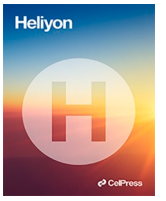
Heliyon 11 (2025) e41259

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Effect of solvent on the development of Poly(2-ethyl-2-oxazoline) nanofibrous scaffolds using electrospinning technique for biomedical applications

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ARTICLE INFO

Keywords:
Scaffolds
Tissue engineering
Electrospinning
Morphology
Wettability
Hydrophilicity

ABSTRACT

The selection of a biomaterial plays a very important role for the development of scaffolds for biomedical applications. Amidst, the development of nanofibrous scaffolds through electrospinning technique by selecting a suitable polymer is of more importance. Poly (2-ethyl-2-oxazoline) (PEOX) is one among the selected polymers that can be employed for electrospinning for the development of scaffolds for biomedical applications. PEOX is a water-soluble polymer which is highly desirable for biomedical applications. At the same time, PEOX is soluble in the mixture of organic solvents as well. In view of this, the present study is the preliminary study of using PEOX for the development of scaffolds by using electrospinning technique and to check its potentiality for biomedical application like tissue engineering for the future research. The PEOX scaffolds were fabricated using electrospinning process using water and organic solvents, and the effect of solvent was studied on the morphology and physical properties of the developed scaffolds. The Scanning Electron Microscopic results of the scaffolds showed a uniform nanofibrous structure in case of aqueous PEOX solution, whereas microfibrous structure was obtained for organic solvent. Wettability of the scaffolds was observed by contact angle measurement, which revealed that the hydrophilicity of the PEOX (aq.) scaffold was higher with the contact angle of 55.2° as compared to PEOX (org.) scaffold with the contact angle of 70.38°. Further, the mechanical strength of the scaffolds was calculated in terms of Young's modulus values and it was observed that the PEOX (org.) demonstrated a higher tensile strength of 1.9 MPa compared to PEOX (aq.) scaffold with 1.02 MPa respectively. The results thus clearly conclude that the nature of solvents greatly affect the electrospinning process of PEOX and thereby the properties of the developed PEOX scaffolds based on the solvent. Further, we can say that the developed PEOX scaffolds possess suitable properties to be employed for biomedical applications like tissue engineering.

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https://doi.org/10.1016/j.heliyon.2024.e41259
Received 14 July 2024; Received in revised form 9 December 2024; Accepted 13 December 2024
Available online 14 December 2024

1. Introduction

Electrospinning is one of the well-known versatile techniques to produce extremely fine diameter fibres using the polymer solution. The polymeric scaffolds so developed from the electrospinning technique possess fibres in the range of hundreds of nanometers to several micrometers. The technique of electrospinning is in high demand by the researchers for its versatility in spinning different types of polymers and producing unique nanofiber features such as high surface area to volume ratio, high porosity, and good structural stability, which on the other side is difficult to obtain by any other fiber-spinning methods [1–3]. In addition, the electrospinning method is an effective strategy to produce nanofibrous scaffolds with suitable structure and morphology to facilitate cellular proliferation and differentiation to mimic the natural extracellular matrix [4,5]. The polymeric fibres developed from electrospinning techniques were proved to be the promising candidates for various biomedical applications including tissue engineering, wound dressing, medical implants, biosensors and drug delivery. Specifically, the scaffolds developed from electrospinning are widely used in reconstructive medicine, for repair and regeneration of tissues like, bone, cartilage, heart, muscle, nerve etc. [6–8].

The process of electrospinning technique depends on the use electrostatic forces to produce nano and micro fibers. The simple setup of electrsopinning requires a high-voltage power supply, a syringe pump, a grounded collector surface and a polymer solution. The morphology of the resulting fibres depends on various parameters like, voltage applied, distance between needle and collector, diameter of the syringe, and the properties of polymeric solution [7,8]. A large number of natural and synthetic polymers have been used so far for the development of nanofibers using electrospinning technique for biomedical applications. These include, Polyaprolactone (PCL), Poly (lactic acid) (PLA), Polyvinyal alcohol (PVA), Poly (lactic-co-glycolic) acid (PLGA), poly (3-hydroxybutyrate) (PHB), chitosan, gelatin, sodium alginate, collagen, silk fibroin, cellulose etc. [7–11]. Among these, electrospining of hydrogels were proved to have more ideal scaffold structure for tissue engineering application [5].

