
Title: Current advances in electrospun gelatin-based scaffolds for tissue engineering applications

Author: Ana A. Aldana Gustavo A. Abraham

PII: S0378-5173(16)30874-2
DOI: http://dx.doi.org/doi:10.1016/j.ijpharm.2016.09.044
Reference: IJP 16086

To appear in: *International Journal of Pharmaceutics*

Received date: 11-7-2016
Revised date: 2-9-2016
Accepted date: 13-9-2016

Please cite this article as: Aldana, Ana A., Abraham, Gustavo A., Current advances in electrospun gelatin-based scaffolds for tissue engineering applications.International Journal of Pharmaceutics http://dx.doi.org/10.1016/j.ijpharm.2016.09.044

# Current advances in electrospun gelatin-based scaffolds for tissue engineering applications

Ana A. Aldana, Gustavo A. Abraham*

Instituto de Investigaciones en Ciencia y Tecnología de Materiales, INTEMA (UNMdP-CONICET), Av. Juan B. Justo 4302, B7608FDQ Mar del Plata, Argentina

* Corresponding author:

Tel.: +54 223 481 6600. Fax: +54 223 481 0046

E-mail address: gabraham@fi.mdp.edu.ar

**Abstract**

The development of biomimetic highly-porous scaffolds is essential for successful tissue engineering. Electrospun nanofibers are highly versatile platforms for a broad range of applications in different research areas. In the biomedical field, micro/nanoscale fibrous structures have gained great interest for wound dressings, drug delivery systems, soft and hard-tissue engineering scaffolds, enzyme immobilization, among other healthcare applications. In this mini-review, electrospun gelatin-based scaffolds for a variety of tissue engineering applications, such as bone, cartilage, skin, nerve, and ocular and vascular tissue engineering, are reviewed and discussed. Gelatin blends with natural or synthetic polymers exhibit physicochemical, biomechanical, and biocompatibility properties very attractive for scaffolding. Current advances and challenges on this research field are presented.

*Keywords:* Gelatin, Electrospun scaffolds, Tissue engineering, Biopolymers

## 1. Introduction

Electrospun nanofibers are highly versatile platforms for a broad range of applications in different areas such as catalysis, nanofluidics, sensors, medicine, energy, environmental engineering, biotechnology, defense and security, and healthcare (Agarwal et al., 2013). In the biomedical field, micro/nanoscale fibrous structures have gained much interest for wound dressings, drug delivery systems, soft and hard-tissue engineering scaffolds, enzyme immobilization, among other healthcare applications (Abrigo et al., 2014; Ravichandran et al., 2012). Tissue engineering approaches usually require fabrication of engineered scaffolds, which aid in the repair and regeneration processes of the damaged tissue. The extracellular matrix (ECM) is composed of proteins and polysaccharides, mainly collagen, hyaluronic acid, proteoglycans, glycosaminoglycans (GAGs), and elastin. This complex mixture provides mechanical and biochemical support to surrounding cells and directs and modulates their behavior. Thus, the creation of biomimetic and functionalized scaffolds as bioactive ECM analogues is essential to construct an *in vivo*-like microenvironment that mimics biological entities and triggers specific cell responses (Agarwal et al., 2009; Sell et al., 2010; Wang et al., 2013; Pelipenko el al., 2015) Controllable fibrous structures with various compositions, fiber dimensions, and fiber architectures emulating the native ECM can be obtained by electrospinning technology. Mechanical, chemical, and biological properties of electrospun materials can also be tailored to replicate the many roles of native ECM (Sell et al., 2007).

Polymeric electrospun scaffolds provide a transitional three-dimensional support for cell adhesion, migration, proliferation, and differentiation. Moreover, the scaffolds should guide the maturation and tissue formation through a complex mechanical and biochemical signaling process. A number of natural and synthetic polymers have been considered to develop tissue engineered scaffolds (Ma, 2004). Nowadays, there is renewed interest in producing biodegradable scaffolds using biopolymers, including carbohydrate and protein-based biomaterials from both animal and

plant origin (Sridhar et al., 2015). Biopolymers offer many advantages such as chemical cues, hydrophilicity, degradation properties, and biocompatibility making them key players in modulating cell behavior. Thus, some naturally occurring polymers, such as collagen, gelatin, elastin, fibrinogen, and laminin contain integrins with binding affinity for cell-surface receptors that initiate cell adhesion. Among the main disadvantages are the weak mechanical properties, rapid biodegradability, and processability issues. Composite nanofibrous scaffolds composed of polymer blends combine the highly favorable and desired biological characteristics of natural polymers and the mechanical performance (i.e., strength and durability) of the synthetic ones. Thus, polymer mixtures can provide a straightforward pathway to design polymer-based scaffolds with different and superior bioactivities. The development of an ECM analogue is highly challenging, and can be possible through the manipulation of natural polymers (Sell et al., 2010).

In this context, gelatin blends with natural or synthetic polymers exhibit physicochemical, biomechanical and biocompatibility properties which are very attractive for scaffolding. This mini-review is focused on electrospun gelatin-based matrices as tissue engineering scaffolds. First, a short description of the electrospinning process is included for those readers not acquainted with the technique. Then, gelatin properties are briefly described. Finally, the review deals with selected biomedical applications of electrospun gelatin-based scaffolds, such as bone, cartilage, skin, nerve, ocular and vascular tissue engineering. A vision of the future research on this topic is then presented.

## 2. Electrospinning process

Electrospinning or electrostatic spinning is a very attractive electrohydrodynamic technique for processing polymer solutions or melts in the form of micro/nanofibrous non-woven scaffolds. When a polymer solution or melt is subjected to a high-voltage, the surface of a pending drop held by its own surface tension forces is electrostatically charged. Once the electric field established between a

spinneret tip and a grounded collector reaches a certain threshold value, the electrostatic forces overcome the surface tension of the solution and produce a microjet from the pendant drop. Before reaching the collector, the liquid jet undergoes stretching and whipping while the solvent evaporates during the process. The micro/nanofibers produced by electrospinning lead to the formation of non-woven mats of either randomly-oriented or aligned fibers (Bhardwaj et al., 2010).

Although many authors refer to electrospinning as a simple technique, the process is complex, and it is governed by a number of parameters that greatly affect fiber formation, size, and morphology. The intrinsic solution properties (polymer structure, molecular weight, concentration, solvent/co-solvent type, viscosity, conductivity, and surface tension), the processing parameters (applied voltage, polymer solution flow rate, nozzle-to-collector distance, position, nozzle diameter and number, collector geometry and type, and collector polarity), and the ambient parameters (temperature, humidity, pressure and air velocity) strongly determine the quality and characteristics of the electrospun fibers and the resulting mats. Therefore, the electrospinning process is not as simple as it appears. In order to produce defect-free continuous fibers with reproducible fiber diameter distribution and orientation these parameters must be accurately controlled. Detailed explanations of each parameter and its influence on the electrospinning process can be found in the literature (Pham et al., 2006; Bhardwaj et al., 2010).

A huge variety of biocompatible synthetic polymers, natural polymers, or blends of both can be electrospun, each one with different physical properties, mechanical performance, biodegradation rate, and cell-material interactions. Table 1 summarizes some of the polymers most commonly used in electrospinning for tissue engineering applications. Many other polymers were electrospun to satisfy different clinical requirements. Polyphosphazenes, polyethyleneimine, poly(propylene carbonate), polydioxanone, poly(glycerol-sebacate), and polyhydroxyalkanoates, are just some examples of the wide range of polymers investigated. Nanofibers can be functionalized through encapsulation, grafting, immobilization, coating, or blending of biologically active compounds such as proteins, enzymes, and growth factors (Zamani et al., 2013; He et al., 2014; Sridhar et al., 2015).

Moreover, nanofibers can be assembled into a variety of arrays or architectures by manipulating their alignment, stacking, or folding (Li et al., 2014). In the last years, the production of advanced nanofibrous scaffolds includes multilayer mats, core/shell structures, compositional gradients, nanocomposite or drug-loaded nanofibers, structured with superior mechanical properties or with enlarged pores for regenerative engineering. All of these have attracted significant research interest and have demonstrated superiority over traditional nanofibrous scaffolds (Jiang et al., 2015).

**Insert Table 1**

## 3. Gelatin: chemical and biological characteristics

Gelatin is a fibrous protein composed of a unique sequence of amino acids obtained from native collagen via hydrolysis that causes to lose the original α-helix conformation by breaking intermolecular bonds. Irrespective of the hydrolysis process that turns collagen into gelatin, both biopolymers have in the primary structure composition as many as 20 different amino acids in variable proportions. This primary structure provides RGD (L-arginine-glycine-L-aspartic acid), a three-amino-acid recognition sequence for integrin mediated cell adhesion. The low-cost collagen derivative exhibits good biocompatibility and biodegradability, and it is also non-immunogenic. Hence, gelatin finds numerous applications in the food and pharmaceutical industries. According to the literature, both collagen and gelatin used as tissue engineered components significantly improve infiltration, adhesion, spreading, and proliferation of cells on resulting scaffolds (Huang et al., 2004; Sajkiewicz and Kołbuk, 2014; Zhang et al., 2009).

Gelatin properties depend on the source of collagen (usually bovine or porcine), age of the animal, type of collagen, type of conversion of collagen to gelatin (acidic vs. basic hydrolysis), as well as on the conditions of final formation of scaffolds (e.g. acidity of solution). Two types of gelatin are generally obtainable, depending on the pre-treatment procedure (prior to extraction process). Acidic pre-treatment (type A) barely affects the amide groups while the alkaline pre-

treatment (type B) targets the amide groups of asparagine and glutamine and hydrolyzes them into carboxyl groups, thus turns many of these residues into aspartate and glutamate. As a protein, gelatin exhibits an amphoteric behavior due to the presence of both acidic and basic functional groups, as a result of existence of amino acids functional groups and terminal amino and carboxyl groups. In acidic medium, that is, in presence of high concentration of $H^+$ ions, gelatin is positively charged. In an alkaline medium, that is, in the presence of $OH^-$ ions, gelatin is negatively charged. At the isoelectric point (IEP), gelatin has a neutral net charge, as an effect of equilibrium between positive charges from $NH_3^+$ ions and negative charges from $COO^-$ ions. IEP is an intrinsic property of gelatin determined by raw materials' pre-treatment and the type of process. The gelatin of type A typically exhibits an IEP in the range 6–9.5, while type B has an IEP in the range 4.5–5.6. Because of electrostatic attraction of opposite charged groups near IEP, some of properties reach their extreme values close to IEP.

## 4. Gelatin-based electrospun materials for tissue engineering

Gelatin is electrospinnable only from solutions in which gelatin adopts a random coil conformation. In aqueous solutions below ca. 30°C, the gelation occurs making electrospinning at room temperature impossible. Thus, water solution needs heating of gel above gel–sol transition point. Moreover, the high surface tension of water solutions complicates electrospinning due to the destabilization of polymer jets and the formation of droplets. Water's high boiling temperature introduces an additional problem. Non-complete water evaporation before reaching the collector leads to fiber fusion and heterogeneities. Another method to obtain nanofibers is by using certain organic solvents. Apart from some hazardous and toxic organic solvents (typically 2,2,2-trifluoroethanol (TFE), trifluoroacetic acid (TFA) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), an effective method to obtain electrospinning solutions is to use acidic solvents like acetic acid and formic acid aqueous solutions (Sajkiewicz and Kołbuk, 2014). An important advantage of gelatin

over other structural proteins (i.e., collagen) is the fact that gelatin has shown no denaturation phenomena due to the interaction with the applied electric field during the electrospinning process (Zeugolis et al., 2008).

Changes on crystal structure and crystallinity of gelatin film and electrospun nanofibers were investigated by X-ray diffractometry (Ki at al., 2005). As shown in Fig. 1, gelatin powder gives a typical XRD pattern of gelatin crystalline structure originated from α-helix and triple helical structure. However, films prepared from aqueous gelatin solution showed a less developed crystal structure and lower crystallinity. Amorphous structures were observed for the film and electrospun nanofibers obtained from gelatin–formic acid solution. Even though α-helical structure was maintained to a certain extent, very low crystallinity was observed from XRD pattern only for the specimens prepared from gelatin–formic acid solution.

