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Solvent-Dependent Morphology and Properties of Electrospun Poly(2-ethyl-2-oxazoline) Nanofibrous Scaffolds for Tissue Engineering

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ABSTRACT

Choosing an appropriate biomaterial is crucial when designing scaffolds intended for biomedical use. In particular, creating nanofibrous architectures by electrospinning requires careful selection of the polymer. Poly (2-ethyl-2-oxazoline) (PEOX) is one such candidate polymer suitable for electrospinning and can be utilized to fabricate scaffolds for medical purposes. PEOX dissolves readily in water, which is a significant advantage for biological systems, and it also exhibits solubility in various organic solvent mixtures. Based on this, the present work provides an initial evaluation of PEOX for scaffold fabrication through electrospinning and investigates its feasibility for future applications in tissue-engineering-related research. PEOX scaffolds were produced using both aqueous and organic solvent systems, and the influence of solvent type on morphology and physical behavior was assessed. Scanning Electron Microscopy revealed that fibres generated from an aqueous PEOX solution formed a consistent nanofibrous network, while those prepared with organic solvents resulted in micro-scale fibres. Wettability, determined using contact angle analysis, indicated higher hydrophilicity for PEOX (aq.) with a contact angle of 55.2°, compared with 70.38° for PEOX (org.). Mechanical characterization through Young's modulus demonstrated that PEOX (org.) achieved a tensile strength of 1.9 MPa, whereas PEOX (aq.) displayed 1.02 MPa. These observations demonstrate that solvent choice significantly impacts the electrospinning behavior of PEOX and the resulting scaffold characteristics. Overall, the fabricated PEOX scaffolds show promising features suitable for biomedical fields such as tissue engineering.

Keywords: Scaffolds, Tissue engineering, Electrospinning, Morphology, Wettability, Hydrophilicity

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Introduction

Electrospinning is widely recognized as a flexible technique for generating extremely thin polymeric fibres from solution. Scaffolds produced by this method typically contain fibres ranging from several hundred nanometers up to a few micrometers. Researchers continue to favor electrospinning due to its ability to process many different polymers and to yield nanofibers with distinct advantages, including large surface-to-volume ratios, substantial porosity, and strong structural integrity—attributes that are difficult to obtain from other fibre-forming approaches [1–3]. Furthermore, electrospun materials can be engineered to possess architectures that support cell adhesion, growth, and lineage-specific differentiation, closely resembling natural extracellular matrix environments [4, 5]. Numerous studies have shown that fibres produced via electrospinning serve effectively in a broad variety of biomedical contexts such as tissue regeneration, wound care, implant surfaces, biosensing, and drug release systems. In regenerative medicine, in particular, these scaffolds are frequently applied to the repair or reconstruction of tissues, including bone, cartilage, cardiac muscle, skeletal muscle, and nerve tissue [6–8].

The electrospinning process relies on electrostatic forces to stretch a polymer solution into nano- or micro-scaled filaments. A typical electrospinning setup includes a high-voltage source, a syringe operated by a pump, a grounded collector, and a polymer solution. Fibre characteristics are governed by several operation parameters—

such as applied voltage, the gap between the needle and collector, syringe diameter—as well as intrinsic properties of the polymer solution [7, 8]. To date, a wide spectrum of natural and synthetic polymers has been processed into nanofibers for biomedical use, including Polycaprolactone (PCL), Poly (lactic acid) (PLA), Polyvinyl alcohol (PVA), Poly (lactic-co-glycolic acid) (PLGA), poly (3-hydroxybutyrate) (PHB), chitosan, gelatin, sodium alginate, collagen, silk fibroin, cellulose, among others [7–11]. Of particular note, hydrogel-based electrospun scaffolds have shown especially beneficial characteristics for tissue engineering applications [5].