In the present study, poly (2-ethyl-2-oxazoline) (PEOX) was used to develop nanofibrous scaffolds using electrospinning. Poly (2-alkyl/aryl-2-oxazoline)-based polymers (PAOX) have drawn a lot of interest in a very recent years, for use in biological applications [12,13]. Poly (oxazolines) have been the subject of a considerable amount of research since 1960's with a significant number of papers focusing on the polymerisation of 2-substituted oxazolines [14]. The use of poly (2-oxazolines) was also documented in adhesive and coating formulations [15–17], as pigment dispersants in inks [12], and in drug and gene delivery applications [13] as reviewed by Nico Adams and U S Schubert [14]. More specifically, the use of poly (oxazolines) for biomedical applications has been prospered since last two decades [18]. Thus, these peptide-based synthetic polymers are one of the examples of current trend that would display tuneable properties, like, biocompatibility and potential bio-functionalities for various biomedical applications. PAOX are highly versatile and they possess high stability under biological conditions owing to their tertiary amide backbone [19,20]. The structural similarity of polyoxazolines with natural polypeptides accounts for their stealth behaviour and excellent biocompatibility. Amidst, poly (2-oxazolines) is the most investigated PAOX derivatives with similar hydrophilicity to poly (ethylene glycol) (PEG) [21]. The biocompatibility, stealth behaviour, non-ionic character, stability, high functionalization possibilities, low dispersity and solubility in water and organic solvents thus find many applications of this polymer [22]. Specifically, for tissue engineering applications, polyoxazolines have proven to be very competitive as hydrophilic and biocompatible substitutes for numerous polymers, such as PEG, poly (vinyl pyrrolidone) (PVP), and poly (N-(2-hydroxypropyl) methacrylamide) (PHPMA) [16,17,23].

The major goal of the present work is to develop highly porous scaffolds using poly (2-ethyl-2-oxazoline), by electrospinning technique and check the properties of the developed scaffolds based on the solvent used for its potentiality for biomedical applications-tissue engineering in particular. A similar research was carried out by Buruaga et al. [24] on the electrospinning of PEOX, wherein they used PEOX with different solvents and varied the electrospinning process parameters to examine the morphology of the electrospun fibers. But no further study was done to evaluate the properties of electrospun fibres for biomedical applications. Next, Hochleiter et al. [25] performed a systematic study on the influence of the processing parameters on fibre formation of PEOX using melt electrospinning writing, wherein the resultant fibres were found to have a large diameters of 8–130 μm. The study of electrospinning of PEOX was also carried out by B. Stubbe et al. [18] wherein a detailed comparison of the electrospinnability of the commercially available Aquazol® and defined PEOX was studied using aqueous solutions. Further, in a recent study by Wojciech Wałach et al. [26] non-woven fibrous mats and three-dimensional moulds were obtained by the processing of poly (2-isopropyl-2-oxazoline) and gradient copolymers of 2-isopropyl- with 2-n-propyl-2-oxazoline using electrospinning and melt extrusion methods. The polymer solution for electrospinning was prepared using water and hexafluoro-2-propanol, and the properties of the scaffolds were determined to evaluate the influence of the processing conditions on the structure and properties of the final material. But the detailed study of physicochemical and morphological properties of PEOX scaffolds developed from electrospinning technique based on different solvents for biomedical applications is not been reported, particularly for tissue engineering.

In the present research work, a preliminary study is carried out by comparing the effect of solvents on the properties of PEOX electrospun scaffolds and check the suitability of both the scaffolds for tissue engineering application by various characterizations. Literature gives the evidence of a large number of electrospun polymeric scaffolds produced using organic solvents during the preparation of polymer solution. For example, if we consider PCL and PLA scaffolds by elecrospinning, both the polymers are soluble in organic solvents, though PLA have limited solubility in water. Here the toxicity of the organic solvents may inhibit the use of fibres for biomedical applications. Further, they are hydrophobic in nature, which may also inhibit the cell growth and cell-proliferation which further limit their use in biomedical applications. On the other hand, PEOX being soluble in wide range of solvents, its water solubility makes it appealing for biomedical applications. In addition, electrospinning is relatively straightforward in case of PEOX due to its water solubility and good rheological properties. Thus, PEOX is one of such unusual polymers that is soluble in water as well as in organic solvents. Hence, in the present study, the effect of solvent was studied for the fabrication of scaffolds by electrospinning method. Further, the effect of solvent was compared with their physiochemical properties and morphological properties of the

developed scaffolds in order to check their suitability for tissue engineering application.