**Insert Fig. 1**

For biomedical applications, the dissolution and loss of three dimensional structures of gelatin scaffolds in aqueous conditions at temperatures typical at in vivo and in vitro conditions is an important challenge to overcome. There are two main methods of gelatin structure stabilization, either chemical crosslinking or physical blending with other polymers.

Several compounds have been applied as chemical crosslinkers for gelatin used for biomedical applications like aldehydes (e.g. glutaraldehyde (GA), carbodiimides, succinimide, and genipin (GEN)). The general problem of chemical crosslinking of gelatin nanofibers is related to changes in the original morphology and the toxicity of unreacted crosslinkers. Sisson et al. have studied different methods of the electrospun gelatin fiber crosslinking, including vapor-phase glutaraldehyde, aqueous phase GEN, and glyceraldehyde, as well as reactive oxygen species from a plasma cleaner (Sisson et al., 2009). This last method was not efficient because the scaffolds degraded after just a few hours in aqueous medium at 37 °C. Glyceraldehyde and GEN were considered good options as cross-linking agents because of their low toxicity and the resistance to dissolution of the cross-linked fibers in cell culture medium at 37 °C. Zhang et al. indicate that 1-

ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) have low cytotoxicity (Zhang et al., 2006). However, this crosslinking leads to curling, conglutination, and to an increase in fiber diameter (decrease in porosity) as a result of the swelling of nanofibers during crosslinking. The most widely used chemical substance for crosslinking of gelatin is GA, although the residuals of GA indicate possible cytotoxic effects on the cells.

The effect of crosslinking introduces a dramatic modification of the mechanical behavior in the as-electrospun mats. Fig. 2 shows as an example, the tensile stress-strain curves recorded from electrospun gelatin and electrospun gelatin crosslinked with GEN (Panzavolta et al., 2011). The Young's modulus and stress at break of uncrosslinked gelatin electrospun mats were 240 and 6 MPa, respectively. GEN addition to the electrospinning solution dramatically reduced the extensibility of the as-electrospun mats, which displayed further remarkable improvements in Young's modulus and stress at break after successive crosslinking up to values of about 990 and 21 MPa, respectively.

**Insert Fig. 2**

**4.1. Bone**

Electrospun nanofibrous scaffolds have attracted considerable interest as potential platforms for bone repair and regeneration (Jang et al., 2009). The beneficial features of nanofibrous matrices were first explored with degradable polymers, either of synthetic or natural origin. Among the synthetic polymeric biomaterials, poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone) (PCL), poly(hydroxyalkanoates) and their copolymers are the most extensively studied systems for bone tissue engineering. A range of bone-bioactive inorganic components, such as calcium phosphates and bioactive glasses, were then introduced within the polymer nanofibers to achieve bone-specific bioactivity and mechanical properties, mimicking the composition, morphological and mechanical functions of the native bone ECM. New strategies involve endowing the nanofiber surface with biofunctionality to regulate cell/surface response. In this approach,

surface-conjugated with proteins/peptides/genes or protein-loaded nanofibers are interesting to obtain electrospun matrices as drug delivery systems.

The hydrophobic nature of many synthetic polymers limits cell affinity and initial cell adhesion. Blending synthetic and natural polymers is another way to improve the hydrophilicity and, then, cell compatibility (Zhang et al., 2005; Kim et al., 2008). This is a feasible approach that reduces the cytotoxic problems associated to the use of crosslinking reagents needed and that provides new biocompatible materials with improved mechanical, physic-chemical, and surface properties. Thus, PCL/gelatin nanofibrous scaffolds were investigated as promising scaffolds for bone-marrow stromal cell culture (Zhang et al., 2005) and PLLA/gelatin scaffolds triggered favorable preosteoblast cells adhesion and supported rapid cell population and growth (Kim et al., 2008). Randomly-oriented and aligned PLGA and PLGA/gelatin scaffolds were fabricated through electrospinning (Meng et al., 2010). The addition of gelatin clearly increased the hydrophilicity of the composite scaffolds, enhanced the adhesion and proliferation of murine calvarial preosteoblasts (MC3T3-E1), and showed a decrease in the mechanical properties. The aligned orientation of nanofibers guided cells' growth along the longitudinal axis of nanofibers, which would provide a beneficial approach for bone regeneration.

Nanofibrous gelatin/apatite composite scaffolds have been fabricated by thermally-induced phase separation technique to mimic both, the physical architecture and the chemical composition of natural bone ECM (Liu et al., 2009). The scaffolds showed excellent biocompatibility and mechanical stability, and apatite incorporation enhanced the osteogenic differentiation.

The use of phenylazide-conjugated poly(acrylic acids) as crosslinker of gelatin was proposed by Lin et al. (Lin et al., 2015). UV irradiation activates phenylazido groups into nitrenes, which form stable covalent bonds with neighboring N-H or C-H of gelatin. Moreover, the biofunctionality of UV-crosslinked scaffolds was further improved by the incorporation of inorganic hydroxyapatite nanoparticles, cell adhesive peptides (RGD) and bone morphogenetic protein-2 (BMP-2). The three

incorporated bio-factors exert a synergistic effect on osteogenesis of mesenchymal stem cells in the GEF scaffolds.

A biomimetic composite nanofibrous membrane of gelatin/$\beta$-tricalcium phosphate (TCP) was developed by Zhang et al. (Zhang et al., 2015). The incorporation of TCP into gelatin nanofibers caused increased cell attachment, proliferation, alkaline phosphatase activity, and osteogenic gene expression in rat bone marrow mesenchymal stem cells (BMSCs). The gelatin/TCP composite nanofibrous mat promotes osteogenesis and neovascularization of BMSCs, therefore this scaffold has a great potential in practical application in orthopedics and dentistry, such as guided bone regeneration membranes in periodontal pockets.

Recently, Rong et al., demonstrated the very good biocompatibility of gelatin/PCL/nanohydroxyapatite and gelatin/PCL/bone powder electrospun scaffolds by seeding adipose-derived stem cells (ADSCs) and studying cell morphology, cell viability, and proliferation (Rong at al., 2016). Fig. 3 revealed the morphologies of ADSCs attached to each scaffold. Cells covering the surface of the porous nanocomposites, and spreading with their pseudopodia, revealed better adhesion and activity. Gelatin/PCL/bone powder scaffold turned to be the best candidate to mimic bone matrix and the microenvironment for osteogenic differentiation of ADSCs.

**Insert Fig. 3**

### 4.2. Cartilage

Cartilage damage specially occurs in osteoarthritis, rheumatoid arthritis, and different traumas. Such defects can lead to important clinical consequences because of the limited intrinsic potential for healing. In order to overcome this limitation of self-repair capacity, repair of cartilage defects is always a great challenge in surgical treatment (Kock et al., 2012; Chung et al., 2008). Scaffolds, as one of the essential elements of three-dimensional (3-D) cartilage construction, play important roles in directing cartilage regeneration. Cartilage is primarily composed of chondrocytes and ECM

proteins such as type II collagen and other GAGs that provide significant tensile and compressive strength. In the last decades, natural and synthetic polymeric electrospun scaffolds have attracted a great deal of attention due to their capability to mimic ECM. One of the applicable electrospun fibrous membranes is the hybrid of gelatin and polycaprolactone (GT/PCL), which is a versatile biomimetic substrate for the engineering of a variety of soft tissues including cartilage (Xue et al., 2013; Zheng et al., 2014). In order to control the shape of the scaffold, Xue et al. and Zheng et al. have developed a sandwich model in which cells are seeded on acellular cartilage sheets layer-by-layer over a titanium alloy mold to generate ear-shaped cartilage (Fig. 4). The authors proved that all the membranes with different GT/PCL ratios presented good biocompatibility with chondrocytes. However, only the GT/PCL 70:30 ear-shaped scaffold formed homogeneous and continuous cartilage with a satisfactory shape and elasticity similar to that of the human ear. The authors suggest that the good hydrophilicity could partially resist gravity and thus keep a relatively homogeneous distribution of cell suspension in the whole scaffold at cell seeding. Meanwhile, the high PCL content was unfavorable for 3-D cartilage regeneration, especially for the cartilage with a complicated shape. Also, a high PCL amount increases the scaffold degradation rate. The engineering of 3-D cartilage in a sandwich model using GT/PCL 70:30 electrospun fibrous membranes was a facile and effective approach, which has the potential to be applied for the engineering of other tissues with complicated 3-D structures.

In another approach, Torricelli et al. have fabricated gelatin-PLLA fibrous scaffolds by co-electrospinning (Torricelli et al., 2014). The authors demonstrate the possibility to modulate the composition of composite PLLA/GT scaffolds by changing the flow rate parameter of the electrospinning process. Therefore, this composition affects the tensile mechanical properties of the composite scaffolds that were in-between those of the pure PLLA and gelatin scaffolds, and varied as a function of PLLA relative content. Chondrocytes seeded on composite scaffolds showed good viability and proliferation rate. The PLLA50GT50 and PLLA70GT30 scaffolds not only promote chondrocyte differentiation as evidenced by differentiation markers, but also the cell infiltration into

scaffolds was an important aspect to consider them suitable for tissue engineering constructs. Moreover, mineralization experiments suggested that potential applications of the scaffolds can be extended to cartilage-bone interface tissue engineering.

In brief, gelatin-based electrospun scaffolds have a great potential to be applied in cartilage tissue engineering. However, the blending and/or crosslinking of gelatin are needed to improve the mechanical and degradation properties of the final scaffold.

**Insert Fig. 4**

**4.3. Skin**

Skin is the largest organ of the human body and it is susceptible to external injury and wounds because it is our first line of defense against environmental harms. The wound-healing cascade is a complicated physiological process which involves a sequence of five typical local events: acute inflammation, chronic inflammation, growth of granulation tissue, foreign body reaction, and remodeling. Depending on the extent and severity of the injury, treatment can be a clinical problem that requires the use of skin grafts or other healing aids. The use of engineered scaffolds is a strategy to promote skin regeneration. Nanofibers which have ideal environmental conditions could be a good solution for the development of skin tissue engineered scaffolds. As it was explained in section 3, the crosslinking of gelatin nanofibers is essential to avoid its solubility in an aqueous solution. GA crosslinked gelatin electrospun scaffold was obtained by Gomes et al. (Gomes et al., 2015). As many works of gelatin based biomaterials, they found that crosslinked gelatin electrospun scaffolds allow *in vitro* adhesion, migration, and proliferation of fibroblasts and the *in vivo* healing of full thickness skin wounds. In spite of the biocompatibility of crosslinked gelatin electrospun scaffolds, a decrease of the cell viability in the gelatin scaffold after 2 days was observed. The authors suggested that some residual GA may have caused apoptosis of some cells thereby slowing population growth.

As a consequence, the alternative pathways to enhance mechanical properties and to control the scaffold degradation rate are still pending. Huang et al. proposed the inclusion of other crosslinking agent, proanthocyanidin (PA) (Huang et al., 2012). PA is a naturally occurring plant metabolite which can stimulate normal skin fibroblast proliferation and increase the synthesis of an ECM, including collagen and fibronectin. The results not only demonstrated the feasibility of utilizing PA as an effective nontoxic crosslinking agent for gelatin, but also showed that the gelatin/PA blend nanofibers significantly promoted drug loading and obtained a constant drug-release profile. Magnesium ascorbyl phosphate (MAP) was used as a model drug. Although GA crosslinking was not avoided, a non-toxic less swellable gelatin/PA/MAP electrospun scaffold was obtained. Furthermore, this scaffold accelerates fibroblast cell proliferation, increases drug loading efficiency, and might suppress melanin formation during the wound healing process. In other approach, alginate dialdehyde (ADA) was chosen as crosslinker by Chen et al. (Chen et al., 2016). This polymer is biodegradable and non-toxic and could react with gelatin in basic media. The authors developed a simultaneous gentamicin and ciprofloxacin-loaded ADA/gelatin electrospun fibers which showed an integral fibrous morphology during cross-linking and a gradual mass loss during four weeks of incubation. The developed matrix showed significant growth inhibitions on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. They studied how the membrane affects the three phases of wound healing: inflammation, new tissue formation and remolding. The wound re-epithelization, blood vessel formation, collagen deposition, and tissue remodeling process are accelerated with a complete healing observed after 21 d. On the other hand, Shan et al. proposed a uncrosslinked silk fibroin (SF)/gelatin electrospun fibers loaded astragaloside IV (AS) as dressing on deep partial-thickness burn wound (Shan et al., 2015). Even the degraded nanofibrous dressing was 80% at 12 h; the degradation rate is the main influencing factor of drug release. The in vitro and in vivo biological studies were carried out to evaluate the nanofibrous matrix as wound dressing. The AS-loaded SF/gelatin nanofibrous dressing promoted cell adhesion and proliferation with good biocompatibility in vitro ($p < 0.01$). This dressing also accelerated wound healing and

inhibited scar formation in vivo by stimulating wound closure ($p < 0.05$), increasing angiogenesis, regulating newly formed types of collagen, and improving collagen organization.