In this investigation, poly (2-ethyl-2-oxazoline) (PEOX) served as the base material for producing nanofibrous scaffolds through electrospinning. In recent years, polymers belonging to the poly (2-alkyl/aryl-2-oxazoline) (PAOX) family have received substantial attention for biomedical use [12, 13]. Research on poly(oxazolines) dates back to the 1960s, with extensive studies centered on the polymerization of 2-substituted oxazolines [14]. Applications of poly(2-oxazolines) have been documented in coating and adhesive formulations [15–17], pigment dispersion for ink systems [12], and as carriers in drug and gene delivery systems [13], as summarized by Nico Adams and U. S. Schubert [14]. Their relevance to biomedical science has grown notably over the past two decades [18]. These peptide-mimetic synthetic polymers represent a contemporary class of materials offering adjustable traits such as biocompatibility and potential biofunctionality suitable for medical technologies. PAOX polymers are exceptionally adaptable and maintain strong stability under physiological conditions due to their tertiary amide backbone [19, 20]. Their resemblance to native polypeptides contributes to stealth characteristics and excellent biocompatibility. Among them, poly(2-oxazolines) remain the most thoroughly explored PAOX species and exhibit hydrophilicity comparable to poly(ethylene glycol) (PEG) [21]. Owing to their biocompatibility, stealth attributes, non-ionic nature, aqueous and organic solubility, narrow dispersity, thermal and chemical stability, and broad functionalization capacity, these polymers have been widely applied [22]. Within tissue engineering, polyoxazolines stand out as hydrophilic, biocompatible substitutes for materials such as PEG, poly(vinyl pyrrolidone) (PVP), and poly(N-(2-hydroxypropyl) methacrylamide) (PHPMA) [16, 17, 23].

The central aim of the present study is to fabricate highly porous scaffolds from poly (2-ethyl-2-oxazoline) via electrospinning and to evaluate how the chosen solvent influences scaffold properties relevant to biomedical applications, specifically tissue engineering. Comparable work was reported by Buruaga *et al*. [24], who produced PEOX fibres from various solvents and modified spinning parameters to analyze resulting fibre morphology; however, they did not extend their work toward biological suitability. Hochleiter *et al*. [25] later examined processing parameters for PEOX in melt-electrospinning writing and observed fibre diameters ranging from 8–130 μm. Another study by B. Stubbe *et al*. [18] compared the electrospinning behavior of commercial Aquazol® with defined PEOX using aqueous media. Most recently, Wojciech Wałach *et al*. [26] generated non-woven fibrous layers and 3D structures from poly(2-isopropyl-2-oxazoline) and gradient copolymers of 2-isopropyl- and 2-n-propyl-2-oxazoline using electrospinning and melt extrusion. Their electrospinning solutions involved water and hexafluoro-2-propanol, and scaffold properties were assessed to determine how processing conditions shaped the final materials. Despite these contributions, a comprehensive evaluation of physicochemical and morphological characteristics of PEOX electrospun scaffolds prepared from different solvent systems for biomedical use—particularly for tissue engineering—has not been documented.

In the present work, we conducted an initial comparison of solvent-dependent behavior in PEOX electrospun scaffolds and assessed the appropriateness of both scaffold types for tissue-engineering contexts through multiple characterization methods. Prior literature demonstrates that many commonly electrospun polymers rely on organic solvents for solution preparation. For instance, electrospinning of PCL and PLA requires organic media, with PLA exhibiting only limited solubility in water. The toxicity associated with organic solvents may restrict the clinical suitability of such fibres. Additionally, their hydrophobic surfaces can hinder cellular adhesion and proliferation, further reducing their biomedical utility. In contrast, PEOX's solubility in both water and organic systems—especially its favorable solubility in water—makes it advantageous for biological applications. Its aqueous processability and favorable rheological behavior simplify electrospinning considerably. As such, PEOX stands out as an uncommon polymer accommodating both solvent classes. Accordingly, this study investigates how solvent choice impacts PEOX scaffold fabrication using electrospinning and compares the resulting physicochemical and morphological features to determine their relevance for tissue engineering.

Materials and Methods

PEOX (Mw = 50,000 Da) was obtained from Sigma Aldrich Chemie, GmbH, Germany. Tetrahydrofuran (THF) and dimethyl formamide (DMF), sourced from Sigma Aldrich, USA, served as the organic solvents. All reagents used were of analytical grade. Distilled water was employed in preparing the polymer solutions.

