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Original Research Article

Biological characterization of a biodegradable scaffold for common bile duct replacement in an experimental model

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ABSTRACT

Background: The nanofiber scaffolds achieved by the electrospinning technique have been used to develop several biological tissues, the nanofibers obtained by electrospinning procure a favorable microenvironment to mimic the extracellular matrix.
Methods: Study type was of experimental. Study conducted at National Autonomous University of Mexico, from May 2018- May 2022. The protocol was approved by the research and ethics commissions of the UNAM school of medicine. A viscoelastic solution of polylactic-co-glycolic acid (PLGA) and polycaprolactone (PCL) in a 70:30 ratio and gelatin (Gel) in an 80:20 ratio was prepared while a dynamic collector was used with the electrospinning technique.
Results: Mechanical and biological tests were carried out on the scaffold obtained by electrospinning; the resultant scaffold achieves good mechanical matching and structural similarity between the graft and the extrahepatic bile duct.
Conclusions: In this study we managed to create a porous, biocompatible scaffold with good cell adhesion and proliferation, potentially applicable to tissue engineering, especially for the replacement of tubular organs such as blood vessels, bile ducts, and urethra.

Keywords: Nanotechnology, Nanofiber, Scaffolds, Electrospinning, Tissue engineering

INTRODUCTION

Nanotechnology came into existence in the year 1974 and today has made its way into almost every field of science as well as, medicine, metals, textiles, waste management, electronics and especially tissue engineering where it has been able to produce biomimetic nanofiber scaffolds.[1] Nanofiber scaffolds are prepared with various types of materials, such as ceramics, metals, natural and synthetic polymers, to create nanofibers and nanopatterns. These scaffolds are had increased its popularity as their biological and topographical properties closely mimic the extracellular matrix (ECM) properties of the tissues.[2] Several techniques are available to develop nanofibers and nanopatterned structures including electrospinning (ES), particulate leaching, lithography, self-assembly, phase separation and freeze drying. The electrospinning technique is highly attractive and widely used in recent years in the pharmaceutical industry and tissue engineering.[3,4] Electrospinning is a fiber spinning technology that roots itself on exposing a viscoelastic solution to a high-voltage electrostatic field to produce a

single charged jet and its deposition on a grounded collector in the form of nonwoven material with nanofibers from micrometer down to nanometer diameters.[5] The nanofiber scaffolds achieved by the electrospinning technique have been used to develop several biological tissues, to name a few, bone, cardiovascular, ligament and blood vessels.[6,7] With this technique, we can also incorporate specific growth factors, proteins or bioactive molecules in the fabricated scaffolds and aim for adequate surface modifications for improved adhesion of the cultured cells.[2,8] In the ES process, a solution of a polymer is prepared in a volatile solvent. This is placed in a syringe fitted with a metal needle and the viscoelastic solution is expelled at a controlled rate. With the application of an electric field, the drops formed overcome the force of surface tension (Taylor cone). In ES, the positive electrode comes from a high voltage power supply and is connected to the needle, the grounded electrode is connected to a flat or dynamic collector, above a critical voltage, a jet of polymer is ejected from the Taylor cone to the grounded collector. In doing so, the jet narrows and the solvent evaporates. Ultimately, this results in dry fibers at the nano-microscale being deposited in the flat or dynamic collector.[4] A number of factors influence the diameter and morphology of electrospun nanofibers, including polymeric solution properties, processing parameters, and ambient conditions, electrospinning process parameters (such as applied voltage, the distance between the needle and the collector, and the flow rate of the polymer solution) are another significant category in the electrospinning fabrication process.[5,9]

METHODS

Study type

The study was of experimental.

Study place

National Autonomous University of Mexico, from May 2018-May 2022. The protocol was approved by the research and ethics commissions of the UNAM school of medicine.

Electrospinning

A viscoelastic solution of polylactic-co-glycolic acid (PLGA) and polycaprolactone (PCL) in a 70:30 ratio and gelatin (Gel) in an 80:20 ratio was prepared while a dynamic collector was used with the electrospinning technique.

Optical microscopy and electron microscope (SEM)

The calibration of the electrospinning parameters was determined using simple optical microscopy, the correct adjustment of the such as the continuity of the fibers and the absence of bulbs. SEM was used to determine the average fiber diameter and pore size.

Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR–ATR)

FTIR-ATR spectroscopy was performed in which the functional groups of the polymers used were determined and the contact angle was analyzed to determine if the surface is hydrophobic or hydrophilic.

Cell culture

First a cell culture of 10×10^4 fibroblasts per square centimeter of the scaffold was applied, keeping it in a tissue incubator at 37ºC with 5% CO_2, following cytotoxicity tests and cell counts were performed after 10 days of culture growth.

In vivo biocompatibility testing

Scaffold sections were implanted into the subcutaneous cell tissue on the back of rabbits, taking samples for histopathological analysis at 10 and 21 days.

Extrahepatic bile duct implant

Once the mechanical and biological studies were completed, an implant was carried out in the extrahepatic bile duct of pigs, currently with a 2-year follow-up.

RESULTS

Electrospinning

A low-cost dynamic collector (Figure 1) was built with a regulated voltage of 19 V to obtain a constant 3800 rpm, with a distance of 10 centimeters and an output flow of 1.2 ml/hr, using a high voltage source. at 14 kV. The scaffold obtained by electrospinning is observed in Figure 2 A.

SEM

Optical microscopy was used to analyze the continuity in the fibers and the absence of bulbs **(**Figure 2). Images of scaffolds were taken by using scanning electron microscope (SEM) and fiber diameter of scaffolds was measured by image J, obtaining an average pore diameter of 2.5 μm and a fiber diameter of 0.50 μm. (Figure 3).

FTIR-ATR

In the electrospun scaffold, the amide I, II, III functional groups characteristic of gelatin were determined, characteristic spectra were found in band 1723 cm^{-1} corresponding to the C=O carbonyl bond in stretching mode, band 2867 cm^{-1} corresponds to a symmetric stretching of methylene (CH_2) and at 2941 cm-1 is the asymmetric stretching band of CH_2.

Figure 1 (A and B): Dynamic collector, the main motor assembly anchored to the dynamic collector and electrical continuity.

