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Tuning Mechanical Properties of the Composite Nanofibers by Changing the Composition of the Polymer Solution

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ABSTRACT: Composite nanofibers are suitable candidates for applications in biomedical engineering. The relationship and the balance between the structural-volumetric characteristics and the mechanical behavior of the nanofibrous composites have been proved but, in this study, we studied the pattern of such relationships between random and aligned nanofibrous composites made up of PLGA and fibrin. After evaluating the fibers' morphology and the mats' porosity, the relationship between these two features and the tensile strength of the mats was investigated. All mats exhibited relatively homogeneous fibers with higher fiber diameters for random fibers than the oriented ones (0.23 to 1.65 µm and 0.34 to 0.58 µm, respectively) and the diameter of the fibers decreased by fibrin percentage. The porosity proportions of the mats were in the range of 78.4% to 81.4% and random mats depicted higher porosity and interconnected pores. The mechanical features of the mats were compatible with natural tissues and the mechanical characteristics of the aligned mats were higher. In aligned mats, fibrin decreased the diameter of the fibers and the porosity proportion, limited the fiber's thickness distribution, and increased the interconnectivity of the pores and all these factors led to lowering the tensile strength and the stiffness of the aligned mats. On the other hand, the porosity features of the random mats did not significantly change by fibrin, but fibrin lowered the diameter of the fibers and limited the fiber's thickness distribution, and these two factors decreased the tensile strength and stiffness of the random mats.

KEYWORDS: Composite mat, electrospinning, elastic modulus, fiber thickness, porosity.

1. INTRODUCTION

Nanofiber mats can be used as supporting structures to guide and shape tissue growth in vivo or in vitro (1). To construct biomedical implants and tissue scaffolds, various synthetic and natural polymers have been utilized in the form of fiber structures, woven networks, hydrogels, and sponges (2) and the type of the utilized material depended on several different criteria (2-4).

As bioactive materials, natural materials encourage cell attachment and growth, are biodegradable, and support cell growth (5, 6), Carbohydrate polymers including alginate, agarose, hyaluronan, and chitosan and protein matrices such as collagen and fibrin have been utilized in making implants and tissue scaffolds (3). Among them, fibrin has ideal characteristics and its precursor (fibrinogen) is obtained from accumulated plasma works as the natural wound-healing matrix (7). Clots and fibrin adhesives obtained from the reaction of fibrinogen and thrombin have been used in in-vivo studies, and after degradation, they were replaced by tissue ECM without any toxic degradation products (3). Similar to collagen, fibrin contains locations for cell attachment (7) and cells can connect to it through the integrin proteins (5) however like other natural materials, it has poor mechanical characteristics (5, 6).

Biocompatible synthetic materials including Polyglycolic acid (PGA), Polylactic acid (PLA), Poly (lactic-co-glycolic acid) (PLGA), polycaprolactone, polystyrene (PS), Poly (L-lactic acid) (PLLA) and Polyethylene glycol (PEG), all received FDA approval and possess good biomechanical properties, ease of design, support cellular function and modulate host tissue biochemical properties (4, 6). They have been widely used in medical applications for years, such as surgical sutures, growth-factor transfer, biosensors, and tissue engineering (4, 5, 8-11). Among them, Poly (lactic-co-glycolic acid) (PLGA) possesses a controllable degradation rate than its homopolymers (8), and it could facilitate the attachment and growth of chondrocytes and improved extracellular matrix (ECM) production (5). Sponges and foams of PLGA are inherently stiffer and harder than PGA and can be processed much easier (3) and there is no linear relationship between the ratio of lactide to glycolide and the mechanical properties of PLGA (8). However, synthetic materials have limitations such as the risk of graft rejection (6), local inflammation and cell death responses (9), and relatively weak cell adhesion and tissue integrity (3).

