dental-pulp tissue regenerative treatment. The selection of suitable
33 scaffold has vital importance to persuade and confer the optimal formation of new dentin matrix
34 and pulp-dentin complex²⁷. For regenerative dentinogenesis optimal conditions for cell adhesion,
35 migration, proliferation and differentiation must be provided. Among dental problems, periodontal
36 diseases are highly prevalent and 90 % of the worldwide population is affected. Periodontitis is
37 one of the periodontal diseases leading to loss of connective tissue and bone support, which is a
38 major cause of tooth loss in adults²⁴¹. The techniques, such as bone graft, guided tissue
39 regeneration (GTR), and stem cell therapy have been used for periodontal tissue regeneration,
40 among these the GTR has become the most promising treatment and has been widely used in
41 clinical treatment for its convenience and effectiveness²⁴². During GTR technique a barrier
42 membrane provide mechanical support to gingival connective tissue on one side and periodontal
43 ligaments on other side²⁴³. The first generation these membranes comprised of stable, non-
44 immunogenic polytetrafluoroethylene (ePTF), a non-resorbable material. However, the significant
45 drawback is related to the risk of disturbing healing with the second surgery necessary to remove
46 the permanent. To address this issue, a second generation of resorbable membranes was developed.
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4 The resorbable membranes can potentially provide better healing as the material resorption and
5 bone ingrowth occur simultaneously. Recently, third generation of membranes have been
6 introduced with bioactivity²⁴⁴. The basic principal of GTR membrane is to restore the architecture
7 and functionality of the periodontal system²⁴⁵. On the basis of this, the ideal periodontal membrane
8 should have two important properties i.e. stiffness and elasticity²⁴⁶. Various types of materials have
9 been tested for their effectiveness as barriers including non-degradable and biodegradable
10 membranes²⁴⁷. A list of commonly used commercial periodontal membranes is given in Table 1.
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22 Several problems have been associated with the use of non-degradable barrier membranes,
23 particularly the need for a secondary surgery to remove the membrane. Furthermore, early
24 exposure to the saliva present in oral environment and subsequent bacterial colonization are
25 common problems resulting in early detachments. To overcome these issues, a variety of synthetic
26 biodegradable materials, such as polylactide, PLA, PCL, and their copolymers or tissue-derived
27 collagens have been used as membrane barriers²⁴⁸⁻²⁵¹. It is suggested that a highly hydrophobic
28 surface, which act as a non-conductive towards protein attachment, should be used an occlusive
29 barrier for gingival epithelial cells in periodontal regeneration. The schematic structure of
30 periodontal membrane is given in Fig. 10²⁵².
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42 Drug loaded biodegradable periodontal membranes were synthesized and found that non-steroidal
43 anti-inflammatory drugs can create an effect on morphology of electrospun fibers and smooth
44 electrospun fibers can be achieved with high drug loaded polymers. Moreover, doxycycline based
45 periodontal membranes stimulated cell proliferation and osteogenesis²⁵³⁻²⁵⁵. Kasaj *et al.*²⁵⁶
46 evaluated the biological effects of various commercially available biodegradable membranes made
47 of collagen and compared it with non-degradable membranes in cultures of human gingival
48 fibroblasts, periodontal ligament fibroblasts and human osteoblast-like cells. It was found that non-
49 degradable membranes limited the cell adhesion and the biodegradable membranes demonstrated
50 to be more suitable to stimulate cellular proliferation compared to non-resorbable membranes as
51 shown in Fig 11.
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(Inset Figure 11)

Chen *et al.*,²⁵⁷ fabricated biodegradable electrospun PLLA/chitosan membrane synthesized by aminolysis method for periodontal regeneration. The membrane was aminolyzed with chitosan to enhance the biocompatibility. The modification of chitosan can promote the hydrophilicity, bioactivity, and degradation rate of PLLA electrospun membrane. The degradation rate of PLLA scaffold increased significantly after chitosan grafting, which was due to introduction of imine groups ($-\text{CH}=\text{N}-$) on PLLA fibres through the modification. The hydrolysis of imine and ester groups led to the degradation of PLLA-CS, which resulted in additional mass loss, while the PLLA degradation was mainly caused by the hydrolysis of ester groups²⁵⁸. During the aminolysis process, the alkaline catalysed degradation of PLA matrix resulted in a decrease of molecular weight²⁵⁹. In vitro degradation study showed that modified membrane (PLLA-Chitosan) degraded quickly compared to pure PLLA and the quantitative analysis showed that after 6 weeks PLLA-Chitosan degraded 20%, whereas pure PLLA showed only 5%. The SEM micrographs (Fig. 12) show the degradation behaviour of electrospun fibres of modified and pure degradable polymers after 2, 4 and 6 weeks. The modification of imine group ($-\text{CH}=\text{N}-$) with PLLA enhanced the degradation process, however, the main degradation was due to the hydrolysis of ester group.

(Inset Figure 12)

In same study, cell culture showed that the modified membrane had a better biocompatibility and promoted cell (MC3T3) proliferation compared with pure PLLA and tendency to prevent fibroblast invasion²⁵⁷. Fig. 13 shows the optical and fluorescence image of PLLA-chitosan membrane after culturing of fibroblast NIH 3T3 on surface and it was observed that after 5 days, the fibroblasts were on top of the electrospun membrane.

(Inset Figure 13)

The poor biocompatibility of pure PLLA was due to the absence of natural recognition sites on polyester surfaces for covalent cell recognition signal molecules, whereas chitosan mimics extra cellular matrix and facilitate the cells to grow and help in functioning. The polyester-based membranes are biocompatible, biodegradable, and easier to handle clinically as well as allowing

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4 tissue integration. Their degradation rate is important as these membranes must function for at
5 least 4–6 weeks to allow successful regeneration of the periodontal system²⁶⁰. Generally, the
6 biodegradation of these polyesters involves non-enzymatic cleavage of PGA and PLA into pyruvic
7 and lactic acids, respectively, which are common end-products of carbohydrate digestion. Milella
8 *et al.*²⁶¹ evaluated both the morphological and mechanical characteristics of commercially
9 available polyester-based membranes. It was observed that the membranes demonstrated initially
10 high strength (12–14MPa), losing their structural and mechanical properties within 4 weeks of
11 incubation in culture medium. The maximum strength after 14 days of exposure decreased
12 significantly (below 1MPa). Collagens are important alternatives to synthetic polymers in
13 GTR/GBR procedures due to their excellent cell affinity and biocompatibility. However, type I
14 collagen may have limitations in its use due to the high cost and poor definition of its commercial
15 sources, which make it difficult to control degradation and mechanical properties. Collagen-based
16 membranes have shown very poor performance *in vivo* as the membrane starts to degrade. The
17 breakage and fragmentation of collagen fibrous membranes started after 7 days of incubation and
18 after 30 days, the degradative behaviour enhanced, and pores were evident as shown in Fig. 14.

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35 Additionally, the risks of disease transmission due to the use of human- or animal-derived collagen
36 may pose regulatory or other limitations, such as religious beliefs, on its use. Biomechanical
37 properties and collagen matrix stability can be enhanced by means of physical/chemical
38 crosslinking, by ultraviolet (UV) radiation, genipin (Gp), and glutaraldehyde^{261, 262}. It was
39 proposed that a natural polymer based membrane has better cell adhesive and biocompatibility
40 properties; however, its mechanical strength is not up to the mark. In contrast, synthetic polymers
41 have desirable mechanical properties, but poor biological properties. Therefore, modifying natural
42 polymers, such as collagen membranes with synthetic polymers may yield GTR barrier membranes
43 with optimal properties. PLA, poly(glycolide-co caprolactone)(PGC) and PLGA was employed
44 and spray coated on collagen membrane which significantly improved its mechanical strength²⁶³.

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55 To date, the chitosan membranes' application is still in the animal assay phase, but the results
56 showed great potential for chitosan materials in GTR procedures. In comparison to other
57 biodegradable membranes, the chitosan membranes are cheaper and possess better tissue healing
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4 effect and showed more cementum and bone formation in animal models. The bacteriostatic
5 property of chitosan may reduce the bacterial contamination and enhance periodontal tissue
6 regeneration. The degradation rate of chitosan membranes manufactured by different methods was
7 evaluated in a number of studies^{264, 265}. Pure chitosan membranes degraded by about 15–40% of
8 their initial weight after 90 days shaking in phosphate buffer saline (PBS). In vivo testing showed
9 that after grafting into rat subcutaneous tissue chitosan membranes maintained their shape and
10 space for bone regeneration for 6 weeks²⁶⁶. The degradation rate of chitosan membranes depends
11 on their molecular weight and the preparation methods, and it fit into the schedule of remodelling
12 of tissue regeneration²⁶⁷.
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23 **3.7- Trachea**