The coaxial electrospinning to obtain nanofibers with poly (3-hydroxybutyric acid) as core and GA crosslinked gelatin as sheath nanofiber scaffold was proposed by Nagiah et al. as a strategy to enhance mechanical properties (Nagiah et al., 2013). The inclusion of PHB prolonged mean-life and enhanced mechanical properties in respect to gelatin nanofibers. In addition, the gelatin/PHB scaffolds supported the adhesion and proliferation of human dermal fibroblasts and keratinocytes.

In order to obtain a smart wound dressing, the inclusion of a shape memory polymer or a multifunctional polymer in gelatin electrospun scaffolds has been developed (Dongargaonkar et al., 2013; Tan et al., 2015). Tan et al. studied a blend of chitosan, gelatin and shape memory polyurethane (Tan et al., 2015). By GA crosslinking and synthetic polymer blending, the gelatin/chitosan/PU electrospun scaffold has desirable mechanical behavior, water vapor transmission ratio, and surface wettability. In addition, the membrane has antibacterial activity, cytocompatibility and hemostatic properties. Particularly, the shape memory function of PU confers good controllability of the tensile force under different strains on the composite electrospun scaffold. Thus, wound healing can possibly benefit through shape fixation-assisted easy processing and shape recovery-assisted closure of cracked wounds, which can be fine-tuned by pre-programming.

Together with the development of smart scaffolds, highly branched star-shaped polyamidoamine (PAMAM) dendrimer G3.5 was covalently conjugated to gelatin via EDC/NHS chemistry and then blends of gelatin and gelatin-dendrimer conjugates mixed with various loading levels of silver acetate were successfully electrospun (Dongargaonkar et al., 2013). The addition of dendrimer provides functional sites for attachment of drugs and their controlled release. The scaffolds were further converted into semi-interpenetrating networks (sIPNs) with photoreactive polyethylene glycol diacrylate (PEG DA575). This strategy successfully improved the structural stability and mechanical properties of the construct without compromising fiber morphology. The sIPNs showed

good swelling capacity owing to porous structures and were permeable to aqueous solutions. They allowed sustained silver release and showed antimicrobial activity against two common types of pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa* possibly through a dual bactericidal mode of action: release and contact killing mechanisms. This work demonstrated that both, sIPN and dendrimer conjugation, enhance properties of gelatin electrospun scaffold to be used in tissue engineering applications.

In order to promote cellular proliferation as well as to facilitate the cellular differentiation process for skin regeneration, biodegradable and biocompatible scaffolds with biophysical microenvironment have been designed. Pezeshki et al. have reported, firstly, the inclusion of a GAG in gelatin nanofibers (Pezeshki-Modaress et al., 2015). The crosslinked gelatin and chondroitin sulfate (CS) nanofibrous scaffolds were synthetized. CS is extensively present in the ECM and affects several cellular performances such as migration, attachment and proliferation of the cells. Also, CS has an effective role in wound healing and tissue regeneration. The presence of GAGs in the solution could increase electrical conductivity due to the presence of cations in salts of chondroitin sulfate. Pezeshki-Modaress et al. studied how the electrospinning parameters affect the diameter of fibers. Also, human dermal fibroblasts were seeded in the scaffolds. The cells attached and spread completely onto the nanofibrous surface and, in addition, the crosslinked gelatin/CS nanofibrous mats could maintain their nanofibrous structure in the culture medium.

Additionally, Bhowmink et al. evaluated the influence of biological cues of sericin-loaded gelatin/hyaluronan/CS electrospun scaffolds to mimic the extracellular microenvironment for dermal tissue engineering applications (Bhowmick et al., 2016). Hyaluronan and CS are both glycosaminglycans. Sericin is a degumming macromolecule protein with antioxidant, antibacterial, UV-light protection, and anticancer properties. The slow and sustain proteins (gelatin/sericin) and GAGs release and the enhanced mechanical properties of electrospun scaffolds suggest that GA crosslinking not only affects scaffold properties but that there is also an interaction between sericin and GAGs with free functional groups of gelatin like -COOH and -$NH_2$ by hydrogen bonds. The

GA crosslinking was essential for decreasing scaffold degradation time. The microbiological study has demonstrated that sericin-loaded electrospun scaffolds promote adhesion and proliferation of fibroblasts, keratinocytes, and hMSCs. *In vitro* results evidently prove that both, cellular interaction with neighboring cells and with cellular microenvironments can synergistically stimulate the differentiation of hMSCs towards epithelial linage. This work showed the importance of biological cues on scaffold designing.

**4.4. Nerve**

Patients who have injuries or traumas in the nervous system often suffer from the loss of sensory or motor function, and neuropathic pains because the nerve has a very limited capacity to regenerate (Chang et al., 2008). In the peripheral nervous system (PNS), direct end-to-end surgical reconnection is a common method of treatment for nerve transection injuries when the injury gap is small. The use of autograft or allograft or xenografts has many limitations, including donor scarcity, multiple surgeries, donor site morbidity, scarring, and the need for an allograft patient to take immunosuppressants indefinitely after surgery to avoid rejection (Cao et al., 2009; Chalfound et al., 2006). Artificial nerve grafts using biocompatible and biodegradable polymers represent an alternative strategy to restore nerve function (Sannino and Madaghiele, 2009).

In this context, some studies were conducted with gelatin-based electrospun scaffolds in order to evaluate the influence of morphological and biological cues on *in vitro* neural cellular behavior. A precedent of gelatin nanofibrous membrane as scaffold for nerve tissue engineering was reported (Alvarez-Perez et al., 2010). Nanoscale PCL electrospun membranes enhanced with gelatin were investigated to validate their biological response under *in vitro* culture of PC-12 nerve cells. The contact angle measurements confirmed the hydrophilic behavior of the membranes, ascribable to the gelatin content. The higher diameter of PCL/gelatin fibers limits the surface/volume ratio and fiber density which, in turn, negatively affect cell recognition. On the other hand, the enhanced

biocompatibility of the composite scaffold demonstrates the main role of gelatin cues, which improves cell affinity compared to synthetic polyester fibers of PCL. Immunostaining and RT-PCR results confirm that gelatin cues also support neuronal differentiation and changes in gene expression during *in vitro* differentiation, directly related to neurite outgrowth. The results corroborated that gelatin incorporation improves all the fundamental biological events of nerve regeneration. However, degradation and mechanical studies must be done to consider them as scaffolds for nerve tissue engineering.

In order to handle the degradation rate and the mechanical properties of gelatin nanofibrous scaffolds, some strategies have been reported. Coaxial electrospun fibers of poly L-lactic acid and gelatin with a degradation rate and mechanical properties similar to peripheral nerve tissue have been developed (Binan et al., 2014). A core/sheath structure made of PLLA and gelatin, respectively, showed fiber diameter and mechanical properties tailored by polymer concentration. With an increase of PLLA and gelatin concentration, the fiber diameter was from 0.8 to 2 μm and the Young's moduli were comprised between 0.1 and 1.2 MPa (peripheral nerve tissue is around 0.45 MPa (Sundback et al., 2005)), depending on the crosslinking percentage. Moreover, the authors have investigated the effect of fiber mats on neural stem-like cell survival and differentiation into motor neuronal lineages through the controlled release of retinoic acid and purmorphamine (Fig. 5). The bioactive material not only enhanced the differentiation into motor neuronal lineages but also promoted neurite outgrowth. They observed the creation of extended, mechanically connected networks of motor neuron cells in the newly generated tissue in vitro. These promising results concluded that a combination of electrospun scaffolds, neural stem cells, and controlled delivery of instructive cues could lead to the development of a better strategy for peripheral nerve injury repair.

**Insert Fig. 5**

Currently, Mehrasa et al. proposed the incorporation of mesoporous silica nanoparticles (MSNPs) as a strategy to improve mechanical properties and degradation rates of aligned

PLGA/gelatin fibrous scaffolds (Mehrasa et al., 2015). Rat pheocromocytoma PC-12 cells were cultivated on PLGA/gelatin/MSNPs scaffolds. The degradation rate and cell adhesion and spreading were enhanced by adding gelatin and MSNPs into PLGA matrix due to the higher hydrophilic behavior of membranes. In addition, the controlled architecture of aligned nanofibrous scaffolds was helpful for cell proliferation, which might eventually be valuable in nerve regeneration, especially for the outgrowth of axons and dendrites. The incorporation of MSNPs on scaffolds improves the Young's module of the PLGA/gelatin scaffolds.

Nerve regeneration in the central nervous system (CNS) is even more challenging than the PNS, since the inhibitory environment formed after injury in the CNS often restricts nerve regeneration (Cao et al., 2009). Indeed, it has been demonstrated that the interaction of cells, either transplanted or migrating endogenous stem cells, with the ECM plays a key role in brain healing and regeneration (Zimmerman and Dours-Zimmermann, 2008). Therefore, the development of new tools is needed to reconstitute the native ECM and the tissue architecture of the damaged CNS. The brain ECM has a unique composition as it contains relatively small amounts of fibrous proteins, such as collagen, laminin and fibronectin, and high amounts of linear polysaccharides, such as GAGs (Novak and Kaye, 2000). Therefore, appropriate biomaterials, to be processed to mimic a three-dimensional instructive microenvironment with specific biochemical cues and to promote cell migration, adhesion and survival, could enhance the success of neural implants (Cao et al., 2009; Zhong and Bellamkonda, 2008). Baiguera et al. proposed a GEN crosslinked gelatin electrospun scaffold incorporating rat decellularized brain extracellular matrix (dBECM) as a potential scaffold for central nerve regeneration (Baiguera et al., 2014). dBECM was extracted successfully from rat brain preserving the ECM structural components necessary for cell attachment and repopulation. And the electrospun crosslinked dBECM-gelatin mat was evaluated as a supportive platform for rat mesenchymal stromal cells (MSCs) and mononuclear cells adhesion and growth. Analyzing the expression of a specific marker for neural cells, the results suggested that the incorporation of dBECM into gelatin crosslinked mats could play a role in triggering the differentiation of MSCs to

neural/glial precursor cells. Thus, this study demonstrated the potential of electrospun gelatin mats, incorporating dBECM, to act as effective scaffolds providing a suitable microenvironment for MSCs adhesion, proliferation, and survival.

### 4.5. Ocular

#### *4.5.1. Retinal tissue engineering*

A variety of ocular disorders are originated by an alteration in the retinal pigment epithelium (RPE). The RPE is anatomically adjacent to the neurosensory retina and it is composed of a monolayer of polarized pigment cells, which lie on Bruch's membrane (BM). This epithelium plays a crucial role in a number of processes (Liu et al., 2014; Lu at al., 2001; Xiang et al., 2014) which include: nourishment of the retinal visual cells, response to distinct extracellular signals, stray light absorption, retinal isomerization during the visual cycle, secretion of neurotrophic factors, phagocytosis of photoreceptor outer segments (POS), and preservation of the blood-retina barrier through tight junctions. RPE dysfunction or cell loss is one of the major pathological changes that leads to a wide range of retinal degenerative diseases, such as age-related macular degeneration and some forms of retinitis pigmentosa, which are the leading causes of blindness worldwide. Unfortunately, there is still no radical treatment either to alleviate the progression of these diseases or to recover the lost vision associated with them. Tissue engineering provides a promising opportunity to improve cell-based RPE therapies. The fundamental concept is to use a scaffold, on which the RPE is pre-cultured, that supports maturation into a functional monolayer, followed by transplantation of these RPE/scaffold complexes underneath the retina (Liu et al., 2014). Recently, electrospun nanofibers designed to bio-mimic the natural basement membranes used for retinal tissue engineering have received considerable attention (Gu et al., 2011). In this context, Xiang et al. have fabricated an ultrathin and porous nanofibrous membrane composed of regenerated wild *Antheraeapernyi* silk fibroin (RWSF), PCL, and gelatin to replicate the BM (Xiang et al., 2014).