2. Materials

PEOX (Mw = 50,000 Da) was purchased from Sigma Aldrich Chemie, GmbH Germany. Tetrahydrofuran (THF) and Dimethyl formamide (DMF) procured from Sigma Aldrich, USA were employed as solvents. Reagent grade chemicals were used during the experimentation. Distilled water was used during the preparation of the solution.

3. Preparation of PEOX solution

In order to study the effect of solvent on the electrospinning process and on the properties of the developed scaffolds, two kinds of solvents were used, i.e., water and a mixture of organic solvents. A series of preliminary experiments were conducted with different weight % of PEOX solution to optimize the concentration of polymer solution for electrospinning. Particularly, the solution parameters and the process parameters were optimized before setting the final parameters for electrospinning. Finally, 20 % by weight of PEOX was set to prepare the solution. In brief, as a first sample, 20 % PEOX aqueous solution was prepared using distilled water as a solvent. Similarly, 20 % PEOX organic solution was prepared using a mixture of THF and DMF in the ratio 8:2 as a second sample. The solution was stirred for 4hrs at room temperature in order to obtain a homogeneous solution. This ratio of the organic solvent mixture was optimized and kept constant. The resulting solutions were filtered and sonicated to be used further for electrospinning process.

4. Development of scaffolds by electrospinning

Several aspects come into play while studying the electrospinning of any polymer, like solution properties, process parameters, and fiber morphology for tissue engineering. In the present study, the porous polymeric nanofibrous scaffolds were fabricated by electrospinning technique using the prepared solutions. The process parameters were varied and optimized for the two polymeric solutions to obtain uniform fibres. In brief, a horizontally fixed syringe pump was used to fill the polymer solution, which was having a metallic needle of 0.55 mm as inner diameter. The rotating drum was used as a collector wrapped with the aluminium foil to collect the electrospun fibres. The schematic illustration of electrospinning set-up is shown in Fig. 1.

The other parameters employed during the elctrospinning process are described in Table 1. The nanofibres were then peeled out and stored in vacuum chamber until further use for the characterizations.

The complete steps involved in the preparation of PEOX scaffolds are schematically presented in Fig. 2.

5. Characterization

5.1. Fourier transform infrared spectroscopy

The nature of bonding in the PEOX (aq.) and PEOX (org.) composite nanofibrous scaffolds was studied using Fourier transform infrared spectroscopy (FTIR) (Nicolet, Impact-410USA). The nanofibres were peeled off from the aluminium foil and grinded thoroughly with KBr for pellet preparation. The IR data of the pellets were then analysed using FTIR spectrometer operating at a range of 400–4000 cm^{-1}.

5.2. X-ray diffraction spectroscopy

The X-ray diffraction study was carried out at RT using a powder X-ray diffractometer (RIGAKU Smartlab, Japan). Ni-filtered Cu-K

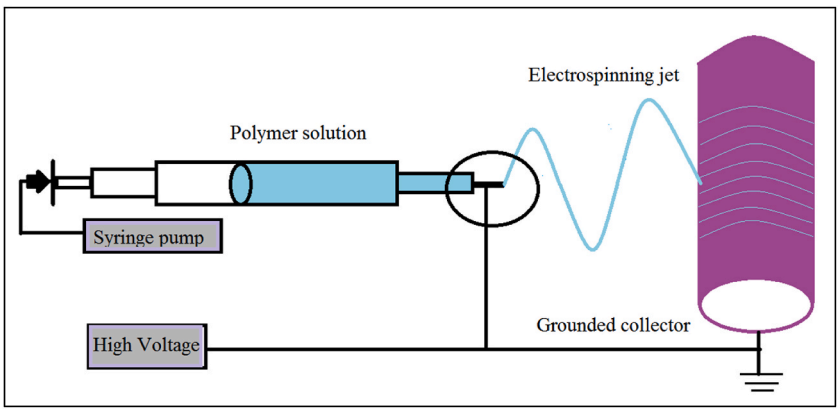

Fig. 1. Schematic illustration of the Electrospinning Set-up.

