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Tuning Fiber Alignment to Achieve Mechanical Anisotropy on Polymeric Electrospun Scaffolds for Cardiovascular Tissue Engineering

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Abstract

Background: Soft tissues are characterized by strong mechanical anisotropy, as a result of internal fiber architecture, matching the needs of mechanical function in each body part. Polymeric grafts, used for diseased tissues replacement, suffer from mechanical mismatch with the tissues replaced and the remaining healthy tissues to be connected. Electrospinning is an attractive technique by which we can produce biodegradable polymeric scaffolds for tissue engineering applications. Fiber characteristics and structural architecture has to be tuned to match mechanically the tissues to be replaced. Furthermore, for the design of fibrous scaffolds, other characteristics, like fiber diameter, porosity and hydrophilicity play an important role as far as cell atraction, function and tissue regeneration are concerned.

Objective: In the present work, we aimed to produce polymeric membranous scaffolds with specific architecture, giving attention to fibers' orientation and hence, controlling the final mechanical behavior to match that of the physiological tissues to be replaced.

Methods: To this end, we used a specifically designed drum collector, with accurate velocity control, and tested different electrospinning parameters (polymeric solution concentrations, transfer rates, rotational speed, etc) to obtain design optimization.

Results: Scanning Electron Microscopy on scaffolds showed a good morphology quality. Fiber orientation was directly related to the drum speed. Tensile testing showed mechanical anisotropy in higher speeds. Young's modulus and Ultimate tensile strength demonstrated strong anisotropy (one order of magnitude larger) in parallel to transverse direction, with regard to drum speed, similar to that of physiologic soft cardiovascular tissues. Scaffold hydrophilicity, expressed by contact angle measurements remained high, although a relation to fiber architecture has been recorded.

Conclusion: Enhancement of membranous anisotropy was attained, one order of magnitude greater for the parallel fibers' direction compared to the transverse one. A similar anisotropy can be found in cardiovascular soft tissues, like human and porcine aortic heart valve leaflets.

Keywords: Electrospinning; Polymeric electrospun scaffolds; Fiber alignment; Rotating drum collector; Mechanical anisotropy; PVA membranes; Cardiovascular tissue engineering

Abbreviations: CDV: Cardiovascular Diseases; EU: European Union; TE: Tissue Engineering; PVA: Polyvinyl Alcohol; RPM: Rounds Per Minute; UTS: Ultimate Tensile Strength; SEM: Scanning Electron Microscopy; P: Parallel; T: Transverse

Introduction

Cardiovascular diseases (CDV) are the first cause of death in the industrialized world and within the first three worldwide. Five million deaths in USA [1] and more than 4 million in Europe (1.9 in EU) [2] annually are caused by CDV. More than 600 thousands vascular implants are used annually in USA for coronary and peripheral vascular surgical repair [1]. Due to limited availability of living materials for transplantation a lot of naturally originated and artificial implant materials have been designed and used in CDV surgery with a limited, however, long-term survival. Tissue engineering (TE) scaffold design is a promising technology for CDV surgical implants as tissue regeneration mechanisms are expected to substitute scaffold material with living functional tissue or organs.

Design and development of safely biodegradable polymeric materials for TE scaffolds, as well as tailoring their functionalities in biomedical applications is a great challenge nowadays [3]. Electrospinning has proved to be a versatile and promising technique to produce biomimetic and functional nanofibrous materials. The exceptional characteristics of electrospun fibers and membranes such as high surface to area ratio, high porosity, flexibility and adjustable pore size distribution make them ideal candidates in biomedical and tissue engineering applications [4-7].

In TE scaffolding, well-aligned micro-nanofibers are often required as they have shown their ability to modulate cell behaviors such as cell shape, migration, differentiation, and extracellular matrix assembly [8-13]. That justifies the increasing demand of oriented or aligned nanofibers over randomly oriented fibers, as alignment can expand existing applications of nanofibers and assist in the development of entirely new ones [14,15].

Use of rotating instead of static collectors (e.g., a mandrel, a wire drum, a wheel, a cone, or a frame) with controlled rotational velocity in an electrospinning setup is the most straightforward and simplest way to fabricate aligned fibers [16-19]. It has been found [20] that fiber

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alignment increased with increasing the drum speed up to a critical level (12.9 m/s). However, the threshold speed for fiber alignment varies from system to system as in some cases onset of fiber alignment was observed at around 3 m/s, whereas other systems require speeds approaching 10 m/s [21]. Additionally, the rotating speed of the collector can affect the diameter of the electrospun fibers, in a manner inversely proportional to the rotating speed [22].