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By compositing different materials, it is possible to resolve the issues mentioned for synthetic and natural polymers (2). Porous fibrous structures filled out with gels are more useful than their single-use (1) and biological and pharmacological compounds could be added to porous mats (12). Combined PLGA network with chondrocyte-containing fibrin glue improved collagen type 2 expression (1) or combining fibrin with polyglycolic acid (PGA-fibrin) increased the differentiation of bovine chondrocytes and depicted tensile strength of above 2.6 MPa (13). In a study published by Norouzi et. al, 10% fibrin containing aligned PLGA/fibrin mats and 20% fibrin containing unaligned electrospun PLGA/fibrin mat exhibited superior cell-mat interaction results and human Adipose-Derived Stem Cells (h-ADSCs) attached and proliferated well on these composite mats (14).

The mechanical characteristic of the implants is a vital factor for their effectiveness (12) and ideal tissue mats possess mechanical features compatible with the anatomical location of the tissue and they enable surgical manipulation during implantation (2, 6). On the microscopic scale, tissue scaffold implants should support cell attachment and distribution, and on a macroscopic scale, their mechanical features ought to be close to the target tissue to protect cells from damage under compressive forces (12) however, they should not prevent applying the natural biomechanical forces to the surrounding tissue (7, 15), and this can be achieved through the precise control of their architecture (12).

The mechanical properties of a tissue implants include the basic strength of the base material and the design features, which can be changed especially during the production process by changing the design parameters, such as porosity, hole size, hole shape, fiber dimensions, and fiber spacing. (1, 4). Fiber and sponge biomaterials with macro holes between 300 and 500 micrometers depicted different mechanical characteristics, and those with less porosity showed a better performance in compression and tension tests, and the shape of the holes was effective on their mechanical properties (4). There is a balance between the porosity and the physical characteristics; as the porosity increases, the degree of strength decreases, so to make a tissue engineering scaffold, a proper balance must be considered between the design parameters (4).

In a study of collagen type 1 cross-linked with Chitosan nanofibers, or collagen combined with hyaluronic acid or collagen combined with Hydroxy Apatite nanoparticles, results showed that the addition of hyaluronic acid significantly reduced the elastic modulus and reinforcing chitosan nanofibers increased the strength of the porous material (1). Norouzi et. al studied PLGA/Fibrin/Lignin electrospun scaffolds and their results indicated that the mechanical properties varied with the composition. The porosity of the scaffolds increased with fibrin and decreased with lignin. Additionally, SEM analysis showed that PLGA/Lignin fibers had the largest diameter, which decreased with lignin content. These variations in fiber size and porosity influenced the mechanical features of the scaffolds, impacting their tensile strength and overall structural integrity (16). Studying the relationship between the structural characteristics of polyurethane elastomer networks and their mechanical characteristics revealed that the elastic modulus of the networks ranged between 0.56 to 3.0 MPa depending on the diameter of the fibers and the degree of fiber alignment (12). Conducted studies on the tensile strength of the cartilage tissue obtained from chondrocyte cell cultures using laboratory and theoretical methods revealed a relationship between the microstructure of the tissue and the macroscopic strength of the material, and a small difference was reported between the theoretical predictions and the laboratory data (1).

In the literature, there is a lack of studies that investigated the relationship between the volumetric mechanical features of the nanofibrous biomaterials and the morphological properties of their fibers on the centimeter scale (mat size) and on the microscale (fiber architecture, fiber orientation, fiber diameter, and fiber rearrangement under tension) (12) and can explain the relationship between the mating structure and material with the mechanical behavior of the mats (15). In this study, we investigated the effect of three structural features of nanofibrous mats made of PLGA and fibrin, including fiber diameter, porosity, and interconnectivity of the pores, on the mechanical characteristics of the composite nanofibers.

2. MATERIALS AND METHODS

2.1. Materials

Poly (lactic-co-glycolic acid) (PLGA) with a copolymer ratio of 85:15 (Lactide:Glycolide) and an inherent viscosity of 2.3 (dl/g) was bought from Corbion (PURASORB PLG 8523, Amsterdam, the Netherlands). Fresh frozen fibrinogen and thrombin, obtained from Iranian Blood Transfusion Organization (IBTO), were used to make fibrin. As the solvent phase, HFIP (CAS #: 920-66-1, Merck, Germany) was used.