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26 Patients suffering from damage of the trachea after tumour formation of excision need permanent
27 treatment^{268, 269}. However, this is a major challenge, in part due to the specialised structure of the
28 tissue. The trachea is a circular segmented architecture of cartilage, interconnected with soft
29 tissues to form the tubular air pipe structure of the respiratory system²⁷⁰. The main function of this
30 fragmented cartilage is to provide sufficient stiffness and flexibility to regulate airflow
31 systematically. Tracheal anatomy reveals an inner surface of columnar epithelium, with cilia that
32 help in trapping extraneous air particles together with goblet cells for exuding mucus to form
33 protection against any external stimuli²⁶⁸. These functions are unique and cannot be modelled
34 through autologous tissue implants. Therefore, tracheal regeneration²⁷¹ is a focus of many
35 biomedical engineers and clinicians. The on-going research in tracheal regeneration uses
36 prosthesis implants, synthetic composites and tissue-engineered constructs²⁷¹⁻²⁷³. But these
37 biomimetic materials are associated with clinical issue e.g., breath impediment, infection and
38 dehiscence, limited epithelialization and vascularization. Tissue engineering strategies²⁷⁴ are not
39 yet able to produce an ideal tracheal implant. Nonetheless, TE holds the potential to realize
40 advanced and optimal tracheal grafts^{29, 275, 276} while considering the following factors such as: (a)
41 the graft should be biodegradable and biocompatible (i.e. it can offer a suitable architecture for
42 cells so they can produce cartilage and soft tissue of the apposite cylindrical contour.) (b) It should
43 stimulate epithelial development (i.e. it has a well-designed epithelial lining that could either be
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4 cultured or migrated from the native trachea), and (c) should facilitate adequate vascularization, to
5 support the volume of tissue required for clinical application²⁶⁹.
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9 The first tracheal tissue engineered product was introduced by Vacanti et al²⁷⁷, who reported a
10 three-dimensional tracheal scaffold prepared from synthetic nonwoven mesh, 100µm thick PGA
11 fibres (15µm in diameter, cut into pieces of 2.5 x 4cm) for replacing large circumferential cervical
12 defects in trachea of rats. Chondrocytes were seeded into engineered cartilage to evaluate their
13 viability, the scaffold allowed the expansion of chondrocytes. Implantation of cell-polymer
14 constructs was reported to produce hyaline cartilage after four weeks in mice. Follow-up
15 histological studies showed that from initial stage samples, an identical cartilage to the natural one
16 was produced, but later the animals suffered from respiratory distress and ultimately died. The
17 collapse of cartilage was supposedly by non-optimized mechanical properties^{269, 277}. In another
18 study by Kojima et al²⁷⁸, they used biodegradable PGA non-woven mesh enfolded in a helical
19 template composed of silicone rubber. For *in vitro* studies, chondrocytes and epithelial cells were
20 isolated and seeded from sheep nasal septum. The cell-polymer construct was implanted into
21 subcutaneous pockets of nude mice. After six weeks of cell growth, epithelial cells were suspended
22 in hydrogel and infused into the implanted tissue construct. Hemotoxylin and eosin staining
23 demonstrated full-grown cartilage, pseudostratified columnar epithelium growth and a separate
24 interface or borderline, connecting tissue-engineered cartilage and epithelium. Furthermore,
25 Safranin-O staining results illustrated ordered circular lobules and angular lacunae respectively,
26 which contained single chondrocytes. The authors concluded that the morphology of the implants
27 resembled native sheep trachea in that the proteoglycan and hydroxyproline content was similar to
28 native cartilage, and therefore had the potential for regeneration of segmental tracheal defects as
29 well as epithelial formation.
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48 Despite the reported success in tracheal restoration by implantation of tissue engineered constructs
49 and transplantation procedures, none of the newly established techniques have resulted in clinical
50 application on a large scale. Developing or regenerating a purposeful tracheal tissue from different
51 cultured cell types is still a major challenge for researchers²⁷⁹. For instance, tracheal fixation in
52 laryngectomized patients and prosthetic voice rehabilitation using tracheoesophageal silicone
53 rubber speech valves and tracheostoma valves has resulted in many complications. Furthermore,
54 animal models used for tracheal research vary widely and in most of the cases, proper scientific
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4 justification for choice of animal is not explained. These issues play a decisive role in tissue
5 engineering and are thoroughly discussed in a review paper by Hallers *et al*²⁷⁹.
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9 With the passage of time, several progressions in tracheal tissue engineering have been made²⁷¹.
10 Rotter *et al.*²⁸⁰ demonstrated the effect of interleukin in tissue-engineered cartilage made from
11 PGA–PLA (PGLA) matrixes. PGLA scaffolds seeded with porcine auricular chondrocytes and
12 unseeded scaffolds as controls were implanted in an autologous immunocompetent pig model.
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14 Histological studies by using haematoxylin and eosin, Safranin, trichrome, and Verhoeff's staining
15 and biochemical studies confirmed that the level of glycosaminoglycan showed acute
16 inflammation. Moreover, homogeneous cartilage development was not observed in any of the
17 samples except in specimens taken after one week of implantation. Furthermore, histological
18 studies revealed acute inflammation around the degrading scaffold, whereas, glycosaminoglycan
19 contents were observed considerably higher in serum free group. These are regarded as inhibiting
20 factors in regeneration of cartilage tissue. Scaffold free cartilages have also been proposed in the
21 literature by Wu *et al.*²⁸¹ and Weidenbecher *et al.*²⁸² respectively. Wu *et al* fabricated cylindrical
22 cartilage using a chondrocyte macro-aggregate. In another study, Weidenbecher *et al.* developed
23 scaffold-free cartilage sheets for fabricating a vascularized neo-trachea in a rabbit model. A
24 tracheal framework was produced by these neo-tracheal tissue engineered constructs after few
25 weeks of harvesting and these neo-tracheas, healthy with well-vascularized supported with
26 integrated layers, but showed limited mechanical strength, thus were unable to reinstate segmental
27 defects and long-term patency in trachea^{281, 282}. In another study²⁸³, composite grafts were
28 fabricated from a biodegradable 3-layered scaffold: a collagen sheet, a PGA mesh, and a
29 copolymer (L-lactide/ ϵ -caprolactone) coarse mesh. Chondrocytes isolated from the auricular
30 cartilage of New Zealand white rabbits were cultured and then seeded onto the biodegradable
31 construct to restore tracheal stenosis. Implantation was carried out in a mid-ventral defect of
32 cervical trachea. In addition, a gelatine sponge for an appropriate supply of basic fibroblast growth
33 factor (b-FGF) on scaffold was also employed. Their findings showed that the biodegradable
34 scaffold was able to regenerate the tracheal architecture up to 3 months after implantation.
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36 Regardless of their success, authors proposed further studies that may establish techniques that
37 could facilitate homogeneous cartilage formation with optimal functional and mechanical
38 properties²⁸³.
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4 Lin *et al.*²⁸⁴ reported a unique approach, as they developed a scaffold based bioreactor system for
5 tissue-engineering of trachea under the influence of controlled fluid flow. A scaffold of poly (3-
6 caprolactone)-type II collagen was seeded with chondrocytes and grown under controlled
7 rotational speed/fluid flow and resulting shear stress in the bioreactor. This procedure enhanced
8 cell proliferation, glycosaminoglycan (GAG) and collagen content in the constructs compared to
9 static culture for the same time. For instance, at a rotation of 15 rpm, a two-fold increase in cell
10 population, 170% increase in GAG content and 240% increase in collagen were achieved. H&E
11 staining provided evidence of neo-cartilage formation along with aligned chondrocytes in direction
12 of fluid flow.
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21 The potential of using transplanted cells from the patients was also carried out by Kobayashi *et*
22 *al.*²⁸⁵. They used synthetic grafts of collagen sponge containing a spiral polypropylene stent and
23 mesh in combination with gingival fibroblasts (GFBs) and adipose-derived stem cells (ASCs) as
24 autologous transplanted cells for tracheal epithelial regeneration. Their studies revealed limited
25 risk of rejection by immune systems and contamination from allotransplant cells but showed
26 sluggish epithelial regeneration²⁸⁵. Tatekawa *et al.*²⁸⁶ reported on the use of a bio absorbable
27 copolymer of caprolactone-lactide sponge sheet reinforced with a poly(glycolic acid) fibre mesh
28 (Cop). Cop incorporated gelatine hydrogel and Cop-gelatine hydrogel with basic fibroblast growth
29 factor were used with an external non-degradable polymer stent. Implantation was carried out in
30 three groups of rabbits and tracheal epithelialization, cartilage formation and vessels were only
31 noticed in bio absorbable copolymer containing gelatine hydrogel (Figure 15).
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47 Their observations revealed that respiratory distress, loss of appetite airway resulted in tracheal
48 collapse as well as dislocations of the copolymer (due to mucosal sloughing) were the reasons of
49 rabbit death. To overcome this situation and to retain long-term survival, the reconstructed trachea
50 was reinforced by external stenting on either side of the trachea²⁸⁶. Interestingly, macromolecules
51 such decorin, a proteoglycan (PG) residing in the complex network of ECM proteins of connective
52 tissues, have also been explored for tissue engineering applications²⁸⁷. Hinderer *et al.*²⁸⁷ introduced
53 a strategy in which decorin was electrospun in 3D fibrillary scaffolds fabricated from
54 biodegradable PCL-gelatine matrices for tracheal tissue regeneration. The electrospun scaffolds
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4 were investigated for cell-matrix-interactions and immune-mediated mechanisms and found low
5 immunogenicity for hPAEC (human primary airway epithelial cells) expansion as shown in Figure
6 16. Their findings revealed possible applications in restoration of the trachea by these functional
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8 3D hybrid scaffolds²⁸⁷.
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17 Different strategies have been executed to advance existing research development in tissue
18 engineering of the trachea^{272, 275}. One such recent example is engineering of a vascularized trachea
19 by utilizing bioresorbable PLGA and PCL scaffold²⁶⁹. In this study, implanted scaffolds were
20 wrapped with pedicled muscle flap over a ring-shaped mould. Furthermore, these muscle enfolded
21 PLGA and PCL scaffolds were seeded with chondrocytes, bone marrow stem cells and co-cultured
22 both cells respectively. Implantation of these engineered scaffolds was done as an ectopic culture
23 over abdominal wall of rabbits and harvested for several weeks. The tissue engineered constructs
24 were harvested after subsequent *in vivo* intra-muscular incubation. It was observed that all the
25 scaffolds preserved adequate cylindrical contours for two weeks. Though, harvesting after four
26 weeks, contraction and deformation in the PLGA scaffolds was observed. After careful detachment
27 of a silicone mould and muscle tissue, a well-encapsulated ring of PLGA and PCL scaffolds were
28 further investigated as shown in Figure 17a. Structural similarities among tissue engineered
29 scaffolds and to native cartilage were evident in PCL scaffolds at the two-observation time-points.
30 Whilst the PLGA scaffolds after four weeks had shrunk and deformed, those at two weeks had not.
31 In addition, a considerable weight loss (22.5%) of PLGA at four weeks was observed, compared
32 to weight loss of PCL at 2 weeks (6.3%) (Figure 17b). Hence, PCL tissue engineered scaffolds due
33 to their adequate porosity maintained tubular scaffold geometry and were considered as more
34 suitable for intra-muscular tracheal tissue engineering as compared to PLGA scaffolds.
35 Histological results further revealed that PCL engineered scaffolds exhibited optimal
36 chondrogenesis with sufficient stiffness to maintain the cylindrical shape and luminal patency
37 comparable to the native trachea.
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3.8- Bones

Bone tissue is a naturally occurring nanocomposite comprising of organic-inorganic molecules compacted together. It consists of a nano-crystalline, rod-like (25-50 nm in length) inorganic ceramics such as hydroxyapatite (HA) $\{Ca_{10}(PO_4)_6(OH)_2\}^{288}$ embedded into collagen fibrils with osteoblasts, osteocytes and osteoclasts as cell components²⁸⁹⁻²⁹¹. Nowadays, synthetic HA has been used in bone regeneration due to its cytocompatibility as well as good osteoinductive and osteoconductive abilities^{292, 293}. Commercial HA and β -tricalcium phosphate (β -TCP)-based ceramic products are used for bone repair, augmentation and replacement, or as fillers in bone and teeth, as well as coatings of orthopaedic and dental implants. However, due to their low mechanical or tensile strength and fracture toughness as compared with natural bone, slow biodegradability in vivo and limited interactions with osteogenic proteins either restrict its use in load-bearing applications or reduce its efficiency in bone tissue regeneration. With the advent of nanotechnology, new horizons in the scientific and industrial research have been accomplished. Research at the nanoscale level enhanced the structure property relationship especially for biomaterial in tissue engineering. HA has been modified and toughened with polymers²⁹⁴, silicon carbide²⁹⁵, alumina²⁹⁶ and titanium materials²⁹⁷. Biodegradable polymers have been explored with HA and a variety of other nano-porous materials²⁹⁸⁻³⁰⁰. HA-PLGA nanocomposite material have been developed which possesses good osteogenic activity²⁹⁰. Bone morphogenetic proteins, such as BMP-7 derived DIF-7c peptide were chemically functionalized onto nano-HA and integrated within the nano-phase of hydroxyapatite-PLGA composite, pristine PLGA and mixed directly into cell culture medium. Experimental studies revealed that HA-PLGA nano composites promoted hMSC adhesion in contrast to pristine PLGA. It was also notable that osteogenic differentiation of hMSCs by nano-hydroxyapatite and nano-hydroxyapatite-PLGA composites was appreciable as compared with direct injection of the DIF-7c peptide into culture media. In a recent study, Chitin-PCL-nHAp (nano-hydroxyapatite) based injectable microgels were prepared for healing major bone defects³⁰¹. It has been observed that addition of nHAp in polymer matrix enhances the mechanical properties. However, biological characteristics of the composite microgels supported material cytocompatibility and protein adsorption. Furthermore, cell culture studies in chitin-PCL-nHAp microgels with adipose derived mesenchymal stem cells (rASCs) from rabbit showed good expressions of alkaline phosphatase, osteopontin, osteocalcin, as well as, migration of rabbit