Amongst polymers that have been successfully electrospun into nanofibers in recent years, polyvinyl alcohol (PVA) is a biodegradable, biocompatible and non-toxic polymer which is ideal for biomedical applications. PVA is a hydrophilic water-soluble synthetic polymer for electrospinning due to the presence of a hydroxyl group in its repeating unit, which makes it cross-linkable by means of its interconnected hydrogen bonding. These characteristics endow the PVA-spun fibers with excellent mechanical properties and chemical resistance. The hydroxyl groups can also help to incorporate biomolecules like collagen, hyaluronic and nucleic acids [23-26]. The combination of hydrophylic (so, cytocompatible) PVA with hydrophobic (less cytocompatible but mechanically strong and more resistant to degradation) polymers may result in a suitable polymer composite for scaffold design.

In the present study, we managed to produce membranous scaffolds with a specific nanofiber architecture, giving special attention to the orientation of the electrospun fibers and hence, controlling the mechanical performance to match that of the physiological tissues to be replaced. To this end, we utilized a low cost, Arduino-driven drum collector with accurate angular velocity control, specifically designed and constructed for our in-house electrospinning system. PVA electrospun membranous scaffolds were designed and produced. Optimization of the scaffold design was achieved by adjusting different electrospinning parameters (polymer concentration, applied voltage, needle to collector distance, drum collector velocity). Morphology and orientation of the fibers were examined by means of Scanning Electron Microscopy (SEM). Tensile testing of electrospun scaffolds as well as contact angle measurements was also performed to assess the mechanical performance and the hydrophilicity of the produced scaffolds.

Materials and Methods

Materials

Polyvinyl Alcohol (Mw 85,000-124,000, 87-89% hydrolyzed) in the form of crystals was provided by Sigma-Aldrich. PVA with high molecular weight is useful in preparing gel which possesses both high strength and modulus. Hence, it is used to produce fibers, films or gels. The specific hydrolysis grade (87-89%) was chosen, as in that range, optimum water solubility occurs.

Electrospinning processing

PVA water solution was prepared by dissolving 10% w/v PVA crystals in 2-distilled water at 90°C to a closed container under stirring for 2-3 hours. Afterwards the solution was left to be cooled at room temperature and stayed overnight under constant stirring. A 10 ml syringe with a plastic tube extension and a 20G (0.9 mm, inner diameter) stainless steel blunt needle was filled with the solution and driven by a syringe pump (New Era Pump Systems Inc. NE-1000), at a feeding rate of 0.5 ml/h. A high DC voltage of 29 kV (Spellman SL300 DC power supply unit) was applied between the metallic needle (+) and the metallic collector surface (earth) at 14 cm distance. A specifically designed and constructed Arduino-driven (Arduino Mega 2560 Rev3 with motor shield Rev3) rotating cylindrical drum with accurate angular velocity control and dimensions of 5 cm in length and 10 cm diameter was used as the earthed collector plate (Figure 1). Voltage and distance was chosen after preliminary experiments to produce thin sheets of fibrous membranes with a good quality of fiber surface morphology. Four different rotational speeds were considered: 500, 1000, 1500 and 2150 RPM which corresponds to the maximum angular velocity reached from our rotating drum system. To create randomly oriented fibers, the rotation speed was set at 500 RPM. Alignment of the fibers was clearly observed at rotation speeds over 1500 RPM.

Morphology

The morphological appearance of the electrospun fibers and their orientation were observed by Scanning Electron Microscopy, SEM (JEOL 6300, Laboratory of Electron Microscopy and Microanalysis (LEMM) of University of Patras, Greece). Rectangular specimens of the fiber mat samples were sputtered with gold prior to SEM observation. Based on these SEM images, the average diameter of the PVA electrospun mats was measured using the scientific software Image J (version 1.51n, National Institutes of Health, USA) and the average values were determined.

