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

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

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

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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

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

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Abstract

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 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.

Key words: Biodegradable Polymers; Skin; Heart; Vascular Arteries; Dental Regeneration; Bone, Cornea

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 implantation[1-3]. Tissue engineering and regenerative medicine exploits properties taken from the life sciences, engineering and physical sciences[4-10], and is therefore not only a highly interdisciplinary field of research, but in practice comprised of many novel and speciality areas[11]. 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 system[7]. 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 organ[14-16]. Regenerated tissues must reinstate, maintain and augment function thereafter. Numerous biomaterials have been used as alternative treatments for damaged tissues or dysfunctional organs,

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

A number of studies have been conducted in the last few decades for developing new and improved bio-mimetic materials for a wide range of biomedical applications[23-26]. Developing physiologically functional artificial tissues and organs is a pre-requisite of tissue engineering, and technological developments in tissue engineering continually underpin progress. One of the earliest approaches, that of cell-seeded scaffolds has led to the current advances, which include new materials and methods of fabrication resulting into well-engineered biocompatible systems[15]. In addition, nano-structuring of biomaterial scaffolds from nanoparticles, nanocomposites and organic-inorganic hybrid polymer materials have showed progress in organ regeneration. Various synthetic and natural polymers and their composite materials have been used to fabricate scaffolds for bone tissue engineering, nerve regeneration, controlled drug release, tooth structure regeneration, guided tissue regeneration, reinforcement of dental composite, bone and cartilage regeneration[27]. Moreover, micro fabrication technologies, such as lithography[28], bio printing[29, 30], micro moulding[31] or photolithography[32] are now becoming more routine and are emerging as powerful tools for the manufacture of biomaterials and tissue engineered constructs. Use of these micro and nanotechnologies not only replicates cell-scale complexities by providing the cells with a microenvironment that mimics the native structure, but also allows obtaining 3D architectures[15, 33-36]. The advances in biomaterials science is also complimented with progresses in cell and molecular biology, in particular induced pluripotent stem cells which makes tissue engineering a highly multi-disciplinary discipline. The challenge however is whether this promise will fulfil the long awaited desire of having readily available methods and facile approaches for regenerating tissues and organs[14].

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 though 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 manner[39]. 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-mediated[41]. Degradation mechanisms include hydrolytic, enzymatic and biodegradation[24]. 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 factors[43]. Sometimes the definition of biodegradation is not accurately described[44], 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

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

2.2- Biodegradable Polymers

Polymeric materials have been used in clinical applications for a number of years[45,46,47], however the clinical and biological requirements vary according to the nature of the application. Numerous techniques have been used to modify and fabricate different compositions to achieve exact requirements for clinical use[48], typically based on control of molecular weight, polydispersity, crystallinity, thermal transition and degradation rate. All of these factors can strongly affect the polymer scaffold properties[49]. There are three general types of biodegradable polymers: synthetic, natural and hybrid materials, which have been gaining recent attention due to their superior characteristics in regenerative therapies. These materials can be produced with high structural precision employing assembly strategies to control properties such as stiffness, degradation and porosity[50]. A wide range of natural and synthetically derived polymers are capable of undergoing degradation, however synthetic biodegradable polymers have found more versatile and diverse biomedical applications, arguably due to a more facile ability to undertake tailorable designs and chemical modifications[51].

The most commonly used synthetic polymers for tissue engineering and drug delivery are aliphatic polymers, and include poly (lactic acid) (PLA) and poly (glycolic acid) (PGA), poly (lactic-co-glycolide) (PLGA), poly (ε-caprolactone) (PCL), poly (p-dioxanone), plus copolymer soft trimethylene carbonate and glycolide. These materials are approved by the U.S. Food and Drug Administration (FDA) for human clinical applications[52]. PLA exists in three forms - D-PLA PDLA, L-PLA (PLLA). Blends of D-PLA and L-PLA (PDLLA), PLA, PGA and PLGA [53-55]have been used clinically to treat patients suffering from damaged or lost organs or tissues and for drug delivery systems [56-59]. These polymers have been demonstrated as being biocompatible and degrading into non-toxic products, with a controllable degradation rate when implanted *in vivo*. Other biodegradable synthetic polymers include poly anhydrides, polyphosphazenes, polyurethanes, poly(glycerol sebacate), synthetic hydrogels and functional synthetic polymers,

with a range of other tissue engineering applications including restorable sutures[60, 61], drug delivery systems[62, 63], artificial skin [64-66], wound healing[62, 67, 68] and orthopaedic implants[69].

These synthetic biodegradable polymer materials can be synthesized by controlling their fundamental building block units to give various properties such as uniformity, which are free from immunogenicity. High molecular weight aliphatic polyesters are mostly synthesized by condensation or ring-opening polymerization. The basic and generic structure of all aliphatic polyesters is very similar and the only difference is the pendant groups, where a change contributes to differences in molecular weight and crystallinity. This directly affects the kinetics of degradation[49]. These synthetic polymers contain chemical bonds in their backbone that undergo breakdown in the presence of water. Polymers having functional groups of esters, ortho-esters, anhydrides, amides, urethane, lactones, and lactams are categorized as polymers that degrade through hydrolysis. However, it is important to clarify that *in vivo*, degradation resulting solely from hydrolysis through water present in tissues is not biodegradation, and should be referred to as hydrolysis or hydrolytic degradation[41].

In addition, synthetic hydrolytically degradable polymers possess number of advantages compared to natural polymers when used in biomedical applications, including tailored-made porosity, degradation time and mechanical characteristics[68]. They are often cheaper than biological scaffolds and can be produced in large quantities under controlled conditions, and have a long shelf life[70]. Synthetic polymers are generally preferred for medical applications, due to manufacturing reproducibility compared to natural polymers, which in contrast have little control over chemical structure. Furthermore, synthetic polymers do not tend to display problems when used as biomedical implants due to this minimal batch-to-batch variation, and are therefore more reproducible[37]. The degradation of synthetic polymers is very much dependent upon the chemical structure of their functional groups. Different polymers show a variable degree of degradation, as some are more water stable than others. Chemical structure therefore plays an important role in materials selection and design for tissue engineering scaffolds. Figure 1 shows the order of hydrolytic degradation of various chemical structures. Based on these observations one can design and select a specific polymer for a required biomedical application. Chemical reactivity depends upon the level of electrophilicity of e.g. a carbonyl moiety (C=O) and stability of the leaving group.

(Insert Figure 01)

Natural biopolymers include polysaccharides(e.g. starch, alginate, chitin/chitosan, hylauronic acid derivatives) or proteins (e.g. soy, collagen, fibrin gels, silk)[71]. They serve as intrinsic templates for cell attachment and growth because of their inherent biocompatibility. However, they also have an ability to stimulate an immune response. The molecular structure of natural polymers is highly organized containing extra cellular ligands that can bind to cell receptors. Although naturally derived polymers are biocompatible, there are some disadvantages including not being available in bulk quantities, being expensive, and difficulty in processing into a desired shape when used as a scaffold for tissue engineering. The degradation rate of both natural and synthetic polymers can vary from patient to patient, because the degradation of natural polymer materials is dependent upon enzyme activity, which is a variable within patients.

Generally, the majority of naturally occurring polymers are degraded under enzymatic conditions[37]. For example, chitin as enzymes are well known for the degradation of chitin (chitinases originating from fungi, bacteria, and plants etc.[72, 73].) Degradative chitinases are divided into two groups; endo- and exo-chitinases. Figure 2(A) shows the pattern of chitin degradation by various catalysts including endo- and exo-chitinases (interestingly, lysozyme is also known to break the β-1,4-linkage in the natural carbohydrate polymers[74, 75]). Chitosanase leads the β-1,4-linkage in the D-glucosamino moieties in the chitosan as shown in Figure 2(B). Hyaluronic acid undergoes catalytic degradation in the presence of mammalian hyaluronidase, assisting in the hydrolysis of 1,4-bonds between the D-glucuronic acid and N-acetyl-D-glucosamine, as shown in Figure 2(C).

Another very common naturally derived polymer is collagen, of which 28 different types have been described. Collagen is found in mammalian connective tissues; consisting of up to 30% of all proteins that are present in the human body that provides strength and flexibility to tissues. The most common, representing about 90%, is type I collagen. Collagen I is abundantly found in tissues, with higher levels found in tendon, skin, bone and fascia. It has been extensively researched for developing biomaterials in tissue engineering[76]. Because of its distinctive physical strength, porosity and biological properties i.e., phylogenetical studies showed a primary sequence and helical structure as well as mild immune-reactive recital[77-79]. Collagens undergo degradation in the presence of collagenases and metalloproteases. Collagenases belong to the family of endopeptidases, and metalloproteases are proteases that require a metallic catalyst for their

activity. These enzymes attack the collagen in the triple helix region, which is composed of polypeptide strands bearing tri-amino acid blocks of glycine-proline-hydroxyproline, and responsible for the repeating helical structure[80] [Figure 2(D)].

(Insert Figure 02)

Hybrid or composite materials behave in such a way that better properties are achieved for scaffold function and have been commonly used for clinical applications[81-83]. Polymeric material blends have been fabricated by the combination of synthetic and natural, natural plus natural and synthetic plus synthetic polymers, to improve the mechanical properties, to improve processability, to lower production costs, or to improve cell compatibility[84]. Bioactive phases increase hydrophilicity and water absorption of the polymer matrix, which can change the degradation behaviour of the polymers by allowing rapid exchange of protons in water from ceramics[85]. Biodegradable hybrid materials may provide a number of benefits, e.g., an enhanced environment for cell seeding, survival, growth, and differentiation due to the osteoconductive function imparted by bioceramics (which increases mechanical properties essential for load bearing applications[86]). The composition, structural and functional versatility of hybrid materials accounts for a range of tuneable physicochemical properties, which are highly suitable for designing organ specific tissue engineering constructs.

3. Organ Specific Regeneration using Biodegradable Materials

3.1 Skin

Skin is the most exposed and largest body organ with an approximate surface area of 1.5-2.0 m^2 in the adult human body and 12-15% by weight. It is a multifaceted organ that is frequently subject to burn and wound injuries. Skin anatomy reveals a three-layered structure: the stratified epithelium or *epidermis*, separated from an underlying tissue stroma or *dermis* and a well-characterized cellar layer of subcutaneous tissue or hypodermis [87]. The functions of skin are to maintain the integumentary system (that includes but is not limited to) protection against any external physical, chemical and biological insults[88], preventing excess water loss from the body and thermoregulation. Skin damage can have a number of causes e.g., traumatic injury, burns,

surgery, non-healing ulcers and chemical injury. These can cause extensive skin loss and require instant treatment to restore structure and function. For several decades, scientists and clinicians have been designing and fabricating new tissue engineering scaffolds to develop artificial skin or wound healing strategies[89].

A noticeable breakthrough is in the development of artificial skin, which is being used in burn patients[90-93]. The plus point of such a development is that artificially grown skin can be stored in tissue banks and be used when required. The major drawback with existing burns treatments using skin grafting is that patients need to wait for a number of weeks while the skin is grown autonomously. The donor site for this is also a new wound and thus a potential site for infection or scarring. In addition, the donor site is limited and so a major limitation in patients with extensive burns. Figure 3 illustrates an example of a patient treated using tissue engineered skin.

(Insert Figure 03)

In 1997, the first tissue engineered skin product TransCyte (Shirepid, California, USA) was launched. This was a non viable productthat was comprised of silicone, a nylon membrane and collagen containing neonatal fibroblasts grown for 17 days to produce a matrix. It was followed by Apligraf® (Organogenesis, Canton,USA) in 1998. Apligraft was used in the chronic woundshealing when such wounds were previously failed in healing by using other methods of treatment. Fifty-six percent of patients treated using Apligraft had full wound healing in comparison with 37% of patients who were treated using standard wound care protocols. Figure 4 shows the appearance of a wound before and after application of Apligraft[94].

(Insert Figure 04)

Dermagraft® (Advanced BioHealing, Westport, Conn) is a synthetic product which either uses polygalactic or polyglycolic acid meshes combined with neonatal fibroblast to enhance wound healing as temporary skin substitutes. It was followed by the development of OrCel® (Ortec International US Inc., New York, USA) in 2001.

According to a research report compiled by ECRI Institute/Evidence-based Practice Centre (EPC) under contract to the Agency for Healthcare Research and Quality (AHRQ, USA)[95] its use is only for chronic wounds such as diabetic foot ulcers, pressure ulcers, and vascular ulcers (including venous ulcers and arterial ulcers)[96]. A wealth of literature reviews elsewhere[97-98] document a range of skin substitutes and techniques investigated for *in vitro* testing and employed as model skin[88, 95, 97-104].

Extra Cellular Matrix (ECM) plays a vital role in tissue engineering. It supports the growth of proliferating cells (in particular, fibroblasts) and serves as a scaffold post injury, and hence is a major component in the process of tissue regeneration. Fibroblasts produce ECM constituents and through it communicate with each other. The ECM can signal to the fibroblasts and control cell phenotype, genetic expression, development, protein expression and the function of these cells. Such interactions are influenced by the microenvironment, which provides a niche for homeostatic modulation of ECM. When considering skin substitutes, development of biomedical materials should ideally aim at mimicking the ECM by incorporating appropriate factors, or pharmacological agents, at physiological quantities and durations[97]. Numerous biomaterials are employed as skin implants, ranging from naturally occurring collagen gels/sponges, alginates, polypeptides, glycol saminoglycans, hyaluronan and fibronectin to synthetic materials e.g., polyvinyl chloride, poly lacetate/glycolate fabrics (PLGA) etc. [88, 97, 105].Researchers are constantly trying to find an ideal skin graft[106]. Huss et al[107] reported on the development of biodegradable polyurethane-urea (PUUR) scaffold for dermis regeneration. After *in vitro* and *in vivo* assessments, the fibrous and porous forms of PUUR scaffold showed biocompatibility with human dermal fibroblasts. Thus, the cells could attach, proliferate and migrate around the biodegradable scaffolds. Porous scaffold discs of dimensions 4 mm diameter, 2 mm-thick) with a polymer solution (of 12% w/w or 9% w/w) were inserted intra-dermally into four volunteer healthy patients. Increased growth of fibroblasts was observed on all materials and after eight weeks, the scaffolds were fully occupied with fibroblasts. Production of procollagen was observed that signified the existence of functional and active cells. The fibroblasts stained immune histochemically for procollagen and von Willebrand factor, demonstrating neocollagenesis and angiogenesis contained within the scaffolds.

These biodegradable materials have shown potential applications in dermal regeneration [91, 107, 108]. Among various biodegradable polymers for skin regeneration, polyurethanes have attracted interest due to their tuneable mechanical properties, biocompatibility and structural adaptability [109]. In a number of other studies [110-112], polyurethane-based dressings are reported which are considered as superior to hydrogels. Furthermore, successful culturing of keratinocytes has also been reported on polyurethane membranes [113]. Nonetheless, biodegradable polyurethanes as dermal scaffolds have not been fully explored. Greenwood *et al.* [109] reported on a study of a dermal skin substitute for restoration of major skin loss caused by burn injury. The authors carried out *in vitro* studies on three derivatives of NovoSorb™ Polynovo Ltd. Australia), a class of biodegradable polyurethane used as a dermal scaffold. Results showed biocompatibility, nominal cytotoxicity on skin cells and facilitation of cell development i.e., growth of human keratinocytes, dermal fibroblasts and microvascular endothelial cells in co-culture. Furthermore, one of the skin substitutes (BTM-2, Biodegradable Temporising Matrix, PolyNovo Ltd. Australia) exhibited a desired degradation profile for a dermal scaffold and was developed into a 3-dimensional porous matrix for further studies. *In-vivo* studies [114] were carried out in both rats and sheep, with subcutaneous implantation of three NovoSorb™ derivatives which revealed no toxic effects. The authors demonstrated an inflammatory response and granulomatous reactions that were comparable to clinically used materials, e.g. sutures and Integra™ (Integra Life Sciences Corporation, NJ, USA) dermal substitutes.

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

Wang et al[115] reported an interesting study for skin restoration and wound healing. They introduced a novel collagen/hyaluronic acid (HA)/gelatine based sponge-like scaffold for human skin regeneration. The scaffold offered an optimal pore size with an average pore diameter of 132.5±8.4μm observed under SEM. The swelling ratio was examined by water absorption and

showed a value of over 20g water/g of dried scaffold. Enzymatic degradation was demonstrated by lysozyme, hyaluronidase and collagenase I assays in a time- and dose-dependent fashion and observed by measuring a reduction in weight. The scaffold degraded gradually to 38.1±2.6% and 36.4±5.1% of original weight after one week using 10,000 and 30,000 U/mL of lysozyme respectively. Similarly, when using 30 U/mL of hyaluronidase, the scaffold maintained about 10% weight after a 5-day examination. With 50 U/mL of hyaluronidase, the scaffold was degraded after 7 days. Furthermore, in 20 U/mL collagenase I, the scaffold degraded almost completely in approximately 3 hours. In contrast, 10 U/mL reported 45% of remaining scaffold in comparison to its starting weight. It was further investigated that with human skin cells growing for 7 days, SEM studies indicated surface degradation of the scaffolds. This was attributed to enzymatic digestion, signifying the biodegradable properties of the scaffolds. Human epidermal keratinocytes, melanocytes and dermal fibroblasts were cultured on the porous scaffold and immunofluorescence microscopy confirmed a normal human skin layer distribution i.e., the scaffold was able to mimic the human epidermis and dermis structures. Furthermore, the authors reported that the amount of collagen was quantified to 50% higher after skin cell seeding, as compared to cells seeded on culture wells. The *in vivo* histological outcomes showed that the scaffold wound healing was faster, with no further inflammation or side effects[115].

Recently, Lagus *et al*[116] in a clinical/histological study compared three different strategies to heal excised burn wounds by using Integra® (Integra LifeSciences Corporation, USA), Split Thickness Skin Graft (STSG) from a donor , and a viscose cellulose sponge Cellonex™ (Vivoxid Ltd, Finland), respectively. Integra®, is a biodegradable porous skin substitute consisting of bovine type I collagen and chondroitin-6-sulphate from shark's cartilage with a temporary epidermal substitute layer made of 0.1mm synthetic polysiloxane matrix. The silicone layer regulates the moisture content from the wound to a permeability value of 0.5 mL/cm^3, reported for the epidermis of human skin[117]. This layer also provides a protective barrier to the host body undergoing a thin split thickness skin graft substitution from infectious microorganisms. It facilitates the formation of a neodermis, autologous extracellular matrix (ECM) and wound bed for a thin STSG. In contrast, Cellonex™ viscose sponge, can be obtained from cellulose. It has shown granular tissue growth on wound beds and is known for its optimal pore-size, open cell-to-cell structures, homogeneity and purity. The sponge contains a viscose cellulose matrix as a main component, which is supported by cotton fibres[118]. The flexible architecture allows the free passage of cells into the

inner parts of the sponge[119]. However, *in vivo* cellulose sponge degradation is believed to be result of chemical, biological, and mechanical interactions[120]. These materials were tested and compared in ten adult patients[116] and results showed that STSGs performed well in muscle fascia, on vascularized Integra® and on wound surfaces possessing a cellulose sponge. Minimal inflammation was observed in Cellonex™ treated areas, in contrast to other materials. Most neutrophils, histiocytes, and lymphocytes were observed with significant differences on days 7 and 14. Entire vascularization of Integra® occurred later, as compared to the other materials (STSG showed most myofibroblasts on day 14). However, it was noted that fibroblasts and myofibroblast number may show a slow increase in Integra®, in contrast to wound beds treated with other materials. Furthermore, it was also revealed that both the maturation of scar tissue and the fibres of Integra® may persist for a year or longer, respectively. From the results, it was concluded that Integra® is a better skin substitute as compared to other materials, but that from the 12 month investigations of histological and immune histochemical outcomes, proposed that three strategies could be clinically adopted[116].

Hypopigmentation is the common problem while using tissue engineered skin grafts to treat burn wounds[121]. A study has been published to investigate the difference between the normal and vitiligo melanocytes in artificial skin grafts. It was observed that skin fibroblasts regulate the pigmentation in tissue engineered skin grafts. Figure 5 shows melanocytes in the epidermis in tissue engineered skin. Melanocyte function depends upon fibroblast presence. In the absence of melanocytes the authors observed no pigmentation. In the presence of fibroblasts and melanocytes (isolated from pale skinned patients) unpigmented skin was observed [Figure 5(A)], whereas in the absence of fibroblasts under same conditions pigmentation arose [Figure 5(B)][122].

(Insert Figure 05)

Biobrane® (Bertek Pharmaceuticals Inc., USA) due to its lower cost, ease of storage, application and fix, and reliable when used according to guidelines and being efficacious in treating partial thickness burns are the main reasons of its popularity in usage. By comparison of Biobrane® and cadaveric allograft for temporizing the acute burn wound, Austin et al. founded that Biobrane® is superior in terms of lower procedural time and associated cost because of mainly the relative ease of its application. Currently Biobrane® is used as an alternative to cadaver allografts as temporizing

dressings after excision of major burn injuries. However, the limitation of this technique is that wound bed must be meticulously prepared to prevent any infection and there is still a lack of existing literature and published clinical protocols proving that it could be a suitable replacement of the human skin allografts, especially in the treatment of full thickness burn wounds. Despite that, Biobrane® is still widely used as a synthetic skin substitute as well known for its success in the definitive management of partial thickness burns in many centres (Fig. 6)[108]

(Insert Figure 06)

Advanced wound healing for diabetic foot ulcers (DFUs) has now started to focus on stem cell therapy for improved healing. Much of the research is still in the starting phases with a paucity of robust clinical trials, but it could prove to be an important method for advance wound healing in difficult patient populations[123, 124].

Adipose stem cells (ASCs) being a type of adult stem cells have been proven to be a useful cell resource for tissue regeneration. Cell therapy plays a major role in regenerative medicine of this century where ASCs holds a key position. These cells have many clinical applications, including fat grafting, overcoming wound healing difficulties, recovery from local tissue ischemia and scar remodeling. Diabetic ulcers and chronic radiation ulcers are notorious for their recurrence. These lesions do not improve over time and tend to become worse. Recently, cell therapy using ASCs has been shown to be a good potential alternative technique because it is less invasive than re-constructive surgery and the cells can be directly placed onto target areas in cutaneous lesions. Sufficient numbers of ASCs can easily be harvested by liposuction and fat tissue digestion. The addition of cells to the defect may reinforce local regeneration capabilities that have been exhausted during the course of prolonged disease processes. The ease of repeating the procedure during the course of regeneration is the main advantage of this type of tissue engineering. This type of cell-based therapy may be a good treatment option for small traumatic defects or skin cancers to avoid more substantial reconstructive surgeries using local flaps[125]. In order to obtain recovery from ischemia, using ASCs is very effective until 4-5 days after the onset of complications and can reduce the area of necrosis in 7 days after the onset as shown in Fig 7[126].

(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 nerve[127]. 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 repair[128]. 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 rate[129]. 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 study[130], 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

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

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

The idea that biomaterials might have electrically conductive properties for nerve repair has been explored largely without success. However, composite materials from the blending of conductive (CPs) and biocompatible polymers are fast emerging as successful biomaterials for the regeneration of the myocardium due to their unique conductive and biological recognition properties and can assure a more efficient electroactive stimulation of cells. Recently, research has been focused on the synthesis of conductive polymers to fulfil basic biocompatibility and biodegradability properties by combining conducting and degradable units[131]. A series of electroactive and biodegradable polymeric materials were prepared by blending PLLA and poly (glycol tetra-aniline) (PGTA). The blended polymers showed good solubility and thermal stability, the cytotoxicity and biocompatibility of the materials were evaluated with positive results obtained. Cell culture results showed that PLLA/PGTA blended materials could accelerate the differentiation of rat C6 glioma cells compared with pure PLLA. They recommended that the 80/20 wt.% PLLA/PGTA blend material showed the best effect and these biodegradable PLLA/PGTA polymer blends are shown to be electroactive[132]. A novel electrically conductive biodegradable poly phosphazene polymer containing aniline pentamer (AP) and glycine ethyl ester (GEE) as side chains was obtained by a nucleophilic substitution reaction. The electrical conductivity of the polymer was ~2×10^{-5} S/cm (i.e. in the semiconducting region) upon protonic-doped experiments. Furthermore, the polymer proved to promote cell adhesion and proliferation *in vitro* using Schwann cells. These polymers also showed good solubility in common organic solvents and good film-forming properties, and consequently potential applications as scaffolds for neuronal and cardiovascular tissue engineering applications[133].