Preparation of PEOX solution

To evaluate how different solvents influence electrospinning behavior and scaffold characteristics, two solvent systems were selected: distilled water and a blend of organic solvents. A set of preliminary trials with various PEOX weight percentages was conducted to determine a suitable concentration for electrospinning. After optimizing both solution-related and process-related parameters, a concentration of 20% (w/w) PEOX was finalized. For the first formulation, an aqueous PEOX solution (20%) was prepared in distilled water. For the second, a 20% PEOX solution was made using a THF–DMF mixture in an 8:2 ratio. Each solution was continuously stirred for 4 hrs at room temperature until homogeneity was achieved. This solvent ratio for the organic system was standardized after optimization. Both solutions were filtered, followed by sonication, before being used for electrospinning.

Development of scaffolds by electrospinning

Numerous considerations affect polymer electrospinning—solution viscosity, processing conditions, and fibre structure are particularly relevant in tissue-engineering applications. In this work, porous nanofibrous PEOX scaffolds were fabricated using the prepared solutions. Process parameters were fine-tuned for each type of solution to ensure uniform fibres. A horizontal syringe pump equipped with a metallic needle of 0.55 mm inner diameter delivered the polymer solutions. A rotating drum covered with aluminium foil functioned as the fibre collector. A schematic overview of the electrospinning arrangement is provided in **Figure 1**. Other operational parameters used during electrospinning are summarized in **Table 1**. After spinning, the resulting nanofibres were removed carefully and stored in a vacuum chamber until characterization. A complete process flowchart for PEOX scaffold preparation is shown in **Figure 2**.

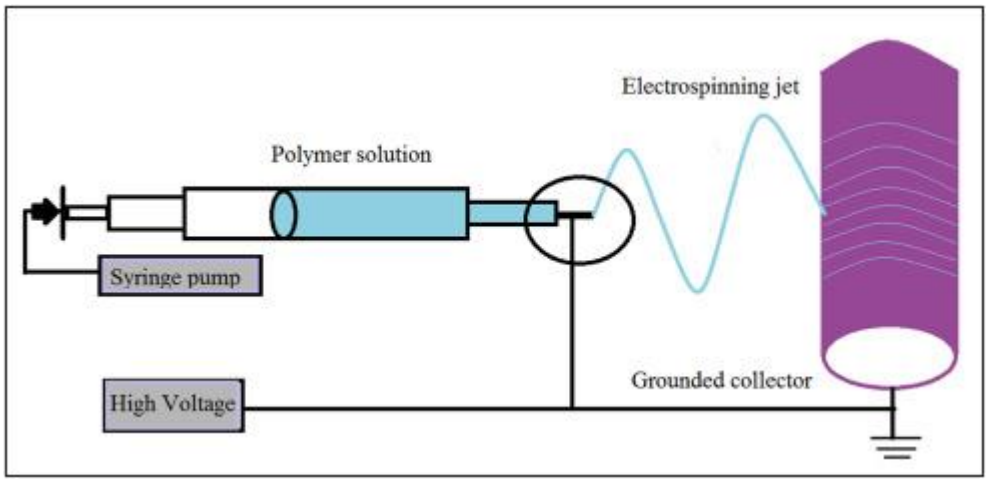


Figure 1. Schematic illustration of the electrospinning set-up.

Table 1. Parameters of electrospinning.