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4 adipose derived mesenchymal stem cells (rASCs). Consequently, chitin-PCL-nHAp microgels
5 could offer an effective injectable material for regenerating a diverse variety and complex bone
6 defects³⁰¹.
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10 In recent years, synthetic biodegradable polymers and their composites have been tuned to
11 fabricate well-aligned and multipurpose tissue engineered constructs^{12, 33, 34, 52, 79, 244, 288, 291, 302-308}.
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14 In a recent study, fibre based biodegradable scaffolds³⁰² such as poly-1-caprolactone/poly(lactic
15 acid (PCL/PLA) composites, containing fibres of PLA in a PCL matrix were developed in cell
16 instructive scaffold fashion for investigating bone osteogenesis. Integration of PLA fibres into the
17 PCL matrix resulted in drastic improvement in mechanical properties. The most interesting aspect
18 of this research is computational fluid dynamic models, which expose the material's capability to
19 exert hydrodynamic forces during in vitro cell culture, as a result, an optimal flow rate was
20 established that enabled specific cellular event to happen. e.g. osteoblast differentiation from
21 hMSCs.
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30 Some natural biodegradable materials, such as collagen, gelatine and silk have also been used in
31 combination with other materials³⁰⁶. Nevertheless, formation and significance of anti-bovine
32 collagen antibodies in many human recipients containing bovine collagen is still a matter of debate
33 and not yet fully understood⁷⁹. Therefore, numerous biodegradable polymers and their composites
34 have been investigated to make hybrid tissue scaffolds for bone and cartilage regeneration^{302, 309}.
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37 Such nanomaterials have remarkable characteristics, such as cell adhesion, interaction and
38 proliferation as compared to the pure synthetic polymers. Collagen has also been used to improve
39 cell interactions with electrospun nano-fibres of bioresorbable PLA, PGA and PCL and their
40 copolymers^{79, 309-311}. Composite biomaterials from biodegradable PLA, PGA and their copolymer
41 PLGA have been employed with bioactive ceramics i.e., bioactive glass particles or HA⁸. Studies
42 showed that such materials stimulate bone regeneration, as well as, offer better mechanical strength
43 and biological concert³⁰³. It was also reported that composites of polymers and Bioglass® are
44 angiogenic i.e., they supported the growth of blood vessels, suggesting a novel approach for
45 providing a vascular supply to implanted materials in bone tissue engineering, which was
46 confirmed by histological studies of resected implants (Figure 18³¹²).
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4 Composite biomaterials from natural, synthetic polymers and nanomaterials are also been used
5 extensively^{34, 291, 305-308}. This includes biomaterials based on gelatine and silk derivatives which
6 have been studied recently. Due to their biological source, biocompatibility, excellent
7 biodegradability and above all its ease of availability at low cost, makes them suitable for tissue
8 engineering^{313, 314}. A number of this class of biomaterials, such as gelatine methacrylate
9 (GelMA)²⁸, interpenetrating GelMA-SF (silk fibroin)²⁸, silk–silk composite scaffold³¹⁵ and CNTs
10 reinforced GelMA composites material³¹⁶ have been employed in tissue engineering applications.
11 All these materials have intrinsic benefits and limitations, such as preparing GelMA is low cost
12 and convenient and it also promotes cell proliferation, migration, natural cell binding and
13 degradation motifs but its use has become limited when rapid degradation is required, or high
14 mechanical stiffness cannot be compromised. In GelMA-SF, SF addition to GelMA system
15 increases physical cross-linking without any chemical modification. Both these factors, such as
16 crosslinking and crystallinity, influence the mechanical and degradation properties of these
17 material²⁸. However, the biocompatibility of silk and its ability to form large porous structures
18 offers a significant advantage and it has been further investigated to fabricate silk-silk macro
19 porous scaffolds³¹⁵. The high interfacial cohesion between SF and macro particles resulted not
20 only in reinforcing mechanical properties but also lowered or restricted the enzymatic degradation
21 of the scaffolds. Use of carbon nanotubes (CNTs) in GelMA also reinforces the mechanical
22 stability due to interaction between peptide chain and CNTs³¹⁶. Cross-linking was not observed
23 resulting in a significant dispersion in the medium.
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41 In preparing an optimal bone graft, the efficiency of materials can also be enhanced by increasing
42 their surface area as can be achieved by producing nanostructures and subsequent functionalization
43 by incorporating nano-fillers in the polymer matrix. For this purpose, an ideal material would
44 instruct mechanical stability to the composite without reducing its bioactivity. In this perspective,
45 one and two dimensional carbon based materials with high chemical inertness and good
46 biocompatibility, such as carbon nanotubes (CNTs), graphene or graphene oxide (GO) can help in
47 enhancing the physical, chemical and biological properties of biomaterials for bone tissue
48 engineering²⁹⁰. Recently, CNTs^{290, 316-318} and GO^{290, 317, 319, 320} have been used as nanofillers and
49 reinforcing agents in synthetic and natural biodegradable polymer matrixes for bone regeneration
50 and tissue engineering applications^{321, 322}. Interestingly, in every case, these nanostructures
51 resulted in improved physical properties, such as resilience, toughness and tensile strength, as well
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4 as good biocompatibility and biodegradation. In addition, no obvious toxic effects in vivo^{215, 317,}
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6 ³²³ were observed. Hence, these types of biomaterials offer great potential in tailor making required
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8 properties when incorporated in polymeric materials, ultimately strengthening material properties
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10 without offsetting its bioactivity/biocompatibility and allowing to be used for bone regeneration^{215,}
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12 ³¹⁷. In terms of its biological efficiency, graphene offers cell adhesion and proliferation i.e., for
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14 osteoblasts^{319, 324, 325}. Furthermore, during tissue formation electrical stimulation of osteoblasts can
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16 be carried out utilising superior electrical conductivity of graphene³²⁶. Besides graphene, graphene
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18 oxide also exhibits promising biological properties by facilitating adhesion and proliferation of
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20 mouse fibroblast cells ³²⁷, providing drug delivery platforms for water insoluble cancer drugs³²⁸
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22 and in biosensors³²⁹. Its multifunctional reinforcing properties in polymer/nanocomposites have
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24 led to the development of synthetic materials with significantly enhanced mechanical strength^{330,}
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26 ³³¹.

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28 Incorporating GO in natural or synthetic polymers are a very effective method for preparing
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30 graphene based polymer nanocomposites. Since GO contains abundant oxygen-containing groups
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32 e.g. hydroxyls, epoxides, diols, ketones and carboxyl on its surface³³⁰, these can promote
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34 interfacial interactions with other materials. Furthermore, it is observed experimentally that by the
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36 addition of very minute amounts, (e.g. 1wt% of GO in polymer matrix) lead to a significant
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38 increase in their physical properties. In a recent study, the reinforcing effects of GO in a gelatine
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40 matrix have been studied in detail, in particular the size and morphology of GO sheets, the degree
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42 of dispersion of the GO sheets in gelatine matrix and the interactions of two phases. Results
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44 obtained in this study indicated that in gelatine-GO composites, an enhancement of the mechanical
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46 properties (tensile strength, Young's modulus and energy at break) of gelatine increases by 84%,
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48 65% and 158%, respectively just by addition of 1 weight% of GO. Furthermore, bio mineralization
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50 and biocompatibility of gelatine was also enhanced. In spite of these attributes, moisture sensitivity
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52 and toughness³¹⁴, use of gelatine based materials for bone tissue engineering have been limited.
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54 Chitosan and epoxy based materials have also been used, but most of the GO-polymer composites
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56 reported in literature exhibit reduced ultimate strain or toughness^{330, 332, 333} so their use as bone
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58 substitutes have been limited to date.
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4- Future Perspectives

In tissue engineering, several biodegradable materials have been examined for organ specific regeneration, as discussed in the relevant sections within this review. Their success totally depends upon the clinical requirements, their physical and biological compatibility with the host tissue as well as various environmental factors. Therefore, extensive research is required to meet the desired goals. For instance, in the case of skin a variety of commercially available skin substitutes are present in order to regenerate skin and to regain its normal structure and function. However, it is obvious that the ideal skin substitute does not exist. The factors hindering implementation of currently available skin substitutes have low mechanical properties, lack of biocompatibility, minimal structural differentiation, limited vascularization and scar development^{88, 97}. Over the last 10 years, tissue-engineering research has been conducted for every important tissue and organ of the body. Hence, optimization of tissue-engineering techniques, including cell harvesting, culture, expansion, as well as polymer material design are prerequisites for success prior to clinical exploitation, as a result of which numerous advancements in regeneration of trachea^{269, 287, 334, 335} and bone³³⁶⁻³⁴¹ have been established. There is however still the need to fabricate organ specific materials and ideal tissue substitutes that can support the regeneration of specific biological tissues. These applications may also include targeted utilization of the resources e.g. biomaterials, cells, tissue, growth factors aimed at either engineering a specific tissue or re-growth of a damaged tissue/organ. Furthermore, development of a physiologically appropriate bioreactors is also essential for tissue regeneration, specifically when tissue engineering is carried out for a complex organ by fabricating tissue engineered constructs and trialled prior to implantation in humans.

Neovascularization is highly desirable process for almost all of tissue engineered products to survive³⁴². The blood vessels supply food and oxygen when scaffolds have been applied to keep them alive. However, to date, the main focus has been on tailoring biocompatibility, mechanical properties or other related characteristics and limited efforts have been carried out to build their angiogenic properties. There is an immediate need for the development of angiogenic biodegradable materials for tissue engineering. Currently a number of strategies including use of growth factors^{343, 344}, stem cells³⁴⁵ and biomolecules (e.g. heparin³⁴⁶) are being investigated to find their role in future tissue engineered commercial products. In addition, there has been significant interest to develop smart functional materials exhibiting conductive, magnetic and optical

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properties. In this regard, the macro- and nanotechnologies have been found to be effective fabrication tools for the manufacturing of such materials. No doubt the stimulus-responsive materials having ability to tailor their properties to specific requirements are the most desired biomaterials for tissue engineering community. For this purpose biocompatible conductive polymeric materials are considered to be the materials of interest and such materials are already being used in fuel cells, electronic devices including capacitors and energy storage devices and these appears to be promising materials for tissue engineering applications as well.