Human RPE cells seeded on the electrospun membranes were evaluated following long-term (up to 12 weeks) co-culture and implanted into the eye of chinchilla rabbits. They could develop Bruch's membrane-mimetic substrates with high porosity and adequate thickness from RWSF, gelatin, and PCL. Gelatin incorporation was fundamental to enhance cell attachment and proliferation due to its hydrophilicity and RGD segments. The feasibility of using these electrospun membranes as alternatives to Bruch's membrane for long-term sustainable culture and growth of functional RPE cells was confirmed. RWSF/PCL/Gelatin membranes not only accelerated RPE cell growth and proliferation without any inflammatory reaction, but also promoted the functionalization of RPE cells. Thus, these electrospun RWSF/PCL/Gelatin membranes could act as a potential prosthetic BM for RPE transplantation. The promising results of this first attempt made of the gelatin-based electrospun membranes as potential scaffolds for retinal tissue engineering and the mechanical and degradation properties will be done to be followed by *in vivo* studies.

*4.5.2. Corneal tissue engineering*

Corneal disease is the second cause of vision loss and affects millions of people worldwide, followed by trachoma and corneal injury. The correction of these affections mainly consists of the use of donor corneal grafts (Gao et al., 2012; Ruberti and Zieske, 2008; Tonsomboon and Oyen, 2013). However, there are serious difficulties since there are few donors who are eligible to donate their corneal tissue and, moreover, it is very hard to obtain quality graft material due to immunological rejection or endothelial decompensation. Therefore, the development of satisfactory corneal substitutes is very important. Corneal ECM consists of an aqueous sugar matrix, containing large water content of up to 80% with a small fraction of GAGs, and orthogonally-aligned collagen nanofibrils (30 nm in diameter) which provides biaxial stiffness and transparency to the native cornea (Ruberti and Zieske, 2008). From this aligned fiber nature, aligned and randomly oriented fibrous gelatin/PLLA scaffolds were studied to guide the growth of corneal stroma cells (Gao et al., 2012). Gao et al. demonstrated that the aligned scaffold not only increases cell viability more

significantly than that in randomly oriented scaffold, but it also provides an external stimulus for the orderly arrangement of cells.

Considering the high percentage of water in the corneal tissue, hydrogels had become the first material of choice for corneal scaffold fabrication. However, the scaffolds not only need to provide optimal conditions for corneal cells and suitable refractive power, but they must also be able to carry the tension induced from high intraocular pressure and eye movements (Ruberti and Zieske, 2008). Currently, the use of electrospun fiber-reinforced hydrogels has emerged as an alternative to substantially improve mechanical properties of the hydrogels. Tonsomboom et al. have developed electrospun gelatin nanofiber-reinforced alginate hydrogels to mimic the microstructure of the corneal ECM (Tonsomboon and Oyen, 2013). They demonstrated that electrospun gelatin nanofibers could manage the mechanical and optic properties of reinforced alginate hydrogels (Fig. 6). Although the fiber alignment of electrospun gelatin mats enhances substantially its mechanical properties (Fig. 7), this enhancement is not evidenced in hydrogels. Probably, the fibers immersed on alginate solution are reordered. The crosslinking of gelatin fibers was necessary to prevent rapid *in vivo* degradation of the scaffolds after being transplanted into the human body, but it is also potentially useful to improve their mechanical properties. Even though the crosslinking with EDC-NHS in ethanol enhanced the mechanical properties of the resulting composite hydrogels, the loss in their optical transparency limits their use as corneal scaffolds. Therefore, the balance between mechanical and optical properties of the final composite hydrogel is still required to match the properties of the corneal native tissue.

**Insert Fig. 6 and Fig. 7**

**4.6. Vascular**

The *in vitro* fabrication of fully functional vascular tissue constructs represents one of the most fundamental challenges in vascular tissue engineering. In this context, aligned and random PLLA/gelatin nanofibrous scaffolds were prepared to enhance cell adhesion sites (Shalumon et al.,

2015). Shalumon et al. observed an increase in viability and proliferation of human umbilical vein endothelial cells (HUVECs) and smooth muscle cells (SMCs) proportional to gelatin content (Fig. 8). Moreover, the aligned fiber morphology helped cells to orient and elongate along their long axis. Electrospun gelatin nanofibrous scaffolds incorporating heparin were recently reported and the obtained matrices were assessed as controlled delivery devices in vascular tissue engineering applications (Wang et al., 2013). The results of the biocompatibility *in vitro* tests carried out using HUVECs indicated good cell viability and proliferation on the gelatin/heparin scaffolds. The results demonstrated that the use of electrospun gelatin fibers as heparin carriers could be promising for vascular tissue applications.

**Insert Fig. 8**

The feasibility of creating scaffolds made of coaxial nanofibers containing a core of polyurethane and a sheath of a mixture of poly(ε-caprolactone)/gelatin was also explored (Gluck et al., 2011). The results showed the advantages of combining both synthetic and natural polymers to create a coaxial scaffold capable of withstanding dynamic culture conditions and of encouraging cellular migration to the interior of the scaffold for tissue engineering applications.

On the other hand, some authors proposed polyurethane/gelatin mixtures to meet the requirements for vascular grafts (Detta et al., 2010; Jia et al., 2013). These studies confirmed that SMCs and HUVECs adhere well to the electrospun polyurethane/gelatin surface and that they proliferate.

Zhang et al. reported a tubular scaffold, featuring a spatially layered structure, by sequential and coelectrospinning of gelatin, elastin and polyglyconate (Maxon®) blends and its *in vitro* degradation profile (Zhang et al., 2009). The degradation of this tri-layered structure affected the mechanical properties of the scaffold containing insoluble elastin more than the one containing soluble elastin. The same authors reported the development of bilayered tubular scaffolds composed of poliglecaprone/polycaprolactone/gelatin and elastin by electrospinning (Zhang et al., 2010). This polymer blend allowed tailoring the mechanical properties and degradation rate of the scaffold. To

prevent early disintegration of protein components, chemical crosslinking was employed. The HUVECs in direct contact with the surface of the scaffold revealed excellent cell-viability.

## 5. Conclusions and future perspectives

Electrospinning is a very attractive, complex, and versatile technique to prepare advanced functional nanofibrous scaffolds for a variety of tissue engineering and drug delivery applications. Current efforts are focused on preparing electrospun scaffolds with controlled multilevel hierarchical structures. The use of gelatin as a biopolymer scaffolding material for tissue engineering applications is directly related to its high biocompatibility, hydrophilicity, and bioactivity associated to specific peptide sequences. Multicomponent fibers containing gelatin are more promising systems than pure gelatin itself, due to the combination of electrospinnable solutions and chemical, structural, and mechanical properties that mimic the important features of natural ECM.

However, there are still many unanswered questions related to the role of higher order gelatin structures in scaffolds bioactivity. The most important one is whether the cells are sensitive to the secondary and higher order structures of gelatin in scaffolds. Challenges also remain to better understand the mechanisms by which nanofiber scaffolds affect cell behavior and tissue regeneration processes. Physiological and mechanotransductory signals need to be further investigated to optimize electrospun scaffold composition and structure.

Literature reporting nanofiber-based delivery of small molecules for regenerating tissues is still very limited. Further research is also needed to investigate the drug choice, loading and delivery method, dosage and pre-clinical animal model studies. While so far only few *in vivo* studies are available that demonstrate clinical potential of nanofibrous scaffolds, most of the studies were of exploratory nature and relied on *in vitro* experiments. Therefore, further advancement in the clinical performance of electrospun matrices will be crucial to move forward the field.

**Acknowledgments**

A.A.Aldana thanks to CONICET for the postdoctoral scholarship. This work was supported by the Argentinean National Agency of Scientific and Technological Promotion (PICT 224), and CONICET (PIP 0089).

**References**

Abrigo, M., McArthur, S.L., Kingshott, P., 2014. Electrospun nanofibers as dressings for chronic wound care: Advances, challenges, and future prospects. Macromol. Biosci. 14, 772-792.

Agarwal, S. Wendorff, J.H, Greiner, A., 2009. Progress in the field of electrospinning for tissue engineering applications. Adv. Mater, 21, 3343-3351.

Agarwal, S., Greiner, A., Wendorff, J.H., 2013. Functional materials by electrospinning of polymers. Progr. Polym. Sci., 38, 963-991.

Alvarez-Perez, M.A., Guarino, V., Cirillo, V., Ambrosio, L., 2010. Influence of Gelatin Cues in PCL Electrospun Membranes on Nerve Outgrowth. Biomacromolecules 11, 2238-2246.

Baiguera, S., Del Gaudio, C., Lucatelli, E., Kuevda, E., Boieri, M., Mazzanti, B., Bianco, A., Macchiarini, P., 2014. Electrospun gelatin scaffolds incorporating rat decellularized brain extracellular matrix for neural tissue engineering. Biomaterials 35, 1205-1214.

Bhardwaj, N., Kundu S.C., 2010. Electrospinning: A fascinating fiber fabrication technique. Biotechnol. Adv. 28, 325-347.

Bhowmick, S., Scharnweber, D., Koul, V., 2016. Co-cultivation of keratinocyte-human mesenchymal stem cell (hMSC) on sericin loaded electrospun nanofibrous composite scaffold (cationic gelatin/hyaluronan/chondroitin sulfate) stimulates epithelial differentiation in hMSCs: In vitro study. Biomaterials 88, 83-96.

Binan, L., Tendey, C., De Crescenzo, G., El Ayoubi, R., Ajji, A., Jolicoeur, M., 2014. Differentiation of neuronal stem cells into motor neurons using electrospun poly-l-lactic acid/gelatin scaffold. Biomaterials 35, 664-674.

Cao, H., Liu, T., Chew, S.Y., 2009. The application of nanofibrous scaffolds in neural tissue engineering. Adv. Drug Deliv. Rev. 61, 1055-1064.

Chalfound, C.T., Wirth, G.A., Evens, G.R., 2006. Tissue engineered nerve constructs: where do we stand? J. Cell Mol. Med. 10, 309-317.

Chang, W.C., Kliot, M., Sretavan, D.W., 2008. Microtechnology and nanotechnology in nerve repair. Neurol. Res. 30, 1053-1062.

Chen, J., Liu, Z., Chen, M., Zhang, H., Li, X. 2016. Electrospun gelatin fibers with a multiple release of antibiotics accelerate dermal regeneration in infected deep burns. Macromol Biosci. doi: 10.1002/mabi.201600108.

Chen, H., Fan, X., Xia, J., Chen, P., Zhou, X., Huang, J., Yu, J., Gu, P., 2011. Electrospun chitosan-graft-poly (ε-caprolactone)/poly (ε-caprolactone) nanofibrous scaffolds for retinal tissue engineering. Int. J. Nanomed., 453-461.

Detta, N., Errico, C., Dinucci, D., Puppi, D., Clarke, D.A., Reilly, G.C., Chiellini, F. 2010. Novel Electrospun polyurethane/gelatin composite for vascular grafts. J. Mater. Sci. Mater. Med. 21, 1761–1769.

Dongargaonkar, A.A., Bowlin, G.L., Yang, H., 2013. Electrospun blends of gelatin and gelatin–dendrimer conjugates as a wound-dressing and drug-delivery platform. Biomacromolecules 14, 4038-4045.

Gao, Y., Yan, J., Cui, X.-j., Wang, H.-y., Wang, Q., 2012. Aligned fibrous scaffold induced aligned growth of corneal stroma cells *in vitro* culture. Chem. Res. Chinese Univer. 28, 1022-1025.