Table 1
Parameters of electrospinning.

Sl. No.	Parameters	PEOX (aq.)	PEOX (org.)
1	Flow rate	3 μl/s	3 μl/s
2	Distance between needle tip and collector	8 cm	8 cm
3	Collector RPM	800	800
4	Voltage applied	14 kV	18.5 kV
5	Diameter of the needle	13.08 mm	13.08 mm
6	Chamber temperature	29°C	30°C
7	Chamber humidity	51 %	66 %

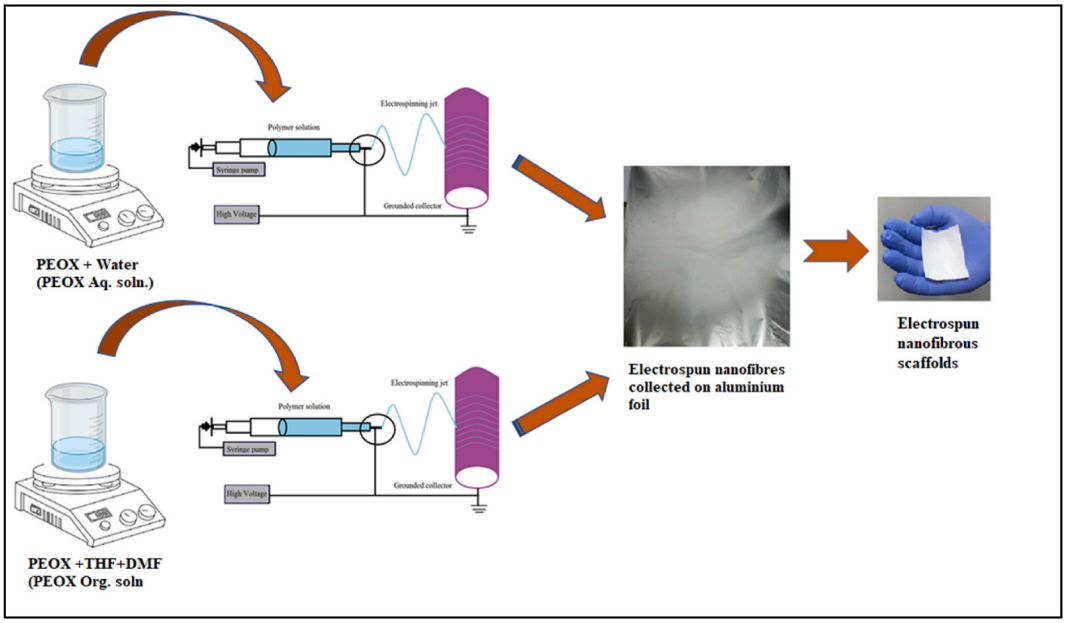

Fig. 2. Illustration of the development of PEOX (aq.) and PEOX (org.) electrospun scaffolds.

radiation was used as the X-ray source (40 kV, 30 mA). Samples were placed on a sample holder, and diffraction was scanned in the reflection mode at an angle of 2θ throughout a range of 10°–80° at a steady speed of 5°/min.

5.3. Scanning electron microscopy

The Scanning electron microscopy (JSM-IT500 JEOL, USA) was used to study the morphology of the electrospun scaffolds. Using Neocoater, a thin layer of gold was sputter-coated onto a small area of nanofibrous mat for approximately 60 s at a process current of 10 mA and a pressure of 10^{-6} mbar. The sputter coated samples were then used to observe the surface morphology using scanning electron microscope.

5.4. Contact angle measurement

The wettability of the nanofibrous scaffolds was measured using Static Contact Angle Goniometer (Kyowa Interface Science Co Ltd., Japan). The sessile drop method was used during the procedure carried at room temperature. Water droplets were placed on the nanofibrous mats, and FAMAS software was used to determine the incident angle. Ten replicates were taken for each PEOX (aq.) and PEOX (org.) scaffold samples to determine the contact angle and the mean values were determined.