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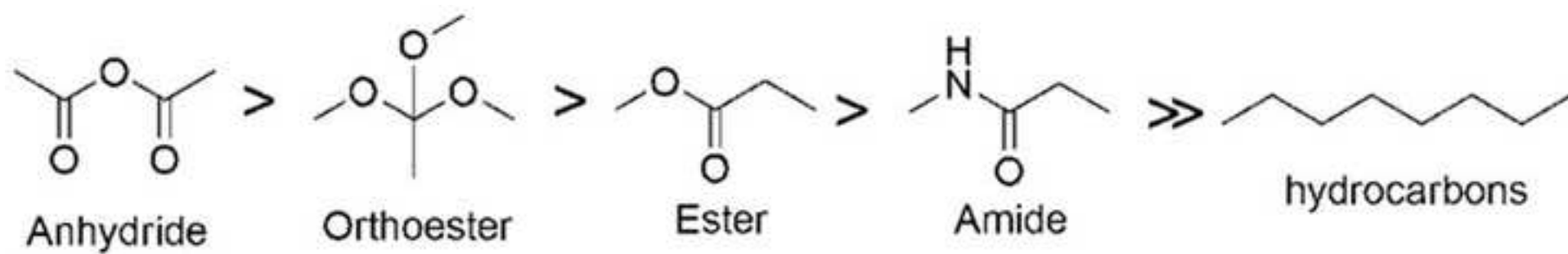
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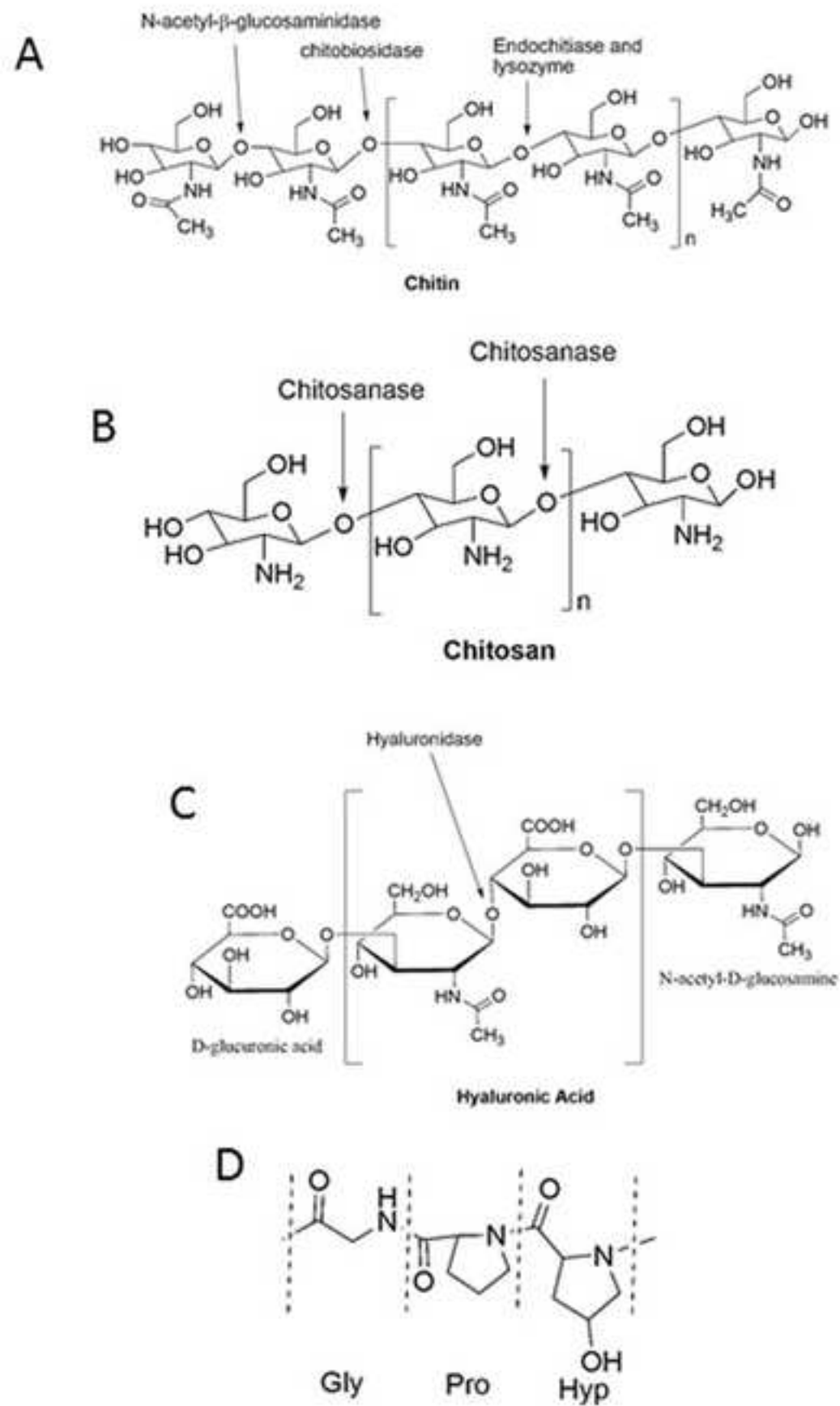
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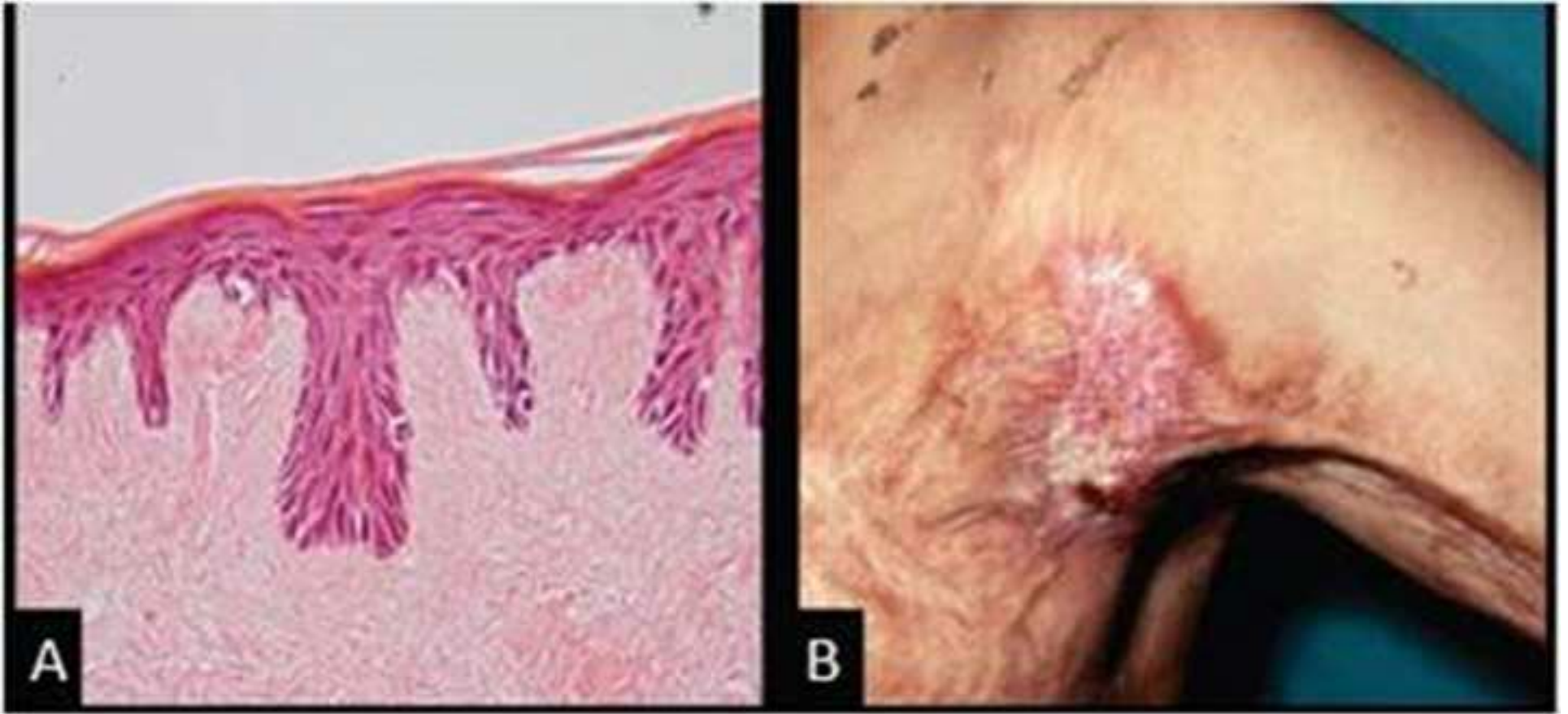
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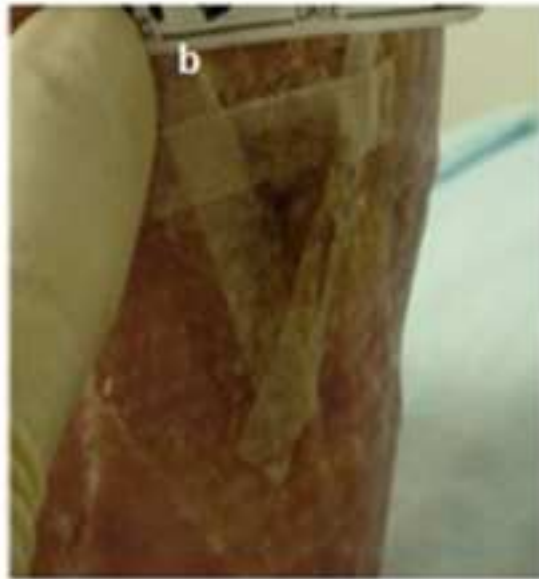
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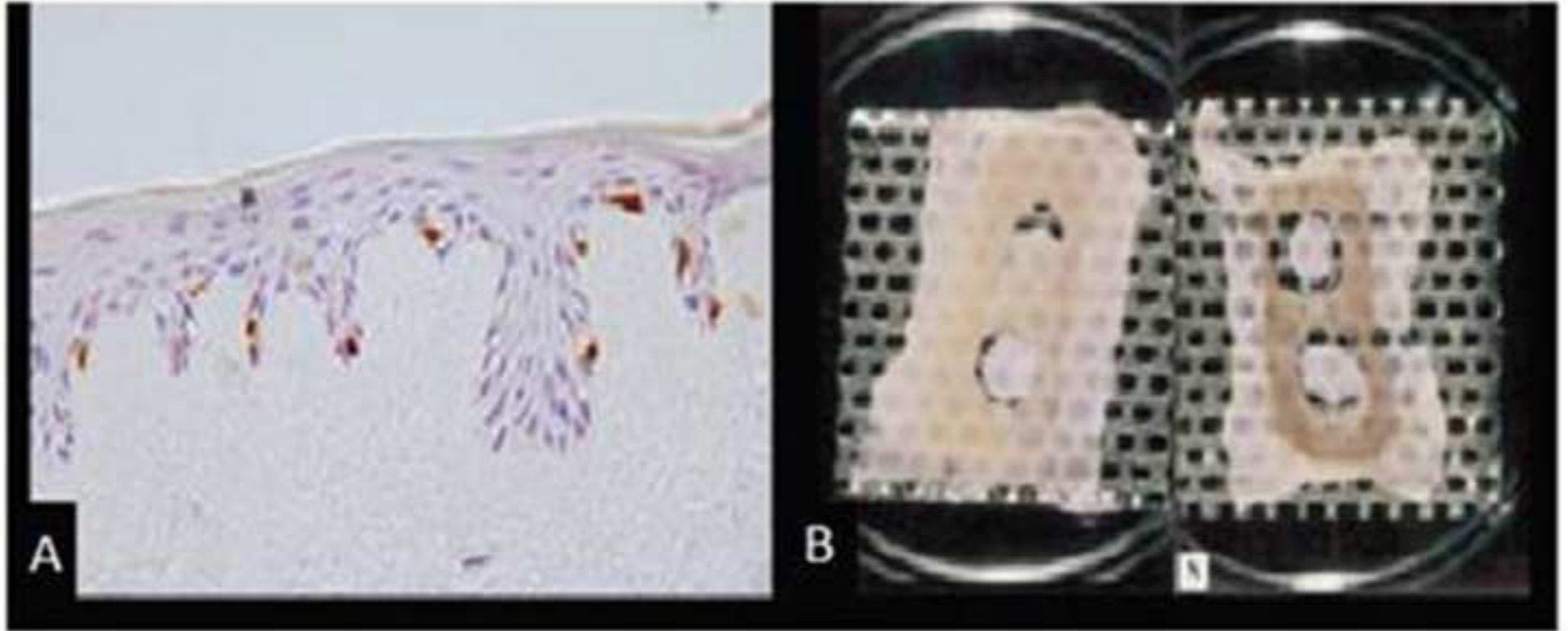
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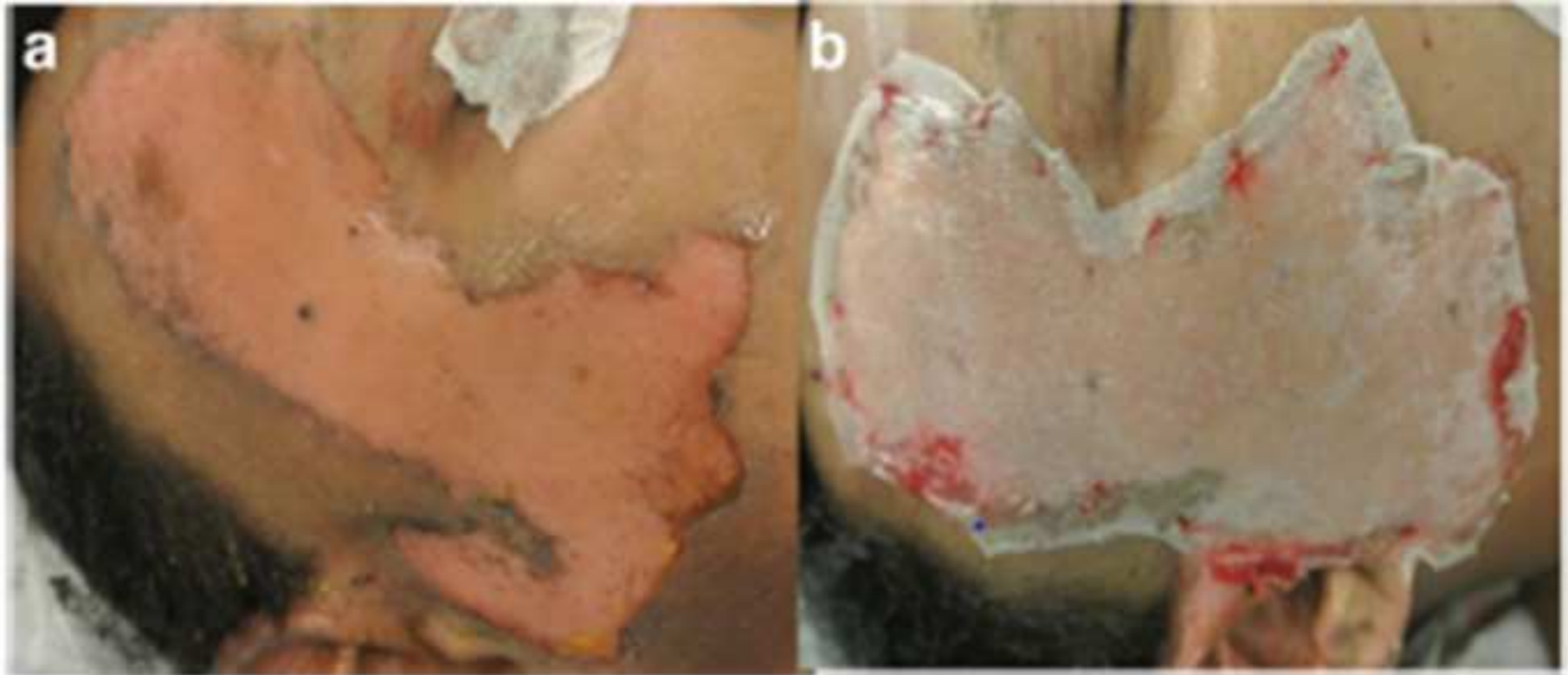