In another study, hyper-branched degradable conducting copolymers were blended with poly caprolactone to construct electroactive tubular porous nerve conduits by a solution-casting/particle-leaching method. Thermal and mechanical properties, hydrophilicity, morphology, toxicity and conductivity (values between 3.4×10^{-6} and 3.1×10^{-7} S/cm were found, depending on the composition) and were determined for blends doped with or without 10 camphor sulfonic acid. The results obtained supported their potential for neural tissue engineering applications[134]. McKeon and group studied several polyaniline and poly(D,L-lactide) (PANi/PDLA) mixtures at different weight percentages and were successfully electrospun from 1,1,1,3,3,3-hexafluoroisopropanol solutions and their conductivity and biocompatibility evaluated. It was claimed that the successful results were only attained when the PANi content reached 25%. Specifically, this scaffold could conduct a current of 5mA and had an electrical conductivity of 0.0437 S/cm. Primary rat muscle cells were able to attach and proliferate over all the new scaffolds, which degraded during the process. The polymer degradation and shrinkage may prevent the blend from being used as the primary component of a biomedical device, but its usefulness as a biocompatible coating on devices such as sensors was proposed[135]. Biodegradable semiconducting melanin films have also been studies for nerve regeneration. Melanins are naturally occurring pigments and exhibit unique electrical/biological properties and were used as melanin thin films to enhanced Schwann cell growth and neurite extension, compared to collagen films *in vitro*. Furthermore, melanin implants were significantly resorbed after 8 weeks[136].

Among natural polymers, collagen[137, 138], chitosan[139] and alginate[140] have been used for constructing nerve guidance channels. Addition of collagen gels to the lumina of nerve conduits speeds the rate of nerve regeneration. A number of collagen based nerve tubes have shown to support regeneration of nerve defects in vivo. However, repair was limited to gaps less than 30mm long[141]. Alginate was employed in tubular and non-tubular repair of a long peripheral nerve defect injury. In vivo studies showed the recovery of 50mm gap of the sciatic nerve of cats, treated by tubular repair or non-tubular repair. In the tabulation group, a nerve conduit consisting of polyglycolic acid mesh tube filled with an alginate sponge was implanted into the gap and the tube was sutured to both nerve stumps. In the non-tabulation group, the nerve defect was repaired by a simple interpolation of two pieces of alginate sponge without any suture. The animals in both groups exhibited similar recovery of locomotor function. After three months, axonal elongation and re-innervation in both the afferent and efferent systems were detected by electrophysiological

examination. Intracellular electrical activity was also recorded, which is directly indicative of continuity of the regenerated nerve and restoration of the spinal reflex circuit. Eight months after surgery, many regenerated myelinated axons with fascicular organization of peri neural (fibroblast) cells were observed within the gap, peroneal and tibial branches were found in both groups, while no alginate residue was found within the regenerated nerves. Morphometric analysis of the axon density and diameter revealed no significant differences between the two groups[142].

3.3 Blood Vessels

There is a substantial patient demand for vascular bypass grafts due to atherosclerosis and related cardiovascular diseases. Vascular disorders are the leading cause of mortality in Western countries. Several studies have been focused on the development of biodegradable vascular grafts able to temporarily substitute the blood vessel and allow for complete regeneration over a predetermined time period. Several biodegradable synthetic polymers[143], and natural polymeric materials like collagen[144] have also been evaluated for developing a successful vascular graft. However, due to the lack of suitable mechanical properties, unsuitable rates of degradation and the poor capacity to create an optimal microenvironment for cell adhesion and differentiation, none of these materials has displayed the required properties for further application in the human body.

However, different methods exist to prepare polymeric vascular grafts, which should allow greater control on both the mechanical properties and the micro- and nanostructures of the product. An ideal artificial graft should be mechanically compatible with the natural arteries and surrounding tissue and should also mimic the extracellular matrix morphology; it should have a nano scale topography (5 to 500nm) with high porosity and adequate pore sizes (5–500μm) to enhance cell attachment and proliferation for the regeneration of the natural tissues. The first tissue-engineered blood vessel substitute was created by Weinberg and Bell in 1986[145]. They generated cultures of bovine endothelial cells; smooth muscle cells (SMCs) and fibroblasts in layers of collagen gel supported by a Dacron mesh. Although physiological pressures were sustained for only 3–6 weeks, they did demonstrate the feasibility of a tissue-engineered graft with human cells. Since then, strategies to create a suitable material for a vascular graft have focused on three areas of research: 1) coatings and surface chemical modifications of synthetic materials; 2) biodegradable scaffolds and 3) biopolymers. Niklason and colleagues have developed a pulsatile bioreactor to remodel PGA scaffolds seeded with bovine smooth muscle and endothelial cells[146]. After a 10-week culture

period, the resulting tissue-engineered vessel displayed a burst pressure of up to 2300mmHg. After 5 weeks, the PGA scaffold had degraded to 15% of its initial mass[147]. Shin'oka *et al* reported the use of PCL-based scaffolds to engineer venous blood vessels. The PCL/PLA copolymer was reinforced with woven PGA and seeded with autologous smooth muscle and endothelial cells harvested from a peripheral vein. After 10 days, the construct was implanted as a pulmonary bypass graft into a 4-year-old child[148]. An alternative strategy to synthetic and degradable scaffold-based vascular grafts is the manipulation of proteins that constitute the architecture of native ECM. Weinberg and Bell first reported the use of collagen gels as substrates for cells in vascular tissue engineering. Since then, Habermehl and colleagues have developed a process to obtain large quantities of collagen from rat tail tendons to allow the scale-up of production[149]. The shortcomings of a relatively stiff collagen-based scaffold have motivated researchers to explore the potential of more elastic fibrin gels in vascular tissue engineering[150]. One such example is the fibrin-based vascular graft developed by Swartz and colleagues, who incorporated bovine SMCs and endothelial cells into the gel[151]. The grafts were implanted in the jugular veins of lambs and remained patent for 15 weeks. Upon histological examination, the constructs were found to contain both collagen and elastin, with the mechanical integrity comparable to that of native coronary arteries. Recent developments in the field of nanotechnology have facilitated vascular tissue-engineering efforts in mimicking the nanostructure of native vasculature, thereby directing mechanical and biologic performance of the bulk material. One such application is electro spinning of synthetic polymers and naturally occurring materials into nanofibres[152,153, 154]. In these studies, use of electro spinning to create nano-fibrous scaffolds composed of collagen-blended degradable PLLA-co-PCL was demonstrated. Results indicated that the blended nano-fibres supported endothelial cell attachment and spreading, and preserved the endothelial cell phenotype[155].

Poly amino acid-graft-polyester copolymers have been functionalized with heparin, for a potential use in tubular structures for vascular regenerative medicine[156]. The fabricated scaffold had morphological characteristics like those of natural extracellular matrix, a suitable rate of degradation in simulated physiological medium (after 60 days approximately 50% of the scaffold degraded), the ability to be easily functionalize and allow endothelial cell adhesion and proliferation**.** Bio functional vascular grafts were synthesized by electrospinning PCL solutions[157]. The obtained fibres showed tensile stresses above 2MPa and up to 7.4MPa and tensile strain at failure values in the range of 200–1200% after ϒ-sterilization. These values are above those for

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

3.4- Heart

Cardiovascular related deaths surpass cancer in general as the leading cause of death worldwide[158]. The report, "Global Atlas on Cardiovascular Disease Prevention and Control" by the WHO has identified cardiac related deaths will continue to increase in future[159]. Increased interdisciplinary research is therefore exploring multidimensional therapeutic aspects of cardiovascular diseases and new materials are continuously being explored. However, current therapies dealing with multifaceted cardiovascular damage lack the potential of intrinsic cardiac tissue regeneration [160]. To date, the exact relationship between the components of engineered biomaterials, the immune system and tissue regeneration has yet to be fully understood. The ultimate goal of tissue engineering is to develop therapeutic strategies that will stabilize, amend and improve cardiovascular anatomy and physiology[161]. Nowadays, cardiac tissue engineering and regenerative medicine (TERM) has become the focal point for the repair of damaged heart tissue[162]. TERM related approaches have shown to minimize the need for ventricular remodelling. Different strategies have been adapted to design and fabricate polymeric scaffolds for heart tissue engineering[163]. One potential application of polymeric scaffolds is the development of efficient degradable heart patches. These heart patches can provide an optimal platform for cellular growth over a period of time[164]. A recent review focuses on the engineering of functional three-dimensional cardiac patches composed of various composite biomaterial including biodegradable materials [165].

A three dimensional fibrin gel construct was reported by Ye *et al*[166], where different concentrations of apportioning (a protease inhibitor) promoted controlled degradation of the autologous scaffold seeded with fibroblasts. Microscopic studies of the developed tissue showed homogenous cell growth with no signs of toxic degradation or inflammatory reaction. However, the feasibility of

forming a cardiovascular graft on the arterial side by the 1mm thick developed tissue appears unlikely. A promising approach in cardiovascular tissue engineering was reported in which fibrin gel was prepared by a non-woven poly glycolic acid (PGA) fibre mesh coated with Polycaprolactone (PCL)[167]. Human saphenous vein cells were seeded onto the fibrin gel and a more mature extracellular matrix was produced in a short time span (days) with a decrease in the loss of soluble collagen. Flanagan *et al* have reported an interesting study where fibrin-based heart valves have been developed in a custom-designed bioreactor. The dynamic conditions were optimized to accelerate the maturation of engineered valves[168]. The experimental findings demonstrated the potential repair and regenerative role of an injectable fibrin glue after a myocardial infarction. This injectable fibrin glue could preserve infarct wall thinning and cardiac function after myocardial infarction in MI-induced rat models. The decisive regenerative features include; increased cell transplant survival, decreased infarct size and an increased blood flow to the ischemic myocardium[169,170].

Another study investigated the use of chitosan to increase the compression modulus of collagen based injectable hydrogel matrices. It has been reported that endothelial cells formed significantly more vascular-like structures on the collagen–chitosan matrix-hydrogels improved the ventricular wall stability and showed an ability to reduce heart dilatation upon myocardial infarction (MI)[171]. Silk protein fibroin of the Indian tropical tasar silkworm *A. mylitta* (AM) has been used by Patra *et al* to develop 3D scaffolds for the *in vitro* engineering of a cardiac patch. The resulting contractile patches were stable and demonstrated spontaneous beating for 20 days[172]. Biosynthetic hydrogels of poly vinyl alcohol– alginate have also been prepared by Thankam *et al*., these consisted of a semi- and full-interpenetrating polymeric network (IPN hydrogel, PAHG) harbouring it suitable for cardiac tissue engineering applications. Its amphiphilic nature and moderate water content favoured cellular migration, growth and long term viability of L929 fibroblasts and H9C2 cardio myoblasts[173]. Another methodology based on the blending of natural polymers i.e. alginate and gelatine, were prepared in the form of films to be used as scaffold for myocardial tissue engineering. Cell culture tests with C2C12 myoblasts, degradation in simulating body fluids, showed best response for alginate/gelatine 20:80 blends[174]. Gelatine and fibrin based tissue engineered heart valve were designed and operated in a bioreactor with enhanced cell attachment and alignment by Kim *et al.*[175].

In 2007, Balguid *et al*. explored the role of collagen content and its cross-links in biomechanical behaviour of human aortic heart valve leaflets and in tissue-engineered constructs. Collagen cross-linked concentration showed a positive linear correlation with the modulus of elasticity, which can enhance biomechanical function[176]. It has been reported that collagen-glycosaminoglycan gels matrices were used for mitral valve tissue engineering. Moreover, addition of chondroitin sulphate (CS) resulted in a more porous model, which enhanced the bioactivity of seeded valve cells and facilitating tissue remodelling[177]. Gelatine/PCL hybrid fibrous scaffolds were synthesized by electro spinning to obtain optimal fibre diameter, pore size and strength, promoting cell seeding and finally development of constructs for cardiovascular tissue regeneration[178].

In another study, Landa *et al*. investigated the use of bioresorbable alginate hydrogel to provide mechanical and physical support to the damaged cardiac tissue after MI[179]. Several recent reports have shown the use of alginate hydrogels in delivery of sequential growth factor VEGF-A(165) and PDGF-BB in a myocardial infarction model[180]. These hydrogels were also able to controls delivery of heat shock protein[181] and serve as a carrier for dual delivery of insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF)[182]. Multi-layered cardiac grafts were fabricated in vitro using biodegradable electrospun nano fibrous PCL meshes with a unique extracellular matrix–like topography by Ishii and his co-workers[183]. In another study, PLLA-co-PCL (PLCL) nano-fibres were encapsulated with vascular endothelial growth factor (VEGF) using two types of protective agents (BSA and dextran) through emulsion electrospinning. In vitro release study demonstrated that the core–shell PLCL–VEGF–DEX nanofibers had potential as sustained-release scaffold for cardiovascular tissue regeneration[184]. Rat smooth muscle cells (SMC) were seeded on biodegradable poly (ε-caprolactone-co-lactide) (PCLA) patches and were checked for cellular penetration in vitro and in vivo. This work permitted the construction of an autologous patch to repair congenital heart defects[185].

(Insert Figure 08)

To date several studies have focused on elastomeric biodegradable poly (glycerol sebacate) (PGS): gelatine nano fibrous scaffolds and poly (glycerol sebacate) PGS/fibrinogen core/shell fibres. These biomaterials exhibited well-defined anisotropy, mimicking the left ventricular myocardium architecture that can be used as constructs for myocardial regeneration and repair[186,187]. The

structural properties of the scaffolds had significant effect on cytoskeletal organization of the cells as shown in Fig 8. Prabhakaran and his research group used a blend of synthetic (PLGA) and natural (gelatine) polymer to obtain PLGA/Gel nano-fibres via electro spinning. Culturing of cardiomyocyte cells on the scaffolds highlighted their potential as biomimetic cardiac patches[188]. In another study, a research group fabricated nano-fibrous scaffolds of electrospun random and aligned PCL/gelatine to mimic structurally the oriented extracellular matrix (ECM), which provide anisotropic wetting and mechanical properties compatible for cardiac regeneration[189]. Composite scaffolds of poly (1, 8-octanediol-*co*-citrate) and PLCL were evaluated for their mechanical and biocompatibility properties. Electrospun scaffolds were elastic and hence provided the necessary mechanical cues required for cardiac tissue repair[190]. An electrospun poly(ethylene glycol) dimethacrylate/poly (L-lactide) PEGDMA/PLA scaffold with biomechanical properties nearly equal to native valve leaflets has also been reported[191]. Sant *et al* synthesized nano fibrous scaffolds made up of blends of poly(glycerol sebacate) PGS prepolymer with PCL to address the mechanical properties relevant to the human aortic valve leaflet[192]. For the regeneration of infarcted myocardium, PGS short fibres were fabricated by co-axial electro spinning, with poly(glycerol sebacate) (PGS) as core material and poly-L-lactic acid (PLLA) as shell material[193]. A mechanically compatible multi-layered scaffold of PCL sandwiched in a gelatine–chitosan hydrogel was developed[194]. This could be used as cardiac patch in tissue engineering applications owing to its ability to sustain cardio-myocyte viability. Conductive nanofibrous scaffolds of melanin, poly(L-lactide-*co*-ε-caprolactone) and gelatine can electrically stimulate cardio myocytes to enhance cell proliferation and therefore are a potential candidate for cardiac patches as demonstrated by Kai *et al*[195].

Scaffolds fabricated from PEG and modified electrospun PCL(ePCL) can serve as a foundation for engineered heart scaffolds[196]. 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 days[201]. The

prospect of limiting myocardial damage and facilitating repair and regeneration was addressed in this study[202].

3.5- Cornea

Pathological conditions associated with cornea are reported as the major cause of vision impairment. Corneal pathology accounts for 4.9 million blind cases worldwide[203]. Anatomically, the transparent corneal layer serves to focus light as it enters the eye. Blindness related to corneal disease include many conditions, e.g. keratoconus, Fuch's dystrophy and Stephen-Johnson syndrome[204 205]. The extracellular matrix (EMC) of the cornea is a highly compact and organized architecture consisting primarily of collagen (types I to V). This EMC is currently under investigation as a prospect therapeutic research area. As estimated by the World Health Organization, corneal diseases are a major cause of vision impairment and blindness, second only to cataracts as the leading cause of blindness[206]. Tissue engineering has been widely explored for its role in regenerative medicine. Recently, significant progress in corneal tissue engineering has been achieved, where researchers have reported on the development of a corneal construct either by employing cellular or acellular based techniques that are biocompatible, with physiological functional for long term endurance[207]. Tissue engineering has focused on developing corneal tissue that can potentially mimic the native cornea. Synthesis of a corneal construct, epithelial and endothelial layers in parallel with a network of nerves have been explored. Reports have identified natural and synthetic polymers as the preferred choice of investigation[205]. In the last few years, intensive research efforts have been focused to determine whether key properties of ECM macromolecules can be replicated within tissue-engineered biosynthetic matrices to influence cellular properties. Tissue engineering of the cornea could overcome shortages of donor corneas for transplantation and improve quality. Hydrogels based corneal implants from concentrated recombinant human type I and type III collagen have promoted stable regeneration of corneal tissue[208]. For example, Madden *et al* reported on one of first successful demonstrations of primary human corneal endothelial cells on fibroin coated with collagen. This step allowed the evaluation of fibroin as a substratum for the transplantation of tissue-constructs for endothelial keratoplasty [209].

A simple corneal substitute was developed from carbodiimides and N-hydroxysuccinimide cross-linked collagen and was found to be suitable for transplantation. This was employed in centres having a shortage of corneas available for implants[210]. An artificial cornea of collagen–chondroitin sulphate foam approximately equal to human cornea thickness seeded with human endothelial cells proved that these collagen-chondroitin sulphate scaffolds are good substrates for artificial cornea construction[211]. Collagen hydrogel matrices were developed by Li *et al* from collagen I cross-linked with a copolymer based on N-isopropyl acryl amide, acrylic acid and acryloxysuccinimide. These hydrogels were found to be non-toxic and allowed epithelial cell overgrowth and optical clarity superior to the human cornea[212]. Fibrillar collagen sponges were used as a substrate for culturing human kerotocyte, epithelial and endothelial cells. This synergy promoted a wound healing in the eye[213]. Liang *et al.* reported on the formation of an *in situ* biodegradable and non-toxic composite hydrogel for corneal endothelium reconstruction[214]. The hydrogel was made by self-cross-linking of water-soluble chitosan and oxidized sodium alginate.

Biodegradable chitosan–PEG hydrogel films (CPHFs) and chitosan; PCL blends with excellent biocompatibility are enviable candidates as substrates for the regeneration and transplantation of CECs (corneal endothelial cell)[215,216]. The transplantation of fibroblast precursors on gelatine hydrogel into the corneal stroma may be a possible treatment for corneal stromal regeneration[217]. Some recent studies revealed fabrication of cross-linked porous gelatine scaffolds for *in vitro* cultivation of corneal endothelial cells (CECs)[218]. These were modified with chondroitin sulphate, which enhanced proliferative and biosynthetic capacity of cultured cells[219]. Cross-linked porous gelatine hydrogel discs were inspected for their potential as cell sheet carriers for corneal endothelial cell therapy. These could efficiently deliver the cell sheet transplants at the site of injury[220]. Lawrence *et al.* reported that silk protein films could support corneal cell functions and were used to reproduce corneal stromal tissue building[221]. Dual layer scaffolds were prepared from Silkworm *(Bombyxmori)* silk fibroin for corneolimbal reconstruction of diseased or damaged ocular surface. These fibroin membranes showed potential as a substrate for human limbal epithelial (L-EC) and limbal mesenchymal stromal cell (L-MSC) cultivation[222]. Porous silk fibroin film were synthesized by mixing of silk fibroin and poly(ethylene glycol) (PEG) followed by the removal of PEG from porous films. These films were used as biocompatible carriers to deliver corneal epithelial cells to ocular surface[223].

Klenkler *et al.* modified polydimethylsiloxane (PDMS) surfaces with epidermal growth factor (EGF) to improve the growth of corneal epithelial cells[224]. The design of artificial limbal stem cell niches for cell delivery to cornea was explored by the fabrication of biodegradable poly(lactic-co-glycolic acid) 50:50 electrospun membranes[225], which have the potential to support the growth of limbal epithelial cells for periods of at least of 2-3 weeks in culture[226, 227]. Biodegradable PLGA membranes containing micro-pockets mimicking an ex vivo 3D cornea model can potentially contribute to the migration of limbal cell from limbal explants [228, 229,230]. Fig 9 reveals degradation of PLGA membranes when placed in media with and without cells and showed that fibres lost integrity over 6 weeks' time. Poly (2-hydroxyethyl methacrylate) (PHEMA) hydrogels were used for functional polymeric artificial corneas (keratoprosthesis)[231].Optically transparent, biocompatible and biodegradable poly(ethylene glycol) (PEG)-based hydrogel films (PHFs) appeared good candidates for regeneration and transplantation of corneal endothelial cells (CECs) by Ozcelik *et al*[232].

(Insert Figure 09)

Recently, much attention has been dedicated to the use of blends of biodegradable polymers to get interpenetrating polymer networks (IPNs). These hydrogels showed better mechanical properties and harnessed greater multi-functionality appropriate for keratoprosthesis[233]. A number of researchers have investigated the synthesis of poly(ethylene glycol)/poly(2-hydroxyethylmethacrylate) (PHEMA)-based IPN hydrogels and chitosan–PCL-blended membranes as carrier for corneal endothelial cell (CEC) transplantation[234,235]. A number of studies have been reported on the pros and cons of using polymers such as collagen and fibrin based carrier materials in limbal stem cell deficiency (LSCD) remedy[204]. In 2010, recombinant human collagen type III (RHCIII) hydrogels that facilitated nerve regeneration and stromal cell were developed as corneal constructs. Although promising, these implants presented some limitations, including sufficient cell division speed to evade infection and fibrosis in some cases.[236] This data identifies the need to develop corneal implant that can be accurately grafted and in parallel allow rapid healing process. Recent studies have highlighted the importance of post fabrication remodelling in achieving positive clinical outcome[237].

Collagen and phospholipids are being used to construct corneal implants that can be further specifically functionalized using printing and laser profile techniques. In a recent study, 500µm

RHCIII–MPC hydrogels were fabricated using 2-methacryloyloxyethyl phosphorylcholine (MPC) that promoted fibronectin printing. The micro-patterns of 30µm size generated in these RHCIII–MPC hydrogels showed optimal mitotic division and cell attachment. *In vivo* studies have yet to replicate these properties exhibited by RHCIII–MPC hydrogels[238]. Biomaterials owing to their therapeutic properties have shown promising avenues in corneal repair and regeneration. At present, research is being focused on corneal regeneration *in vitro* and *in vivo* using polymer (gelatine, alginate and chitosan) based hydrogels, and constructs[239]. A recent study has shown that primary human corneal keratocytes were more compatible with silk fibroin films fabricated by centrifugal force. Bombyx mori cocoons were used to retrieve silk fibroin (SF). SF has been used in corneal tissue engineering and approved by FDA for soft tissue repair. SF films prepared by centrifugal force had smooth surfaces, transparency and elasticity, rendering favourable environment for cell growth[240].

3.6- Dental and Oral Structure

The basic knowledge about the biology of the oral and tooth structure as well as the information about fundamentals of materials and techniques applied to tissues, constitute the basis for restorative dentistry and help in creating biological approaches to tissue regeneration. A suitable inductive carrier is essential for dental-pulp tissue regenerative treatment. The selection of suitable scaffold has vital importance to persuade and confer the optimal formation of new dentin matrix and pulp-dentin complex[27]. For regenerative dentinogenesis optimal conditions for cell adhesion, migration, proliferation and differentiation must be provided. Among dental problems, periodontal diseases are highly prevalent and 90 % of the worldwide population is affected. Periodontitis is one of the periodontal diseases leading to loss of connective tissue and bone support, which is a major cause of tooth loss in adults[241]. The techniques, such as bone graft, guided tissue regeneration (GTR), and stem cell therapy have been used for periodontal tissue regeneration, among these the GTR has become the most promising treatment and has been widely used in clinical treatment for its convenience and effectiveness[242]. During GTR technique a barrier membrane provide mechanical support to gingival connective tissue on one side and periodontal ligaments on other side[243]. The first generation these membranes comprised of stable, non-immunogenic polytetrafluorothylene (ePTF), a non-resorbable material. However, the significant drawback is related to the risk of disturbing healing with the second surgery necessary to remove the permanent. To address this issue, a second generation of resorbable membranes was developed.

The resorbable membranes can potentially provide better healing as the material resorption and bone ingrowth occur simultaneously. Recently, third generation of membranes have been introduced with bioactivity[244]. The basic principal of GTR membrane is to restore the architecture and functionality of the periodontal system[245]. On the basis of this, the ideal periodontal membrane should have two important properties i.e. stiffness and elasticity[246]. Various types of materials have been tested for their effectiveness as barriers including non-degradable and biodegradable membranes[247]. A list of commonly used commercial periodontal membranes is given in Table 1.

(Insert Table 1)

Several problems have been associated with the use of non-degradable barrier membranes, particularly the need for a secondary surgery to remove the membrane. Furthermore, early exposure to the saliva present in oral environment and subsequent bacterial colonization are common problems resulting in early detachments. To overcome these issues, a variety of synthetic biodegradable materials, such as polylactide, PLA, PCL, and their copolymers or tissue-derived collagens have been used as membrane barriers[248-251]. It is suggested that a highly hydrophobic surface, which act as a non-conductive towards protein attachment, should be used an occlusive barrier for gingival epithelial cells in periodontal regeneration. The schematic structure of periodontal membrane is given in Fig. 10 [252].