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2.2. Preparation of Polymer Solution Plga/Fibrin

Polymer solutions consisting of PLGA and PLGA-fibrin mixtures with different ratios of PLGA to fibrin (9:1, 8:2, 7:3) were prepared. In order to synthesize fibrin, fibrinogen, and thrombin were mixed in a ratio of 1:1 and placed at room temperature for a few minutes to obtain solid fibrin. The specified proportions of PLGA and fibrin were dissolved in 1,1,1,3,3,3,3-Hexafluoro-2propanol to obtain 10% (w/v) polymer solutions. The polymer solutions were then stirred for 5 hours using a magnetic stirrer to achieve a uniform and non-agglomerated polymer solution.

2.3. Mat Electrospinning

The electrospinning parameters were optimized using pretests. The polymer injection peed was set at 0.6 ml/hour, the distance between the tip of the needle and the collecting mandrel was 16 cm, the voltage was 20 kV, the device transverse movement speed was 10 cm/min, and the rotation speed of the collecting mandrel (to produce aligned mats) was 25 rpm. To dry the mats and remove the solvent from the fibers, mats were placed in a vacuum oven for 24 hours at a temperature of 30 °C.

2.4. Characterization Of Mats

To characterize the morphology of the mats, the samples were covered with a thin layer of gold, and then SEM micrographs were captured (ZEISS SIGMAVP). To study the chemical composition of the fibers, Fourier-transform infrared spectroscopy (FTIR) analysis was conducted (FTIR6300 MHZ device, Jasco Inc.). SEM micrographs were then processed using ImageJ software (Wayne Rasband, National Institutes of Health, USA) to evaluate the diameter of the fibers and for each mat, an average of 150 measurements was reported. The porosity of the mats and the internal correlation of the porosities were studied using another image processing software (17). The mechanical properties of the mats were studied based on the ASTM D 638 standard. Samples with dimensions of 1*3 cm, and a thickness of 0.2 mm were tested using a tensile strength testing machine (Zwick/Roell Z050 model) with a load of 50 Newtons, the jaw speed 10 mm/min. From the obtained from the stress-strain diagrams, the tensile strength, elastic modulus, and elongation at the breaking point were measured and the average of the values were reported.

2.5. Statistical Studies

To analyze the obtained results, SPSS software (V20) is used and the results were reported in the form of mean ± standard deviation (Mean ± SD) and to check the significance of the differences, One-way analysis of variance (ANOVA) at the significant level of $p>0.05$ was used.

3. RESULTS AND DISCUSSION

3.1. Fiber Morphology

Results of the morphological studies (Figure 1) revealed that the mats consisted of homogeneous fibers with diameters ranging from 0.23 to 1.65 µm (random) and 0.34 to 0.58 µm (aligned) without any beads. The diameter of the fibers is in the range of the previous studies on PLGA-fibrin (14, 18) but is comparable to that of the pure fibrin fibers in another study (19).

