Title

Modified Poly(ε-caprolactone) with Tunable Degradability and Improved Biofunctionality for Regenerative Medicine.

Journal

ACS Materials Au, 3(5)

Authors

Shen, Jun

Yuan, Weihao

Badv, Maryam

et al.

Publication Date

2023-09-13

DOI

10.1021/acsmaterialsau.3c00027

Peer reviewed

ACS MATERIALS Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

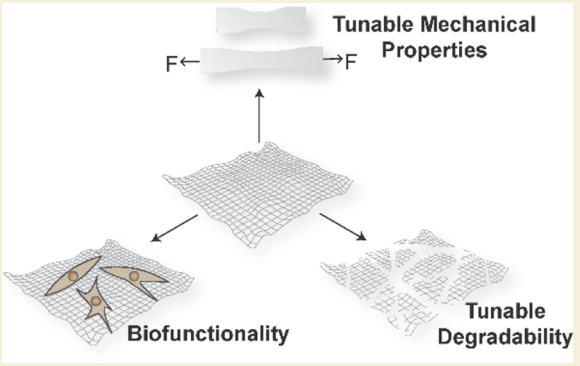
pubs.acs.org/materialsau
Article

Modified Poly(ε-caprolactone) with Tunable Degradability and Improved Biofunctionality for Regenerative Medicine

Jun Shen, Weihao Yuan, Maryam Badv,* Alireza Moshaverinia,* and Paul S. Weiss*

Cite This: ACS Mater. Au 2023, 3, 540−547
Read Online

ABSTRACT: The use of poly(ε-caprolactone) (PCL) for biomedical applications is well established, particularly for permanent implants, due to its slow degradation rate, suitable mechanical properties, and biocompatibility. However, the slow degradation rate of PCL limits its application for short-term and temporary biomedical applications where bioabsorbability is required. To enhance the properties of PCL and to expand its biomedical applications, we developed an approach to produce PCL membranes with tunable degradation rates, mechanical properties, and biofunctional features. Specifically, we utilized electrospinning to create fibrous PCL membranes, which were then chemically modified using potassium permanganate to alter their degradability while having minimal impact on their fibrous morphology. The effects of the chemical treatments were investigated by treating the samples for different time periods ranging from 6 to 48 h. After the 48 h treatment, the membrane degraded by losing 25% of its mass over 12 weeks in degradation studies, while maintaining its mechanical strength and exhibiting superior biofunctional features. Our results suggest that this approach for developing PCL with tailored properties could have significant potential for a range of biomedical applications.



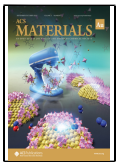
KEYWORDS: *Poly(ε-caprolactone) (PCL), electrospinning, biodegradable membranes, biofunctional membranes, tissue engineering*

INTRODUCTION

There is growing interest in developing resorbable materials for use in tissue engineering and regenerative medicine. Poly(ε-caprolactone) (PCL) is a biocompatible polymer that has been approved by the U.S. Food and Drug Administration (FDA) for clinical use. Poly(ε-caprolactone) is widely applied in long-term surgical implants[1−4] and slow-releasing drug-delivery systems[5−8] due to its biocompatibility, biodegradability, and suitable mechanical properties. In tissue engineering, PCL has been studied for applications that require slowly degrading materials, for example, guided bone regeneration[9−11] and vascular grafts.[12−14] Researchers have also explored ways to modify PCL to increase its degradation rate.[15,16] One approach is to make copolymers or to blend PCL with other polymers, such as polylactic acid,[17−19] poly(*N*-(2-hydroxypropyl)-methacrylamide),[20] alginate,[21,22] starch,[23,24] and zein.[25] However, blending polymers can result in phase separation, degrading the mechanical properties of the resulting material.[15,16] In addition, blending may lead to loss of the desired characteristics of the original system, such as solubility, biocompatibility, mechanical properties, etc. Alternatively, surface modification can be used to modify the surface properties of PCL[26−29] without changing its bulk properties significantly.[29] Oxygen plasma treatment is a common method to modify electrospun PCL scaffolds for mimicking the extracellular matrix (ECM). Surface plasma oxidation is able to decrease the hydrophobicity of PCL, thus enhancing cell adhesion on sample surfaces. However, plasma treatment introduces unexpected topographical and mechanical changes to PCL scaffolds by partially melting PCL nanofibers in scaffolds, as well as significant chemical changes.[28]

Here, we present a one-step method to modify PCL by using potassium permanganate ($KMnO_4$), which causes hydrolytic chain scission while introducing hydrophilic functional groups to PCL chains. This modification increases the water uptake and degradation rate of PCL without compromising its fibrous properties, making it more suitable for biomedical applications where bioadsorption is required. We first fabricated PCL membranes using electrospinning, which is widely used in biomedical applications due to its ability to create highly porous structures with high surface-area-to-volume ratios. We then chemically modified these membranes using $KMnO_4$ to produce a PCL material with tunable degradability. This

Received: April 17, 2023
Revised: June 19, 2023
Accepted: June 21, 2023
Published: July 10, 2023