(Insert Figure 10)

Drug loaded biodegradable periodontal membranes were synthesized and found that non-steroidal anti-inflammatory drugs can create an effect on morphology of electrospun fibers and smooth electrospun fibers can be achieved with high drug loaded polymers. Moreover, doxycycline based periodontal membranes stimulated cell proliferation and osteogenesis[253-255]. Kasaj *et al.*[256] evaluated the biological effects of various commercially available biodegradable membranes made of collagen and compared it with non-degradable membranes in cultures of human gingival fibroblasts, periodontal ligament fibroblasts and human osteoblast-like cells. It was found that non-degradable membranes limited the cell adhesion and the biodegradable membranes demonstrated to be more suitable to stimulate cellular proliferation compared to non-resorbable membranes as shown in Fig 11.

(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 (–CH=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 groups[258]. During the aminolysis process, the alkaline catalysed degradation of PLA matrix resulted in a decrease of molecular weight[259]. 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 (-CH=N-) with PLLA enhanced the degradation process, however, the main degradation was due to the hydrolysis of ester group.

(Insert 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 invasion[257]. 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.

(Insert 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

tissue integration. Their degradation rate is important as these membranes must function for at least 4–6 weeks to allow successful regeneration of the periodontal system[260]. Generally, the biodegradation of these polyesters involves non-enzymatic cleavage of PGA and PLA into pyruvic and lactic acids, respectively, which are common end-products of carbohydrate digestion. Milella *et al.*[261] evaluated both the morphological and mechanical characteristics of commercially available polyester-based membranes. It was observed that the membranes demonstrated initially high strength (12–14MPa), losing their structural and mechanical properties within 4 weeks of incubation in culture medium. The maximum strength after 14 days of exposure decreased significantly (below 1MPa). Collagens are important alternatives to synthetic polymers in GTR/GBR procedures due to their excellent cell affinity and biocompatibility. However, type I collagen may have limitations in its use due to the high cost and poor definition of its commercial sources, which make it difficult to control degradation and mechanical properties. Collagen-based membranes have shown very poor performance *in vivo* as the membrane starts to degrade. The breakage and fragmentation of collagen fibrous membranes started after 7 days of incubation and after 30 days, the degradative behaviour enhanced, and pores were evident as shown in Fig. 14.

(Insert Figure 14)

Additionally, the risks of disease transmission due to the use of human- or animal-derived collagen may pose regulatory or other limitations, such as religious beliefs, on its use. Biomechanical properties and collagen matrix stability can be enhanced by means of physical/chemical crosslinking, by ultraviolet (UV) radiation, genipin (Gp), and glutaraldehyde [261, 262]. It was proposed that a natural polymer based membrane has better cell adhesive and biocompatibility properties; however, its mechanical strength is not up to the mark. In contrast, synthetic polymers have desirable mechanical properties, but poor biological properties. Therefore, modifying natural polymers, such as collagen membranes with synthetic polymers may yield GTR barrier membranes with optimal properties. PLA, poly(glycolide-co caprolactone)(PGC) and PLGA was employed and spray coated on collagen membrane which significantly improved its mechanical strength[263].

To date, the chitosan membranes' application is still in the animal assay phase, but the results showed great potential for chitosan materials in GTR procedures. In comparison to other biodegradable membranes, the chitosan membranes are cheaper and possess better tissue healing

effect and showed more cementum and bone formation in animal models. The bacteriostatic property of chitosan may reduce the bacterial contamination and enhance periodontal tissue regeneration. The degradation rate of chitosan membranes manufactured by different methods was evaluated in a number of studies[264, 265]. Pure chitosan membranes degraded by about 15–40% of their initial weight after 90 days shaking in phosphate buffer saline (PBS). In vivo testing showed that after grafting into rat subcutaneous tissue chitosan membranes maintained their shape and space for bone regeneration for 6 weeks[266]. The degradation rate of chitosan membranes depends on their molecular weight and the preparation methods, and it fit into the schedule of remodelling of tissue regeneration[267].

3.7- Trachea

Patients suffering from damage of the trachea after tumour formation of excision need permanent treatment [268, 269]. However, this is a major challenge, in part due to the specialised structure of the tissue. The trachea is a circular segmented architecture of cartilage, interconnected with soft tissues to form the tubular air pipe structure of the respiratory system[270]. The main function of this fragmented cartilage is to provide sufficient stiffness and flexibility to regulate airflow systematically. Tracheal anatomy reveals an inner surface of columnar epithelium, with cilia that help in trapping extraneous air particles together with goblet cells for exuding mucus to form protection against any external stimuli[268]. These functions are unique and cannot be modelled through autologous tissue implants. Therefore, tracheal regeneration[271] is a focus of many biomedical engineers and clinicians. The on-going research in tracheal regeneration uses prosthesis implants, synthetic composites and tissue-engineered constructs[271-273]. But these biomimetic materials are associated with clinical issue e.g., breath impediment, infection and dehiscence, limited epithelialization and vascularization. Tissue engineering strategies[274] are not yet able to produce an ideal tracheal implant. Nonetheless, TE holds the potential to realize advanced and optimal tracheal grafts[29, 275, 276] while considering the following factors such as: (a) the graft should be biodegradable and biocompatible (i.e. it can offer a suitable architecture for cells so they can produce cartilage and soft tissue of the apposite cylindrical contour.) (b) It should stimulate epithelial development (i.e. it has a well-designed epithelial lining that could either be

cultured or migrated from the native trachea), and (c) should facilitate adequate vascularization, to support the volume of tissue required for clinical application[269].

The first tracheal tissue engineered product was introduced by Vacanti et al[277], who reported a three-dimensional tracheal scaffold prepared from synthetic nonwoven mesh, 100µm thick PGA fibres (15µm in diameter, cut into pieces of 2.5 x 4cm) for replacing large circumferential cervical defects in trachea of rats. Chondrocytes were seeded into engineered cartilage to evaluate their viability, the scaffold allowed the expansion of chondrocytes. Implantation of cell-polymer constructs was reported to produce hyaline cartilage after four weeks in mice. Follow-up histological studies showed that from initial stage samples, an identical cartilage to the natural one was produced, but later the animals suffered from respiratory distress and ultimately died. The collapse of cartilage was supposedly by non-optimized mechanical properties [269, 277]. In another study by Kojima et al[278], they used biodegradable PGA non-woven mesh enfolded in a helical template composed of silicone rubber. For *in vitro* studies, chondrocytes and epithelial cells were isolated and seeded from sheep nasal septum. The cell-polymer construct was implanted into subcutaneous pockets of nude mice. After six weeks of cell growth, epithelial cells were suspended in hydrogel and infused into the implanted tissue construct. Hemotoxylin and eosin staining demonstrated full-grown cartilage, pseudostratified columnar epithelium growth and a separate interface or borderline, connecting tissue-engineered cartilage and epithelium. Furthermore, Safranin-O staining results illustrated ordered circular lobules and angular lacunae respectively, which contained single chondrocytes. The authors concluded that the morphology of the implants resembled native sheep trachea in that the proteoglycan and hydroxyproline content was similar to native cartilage, and therefore had the potential for regeneration of segmental tracheal defects as well as epithelial formation.

Despite the reported success in tracheal restoration by implantation of tissue engineered constructs and transplantation procedures, none of the newly established techniques have resulted in clinical application on a large scale. Developing or regenerating a purposeful tracheal tissue from different cultured cell types is still a major challenge for researchers[279]. For instance, tracheal fixation in laryngectomized patients and prosthetic voice rehabilitation using tracheoesophageal silicone rubber speech valves and tracheostoma valves has resulted in many complications. Furthermore, animal models used for tracheal research vary widely and in most of the cases, proper scientific

justification for choice of animal is not explained. These issues play a decisive role in tissue engineering and are thoroughly discussed in a review paper by Hallers *et al*[279].

With the passage of time, several progressions in tracheal tissue engineering have been made[271]. Rotter *et al.*[280] demonstrated the effect of interleukin in tissue-engineered cartilage made from PGA–PLA (PGLA) matrixes. PGLA scaffolds seeded with porcine auricular chondrocytes and unseeded scaffolds as controls were implanted in an autologous immunocompetent pig model. Histological studies by using haematoxylin and eosin, Safranin, trichrome, and Verhoeff's staining and biochemical studies confirmed that the level of glycosaminoglycan showed acute inflammation. Moreover, homogeneous cartilage development was not observed in any of the samples except in specimens taken after one week of implantation. Furthermore, histological studies revealed acute inflammation around the degrading scaffold, whereas, glycosaminoglycan contents were observed considerably higher in serum free group. These are regarded as inhibiting factors in regeneration of cartilage tissue. Scaffold free cartilages have also been proposed in the literature by Wu *et al.* [281] and Weidenbecher *et al.*[282] respectively. Wu *et al* fabricated cylindrical cartilage using a chondrocyte macro-aggregate. In another study, Weidenbecher *et al.* developed scaffold-free cartilage sheets for fabricating a vascularized neo-trachea in a rabbit model. A tracheal framework was produced by these neo-tracheal tissue engineered constructs after few weeks of harvesting and these neo-tracheas, healthy with well-vascularized supported with integrated layers, but showed limited mechanical strength, thus were unable to reinstate segmental defects and long-term patency in trachea[281, 282]. In another study[283], composite grafts were fabricated from a biodegradable 3-layered scaffold: a collagen sheet, a PGA mesh, and a copolymer (L-lactide/ε-caprolactone) coarse mesh. Chondrocytes isolated from the auricular cartilage of New Zealand white rabbits were cultured and then seeded onto the biodegradable construct to restore tracheal stenosis. Implantation was carried out in a mid-ventral defect of cervical trachea. In addition, a gelatine sponge for an appropriate supply of basic fibroblast growth factor (b-FGF) on scaffold was also employed. Their findings showed that the biodegradable scaffold was able to regenerate the tracheal architecture up to 3 months after implantation. Regardless of their success, authors proposed further studies that may establish techniques that could facilitate homogeneous cartilage formation with optimal functional and mechanical properties[283].

Lin *et al.*[284] reported a unique approach, as they developed a scaffold based bioreactor system for tissue-engineering of trachea under the influence of controlled fluid flow. A scaffold of poly (3-caprolactone)-type II collagen was seeded with chondrocytes and grown under controlled rotational speed/fluid flow and resulting shear stress in the bioreactor. This procedure enhanced cell proliferation, glycosaminoglycan (GAG) and collagen content in the constructs compared to static culture for the same time. For instance, at a rotation of 15 rpm, a two-fold increase in cell population, 170% increase in GAG content and 240% increase in collagen were achieved. H&E staining provided evidence of neo-cartilage formation along with aligned chondrocytes in direction of fluid flow.

The potential of using transplanted cells from the patients was also carried out by Kobayashi *et al.*[285]. They used synthetic grafts of collagen sponge containing a spiral polypropylene stent and mesh in combination with gingival fibroblasts (GFBs) and adipose-derived stem cells (ASCs) as autologous transplanted cells for tracheal epithelial regeneration. Their studies revealed limited risk of rejection by immune systems and contamination from allotransplant cells but showed sluggish epithelial regeneration[285]. Tatekawa *et al.*[286] reported on the use of a bio absorbable copolymer of caprolactone-lactide sponge sheet reinforced with a poly(glycolic acid) fibre mesh (Cop). Cop incorporated gelatine hydrogel and Cop-gelatine hydrogel with basic fibroblast growth factor were used with an external non-degradable polymer stent. Implantation was carried out in three groups of rabbits and tracheal epithelialization, cartilage formation and vessels were only noticed in bio absorbable copolymer containing gelatine hydrogel (Figure 15).

(Insert Figure 15)

Their observations revealed that respiratory distress, loss of appetite airway resulted in tracheal collapse as well as dislocations of the copolymer (due to mucosal sloughing) were the reasons of rabbit death. To overcome this situation and to retain long-term survival, the reconstructed trachea was reinforced by external stenting on either side of the trachea[286]. Interestingly, macromolecules such decorin, a proteoglycan (PG) residing in the complex network of ECM proteins of connective tissues, have also been explored for tissue engineering applications[287]. Hinderer *et al*[287] introduced a strategy in which decorin was electrospun in 3D fibrillary scaffolds fabricated from biodegradable PCL-gelatine matrices for tracheal tissue regeneration. The electrospun scaffolds

were investigated for cell-matrix-interactions and immune-mediated mechanisms and found low immunogenicity for hPAEC (human primary airway epithelial cells) expansion as shown in Figure 16. Their findings revealed possible applications in restoration of the trachea by these functional 3D hybrid scaffolds[287].

(Insert Figure 16)

Different strategies have been executed to advance existing research development in tissue engineering of the trachea[272, 275]. One such recent example is engineering of a vascularized trachea by utilizing bioresorbable PLGA and PCL scaffold[269]. In this study, implanted scaffolds were wrapped with pedicled muscle flap over a ring-shaped mould. Furthermore, these muscle enfolded PLGA and PCL scaffolds were seeded with chondrocytes, bone marrow stem cells and co-cultured both cells respectively. Implantation of these engineered scaffolds was done as an ectopic culture over abdominal wall of rabbits and harvested for several weeks. The tissue engineered constructs were harvested after subsequent *in vivo* intra-muscular incubation. It was observed that all the scaffolds preserved adequate cylindrical contours for two weeks. Though, harvesting after four weeks, contraction and deformation in the PLGA scaffolds was observed. After careful detachment of a silicone mould and muscle tissue, a well-encapsulated ring of PLGA and PCL scaffolds were further investigated as shown in Figure 17a. Structural similarities among tissue engineered scaffolds and to native cartilage were evident in PCL scaffolds at the two-observation time-points. Whilst the PLGA scaffolds after four weeks had shrunk and deformed, those at two weeks had not. In addition, a considerable weight loss (22.5%) of PLGA at four weeks was observed, compared to weight loss of PCL at 2 weeks (6.3%) (Figure 17b). Hence, PCL tissue engineered scaffolds due to their adequate porosity maintained tubular scaffold geometry and were considered as more suitable for intra-muscular tracheal tissue engineering as compared to PLGA scaffolds. Histological results further revealed that PCL engineered scaffolds exhibited optimal chondrogenesis with sufficient stiffness to maintain the cylindrical shape and luminal patency comparable to the native trachea.

(Insert Figure 17)

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 components[289-291]. 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 polymers[294], silicon carbide[295], alumina[296] and titanium materials[297]. Biodegradable polymers have been explored with HA and a variety of other nano-porous materials [298-300]. HA-PLGA nanocomposite material have been developed which possesses good osteogenic activity[290]. 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-hydoxyapatite) based injectable microgels were prepared for healing major bone defects[301]. 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

adipose derived mesenchymal stem cells (rASCs). Consequently, chitin-PCL-nHAp microgels could offer an effective injectable material for regenerating a diverse variety and complex bone defects[301].

In recent years, synthetic biodegradable polymers and their composites have been tuned to fabricate well-aligned and multipurpose tissue engineered constructs [12, 33, 34, 52, 79, 244, 288, 291, 302-308]. In a recent study, fibre based biodegradable scaffolds[302] such as poly-1-caprolactone/polylactic acid (PCL/PLA) composites, containing fibres of PLA in a PCL matrix were developed in cell instructive scaffold fashion for investigating bone osteogenesis. Integration of PLA fibres into the PCL matrix resulted in drastic improvement in mechanical properties. The most interesting aspect of this research is computational fluid dynamic models, which expose the material's capability to exert hydrodynamic forces during in vitro cell culture, as a result, an optimal flow rate was established that enabled specific cellular event to happen. e.g. osteoblast differentiation from hMSCs.

Some natural biodegradable materials, such as collagen, gelatine and silk have also been used in combination with other materials[306]. Nevertheless, formation and significance of anti-bovine collagen antibodies in many human recipients containing bovine collagen is still a matter of debate and not yet fully understood[79]. Therefore, numerous biodegradable polymers and their composites have been investigated to make hybrid tissue scaffolds for bone and cartilage regeneration [302, 309]. Such nanomaterials have remarkable characteristics, such as cell adhesion, interaction and proliferation as compared to the pure synthetic polymers. Collagen has also been used to improve cell interactions with electrospun nano-fibres of bioresorbable PLA, PGA and PCL and their copolymers [79, 309-311]. Composite biomaterials from biodegradable PLA, PGA and their copolymer PLGA have been employed with bioactive ceramics i.e., bioactive glass particles or HA[8]. Studies showed that such materials stimulate bone regeneration, as well as, offer better mechanical strength and biological concert[303]. It was also reported that composites of polymers and Bioglass® are angiogenic i.e., they supported the growth of blood vessels, suggesting a novel approach for providing a vascular supply to implanted materials in bone tissue engineering, which was confirmed by histological studies of resected implants (Figure 18[312]).

(Insert Figure 18)

Composite biomaterials from natural, synthetic polymers and nanomaterials are also been used extensively[34, 291, 305-308]. This includes biomaterials based on gelatine and silk derivatives which have been studied recently. Due to their biological source, biocompatibility, excellent biodegradability and above all its ease of availability at low cost, makes them suitable for tissue engineering[313, 314]. A number of this class of biomaterials, such as gelatine methacrylate (GelMA)[28], interpenetrating GelMA-SF (silk fibroin)[28], silk–silk composite scaffold[315] and CNTs reinforced GelMA composites material[316] have been employed in tissue engineering applications. All these materials have intrinsic benefits and limitations, such as preparing GelMA is low cost and convenient and it also promotes cell proliferation, migration, natural cell binding and degradation motifs but its use has become limited when rapid degradation is required, or high mechanical stiffness cannot be compromised. In GelMA-SF, SF addition to GelMA system increases physical cross-linking without any chemical modification. Both these factors, such as crosslinking and crystallinity, influence the mechanical and degradation properties of these material[28]. However, the biocompatibility of silk and its ability to form large porous structures offers a significant advantage and it has been further investigated to fabricate silk-silk macro porous scaffolds[315]. The high interfacial cohesion between SF and macro particles resulted not only in reinforcing mechanical properties but also lowered or restricted the enzymatic degradation of the scaffolds. Use of carbon nanotubes (CNTs) in GelMA also reinforces the mechanical stability due to interaction between peptide chain and CNTs[316]. Cross-linking was not observed resulting in a significant dispersion in the medium.

In preparing an optimal bone graft, the efficiency of materials can also be enhanced by increasing their surface area as can be achieved by producing nanostructures and subsequent functionalization by incorporating nano-fillers in the polymer matrix. For this purpose, an ideal material would instruct mechanical stability to the composite without reducing its bioactivity. In this perspective, one and two dimensional carbon based materials with high chemical inertness and good biocompatibility, such as carbon nanotubes (CNTs), graphene or graphene oxide (GO) can help in enhancing the physical, chemical and biological properties of biomaterials for bone tissue engineering[290]. Recently, CNTs[290, 316-318] and GO[290, 317, 319, 320]have been used as nanofillers and reinforcing agents in synthetic and natural biodegradable polymer matrixes for bone regeneration and tissue engineering applications[321, 322]. Interestingly, in every case, these nanostructures resulted in improved physical properties, such as resilience, toughness and tensile strength, as well

as good biocompatibility and biodegradation. In addition, no obvious toxic effects in vivo[215, 317, 323] were observed. Hence, these types of biomaterials offer great potential in tailor making required properties when incorporated in polymeric materials, ultimately strengthening material properties without offsetting its bioactivity/biocompatibility and allowing to be used for bone regeneration[215, 317]. In terms of its biological efficiency, graphene offers cell adhesion and proliferation i.e., for osteoblasts[319, 324, 325]. Furthermore, during tissue formation electrical stimulation of osteoblasts can be carried out utilising superior electrical conductivity of graphene[326]. Besides graphene, graphene oxide also exhibits promising biological properties by facilitating adhesion and proliferation of mouse fibroblast cells [327], providing drug delivery platforms for water insoluble cancer drugs[328] and in biosensors[329]. Its multifunctional reinforcing properties in polymer/nanocomposites have led to the development of synthetic materials with significantly enhanced mechanical strength[330, 331].

Incorporating GO in natural or synthetic polymers are a very effective method for preparing graphene based polymer nanocomposites. Since GO contains abundant oxygen-containing groups e.g. hydroxyls, epoxides, diols, ketones and carboxyl on its surface[330], these can promote interfacial interactions with other materials. Furthermore, it is observed experimentally that by the addition of very minute amounts, (e.g. 1wt% of GO in polymer matrix) lead to a significant increase in their physical properties. In a recent study, the reinforcing effects of GO in a gelatine matrix have been studied in detail, in particular the size and morphology of GO sheets, the degree of dispersion of the GO sheets in gelatine matrix and the interactions of two phases. Results obtained in this study indicated that in gelatine-GO composites, an enhancement of the mechanical properties (tensile strength, Young's modulus and energy at break) of gelatine increases by 84%, 65% and 158%, respectively just by addition of 1 weight% of GO. Furthermore, bio mineralization and biocompatibility of gelatine was also enhanced. In spite of these attributes, moisture sensitivity and toughness[314], use of gelatine based materials for bone tissue engineering have been limited. Chitosan and epoxy based materials have also been used, but most of the GO-polymer composites reported in literature exhibit reduced ultimate strain or toughness[330, 332, 333] so their use as bone substitutes have been limited to date.

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 bone[336-341] 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 survive [342]. 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 cells[345] and biomolecules (e.g. heparin[346]) 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

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.

References:

1. Wilson RS. New absorbable explants using gelatin and synthetic materials. *Trans Am Ophthalmol Soc.* 1983; **81**:966.
2. Yannas IV. Tissue and organ regeneration in adults. Springer Science & Business Media.
3. Kuchler-Bopp S, Larrea A, Petry L, et al. Promoting bioengineered tooth innervation using nanostructured and hybrid scaffolds. *Acta Biomater.* 2017; **50**:493-501.
4. Khademhosseini A, Langer R, Borenstein J, et al. Microscale technologies for tissue engineering and biology. *Proc Natl Acad Sci U S A.* 2006; **103**(8):2480-2487.
5. Santos MI, Reis RL. Vascularization in Bone Tissue Engineering: Physiology, Current Strategies, Major Hurdles and Future Challenges. *Macromol Biosci.* 2010; **10**(1):12-27.
6. Hubbell JA, Langer R. Tissue Engineering. *Chem Eng News* 1995; **73**(11):42-54.
7. Langer R, Vacanti J. Tissue engineering. *Science.* 1993; **260**(5110):920-926.
8. Guo B, Lei B, Li P, et al. Functionalized scaffolds to enhance tissue regeneration. *Regen biomater.* 2015; **2**(1):47-57.
9. Bacelar AH, Cengiz IF, Silva-Correia J, et al. "Smart" Hydrogels in Tissue Engineering and Regenerative Medicine Applications. *Handbook of intelligent scaffolds for tissue engineering and regenerative medicine.* 2017; (2):327-361.
10. Chen B, Liebman C, Rabbani P, et al. Stem Cell Tissue Engineering and Regenerative Medicine. First ed. Mrignayani Kotecha RLM, Jeremy J. Mao, editor. Date|. John Wiley & Sons, Inc.
11. Gomes ME, Rodrigues MT, Domingues RM, et al. Tissue Engineering and Regenerative Medicine: New Trends and Directions—A Year in Review. *Tissue Engineering Part B: Reviews.* 2017; **23**(3):211-224.
12. Apicella A, Apicella D, Syed J, et al. Innovative Biomaterials in Bone Tissue Engineering and Regenerative Medicine. MSCs and Innovative Biomaterials in Dentistry. p. 63-84 2017: Springer.
13. Vial S, Reis RL, Oliveira JM. Recent advances using gold nanoparticles as a promising multimodal tool for tissue engineering and regenerative medicine. *Curr Opin Solid State Mater Sci.* 2017; **21**(2):92-112.
14. Balakrishnan B, Banerjee R. Biopolymer-Based Hydrogels for Cartilage Tissue Engineering. *Chem Rev.* 2011; **111**(8):4453-4474.
15. Gauvin R, Khademhosseini A. Microscale Technologies and Modular Approaches for Tissue Engineering: Moving toward the Fabrication of Complex Functional Structures. *ACS Nano.* 2011; **5**(6):4258-4264.
16. Ma PX. Biomimetic Materials for Tissue Engineering. *Adv Drug Del Rev.* 2008; **60**(2):184-198.
17. Van Vlierberghe S, Dubruel P, Schacht E. Biopolymer-Based Hydrogels As Scaffolds for Tissue Engineering Applications: A Review. *Biomacromolecules.* 2011; **12**(5):1387-1408.
18. Pacelli S, Basu S, Whitlow J, et al. Strategies to develop endogenous stem cell-recruiting bioactive materials for tissue repair and regeneration. *Adv Drug Deliv Rev.* 2017; **120**:50-70.
19. Ravnic DJ, Leberfinger AN, Koduru SV, et al. Transplantation of Bioprinted Tissues and Organs: Technical and Clinical Challenges and Future Perspectives. *Ann Surg.* 2017; **266**(1):48-58.
20. Wu W, Cheng R, das Neves J, et al. Advances in biomaterials for preventing tissue adhesion. *J Controlled Release.* 2017; **261**:318-336.
21. Jakus AE, Laronda MM, Rashedi AS, et al. "Tissue Papers" from Organ-Specific Decellularized Extracellular Matrices. *Adv Funct Mater.* 2017; **27**:1700992.
22. Peng G, Liu H, Fan Y. Biomaterial scaffolds for reproductive tissue engineering. *Ann Biomed Eng.* 2017; **45**(7):1592-1607.
23. Haupt K. Biomaterials: Plastic antibodies. *Nat Mater.* 2010; **9**(8):612-4.