5.5. Mechanical testing

The mechanical properties of nanofibrous scaffolds were analysed at ambient temperature using a Universal Tensile Testing machine (UTM) (DakSystemInc. Mumbai, India). The sample mats were cut in a rectangular dimension of 2 cm x10 cm for both the kind of samples. A thickness gauge was used to determine the thickness of these samples. The scaffold samples were mounted at the jaws of the UTM and stretched at a rate of 0.1 mm/min from either ends with a 1 KN load. The stress-strain curves were plotted for all the scaffold

samples and the average Young's modulus value was calculated.

6. Results and discussion

6.1. FTIR spectroscopy

As discussed in the previous section, PEOX is soluble in both water and organic solvents. Accordingly, the scaffolds were fabricated in the present study using water as one sample of scaffolds and using mixture of organic solvents as another sample of scaffolds. The similar nanofibrous mats were obtained in both the cases using electrospinning method. Thus, in order to observe if the solvent could affect in any kind of variations in the PEOX bonds with respect to water or organic solvent mixture, FTIR analysis was done. The FTIR spectra of PEOX (org.) and PEOX (aq.) scaffolds are shown in Fig. 3. As expected, it was observed that almost similar absorption bands were obtained for both the samples irrespective of the solvents used. Strong absorption bands at 3436 cm^{-1} are seen in both PEOX samples, suggesting stretching vibrations of OH. The peaks located at 1631 cm^{-1} and 2929 cm^{-1} are attributed to amide C=O stretching and $-CH_2$ asymmetric stretching, in that order. The two peaks at 1442 and 1035 cm^{-1} are ascribed to the respective stretching of CH_3 and the bending of C−N.

6.2. X-ray diffraction spectroscopy

To analyse the effect of solvent into the crystalline structure of nanofibrous PEOX (aq.) and PEOX (org.) scaffolds, XRD analysis has been carried out and the resulting spectra is shown in Fig. 4. The XRD patterns revealed two sharp intense diffraction peak at $2\theta = 14.2^{\circ}$ and 17.25° in both the cases irrespective of the solvents used, which shows the crystalline nature of the developed PEOX nanofibers. PEOX is generally considered to be amorphous in nature, but the clear intense peaks in the XRD reveals that the crystallinity has been introduced into the developed electrospun nanofibres during the process of electrospinning under high electric field.

6.3. Scanning electron microscopy

The morphological variables of the scaffolds including pore size and porosity are critical parameters in determining tissue engineered constructs. These parameters, in particular, play an important role in cell attachment, cell infiltration, and cell migration, thus have a direct impact on vascularisation and osteoconduction particularly for bone tissue engineering. The porous interconnected structure facilitates the cell in-growth by supporting the transport of nutrient molecules within the scaffolds. The mechanical stability and rate of degradation of the scaffolds also depends on the porosity of the scaffolds. The scanning electron microscopic images of PEOX nanofibres in two different solvents are shown in Fig. 5. The effect of solvent can be clearly seen in the morphology of the developed fibres. It was observed that for the PEOX scaffolds with water as a solvent produced some kind of agglomerations and a minimum number of bead formation in the SEM images. Whereas, in case of PEOX (org.) scaffolds, the fibres are found to exhibit clearly fine, smooth and uniform fibres with no agglomerations.

Fig. 6 shows the histogram analysis of the diameter measured on a couple of fibres using image J software. The histogram analysis of the fibres showed that the fibres lie in the range of several hundred nanometers (200 nm–600nm) for PEOX (aq.) scaffolds, whereas, the fibres were in the range of 8–14 μm for PEOX (org.) scaffold. Thus, an increase in fibre diameter was observed from nanometers to micrometers in case of organic solvent mixture. This can be attributed to the increased viscosity of the organic solution.

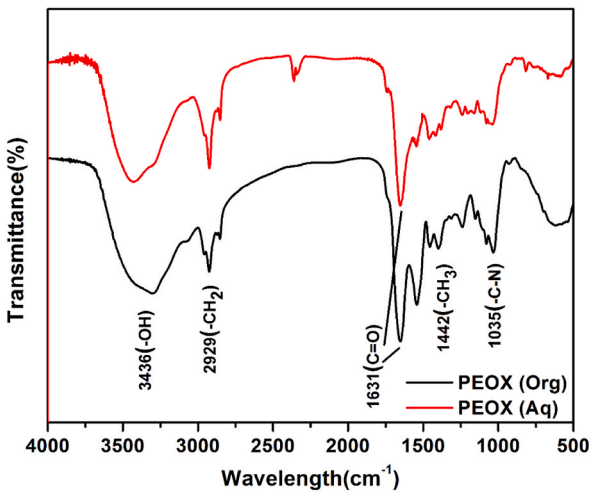

Fig. 3. The FTIR spectra of nanofibrous scaffolds.

