The Review on Electrospun Gelatin Fiber Scaffold

Jianchao Zhan and Ping Lan*

Department of Materials and Textile Engineering, Jiaxing University, 118 Jiahang Road, Jiaxing, P.R. China

Abstract: The fabrication of the Guided Tissue Regeneration (GTR) membrane materials have become the key technique of the tissue engineering scaffold study. The cells adhere well on the fibers whose dimension is below their own so that the porous three dimension scaffold material can mimic the strueture of the natural extracellular matrix better and have the potential to be an ideal GTR membrane material. Gelatin, a kind of protein obtained from hydrolyzed and denatured animal skin, is a condensation polymer of a variety of amino acids and so it is a kind of bio-polymer with good water-solubility. Gelatin fiber mats with submicro and nanometer scale can simulate extracellular matrix structure of the human tissues and organs and can be used widely in the tissue engineering field because of their excellent bio-affinity. Electrospinning is a very attractive method for preparing polymer or composite nanofibers and so electrospinning technique was developed to prepare nanofibrous gelatin matrix. The electrospun of gelatin to fabricate the scaffold material has obtained more attention recently because of its biocompatibility, high surface area-to-volume ratio, degradability and less immunogenic property. The structure and performance of the electrospinning gelatin fiber mats which were manufactured by different solvents, electrospinning process, cross-linking process were reviewed. The properties and application of the two-component and multicomponent gelatin fiber mats were analyzed.

Keywords: Electrospinning, gelatin, scaffold, tissue engineering, membrane materials, nanofiber.

1. INTRODUCTION

Scaffolds for tissue engineering can act as a substitute for the extracellular matrix (ECM) and provide a substrate for cellular adhesion and organization. The chemistry and structure of the ECM can regulate cell proliferation, differentiation, and maturation; thus, tissue engineering scaffolds should have a strong resemblance to the natural ECM, which is comprised of nanometer-diameter protein fibers [1].

Gelatin, a kind of protein obtained from hydrolyzed and denatured animal skin, is a kind of bio-polymer with good water-solubility. Gelatin fiber mats with submicro and nanometer scale can simulate ECM structure of the human tissues and organs and can be used widely in the tissue engineering field because of its excellent bio-affinity, biological origin, biocompatibility, non-immunogenicity, biodegradability and commercial availability.

Electrospinning process is one of the most convenient and effective methods for preparation of nanoscale gelatin fibers, which can produce a highly porous nonwoven mat with a high surface-to-volume ratio and porosity. The gelatin mat provides space for cell and tissue to grow and so the electrospun gelatin and gelatin-based scaffolds have been engineered for a variety of biomedical applications, such as bone regeneration, skin tissue engineering, nerve tissue engineering, cardiac tissue engineering, tubular scaffold, drug delivery and so on.

*Address corresponding to this author at the Department of Materials and Textile Engineering, Jiaxing University, 118 Jiahang Road, Jiaxing, P.R. China; E-mail: lanping007@126.com

Electrospun gelatin nanofiber matrices in various forms including thick nanofiber sheets, tubular structures, and as a coating material have been used in a variety of biomedical applications.The present review will summarize the electrospun gelatin matrices and their potential applications in the field of tissue engineering. The review is broadly divided into different categories where electrospun gelatin matrices are used as bone regeneration, skin tissue engineering, nerve tissue engineering and tubular scaffold.

2. CROSS-LINKING OF ELECTROSPUN GELATIN NANOFIBERS

The electrospun nanofibers of gelatin still is constrained by fast degradation, total dissolution, or weak mechanical properties. Thus, further treatment to improve these drawbacks such as cross-linking is required. The treatment can improve not only the water-resistant ability but also the thermo-mechanical performance of the treated nanofiber, leading to an enhanced mechanical strength.

Cross-linking can be performed *via* several methods including physical cross-linking such as dehydrothermal treatment (DHT), plasma treatment and ultraviolet (UV) treatment, and chemical cross-linking by some cross-linking agents such as glutaraldehyde (GA) and 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC). Generally, physical treatment results in a low cross-linking degree because the reaction occurs only at the surface of the materials. Chemical treatment provides a higher extent of cross-linking but sometimes changes the material structure [2].

E-ISSN: 1929-5995/12

Table 1: Groups of Gelatin Fiber Mats Treated with Various Physical and Chemical Cross-Linking Techniques

Group	Crossinglinking	Process
1	DHT	by dehydrothermal treatment at 140°C for 48 h
2	PLAS	By pulsed inductively coupled plasma (PICP) treatment under argon gas (Ar) at a pressure of 5 Pa and for 1 pulse .
3	DHT/PLAS	the DHT treated gelatin fiber mats that were further cross-linked *via* PICP technique at the same conditions described for group#2 .
4	DHT/EDCw	the DHT treated gelatin fiber mats (group #1) that were further cross-linked by immersion into 14 mM EDC/5.5 mM NHS for 2 h in water
5	DHT/EDCe	the DHT treated gelatin fiber mats (group #1) that were further cross-linked by immersion into 14 mM EDC/5.5 mM NHS for 2 h in absolute ethanol
6	DHT/sEDCw	treated similar to group #4 except that the fiber mat was sprayed with 14mM EDC/5.5mM NHS in water and left to dry for 5 cycles before immersion in the same EDC solution for 2 h
7	DHT/sEDCe	by the same method as for group #6 but the EDC/NHS in absolute ethanol was used instead of DHT/sEDCw.
8	DHT/vGA	the DHT treated gelatin fiber mat that was further incubated in the vapor of GA (0.06% in 75/25 of Acetone/HCl) under dark vacuum at 4°C for 48 h (DHT/vGA).

J.R. *et al*. studied the influences of various cross-linking techniques on the degradation rate of electrospun type A and type B gelatin fiber mats (Table **1**). The structures of electrospun gelatin fiber mats before and after cross-linking with different techniques are shown in Figures **1** and **2** [3].

They draw the following conclusion:

(1) The cross-linking by DHT did not affect the morphology of the fiber mats, as can be observed in Figures (**1a**, **b**) and (**2a**, **b**), which was similar to the PLAS and DHT/PLAS treatments, the fibers at the surface melted while the fibers inside remained the same, as seen in Figures (**1c**, **d**) and (**2c**, **d**).

(2) The chemical cross-linking by immersion in EDC/NHS after DHT treatment caused fibers to become swollen and some small interconnected pores among the swollen fibers remained, as can be observed in Figures (**1e**, **f**) and (**2e**, **f**).

(3) The spraying technique could be used to induce the gelatin fiber mats with a basket-weave structure as presented in Figures (**1g**, **h**) and (**2g**, **h**).

(4) The structures of the gelatin fiber mats obtained from the cross-linking using the vapor of GA after DHT treatment were different from the others. The fibers fused and merged. In this case, the interconnected pores could not be observed, as demonstrated in Figures (**1i**) and (**2i**).

In the same study, they found that both type A and B gelatin, the fiber mats cross-linked by DHT exhibited the fastest degradation; other combinations of DHT and chemical cross-linking techniques reduced the degradation of gelatin fiber mats [3].

3. ELECTROSPUN GELATIN NANOFIBERS AS A SCAFFOLD FOR BONE REGENERATION

Natural bone is a composite material composed of a collagen matrix reinforced with hydroxyapatite (HA) crystals, which forms *via* the biological mineralization process [4, 5]. This mineralization generates well-ordered bone building blocks through hierarchical self-assembly, in which nanosized apatite crystals grow on an organic matrix rich in collagen nanofibers. When human bone is traumatically fractured, this bone formation process is inhibited. The large gaps between the broken bone segments confine the osteoblasts to areas that contain nutrients near vascularized tissue. These cells will not be able to secrete collagen fibrils to initiate the bone reformation process. It is necessary for a porous scaffolding material to be implanted into the defect to allow vascularization to occur, thus providing osteoblasts with sufficient nutrients to move through the defect [6]. The structural characteristics of nanofibrous membranes are necessary to enhance the osteogenic cell attachment and to expedite the tissue ingrowth both *in vitro* and *in vivo*. Electrospun gelatin, in the form of nanofibrous structure, have recently gained much interest for bone tissue engineering [7-10].