24. Misra SK, Valappil SP, Roy I, et al. Polyhydroxyalkanoate (PHA)/Inorganic Phase Composites for Tissue Engineering Applications. *Biomacromolecules.* 2006; **7**(8):2249-2258.
25. Gupta A, Dixit A, Sales KM, et al. Tissue Engineering of Small IntestineCurrent Status. *Biomacromolecules.* 2006; **7**(10):2701-2709.
26. Rosellini E, Barbani N, Giusti P, et al. Molecularly imprinted nanoparticles with recognition properties towards a laminin H-Tyr-Ile-Gly-Ser-Arg-OH sequence for tissue engineering applications. *Biomed Mater.* 2010; **5**(6):065007.
27. Khan AS, Hussain AN, Sidra L, et al. Fabrication and in vivo evaluation of hydroxyapatite/carbon nanotube electrospun fibers for biomedical/dental application. *Materials Science and Engineering: C.* 2017; **80**:387-396.
28. Xiao W, He J, Nichol JW, et al. Synthesis and characterization of photocrosslinkable gelatin and silk fibroin interpenetrating polymer network hydrogels. *Acta Biomater.* 2011; **7**(6):2384-2393.
29. Park JH, Hong JM, Ju YM, et al. A novel tissue-engineered trachea with a mechanical behavior similar to native trachea. *Biomaterials.* 2015; **62**(0):106-115.
30. Murphy SV, Atala A. 3D bioprinting of tissues and organs [Research]. *Nat Biotech.* 2014; **32**(8):773-785.
31. Koroleva A, Gill AA, Ortega I, et al. Two-photon polymerization-generated and micromolding-replicated 3D scaffolds for peripheral neural tissue engineering applications. *Biofabrication.* 2012; **4**(2):025005.
32. Vasiev I, Greer AIM, Khokhar AZ, et al. Self-folding nano- and micropatterned hydrogel tissue engineering scaffolds by single step photolithographic process. *Microelectron Eng.* 2013; **108**(0):76-81.
33. Cunniffe GM, Gonzalez-Fernandez T, Daly A, et al. Three-Dimensional Bioprinting of Polycaprolactone Reinforced Gene Activated Bioinks for Bone Tissue Engineering. *Tissue Eng Part A.* 2017; **23**:891-900.
34. Murphy C, Kolan K, Li W, et al. 3D bioprinting of stem cells and polymer/bioactive glass composite scaffolds for bone tissue engineering. *International Journal of Bioprinting.* 2017; **3**(1):1-11.
35. Costa RM, Rauf S, Hauser CA. Towards biologically relevant synthetic designer matrices in 3D bioprinting for tissue engineering and regenerative medicine. *Current Opinion in Biomedical Engineering.* 2017; **2**:90-98.
36. Nguyen D, Hägg DA, Forsman A, et al. Cartilage Tissue Engineering by the 3D Bioprinting of iPS Cells in a Nanocellulose/Alginate Bioink. *Scientific Reports.* 2017; **7**(1):658.
37. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. *Prog Polym Sci.* 2007; **32**(8):762-798.
38. Tian H, Tang Z, Zhuang X, et al. Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. *Prog Polym Sci.* 2012; **37**(2):237-280.
39. Kim B-S, Baez CE, Atala A. Biomaterials for tissue engineering. *World J Urol.* 2000; **18**(1):2-9.
40. Bergsma JE, Rozema FR, Bos RRM, et al. Biocompatibility and degradation mechanisms of predegraded and non-predegraded poly(lactide) implants: an animal study. *J Mater Sci Mater Med.* 1995; **6**(12):715-724.
41. Vert M, Hellwich K-H, Hess M, et al. Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). *Pure Appl Chem.* 2012; **84**(2):377-410.
42. Jones RG, Wilks ES, Metanomski WV, et al. Compendium of Polymer Terminology and Nomenclature: IUPAC Recommendations, 2008. Royal Society of Chemistry Cambridge.
43. Amass W, Amass A, Tighe B. A review of biodegradable polymers: uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. *Polym Int.* 1998; **47**(2):89-144.

44. McNaught AD. Compendium of chemical terminology. Vol. 1669. Blackwell Science Oxford.
45. Navarro M, Michiardi A, Castaño O, et al. Biomaterials in orthopaedics. *J R Soc Interface.* 2008; **5**(27):1137-1158.
46. Khan A, Wong F, McKay I, et al. Structural, mechanical, and biocompatibility analyses of a novel dental restorative nanocomposite. *J Appl Polym Sci.* 2013; **127**(1):439-447.
47. Khan AF, Afzal A, Chaudhary AA, et al. (Hydroxypropyl) methylcellulose Mediated Synthesis of Highly Porous Composite Scaffolds for Trabecular Bone Repair Applications. *Sci Adv Mater.* 2015; **7**(6):1177-1186.
48. Khan AS, Aziz MS, Paul D, et al. Synthesis and in-vitro analysis of degradative resistance of a novel bioactive composite. *Journal of Bionanoscience.* 2008; **2**(2):75-88.
49. Sabir M, Xu X, Li L. A review on biodegradable polymeric materials for bone tissue engineering applications. *J Mat Sci.* 2009; **44**(21):5713-5724.
50. Gomes ME, Reis R. Biodegradable polymers and composites in biomedical applications: from catgut to tissue engineering. Part 2 Systems for temporary replacement and advanced tissue regeneration. *Int Mater Rev.* 2004; **49**(5):274-285.
51. Gomes ME, Reis R. Biodegradable polymers and composites in biomedical applications: from catgut to tissue engineering. Part 1 Available systems and their properties. *Int Mater Rev.* 2004; **49**(5):261-273.
52. Guo B, Ma PX. Synthetic biodegradable functional polymers for tissue engineering: a brief review. *Science China Chemistry.* 2014; **57**(4):490-500.
53. Rich J, Korhonen H, Hakala R, et al. Porous Biodegradable Scaffold: Predetermined Porosity by Dissolution of Poly(ester-anhydride) Fibers from Polyester Matrix. *Macromol Biosci.* 2009; **9**(7):654-660.
54. Rhim J-W, Mohanty AK, Singh SP, et al. Effect of the processing methods on the performance of polylactide films: Thermocompression versus solvent casting. *J Appl Polym Sci.* 2006; **101**(6):3736-3742.
55. Gentile P, Chiono V, Carmagnola I, et al. An Overview of Poly (lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering. *Int J Mol Sci.* 2014; **15**(3):3640-3659.
56. Ma PX. Scaffolds for tissue fabrication. *Mater Today.* 2004; **7**(5):30-40.
57. Vacanti JP, Langer R. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. *The Lancet.* 1999; **354**:S32-S34.
58. Chang L, Liu J, Zhang J, et al. pH-sensitive nanoparticles prepared from amphiphilic and biodegradable methoxy poly (ethylene glycol)-block-(polycaprolactone-graft-poly (methacrylic acid)) for oral drug delivery. *Polymer Chemistry.* 2013; **4**(5):1430-1438.
59. E McNeil S, R Griffiths H, Perrie Y. Polycaprolactone fibres as a potential delivery system for collagen to support bone regeneration. *Curr Drug Del.* 2011; **8**(4):448-455.
60. Pillai CKS, Sharma CP. Review Paper: Absorbable Polymeric Surgical Sutures: Chemistry, Production, Properties, Biodegradability, and Performance. *J Biomater Appl.* 2010; **25**(4):291-366.
61. Lee HS, Park SH, Lee JH, et al. Antimicrobial and biodegradable PLGA medical sutures with natural grapefruit seed extracts. *Mater Lett.* 2013; **95**(0):40-43.
62. Shin H, Jo S, Mikos AG. Biomimetic materials for tissue engineering. *Biomaterials.* 2003; **24**(24):4353-4364.
63. Wen X, Tresco PA. Fabrication and characterization of permeable degradable poly(DL-lactide-co-glycolide) (PLGA) hollow fiber phase inversion membranes for use as nerve tract guidance channels. *Biomaterials.* 2006; **27**(20):3800-3809.
64. Bhardwaj N, Sow WT, Devi D, et al. Silk fibroin-keratin based 3D scaffolds as a dermal substitute for skin tissue engineering. *Integr Biol.* 2015; **7**(1):53-63.

65. Nguyen X-T, Van Toi V, Nguyen T-H. Development of a New Injectable PVA–Ag NPs/ Chitosan Hydrogel for Wound Dressing Application. 5th International Conference on Biomedical Engineering in Vietnam. In: Toi VV, Lien Phuong TH, editors. IFMBE Proceedings. Vol. 46. p. 321-324 2015: Springer International Publishing.
66. Kanitkar A, Smoak M, Chen C, et al. Synthesis of novel polyesters for potential applications in skin tissue engineering. *J Chem Technol Biotechnol.* 2016; **91**:733-741.
67. Bendix D. Chemical synthesis of polylactide and its copolymers for medical applications. *Polym Degrad Stab.* 1998; **59**(1):129-135.
68. Rehman IU. Biodegradable polyurethanes: biodegradable low adherence films for the prevention of adhesions after surgery. *J Biomater Appl.* 1996; **11**(2):182-257.
69. Sitharaman B, Shi X, Tran LA, et al. Injectable in situ cross-linkable nanocomposites of biodegradable polymers and carbon nanostructures for bone tissue engineering. *J Biomater Sci Polym Ed.* 2007; **18**(6):655-671.
70. Dhandayuthapani B, Yoshida Y, Maekawa T, et al. Polymeric scaffolds in tissue engineering application: a review. *International Journal of Polymer Science.* 2011; **2011**:290602.
71. Swetha M, Sahithi K, Moorthi A, et al. Biocomposites containing natural polymers and hydroxyapatite for bone tissue engineering. *Int J Biol Macromol.* 2010; **47**(1):1-4.
72. Rogalski J, Krasowska B, Glowiak G, et al. Purification and some properties of extracellular chitinases produced by Trichoderma viride F-19. *Acta Microbiol Pol.* 1997; **46**(4).
73. Tronsmo A, Harman GE. Detection and Quantification of N-Acetyl-β-D-glucosaminidase, Chitobiosidase, and Endochitinase in Solutions and on Gels. *Anal Biochem.* 1993; **208**(1):74-79.
74. Felse P, Panda T. Production of microbial chitinases–A revisit. *Bioprocess Eng.* 2000; **23**(2):127-134.
75. Bolar JP, Norelli JL, Harman GE, et al. Synergistic activity of endochitinase and exochitinase from Trichoderma atroviride (T. harzianum) against the pathogenic fungus (Venturia inaequalis) in transgenic apple plants. *Transgenic Res.* 2001; **10**(6):533-543.
76. van der Rest M, Dublet B, Champliaud MF. Fibril-associated collagens. *Biomaterials.* 1990; **11**:28-31.
77. Pachence JM. Collagen-based devices for soft tissue repair. *J Biomed Mater Res.* 1996; **33**(1):35-40.
78. Anselme K, Bacques C, Charriere G, et al. Tissue reaction to subcutaneous implantation of a collagen sponge. A histological, ultrastructural, and immunological study. *J Biomed Mater Res.* 1990; **24**(6):689-703.
79. Zhang Z, Ortiz O, Goyal R, et al. Chapter 23 - Biodegradable Polymers. Principles of Tissue Engineering (Fourth Edition). In: Lanza R, Langer R, Vacanti J, editors. p. 441-473 2014 Boston: Academic Press.
80. Charvolin J, Sadoc J-F. About collagen, a tribute to Yves Bouligand. *Interface focus.* 2012; **2**(5):567-574.
81. Khan AS, Aamer S, Chaudhry AA, et al. Synthesis and characterizations of a fluoride-releasing dental restorative material. *Mat Sci Eng C.* 2013; **33**(6):3458-3464.
82. Khan AS, Ahmed Z, Edirisinghe MJ, et al. Preparation and characterization of a novel bioactive restorative composite based on covalently coupled polyurethane–nanohydroxyapatite fibres. *Acta Biomater.* 2008; **4**(5):1275-1287.
83. Talal A, McKay I, Tanner K, et al. Effects of hydroxyapatite and PDGF concentrations on osteoblast growth in a nanohydroxyapatite-polylactic acid composite for guided tissue regeneration. *J Mater Sci Mater Med.* 2013; **24**(9):2211-2221.

84. Cai Q, Yang J, Bei J, et al. A novel porous cells scaffold made of polylactide–dextran blend by combining phase-separation and particle-leaching techniques. *Biomaterials.* 2002; **23**(23):4483-4492.
85. Maquet V, Boccaccini AR, Pravata L, et al. Porous poly(α-hydroxyacid)/Bioglass® composite scaffolds for bone tissue engineering. I: preparation and in vitro characterisation. *Biomaterials.* 2004; **25**(18):4185-4194.
86. Wei G, Ma PX. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials.* 2004; **25**(19):4749-4757.
87. Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. *Eur J Dermatol.* 2002; **12**(4):390-9.
88. Mansbridge J. Chapter 81 - Tissue-Engineered Skin Products. Principles of Tissue Engineering (Fourth Edition). In: Lanza R, Langer R, Vacanti J, editors. p. 1697-1715 2014 Boston: Academic Press.
89. Shahzadi L, Yar M, Jamal A, et al. Triethyl orthoformate covalently cross-linked chitosan-(poly vinyl) alcohol based biodegradable scaffolds with heparin-binding ability for promoting neovascularisation. *J Biomater Appl.* 2016; **31**(4):582-593.
90. MacNeil S. Progress and opportunities for tissue-engineered skin. *Nature.* 2007; **445**(7130):874-880.
91. Vig K, Chaudhari A, Tripathi S, et al. Advances in Skin Regeneration Using Tissue Engineering. *Int J Mol Sci.* 2017; **18**(4):789.
92. Jeon EY, Choi B-H, Jung D, et al. Natural healing-inspired collagen-targeting surgical protein glue for accelerated scarless skin regeneration. *Biomaterials.* 2017; **134**:154-165.
93. Bhardwaj N, Chouhan D, Mandal B. Tissue engineered skin and wound healing: current strategies and future directions. *Curr Pharm Des.* 2017; **23**(24):3455-3482.
94. Papuga AY, Lukash LL. Different types of biotechnological wound coverages created with the application of alive human cells. *Biopolym Cell.* 2015; **31**(2):83-96.
95. David L. Snyder, Nancy Sullivan, Karen M. Schoelles. Skin Substitutes for Treating Chronic Wounds.: Agency for Healthcare Research and Quality MD USA; 2012.
96. Blakely M. The Use of Best Practice in the Treatment of a Complex Diabetic Foot Ulcer: A Case Report. *Healthcare.* 2016; **4**(1):18.
97. Kamel RA, Ong JF, Eriksson E, et al. Tissue Engineering of Skin. *J Am Coll Surg.* 2013; **217**(3):533-555.
98. Supp DM, Boyce ST. Engineered skin substitutes: practices and potentials. *Clin Dermatol.* 2005; **23**(4):403-412.
99. Balasubramani M, Kumar TR, Babu M. Skin substitutes: a review. *Burns.* 2001; **27**(5):534-544.
100. Jones I, Currie L, Martin R. A guide to biological skin substitutes. *Br J Plast Surg.* 2002; **55**(3):185-193.
101. Shakespeare PG. The role of skin substitutes in the treatment of burn injuries. *Clin Dermatol.* 2005; **23**(4):413-418.
102. Shakespeare P. Burn wound healing and skin substitutes. *Burns.* 2001; **27**(5):517-522.
103. Metcalfe AD, Ferguson MWJ. Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. *J R Soc Interface.* 2007; **4**(14):413-437.
104. Groeber F, Holeiter M, Hampel M, et al. Skin tissue engineering — In vivo and in vitro applications. *Adv Drug Del Rev.* 2011; **63**(4–5):352-366.
105. Ru C, Wang F, Pang M, et al. Suspended, Shrinkage-Free, Electrospun PLGA Nanofibrous Scaffold for Skin Tissue Engineering. *ACS applied materials & interfaces.* 2015; **7**(20):10872-10877.

106. Ramos DM, Subramanian A, Aravamudhan A, et al. Nanotissue Engineering of Skin Cells. Stem-Cell Nanoengineering. p. 315-326 2015: John Wiley & Sons, Inc.
107. Huss FRM, Nyman E, Gustafson C-J, et al. Characterization of a new degradable polymer scaffold for regeneration of the dermis: In vitro and in vivo human studies. *Organogenesis.* 2008; **4**(3):195-200.
108. Chua AWC, Khoo YC, Tan BK, et al. Skin tissue engineering advances in severe burns: review and therapeutic applications. *Burns & trauma.* 2016; **4**(1):3.
109. Greenwood JE LA, Dearman BL, Moore TG. Evaluation of NovoSorb™ novel biodegradable polymer for the generation of a dermal matrix Part 1: In-vitro *Wound Practice & Research: Journal of the Australian Wound Management Association.* 2010; **18**(1):14-22.
110. Akita S, Akino K, Imaizumi T, et al. A polyurethane dressing is beneficial for split-thickness skin-graft donor wound healing. *Burns.* 2006; **32**(4):447-451.
111. Cho YS, Lee JW, Lee JS, et al. Hyaluronic acid and silver sulfadiazine-impregnated polyurethane foams for wound dressing application. *J Mater Sci Mater Med.* 2002; **13**(9):861-865.
112. Martineau L, Shek PN. Evaluation of a bi-layer wound dressing for burn care: I. Cooling and wound healing properties. *Burns.* 2006; **32**(1):70-76.
113. Phan TT, Lim IJ, Tan EK, et al. Evaluation of cell culture on the polyurethane-based membrane (TegadermTM): implication for tissue engineering of skin. *Cell Tissue Banking.* 2005; **6**(2):91-97.
114. Greenwood JE LA, Dearman BL , Moore TG. Evaluation of NovoSorb™ novel biodegradable polymer for the generation of a dermal matrix Part 2: In-vivo Studies. *Wound Practice & Research: Journal of the Australian Wound Management Association.* 2010; **18** (1):24-34.
115. Wang H-M, Chou Y-T, Wen Z-H, et al. Novel Biodegradable Porous Scaffold Applied to Skin Regeneration. *PLoS ONE.* 2013; **8**(6):e56330.
116. Lagus H, Sarlomo-Rikala M, Bohling T, et al. Prospective study on burns treated with Integra(R), a cellulose sponge and split thickness skin graft: comparative clinical and histological study--randomized controlled trial. *Burns.* 2013; **39**(8):1577-87.
117. Berger A, Tanzella U, Machens HG, et al. Der Einsatz von Integra bei primären Verbrennungswunden und instabilen sekundären Narben. *Der Chirurg.* 2000; **71**(5):558-563.
118. Martson M, Viljanto J, Laippala P, et al. Connective tissue formation in subcutaneous cellulose sponge implants in the rat. The effect of the size and cellulose content of the implant. *Eur Surg Res.* 1998; **30**(6):419-25.
119. Viljanto J. Cellstic: A device for wound healing studies in man. Description of the method. *J Surg Res.* 1976; **20**(2):115-119.
120. Märtson M, Viljanto J, Hurme T, et al. Is cellulose sponge degradable or stable as implantation material? An in vivo subcutaneous study in the rat. *Biomaterials.* 1999; **20**(21):1989-1995.
121. Swope VB, Supp AP, Schwemberger S, et al. Increased expression of integrins and decreased apoptosis correlate with increased melanocyte retention in cultured skin substitutes. *Pigm Cell Res.* 2006; **19**(5):424-433.
122. Hedley SJ, Layton C, Heaton M, et al. Fibroblasts Play a Regulatory Role in the Control of Pigmentation in Reconstructed Human Skin from Skin Types I and II. *Pigm Cell Res.* 2002; **15**(1):49-56.
123. You H-J, Han S-K. Cell therapy for wound healing. *J Korean Med Sci.* 2014; **29**(3):311-319.
124. Mathioudakis N, Hicks CW, Canner JK, et al. The Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system predicts wound healing but not major amputation in patients with diabetic foot ulcers treated in a multidisciplinary setting. *J Vasc Surg.* 2017; **65**(6):1698-1705. e1.

125. Jamal A, Shahzadi L, Ahtzaz S, et al. Identification of anti-cancer potential of doxazocin: Loading into chitosan based biodegradable hydrogels for on-site delivery to treat cervical cancer. *Mat Sci Eng C.* 2018; **82**:102-109.
126. Kim Y-J, Jeong J-H. Clinical application of adipose stem cells in plastic surgery. *J Korean Med Sci.* 2014; **29**(4):462-467.
127. Wang S, Wan ACA, Xu X, et al. A new nerve guide conduit material composed of a biodegradable poly(phosphoester). *Biomaterials.* 2001; **22**(10):1157-1169.
128. Wan ACA, Mao H-Q, Wang S, et al. Fabrication of poly(phosphoester) nerve guides by immersion precipitation and the control of porosity. *Biomaterials.* 2001; **22**(10):1147-1156.
129. Bini TB, Gao S, Xu X, et al. Peripheral nerve regeneration by microbraided poly(L-lactide-co-glycolide) biodegradable polymer fibers. *J Biomed Mater Res Part A.* 2004; **68A**(2):286-295.
130. B BT, Gao S, Wang S, et al. Development of fibrous biodegradable polymer conduits for guided nerve regeneration. *J Mater Sci Mater Med.* 2005; **16**(4):367-375.
131. Elena Llorense EA, María del Mar Pérez-Madrigale, Luís Javier del Vallee, Carlos Alemán and Jordi Puiggalí. Nanomembranes and Nanofibers from Biodegradable Conducting Polymers. *Polymers.* 2013; **5**(3):1115-1157.
132. Liu Y, Hu J, Zhuang X, et al. Preparation and Characterization of Biodegradable and Electroactive Polymer Blend Materials Based on mPEG/Tetraaniline and PLLA. *Macromol Biosci.* 2011; **11**(6):806-813.
133. Zhang Q, Yan Y, Li S, et al. The synthesis and characterization of a novel biodegradable and electroactive polyphosphazene for nerve regeneration. *Mater Sci Eng C.* 2010; **30**(1):160-166.
134. Guo B, Sun Y, Finne-Wistrand A, et al. Electroactive porous tubular scaffolds with degradability and non-cytotoxicity for neural tissue regeneration. *Acta Biomater.* 2012; **8**(1):144-153.
135. McKeon KD, Lewis A, Freeman JW. Electrospun poly(D,L-lactide) and polyaniline scaffold characterization. *J Appl Polym Sci.* 2010; **115**(3):1566-1572.
136. Bettinger CJ, Bruggeman JP, Misra A, et al. Biocompatibility of biodegradable semiconducting melanin films for nerve tissue engineering. *Biomaterials.* 2009; **30**(17):3050-3057.
137. Krarup C AS, Madison RD. Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve. *Ann Neurol.* 2002; **5**(1):69-81.
138. McLaughlin CR, Acosta MC, Luna C, et al. Regeneration of functional nerves within full thickness collagen–phosphorylcholine corneal substitute implants in guinea pigs. *Biomaterials.* 2010; **31**(10):2770-2778.
139. Yamaguchi I, Itoh S, Suzuki M, et al. The chitosan prepared from crab tendon I: the characterization and the mechanical properties. *Biomaterials.* 2003; **24**(12):2031-2036.
140. Hashimoto T, Suzuki Y, Suzuki K, et al. Review Peripheral nerve regeneration using non-tubular alginate gel crosslinked with covalent bonds. *J Mater Sci Mater Med.* 2005; **16**(6):503-509.
141. Yoshii S, Oka M, lkeda N, et al. Bridging a peripheral nerve defect using collagen filaments. *Journal of Hand Surgery.* **26**(1):52-59.
142. Wu Sufan YS, Masao Tanihara, et al. Sciatic Nerve Regeneration Through Alginate With Tubulation or Nontubulation Repair in Cat *J Neurotrauma.* 2001; **18**(3):329-338.
143. Vaz CM, van Tuijl S, Bouten CVC, et al. Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique. *Acta Biomater.* 2005; **1**(5):575-582.
144. Li M, Mondrinos MJ, Gandhi MR, et al. Electrospun protein fibers as matrices for tissue engineering. *Biomaterials.* 2005; **26**(30):5999-6008.
145. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. *Science.* 1986; **231**(4736):397-400.
146. Niklason LE, Gao J, Abbott WM, et al. Functional Arteries Grown in Vitro. *Science.* 1999; **284**(5413):489-493.