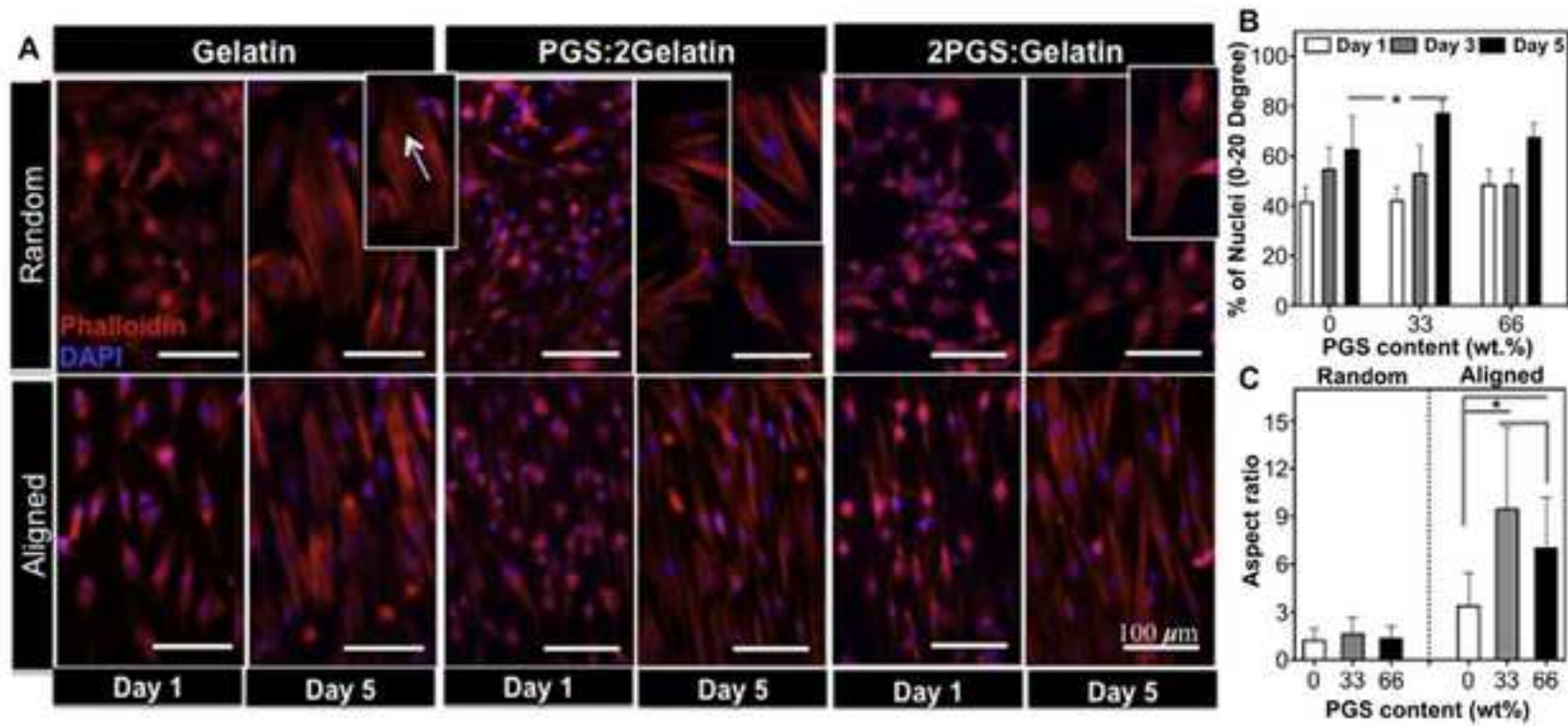


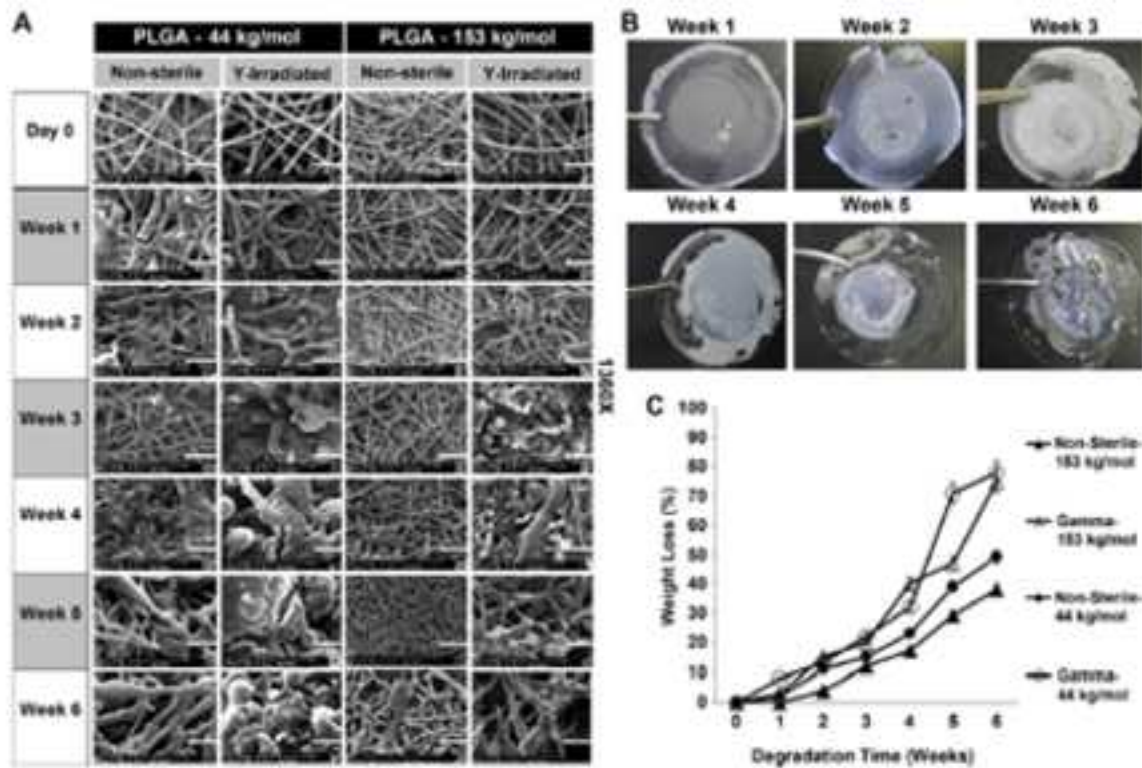


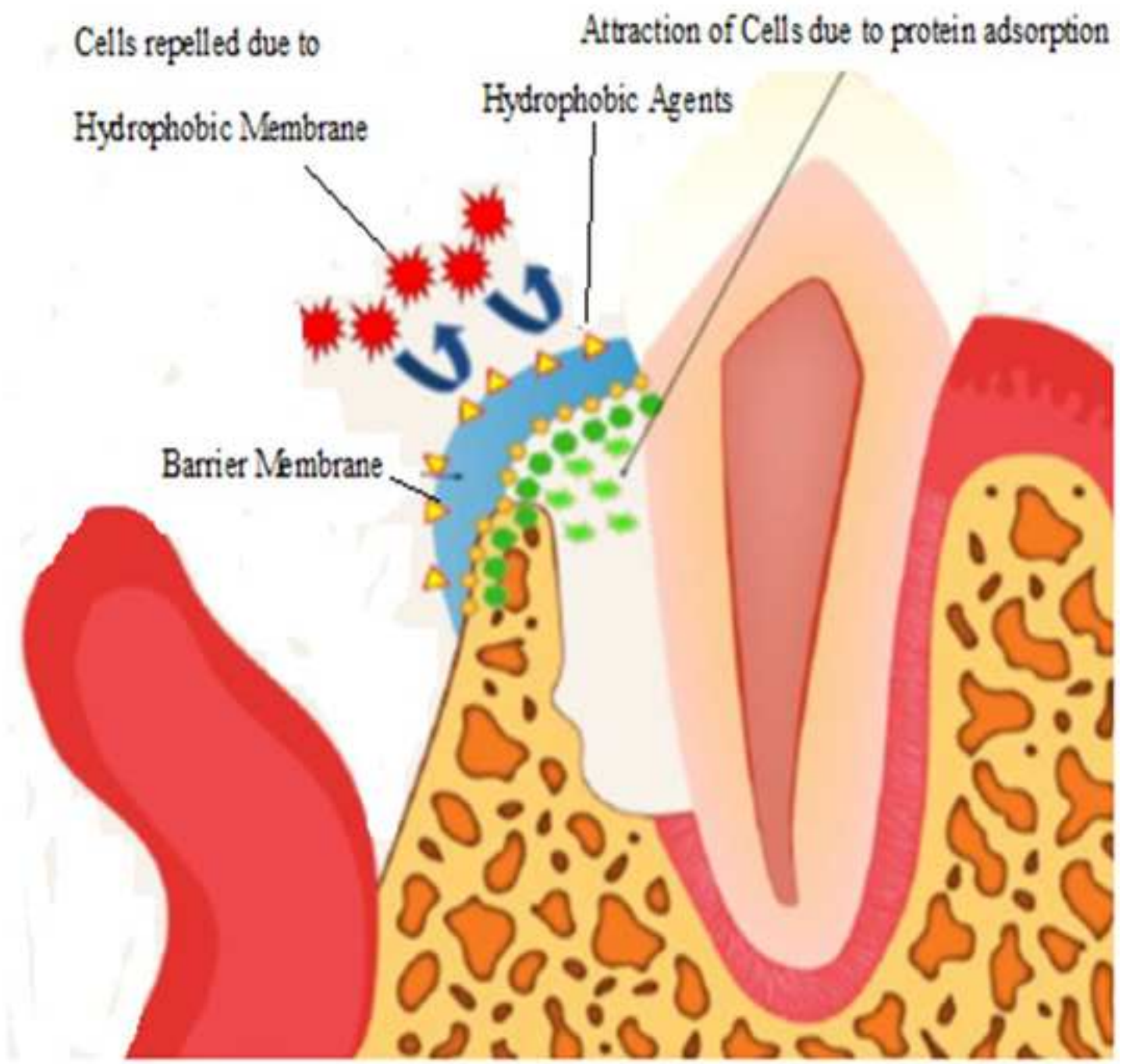


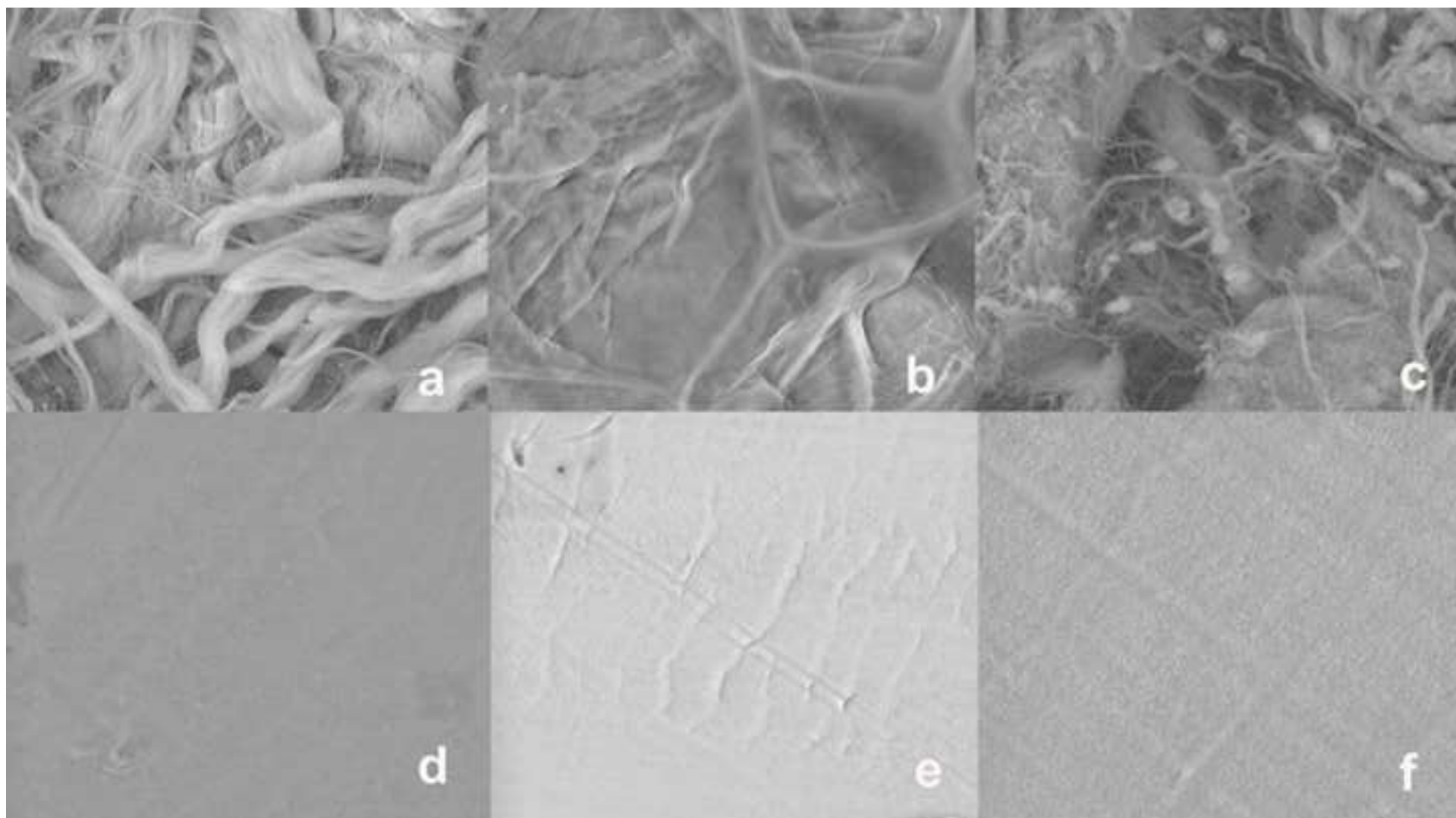


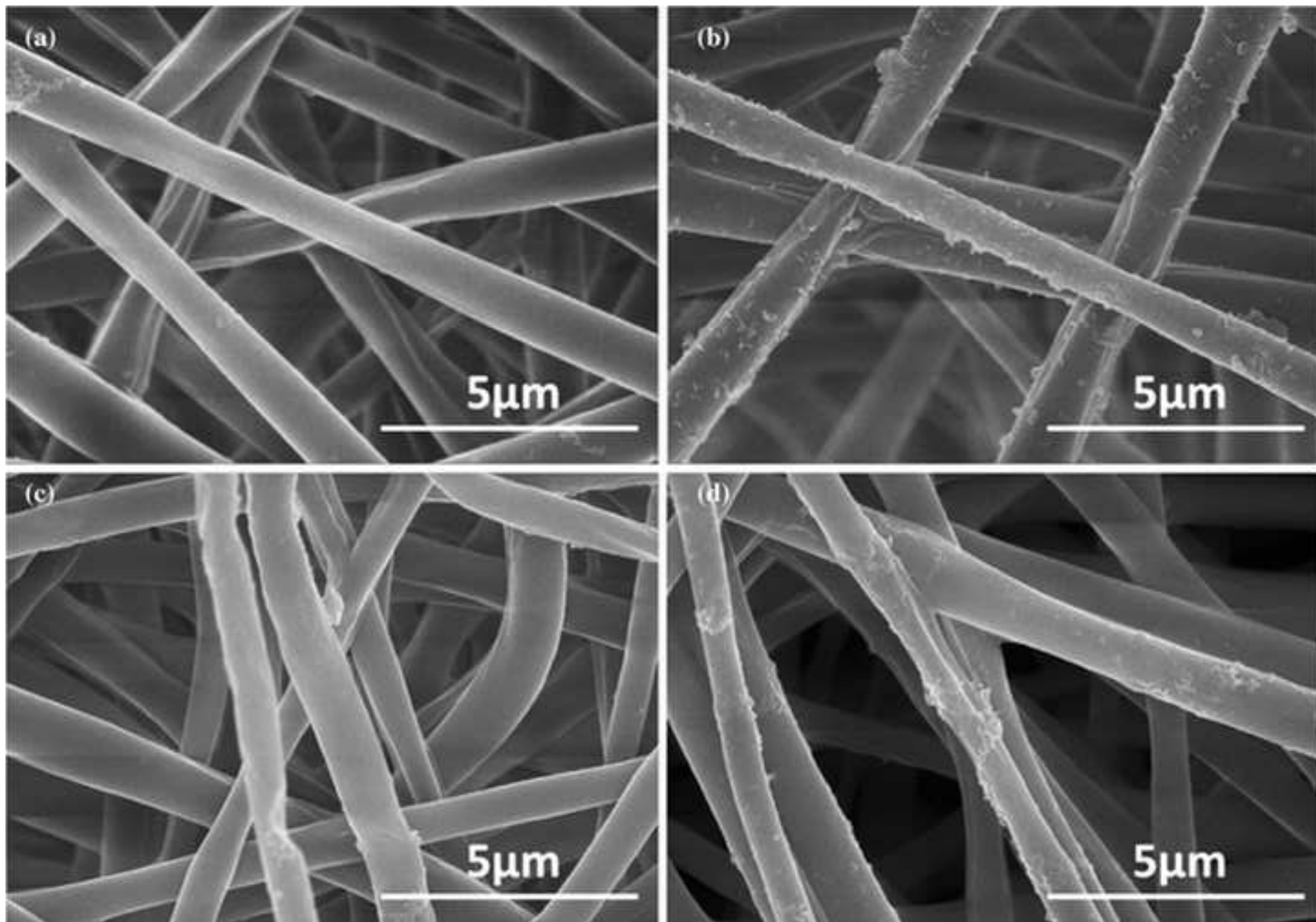