Mechanical Characterization

Mechanical Testing of the produced fiber mats was undertaken using a bench type miniature tensile tester (MiniMat 2000, Rheometric Scientific Inc.) equipped with a 200 Nt load cell, at a constant rate of 10 mm/min until breakage. To assess the anisotropy of the scaffolds rectangular specimens 20 × 5 mm were cut along the directional axis of the fibers (the direction of perimeter of the drum) and perpendicularly to fiber axis. Five (5) samples were tested for each case and the Young's modulus and Ultimate Tensile Stress at Fracture (UTS) were assessed.

Contact angle measurements

It is well known that PVA demonstrates a fully hydrophilic behavior in contact with water. To determine possible alterations associated with electrospinning procedure and fiber morphology and orientation, contact angle measurements (sessile drop method) were performed at room temperature in a contact angle meter (CAM 101, KSV instruments Ltd.) using ultrapure water as the test liquid. The contact angles were measured during a short time (at 20, 40, 60, 80 and

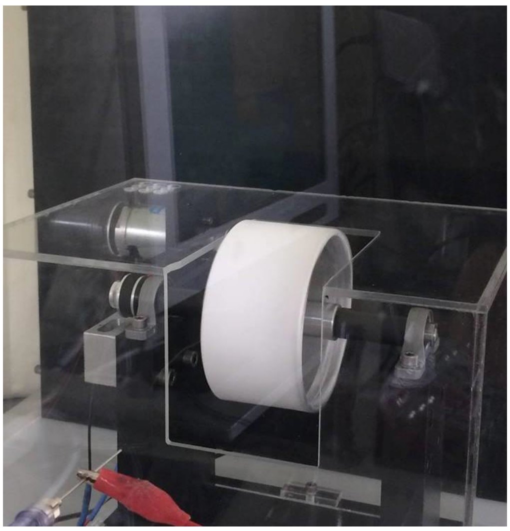

Figure 1: Rotating fiber collector setup.

100 ms) by depositing water droplets onto the sample surfaces. At least 3 measurements for each rotational speed category were performed in different sample locations and the average contact angle for each time was calculated.

Statistical analysis

Statistical comparison of the results obtained from the mechanical and the contact angle testing was performed by using analysis of variance (one-way ANOVA), followed by Tukey and Bonferonni post-hoc tests for the comparison of means from different groups, at the significance level of $p<0.05$. For the data analysis, the SPSS Statistical Software Package (Version 25.0. Armonk, NY: IBM Corp.) was used.

Results and Discussion

Electrospun scaffold morphology

The combination of the electrospinning parameters (DC voltage, feeding rate, needle to collector distance) was found to maintain a smooth electrospinning process, leading to smooth scaffold surface for all the drum velocities. After a period of three (3) hours, a membranous scaffold was produced covering the drum collector surface. Membrane thickness for all velocities was measured at different points using a high precision micrometer and found to be 0.07 mm (±0.01 mm).

SEM images at 1000x and 1700x (inserted frames) of the electrospun fibers for the velocities considered (500, 1000, 1500 and 2150 RPM) are shown in Figure 2a-2d. It can be observed that smooth, uniform, defect-free and round-shaped fibers were produced for all cases. For the lowest velocity of 500 and 1000 RPM the fibers exhibited a random orientation whereas for RPM 1500 and 2150 RPM images demonstrated a dominantly parallel alignment of the fibers, more organized and more uniform for the greater velocity. Furthermore, fibers presented a higher surface density (fibers/image surface area) for the velocities of 1500 and 2150 RPM. The Image J software package was used for the measurement of the average fiber diameter (Figure 3). The results showed that by increasing the velocity of the rotating collector we achieved a decrease in the average fiber diameter. It seems that the diameter of the electrospun fibers is inversely proportional to the rotating speed.

Mechanical properties of electrospun scaffolds

The tensile properties of the scaffolds were assessed, and the results are presented in Figure 4a and 4b for the different drum collector velocities considered. For each velocity, the Young's modulus and UTS were calculated parallel (P) (blue bar) and transversely (T) (red bar) to the rotation direction. Statistical analysis shows statistical differences of the means among the different groups.