147. Watanabe M, Shin'oka T, Tohyama S, et al. Tissue-engineered vascular autograft: inferior vena cava replacement in a dog model. *Tissue Eng.* 2001; **7**(4):429-439.
148. Shin'oka T, Imai Y, Ikada Y. Transplantation of a Tissue-Engineered Pulmonary Artery. *N Engl J Med.* 2001; **344**(7):532-533.
149. Habermehl J, Skopinska J, Boccafoschi F, et al. Preparation of Ready-to-use, Stockable and Reconstituted Collagen. *Macromol Biosci.* 2005; **5**(9):821-828.
150. Cummings CL, Gawlitta D, Nerem RM, et al. Properties of engineered vascular constructs made from collagen, fibrin, and collagen–fibrin mixtures. *Biomaterials.* 2004; **25**(17):3699-3706.
151. Swartz DD, Russell JA, Andreadis ST. Engineering of fibrin-based functional and implantable small-diameter blood vessels. *Am J Physiol Heart Circ Physiol.* 2005; **288**(3):H1451-H1460.
152. Lee SJ, Yoo JJ, Lim GJ, et al. In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. *J Biomed Mater Res A.* 2007; **83A**(4):999-1008.
153. Stankus JJ, Soletti L, Fujimoto K, et al. Fabrication of cell microintegrated blood vessel constructs through electrohydrodynamic atomization. *Biomaterials.* 2007; **28**(17):2738-2746.
154. Barnes CP, Sell SA, Boland ED, et al. Nanofiber technology: Designing the next generation of tissue engineering scaffolds. *Adv Drug Del Rev.* 2007; **59**(14):1413-1433.
155. Ravi S, Chaikof EL. Biomaterials for vascular tissue engineering. *Regenerative Medicine.* 2009; **5**(1):107-120.
156. Calogero Fiorica SR, Fabio S. Palumbo, et al. A Fibrillar Biodegradable Scaffold for Blood Vessels Tissue Engineering. *CET chemical engineering transactions.* 2012; **27**:403-408.
157. Nottelet B, Pektok E, Mandracchia D, et al. Factorial design optimization and in vivo feasibility of poly(ε-caprolactone)-micro- and nanofiber-based small diameter vascular grafts. *J Biomed Mater Res A.* 2009; **89A**(4):865-875.
158. Kawaguchi N, Hatta K, Nakanishi T. 3D-culture system for heart regeneration and cardiac medicine. *Biomed Res Int.* 2013; **2013**:895967.
159. Forouzanfar MH, Moran AE, Flaxman AD, et al. Assessing the Global Burden of Ischemic Heart Disease. *Global Heart.* 2014; **7**(4):331-342.
160. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science.* 2009; **324**(5923):98-102.
161. Ruvinov E, Dvir T, Leor J, et al. Myocardial repair: from salvage to tissue reconstruction. *Expert Rev Cardiovasc Ther.* 2008; **6**(5):669-86.
162. Shahabipour F, Banach M, Johnston TP, et al. Novel approaches toward the generation of bioscaffolds as a potential therapy in cardiovascular tissue engineering. *Int J Cardiol.* 2017; **228**:319-326.
163. Puppi D, Pirosa A, Lupi G, et al. Design and fabrication of novel polymeric biodegradable stents for small caliber blood vessels by computer-aided wet-spinning. *Biomedical Materials* 2017; **12**(3):035011.
164. Silvestri A, Boffito M, Sartori S, et al. Biomimetic materials and scaffolds for myocardial tissue regeneration. *Macromol Biosci.* 2013; **13**(8):984-1019.
165. Shapira A, Feiner R, Dvir T. Composite biomaterial scaffolds for cardiac tissue engineering. *Int Mater Rev.* 2016; **61**(1):1-19.
166. Ye Q, Zünd G, Benedikt P, et al. Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. *Eur J Cardiothorac Surg.* 2000; **17**(5):587-591.
167. Mol A, van Lieshout MI, Dam-de Veen CG, et al. Fibrin as a cell carrier in cardiovascular tissue engineering applications. *Biomaterials.* 2005; **26**(16):3113-3121.
168. Flanagan TC, Cornelissen C, Koch S, et al. The in vitro development of autologous fibrin-based tissue-engineered heart valves through optimised dynamic conditioning. *Biomaterials.* 2007; **28**(23):3388-3397.

169. Christman KL, Fok HH, Sievers RE, et al. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng.* 2004; **10**(3-4):403-409.
170. Christman KL, Vardanian AJ, Fang Q, et al. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J Am Coll Cardiol.* 2004; **44**(3):654-660.
171. Deng C, Zhang P, Vulesevic B, et al. A collagen–chitosan hydrogel for endothelial differentiation and angiogenesis. *Tissue Engineering Part A.* 2010; **16**(10):3099-3109.
172. Patra C, Talukdar S, Novoyatleva T, et al. Silk protein fibroin from Antheraea mylitta for cardiac tissue engineering. *Biomaterials.* 2012; **33**(9):2673-2680.
173. Gnanaprakasam Thankam F, Muthu J, Sankar V, et al. Growth and survival of cells in biosynthetic poly vinyl alcohol–alginate IPN hydrogels for cardiac applications. *Colloids Surf B Biointerfaces.* 2013; **107**:137-145.
174. Rosellini E, Cristallini C, Barbani N, et al. Preparation and characterization of alginate/gelatin blend films for cardiac tissue engineering. *J Biomed Mater Res A.* 2009; **91**(2):447-453.
175. Kim D-H, Smith JT, Chilkoti A, et al. The effect of covalently immobilized rhIL-1ra-ELP fusion protein on the inflammatory profile of LPS-stimulated human monocytes. *Biomaterials.* 2007; **28**(23):3369-3377.
176. Balguid A, Rubbens MP, Mol A, et al. The role of collagen cross-links in biomechanical behavior of human aortic heart valve leaflets-relevance for tissue engineering. *Tissue Eng.* 2007; **13**(7):1501-1511.
177. Flanagan TC, Wilkins B, Black A, et al. A collagen-glycosaminoglycan co-culture model for heart valve tissue engineering applications. *Biomaterials.* 2006; **27**(10):2233-2246.
178. Heydarkhan-Hagvall S, Schenke-Layland K, Dhanasopon AP, et al. Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering. *Biomaterials.* 2008; **29**(19):2907-2914.
179. Landa N, Miller L, Feinberg MS, et al. Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat. *Circulation.* 2008; **117**(11):1388-1396.
180. Hao X, Silva EA, Månsson-Broberg A, et al. Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. *Cardiovasc Res.* 2007; **75**(1):178-185.
181. Lee J, Tan CY, Lee S-K, et al. Controlled delivery of heat shock protein using an injectable microsphere/hydrogel combination system for the treatment of myocardial infarction. *J Controlled Release.* 2009; **137**(3):196-202.
182. Ruvinov E, Leor J, Cohen S. The promotion of myocardial repair by the sequential delivery of IGF-1 and HGF from an injectable alginate biomaterial in a model of acute myocardial infarction. *Biomaterials.* 2011; **32**(2):565-578.
183. Ishii O, Shin M, Sueda T, et al. In vitro tissue engineering of a cardiac graft using a degradable scaffold with an extracellular matrix–like topography. *J Thorac Cardiovasc Surg.* 2005; **130**(5):1358-1363.
184. Tian L, Prabhakaran MP, Ding X, et al. Emulsion electrospun vascular endothelial growth factor encapsulated poly (l-lactic acid-co-ε-caprolactone) nanofibers for sustained release in cardiac tissue engineering. *J Mater Sci.* 2012; **47**(7):3272-3281.
185. Ozawa T, Mickle DA, Weisel RD, et al. Optimal biomaterial for creation of autologous cardiac grafts. *Circulation.* 2002; **106**(12):176-182.
186. Kharaziha M, Nikkhah M, Shin S-R, et al. PGS: Gelatin nanofibrous scaffolds with tunable mechanical and structural properties for engineering cardiac tissues. *Biomaterials.* 2013; **34**(27):6355-6366.

187. Ravichandran R, Venugopal JR, Sundarrajan S, et al. Expression of cardiac proteins in neonatal cardiomyocytes on PGS/fibrinogen core/shell substrate for cardiac tissue engineering. *Int J Cardiol.* 2013; **167**(4):1461-1468.
188. Prabhakaran MP, Kai D, Ghasemi-Mobarakeh L, et al. Electrospun biocomposite nanofibrous patch for cardiac tissue engineering. *Biomedical Materials.* 2011; **6**(5):055001.
189. Kai D, Prabhakaran MP, Jin G, et al. Guided orientation of cardiomyocytes on electrospun aligned nanofibers for cardiac tissue engineering. *Journal of Biomedical Materials Research Part B: Applied Biomaterials.* 2011; **98**(2):379-386.
190. Prabhakaran MP, Nair AS, Kai D, et al. Electrospun composite scaffolds containing poly (octanediol-co-citrate) for cardiac tissue engineering. *Biopolymers.* 2012; **97**(7):529-538.
191. Hinderer S, Seifert J, Votteler M, et al. Engineering of a bio-functionalized hybrid off-the-shelf heart valve. *Biomaterials.* 2014; **35**(7):2130-2139.
192. Sant S, Hwang CM, Lee SH, et al. Hybrid PGS–PCL microfibrous scaffolds with improved mechanical and biological properties. *J Tissue Eng Reg Med.* 2011; **5**(4):283-291.
193. Ravichandran R, Venugopal JR, Sundarrajan S, et al. Minimally invasive injectable short nanofibers of poly (glycerol sebacate) for cardiac tissue engineering. *Nanotechnology.* 2012; **23**(38):385102.
194. Pok S, Myers JD, Madihally SV, et al. A multilayered scaffold of a chitosan and gelatin hydrogel supported by a PCL core for cardiac tissue engineering. *Acta Biomater.* 2013; **9**(3):5630-5642.
195. Kai D, Prabhakaran MP, Jin G, et al. Biocompatibility evaluation of electrically conductive nanofibrous scaffolds for cardiac tissue engineering. *J Mater Chem B.* 2013; **1**(17):2305-2314.
196. Tseng H, Puperi DS, Kim EJ, et al. Anisotropic poly (ethylene glycol)/polycaprolactone (PEG/PCL) hydrogel-fiber composites for heart valve tissue engineering. *Tissue Eng Part A.* 2014; **20**:2634-2645.
197. Fong P, Shin'oka T, Lopez-Soler RI, et al. The use of polymer based scaffolds in tissue-engineered heart valves. *Progress in Pediatric cardiology.* 2006; **21**(2):193-199.
198. Martin DP, Williams SF. Medical applications of poly-4-hydroxybutyrate: a strong flexible absorbable biomaterial. *Biochem Eng J.* 2003; **16**(2):97-105.
199. Vunjak-Novakovic G, Lui KO, Tandon N, et al. Bioengineering heart muscle: a paradigm for regenerative medicine. *Annu Rev Biomed Eng.* 2011; **13**:245-67.
200. Hirt MN, Hansen A, Eschenhagen T. Cardiac tissue engineering: state of the art. *Circ Res.* 2014; **114**(2):354-67.
201. Elamparithi A, Punnoose AM, Kuruvilla S. Electrospun type 1 collagen matrices preserving native ultrastructure using benign binary solvent for cardiac tissue engineering. *Artif Cells Nanomed Biotechnol.* 2016; **44**:1318-1325.
202. Ruvinov E, Cohen S. Alginate biomaterial for the treatment of myocardial infarction: Progress, translational strategies, and clinical outlook: From ocean algae to patient bedside. *Adv Drug Deliv Rev.* 2016; **96**:54-76.
203. Avadhanam VS, Smith HE, Liu C. Keratoprostheses for corneal blindness: a review of contemporary devices. *Clin Ophthalmol.* 2015; **9**:697-720.
204. Feng Y, Borrelli M, Reichl S, et al. Review of alternative carrier materials for ocular surface reconstruction. *Curr Eye Res.* 2014; **39**(6):541-52.
205. Ghezzi CE, Rnjak-Kovacina J, Kaplan DL. Corneal Tissue Engineering: Recent Advances and Future Perspectives. *Tissue Eng Part B Rev.* 2015; **21**:278-289.
206. Li F, Carlsson D, Lohmann C, et al. Cellular and nerve regeneration within a biosynthetic extracellular matrix for corneal transplantation. *Proceedings of the National Academy of Sciences.* 2003; **100**(26):15346-15351.

207. Shah A, Brugnano J, Sun S, et al. The development of a tissue-engineered cornea: biomaterials and culture methods. *Pediatr Res.* 2008; **63**(5):535-44.
208. Gao X, Liu W, Han B, et al. Preparation and properties of a chitosan-based carrier of corneal endothelial cells. *J Mater Sci Mater Med.* 2008; **19**(12):3611-3619.
209. Madden PW, Lai JN, George KA, et al. Human corneal endothelial cell growth on a silk fibroin membrane. *Biomaterials.* 2011; **32**(17):4076-4084.
210. Liu Y, Gan L, Carlsson DJ, et al. A simple, cross-linked collagen tissue substitute for corneal implantation. *Invest Ophthalmol Visual Sci.* 2006; **47**(5):1869-1875.
211. Vrana NE, Builles N, Justin V, et al. Development of a reconstructed cornea from collagen–chondroitin sulfate foams and human cell cultures. *Invest Ophthalmol Visual Sci.* 2008; **49**(12):5325-5331.
212. Li F, Griffith M, Li Z, et al. Recruitment of multiple cell lines by collagen-synthetic copolymer matrices in corneal regeneration. *Biomaterials.* 2005; **26**(16):3093-3104.
213. Orwin EJ, Hubel A. In vitro culture characteristics of corneal epithelial, endothelial, and keratocyte cells in a native collagen matrix. *Tissue Eng.* 2000; **6**(4):307-319.
214. Liang Y, Liu W, Han B, et al. An in situ formed biodegradable hydrogel for reconstruction of the corneal endothelium. *Colloids Surf B Biointerfaces.* 2011; **82**(1):1-7.
215. Liu H, Xi P, Xie G, et al. Simultaneous Reduction and Surface Functionalization of Graphene Oxide for Hydroxyapatite Mineralization. *J Phy Chem C.* 2012; **116**(5):3334-3341.
216. Ozcelik B, Brown KD, Blencowe A, et al. Ultrathin chitosan–poly (ethylene glycol) hydrogel films for corneal tissue engineering. *Acta Biomater.* 2013; **9**(5):6594-6605.
217. Mimura T, Amano S, Yokoo S, et al. Tissue engineering of corneal stroma with rabbit fibroblast precursors and gelatin hydrogels. *Mol Vis.* 2008; **14**:1819.
218. Lai J-Y, Ma DH-K, Lai M-H, et al. Characterization of cross-linked porous gelatin carriers and their interaction with corneal endothelium: Biopolymer concentration effect. *PLoS ONE.* 2013; **8**(1):e54058.
219. Lai J-Y, Li Y-T, Cho C-H, et al. Nanoscale modification of porous gelatin scaffolds with chondroitin sulfate for corneal stromal tissue engineering. *Int J Nanomed.* 2012; **7**:1101-1114.
220. Lai J-Y, Li Y-T. Functional assessment of cross-linked porous gelatin hydrogels for bioengineered cell sheet carriers. *Biomacromolecules.* 2010; **11**(5):1387-1397.
221. Lawrence BD, Marchant JK, Pindrus MA, et al. Silk film biomaterials for cornea tissue engineering. *Biomaterials.* 2009; **30**(7):1299-1308.
222. Bray LJ, George KA, Hutmacher DW, et al. A dual-layer silk fibroin scaffold for reconstructing the human corneal limbus. *Biomaterials.* 2012; **33**(13):3529-3538.
223. Higa K, Takeshima N, Moro F, et al. Porous silk fibroin film as a transparent carrier for cultivated corneal epithelial sheets. *J Biomater Sci Polym Ed.* 2011; **22**(17):2261-2276.
224. Klenkler B, Griffith M, Becerril C, et al. EGF-grafted PDMS surfaces in artificial cornea applications. *Biomaterials.* 2005; **26**(35):7286-7296.
225. Ortega Í, Ryan AJ, Deshpande P, et al. Combined microfabrication and electrospinning to produce 3-D architectures for corneal repair. *Acta Biomater.* 2013; **9**(3):5511-5520.
226. Deshpande P, McKean R, Blackwood KA, et al. Using poly (lactide-co-glycolide) electrospun scaffolds to deliver cultured epithelial cells to the cornea. *Regenerative Medicine.* 2010; **5**(3):395-401.
227. Sefat F, McKean R, Deshpande P, et al. Production, Sterilisation and Storage of Biodegradable Electrospun PLGA Membranes for Delivery of Limbal Stem Cells to the Cornea. *Procedia Engineering.* 2013; **59**:101-116.

228. Ortega I, McKean R, Ryan AJ, et al. Characterisation and evaluation of the impact of microfabricated pockets on the performance of limbal epithelial stem cells in biodegradable PLGA membranes for corneal regeneration. *Biomaterials Science.* 2014; **2**:723-734.
229. Deshpande P, Ramachandran C, Sangwan VS, et al. Cultivation of limbal epithelial cells on electrospun poly (lactide-co-glycolide) scaffolds for delivery to the cornea. Corneal Regenerative Medicine,Methods and Protocols, Methods in Molecular Biology,. In: Wright B, Connon, Che J editor. Vol. 1014. p. 179-185 2013 New York: Springer.
230. Deshpande P, Ramachandran C, Sefat F, et al. Simplifying corneal surface regeneration using a biodegradable synthetic membrane and limbal tissue explants. *Biomaterials.* 2013; **34**(21):5088-5106.
231. Chirila TV. An overview of the development of artificial corneas with porous skirts and the use of PHEMA for such an application. *Biomaterials.* 2001; **22**(24):3311-3317.
232. Ozcelik B, Brown KD, Blencowe A, et al. Biodegradable and Biocompatible Poly (Ethylene Glycol)-based Hydrogel Films for the Regeneration of Corneal Endothelium. *Advanced Healthcare Materials.* 2014; **3**:1496-1507.
233. Myung D, Farooqui N, Zheng LL, et al. Bioactive interpenetrating polymer network hydrogels that support corneal epithelial wound healing. *Journal of Biomedical Materials Research Part A.* 2009; **90**(1):70-81.
234. Park S, Nam SH, Koh WG. Preparation of collagen-immobilized poly (ethylene glycol)/poly (2-hydroxyethyl methacrylate) interpenetrating network hydrogels for potential application of artificial cornea. *J Appl Polym Sci.* 2012; **123**(2):637-645.
235. Young T-H, Wang I, Hu F-R, et al. Fabrication of a Bioengineered Corneal Endothelial Cell Sheet Using Chitosan/Polycaprolactone Blend Membranes. *Colloids Surf B Biointerfaces.* 2014; **116**:403-410.
236. Fagerholm P, Lagali NS, Merrett K, et al. A biosynthetic alternative to human donor tissue for inducing corneal regeneration: 24-month follow-up of a phase 1 clinical study. *Sci Transl Med.* 2010; **2**(46):46ra61.
237. Jarade EF, Slim E, Cherfan C, et al. Mathematical analysis of corneal remodelling after intracorneal ring surgery in keratoconus. *International journal of ophthalmology.* 2017; **10**(3):348.
238. Mirazul Islam M, Cepla V, He C, et al. Functional fabrication of recombinant human collagen-phosphorylcholine hydrogels for regenerative medicine applications. *Acta Biomater.* 2015; **12**:70-80.
239. Tsai IL, Hsu CC, Hung KH, et al. Applications of biomaterials in corneal wound healing. *J Chin Med Assoc.* 2015; **78**(4):212-217.
240. Lee MC, Kim DK, Lee OJ, et al. Fabrication of silk fibroin film using centrifugal casting technique for corneal tissue engineering. *J Biomed Mater Res B Appl Biomater.* 2016; **104B**:508-514.
241. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *The Lancet.* 2005; **366**(9499):1809-1820.
242. Villar CC, Cochran DL. Regeneration of Periodontal Tissues: Guided Tissue Regeneration. *Dent Clin North Am.* 2010; **54**(1):73-92.
243. Minabe M. A critical review of the biologic rationale for guided tissue regeneration. *J Periodontol.* 1991; **62**(3):171-179.
244. Karfeld-Sulzer LS, Ghayor C, Siegenthaler B, et al. Comparative study of NMP-preloaded and dip-loaded membranes for guided bone regeneration of rabbit cranial defects. *Journal of tissue engineering and regenerative medicine.* 2017; **11**(2):425-433.
245. Bottino MC, Pankajakshan D, Nör JE. Advanced Scaffolds for Dental Pulp and Periodontal Regeneration. *Dent Clin North Am.* 2017; **61**(4):689-711.

246. Bottino MC, Thomas V, Janowski GM. A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. *Acta Biomater.* 2011; **7**(1):216-224.
247. Kellomäki M, Niiranen H, Puumanen K, et al. Bioabsorbable scaffolds for guided bone regeneration and generation. *Biomaterials.* 2000; **21**(24):2495-2505.
248. Park JK, Yeom J, Oh EJ, et al. Guided bone regeneration by poly(lactic-co-glycolic acid) grafted hyaluronic acid bi-layer films for periodontal barrier applications. *Acta Biomater.* 2009; **5**(9):3394-3403.
249. Gentile P, Chiono V, Tonda-Turo C, et al. Polymeric membranes for guided bone regeneration. *Biotechnology Journal.* 2011; **6**(10):1187-1197.
250. Behring J, Junker R, Walboomers XF, et al. Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. *Odontology.* 2008; **96**(1):1-11.
251. Yamada M, Kojima N, Att W, et al. Improvement in the osteoblastic cellular response to a commercial collagen membrane and demineralized freeze-dried bone by an amino acid derivative: an in vitro study. *Clin Oral Implants Res.* 2011; **22**(2):165-172.
252. Sheikh Z, Khan AS, Roohpour N, et al. Protein adsorption capability on polyurethane and modified-polyurethane membrane for periodontal guided tissue regeneration applications. *Mater Sci Eng C.* 2016; **68**:267-275.
253. Yar M, Farooq A, Shahzadi L, et al. Novel meloxicam releasing electrospun polymer/ceramic reinforced biodegradable membranes for periodontal regeneration applications. *Mater Sci Eng C Mater Biol Appl.* 2016; **64**:148-56.
254. Farooq A, Yar M, Khan AS, et al. Synthesis of piroxicam loaded novel electrospun biodegradable nanocomposite scaffolds for periodontal regeneration. *Mater Sci Eng C Mater Biol Appl.* 2015; **56**:104-13.
255. Trajano VC, Costa KJ, Lanza CR, et al. Osteogenic activity of cyclodextrin-encapsulated doxycycline in a calcium phosphate PCL and PLGA composite. *Mater Sci Eng C Mater Biol Appl.* 2016; **64**:370-5.
256. Kasaj A, Reichert C, Gotz H, et al. In vitro evaluation of various bioabsorbable and nonresorbable barrier membranes for guided tissue regeneration. *Head Face Med.* 2008; **4**(1):22-29.
257. Chen S, Hao Y, Cui W, et al. Biodegradable electrospun PLLA/chitosan membrane as guided tissue regeneration membrane for treating periodontitis. *J Mater Sci.* 2013; **48**(19):6567-6577.
258. Kim YH, Park JH, Lee M, et al. Polyethylenimine with acid-labile linkages as a biodegradable gene carrier. *J Controlled Release.* 2005; **103**(1):209-219.
259. Cui W, Li X, Chen J, et al. In Situ Growth Kinetics of Hydroxyapatite on Electrospun Poly(dl-lactide) Fibers with Gelatin Grafted. *Cryst Growth Des.* 2008; **8**(12):4576-4582.
260. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials – biological foundation and preclinical evidence: A systematic review. *J Clin Periodontol.* 2008; **35**:106-116.
261. Milella E, Ramires PA, Brescia E, et al. Physicochemical, mechanical, and biological properties of commercial membranes for GTR. *J Biomed Mater Res.* 2001; **58**(4):427-435.
262. Bottino MC, Thomas V, Schmidt G, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration—A materials perspective. *Dent Mater.* 2012; **28**(7):703-721.
263. Liu X, Ma L, Mao Z, et al. Chitosan-Based Biomaterials for Tissue Repair and Regeneration. Chitosan for Biomaterials II. In: Jayakumar R, Prabaharan M, Muzzarelli RAA, editors. Advances in Polymer Science. Vol. 244. p. 81-127 2011: Springer Berlin Heidelberg.
264. Ku Y, Shim IK, Lee JY, et al. Chitosan/poly (L-lactic acid) multilayered membrane for guided tissue regeneration. *Journal of Biomedical Materials Research Part A.* 2009; **90**(3):766-772.