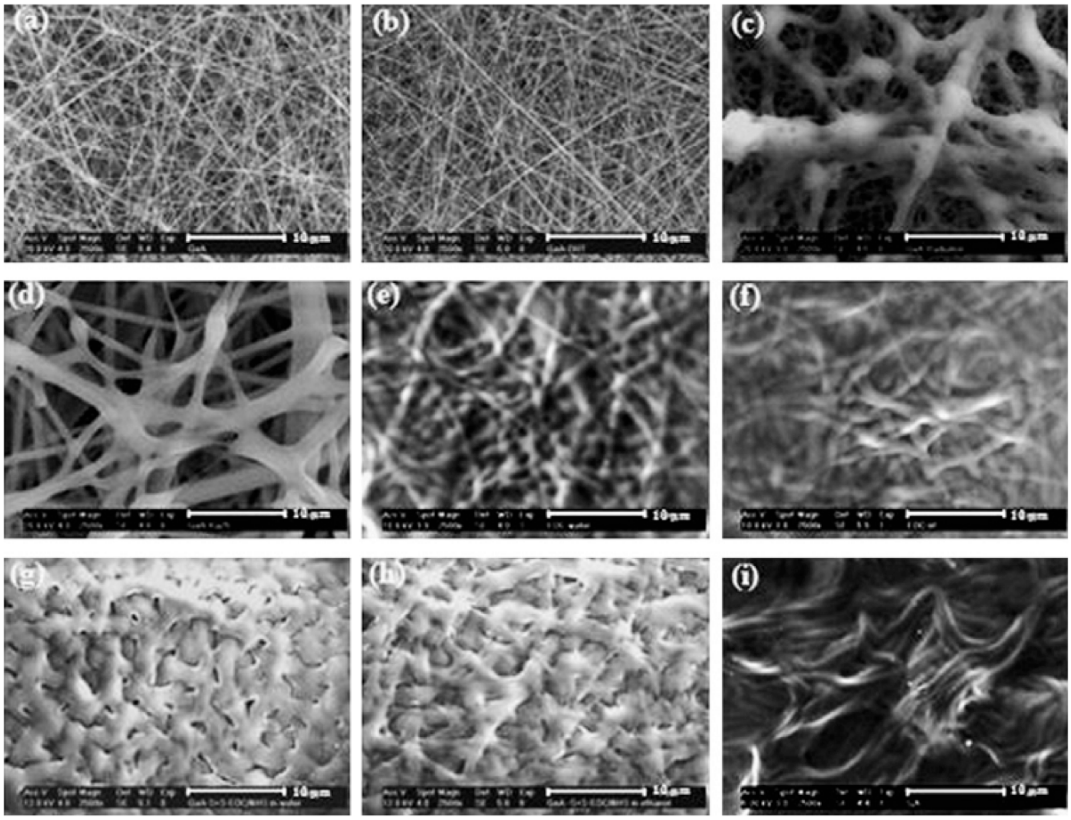


Figure 1: SEM photographs of electrospun type A gelatin fiber mats before and after cross-linking with different techniques (**a**) non-cross-linked, (**b**) DHT, (**c**) PL AS, (**d**) DHT/PLAS, (**e**) DHT/EDCw, (**f**) DHT/EDCe, (**g**) DHT/sEDCw, (**h**) DHT/sEDCe and (**i**) DHT/vGA (scale bar = 10 μm) [3].

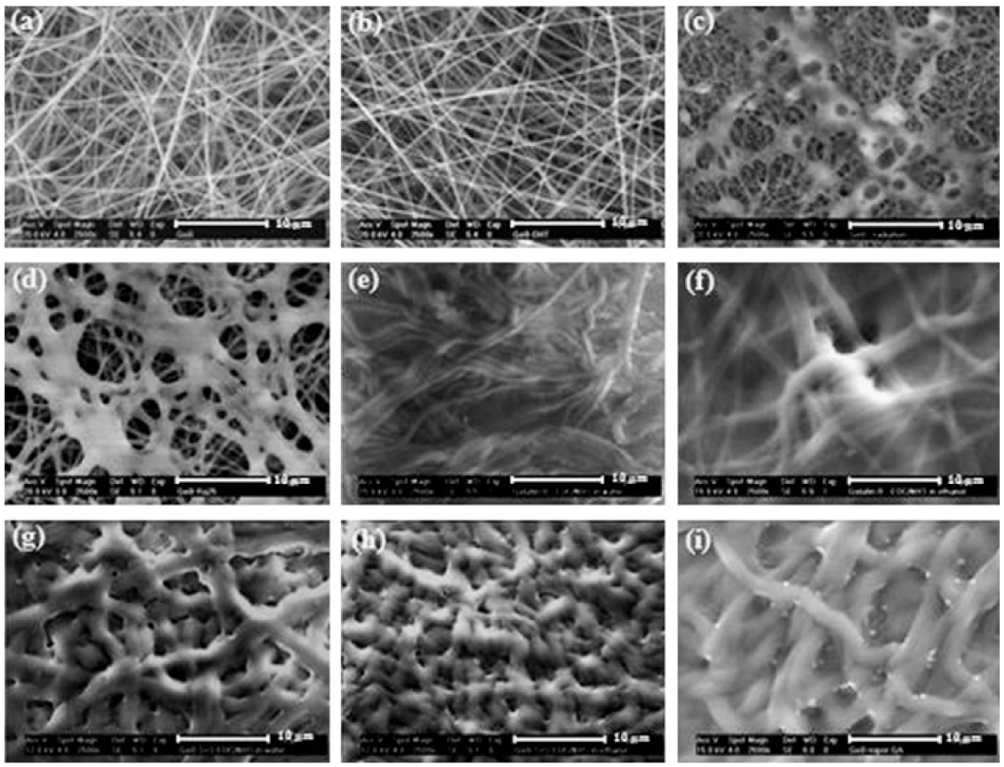


Figure 2: SEM photographs of electrospun type B gelatin fiber mats before and after cross-linking with different techniques (**a**) non-cross-linked, (**b**) DHT, (**c**) PL AS, (**d**) DHT/PLAS, (**e**) DHT/EDCw, (**f**) DHT/EDCe, (**g**) DHT/sEDCw, (**h**) DHT/sEDCe and (**i**) DHT/vGA (scale bar = 10 μm) [3].

For the production of composite nanofibrous membranes covered on not only the external surface but also the inner side with high concentration of inorganic crystals and having characteristics quite similar to natural bone ECM, Choi M O *et al*. have developed a new method that gelatin was first dissolved in TFE and then mixed with 0.3 $MCaCl_2$ or 0.3 M Na_2HPO_4 solution at a volume ratio of 1:1 to obtain a concentration of 12% (w/v) solution and then was electrospun, the gelatin nanofibers including Ca^{2+} ions (GEL-Ca) or PO_3^{-4} ions(GEL-P) was placed in previously prepared counterion solution, 0.5 M Na_2HPO_4 (GEL-Ca-P) or 0.5$MCaCl_2$ (GEL-P-Ca) [11].

Choi M O *et al*. referred that only external layers of GEL-P-Ca were mineralized, the Ca-P crystals formed to the inside of membrane, inducing to the biomimetic mineralization of entire layers of membrane and the increase of membrane thickness, as can be observed in Figure **3**. This is due to the remaining amount of Ca^{2+}or PO_3^{-4} ion precursors in the polymer membrane during the mineralization process. Ca^{2+} ions showed the strong ionic interactions with anionic residues of gelatin and were homogenously distributed on the membrane [12].

The essential requirement for an artificial material to bond to living bone is the formation of bone-like apatite on its surface when implanted in the living body, and that this *in vivo* apatite formation can be reproduced in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [13]. This means that *in vivo* bone bioactivity of a material can be predicted from the apatite formation on its surface in SBF. In the same study, they found that after 48 h of immersion in SBF, the deposition of apatite occurred at some sites on the surface of GEL-P-Ca composite membrane and only small amount of spherical-like particles and the whole surfaces of GEL-Ca-P membrane were fully covered by a layer of apatite which consisted of nanosheets self-assembled to form three-dimensional structures and the underlying nanofibers were not observable and the pores of membrane were clogged due to considerable crystal growth, as can be observed in Figure **4**. Higher amount of Ca-P crystals probably accelerated the significant level of apatite formation on the surface of GEL-Ca-P since the exposure of Ca-P crystals on the membrane surface could provide nucleation sites for apatite formation or growth, resulting that GEL-Ca-P maybe reveals the improved *in vivo* bone bioactivity as compared to GEL-P-Ca [14].