Sl. No.	Electrospinning Parameter	PEOX (Aqueous Solution)	PEOX (Organic Solution)
1	Flow rate	3 µL/s	3 µL/s
2	Tip-to-collector distance	8 cm	8 cm
3	Collector rotation speed	800 rpm	800 rpm
4	Applied voltage	14 kV	18.5 kV
5	Needle inner diameter	13.08 mm	13.08 mm
6	Chamber temperature	29 °C	30 °C
7	Relative humidity in chamber	51%	66%

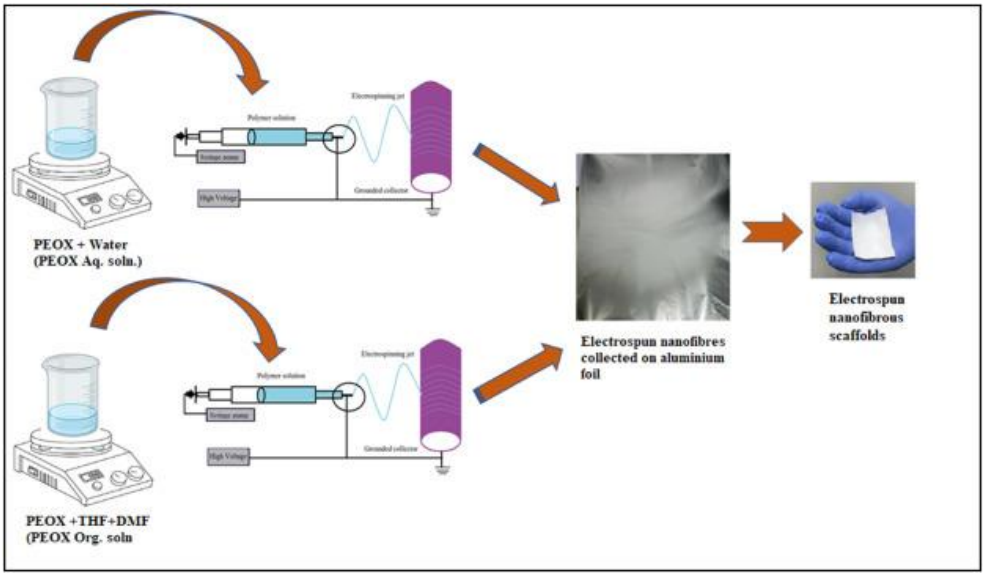


Figure 2. Illustration of the development of PEOX (aq.) and PEOX (org.) electrospun scaffolds.

Characterization

Fourier transform infrared spectroscopy

Bonding features in PEOX (aq.) and PEOX (org.) nanofibrous scaffolds were analyzed by FTIR (Nicolet, Impact-410 USA). Fibres were detached from aluminium foil and finely mixed with KBr to form pellets. Spectra were recorded in the 400–4000 cm^{-1} region.

X-ray diffraction spectroscopy

XRD patterns were recorded at room temperature (RT) using a powder diffractometer (RIGAKU Smartlab, Japan). Ni-filtered Cu–Kα radiation (40 kV, 30 mA) served as the source. Samples were mounted on a holder, and scans were taken in reflection mode over $2\theta = 10°–80°$ at 5°/min.

Scanning electron microscopy

Morphological examination was carried out using SEM (JSM-IT500 JEOL, USA). Prior to imaging, each nanofibrous specimen was coated with a thin gold layer using a Neocoater for approximately 60 s at 10 mA and 10^{-6} mbar. The sputter-coated sections were then analyzed under the electron microscope.

Contact angle measurement

Wettability of the scaffolds was assessed using a static contact angle goniometer (Kyowa Interface Science Co. Ltd., Japan). The sessile drop technique was used at room temperature. Water droplets were placed on the scaffold surface, and the incident angle was measured using FAMAS software. For each of the PEOX (aq.) and PEOX (org.) samples, 10 measurements were collected and averaged.

Mechanical testing

Mechanical behavior was evaluated at ambient temperature with a Universal Tensile Testing Machine (UTM) (DakSystem Inc., Mumbai, India). Rectangular strips (2 cm × 10 cm) were cut from each scaffold. Thickness was determined using a thickness gauge. Samples were clamped in the UTM grips and pulled at 0.1 mm/min under a 1 kN load. Stress–strain curves were produced for all samples, and mean Young's modulus values were calculated.