265. Lee E-J, Shin D-S, Kim H-E, et al. Membrane of hybrid chitosan–silica xerogel for guided bone regeneration. *Biomaterials.* 2009; **30**(5):743-750.
266. Shin S-Y, Park H-N, Kim K-H, et al. Biological evaluation of chitosan nanofiber membrane for guided bone regeneration. *J Periodontol.* 2005; **76**(10):1778-1784.
267. Xu C, Lei C, Meng L, et al. Chitosan as a barrier membrane material in periodontal tissue regeneration. *Journal of Biomedical Materials Research Part B: Applied Biomaterials.* 2012; **100**(5):1435-1443.
268. Knight DA, Holgate ST. The airway epithelium: Structural and functional properties in health and disease. *Respirology.* 2003; **8**(4):432-446.
269. Tsao C-K, Ko C-Y, Yang S-R, et al. An ectopic approach for engineering a vascularized tracheal substitute. *Biomaterials.* 2014; **35**(4):1163-1175.
270. Karp P, Moninger T, Pary Weber S, et al. An In Vitro Model of Differentiated Human Airway Epithelia. Epithelial Cell Culture Protocols. In: Wise C, editor. Methods In Molecular Medicine™. Vol. 188. p. 115-137 2002 New York: Humana Press.
271. Kanemaru S-i, Omori K, Yamashita M, et al. Regeneration of the Trachea. Regenerative Medicine in Otolaryngology. In: Ito J, editor. p. 225-234 2015: Springer Japan.
272. Delaere P, Molitor M. TRACHEAL ALLOTRANSPLANTATION AND REGENERATION. *Acta Chir Plast.* 2017; **58**(1):29-38.
273. Wang J, Sun B, Tian L, et al. Evaluation of the potential of rhTGF-β3 encapsulated P (LLA-CL)/collagen nanofibers for tracheal cartilage regeneration using mesenchymal stems cells derived from Wharton's jelly of human umbilical cord. *Mater Sci Eng C.* 2017; **70**:637-645.
274. Teoh GZ, Crowley C, Birchall MA, et al. Development of resorbable nanocomposite tracheal and bronchial scaffolds for paediatric applications. *Br J Surg.* 2015; **102**(2):e140-e150.
275. Shin YS. Tissue-Engineered Tracheal Regeneration: Current Status. *Korean Journal of Otorhinolaryngology-Head and Neck Surgery.* 2017; **60**(4):153-157.
276. Ghorbani F, Moradi L, Shadmehr MB, et al. In-vivo characterization of a 3D hybrid scaffold based on PCL/decellularized aorta for tracheal tissue engineering. *Mater Sci Eng C.* 2017; **81**:74-83.
277. Vacanti CA, Paige KT, Kim WS, et al. Experimental tracheal replacement using tissue-engineered cartilage. *J Pediatr Surg.* 1994; **29**(2):201-205.
278. Kojima K, Bonassar LJ, Roy AK, et al. A composite tissue-engineered trachea using sheep nasal chondrocyte and epithelial cells. *FASEB J.* 2003; **17**(8):823-8.
279. ten Hallers EJO, Rakhorst G, Marres HAM, et al. Animal models for tracheal research. *Biomaterials.* 2004; **25**(9):1533-1543.
280. Rotter N, Ung F, Roy AK, et al. Role for interleukin 1alpha in the inhibition of chondrogenesis in autologous implants using polyglycolic acid-polylactic acid scaffolds. *Tissue Eng.* 2005; **11**(1-2):192-200.
281. Wu W, Cheng X, Zhao Y, et al. Tissue Engineering of Trachea-like Cartilage Grafts by Using Chondrocyte Macroaggregate: Experimental Study in Rabbits. *Artif Organs.* 2007; **31**(11):826-834.
282. Weidenbecher M, Tucker HM, Awadallah A, et al. Fabrication of a Neotrachea Using Engineered Cartilage. *The Laryngoscope.* 2008; **118**(4):593-598.
283. Komura M, Komura H, Kanamori Y, et al. An animal model study for tissue-engineered trachea fabricated from a biodegradable scaffold using chondrocytes to augment repair of tracheal stenosis. *J Pediatr Surg.* 2008; **43**(12):2141-2146.
284. Lin C-H, Hsu S-h, Huang C-E, et al. A scaffold-bioreactor system for a tissue-engineered trachea. *Biomaterials.* 2009; **30**(25):4117-4126.

285. Kobayashi K, Suzuki T, Nomoto Y, et al. A tissue-engineered trachea derived from a framed collagen scaffold, gingival fibroblasts and adipose-derived stem cells. *Biomaterials.* 2010; **31**(18):4855-4863.
286. Tatekawa Y, Ikada Y, Komuro H, et al. Experimental Repair of Tracheal Defect Using a Bioabsorbable Copolymer. *J Surg Res.* 2010; **160**(1):114-121.
287. Hinderer S, Schesny M, Bayrak A, et al. Engineering of fibrillar decorin matrices for a tissue-engineered trachea. *Biomaterials.* 2012; **33**(21):5259-5266.
288. Hutmacher DW, Schantz JT, Lam CXF, et al. State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J Tissue Eng Regen Med.* 2007; **1**(4):245-260.
289. Weiner S, Wagner HD. THE MATERIAL BONE: Structure-Mechanical Function Relations. *Annu Rev Mater Sci.* 1998; **28**(1):271-298.
290. Perán M, García MA, López-Ruiz E, et al. Functionalized Nanostructures with Application in Regenerative Medicine. *Int J Mol Sci.* 2012; **13**(3):3847-3886.
291. Diaz-Gomez L, García-González CA, Wang J, et al. Biodegradable PCL/fibroin/hydroxyapatite porous scaffolds prepared by supercritical foaming for bone regeneration. *Int J Pharm.* 2017; **527**:115-125.
292. Dorozhkin S. Calcium orthophosphate-based biocomposites and hybrid biomaterials. *Journal of Materials Science.* 2009; **44**(9):2343-2387.
293. Haider A, Haider S, Han SS, et al. Recent advances in the synthesis, functionalization and biomedical applications of hydroxyapatite: a review. *Rsc Advances.* 2017; **7**(13):7442-7458.
294. Bonfield W, Grynpas MD, Tully AE, et al. Hydroxyapatite reinforced polyethylene — a mechanically compatible implant material for bone replacement. *Biomaterials.* 1981; **2**(3):185-186.
295. Suchanek W, Yoshimura M. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. *J Mater Res.* 1998; **13**(1):94-117.
296. Li J, Fartash B, Hermansson L. Hydroxyapatite—alumina composites and bone-bonding. *Biomaterials.* 1995; **16**(5):417-422.
297. Zheng X, Huang M, Ding C. Bond strength of plasma-sprayed hydroxyapatite/Ti composite coatings. *Biomaterials.* 2000; **21**(8):841-849.
298. Brown A, Zaky S, Ray Jr H, et al. Porous magnesium/PLGA composite scaffolds for enhanced bone regeneration following tooth extraction. *Acta Biomater.* 2015; **11**(0):543-553.
299. Mochalin VN, Gogotsi Y. Biomedical Applications of Diamond Nanoparticles. *Meeting Abstracts.* 2015; **MA2015-01**(5):766.
300. Araujo-Pires AC, Mendes VC, Ferreira-Junior O, et al. Investigation of a Novel PLGA/CaP Scaffold in the Healing of Tooth Extraction Sockets to Alveolar Bone Preservation in Humans. *Clin Implant Dent Relat Res.* 2016; **18**(3):559-570.
301. Arun Kumar R, Sivashanmugam A, Deepthi S, et al. Injectable Chitin-Poly(ε-caprolactone)/Nanohydroxyapatite Composite Microgels Prepared by Simple Regeneration Technique for Bone Tissue Engineering. *ACS applied materials & interfaces.* 2015; **7**(18):9399-9409.
302. Guarino V, Urciuolo F, Alvarez-Perez MA, et al. Osteogenic differentiation and mineralization in fibre-reinforced tubular scaffolds: theoretical study and experimental evidences. *J R Soc Interface.* 2012; **9**(74):2201-2212.
303. Rezwan K, Chen QZ, Blaker JJ, et al. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials.* 2006; **27**(18):3413-3431.
304. Dang M, Koh AJ, Jin X, et al. Local pulsatile PTH delivery regenerates bone defects via enhanced bone remodeling in a cell-free scaffold. *Biomaterials.* 2017; **114**:1-9.

305. Hajiali F, Tajbakhsh S, Shojaei A. Fabrication and properties of polycaprolactone composites containing calcium phosphate-based ceramics and bioactive glasses in bone tissue engineering: a review. *Polymer Reviews.* 2018; **58**:164-207.
306. Ishiko-Uzuka R, Anada T, Kobayashi K, et al. Oriented bone regenerative capacity of octacalcium phosphate/gelatin composites obtained through two-step crystal preparation method. *J Biomed Mater Res Part B.* 2017; **105**(5):1029-1039.
307. Nazeer MA, Yilgör E, Yilgör I. Intercalated chitosan/hydroxyapatite nanocomposites: Promising materials for bone tissue engineering applications. *Carbohydr Polym.* 2017; **175**:38-46.
308. Yao Q, Cosme JG, Xu T, et al. Three dimensional electrospun PCL/PLA blend nanofibrous scaffolds with significantly improved stem cells osteogenic differentiation and cranial bone formation. *Biomaterials.* 2017; **115**:115-127.
309. Ito T, Hashimoto Y, Baba S, et al. Bone Regeneration With a Collagen Model Polypeptides/α-Tricalcium Phosphate Sponge in a Canine Tibia Defect Model. *Implant Dent.* 2015; **24**(2):197-203.
310. Chen G, Sato T, Tanaka J, et al. Preparation of a biphasic scaffold for osteochondral tissue engineering. *Mater Sci Eng C.* 2006; **26**(1):118-123.
311. Hsu S-h, Chang S-H, Yen H-J, et al. Evaluation of Biodegradable Polyesters Modified by Type II Collagen and Arg-Gly-Asp as Tissue Engineering Scaffolding Materials for Cartilage Regeneration. *Artif Organs.* 2006; **30**(1):42-55.
312. Day RM, Maquet V, Boccaccini AR, et al. In vitro and in vivo analysis of macroporous biodegradable poly(D,L-lactide-co-glycolide) scaffolds containing bioactive glass. *J Biomed Mater Res Part A.* 2005; **75A**(4):778-787.
313. Askarzadeh K OF, Moztarzadeh F. Fabrication and Characterization of a Porous Composite Scaffold Based on Gelatin and Hydroxyapatite for Bone Tissue Engineering. *Iranian Polymer Journal.* 2005; **14**(6):511-520.
314. Wan C, Frydrych M, Chen B. Strong and bioactive gelatin-graphene oxide nanocomposites. *Soft Matter.* 2011; **7**(13):6159-6166.
315. Rajkhowa R, Gil ES, Kluge J, et al. Reinforcing Silk Scaffolds with Silk Particles. *Macromol Biosci.* 2010; **10**(6):599-611.
316. Shin SR, Bae H, Cha JM, et al. Carbon Nanotube Reinforced Hybrid Microgels as Scaffold Materials for Cell Encapsulation. *ACS Nano.* 2011; **6**(1):362-372.
317. Goncalves G, Cruz SMA, Ramalho A, et al. Graphene oxide versus functionalized carbon nanotubes as a reinforcing agent in a PMMA/HA bone cement. *Nanoscale.* 2012; **4**(9):2937-2945.
318. Siqueira IAWB, Corat MAF, Cavalcanti BdN, et al. In Vitro and in Vivo Studies of Novel Poly(d,l-lactic acid), Superhydrophilic Carbon Nanotubes, and Nanohydroxyapatite Scaffolds for Bone Regeneration. *ACS Appl Mater Interf.* 2015; **7**(18):9385-9398.
319. Neelgund GM, Oki A, Luo Z. In situ deposition of hydroxyapatite on graphene nanosheets. *Mater Res Bull.* 2013; **48**(2):175-179.
320. Gao F, Xu C, Hu H, et al. Biomimetic synthesis and characterization of hydroxyapatite/graphene oxide hybrid coating on Mg alloy with enhanced corrosion resistance. *Mater Lett.* 2015; **138**(0):25-28.
321. Wu C, Xia L, Han P, et al. Graphene-oxide-modified β-tricalcium phosphate bioceramics stimulate in vitro and in vivo osteogenesis. *Carbon.* 2015; **93**(0):116-129.
322. Manitha N, Nancy D, Amit GK, et al. Graphene oxide nanoflakes incorporated gelatin–hydroxyapatite scaffolds enhance osteogenic differentiation of human mesenchymal stem cells. *Nanotechnology.* 2015; **26**(16):161001.

323. Ali-Boucetta H, Bitounis D, Raveendran-Nair R, et al. Purified Graphene Oxide Dispersions Lack In Vitro Cytotoxicity and In Vivo Pathogenicity. *Adv Healthc Mater.* 2013; **2**(3):433-441.
324. Agarwal S, Zhou X, Ye F, et al. Interfacing Live Cells with Nanocarbon Substrates. *Langmuir.* 2010; **26**(4):2244-2247.
325. Kalbacova M, Broz A, Kong J, et al. Graphene substrates promote adherence of human osteoblasts and mesenchymal stromal cells. *Carbon.* 2010; **48**(15):4323-4329.
326. Supronowicz PR, Ajayan PM, Ullmann KR, et al. Novel current-conducting composite substrates for exposing osteoblasts to alternating current stimulation. *J Biomed Mater Res.* 2002; **59**(3):499-506.
327. Chen H, Müller MB, Gilmore KJ, et al. Mechanically Strong, Electrically Conductive, and Biocompatible Graphene Paper. *Adv Mater.* 2008; **20**(18):3557-3561.
328. Liu Z, Robinson JT, Sun X, et al. PEGylated Nanographene Oxide for Delivery of Water-Insoluble Cancer Drugs. *J Am Chem Soc.* 2008; **130**(33):10876-10877.
329. Yang W, Ratinac KR, Ringer SP, et al. Carbon Nanomaterials in Biosensors: Should You Use Nanotubes or Graphene? *Angew Chem Int Ed.* 2010; **49**(12):2114-2138.
330. Kim H, Abdala AA, Macosko CW. Graphene/Polymer Nanocomposites. *Macromolecules.* 2010; **43**(16):6515-6530.
331. RamanathanT, Abdala AA, StankovichS, et al. Functionalized graphene sheets for polymer nanocomposites. *Nat Nano.* 2008; **3**(6):327-331.
332. Kuilla T, Bhadra S, Yao D, et al. Recent advances in graphene based polymer composites. *Prog Polym Sci.* 2010; **35**(11):1350-1375.
333. Xu Y, Hong W, Bai H, et al. Strong and ductile poly(vinyl alcohol)/graphene oxide composite films with a layered structure. *Carbon.* 2009; **47**(15):3538-3543.
334. Acocella F, Brizzola S. 12 - Tracheal tissue regeneration. Electrospinning for Tissue Regeneration. In: Bosworth LA, Downes S, editors. p. 242-279 2011: Woodhead Publishing.
335. Walles T. Tracheobronchial bio-engineering: Biotechnology fulfilling unmet medical needs. *Adv Drug Del Rev.* 2011; **63**(4–5):367-374.
336. Chen K-Y, Lin K-C, Chen Y-S, et al. A Novel Porous Gelatin Composite Containing Naringin for Bone Repair. *Evid Based Complementary Alternat Med.* 2013; **2013**:10.
337. Shah NJ, Hong J, Hyder MN, et al. Osteophilic Multilayer Coatings for Accelerated Bone Tissue Growth. *Adv Mater.* 2012; **24**(11):1445-1450.
338. Chai YC, Carlier A, Bolander J, et al. Current views on calcium phosphate osteogenicity and the translation into effective bone regeneration strategies. *Acta Biomater.* 2012; **8**(11):3876-3887.
339. Lü L-X, Zhang X-F, Wang Y-Y, et al. Effects of Hydroxyapatite-Containing Composite Nanofibers on Osteogenesis of Mesenchymal Stem Cells In vitro and Bone Regeneration In vivo. *ACS applied materials & interfaces.* 2012; **5**(2):319-330.
340. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. *Trends Biotechnol.* 2012; **30**(10):546-554.
341. Sinha Ray S. Polylactide-Based Bionanocomposites: A Promising Class of Hybrid Materials. *Acc Chem Res.* 2012; **45**(10):1710-1720.
342. Dew L, MacNeil S, Chong CK. Vascularization strategies for tissue engineers. *Regen Med.* 2015; **10**(2):211-224.
343. des Rieux A, Ucakar B, Mupendwa BPK, et al. 3D systems delivering VEGF to promote angiogenesis for tissue engineering. *J Controlled Release.* 2011; **150**(3):272-278.
344. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface.* 2011; **8**(55):153-170.

345. Matsuda K, Falkenberg KJ, Woods AA, et al. Adipose-derived stem cells promote angiogenesis and tissue formation for in vivo tissue engineering. *Tissue Eng Part A.* 2013; **19**(11-12):1327-1335.
346. Easton CD, Bullock A, Gigliobianco G, et al. Application of layer-by-layer coatings to tissue scaffolds–development of an angiogenic biomaterial. *J Mater Chem B.* 2014; **2**(34):5558-5568.

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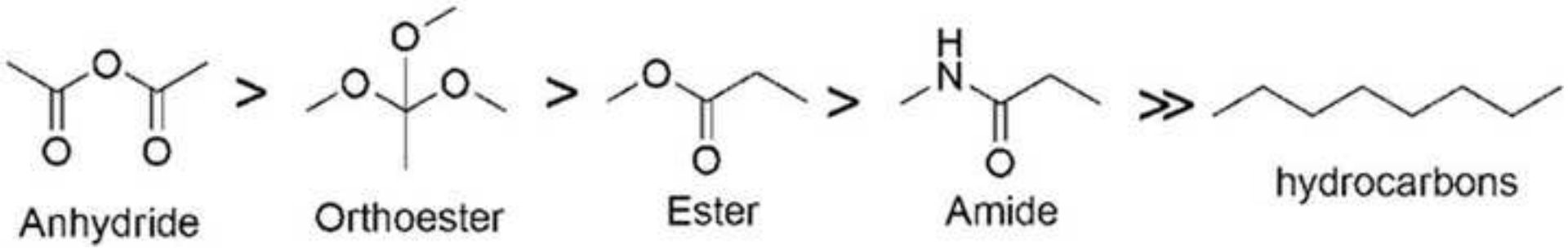

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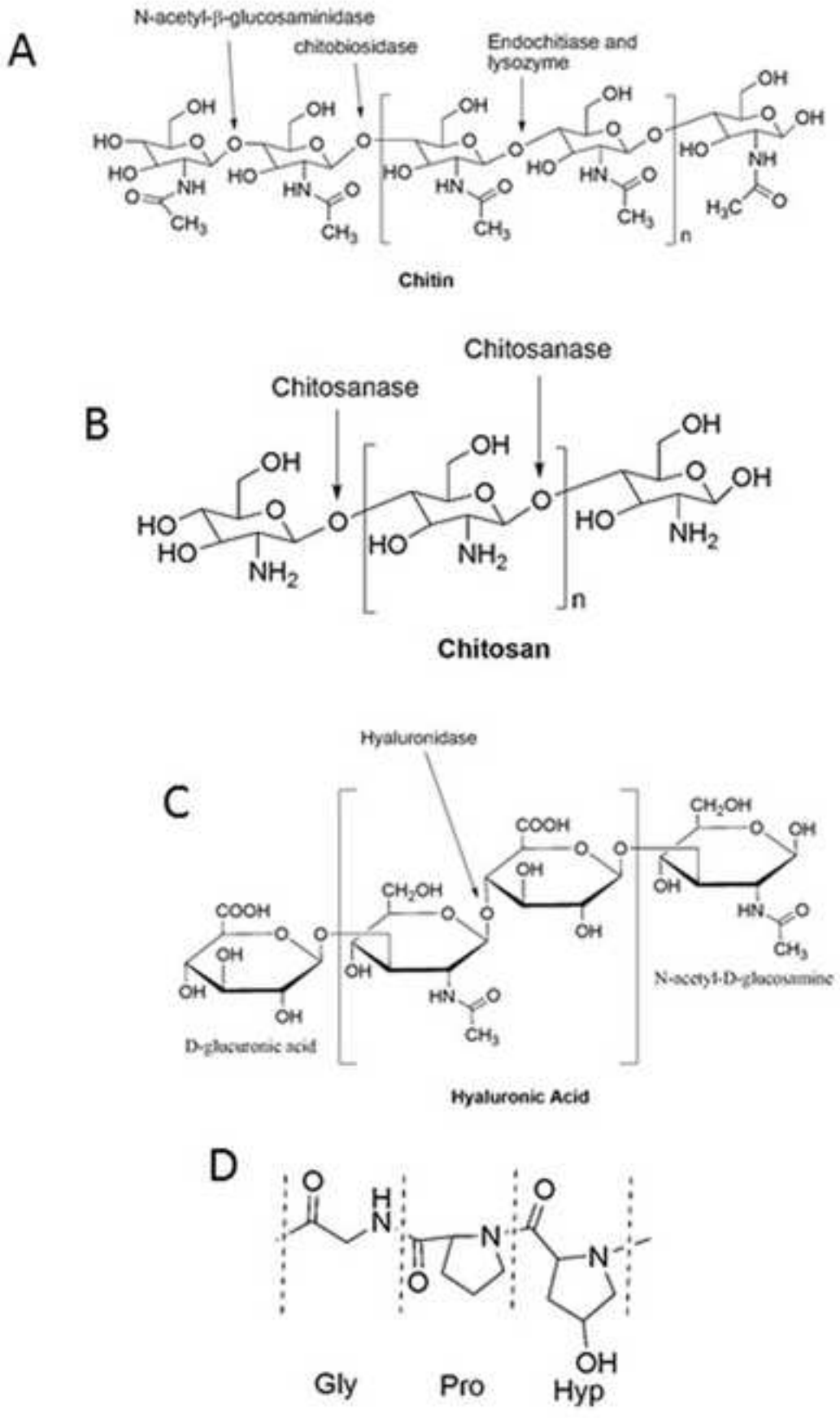

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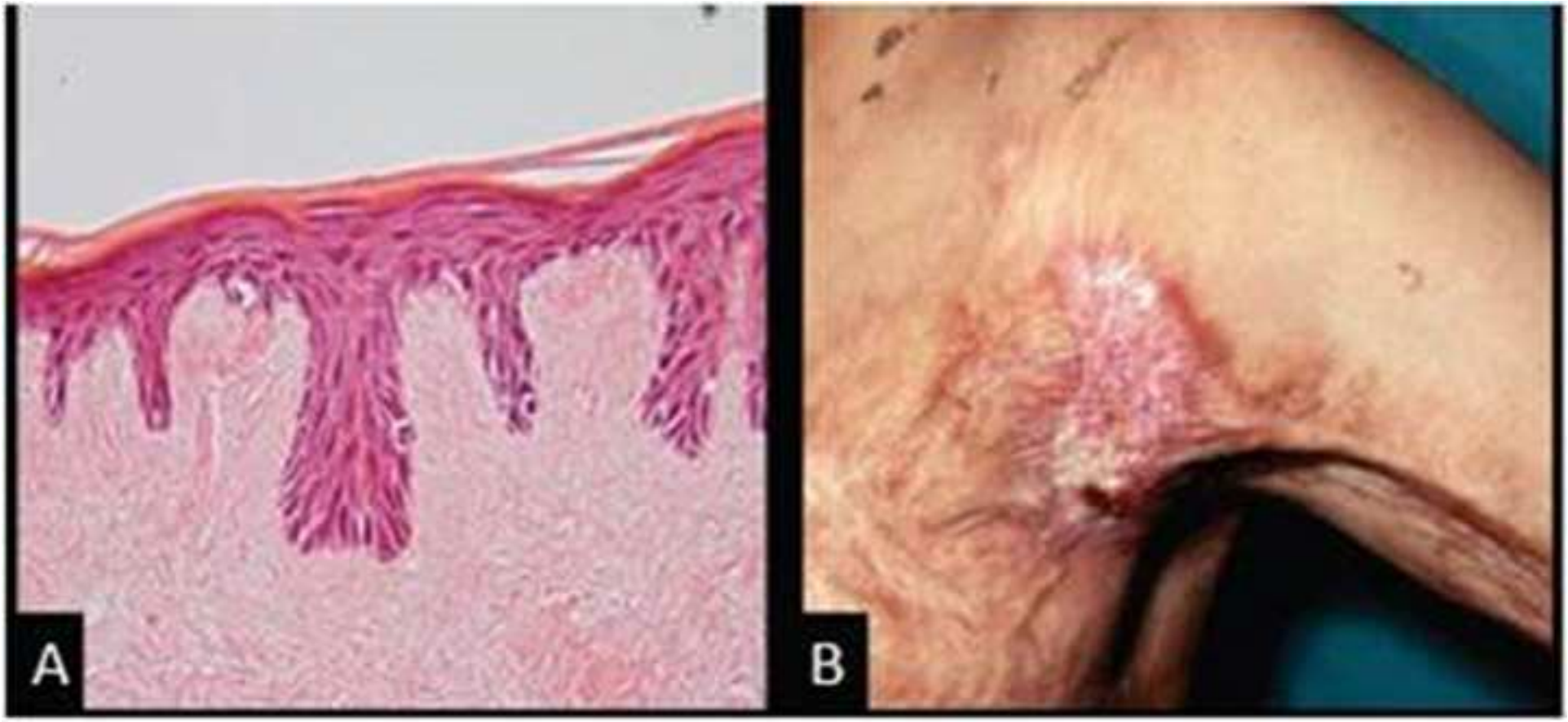