Gluck, J.M., Rahgozar, P., Ingle, N.P., Petrosian, A., Cline, M.G., Jordan, M.C., Roos, K.P.,

Gomes, S.R., Rodrigues, G., Martins, G.G., Roberto, M.A., Mafra, M., Henriques, C.M.R., Silva, J.C., 2015. *In vitro* and *in vivo* evaluation of electrospun nanofibers of PCL, chitosan and gelatin: A comparative study. Mat. Sci. Eng. C 46, 348-358.

He, Ch., Nie, W., Feng, W. 2014. Engineering of biomimetic nanofibrous matrices for drug delivery and tissue engineering. J. Mater. Chem. B., 2, 7828-7848.

Huang, C.-H., Chi, C.-Y., Chen, Y.-S., Chen, K.-Y., Chen, P.-L., Yao, C.-H., 2012. Evaluation of proanthocyanidin-crosslinked electrospun gelatin nanofibers for drug delivering system. Mat. Sci. Eng. C 32, 2476-2483.

Huang, Z.-M., Zhang, Y.Z., Ramakrishna, S., Lim, C.T., 2004. Electrospinning and mechanical characterization of gelatin nanofibers. Polymer 45, 5361-5368.

Jang, J.-H., Castano, O., Kim, H.-W. 2009. Electrospun materials as potential platforms for bone tissue engineering. Adv. Drug Deliv. Rev., 61, 1065–1083

Jia, L., Prabhakaran, M.P., Qin, X., Kai, D., Ramakrishna, S. 2013. Biocompatibility evaluation of protein-incorporated electrospun polyurethane-based scaffolds with smooth muscle cells for vascular tissue engineering. J. Mater. Sci. 48, 5113–5124.

Jiang T., Carbone, E.J., Lo, K.W-H., Laurencin, C.T. 2015. Electrospinning of polymer nanofibers for tissue regeneration. Prog. Polym. Sci., 46, 1-24.

Ki, Ch.S., Baek, D.H., Gang, K.D., Lee, K.H., Um, I.Ch., Park, Y.H. 2005. Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. Polymer, 46, 5094–5102.

Kim, H.W., Yu, H.S., Lee, H.H. 2008. Nanofibrous matrices of poly(lactic acid) and gelatin polymeric blends for the improvement of cellular responses, J. Biomed. Mater. Res., 87A, 25–32.

Li, H., Xu, Y., Xu, H., Chang J. 2014. Electrospun membranes: control of the structure and structure related applications in tissue regeneration and drug delivery. J. Mater. Chem. B., 2, 5492-5510.

Lin, W.H., Yu, J., Chen, G., Tsai, W.B., 2016. Fabrication of multi-biofunctional gelatin-based electrospun fibrous scaffolds for enhancement of osteogenesis of mesenchymal stem cells. Coll. Surf. B Biointerfaces. 138, 26-31.

Liu, X., Smith, L.A., Hu, J., Ma, P.X. 2009. Biomimetic nanofibrous gelatin/apatite composite scaffolds for bone tissue engineering. Biomaterials, 30, 2252-2258.

Liu, Z., Yu, N., Holz, F.G., Yang, F., Stanzel, B.V., 2014. Enhancement of retinal pigment epithelial culture characteristics and subretinal space tolerance of scaffolds with 200 nm fiber topography. Biomaterials 35, 2837-2850.

Lu, L., Yaszemski, M.J., Mikos, A.G., 2001. Retinal pigment epithelium engineering using synthetic biodegradable polymers. Biomaterials 22, 3345-3355.

Ma, P.X., 2004. Scaffolds for tissue fabrication. Mater. Today 7, 30-40.

Maclellan, W.R., Shemin, R.J., Heydarkhan-Hagvall, S. 2011. Hybrid coaxial electrospun nanofibrous scaffolds with limited immunological response created for tissue engineering, J. Biomed. Mater. Res. B Appl. Biomater. 99B, 180–190.

Mehrasa, M., Asadollahi, M.A., Ghaedi, K., Salehi, H., Arpanaei, A., 2015. Electrospun aligned PLGA and PLGA/gelatin nanofibers embedded with silica nanoparticles for tissue engineering. Int. J. Biol. Macromol. 79, 687-695.

Meng, Z.X., Wang, Y.S., Ma, C., Zheng, W., Li, L., Zheng, Y.F. 2010. Electrospinning of PLGA/gelatin randomly-oriented and aligned nanofibers as potential scaffold in tissue engineering. Mater. Sci. Eng., C, 30, 1204–1210.

Nagiah, N., Madhavi, L., Anitha, R., Anandan, C., Srinivasan, N.T., Sivagnanam, U.T., 2013. Development and characterization of coaxially electrospun gelatin coated poly (3-hydroxybutyric acid) thin films as potential scaffolds for skin regeneration. Mat. Sci. Eng. C 33, 4444-4452.

Novak, U., Kaye, A.H., 2000. Extracellular matrix and the brain: components and function. J. Clin. Neurosci. 7, 280-290.

Panzavolta, S., Gioffrè, M., Focarete, M.L., Gualandi, Ch., Foroni, L., Bigi, A. 2011. Electrospun gelatin nanofibers: Optimization of GEN cross-linking to preserve fiber morphology after exposure to water. Acta Biomaterialia, 7, 1702–1709.

Pelipenko, J., Kocbek, P., Kristl J., 2015. Critical attributes of nanofibers: Preparation, drug loading, and tissue regeneration. Int. J. Pharm. 484, 57-74.

Pezeshki-Modaress, M., Mirzadeh, H., Zandi, M., 2015. Gelatin–GAG electrospun nanofibrous scaffold for skin tissue engineering: Fabrication and modeling of process parameters. Mater. Sci. Eng. C 48, 704-712.

Pham, Q.P., Sharma, U., Mikos, A.G. 2006. Electrospinning of polymeric nanofibers for tissue engineering applications: A review. Tissue Eng., 12, 1197-1211.

Ravichandran, R., Sundarrajan, S., Venugopal, J.R., Mukherjee, S., Ramakrishna, S., 2012. Advances in polymeric systems for tissue engineering and biomedical applications. Macromol. Biosci. 12, 286-311.

Rong, D., Chen, P., Yang, Y., Li, Q., Wan, W., Fang X., Zhang, J., Han, Z., Tian J., Ouyang, J. 2016. Fabrication of gelatin/PCL electrospun fiber mat with bone powder and the study of its biocompatibility. J. Funct. Biomater. 7, 6.

Ruberti, J.W., Zieske, J.D., 2008. Prelude to corneal tissue engineering – Gaining control of collagen organization. Progr. Retinal Eye Res. 27, 549-577.

Sajkiewicz, P., Kołbuk, D., 2014. Electrospinning of gelatin for tissue engineering – molecular conformation as one of the overlooked problems. J. Biomater. Sci. Polym. Edn. 25, 2009-2022.

Sannino, A., Madaghiele, M., 2009. J. Cell Plast. 45, 137.

Sell, S., Barnes, C., Smith, M., McClure, M., Madurantakam, P., Grant, J., McManus, M., Bowlin, G., 2007. Extracellular matrix regenerated: tissue engineering via electrospun biomimetic nanofibers. Polym. Int. 56, 1349-1360.

Sell, S.A., Wolfe, P.S., Garg, K., McCool, J.M., Rodriguez, I.A., Bowlin, G.L., 2010. The use of natural polymers in tissue engineering: A focus on electrospun extracellular matrix analogues. Polymers, 2, 522-553.

Shalumon, K.T., Deepthi, S., Anupama, M.S., Nair, S.V., Jayakumar, R., Chennazhi, K.P. 2015. Fabrication of poly (L-lactic acid)/gelatin composite tubular scaffolds for vascular tissue engineering. Int. J. Biol. Macromol. 72, 1048-1055.

Shan, Y.H., Peng, L.H., Liu, X., Chen, X., Xiong, J., Gao, J.Q., 2015. Silk fibroin/gelatin electrospun nanofibrous dressing functionalized with astragaloside IV induces healing and anti-scar effects on burn wound. Int. J. Pharm. 479, 291-301.

Sisson, K., Zhang, C., Farach-Carson, M.C., Chase, D.B., Rabolt, J.F., 2009. Evaluation of Cross-Linking Methods for Electrospun Gelatin on Cell Growth and Viability. Biomacromolecules 10, 1675-1680.

Sridhar, R., Lakshminarayanan, R., Madhaiyan, K., Barathi, V.A., Lim, K.H.Ch., Ramakrishna, S. 2015. Electrosprayed nanoparticles and electrospun nanofibers based on natural materials: applications in tissue regeneration, drug delivery and pharmaceuticals. Chem. Soc. Rev. 44, 790-814.

Sundback, C.A., Shyu, J.Y., Wang, Y., Faquin, W.C., Langer, R.S., Vacanti, J.P., Hadlock, T.A., 2005. Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material. Biomaterials 26, 5454-5464.

Tan, L., Hu, J., Huang, H., Han, J., Hu, H., 2015. Study of multi-functional electrospun composite nanofibrous mats for smart wound healing. Int. J. Biol. Macromol. 79, 469-476.

Tonsomboon, K., Oyen, M.L., 2013. Composite electrospun gelatin fiber-alginate gel scaffolds for mechanically robust tissue engineered cornea. J. Mech. Behavior Biomed. Mater. 21, 185-194.

Torricelli, P., Gioffrè, M., Fiorani, A., Panzavolta, S., Gualandi, C., Fini, M., Focarete, M.L., Bigi, A., 2014. Co-electrospun gelatin-poly(l-lactic acid) scaffolds: Modulation of mechanical properties and chondrocyte response as a function of composition. Mat. Sci. Eng. C 36, 130-138.

Wang, H., Feng, Y., Fang, Z., Xiao, R., Yuan, W., Khan, M. 2013. Fabrication and characterization of electrospun gelatin-heparin nanofibers as vascular tissue engineering. Macromol. Res., 21, 860-869

Wang, X., Ding, B., Li, B., 2013. Biomimetic electrospun nanofibrous structures for tissue engineering. Mater. Today, 16, 229-241.

Xiang, P., Wu, K.-C., Zhu, Y., Xiang, L., Li, C., Chen, D.-L., Chen, F., Xu, G., Wang, A., Li, M., Jin, Z.-B., 2014. A novel Bruch's membrane-mimetic electrospun substrate scaffold for human retinal pigment epithelium cells. Biomaterials 35, 9777-9788.

Xue, J., Feng, B., Zheng, R., Lu, Y., Zhou, G., Liu, W., Cao, Y., Zhang, Y., Zhang, W.J., 2013. Engineering ear-shaped cartilage using electrospun fibrous membranes of gelatin/polycaprolactone. Biomaterials 34, 2624-2631.

Zamani, M., Prabhakaran M.P., Ramakrishna, S., 2013. Advances in drug delivery via electrospun and electrosprayed nanomaterials. Int. J. Nanomedicine, 8, 2997-3017.

Zeugolis, D.I., Khew, S.T., Yew, E.S.Y., Ekaputra, A.K., Tong, Y.W., Yung, L.-Y.L., Hutmacher, D.W., Sheppard, C., Raghunath, M., 2008. Electro-spinning of pure collagen nano-fibres – Just an expensive way to make gelatin? Biomaterials 29, 2293-2305.

Zhang, S., Huang, Y., Yang, X., Mei, F., Ma, Q., Chen, G., Ryu, S., Deng, X., 2009. Gelatin nanofibrous membrane fabricated by electrospinning of aqueous gelatin solution for guided tissue regeneration. J. Biomed. Mater. Res. 90A, 671-679.

Zhang, X., Thomas, V., Vohra, Y.K. 2009. In vitro biodegradation of designed tubular scaffolds of electrospun protein/polyglyconate blend fibers. J. Biomed. Mater. Res. Appl. Biomater. 89B, 135–147.

Zhang, X., Thomas, V., Vohra, Y.K. 2010. Two ply tubular scaffolds comprised of proteins/poliglecaprone/polycaprolactone fibers. J. Mater. Sci. Mater. Med. 21, 541-549.

Zhang, X., Meng S., Huang, Y., Xu, M., He, Y., Lin, H., Han, J., Chai, Y., Wei, Y., Deng, X, 2015. Electrospun gelatin/b-TCP composite nanofibers enhance osteogenic differentiation of bmscs and in vivo bone formation by activating Ca (2+) -sensing receptor signaling. Stem Cells Int., ID 507154.