4. ELECTROSPUN GELATIN NANOFIBERS AS TUBULAR SCAFFOLD

Large numbers of patients suffer from cardiovascular diseases, most of which need proper vascular grafts [15]. Presently, autologous vascular grafts and allografts are widely used for the reconstruction of small-diameter vessels. However,

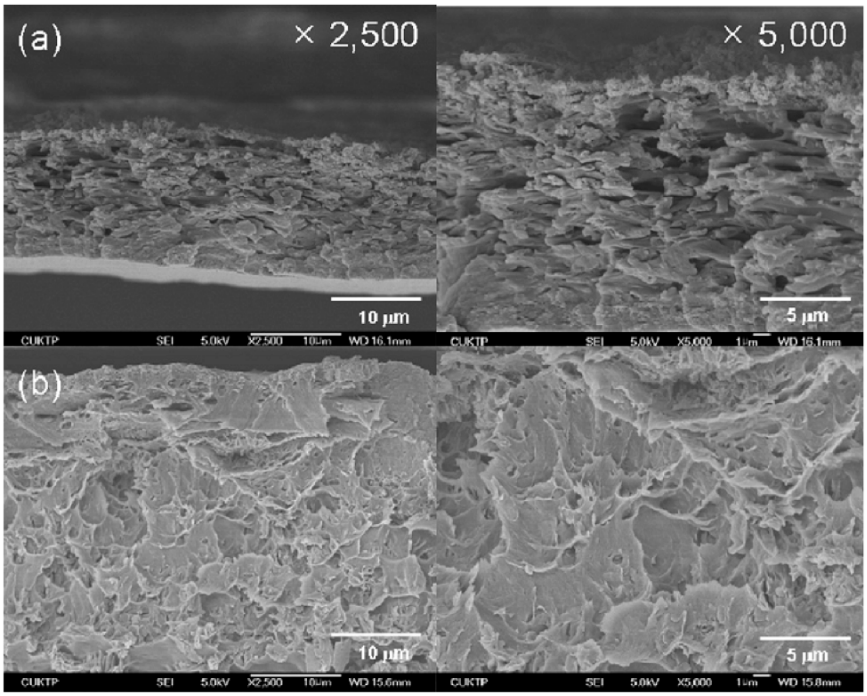


Figure 3: Cross-sectional SEM images of (**a**) GEL-P-Ca and (**b**) GEL-Ca-P composite membranes [11].

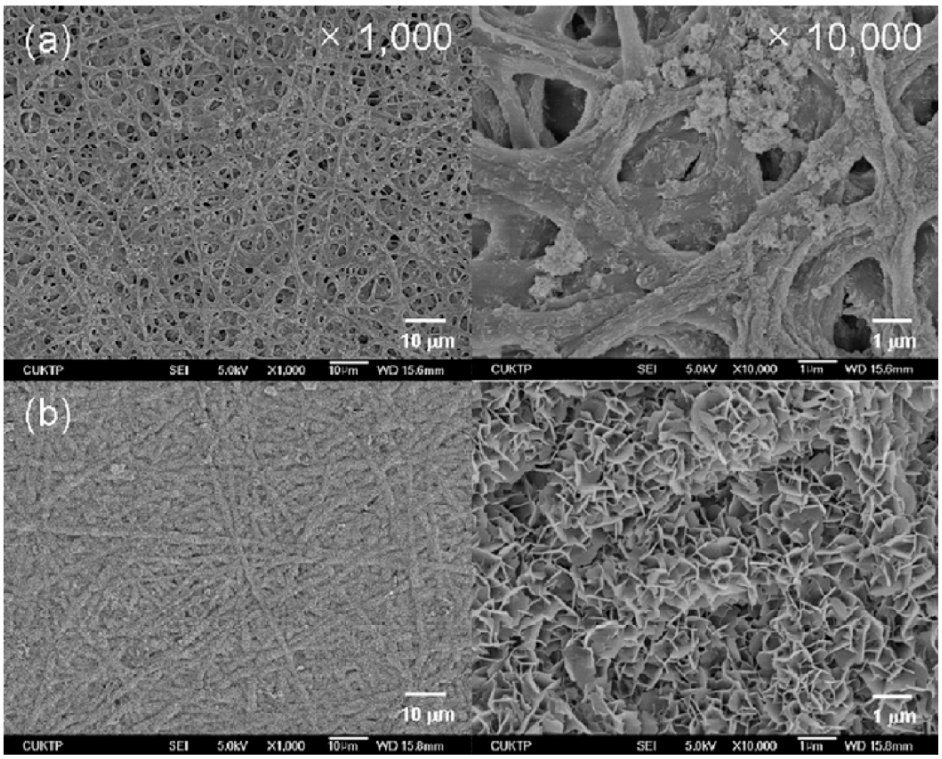


Figure 4: SEM micrographs of (**a**) GEL-P-Ca and (**b**) GEL-Ca-P composite membranes after immersion in SBF for 48 h [11].

their clinical utility is limited by the potential immunogenic response and the origin of suitable vessels [16]. With the development of tissue engineering, tissue engineering blood vessels have emerged as a promising approach to address the shortage of current therapies [17-19]. Tissue engineering blood vessel attempts to fabricate functional small-diameter grafts by combining cells with the scaffold materials under suitable culture conditions, resulting in a tubular scaffold that can be used *in vivo* [20]. A tissue-engineered blood vessel scaffold should be biocompatible, have appropriate mechanical properties, and be readily available in a variety of sizes for grafting applications. Numerous fabrication technique have been used to produce vascular scaffolds [21-23]. Recently, electrospinning technology has gained much attention because it can provide a biomimetic environment with nanoscale to microscale diameter fibers, and it may be easy to form a tubular scaffold with a desirable diameter. Scaffolds fabricated by electrospinning natural and synthetic polymers have been applied widely to biomedical areas [24-26].

4.1. Electrospun Gelatin-bFGF Tubular Scaffold

Current therapeutic angiogenesis strategies are focused on the development of biologically responsive scaffolds that can deliver multiple angiogenic cytokines and cells in ischemic regions. Montero R.B. *et al*. referred that scaffold architecture with respect to nanofiber alignment (random vs. aligned) had a pronounced effect on individual cell morphology, scaffolds with aligned fiber configuration (loaded with 100 ng of bFGF) had a 28% increase in sprout length in comparison to scaffolds with random fiber configuration, as can be observed in Figure **5** [27].

4.2. Electrospun PLA/SF-Gelatin Composite Tubular Scaffold

Wang *et al*. fabricated a tubular scaffold composed of an aligned polylactide (PLA) fiber outside layer and a randomly oriented silk fibroin(SF)-gelatin fiber inner layer (PLA/SF-gelatin) by electrospinning. Wang *et al*. referred that the scaffold has less inflammation and no significant rejection and the scaffolds had good biocompatibility and can be biodegraded gradually *in vivo*, as can be observed in Figure **6**. The new tissue after implantation of months 1and 2, and macrophages and lymphocytes were not found (Figure **6a**, **b**). At month 3 the scaffolds could guide the formation of connective vascular network and the shape of the implants became smaller (Figure **6c**) [28].

4.3. Electrospun PGE Tubular Scaffold

Han J.J. *et al*. fabricated the PLGA/gelatin/elastin (PGE) matrix by electrospinning and referred that all

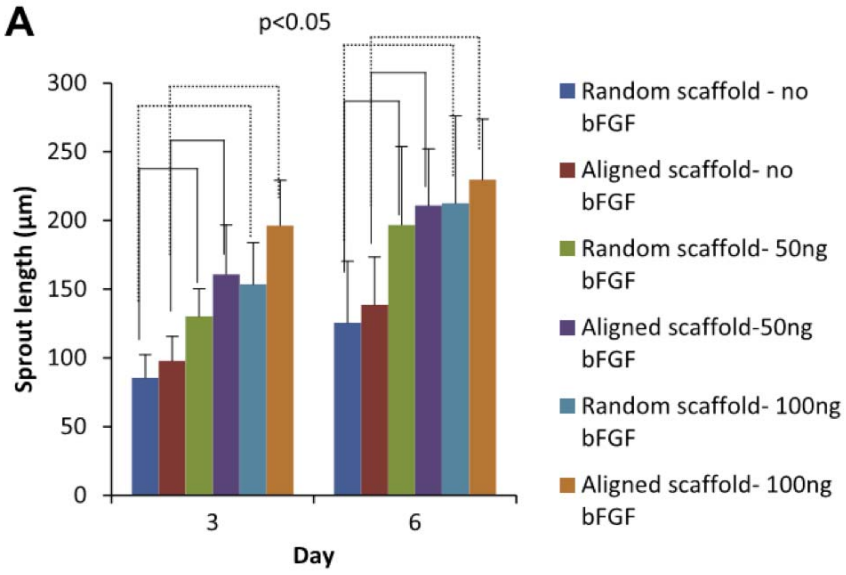


Figure 5: Comparison of average length of HUVEC sprouting with respect to bFGF release between scaffolds with aligned fiber orientation and scaffolds with random fiber orientation. Statistical significance was determined at $p < 0.05$. A solid line denotes statistical significance between the control (no bFGF) and scaffolds loaded with 50 ng bFGF. A dotted line denotes statistical significance between the control (no bFGF) and scaffolds loaded with 100 ng bFGF [27].