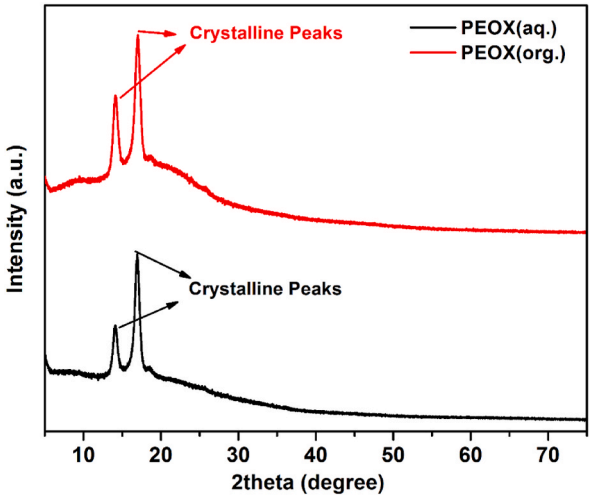

Fig. 4. XRD spectra of nanofibrous scaffolds.

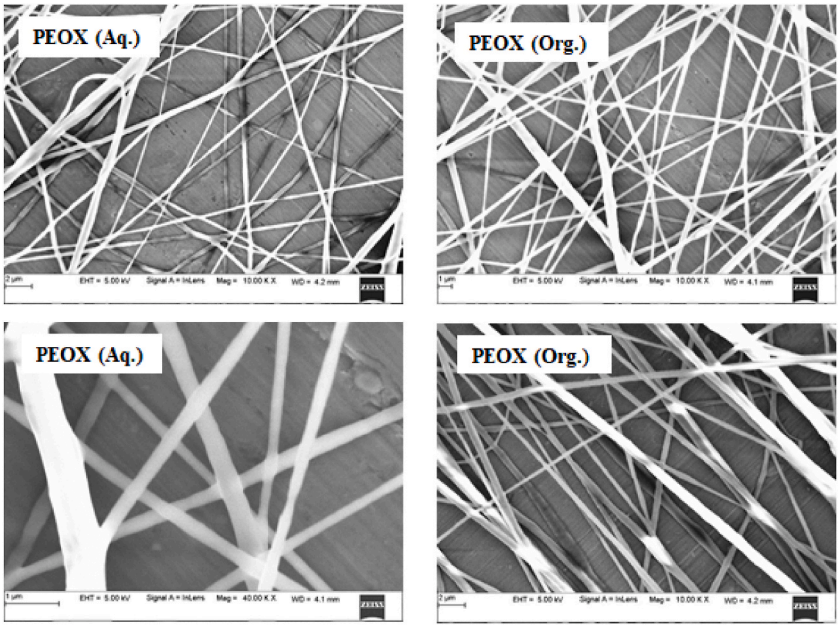

Fig. 5. SEM images of nanofibrous scaffolds.

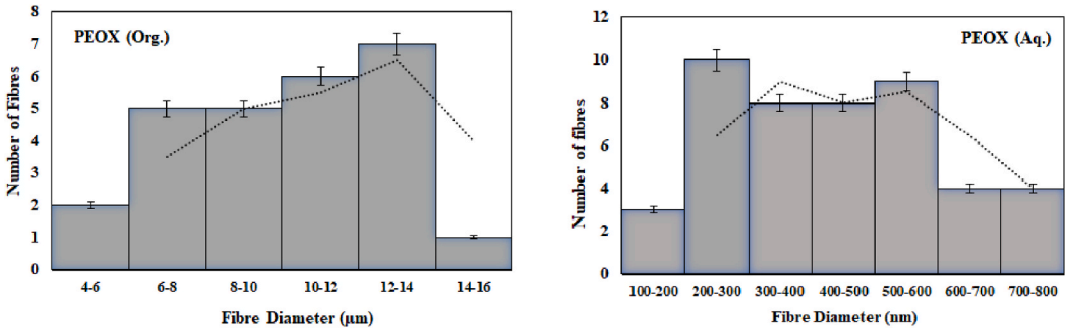

Fig. 6. Histogram analysis of nanofibres.