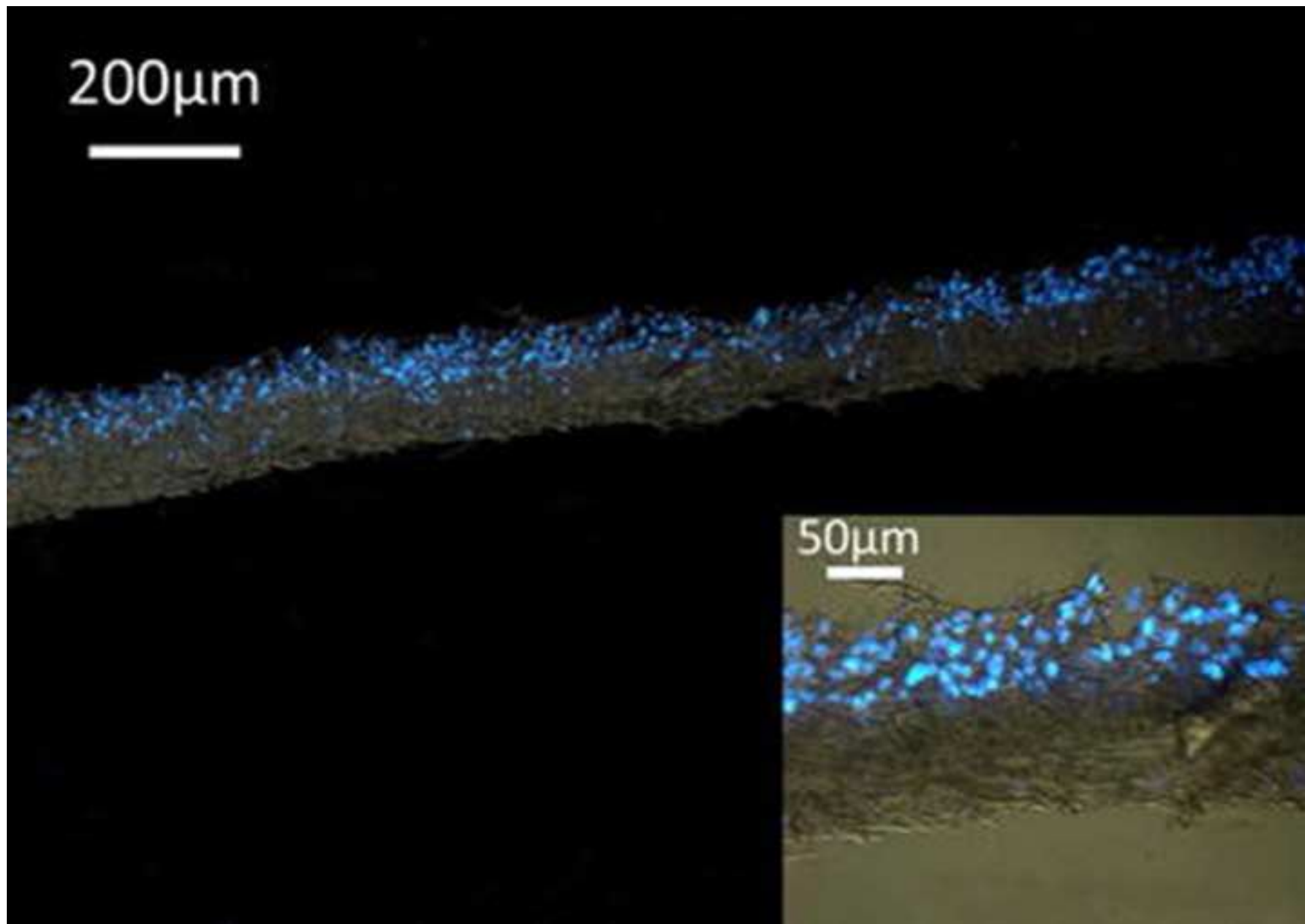


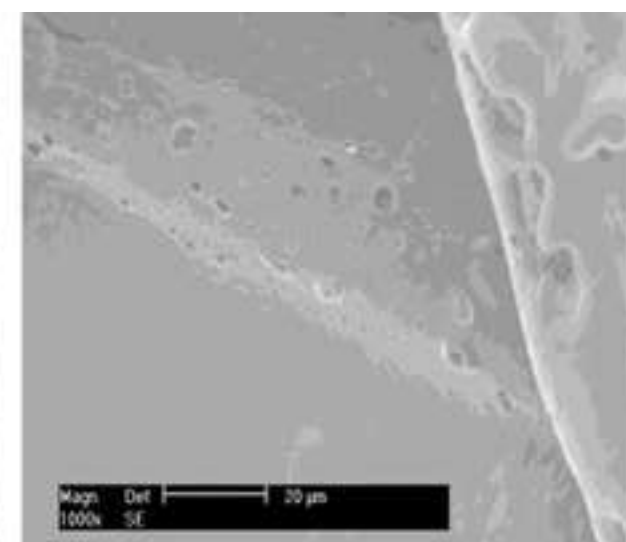
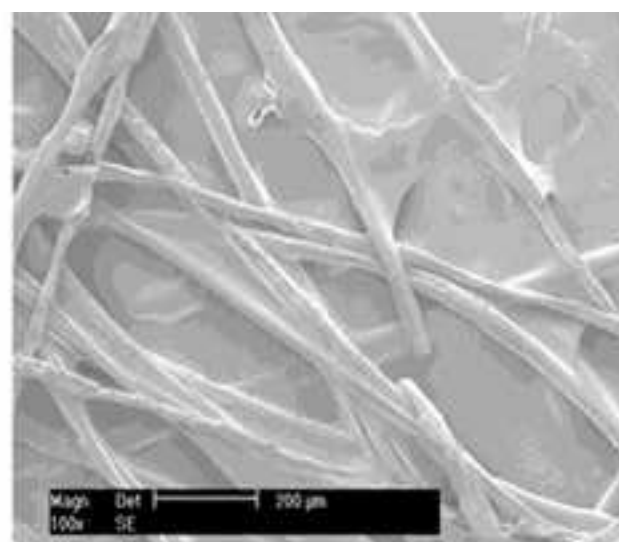
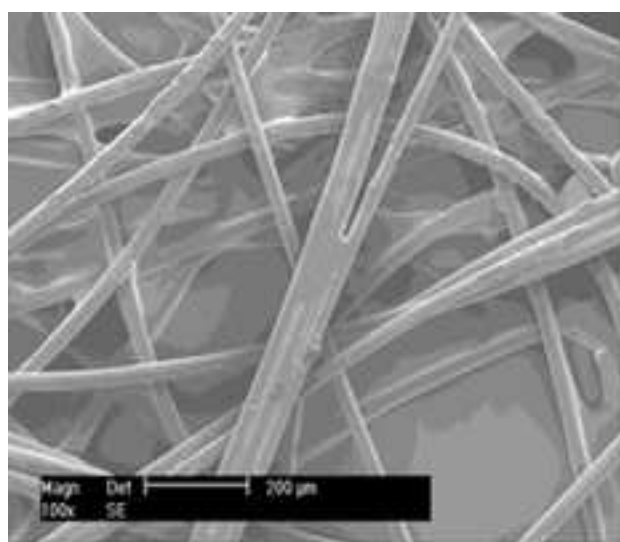