Major differences in the mechanical testing results were found between P and T direction for all considered velocities, especially for 1500 (63% difference in Young's modulus and 79% in UTS values) and 2150 RPM (87% difference in Young's modulus and 79% in UTS values) where fiber alignment parallel to rotation is clearly observed. Scaffold anisotropy is clearly pronounced at higher speeds, been as a confirmation that successful electrospinning of a parallel fiber alignment has been achieved. Mechanical testing results for the drum velocity of 1000 RPM showed also significant differences in the Young's modulus (32% difference) and UTS (70% difference) values, although no parallel fiber alignment was shown at the surface inspection of the SEM images. This is evidence that parallel fiber alignment has also been achieved in bulk scaffold material for that specific speed of the drum collector. As for the 500 RPM, no significant differences were observed between the two directions considered as far as the Young's modulus is concerned, which confirms that random orientation of the fibers at this rotational speed is dominant. Strain at fracture ranged within 60-120% parallel and 20-80% perpendicular to rotation direction, which is in agreement with the results from similar studies [27]. A direct correlation between the drum collector speed and the strain at fracture is not obvious. This can be ascribed to variations of different mechanisms involved in the structural integrity of the fibers within the polymer membrane, gradually diminished (and possibly permanently micro damaged) at great membrane (and fiber) deformations, reaching nearly 100% of the unloaded length. Further investigation and analysis of such mechanisms is beyond the scope of this study.

When samples from parallel direction are compared, the ones corresponding to 1500 (Young's modulus: 254.26±38.27 MPa, UTS: 12.10±1.94 MPa) and 2150 RPM (Young's modulus: 278.66 MPa±44.18, UTS: 11.25±1.18 MPa) exhibited higher Young's modulus and UTS compared to those at 500 (Young's modulus: 147.27±22.62 MPa, UTS: 7.47±0.93 MPa) and 1000 RPM (Young's modulus: 208.46±41.98 MPa, UTS: 9.01±1.20 MPa). That agrees with results from other studies where aligned fiber mats were compared to randomly oriented ones [28-32].

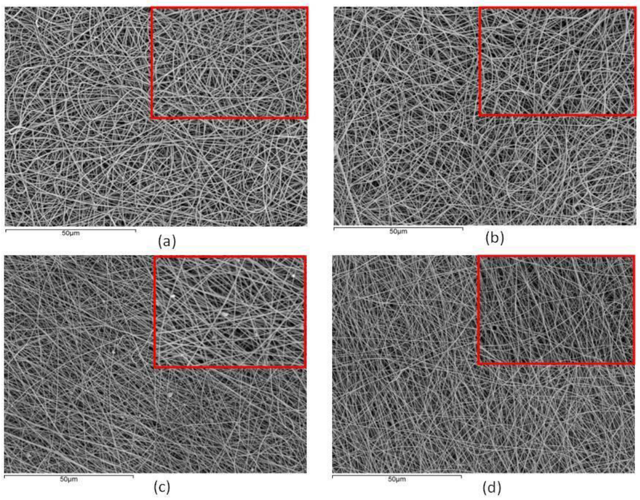

Figure 2: SEM images of electrospun scaffolds for (a) 500 (b) 1000 (c) 1500 and (d) 2150 RPM.

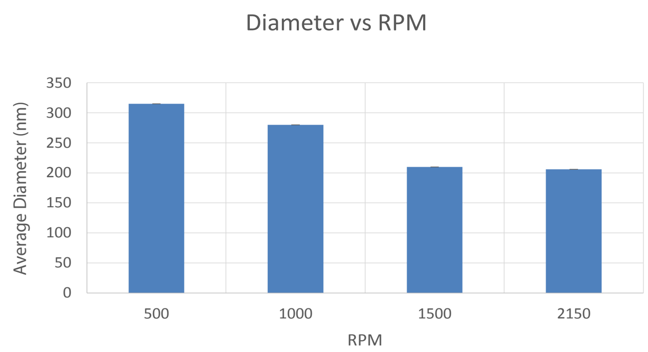

Figure 3: Average diameter of the electrospun scaffolds vs. drum rotation speed (mean ± SDEV, n=30).