6.4. Contact angle measurement

The interconnected porous structure of the scaffolds defines the wettability of the samples. The hydrophilic property/wettability of the scaffolds are important for the cell growth and cell infiltration. The hydrophilic nature of the scaffolds provides the suitable environment which enhances the cell growth and proliferation. Thus, the wettability of the developed scaffolds was determined using sessile drop method by measuring the water contact angle upon the samples as shown in Fig. 7. It was found that, the scaffolds exhibited a lower contact angle values of $<90^{O}$, irrespective of the solvent used, thereby showing a hydrophilic nature of PEOX scaffolds. In addition, PEOX (aq.) scaffolds demonstrated highly hydrophilic behaviour with a very lower contact angle of 55.2° and PEOX (org.) scaffolds showed a considerably higher contact angle of 70.38°. The significantly lower contact angle values of PEOX (aq.) scaffolds is attributed to the use of aqueous solvent during solution preparation and the morphology of the scaffolds with nano ranged fibres. Thus, this leads to a hydrophilic nature of the developed nanofibres. These results reveal that the developed scaffolds could act as a suitable candidate for cell growth and cell proliferation for tissue engineering applications.

6.5. Mechanical testing

The mechanical strength of the polymeric scaffolds plays a vital role which supports the cell growth and tissue organisation over the scaffolds. According to the requirement of the defect site, the mechanical properties of the scaffolds should be tailored. The mean stress-strain curves of the scaffolds were plotted as shown in Fig. 8 (a). The curves consist of a linear elastic region, a plastic region of roughly constant stress and a region of steeply rising stress. From the curves, it was observed that the PEOX (org.) scaffold possessed a higher stress compared to PEOX (aq.). Further, the Young's modulus values were determined from the slope of the stress-strain curves and the mechanical strength of the scaffolds was assessed using a Universal Testing Machine.

Fig. 8 (b) shows the tensile strength of the developed scaffolds in terms of Young's modulus values. The Young's modulus for PEOX (org.) and PEOX (aq.) was observed to be 1.9 MPa and 1.02 MPa respectively. The effect of solvents during the fabrication of scaffold was clearly found to influence the mechanical properties of the resulting scaffolds. The use of organic solvent has increased the mechanical strength of the scaffolds whereas; the aqueous solvent has marginally reduced the mechanical properties. This is again attributed to the microfibers of the PEOX (org.) scaffolds which possess significantly greater mechanical strength as compared to the nanofibers of PEOX (aq.) scaffolds.

7. Conclusions

In this study, the electrospinning process was used to fabricate PEOX nanofibrous scaffolds. The effect of solvent was studied for the first time in literature for the electrospining of PEOX solution by using water and a mixture of organic solvents, and the physico-chemical properties of the developed scaffolds were compared for biomedical application. The process parameters of electrospinning were varied and optimized to obtain uniform fibres for both the kind of solvents. It was observed that a relatively high potential voltage was required to electrospin PEOX solution with organic solvent than compared to aqueous solution. To understand the effect of solvent on the properties of scaffolds, the physicochemical properties were examined for both the kinds of developed scaffolds. The SEM images revealed that both the scaffolds showed porous interconnected network structure with uniform non-beaded homogenous fibres. The effect of solvent was clearly observed by measuring the fibre diameters of the scaffolds. It was noted that the use of water as a solvent produced nanofibres in the range of several hundred nanometers whereas, organic solvent produced microfibrous scaffolds. This influenced the wettability of the resulting scaffolds wherein PEOX (aq.) scaffolds demonstrated highly hydrophilic nature when compared to PEOX (org.) scaffolds. This infers that the hydrophilicity of the scaffolds was greatly affected by the use of solvent. Further, the solvent effect was also observed in their mechanical properties of both the scaffolds, wherein PEOX (org.) scaffold exhibited a higher Young's modulus of 1.9 MPa and PEOX (aq.) showed a slightly lower modulus value of 1.02 MPa respectively, based on the stress-strain curves of the scaffolds. Thus, it can be concluded that the structural morphology and the physical properties of the scaffolds are greatly influenced by the nature of the solvent used during the electrospinning process. Further, an attempt was made to carry out the biodegradation study of the scaffolds using phosphate buffer saline solution (PBS). The study was carried out for PEOX (org.) scaffolds, but could not able to do with the PEOX (aq.) scaffold samples due to instability of scaffolds in the PBS solution. Thus, due to the unavailability of complete data, the biodegradation study is not mentioned in the experimental and result section. Based on these results, we observed that PEOX scaffolds with organic solvents possessed all the necessary features suitable for tissue engineering application and hence would be the better option for tissue engineering applications. Hence, in light of these findings, the next phase of this work will be to examine PEOX-based electrospun scaffolds utilizing an organic solvent for tissue engineering applications.