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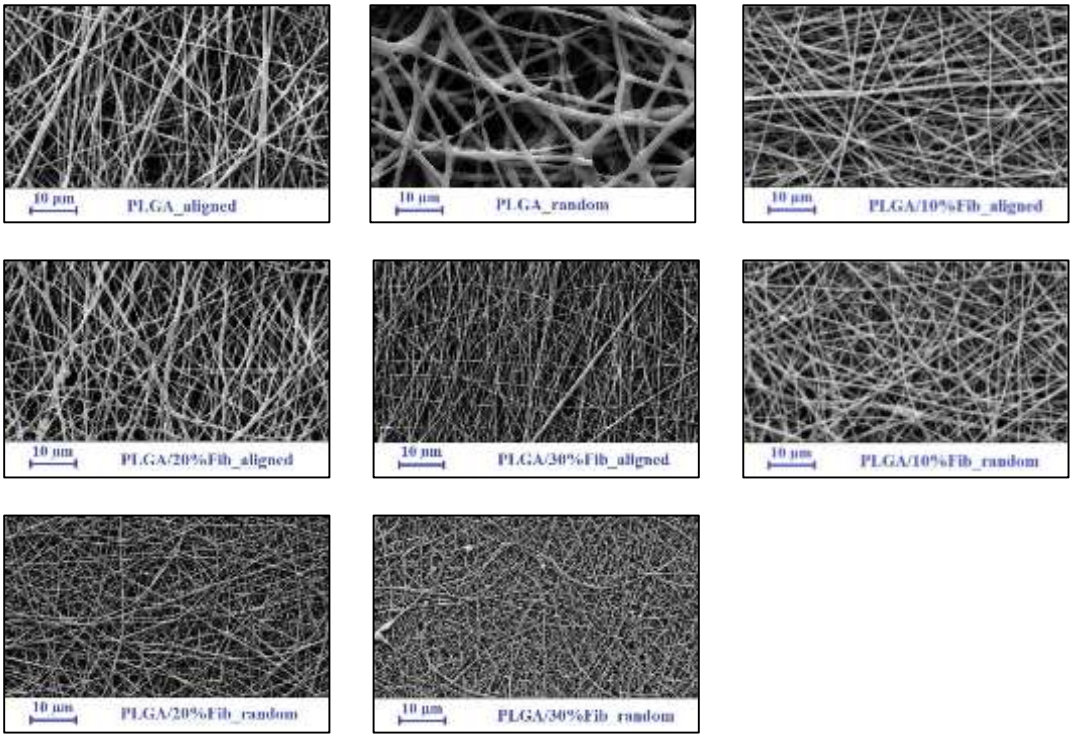

Figure 1: SEM micrograph images of aligned and random electrospun mats.

Figure 2 shows that in almost all cases, the diameter of the fibers in aligned nanofibrous mats was smaller than that of the random ones. This is because of the effect of rotation of the collecting mandrel on stretching the fibers of the aligned mats (20). The highest average fiber diameter owned by the PLGA_random mat (1.65 ± 0.67µm) and the lowest fiber diameter belongs to PLGA/30%Fib_random (0.23 ± 0.07 µm). By increasing the proportion of fibrin, aligned and random mats depicted a narrower distribution of the fiber diameter and thus more homogeneous fibers presented in mats which are contrary to the results of the study of Razavi et al. on PLGA/chitosan nanofibrous mats (21).

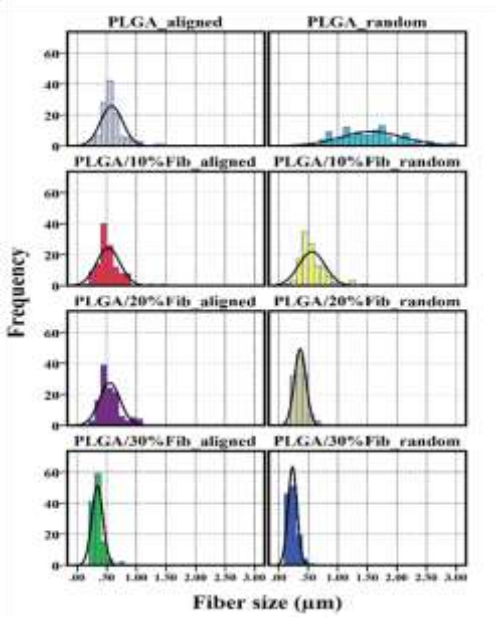

Figure 2: Diameter distribution diagrams of aligned and random electrospun nanofibrous mats.

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Figure 3 shows that with the addition of fibrin, the diameter of the fibers gradually decreased, and the results of the Scheffe follow-up test indicated that every 10% increase in fibrin proportion significantly reduced the diameter of fibers in random mats ($p<0.05$) while the diameter of the random mats did not change significantly.