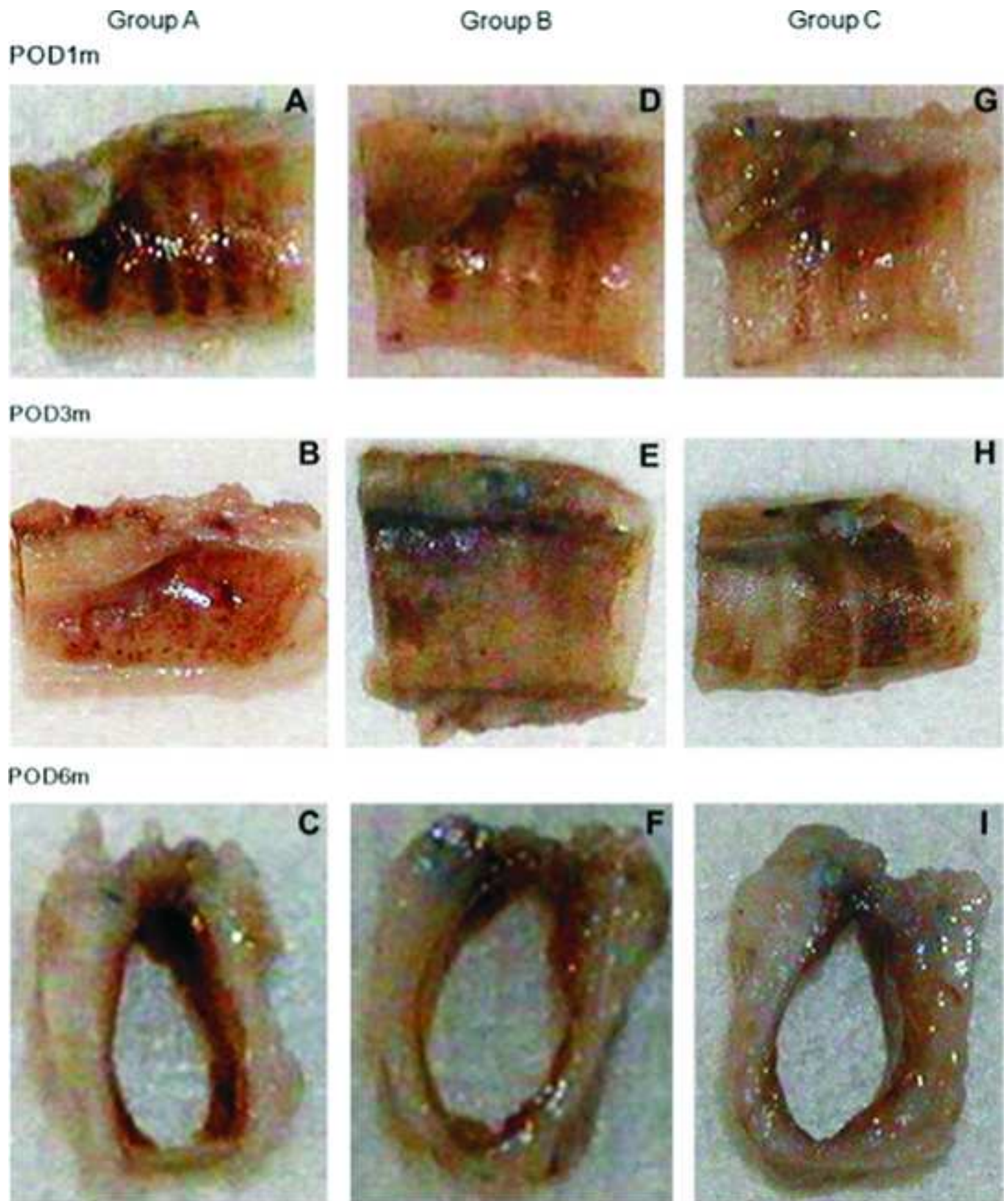


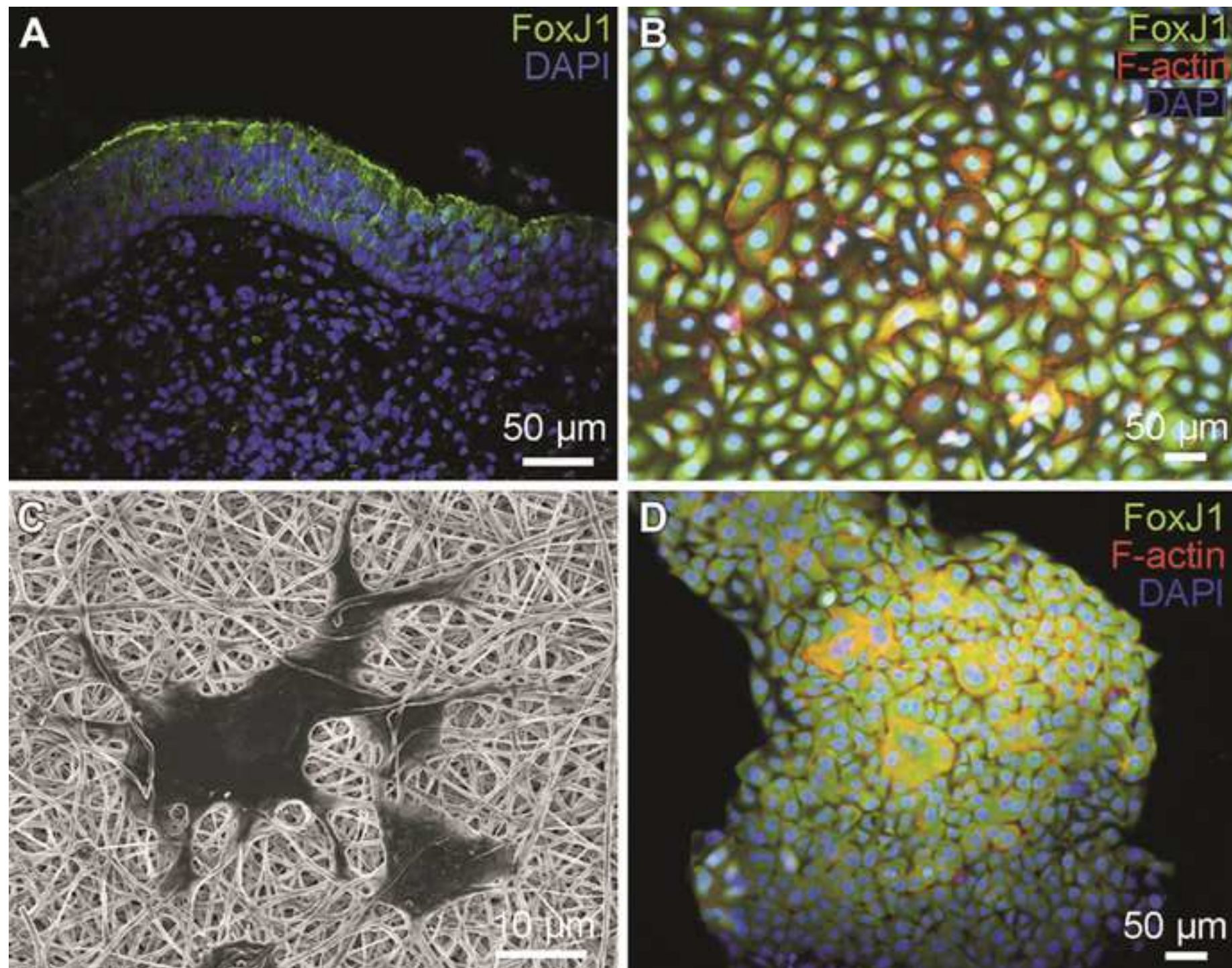


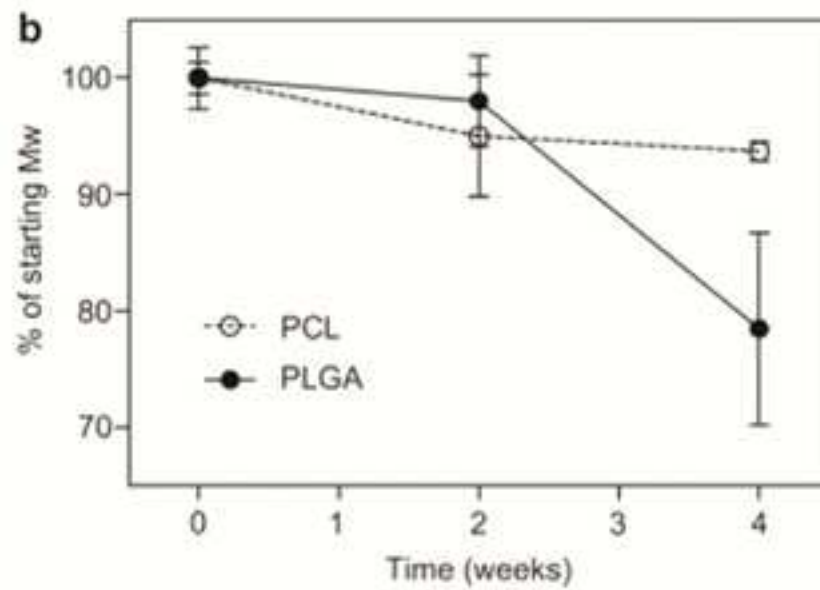
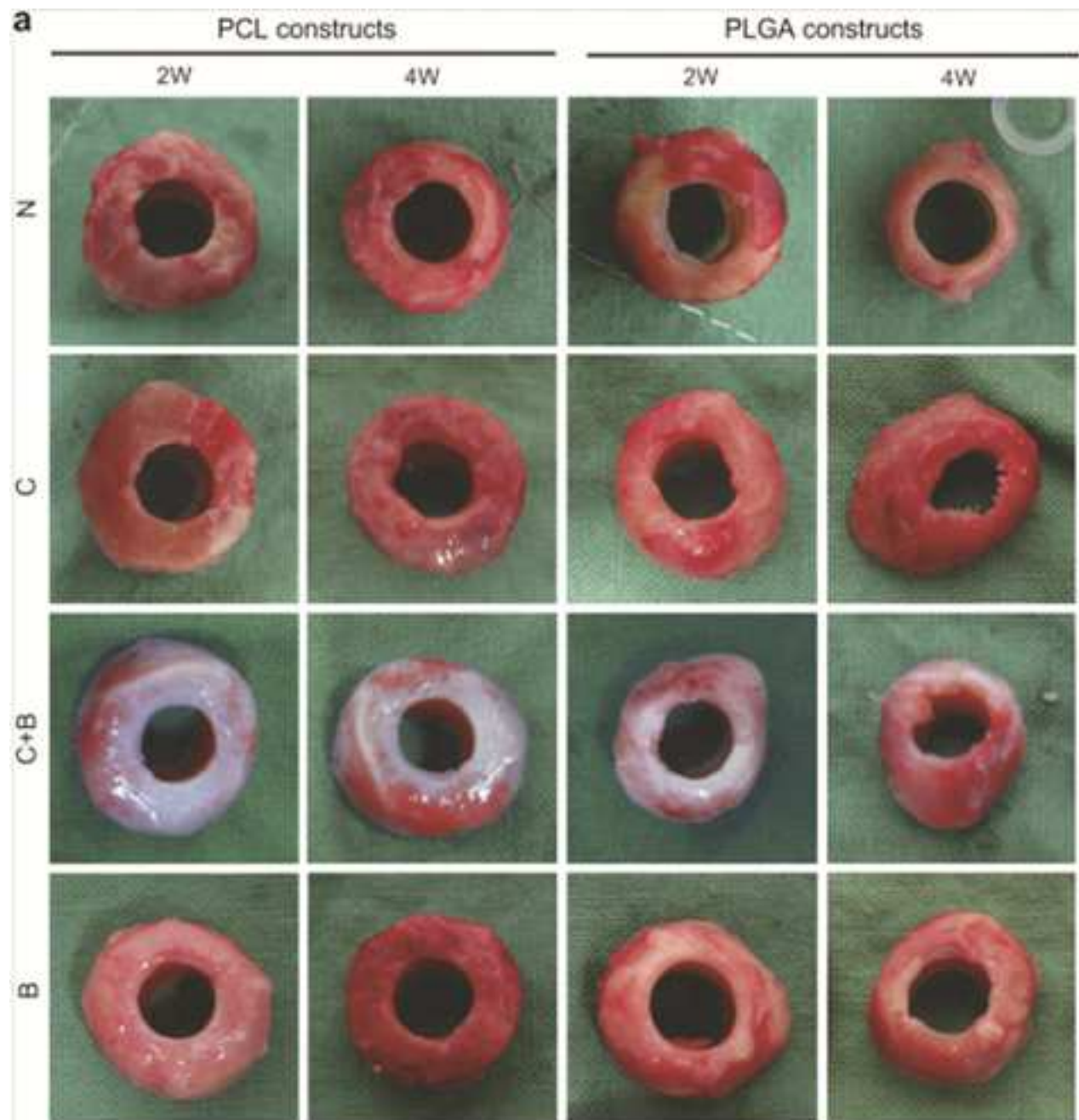
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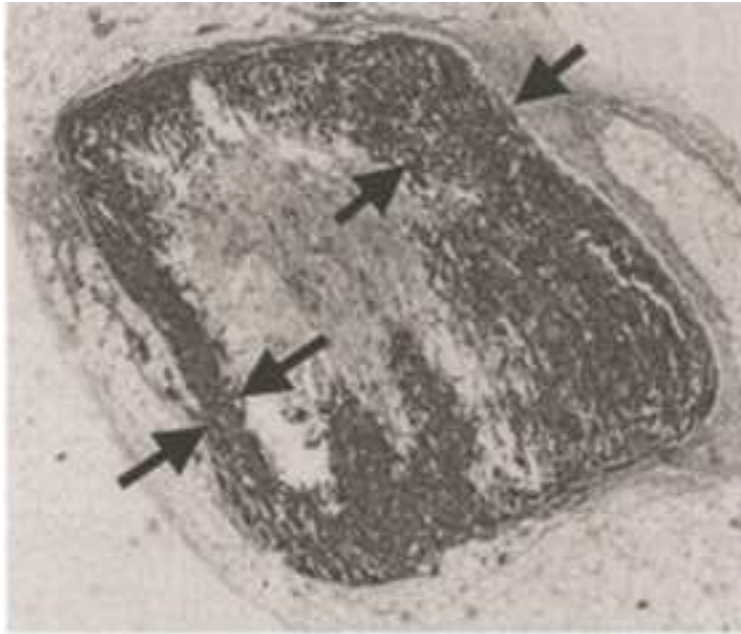
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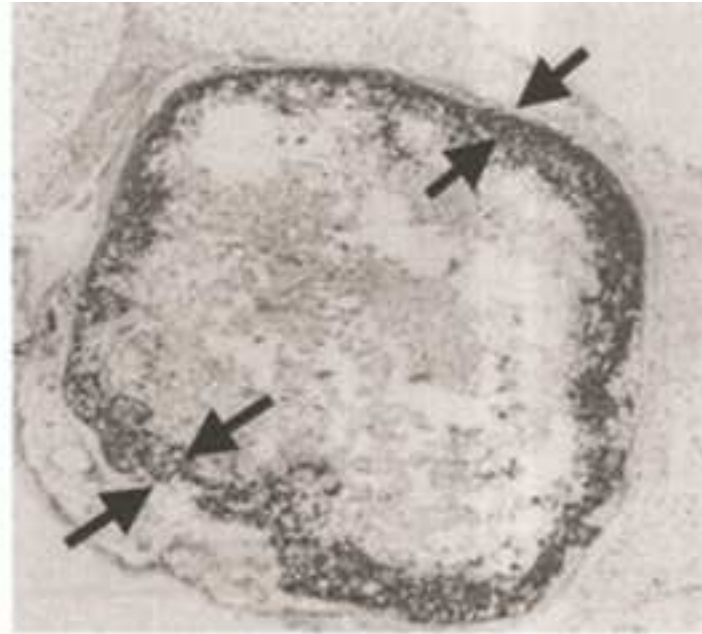