Zhang, Y., Ouyang, H., Lim, C.T., Ramakrishna, S., Huang, Z.M. 2005. Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds, J. Biomed. Mater. Res. Appl. Biomater. 72, 156–165.

Zhang, Y.Z., Venugopal, J., Huang, Z.M., Lim, C.T., Ramakrishna, S., 2006. Crosslinking of the electrospun gelatin nanofibers. Polymer 47, 2911-2917.

Zheng, R., Duan, H., Xue, J., Liu, Y., Feng, B., Zhao, S., Zhu, Y., Liu, Y., He, A., Zhang, W., Liu, W., Cao, Y., Zhou, G., 2014. The influence of gelatin/PCL ratio and 3-D construct shape of electrospun membranes on cartilage regeneration. Biomaterials 35, 152-164.

Zhong, Y., Bellamkonda, R.V., 2008. Biomaterials for the central nervous system. J. Royal Soc. Interface 5, 957-975.

Zimmermann, D.R., Dours-Zimmermann, M.T., 2008. Extracellular matrix of the central nervous system: from neglect to challenge. Histochem. Cell Biol. 130, 635-653.

**Figure Captions:**

Fig. 1. X-ray diffractograms of (a) gelatin powder, (b) gelatin film cast from gelatin aqueous solution, (c) gelatin film cast from gelatin–formic acid solution, and (d) electrospun gelatin nanofiber. (Ki et al., 2005)

Fig. 2. Typical stress–strain curves recorded from as-electrospun (a) gelatin and (b) gelatin-GEN mats, (c) gelatin mats crosslinked with 5% w/v GEN solution during 7 days and (d) gelatin-GEN mats crosslinked with 5% w/v GEN solution during 7 days. (Panzavolta et al., 2011)

Fig. 3. SEM micrographs of ADSCs attached on scaffolds gelatin/PCL (A); gelatin/PCL/nanohydroxyapatite (B); and gelatin/PCL/bone powder (C). Scale bar: 20 μm. (Rong et al., 2016)

Fig. 4. Engineering ear-shaped cartilage. A: Tailored electrospun GT/PCL membrane; B: Titanium alloy ear-shaped mold. C: Gross view of ear-shaped cell-scaffold constructs immediately after stacking (0 h). D: Cell scaffold constructs after 2 weeks of culture in vitro (2 weeks). E: Subcutaneous implantation in nude mice; F-H: Engineered ear-shaped cartilage after 6 weeks of in vivo incubation. I: The similarity analysis of engineered ear compared to the titanium mold. (Xue et al., 2013)

Fig. 5. Morphological evaluation. Cell morphology on 40x images obtained with TUJ-1 staining and SEM images. Cells cultured on pure fibers (A, C, E, G) or RA- and purmorphamine-loaded fibers (B, D, F, H) at days 2 (A, B, G), 7 (C, D), and 14 (E, F, H). (Binan et al., 2014)

Fig. 6. Structure of electrospun gelatin fibers in the composite hydrogels. (a) Confocal image of aligned gelatin fibers in the electrospun mat.; confocal images of the composite hydrogel produced from aligned gelatin fibers at (b) the surface, (c) at 5 μm, (d) at 10 μm and (e) at 15 μm depth. Scale bars are equal to 5 μm. (Tonsomboon and Oyen, 2013)

Fig. 7. A comparison of stress–strain curves of (a) randomly-oriented and (b) aligned electrospun gelatin mats (3100 RPM) under two different loading orientations. (Tonsomboon and Oyen, 2013)

Fig. 8. Fluorescent microscope images of Texas Red/DAPI stained SMCs showing attachment of SMCs on the surface of the scaffolds after 120 h (A) PLLA alone, (B) PLLA with 5% gelatin, (C) PLLA with 10% gelatin, (D) PLLA with 20% gelatin, for (i) nonwoven mats and (ii) aligned nanofibrous scaffolds. (Shalumon et al., 2015)

Table 1. Synthetic and natural polymers and their properties for tissue engineering.

**Table 1**

| Polymers | Surface properties | | Integrin binding | Biodegradability | | Mechanical performance | |
|---|---|---|---|---|---|---|---|
| | Hydrophobic | Hydrophilic | | Hydrolytic | Enzymatic | Good | Poor |
| **Synthetic** | | | | | | | |
| PGA | x | | | x | | x | |
| PLLA | x | | | x | | x | |
| PLGA | x | | | x | | x | |
| PCL | x | | | x | | x | |
| SPEU | x | x | | x | | x | |
| PVA | | x | | x | | | x |
| PEO | | x | | x | | | x |
| PVP | | x | | x | | | x |
| **Natural** | | | | | | | |
| Collagen | | x | x | | x | | x |
| Gelatin | | x | x | x | x | x | |
| Silk fibroin | | x | | | x | x | |
| Fibrinogen | | x | x | | x | x | |
| Elastin | | x | x | | x | | x |
| Laminin | | x | x | | x | | x |
| Soy protein | | x | x | | x | | x |
| Chitosan | | x | | | x | x | |
| Alginate | | x | | | x | | x |
| Hyaluronic acid | | x | | | x | | x |
| Cellulose acetate | | x | | x | | x | |
| Starch | | x | | | x | | x |

PGA: poly(glycolide); PLLA: poly(lactic acid), PLGA: poly(lactide-co-glycolide); PEO: poly(ethylene oxide); PVA: poly(vinyl alcohol); PCL: poly(caprolactone); SPEU: segmented poly(esterurethane), PVP: poly(vinylpyrrolidone)

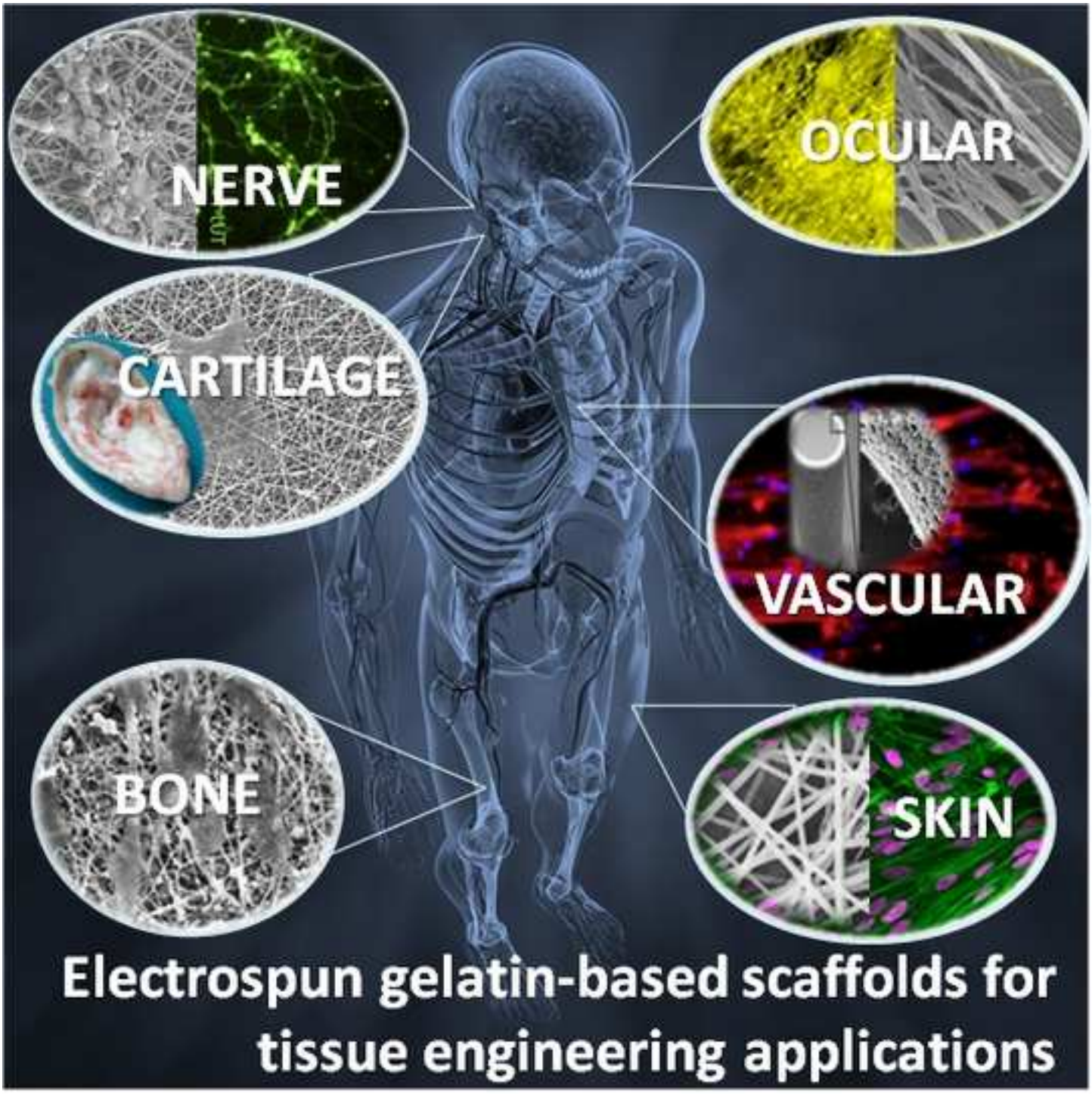
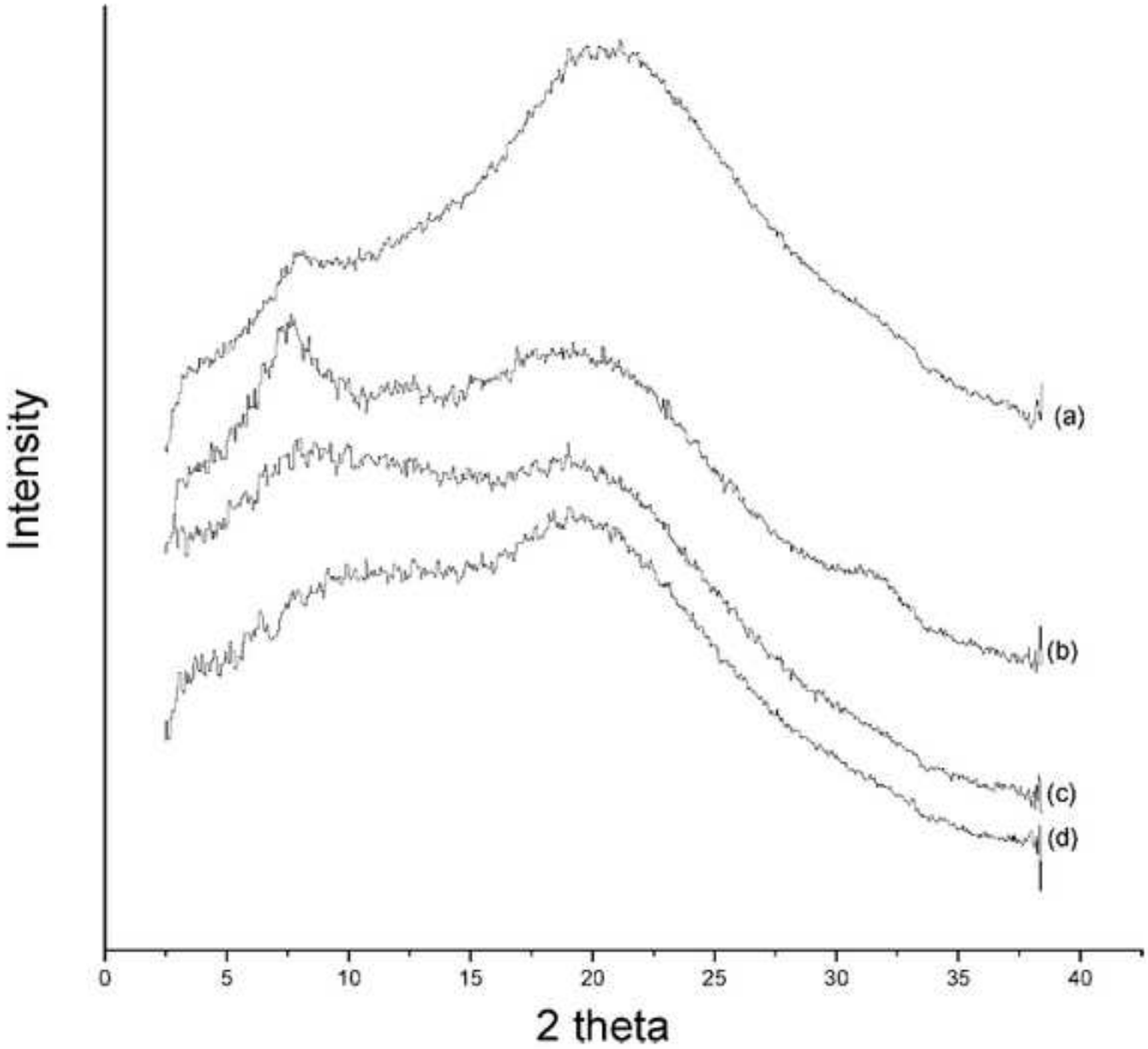