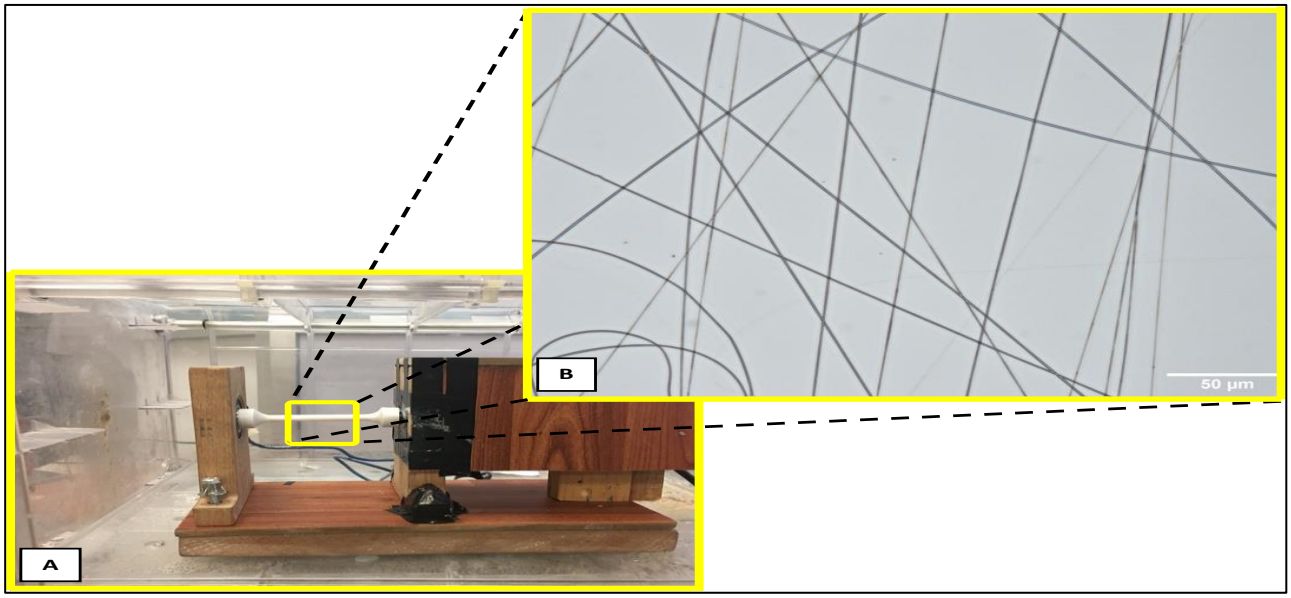

Figure 2 (A and B): Polymeric fibers deposited in the dynamic collector. The macroscopic collection with a length of 10 centimeters and an internal diameter of 6 millimeters. Control by optical microscopy where continuous fibers and absence of bulbs can be seen.

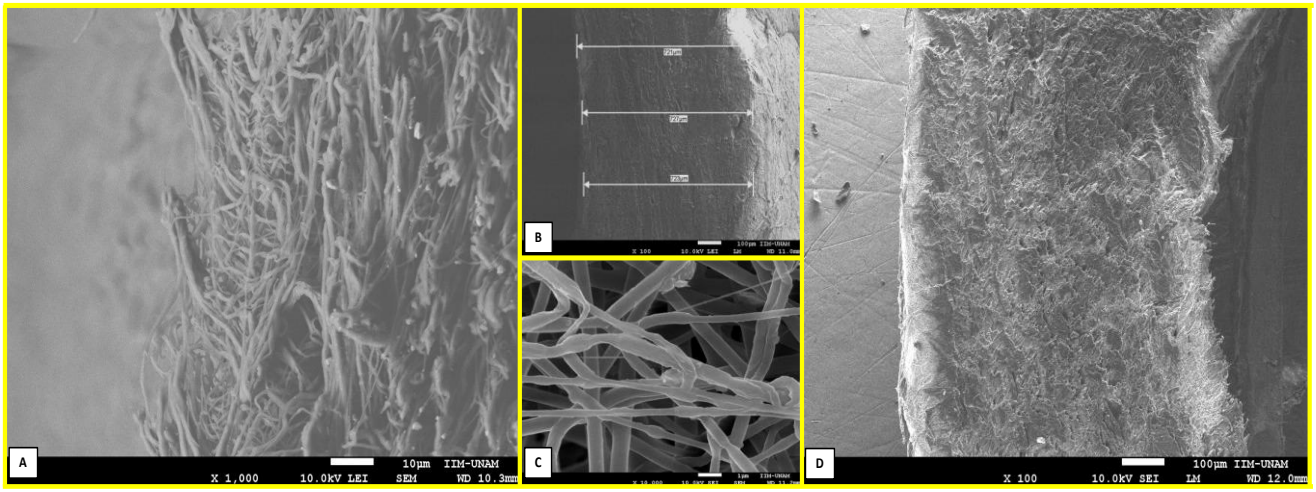

Figure 3 (A-D): Electron microscope. Fibers of the electrospun scaffold, 3B cross section with an average of 723 µm, 3C image obtained for the analysis of pores and fibers performed, 3D cross section at 100 µm.