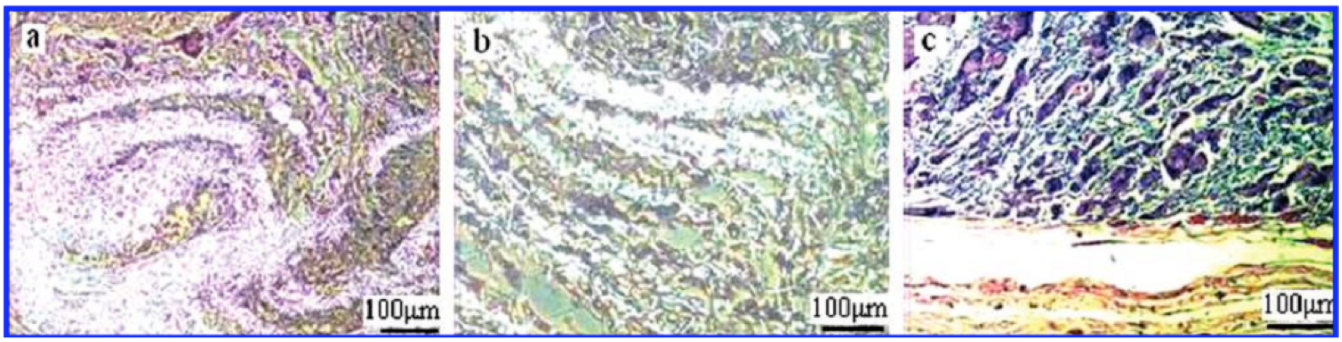


Figure 6: Representative histological photomicrographs of the subcutaneous implants: (**a-c**) PLA/SF-gelatin at 1, 2, and 3 months, respectively [28].

PGE scaffolds support the attachment and metabolization of human endothelial cells (ECs) and bovine aortic smooth muscle cells (SMCs) with some variances in EC morphology and cytoskeletal spreading observed at 48 h postseeding, whereas no morphologic differences were observed at confluence (day 8), as can be observed in Figure **7**. As shown in Figure **7A**, ECs cultured on PGE131 appeared more elongated, whereas on PGE121, that is, a slightly“stiffer”scaffold with smaller fibers containing less of the natural proteins, the cells remained more rounded (Figure **7A**). ECs cultured on rigid glass surfaces appeared more“typical”EC: parsley-shaped with a significantly bigger cell size (Figure **7**). ECs cultured for 48 h on PGE121, PGE131, and glass were significantly different from each other in terms of cell area and shape factor (Figure **7C**, **D**). However, upon confluence at day8, the EC monolayers displayed similar morphology, regardless of which scaffold they were cultured on (Figure **7A**, lower row) [29].

4.4. PU/Gelatin-Heparin Bi-Layer Electrospun Membrane

Wang *et al*. fabricated the tubular scaffolds (inner diameter 4 mm, length 3cm) which are composed of a polyurethane (PU) fibrous outer-layer and a gelatin-heparin fibrous inner-layer by electrospinning technology. They referred that the scaffolds achieved a breaking strength (3.7±0.13 MPa) and an elongation at break (110±8%) that are appropriate for artificial blood vessels. When the scaffolds were immersed in water for 1 h, the breaking strength decreased slightly to 2.2±0.3 MPa, but the elongation at break increased to 145±21%. In the same study, they found that the

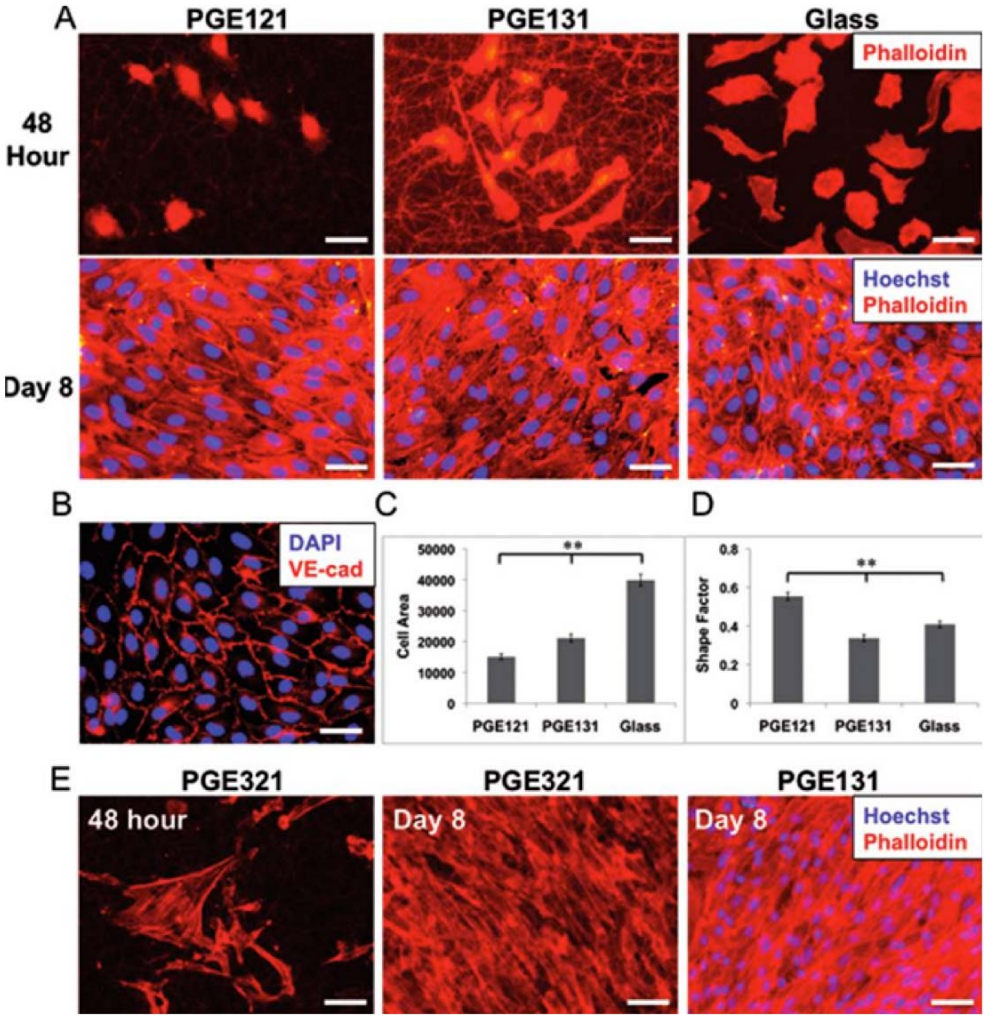


Figure 7: (**A**) Morphology of EA.hy926 endothelial cells on PGE fibrous scaffolds or glass coverslips at 48 h postseeding and at confluence (day8). Staining for nuclei-hoechst (blue) and actin cytoskeleton-phalloidin (red). (**B**) Immuofluorescence staining for monolayer formation on PGE121 .Staining for nuclei-DAPI and intercellular junctions-VE-cadherin. (**C**) Cell area analysis 48 h postseeding (cell area expressed as number of pixels). (**D**) Shape factor analysis 48 h postseeding (shape factor: 0) line, 1) circle). (**E**) Morphology of bovine aortic smooth muscle cells on PGE fibrous scaffolds. Staining as in panel **A**. (**C**, **D**) Data are expressed as mean (SE, representative from three experiments,n = 90. **: P < 0.01. Scale bar =50μm [29].

gelatin- heparin fibrous scaffolds showed a significant suppression of platelet adhesion and heparin was released from the scaffolds at a fairly uniform rate during the period of 2nd day to 9th day. The scaffolds are expected to mimic the complex matrix structure of native arteries, and to have good biocompatibility as an artificial blood vessel owing to the heparin release [30].