**Table 1**

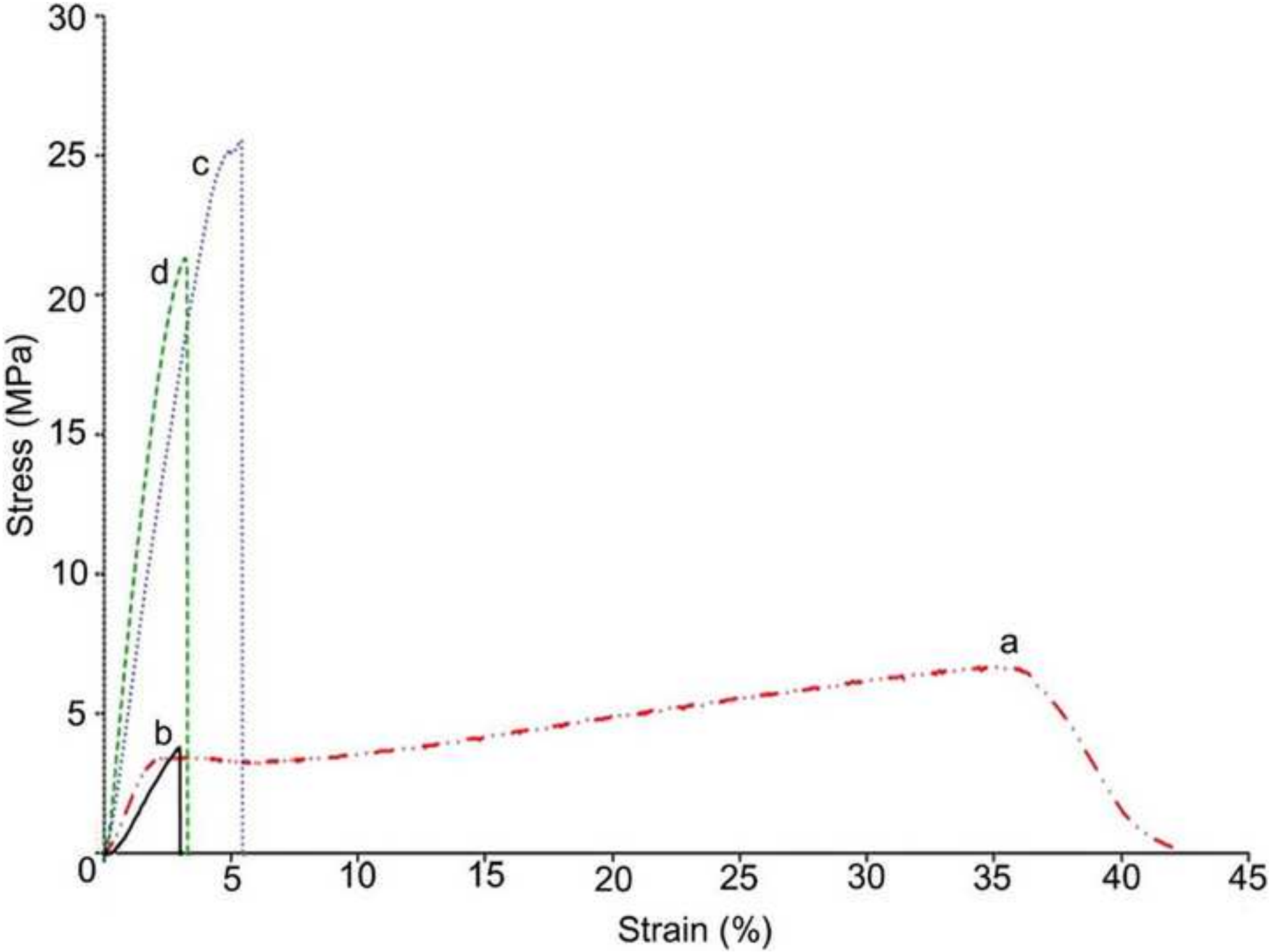
| Polymers | Surface properties | | Integrin binding | Biodegradability | | Mechanical performance | |
|---|---|---|---|---|---|---|---|
| | Hydrophobic | Hydrophilic | | Hydrolytic | Enzymatic | Good | Poor |
| **Synthetic** | | | | | | | |
| PGA | x | | | x | | x | |
| PLLA | x | | | x | | x | |
| PLGA | x | | | x | | x | |
| PCL | x | | | x | | x | |
| SPEU | x | x | | x | | x | |
| PVA | | x | | x | | | x |
| PEO | | x | | x | | | x |
| PVP | | x | | x | | | x |
| **Natural** | | | | | | | |
| Collagen | | x | x | | x | | x |
| Gelatin | | x | x | x | x | x | |
| Silk fibroin | | x | | | x | x | |
| Fibrinogen | | x | x | | x | x | |
| Elastin | | x | x | | x | | x |
| Laminin | | x | x | | x | | x |
| Soy protein | | x | x | | x | | x |
| Chitosan | | x | | | x | x | |
| Alginate | | x | | | x | | x |
| Hyaluronic acid | | x | | | x | | x |
| Cellulose acetate | | x | | x | | x | |
| Starch | | x | | | x | | x |

PGA: poly(glycolide); PLLA: poly(lactic acid), PLGA: poly(lactide-co-glycolide); PEO: poly(ethylene oxide); PVA: poly(vinyl alcohol); PCL: poly(caprolactone); SPEU: segmented poly(esterurethane), PVP: poly(vinylpyrrolidone)

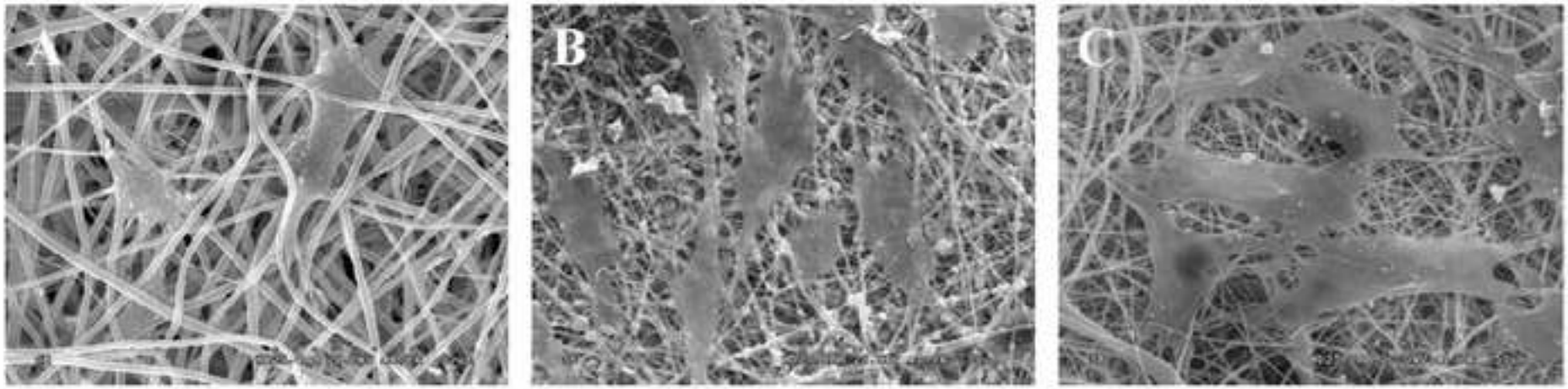
Figure(1)



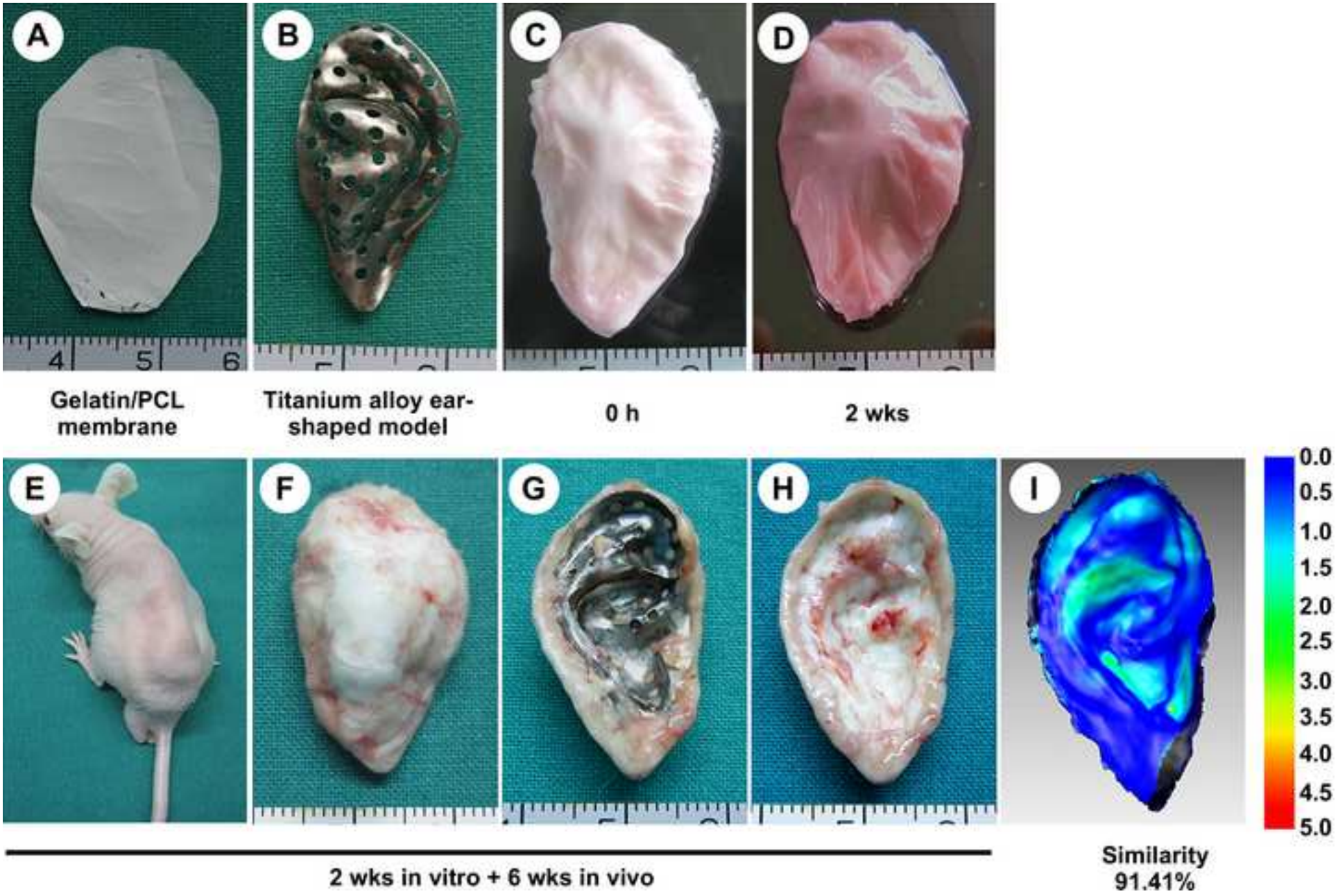
Figure(2)