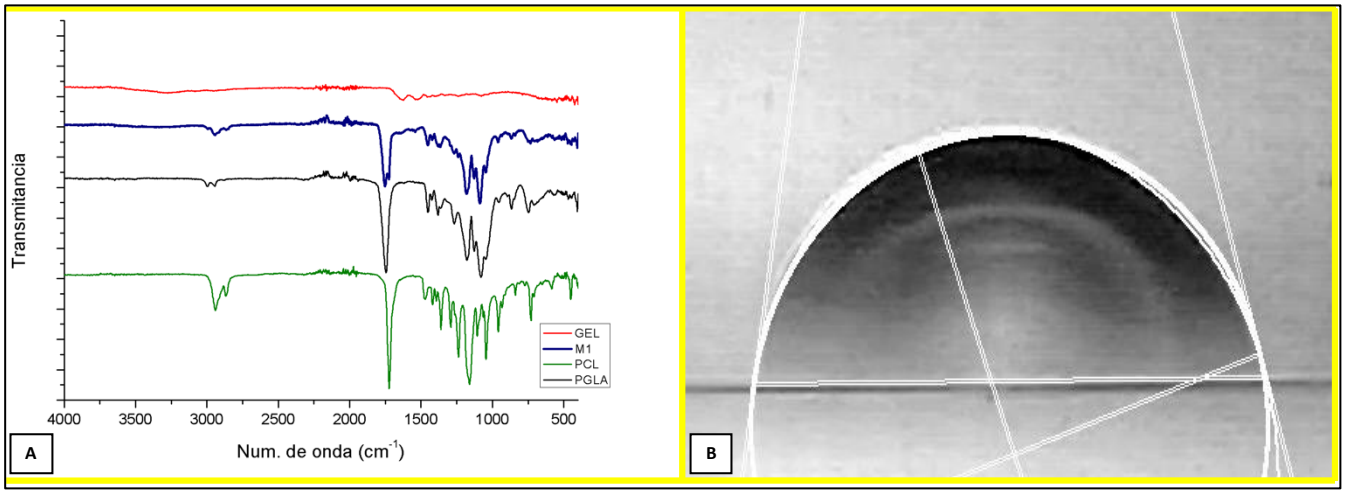

Figure 4 (A and B): Spectroscopy and contact angle. The presence of specific bands in the functional groups of each polymer and in blue the different peaks in bands that represent a copolymer composed of PGLA-PCL-Gel are observed. The contact angle that extends to 42º at 300 seconds.

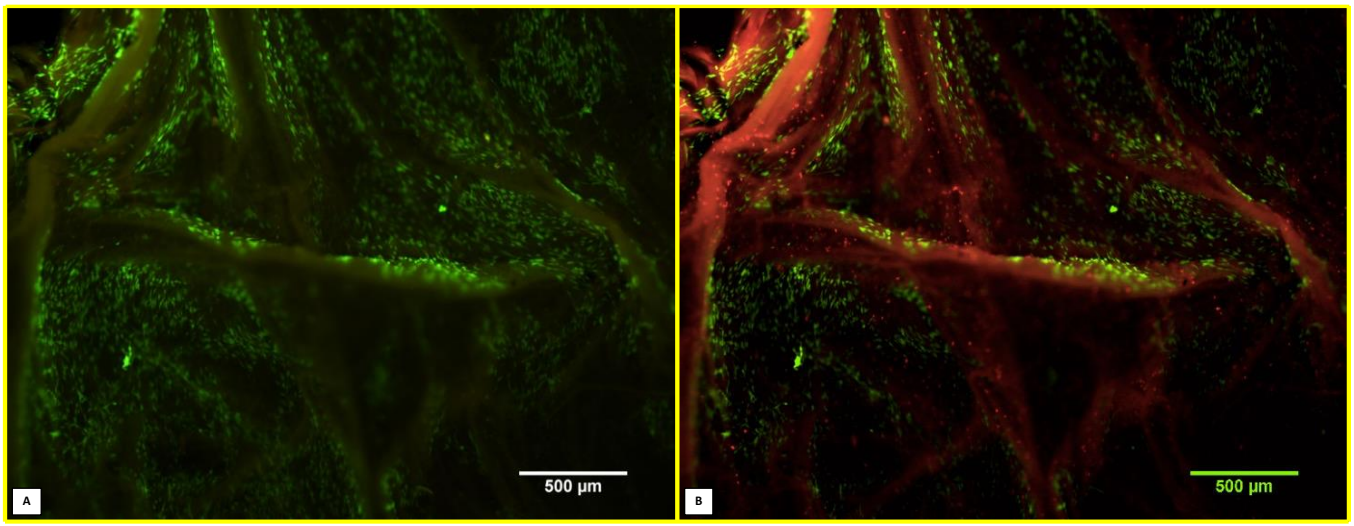

Figure 5 (A and B): Kit live dead. Marks fluorescence of living and dead fibroblasts in green with ethidium homodimer (cell permeability).