CRediT authorship contribution statement

Nandini A. Pattanashetti: Project administration, Methodology, Formal analysis, Data curation. **Mahadevappa Y. Karidur-aganavar:** Supervision, Resources, Investigation. **Arjun Sunil Rao:** Visualization, Formal analysis, Conceptualization. **Amruta Savadi:** Writing – original draft, Software. **Maruti Pali:** Writing – original draft, Visualization, Validation, Conceptualization. **Sid-dharth Sonavane:** Visualization, Project administration, Methodology, Investigation, Data curation. **Sunita:** Supervision, Software, Resources, Project administration, Methodology.

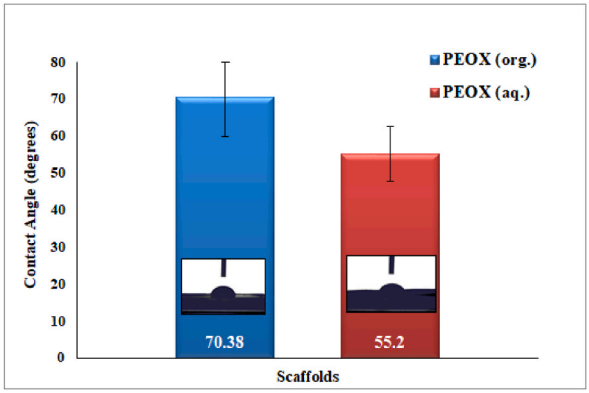

Fig. 7. The contact angle measurements of scaffolds.

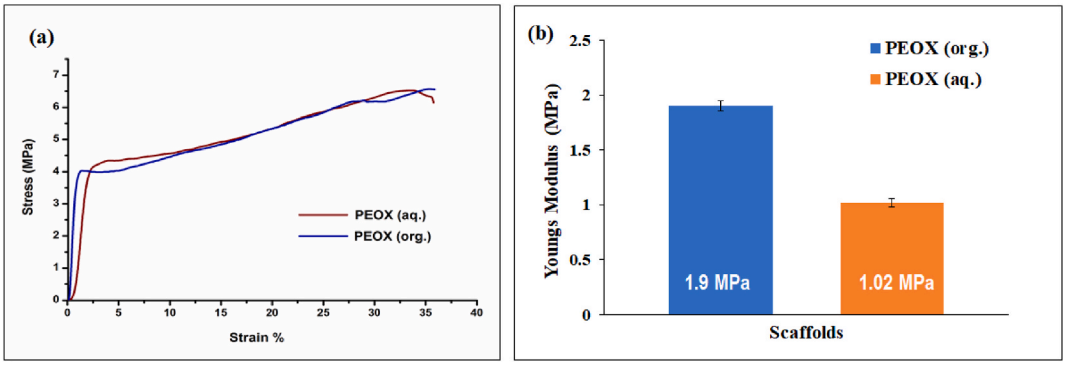

Fig. 8. The Mechanical properties of the nanofibrous scaffolds representing (a) Stress-strain curves of the scaffolds, (b) Young's Modulus values of the scaffolds.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability statement

All data generated or analysed during this study are included in this published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors wish to thank the Sophisticated Analytical Instrumentation Facilities (SAIF) in University Scientific and Instruments Centre (USIC), Karnatak University Dharwad, India for providing the instrumental facilities during the experimental work of the present study.

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