Results and Discussion

FTIR spectroscopy

As noted earlier, PEOX dissolves readily in both aqueous and organic media; therefore, two scaffold variants were produced—one from water and the other from an organic solvent mixture. Comparable nanofibrous layers were generated in each case via electrospinning. To determine whether the solvent system introduced any detectable

differences in the chemical bonding of PEOX, FTIR examination was performed. The spectra for PEOX (org.) and PEOX (aq.) scaffolds are displayed in **Figure 3**. As anticipated, both samples exhibited nearly identical absorption features independent of the solvent. A strong band at 3436 cm^{-1} appeared in each scaffold, corresponding to O–H stretching. Absorptions at 1631 cm^{-1} and 2929 cm^{-1} were assigned to amide C=O stretching and asymmetric CH_2 stretching, respectively. Additional peaks at 1442 cm^{-1} and 1035 cm^{-1} were attributed to CH_3 stretching and C–N bending.

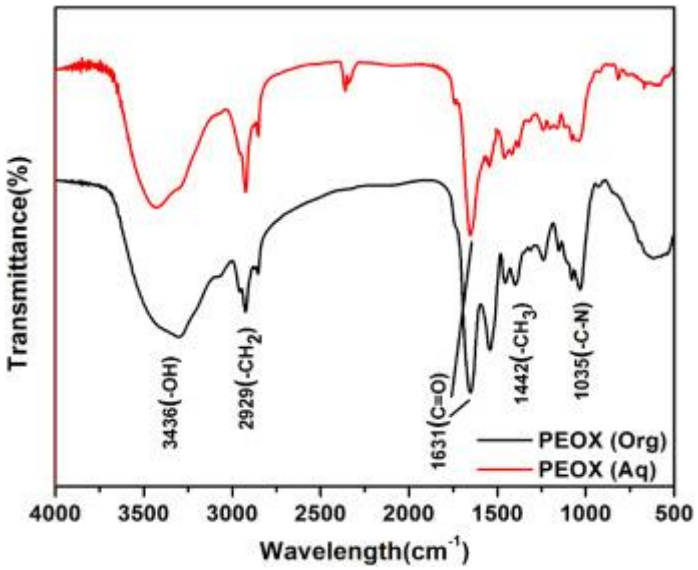


Figure 3. FTIR spectra of nanofibrous scaffolds.

X-ray diffraction spectroscopy

XRD was employed to determine whether the solvent choice influenced the crystalline arrangement of the PEOX (aq.) and PEOX (org.) nanofibres. The resulting diffractograms, presented in **Figure 4**, feature two well-defined reflections at 2θ = 14.2° and 17.25° for both scaffold types, implying that the fibres possess a crystalline component. Although PEOX is typically described as amorphous, the distinct peaks suggest that crystallinity was induced during electrospinning under high-voltage conditions.

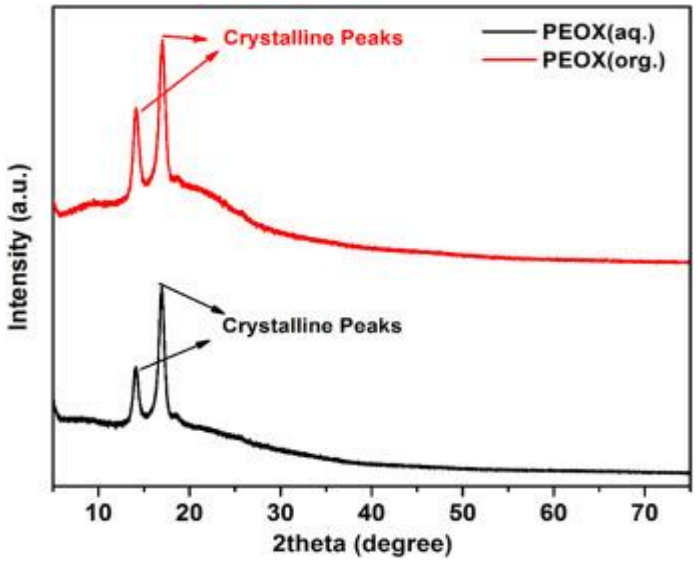


Figure 4. XRD spectra of nanofibrous scaffolds.

Scanning electron microscopy

Pore architecture and porosity are essential considerations for tissue-engineered scaffolds, since they directly affect cell adhesion, migration, infiltration, as well as processes such as vascular formation and bone integration.