5. ELECTROSPUN PCL/GELATIN NANOFIBERS AS NEVER TISSUE ENGINEERING

Nerve tissue engineering is one of the most promising methods to restore nerve systems in human health care. Scaffold design has pivotal role in nerve tissue engineering. In recent years, electrospinning has emerged as a leading technique for neural tissue engineering due to its ability to create fine, random, aligned, and densely packed fibers that mimic, geometrically and topologically, the native state of the extracellular matrix and its complex supramolecular assemblies [31-32]. It has recently been demonstrated that electrospun membranes comprising biodegradable polymers, such as polylactid acid or polydioxanone, exhibit favorable interactions with neuronal cells, promoting adhesion and supporting cell differentiation [33, 34]. Other studies on Schwann cells seeded onto polycaprolactone-based electrospun membranes showed important evidence of cell morphology and proliferation, underlining the ability of cells to form bipolar spreading onto the nanofibrous surface [35] and acting as a positive cue to elongate neurite outgrowth [36].

Polymer blending is one of the most effective methods for providing new, desirable biocomposites for

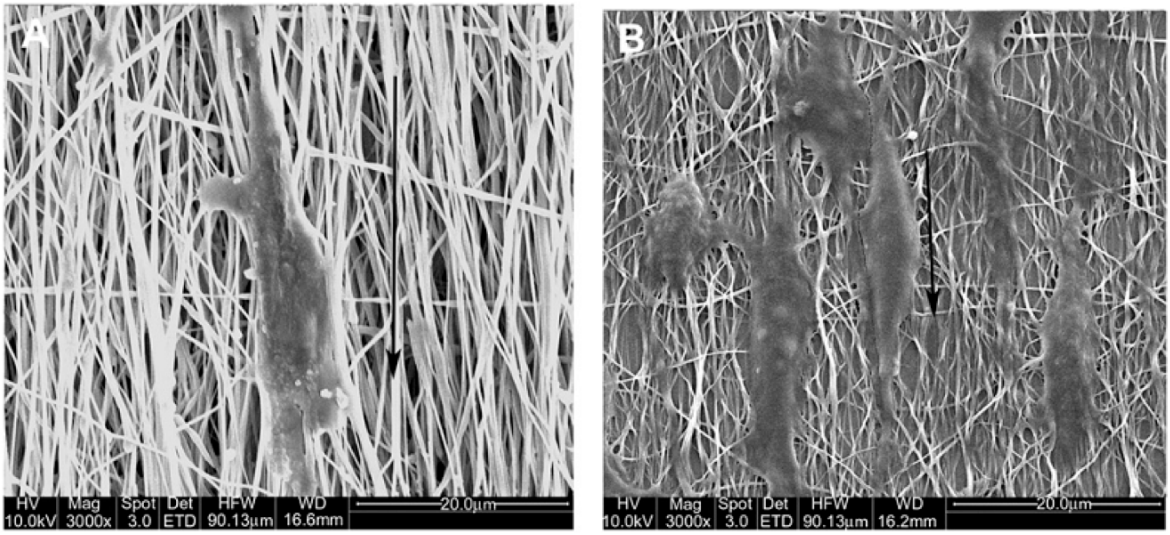


Figure 8: Morphology of C17.2 cells on aligned (**A**) PCLand (**B**) PCL/gelatin (70:30) nanofibers after 6 days of cell culture [37].

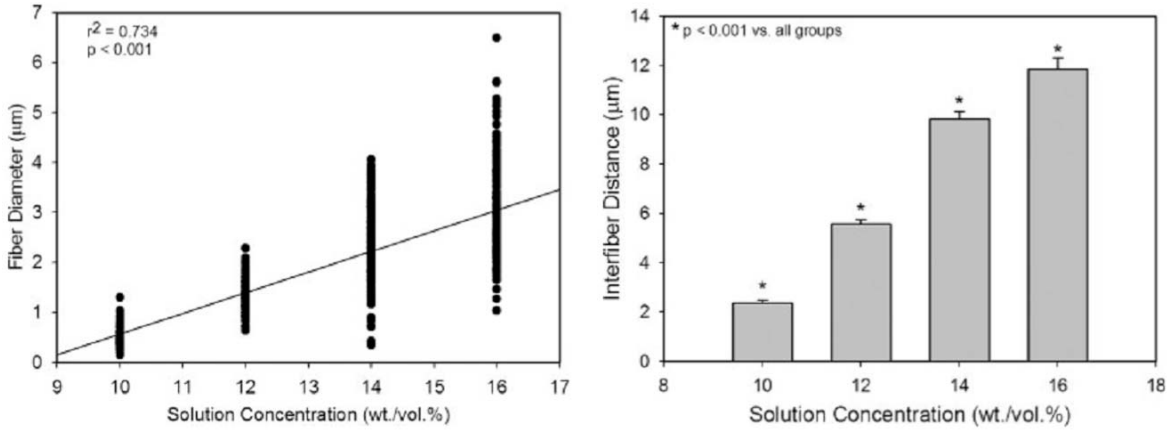
tissue-engineering applications. L. Ghasemi-Mobarakeh *et al*. explored the electrospinning PCL/Gelatin scaffolds for nerve regeneration and they referred that the biocomposite of PCL/gelatin 70:30 nanofibrous scaffolds enhanced the nerve differentiation and proliferation compared to PCL nanofibrous scaffolds and acted as a positive cue to support neurite outgrowth. In the same study they also found that the direction of nerve cell elongation and neurite outgrowth on aligned nanofibrous scaffolds is parallel to the direction of fibers, as can be observed in Figure **8** [37].

6. ELECTROSPUN GELATIN NANOFIBERS FOR SKIN BIOENGINEERING

The majority of bioengineered skin substitutes are comprised of freeze-dried bio-polymer sponges populated with dermal fibroblasts alone [38, 39] or in conjunction with keratinocytes [40, 41]. However, freeze-drying is a labor intensive, costly process that can produce sponges with significant structural heterogeneity [42, 43]. To overcome these difficulties, electrospinning has been used to generate nonwoven, homogeneous fibrous scaffolds from a wide variety of synthetic and natural polymers. Electrospun gelatin and gelatin blends are widely used as a dressing for wound healing [44-48] and as a scaffold for dermal tissue engineering.

6.1. Gelatin Nanofibers

The potential of electrospun gelatin as a scaffolding material for dermal and epidermal tissue regeneration was evaluated by H. M. Powell *et al*. They referred that solution concentration was a significant predictor of fiber diameter, interfiber distance, and porosity with higher solution concentration correlated with larger



(**a**). Scatter plot of fiber diameter (**b**). Mean interfiber distance

Figure 9: Function of solution concentration to fiber diameter (**a**) and interfiber distance (**b**) [49].