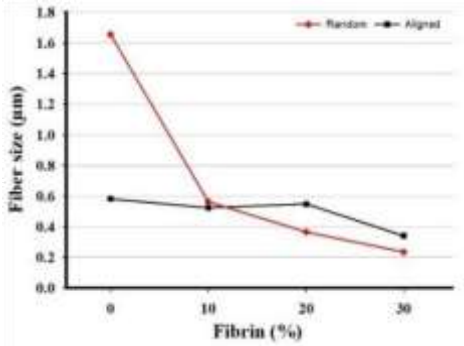

Figure 3: The influence of the changing percentage of fibrin on the diameter of the aligned and random fibers.

3.2. The Porosity of the Mats

Mats with more than 80% porosity ratios are considered ideal candidates for several biomedical and tissue engineering applications and they can facilitate oxygen, nutrition, and waste exchange and support cell growth and proliferation (22) and the size of porosities is also an important factor in cell growth and adhesion (23).

Graphs in Figure 4 showed that all mats exhibited suitable porosity (78.4 to 91.4%) and the internal correlation of their pores was between 25.1 to 33.8%. These porosity results are lower compared to some studies in the literature (24, 25) but they are higher compared to some other studies (21, 26).

Statistical analysis revealed that except in the case of random and aligned PLGA/10%Fibrin mats, the porosity of the random mats was higher than aligned mats and the differences between counterpart aligned and random mats were statistically significant in both two features ($p<0.05$).

According to Figure 4, by increasing the percentage of the fibrin, the porosity of the aligned mats slightly decreased while the porosity of the random mats fluctuated between 91.4 to 81.2%. On the other hand, by increasing the proportion of fibrin, the interconnectivity of the pores in aligned mats fluctuated between 25.1 to 33.8% while in random mats, by adding more than 10% fibrin, the interconnectivity of the pores gradually dropped. In a study on PCL/gelatin nanofibers, gelatin-containing fibers overlapped each other which led to enhancing the interaction of the fibers and reduced the porosity of the mats (27) or in another study, the addition of Hydroxyapatite (HA) in the form of nano-sized powder to the PLGA polymer solution led to a drastic reduction in the porosity of the mats (26).

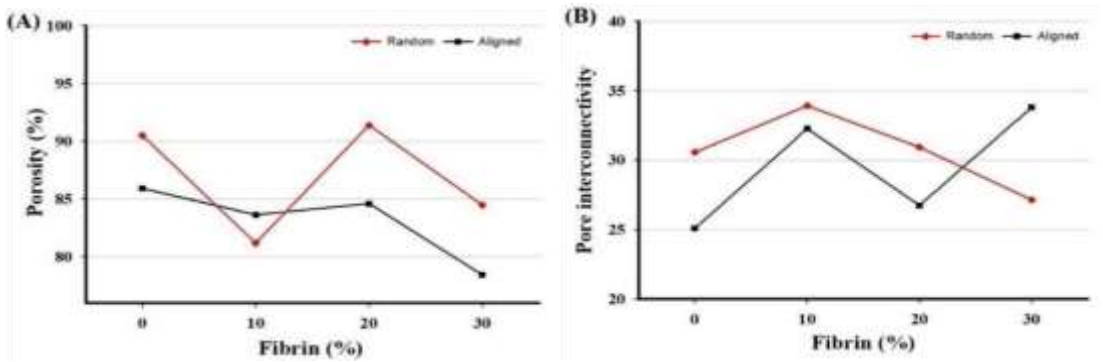

Figure 4: The influence of changing the percentage of fibrin on the porosity (A) and the internal connection of the porosities (B) in aligned and random mats.

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3.3. Mechanical Results

Based on the results of the mechanical studies (Table 1), aligned mats depicted stronger mechanical features than their random counterparts and this is in line with the results of the previous studies (21, 23).