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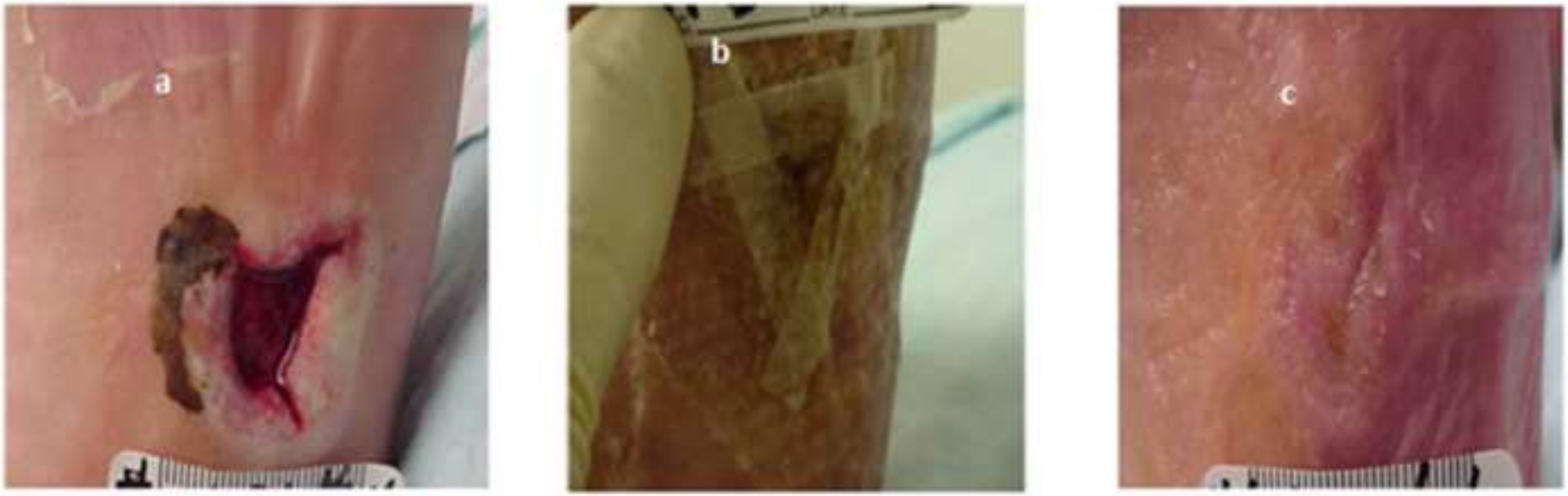

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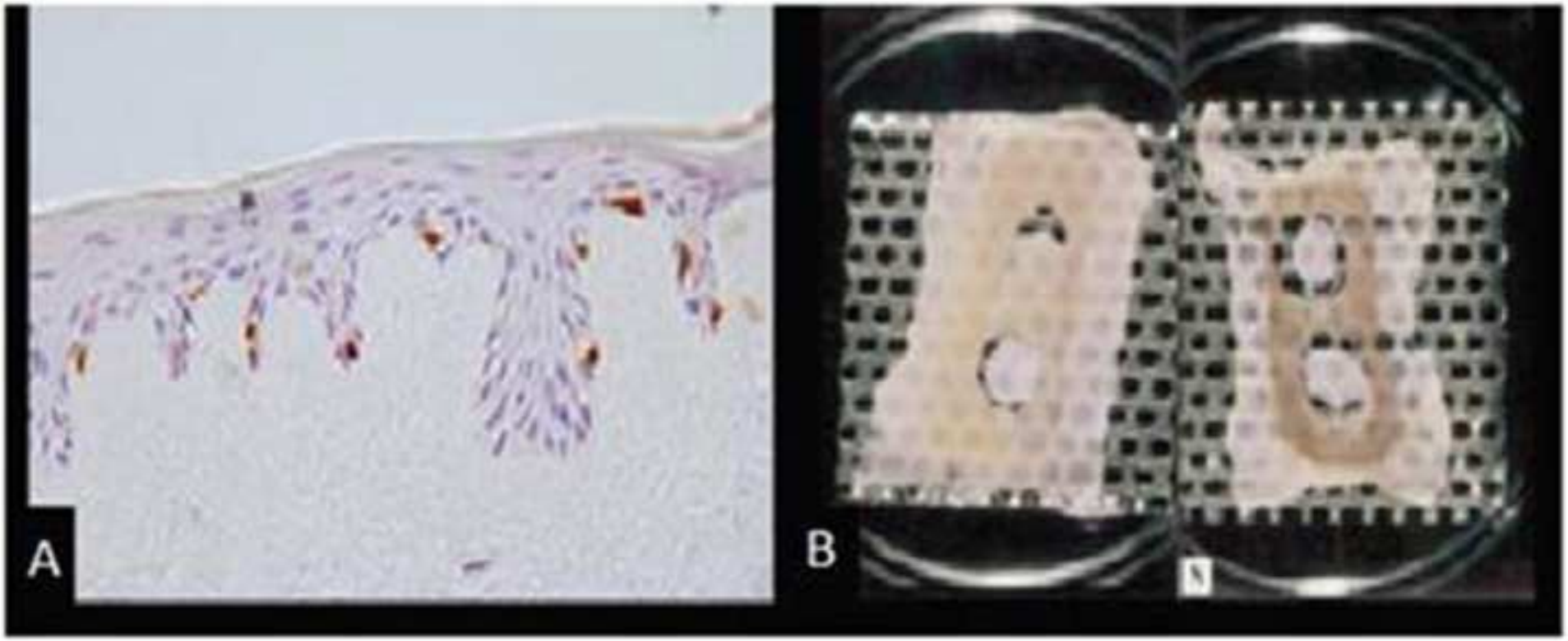

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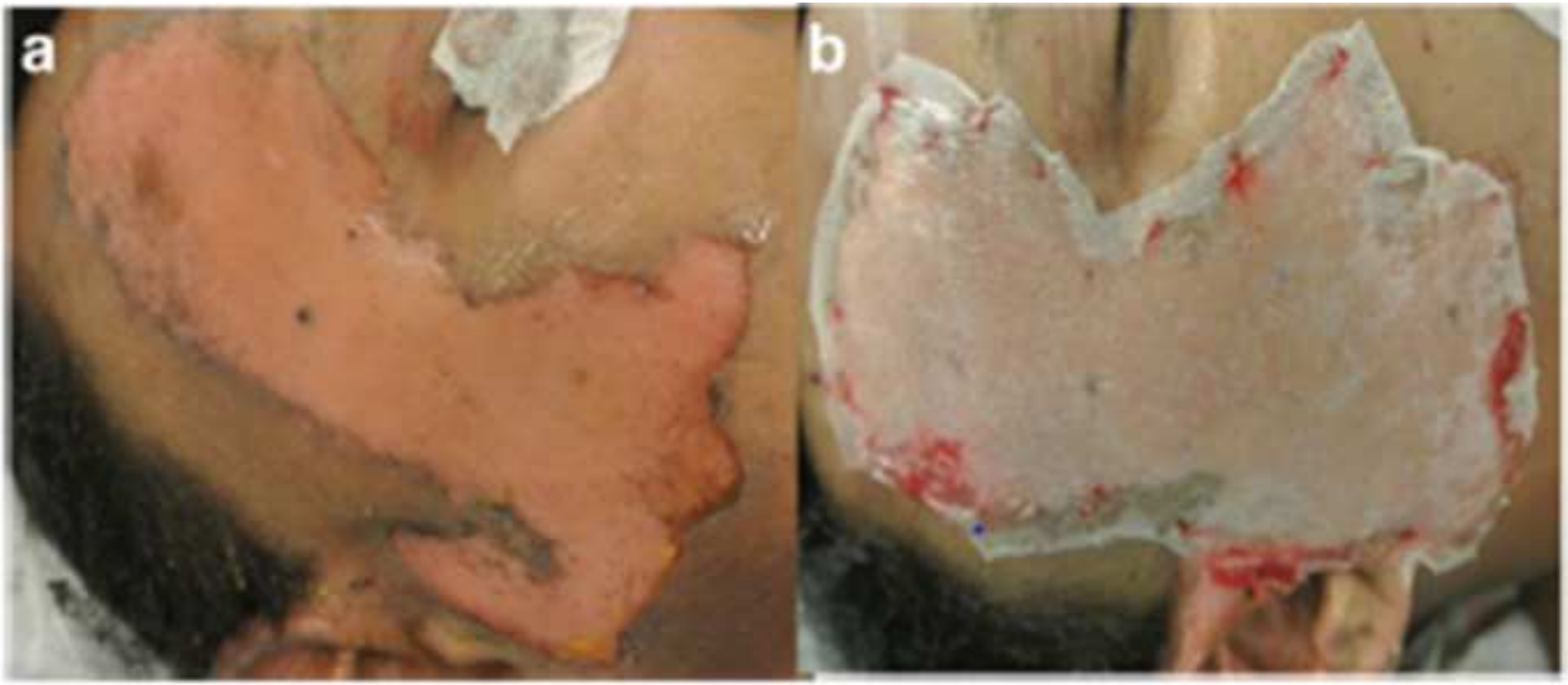

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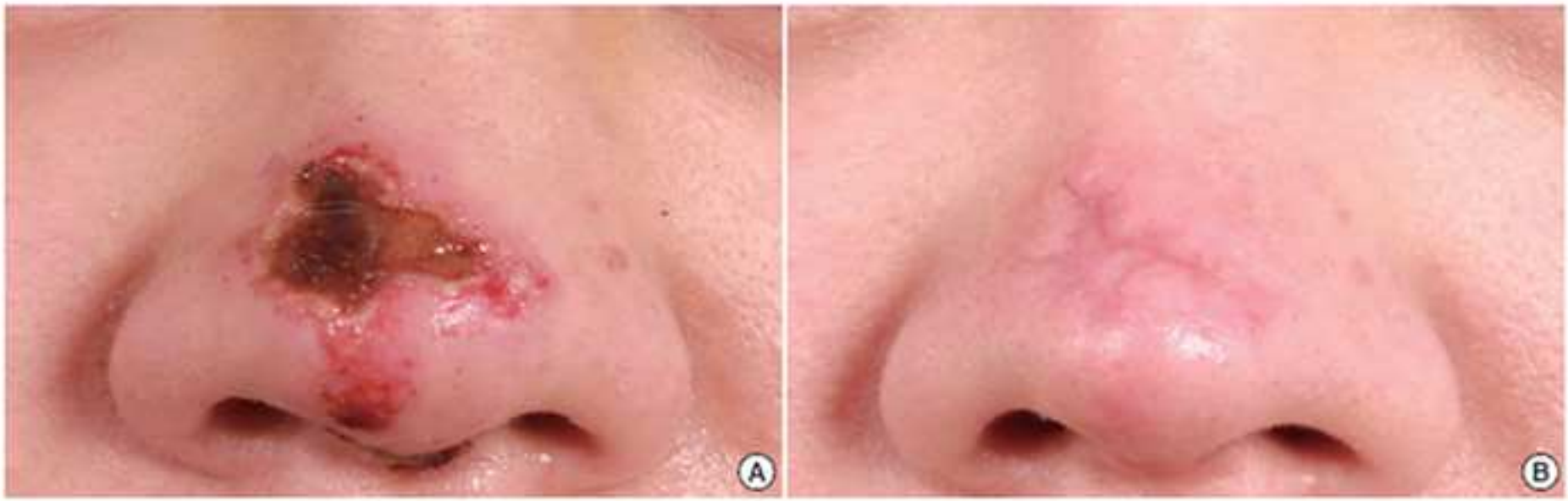

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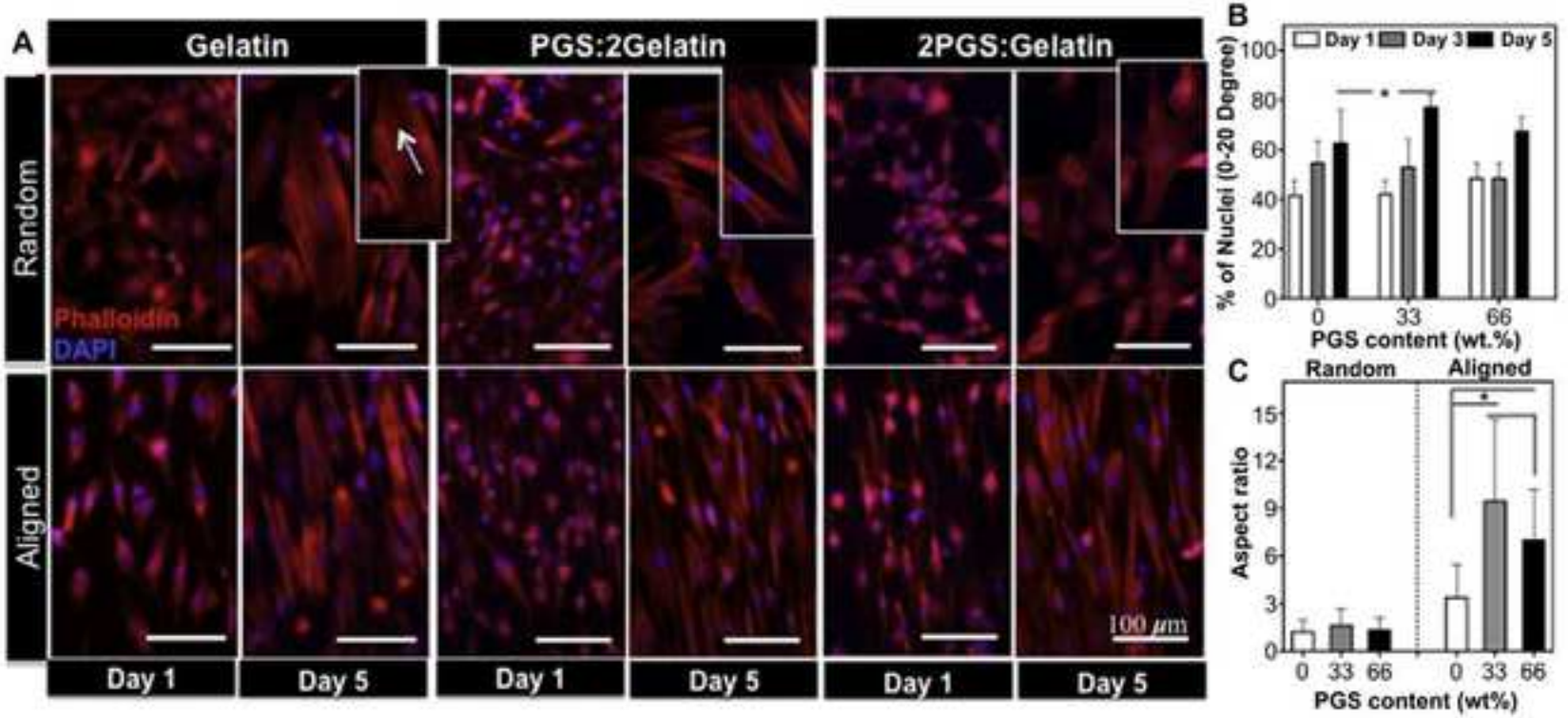

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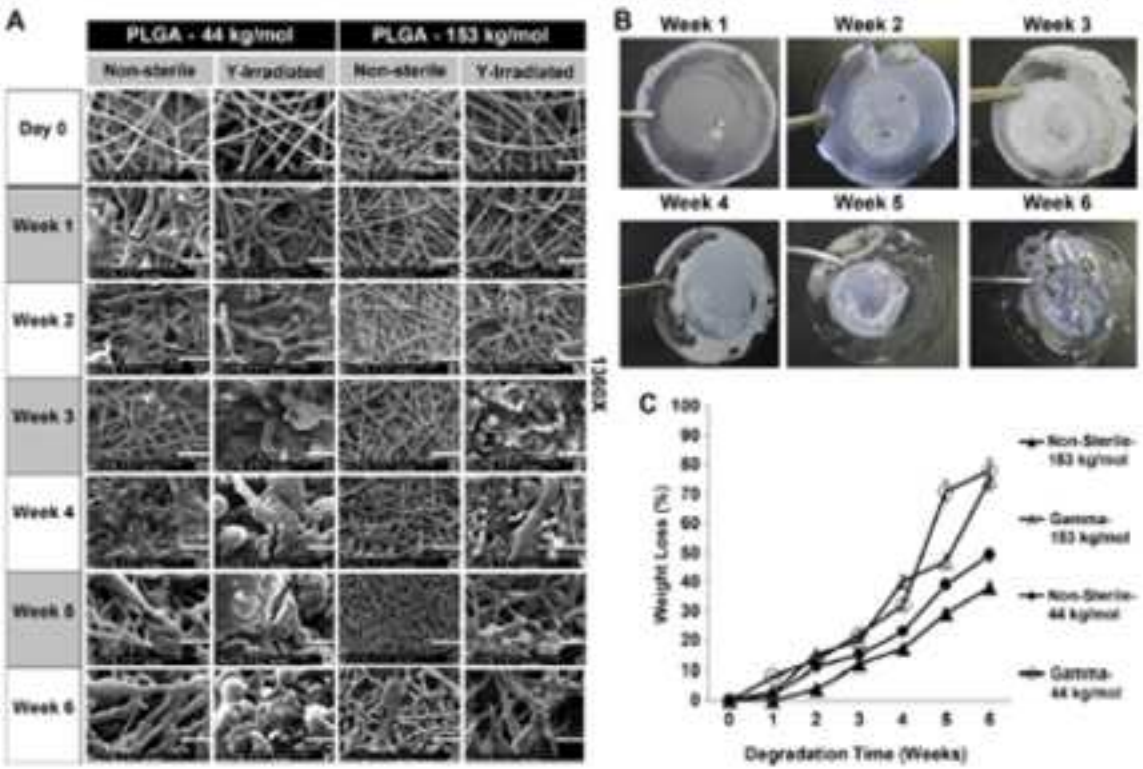

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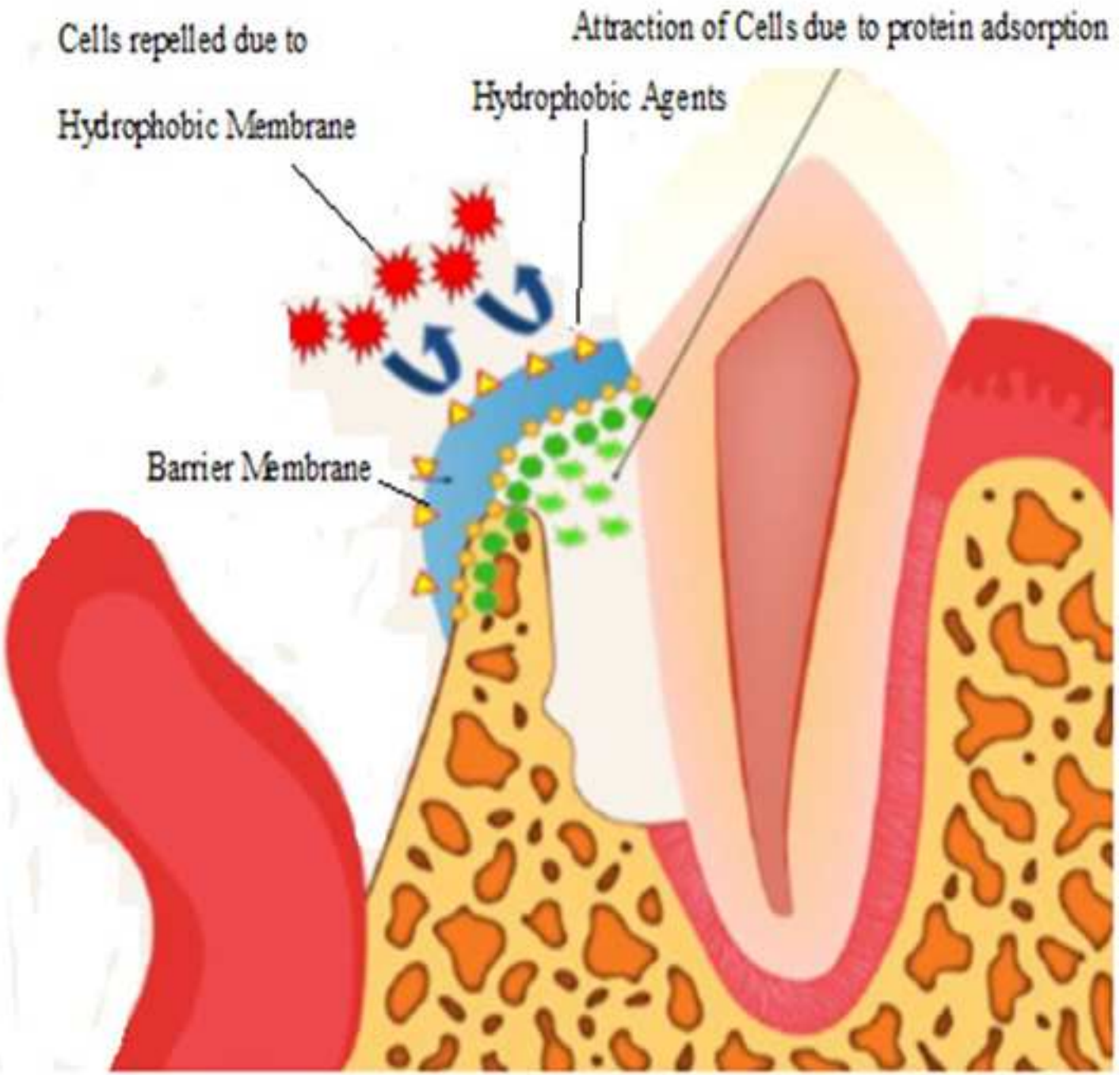

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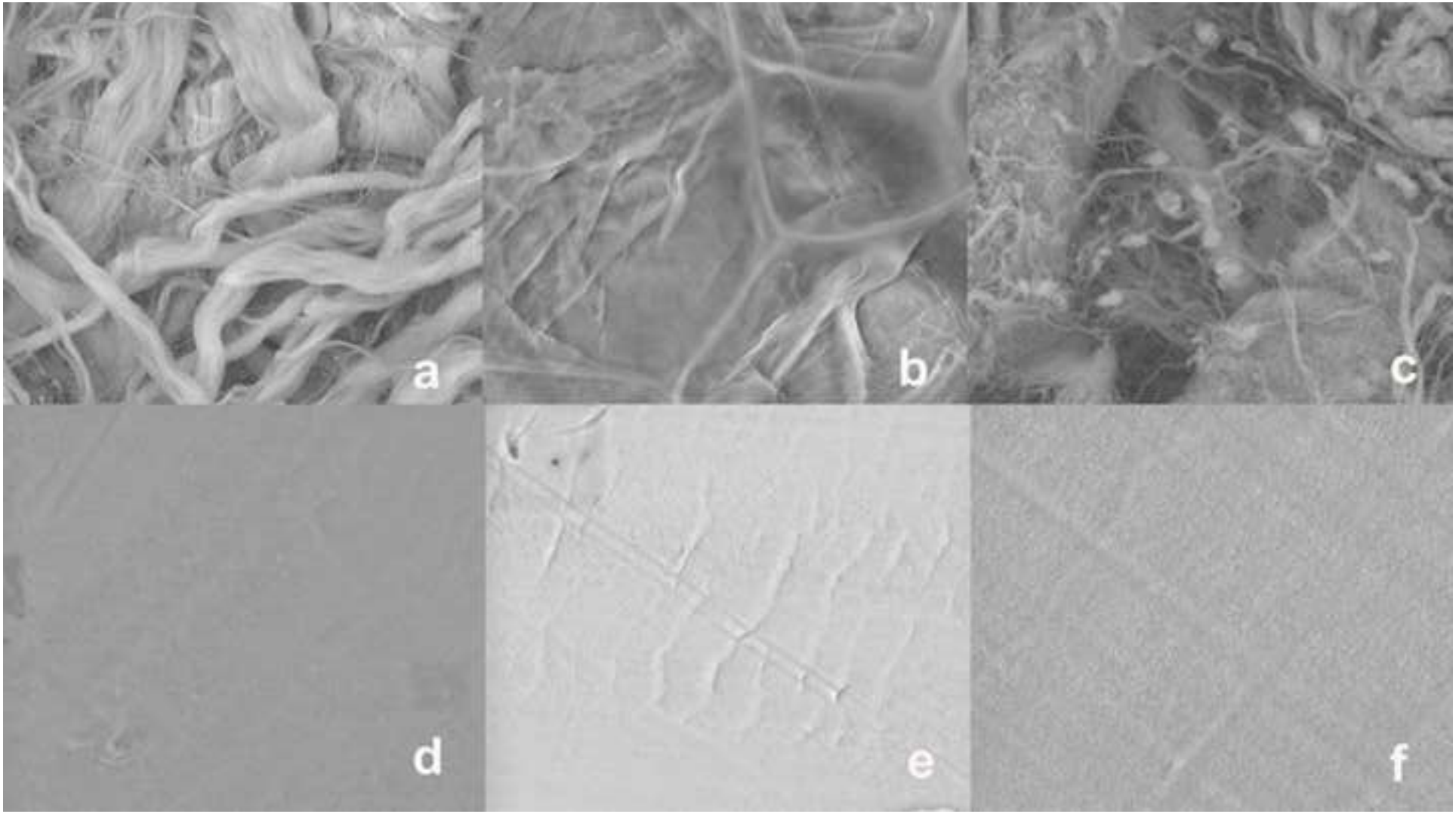