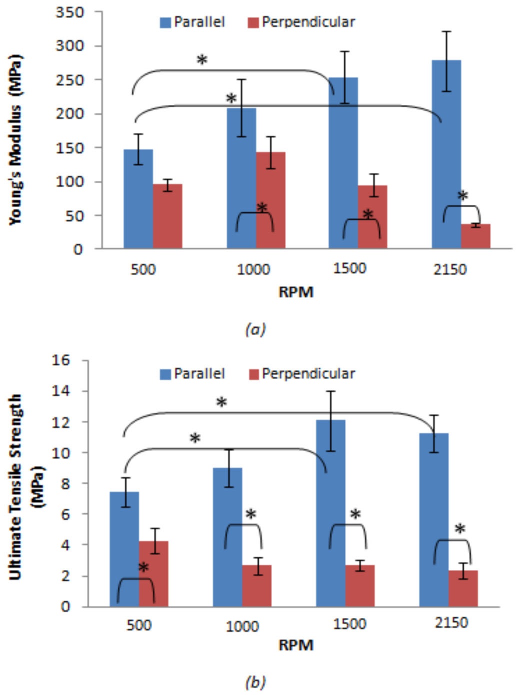

Figure 4: Tensile properties of the electrospun scaffolds a) Young's modulus, b) Ultimate Tensile Strength (mean ± SDEV, n=5), *p<0.05.

Regarding the transverse direction, the Young's modulus seems to decrease as the collector velocity increases (over 90% decrease between randomly oriented fibers at 500 RPM and strongly aligned fibers at 2150 RPM). That is something expected, as the dominant direction of the fibers, mainly contributing in the membrane elasticity, changed from random to parallel direction, as rotational speed increased. That is not the case, regarding the UTS differences. It must be noted however that Young's modulus is computed at the linear part of the stress-strain curves, far away from the very long strains at fracture where UTS is computed.

Contact angle measurements

Contact angle measurements for the different rotational speeds at discrete times are presented in Figure 5. Values started from around 90 degrees at time zero minimized very fast at 80 ms to avg 25 deg and zeroed at 100 ms, verifying that fiber arrangement didn't change the strong hydrophilic character of PVA. However small, but statistically significant differences were observed comparing contact angles for different groups at different time values. It seems that, as rotational speed increases (and fibers get aligned to a parallel direction), lowering of the contact angle is demonstrated. This is more evident in shorter times, with a tendency to disappear as time approaches 100 ms. That is not something uncommon as in a similar study of Areias et al. on PLLA fiber orientation, it was found that aligned fiber mats were more hydrophobic than randomly oriented ones [33]. That can be possibly ascribed to the smaller inter-fiber spacing leading to smaller void sizes and therefore smaller porosity. This hypothesis is also supported by the SEM images of the scaffolds in our study where we observed that for higher rotational speeds the fibers presented a higher membrane surface density compared to fibers collected at lower RPM.

Conclusions

In this study we aimed to produce electrospun PVA membranous scaffolds with aligned fiber orientation at different rotational speeds

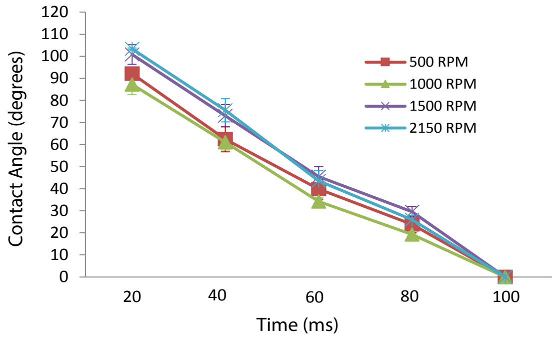

Figure 5: Contact angles of the electrospun scaffolds as a function of time (mean ± SDEV, n=3).

and hence, to control their mechanical performance in an approach to match the material topography and mechanics of physiological soft tissues to be replaced. To this end, we utilized a low cost, Arduino-driven drum collector with accurate angular velocity control, specifically designed and constructed for our in-house electrospinning system. The produced scaffolds presented a random fiber orientation at lower speeds while at higher speeds; fiber alignment was achieved with improved mechanical performance. Enhancement of membranous anisotropy was attained as a result, one order of magnitude greater for the parallel fibers' direction compared to the transverse ones. A similar anisotropy can be found in cardiovascular soft tissues, like human and porcine aortic heart valve leaflets and rabbit infrarenal abdominal aortic wall [34,35]. Scaffolds electrospun at the highest speed (2150 RPM) exhibited a more hydrophobic behavior compared to scaffolds produced at lower speeds. Further work on the design of an anisotropic hydrophobic/hydrophilic polymeric fibrous composite is in progress.

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