The aligned pure PLGA mat exhibited the highest tensile strength and elastic modulus while the lowest tensile strength and elastic modulus belonged to the PLGA/20%Fib_random mat. The Young's modulus of all members of the aligned mat group was significantly higher than their counterpart random mats ($p<0.05$). Similarly, the tensile strength of the aligned mats was higher than random ones, and except for PLGA/10%Fib_aligned mat, the differences between the interconnectivity of the pores between mats were statistically significant. In addition, although fibrin-containing mats exhibited lower mechanical characteristics than pure mats, by increasing the proportion of the fibrin, we could not see a trend (constant decrease or increase trend) in the mechanical features of the mats in both groups.

Results of the mechanical studies indicated that the modulus of elasticity of the mats was in the range of most natural tissues such as kidney, liver, cardiovascular, cartilage and skin (28-31), which means that the studied mats are suitable candidates for various applications in the human body.

Table 1: Results of The Tensile Strength of The Mats.

Nomenclature	*Heading1 Elastic modulus (MPa)*	*Tensile strength (MPa)*	*PLGA/Fibrin ratio*	*(W/V) %*
PLGA_aligned	20.5 ± 2.1*	89.6 ± 8.1*	10:0	10
PLGA/10%Fib_aligned	6.4 ± 0.2*	39.8 ± 2.5	9:1	10
PLGA/20%Fib_aligned	10 ± 1.2*	86.7 ± 2*	8:2	10
PLGA/30%Fib_aligned	8.2 ± 1.5*	65.6 ± 9.6*	7:3	10
PLGA_random	6.5 ± 0.4	20 ± 0.9	10:0	10
PLGA/10%Fib_random	3.2 ± 0.8	23.1 ± 4.7	9:1	10
PLGA/20%Fib_random	2.1 ± 1	11.1 ± 1.4	8:2	10
PLGA/30%Fib_random	3 ± 0.6	16.9 ± 0.6	7:3	10

* The difference between the aligned and the random mats is significant at the level of 0.05.

Based on the results of this study, the diameter of the fibers (average and distribution) and the porosity and the interconnectivity of the pores in aligned and random mats were affected by the composition of the nanofibers. Figures 5 and 6 show the relationship between these three factors and the elastic modulus of the mats. Studying the effect of the diameter of the fibers on the elastic modulus of the mats (Figure 5) indicated that in general, by increasing the diameter of the fibers in both aligned and random mats, their elastic modulus went up. Random mats with fiber diameters in the range of 0.2 to 1.6 µm exhibited mechanical features compatible with most of the soft tissues up to cartilage and the aligned mats with fiber diameters between 0.4 to 0.6 µm depicted higher mechanical characteristics even up to the range of aortic valve.

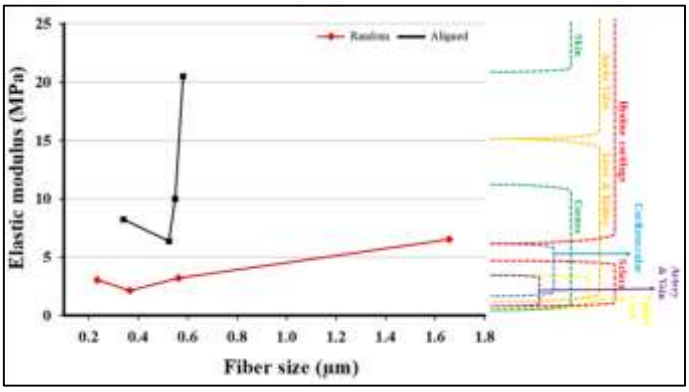

Figure 5: Change of the elastic modulus of aligned and random mats as a result of changing the diameter of the fibers.

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Results of the relationship between elastic modulus and the porosity and pore interconnectivity of the mats (Figure 6) indicated that by increasing the porosity and the pore interconnectivity of the random mats, their tensile strength did not change significantly while changing these two features had a significant influence on the elastic modulus of the aligned mats.