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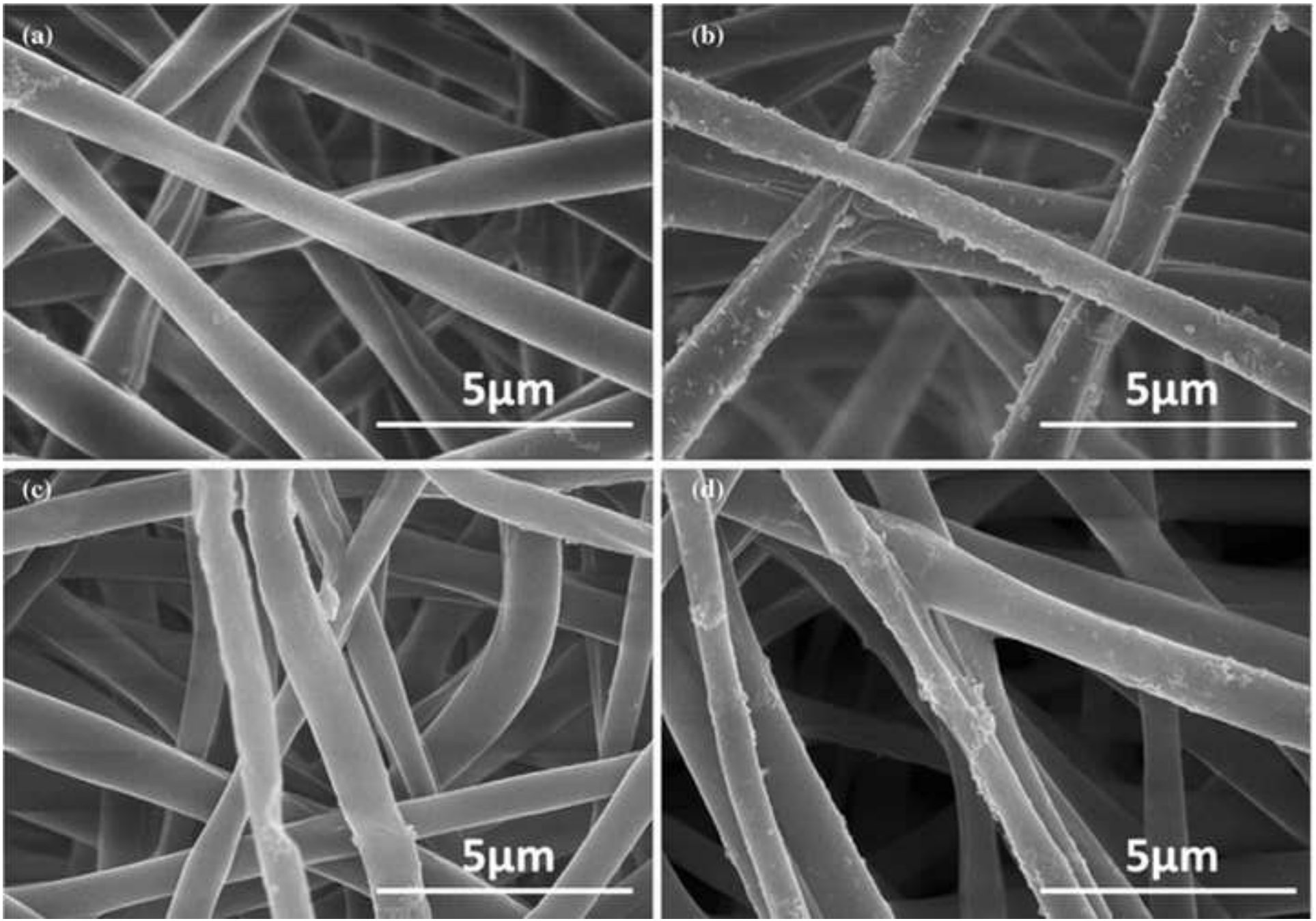

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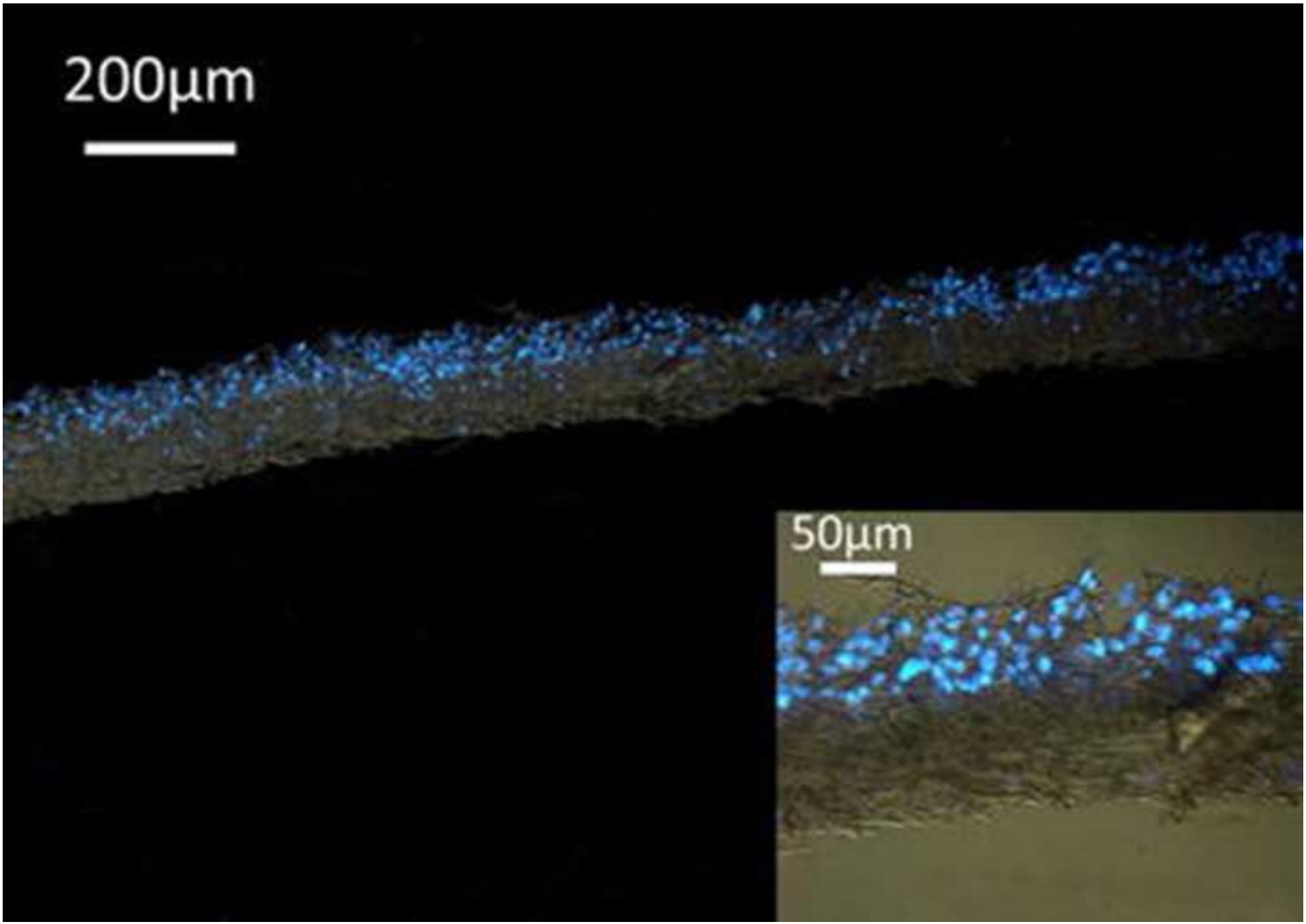

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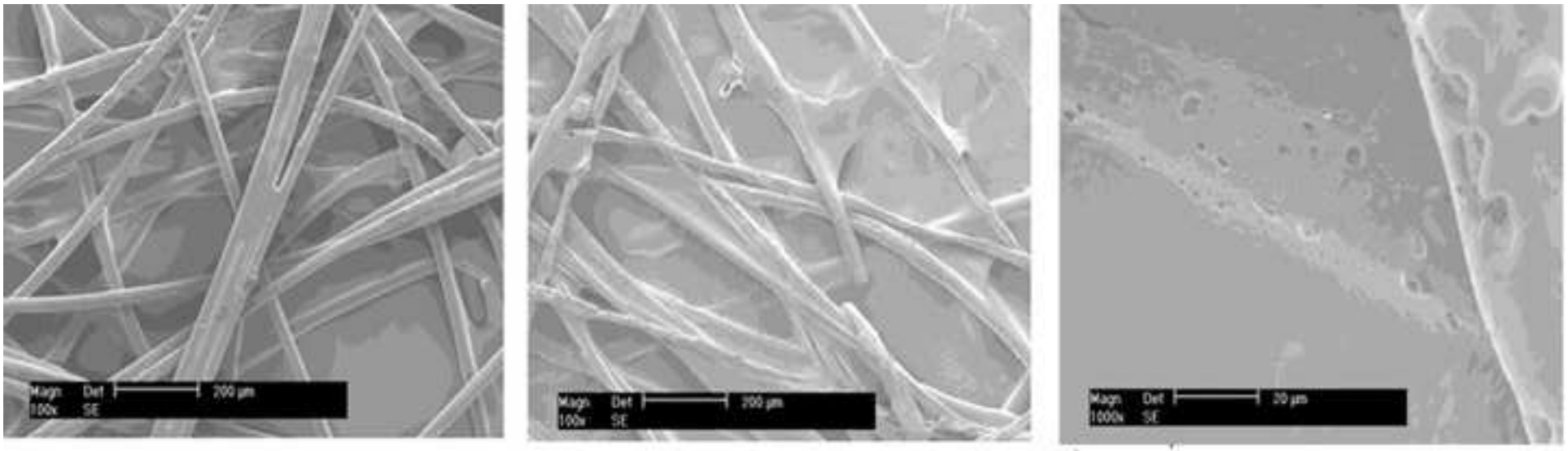

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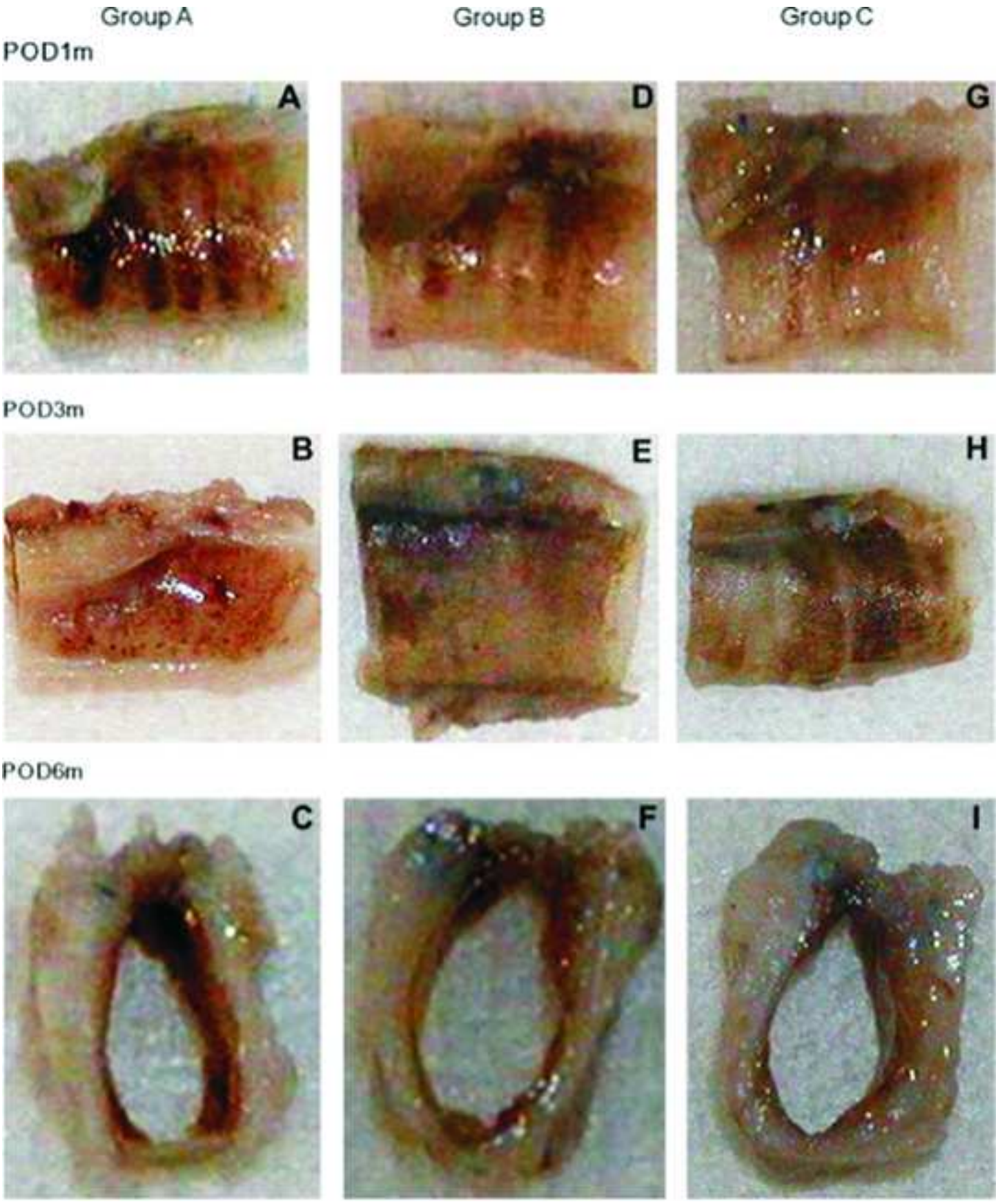

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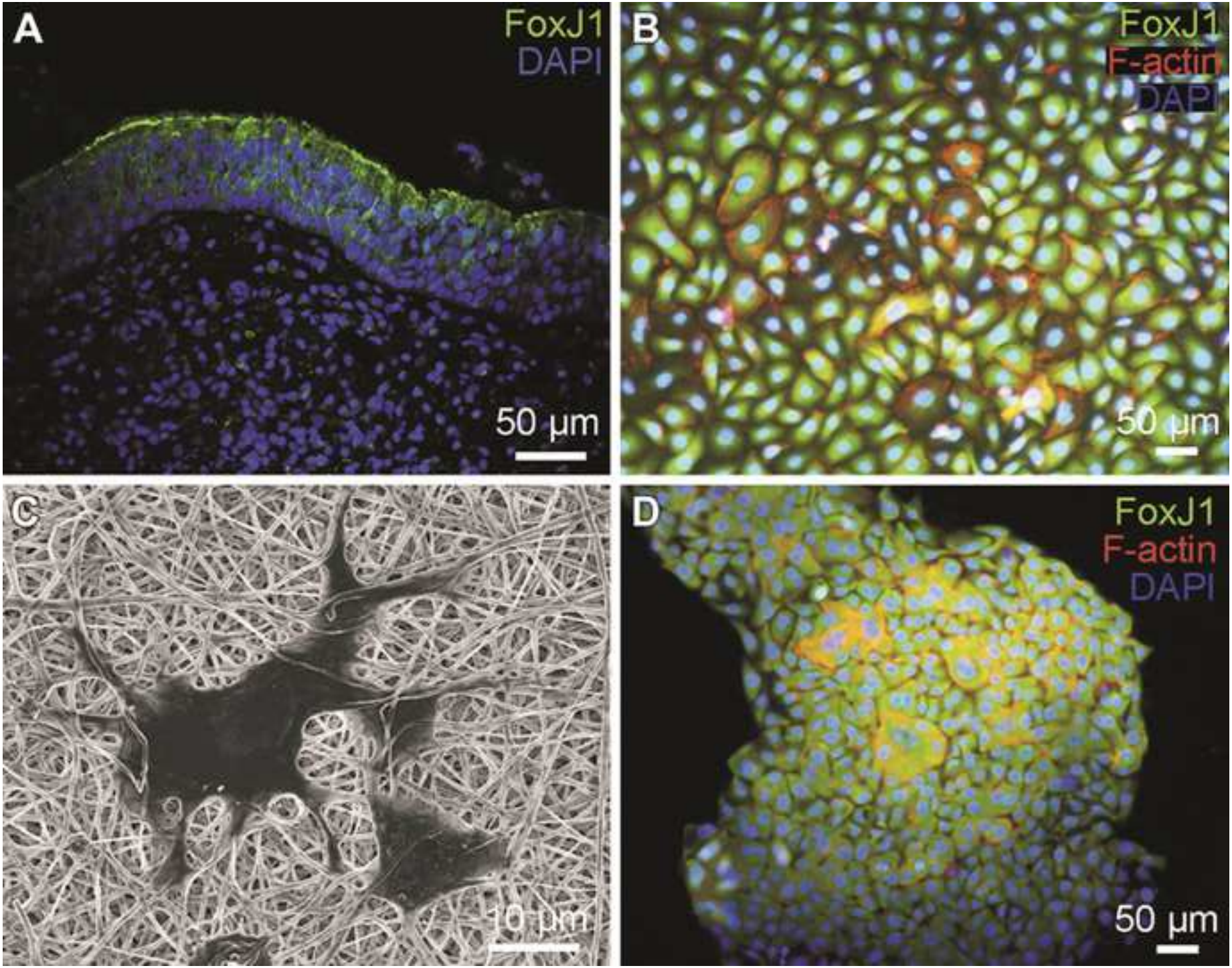

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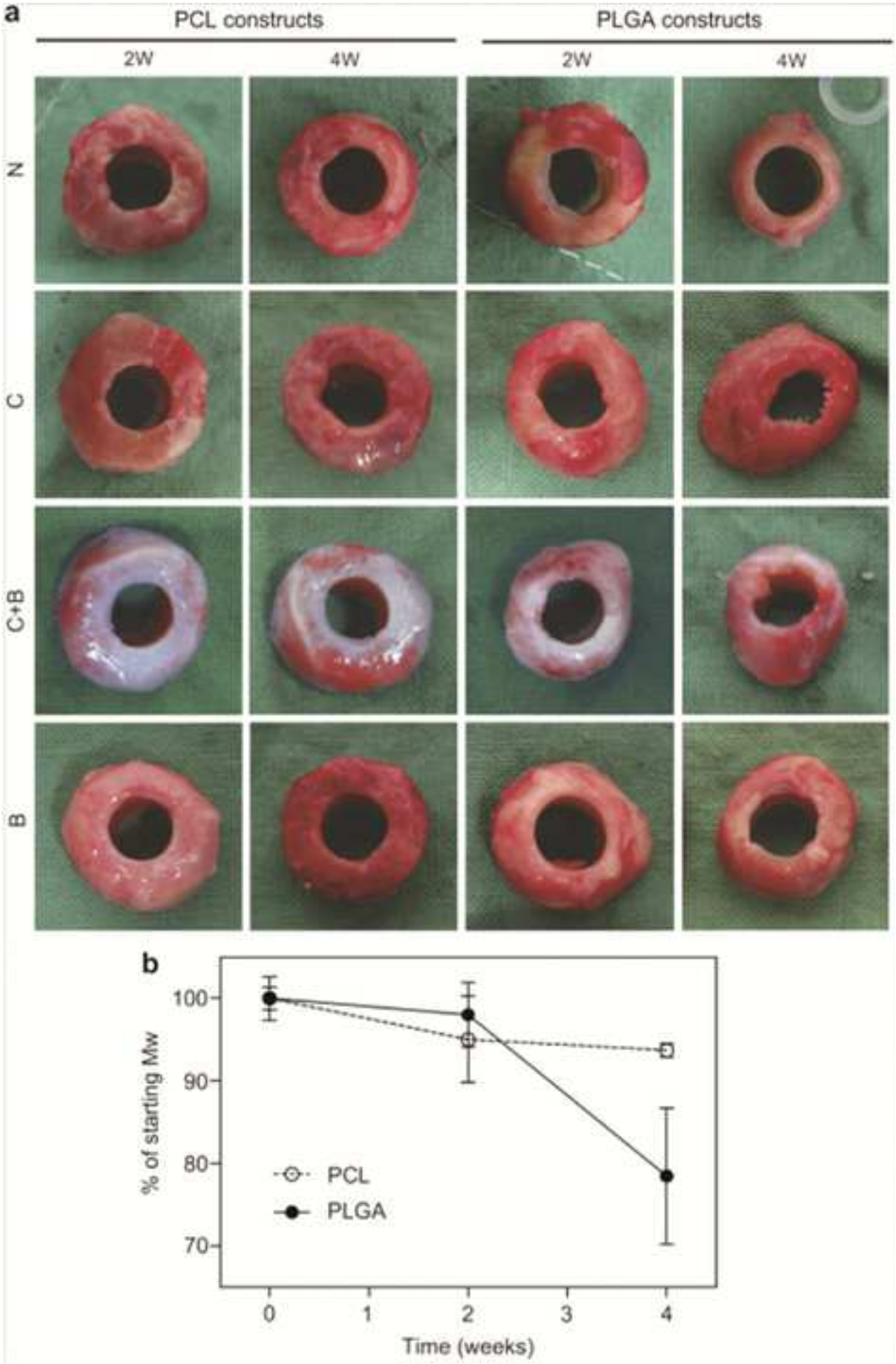

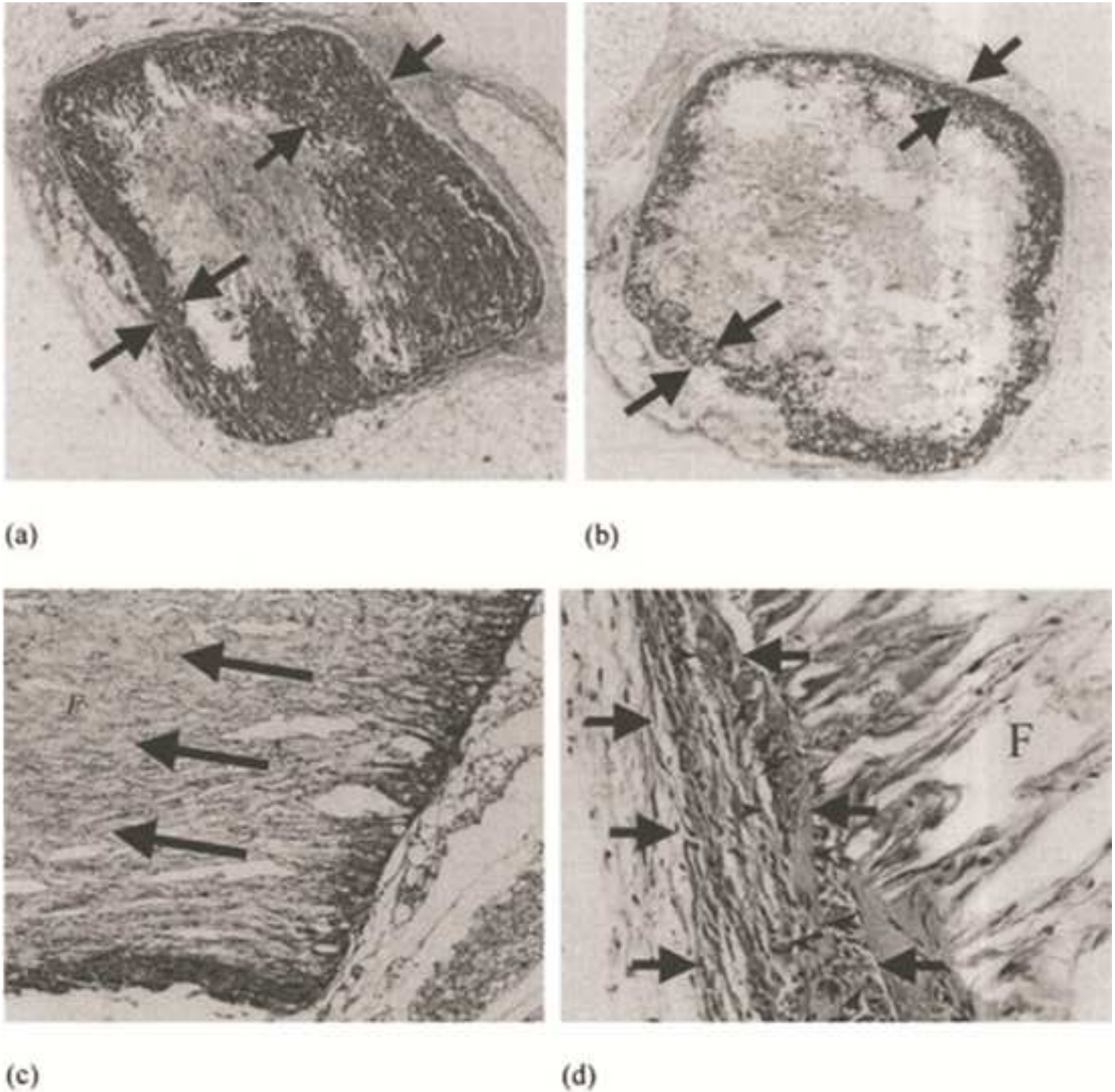

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9. Figure 9. Breakdown of PLGA membranes over 6 weeks. In these experiments membranes were placed in culture media and their morphology and mass examined over 6 weeks. A shows SEM of the fibres of non-sterilised and g-irradiated for 44 kg/mol and 153 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*)
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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

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).