Highly interconnected pores support nutrient transport and guide tissue ingrowth, while porosity also influences scaffold mechanics and degradation. SEM micrographs of PEOX fibres produced from the two solvent types are presented in **Figure 5**. Solvent selection had a pronounced impact on fibre morphology: PEOX (aq.) scaffolds displayed areas of fibre clustering and limited bead formation, whereas PEOX (org.) scaffolds produced smooth, homogeneous, bead-free fibres without visible agglomeration.

Figure 6 illustrates the histogram analysis of fibre diameter using ImageJ. The aqueous PEOX scaffolds yielded fibre diameters within 200–600 nm, while the organic-solvent fibres ranged from 8–14 µm. The transition from nanoscale to microscale diameters in the organic system may be attributed to the higher viscosity of the THF–DMF solution.

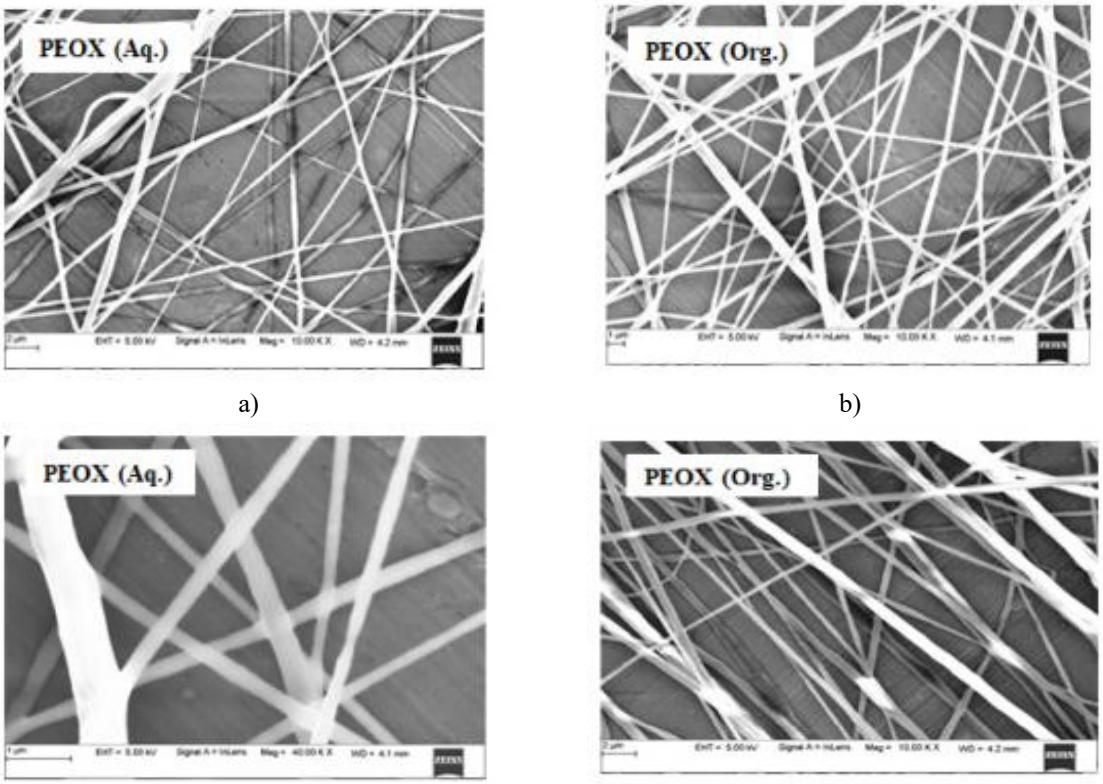


Figure 5. SEM images of nanofibrous scaffolds.