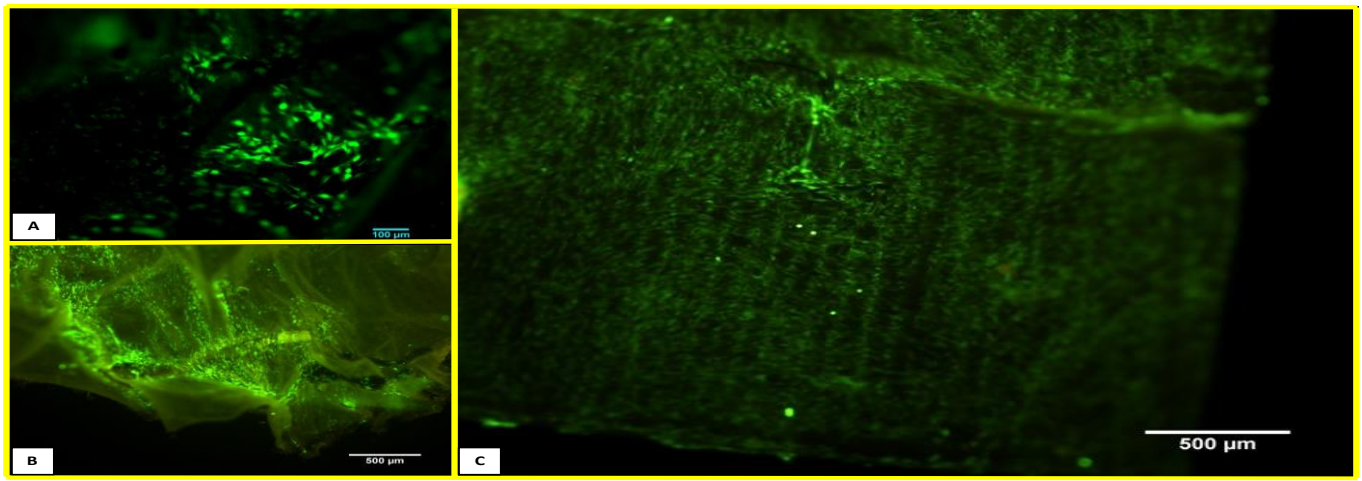

Figure 6 (A-C): Carry out cell count, cells were counted with image J program, 100 µm and 500 µm.