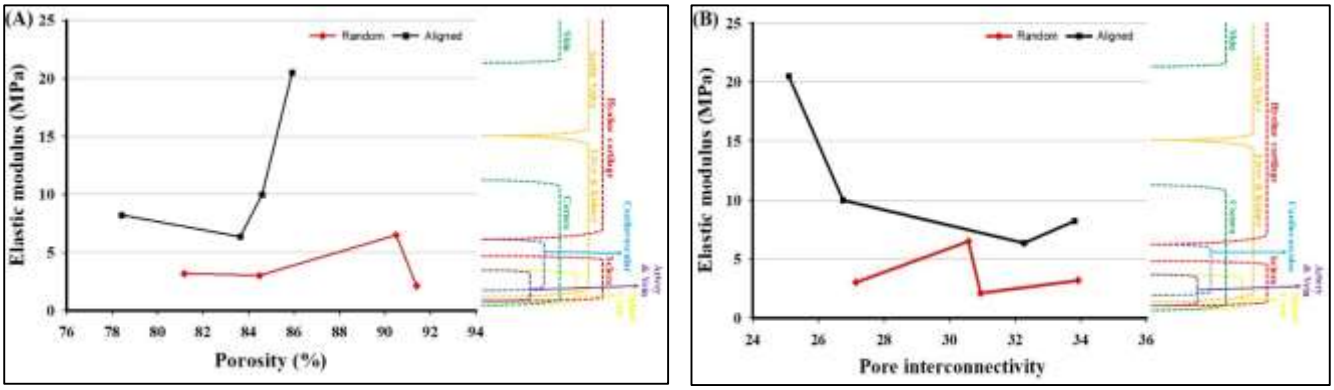

Figure 6: Change of the elastic modulus of aligned and random mats as a result of changing the porosity (A) and the interconnectivity of the pores (B).

In general, in random mats, fibrin decreased the diameter of the fibers and the porosity and the interconnectivity of the pores, but these two factors did not show a significant influence on the elasticity of the mats. However, in terms of aligned mats, fibrin lowered the diameter of the fibers and the porosity of the mats while the interconnectivity of the pores enhanced, and these two phenomenon decreased the tensile strength of the mats (18, 27). This is in line with the results of a study on the effect of the addition of gelatin and silica nanoparticles to PLGA on decreasing the porosity of the achieved mats (32) while in two other studies, adding chitosan and hydroxyapatite to PLGA increased the mechanical strength of this material (21, 26). Of course, the influence of the environmental factors such as the temperature and the humidity (33), the alignment ratio of the fibers (18), and the proportion of the transverse branches between the fibers, on the mechanical features of the mats should be also considered.

4. CONCLUSION

This study successfully produced aligned and random nanofibrous mats from PLGA and fibrin. The structural features, such as mat porosity and fiber diameter, were investigated for their effect on the mechanical properties of the mats. Aligned and random mats exhibited significant differences in fiber diameter. Addition of 10% fibrin decreased fiber diameter in both types of mats due to lower polymer solution viscosity, leading to limited diameter distribution. All mats showed high porosity, with fibrin reducing porosity in both aligned and random mats, as well as affecting pore correlation in random mats. Tensile strength and young modulus of the mats fell within the range of natural tissues, with aligned mats demonstrating higher tensile strength compared to random mats. In aligned mats, increasing fiber diameter significantly increased elastic modulus, while in random mats, it only had a slight effect on modulus of elasticity. Porosity and pore interconnectivity minimally influenced elastic modulus in random mats, but significantly impacted aligned mats—increasing with porosity and decreasing with interconnectivity. Overall, in random mats, increasing fibrin content affected fiber diameter and distribution but had limited impact on porosity, ultimately lowering mat stiffness. Conversely, in aligned mats, fibrin affected multiple factors—decreasing fiber diameter, porosity, and increasing pore interconnectivity—all contributing to decreased tensile strength. The consideration of environmental factors, fiber alignment ratio, and proportion of transverse branches between fibers is crucial when evaluating the mechanical properties of the mats. Further exploration in these areas would significantly enhance our comprehension of nanofibrous mat behavior and unlock its full potential for various applications in tissue engineering.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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