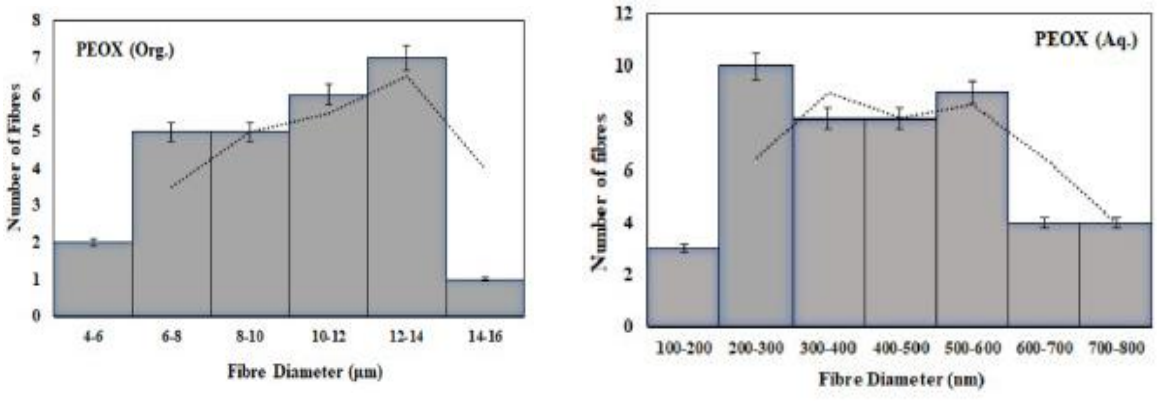


Figure 6. Histogram analysis of nanofibres.

Contact angle measurement

The wettability of the scaffolds is governed by their porosity and interconnected fibrous structure. Hydrophilic surfaces generally promote improved cellular adhesion and proliferation. The contact angle values, obtained using

the sessile drop technique and shown in **Figure 7**, indicate that both scaffold types possessed contact angles below 90°, confirming their hydrophilic character. PEOX (aq.) scaffolds exhibited a greatly reduced contact angle of 55.2°, whereas PEOX (org.) scaffolds showed a comparatively higher value of 70.38°. The pronounced hydrophilicity of the aqueous scaffold is linked to the use of water as the solvent and its nanoscale fibre morphology. These findings suggest that the produced scaffolds offer conditions favorable for cellular attachment and propagation in tissue engineering contexts.

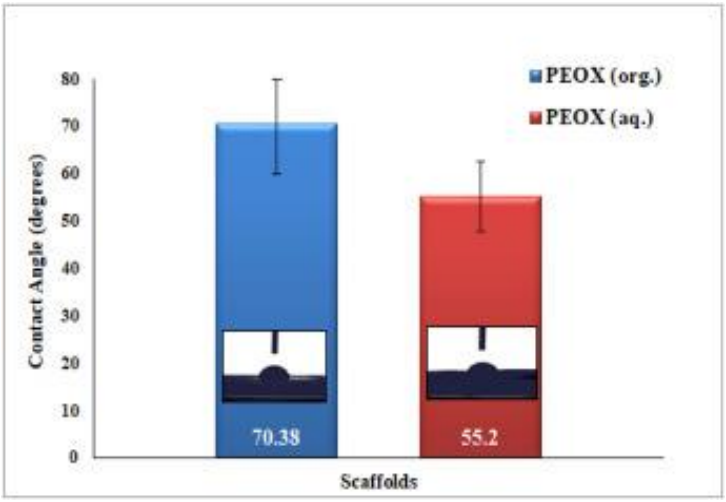
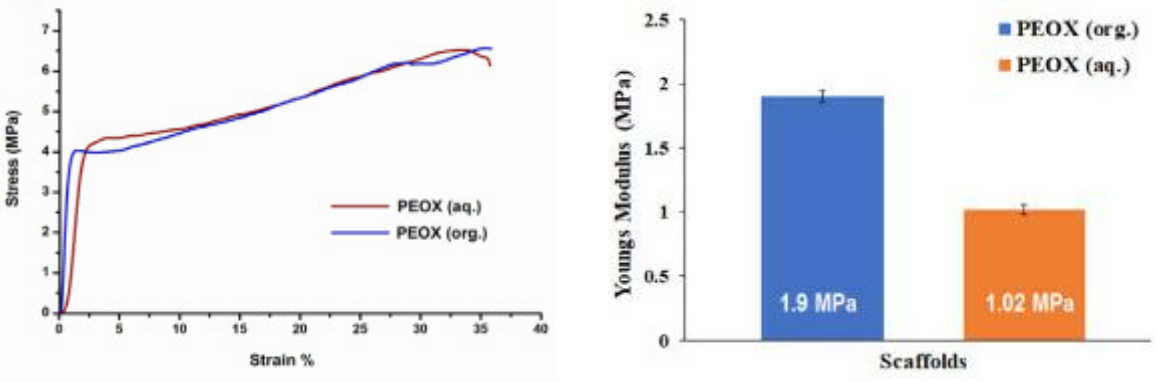