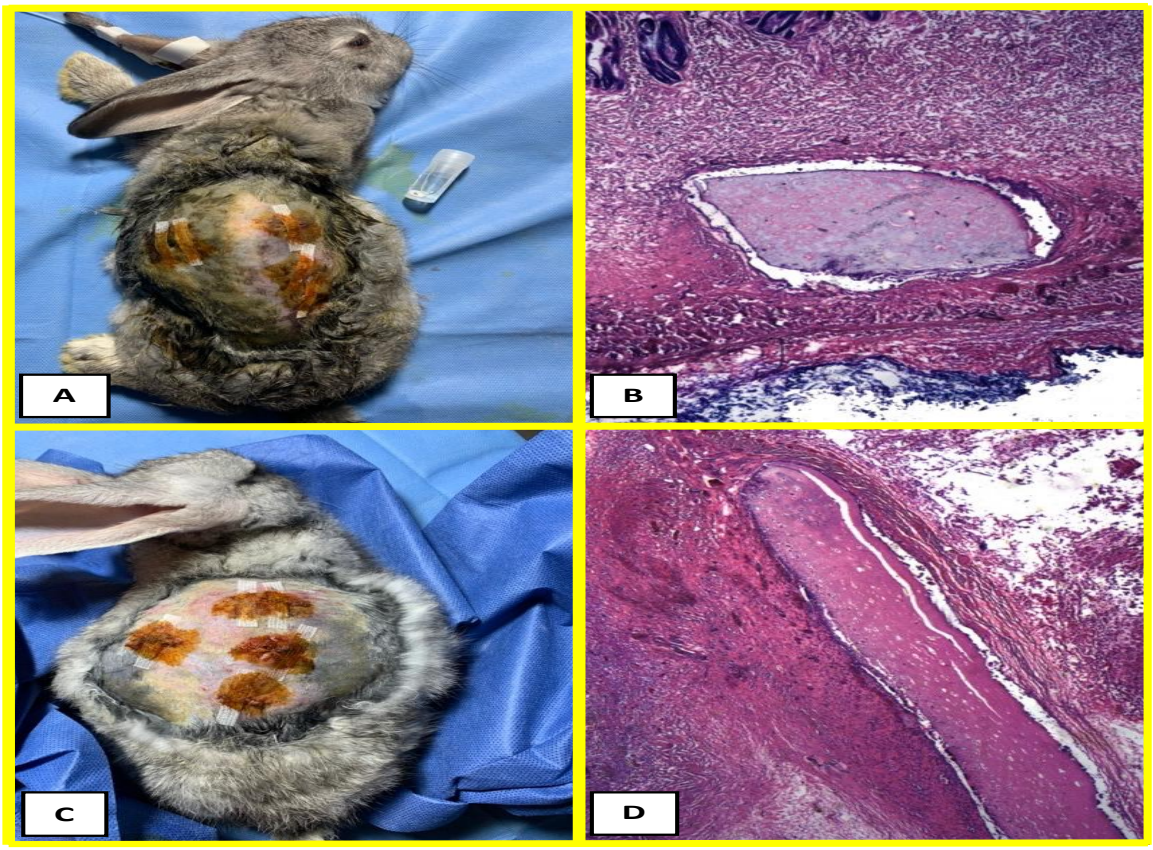

Figure 7 (A-D): Biocompatibility, *in vivo* implant section of scaffold at 10 days where in epithelialization of scaffold with little inflammation is observed, on day 23 and complete epithelium coverage is observed without dermal reaction, inflammation/ interstitial fibrosis.

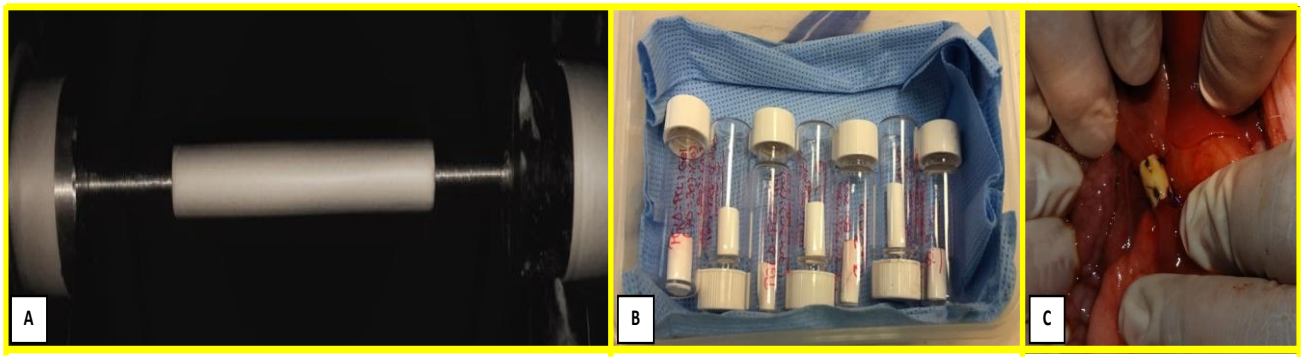

Figure 8 (A-C): Prosthesis obtained by electrospinning placed in extrahepatic bile duct of porcine. Prosthesis obtained by electrospinning in a dynamic manifold, which is later sectioned into necessary lengths, to be implanted in extrahepatic bile duct of porcine.

To determine the contact angle, a drop of distilled water was deposited on the scaffold, the theoretical contact angle of 61.1º at 60 seconds and 42º at 300 seconds (hydrophilic surface) was determined (Figure 4).

Cell culture

Evaluation of the cytotoxicity of the scaffold was carried out using the live dead kit with calcein/ethideo homodimer, cell viability was determined by calcein fluorescence and cell death indicated by cell permeability using ethideo homodimer (Figure 5). The cell count was carried out in 500 μm^2 of different samples with the ImageJ program, the average percentage of cell viability was determined at 10 days (Figure 6). The average percentage of cell viability at 10 days was 93.5%, with a mortality rate at 10 days of 6.5%.

In vivo biocompatibility testing

Sections of the scaffold were implanted on the back of rabbits, no dermal reaction to the scaffold with little

inflammation was covering epithelium without interstitial fibrosis was observed on days 10 and 21.

Extrahepatic bile duct implant

The latest analysis of the research consists of implantation in the extrahepatic bile duct in pigs (Figure 8), currently the 2-year follow-up continues.