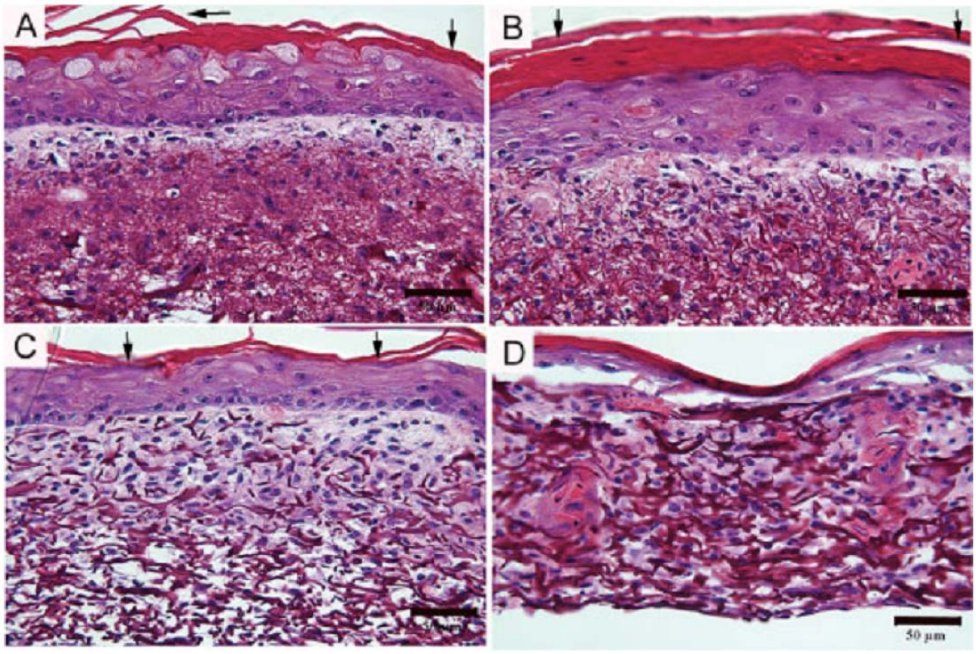


Figure 10: H&E stained cross-sections of skin substitutes made with (**A**) 10, (**B**) 12, (**C**) 14, and (**D**) 16 wt/vol % gelatin scaffolds after 14 days in culture. Note the well-stratified dermal and epidermal layers in the 10, 12, and 14% groups and lack of a well-formed epidermis in the 16 wt % group. Arrows point to cornified layers formed in the 10, 12, and 14% group. Scale bar¼ 50µm [49].

fiber diameters and interfiber distances, as can be observed in Figure **9** [49].

In the same study, they also found that interfiber distances between 5 and 10µm appear to yield the most favorable skin substitute *in vitro*, as can be observed in Figure **10**. A thick epithelium with basal keratinocytes was present in the 10, 12, and 14% groups (Figure **10A-C**) but not evident in the 16% gro up. The 16% group was not well stratified and only a thin epithelium existed (Figure **10D**).

M. Dubsky *et al*. Referred that gelatin nanofibers produced by needleless technology accelerate wound healing and be suitable as a scaffold for cell transfer and skin regeneration. Compared to control wounds covered with gauze, epithelialization was considerably faster after gelatin treatment, which resulted in

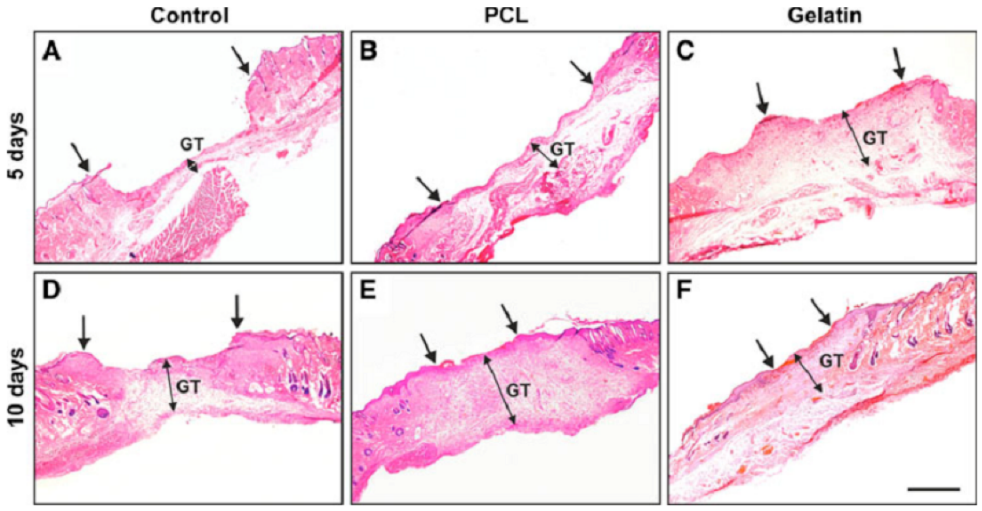


Figure 11: Representative histology (H&E staining) on days 5 (**a** , **b**, **c**) and 10 (**d**, **e**, **f**). Arrows show the thickness of the GT and the edge of the ulcer where the new epidermis has not been created. A large epithelial gap (EG) and a thin GT are seen in the control (**a**) and PCL-treated groups on day 5 (**b**). Gelatin-treated wounds (**c**) on day 5 showed a smaller EG and a thicker GT in comparison with controls. A shorter EG is seen in gelatin-(**f**) on day 10. Scale bar: 1mm [50].

significantly shorter linear and polygonal epithelial gaps on days 5 and10 and a significantly thicker layer of GT (granulation tissue) on day 5, as can be observed in Figure **11** [50].

6.2. Chitosan/Gelatin Blend Nanofibers

Chitosan with its antimicrobial properties and gelatin with its cell adhesion property offer a unique combination that has immense potential to serve as a scaffold for skin tissue engineering. J. Jafari *et al*. referred that chitosan/gelatin ratio of 30 /70 was identified as the optimum ratio for production of fibers with uniform morphologies and minimum defects. In the assessment of in-vitro biocompatibility of the scaffolds with human skin fibroblasts study, they also found the excellent biocompatibility of these scaffolds and their high capacity to support cell attachment and proliferation, as can be observed in Figure **12** [51].

6.3. PLACL-Gelatin Blend Nanofibrous Scaffolds

Poly(L-lactic acid)-co-poly(ε-caprolactone) (PLACL), a synthetic copolymer of PCL and PLLA [52], because of its beneficial features like biodegradability and non-toxicity, has been used as substrates for the culture of human dermal fibroblasts. The use of collagen requires a cross-linking agent and thus could lead to cytotoxicity [53]. Also, collagen has poor mechanical properties and occurs in composites such as collagen-glycosaminoglycans which deter skin regeneration [54]. Use of gelatin overcomes these problems, and in combination with a synthetic polymer like PLACL, gives a 'bioartifical polymer' with enhanced biocompatibility and chemical properties.

Chandrasekaran A.R. *et al*. fabricated the PLACL/gelatin nanofibrous scaffolds by the electrospinning process and they referred that the tensile properties of PLACL-G (3:1wt%)-blended nanofibres were higher than that of PLACL nanofibres [55]. Addition of 10-39 wt% of gelatin has previously resulted in enhanced tensile properties of PLACL-G scaffolds [56]. In the same study, they also found that PLACL-G plasma-treated nanofibrous scaffolds were more suitable for growth of fibroblasts as compared to PLACL scaffolds, attaining a significant level of increase in cell proliferation after day 6 and day 9 of culture and a percent level of increase up to 40% after day 9 of culture, as can be observed in Figure **13**. The percentages of cell proliferation increase on PLACL-P and PLACL-G were only 10% and 14%, respectively. Furthermore, a significant level of increase in proliferation was seen in PLACL-G-P showing that the fibres with gelatin assisted increased proliferation of the fibroblasts. A large number of interconnected pores and the rough surface of the nanofibrous membrane support the proliferation of fibroblasts and quicker regeneration of skin tissue [57]. In addition to the porous nature and suitable mechanical properties, molecular signals from the nanofibres may also guide cells entering the cell substrate by their amoeboid movement [58]. The hydrophilic nature of the PLACL-G scaffolds is another reason for better adhesion and proliferation of fibroblasts.

The PLACL-G scaffolds in comparison with the previous scaffolds of PCL-collagen and PLLA will serve as better tissue engineering scaffolds in the longer run because of the relatively low cost and biological origin

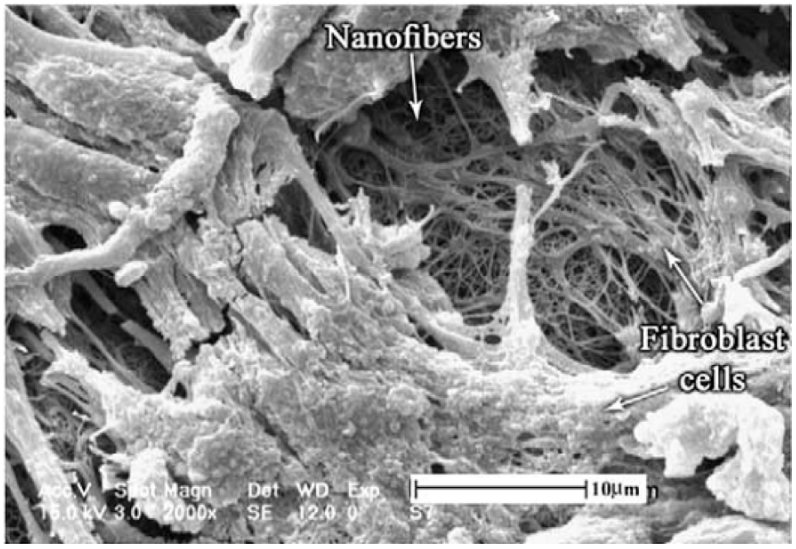


Figure 12: SEM micrographs of fibroblast cells attached to nanofibrous scaffold with chitosan-gelatin ratio of 30/70 [51].