Figure 7. Contact angle measurements of scaffolds.

Mechanical testing

The mechanical performance of polymer scaffolds is crucial, as it provides support for cellular activities and tissue formation. Depending on the characteristics of the defect site, the scaffold's mechanical behavior must be appropriately adjusted. The averaged stress–strain profiles of the samples are illustrated in **Figure 8a**. These plots display an initial elastic zone, followed by a nearly constant-stress plastic region, and finally a segment where the stress rises sharply. From these graphs, it became clear that the PEOX (org.) construct exhibited greater stress values than the PEOX (aq.) counterpart. Young's modulus values were calculated from the linear portions of the curves, and the overall mechanical performance was evaluated using a Universal Testing Machine. **Figure 8b** presents the Young's modulus values for both scaffolds. The modulus for PEOX (org.) was measured at 1.9 MPa, while that of PEOX (aq.) was 1.02 MPa. The data show that the solvent used during fabrication significantly impacted the mechanical outcome. Incorporating an organic solvent enhanced mechanical strength, whereas employing water resulted in slightly diminished mechanical properties. This difference is attributed to the microfiber structure of PEOX (org.) scaffolds, which confers higher strength compared to the nanofibrous architecture of PEOX (aq.) scaffolds.



a) b)

Figure 8. Mechanical characteristics of the nanofibrous scaffolds: (a) Stress–strain curves, (b) Young's modulus values.

Conclusion

This work employed electrospinning to create PEOX-based nanofibrous scaffolds. For the first time, the influence of solvent choice—water versus an organic solvent mixture—was systematically examined for PEOX electrospinning, and the resultant scaffolds were evaluated for biomedical applicability. Electrospinning conditions were altered and optimized to generate uniform fibers with both solvent systems. It was found that electrospinning PEOX with an organic solvent required a noticeably higher applied voltage compared with the aqueous formulation. To assess how the solvent affected the scaffold characteristics, several physicochemical properties were analyzed. SEM observations indicated that both types of scaffolds displayed porous, interconnected structures composed of smooth, bead-free fibers. A clear impact of solvent selection was evident in the measured fiber diameters: water-based processing produced fibers within the nanometer scale, while the organic solvent yielded micro-scale fibers. This variation influenced wettability, with PEOX (aq.) samples showing much stronger hydrophilic behavior than PEOX (org.) samples. These findings suggest that solvent choice strongly governs scaffold hydrophilicity. Mechanical testing likewise showed solvent-related differences, with PEOX (org.) achieving a higher Young's modulus of 1.9 MPa, compared to 1.02 MPa for PEOX (aq.), based on the collected stress–strain data. Overall, the morphology and physical attributes of the scaffolds were significantly shaped by the type of solvent used during electrospinning.

An initial biodegradation assessment was attempted using phosphate-buffered saline (PBS). While the study could be completed for PEOX (org.), the PEOX (aq.) scaffolds disintegrated in the PBS medium, preventing data collection; therefore, biodegradation results are not included. Taken together, the findings indicate that PEOX scaffolds fabricated with organic solvents exhibited favorable characteristics for tissue engineering, making them a more suitable choice for future applications. Consequently, upcoming work will focus on developing electrospun PEOX scaffolds using organic solvents for tissue engineering purposes.

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Conflict of Interest: None

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Ethics Statement: None

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