DISCUSSION

The nanofibers obtained by electrospinning procure a favorable microenvironment to mimic the extracellular matrix. This uniqueness has been widely valued by researchers due to the multiple and potential applications in biomedical engineering, biomedicine, and tissue engineering.[10] Electrospun nanofiber scaffolds, as a fundamental component of tissue engineering, have the potential to become a wide range of tissues, including tendon, vascular, neuron, bone, and cartilage, just to name a few.[11] Clinically, there is a significant unmet need for off-the-shelf tubular grafts, and ES has been proven to be a versatile technique to prepare nanofibrous scaffolds for such grafts. Small diameter vascular grafts (6 mm) for the bypass or replacement of diseased or damaged blood vessels can be obtained by using a suitably sized rotating drum as the collector in ES. The resultant scaffold achieves good mechanical matching and structural similarity between the graft and the native vessels or extrahepatic bile duct.[12]

Limitations

Due to the experimental nature of this research, only the mechanical and biological properties of a scaffold with specific polymers were evaluated, the biochemical and molecular behavior could be evaluated with other concentrations or different polymers. The experimental clinical postoperative follow-up in porcine models is still ongoing, we do not have conclusive results in vivo.

CONCLUSION

The electrospinning process is a promising route for the next generation of polymer-based fibers with diameters on the nano to micron-scale, in this study we managed to create a porous, biocompatible scaffold with good cell adhesion and proliferation, potentially applicable to tissue engineering, especially for the replacement of tubular organs such as blood vessels, bile ducts, and urethra.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee by UNAM School of Medicine, with registration number FM/DI/047/2018.

REFERENCES

1. Anbusagar N, Palanikumar K, Ponshanmugakumar A. Preparation and properties of nano-polymer advanced composites: A review. Materials Sci. 2018:27-73.
2. Vasudevan A, Tripathi D, Sundarrajan S, Reddy J, Ramakrishna S, Kaur S. Evolution of Electrospinning in Liver Tissue Engineering. Biomimetics. 2022;7:149.
3. Moreira A, Lawson D, Onyekuru L, Dziemidowicz K, Angkawinitwong U, Costa PF et al. Protein encapsulation by electrospinning and electrospraying. J Controlled Rel. 2021;1172-97.
4. Dziemidowicz K, Sang Q, Wu J, Zhang Z, Zhou F, Lagaron J et al. Electrospinning for healthcare: recent advancements. J Mater Chem B. 2021;9:939-51.
5. Topuz F, Uyar T. Electrospinning of cyclodextrin nanofibers: the effect of process parameters. J Nanomaterials. 2020;1-10.
6. Aghajanpoor M, Hashemi-Najafabadi S, Baghaban-Eslaminejad M, Bagheri F, Mohammad Mousavi S, Azam Sayyahpour F. The effect of increasing the pore size of nanofibrous scaffolds on the osteogenic cell culture using a combination of sacrificial agent electrospinning and ultrasonication. J Biomed Mater Res Part A. 2017;105:1887-99.
7. Kitsuka T, Hama R, Ulziibayar A, Matsuzaki Y, Kelly J, Shinoka, T. Clinical Application for Tissue Engineering Focused on Materials. Biomedicines. 2022;10:1439.
8. Baskapan B, Callanan A. Electrospinning Fabrication Methods to Incorporate Laminin in Polycaprolactone for Kidney Tissue Engineering. Tissue Eng Regen Med. 2022;19:73-82.
9. Sun B, Long YZ, Zhang HD. Advances in three dimensional nanofibrous macrostructures via electrospinning. Progress in Polymer Science. 2014;39(5):862-90.
10. Keirouz A, Chung M, Kwon J, Fortunato G, Radacsi N. 2D and 3D electrospinning technologies for the fabrication of nanofibrous scaffolds for skin tissue engineering: A review. WIREs Nanomed Nanobiotechnol. 2020;12:e1626.
11. Owida HA, Al-Nabulsi JI, Alnaimat F, Al-Ayyad M, Turab NM, Al Sharah A et al. Recent Applications of Electrospun Nanofibrous Scaffold in Tissue Engineering. Appl Bionics Biomecha. 2022;1-15.
12. Zhu J, Chen D, Du J, Chen X, Wang J, Zhang H et al. Mechanical matching nanofibrous vascular scaffold with effective anticoagulation for vascular tissue engineering. Composites Part B: Engineering. 2020;186:107788.

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