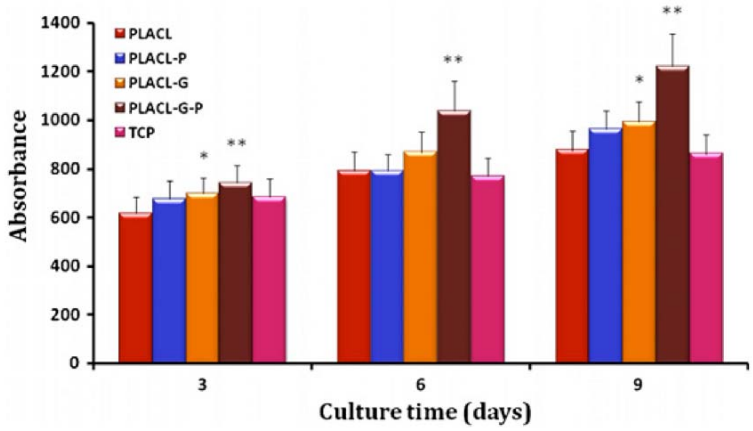


Figure 13: MTS assay for proliferation of fibroblasts on nanofibrous scaffolds and TCP. Bar represents mean standard deviation. Asterisks indicate the significant level of proliferation obtained by the t-test. *P < 0.05, **P < 0.001 [55].

of gelatin in addition to its water retention properties which are typically required for light-to-moderate exudates wounds.

7. FUTURE DEVELOPMENTS

Electrospun gelatin scaffolds for tissue regeneration is an ever expanding area, wheras the products that meet the requirement are far and few. In future, better approaches would be to devise nanofibrous scaffolds which are capable of supporting the tissues in their natural environment and possess controlled surface topography as well as structural morphology.

1. Electrospinning gelatin nanofiber. Polymer blending is one of the most effective methods for providing new, desirable biocomposites for tissue-engineering applications. It is the orientation to electrospin gelatin blending with other tissue engineering materials nanofibers. It is also important to fabricate bFGF-loaded nanofibrous scaffolds with patterned fiber architecture.

2. Electrospinning gelatin scaffold. In order to fully realize the potential of electrospun gelatin nanofibers, it is important to fabricate fibrous assemblies with controllable three-dimensional (3D) microstructures as the fiber arrangement will significantly affect the performance of scaffold.

    - One such approach could be to formulate nonwoven 3D scaffolds for tissue regeneration.

3. Animal experiment and clinical application. The current study of electrospinning gelatin scaffold seldom come down to animal experiment, not to mention clinical application. The future direction of the research will have turned from basic research to animal experiment and clinical application.

ACKNOWLEDGEMENTS

This work was financially supported by the Technology Bureau of Jiaxing City (2011AY1026, MTC2012-006, 2012AY1024) and Science and Technology Agency of Zhejiang Province (2012R10012-09, 2012R10012-06).

REFERENCES

[1] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005; 23: 47. http://dx.doi.org/10.1038/nbt1055

[2] Chaitaworn N. Master Thesis, Faculty of Engineering, Chulalongkorn University 2009.

[3] Ratanavaraporn J, Rangkupan RH, Jeeratawatchai H, Kanokpanont S, Damrongsakkul SP. Influences of physical and chemical cross-linking techniques on electrospun type A and B gelatin fiber mats. Int J Biol Macromol 2010; 47: 431-38. http://dx.doi.org/10.1016/j.ijbiomac.2010.06.008

[4] Hutchens SA, Benson RS, Evans BR, O'Neill HM, Rawn CJ. Biomaterials 2006; 27: 4661-70. http://dx.doi.org/10.1016/j.biomaterials.2006.04.032

[5] Zimmermann KA, Leblanc JM, Sheets KT, Fox RW, Gatenholm P. Materials Sci Eng C 2011; 31: 43-49. http://dx.doi.org/10.1016/j.msec.2009.10.007

[6] Will J, Melcher R, Treul C, Travitzky N, Kneser U, Polykandriotis E, *et al*. J Mater Sci Mater Med 2008; 19: 2781-90. http://dx.doi.org/10.1007/s10856-007-3346-5

[7] Gupta D, Venugopal J, Mitra S, Giri Dev VR, Ramakrishna S. Biomaterials 2009; 30: 2085-94. http://dx.doi.org/10.1016/j.biomaterials.2008.12.079

[8] Yang FY, Both SK, Yang X, Walboomers XF, Jansen JA. Acta Biomater 2009; 5: 3295-304. http://dx.doi.org/10.1016/j.actbio.2009.05.023

[9] Jose MV, Thomas V, Johnson KT, Dean DR, Nyairo E. Acta Biomater 2009; 5: 305-15. http://dx.doi.org/10.1016/j.actbio.2008.07.019

[10] Sisson K, Zhang C, Farach-Carson MC, Chase DB, Rabolt JF. Fiber diameters control osteoblastic cell migration and differentiation in electrospun gelatin. J Biomed Mater Res 2010; 94A: 1312-20.

[11] Choi MO, Kim YJ. Fabrication of gelatin/calcium phosphate composite nanofibrous membranes by biomimetic mineralization. Int J Biol Macromol 2012; 50: 1188-94. http://dx.doi.org/10.1016/j.ijbiomac.2012.04.001

[12] Kim YJ. Polymer (Korea) 2008; 32: 409-14.

[13] Kokubo T. Biomaterials 1991; 12: 155-63. http://dx.doi.org/10.1016/0142-9612(91)90194-F

[14] Yang FY, Both SK, Yang X, Walboomers XF, Jansen JA. Acta Biomater 2009; 5: 3295-304. http://dx.doi.org/10.1016/j.actbio.2009.05.023

[15] Inoguchi H, Keun K, Inoue E, Takamizawa K, Maehara Y, Matsuda T. Biomaterials 2006; 27: 1470-78. http://dx.doi.org/10.1016/j.biomaterials.2005.08.029

[16] He W, Ma Z, Yong T, Teo WE, Ramakrishna S. Biomaterials 2005; 26: 7606-15. http://dx.doi.org/10.1016/j.biomaterials.2005.05.049

[17] Puskas JE, Chen Y. Biomacromolecules 2004; 5(4): 1141-54. http://dx.doi.org/10.1021/bm034513k

[18] Sayers RD, Raptis S, Berce M, Miller JH. Br J Surg 1998; 85: 934-38. http://dx.doi.org/10.1046/j.1365-2168.1998.00765.x

[19] Nerem RM, Seliktar D. Annu Re V Biomed Eng 2001; 3: 225-43. http://dx.doi.org/10.1146/annurev.bioeng.3.1.225

[20] Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R. Science 1999; 284: 489-93. http://dx.doi.org/10.1126/science.284.5413.489

[21] Mikos AG, Temenoff JS. J Biotechnol 2000; 3: 114-19.