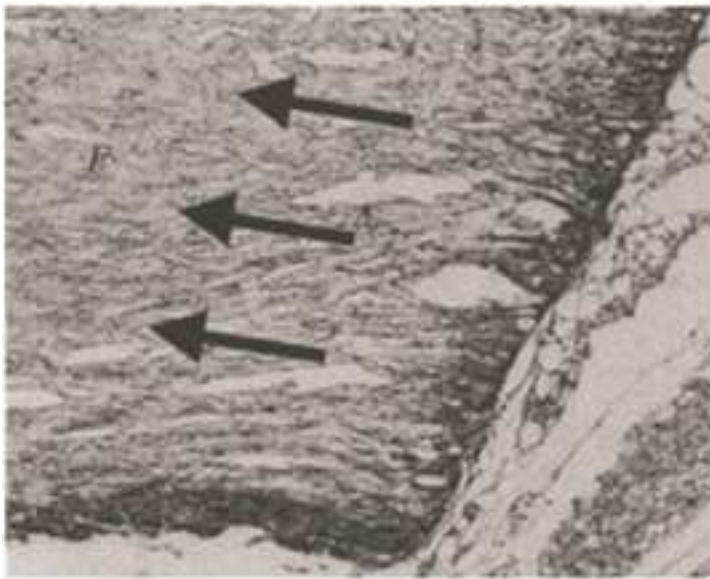




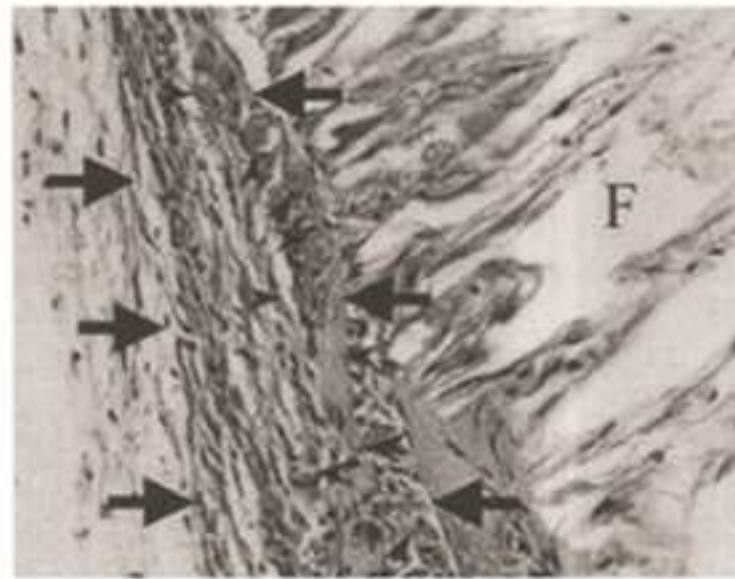
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List of Figure captions

- 1
2
3
4 1. Figure 1: Rate order of hydrolytic degradation of chemical functional groups present in
5 commonly used synthetic polymers.
6
- 7
8 2. Figure 2 (A) Enzymatic degradation patterns of chitin; (B) Endo-hydrolysis by
9 chitosanase in chitosan; (C) Mammalian hyaluronidases (EC# 3.2.1.35) catalyzed
10 degradation of hyaluronic acid; (D) The amino acids glycine, proline and
11 hydroxylproline base triple helix region which cleaves as a result of collagenases action.
12
- 13
14 3. Figure 3. Burn victim's wounds healing by using tissue engineered skin. (A) Wound
15 healed by using patient's own keratinocytes and fibroblasts grown in the laboratory;
16 (B) wound after two months of surgery. (Ref ⁹⁰= Reused with permissions, Copyrights
17 @Springer Nature)
18
- 19
20 4. Figure 4 :(a) Appearance of wound prior to application of apligraf; (b) application of
21 apligraf; (c) appearance of wound after application. (Reprinted as an open access for
22 unrestricted noncommercial use ⁹⁶.
23
- 24
25 5. Figure 5. Tissue engineered skin in :(A) melanocytes can be seen in dermis; (B)
26 showing the effect of combining fibroblasts and melanocytes on pigmentation. (Ref ⁹⁰=
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30 6. Figure 6. Application of Biobrane. a). Before application b). After
31 application(Reprinted as an open access for unrestricted non commercial use¹⁰⁸.
32
- 33
34 7. Figure 7:ASC-based cell therapy for a case of acute skin necrosis in the nose dorsum
35 due to inadvertent arterial injection of hyaluronic acid (HA) filler: (A) Before stem cell
36 therapy (seven days after HA filler injection); (B) Three weeks after stem cell
37 therapy.(Reprinted as an open access for unrestricted non commercial use)¹²⁶
38
- 39
40 8. Figure 8: Effects of scaffold composition and architecture on the morphology,
41 alignment and elongation of CFs. (A) F-actin filaments and nuclei stained CFs with
42 phalloidin and DAPI on the random and aligned scaffolds. Main panels indicate the
43 orientation and morphology of cells. Insets: higher magnification images showing
44 elongated stress fibers within the cells cytoplasm increasing with lower PGS content.
45 Quantified (B) alignment and (C) elongation of CFs on each scaffold. Cell alignment
46 was significantly higher on A(PGS:2Gelatin) scaffold compared to A(Gelatin)scaffold
47 (*: $P < 0.05$). (Adapted from ¹⁸⁶ Elsevier).
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9. Figure 9. Breakdown of PLGA membranes over 6 weeks. In these experiments membranes were placed in culture media and their morphology and mass examined over 6 weeks. A shows SEM of the fibres of non-sterilised and g-irradiated for 44 kg/mol and 153 kg/mol of PLGA over 6 weeks. B shows the visual appearance of g-irradiated PLGA (molecular weight 44 kg/mol) over 6 weeks when cultured with rLEC cells. Membranes plus cells are shown on glass coverslips. C shows percentage weight loss of the membranes both nonsterile and g-irradiated comparing low (44 kg/mol) and high (153 kg/mol) molecular weight membranes over 6 weeks of culture with rLEC cells. (adapted from reference ²³⁰ *Elsevier*)
 10. Figure 10. Schematic structure of periodontal membrane (Redraw from Sheikh et al).²⁵²
 11. Figure 11. SEM images showing the cell adhesion on degradable (a-c) and on non-degradable (d-f) polymeric network and images confirmed that more cell adhesion was found on degradable polymers (Courtesy from Kasaj et al).²⁵⁶
 12. Figure 12. The images showing PLLA-Chitosan modified electrospun fibers (a) before immersion, (b) after 2 weeks immersion in PBS, (c) 4 weeks and (d) 6 weeks (Courtesy from Chen et al).²⁵⁷
 13. Figure 13: Presence of fibroblast on top surface of PLLA-chitosan electro spun membrane after 5 days culturing (courtesy from Chen et al). ²⁵⁷
 14. Figure 14. SEM images of collagen membrane (a) surface analysis, (b) after 30 days incubation in DMEM at 100x, and (c) at 1000x (Courtesy from Milella et al).²⁶¹
 15. Figure 15: Implant samples in formalin at 1, 3, and 6 months postoperatively in the Cop alone group, the Cop-incorporating gelatine hydrogel group, and the Cop-incorporating gelatine hydrogel containing b-FGF group. (A), (D), (G): postoperative 1 month, (B) (E) (H): postoperative 3 months, (C) (F) (I): postoperative 6 months. After implantation of Cop alone (group A), the implanted copolymer became thicker than the original copolymer at one and three months after defect repair, but the thickness of the artificial implant was comparable to original copolymer as a result of degradation at 6 months postoperatively (A) (B) (C). After implantation of Cop-incorporating gelatine hydrogel (group B), the implanted copolymer became thicker than the original copolymer 1 month postoperatively, but the thickness of the artificial implant was almost equal to that of the original copolymer through degradation at 6 months postoperatively (D) (E) (F). After implantation of Cop-incorporating gelatine hydrogel containing b-FGF (group C), the change of wall thickness after implantation with b-FGF was similar to

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that without b-FGF (G) (H) (I). (Ref ²⁸⁶= Reused with permissions, Copyrights @Elsevier)

16. Figure 16 (A) FOXJ1 (green) expressed in the native trachea (B) and isolated hPAECs. Cell nuclei are visualized with DAPI (blue). F-actin labels the cell cytoskeleton (red). (C) SEM image of hPAECs appended to PCL/G/DCN scaffold. (D) Triple-immunofluorescence staining of hPAECs that was cultured for 7 days on the PCL/G/DCN matrix (FOXJ1 (green); F-actin (red); DAPI (blue)). (Ref=²⁸⁷ Reused with permissions, Copyright @ Elsevier)

17. Figure 17: (a) The tissue-engineered PCL and PLGA constructs in the shape of a ring were harvested at two (2W) and four weeks (4W) of implantation (N, no cells; C, chondrocytes; B, bone marrow stem cells) (b) The degradation of the polymer scaffolds (n = 3) in vivo was measured by gel permeation chromatography. The degradation behavior was recorded as a percent of the starting molecular weight (Mw). The error bars represent the mean standard deviation (SD). (Ref ²⁶⁹ = Reused with permissions copyright@ Elsevier).

18. Figure 18: Histology of the resected foam implants demonstrates that even after 6 weeks of implantation, the foams have failed to become completely infiltrated by fibrovascular tissue from the surrounding tissue. However, the TIPS foams (a) revealed greater tissue infiltration (between arrows) compared with compression-molded foams (b), but this was dependent on the pore orientation of the foam (F) at the site of implantation (c), with the greatest extent of infiltration occurring along the axis of the pores (arrows). (d) Quantitative assessment of the implanted foams included measuring the area of granulation tissue (between arrows) surrounding the foam (F) and counting the number of blood vessels in the granulation tissue (arrow heads). [(a) TIPS foam + 0% Bioglass® after 6 weeks of implantation (original magnification ×20); (b) compression-molded foam + 0% Bioglass® after 6 weeks of implantation (original magnification ×20); (c) TIPS foam + 0% Bioglass® after 1 week of implantation (original magnification ×100); (d) TIPS foam + 0.1% Bioglass® after 2 weeks of implantation (original magnification ×200).] (Ref ³¹² = Reused with permissions, Copyright © 2005 Wiley Periodicals, Inc).