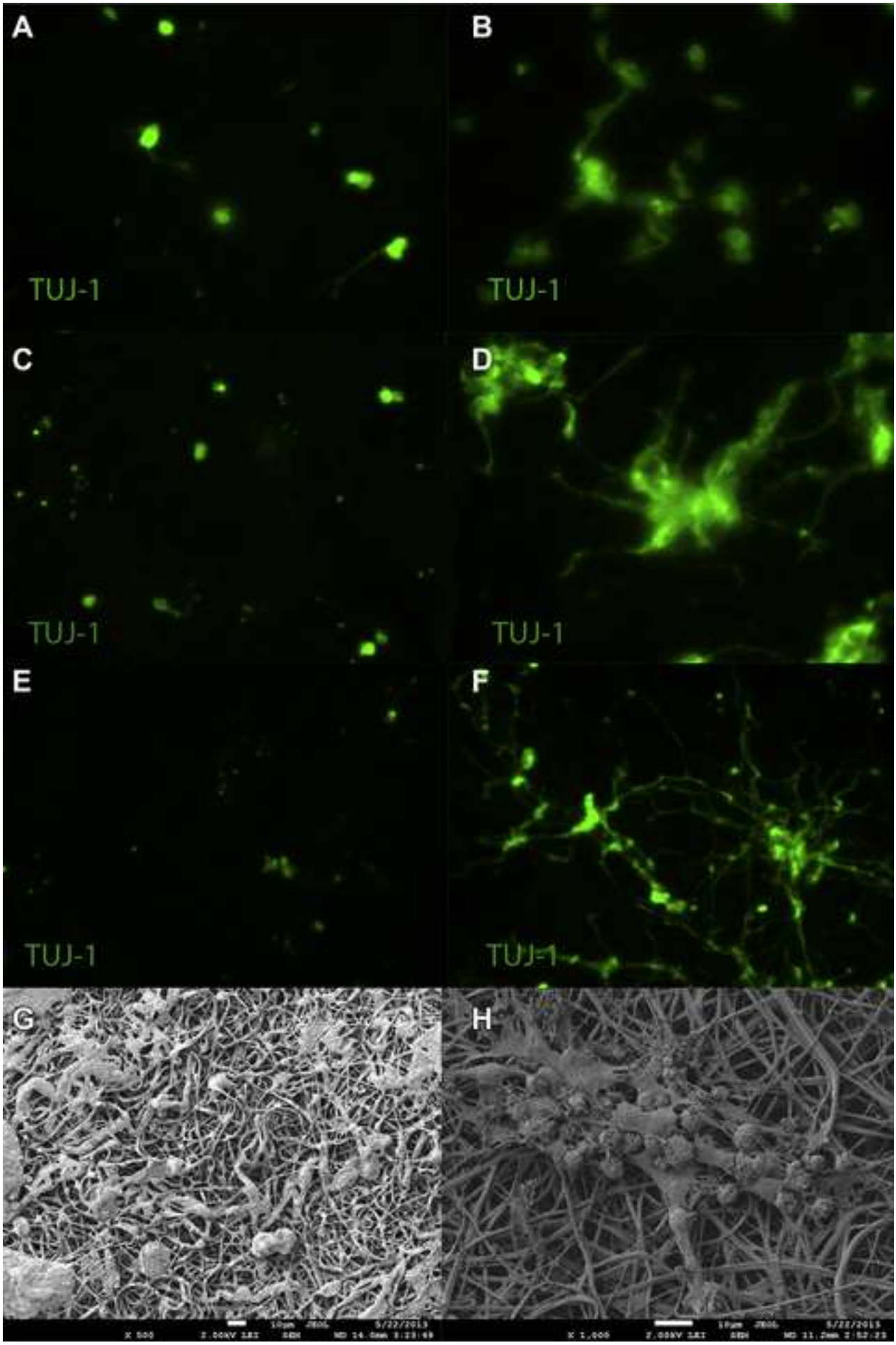


**Figure(3)**

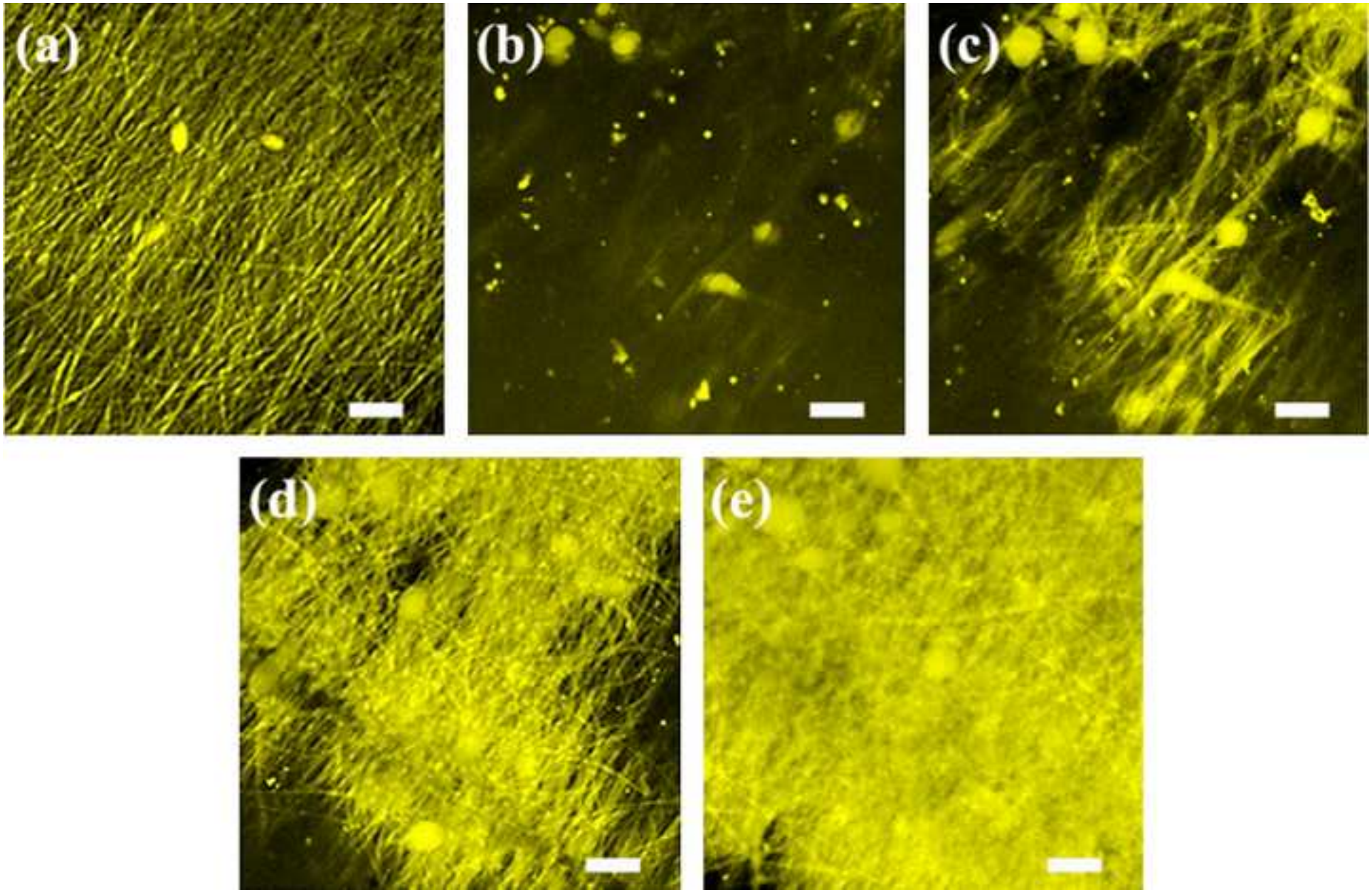


Figure(4)

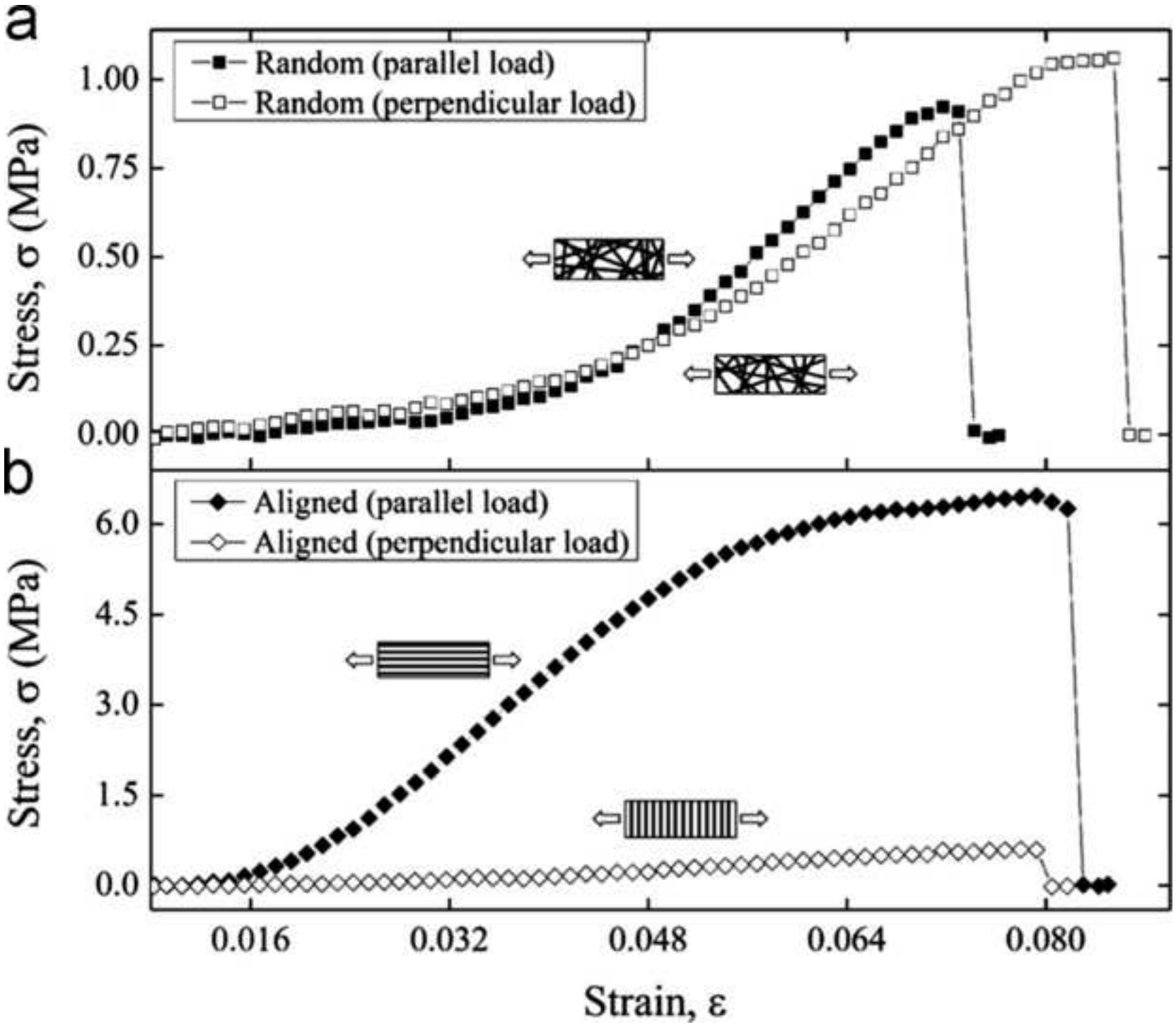

A
B
C
D
Gelatin/PCL membrane
Titanium alloy ear-shaped model
0 h
2 wks
E
F
G
H
I
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
2 wks in vitro + 6 wks in vivo
Similarity 91.41%



Figure(6)



Figure(7)


a
Random (parallel load)
Random (perpendicular load)
Stress, σ (MPa)
1.00
0.75
0.50
0.25
0.00
b
Aligned (parallel load)
Aligned (perpendicular load)
Stress, σ (MPa)
6.0
4.5
3.0
1.5
0.0
0.016
0.032
0.048
0.064
0.080
Strain, ε