[22] Yang S, Leong KF, Du Z, Chua CK. Tissue Eng 2001; 7: 679-89. http://dx.doi.org/10.1089/107632701753337645

[23] Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Trends Biotechnol 2003; 21: 157-61. http://dx.doi.org/10.1016/S0167-7799(03)00033-7

[24] Kriegel C, Kit KM, McClements D. J Polymer 2009; 50: 189-200. http://dx.doi.org/10.1016/j.polymer.2008.09.041

[25] Kowalczyk T, Nowicka A, Elbaum D, Kowalewski TA. Biomacromolecules 2008; 9(7): 2087-90. http://dx.doi.org/10.1021/bm800421s

[26] Pham QP, Sharma U, Mikos AG. Biomacromolecules 2006; 7(10): 2796-805. http://dx.doi.org/10.1021/bm060680j

[27] Montero RB, Vial X, Nguyen DT, Farhand S, Reardon M, Pham SM, *et al*. bFGF-containing electrospun gelatin scaffolds with controlled nano-architectural features for directed angiogenesis. Acta Biomater 2012; (8): 1778-91. http://dx.doi.org/10.1016/j.actbio.2011.12.008

[28] Wang SD, Zhang YZ, Yin GB, Wang HG, Dong ZH. Electrospun Polylactide /Silk Fibroin-Gelatin Composite Tubular Scaffolds for Small-Diameter Tissue Engineering Blood Vessels. J Appl Polym Sci 2009; 113: 2675-82. http://dx.doi.org/10.1002/app.30346

[29] Han JJ, Lazarovici P, Pomerantz C, Chen XS, Wei Y, Lelkes PI. Co-Electrospun Blends of PLGA, Gelatin, and Elastin as Potential Nonthrombogenic Scaffolds for Vascular Tissue Engineering. Biomacromolecules 2011; 12: 399-408. http://dx.doi.org/10.1021/bm101149r

[30] Wang HY, Feng YK, Behl M, Lendlein A, Zhao HY , Xiao RF, *et al*. Hemocompatible polyurethane/gelatin-heparin nanofibrous scaffolds formed by a bi-layer electrospinning technique as potential artificial blood vessels. Front Chem Sci Eng 2011; 5(3): 392-400. http://dx.doi.org/10.1007/s11705-011-1202-0

[31] Teo WE, He W, Ramakrishna S. Biotechnol J 2006; 1: 918-29. http://dx.doi.org/10.1002/biot.200600044

[32] Kumbar SG, James R, Nukavarapu SP, Laurencin CT. Biomed Mater 2008; 3: 1-15. http://dx.doi.org/10.1088/1748-6041/3/3/034002

[33] Yang F, Murugan R, Wang S, Ramakrishna S. Biomaterials 2005; 26: 2603-10. http://dx.doi.org/10.1016/j.biomaterials.2004.06.051

[34] Yang F, Xu CY, Koktaki M, Wang S, Ramakrishna SJ. Biomater Sci Polym Ed 2004; 15: 1483-97. http://dx.doi.org/10.1163/1568562042459733

[35] Prabhakaran MP, Venugopal J, Chyan TT, Hai LB, Chan CK, Yu-Tang AL, Ramakrishna S. Tissue Eng Part A 2008; 14: 1-11. http://dx.doi.org/10.1089/ten.tea.2007.0393

[36] Ghasemi-Mobarakeh L, Prabhakaran MP, Morshed M, Nasr-Esfahani MH, Ramakrishna S. Biomaterials 2008; 29: 4532-39. http://dx.doi.org/10.1016/j.biomaterials.2008.08.007

[37] Mobarakeh LG, Prabhakaran MP, Morshed M, Esfahani MHN, Ramakrishna S. Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. Biomaterials 2008; (29): 4532-39. http://dx.doi.org/10.1016/j.biomaterials.2008.08.007

[38] Yannas IV, Burke JF. Design of an artificial skin. I. Basic design principles. J Biomed Mater Res 1980; 14: 65-81. http://dx.doi.org/10.1002/jbm.820140108

[39] Heimbach D, Luterman A, Burke JF, Cram A, Herndon D, Hunt J. Artificial dermis for major burns: A multi-center randomized clinical trial. Surgery 1988; 208: 313-20. http://dx.doi.org/10.1001/jama.1989.03430150093032

[40] Hansbrough JF, Boyce ST, Cooper ML, Foreman TJ. Burn wound closure with cultured autologous keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. JAMA 1989; 262: 2125-30.

[41] Boyce ST, Kagan RJ, Greenhalgh DG, Warner P, Yakuboff KP, Palmieri T, *et al*. Cultured skin substitutes reduce requirements for harvesting of skin autograft for closure of excised, full-thickness burns. J Trauma 2006; 60: 821-29.

[42] Erdag G, Sheridan R. Fibroblasts improve performance of cultured composite skin substitutes on athymic mice. Burns 2004; 30: 322-28. http://dx.doi.org/10.1016/j.burns.2003.12.007

[43] Freyman TJ, Yannas IV, Yokoo R, Gibson LJ. Fibroblasts con-traction of a collagen-GAG matrix. Biomaterials 2001; 22: 2883-91. http://dx.doi.org/10.1016/S0142-9612(01)00034-5

[44] O'Brien F, Harley B, Yannas I, Gibson L. Influence of freezing rate on pore structure in freeze-dried collagen-GAG scaffolds. Biomaterials 2004; 25: 1077-86. http://dx.doi.org/10.1016/S0142-9612(03)00630-6

[45] Zhang Y, Venugopal J, Huang Z-M, Lim C, Ramakrishna S. Crosslinking of the electrospun gelatin nanofibers. Polymer 2006; 47: 2911-17. http://dx.doi.org/10.1016/j.polymer.2006.02.046

[46] Ulubayram K, Cakar A, Korkusuz P, Ertan C, Hasirci N. EGF containing gelatin-based wound dressings. Biomaterials 2001; 22: 1345-56. http://dx.doi.org/10.1016/S0142-9612(00)00287-8

[47] Neumann P, Zur B, Ehernreich Y. Gelatin-based sprayable foam as a skin substitute. J Biomed Mater Res 1981; 15: 9-18. http://dx.doi.org/10.1002/jbm.820150105

[48] Wang T-W, Wu H-C, Huang Y-C, Sun J-S, Lin F-H. Biomimetic bilayered gelatin-chondroitin sulfate-hyaluronic acid biopolymer as a scaffold for skin equivalent tissue engineering. Artif Organs 2006; 30: 141-49. http://dx.doi.org/10.1111/j.1525-1594.2006.00200.x

[49] Powell HM, Boyce ST. Fiber density of electrospun gelatin scaffolds regulates morphogenesis of dermal-epidermal skin substitutes. Wiley Periodicals, Inc. 2007.

[50] J Mater Sci Mater Med 2012; 23: 931-41. http://dx.doi.org/10.1007/s10856-012-4577-7

[51] Jafari J, Emami SH, Samadikuchaksaraei A, Bahar MA, Gorjipour F. Electrospun chitosan- gelatin nanofiberous scaffold: Fabrication and *in vitro* evaluation. Bio-Med Mater Eng 2011; (21): 99-112.

[52] Lemmouchi Y, Schacht E. Preparation and *in vitro* evaluation of biodegradable poly(ε-caprolactone-co-D,L lactide)(X-Y) devices containing trypanocidal drugs. J Control Release 1997; 45(2): 27-33.

[53] Venugopal JR, Low S, Choon AT, Kumar AB, Ramakrishna S. Nanobioengineered electrospun composite nanofibres and osteoblasts for bone regeneration. Artif Organs 2008; 32: 388-97. http://dx.doi.org/10.1111/j.1525-1594.2008.00557.x

[54] Wahl DA, Czernuszka JT. Collagen-hydroxyapatite composites for hard tissue repair. Eur Cell Mater 2006; 1143-56.

[55] Chandrasekaran AR, Venugopal J, Sundarrajan S, Ramakrishna S. Fabrication of a nanofibrous scaffold with improved bioactivity for culture of human dermal fibroblasts for skin regeneration. Biomed Mater 2011; 6: 015001. http://dx.doi.org/10.1088/1748-6041/6/1/015001

[56] Lee J, Tae G, Kim YH, Park IS, Sang-Heon K, Kim SH. The effect of gelatin incorporation into electrospun poly(l-lactide-co-caprolactone) fibres on mechanical properties and cytocompatibility. Biomaterials 2008; 29: 1872-79. http://dx.doi.org/10.1016/j.biomaterials.2007.12.029

[57] Murugan R, Ramakrishna S. Design strategies of tissue engineering scaffolds with controlled fibre orientation. Tissue Eng 2007; 13: 1845-66. http://dx.doi.org/10.1089/ten.2006.0078

[58] Venugopal JR, Low S, Choon AT, Kumar AB, Ramakrishna S. Nanobioengineered electrospun composite nanofibres and osteoblasts for bone regeneration. Artif Organs 2008; 32: 388-97. http://dx.doi.org/10.1111/j.1525-1594.2008.00557.x

Received on 17-09-2012 Accepted on 18-11-2012 Published on 31-12-2012

DOI: http://dx.doi.org/10.6000/1929-5995.2012.01.02.1