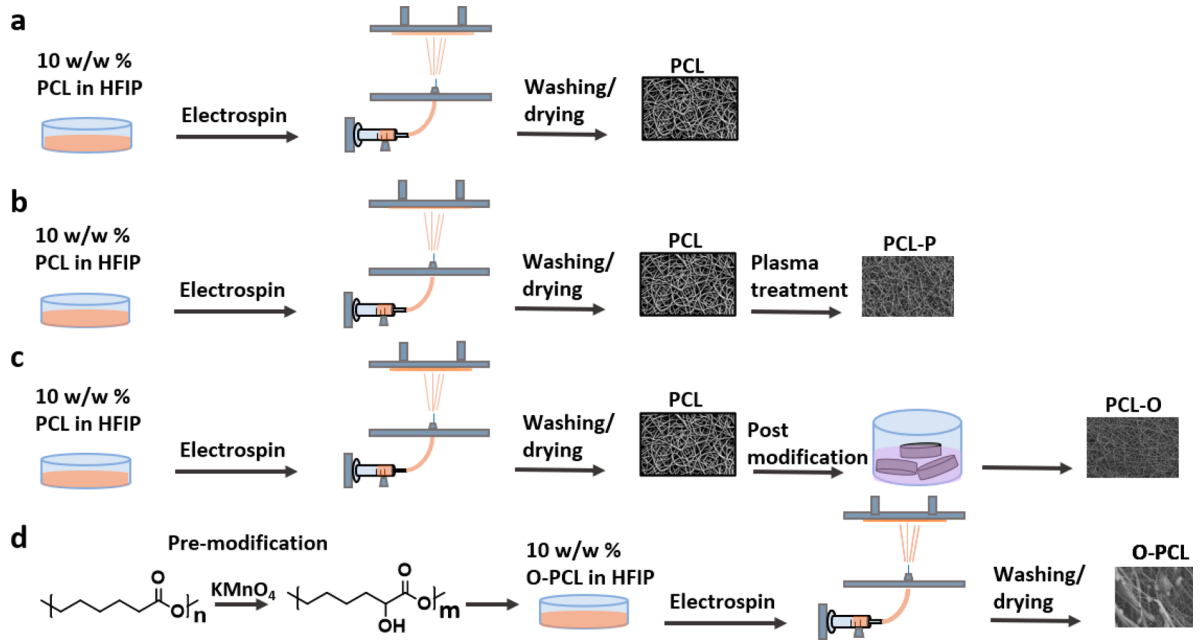


Figure 1. Schematic of the fabrication of poly(ε-caprolactone) (PCL) membranes. (a) Poly(ε-caprolactone) is dissolved in hexafluoroisopropanol and then electrospun into fibrous membranes. (b) Electrospun PCL membranes are plasma-treated to produce hydrophilic PCL-P. (c) Chemically modified PCL membranes (PCL-O) are created by conducting postmodification on electrospun PCL membranes. (d) Premodified PCL membranes (O-PCL) are electrospun from PCL that has been treated with potassium permanganate. HFIP: hexafluoroisopropanol.

treatment endows the polymer with a targeted degradation time frame to match the tissue healing timeline to help tissues restore their function.[30] We characterized the modified PCL using chemical and mechanical tests and evaluated its biofunctional and cytotoxic properties in vitro. Our findings demonstrate that PCL modified with our method is a promising material for use in resorbable implants for regenerative medicine applications. The one-step method presented offers a simple and effective way to modify PCL without sacrificing its desirable characteristics and may pave the way for new developments in the field of regenerative medicine.

RESULTS AND DISCUSSION

Nanofibrous poly(ε-caprolactone) (PCL) membranes were fabricated by electrospinning, which requires sufficient topological entanglement between electrospun molecules to allow for the formation of a continuous jet during the process. As a result, electrospinnable molecules typically have high molecular weights.[31,32] For polymers lacking sufficient chain entanglement, higher concentrations are required, but the high viscosity of highly concentrated polymer solutions can prevent fiber formation.[32−34] Polycaprolactone with a molecular weight of 80,000 g/mol is widely used for electrospinning in tissue engineering applications, but it has a degradation time of two to three years. In contrast, PCL with a low molecular weight has a shorter degradation time but may not be suitable for producing nanofibers using electrospinning. To overcome this challenge, we first fabricated PCL electrospun membranes by dissolving PCL in hexafluoroisopropanol (HFIP). We then chemically modified the electrospun PCL membranes with sulfuric acid and potassium permanganate, an inorganic oxidizer commonly used as a disinfectant in superficial wounds and water treatment. During this process, saturated hydrocarbons in the PCL backbone were oxidized, producing functional groups such as hydroxyl, vinyl, carbonyl, and carboxyl.[35] Hydrolysis and degradation were also expected to occur with the presence of potassium permanganate and sulfuric acid, lowering the molecular weight of PCL. The degree of oxidation and degradation of PCL was tuned by varying the length of the reaction time. We treated electrospun PCL membranes for 6, 18, 24, 36, and 48 h, producing "PCL-O" materials, including PCL-6, PCL-18, PCL-24, PCL-36, and PCL-48, respectively. We fabricated membranes that were chemically modified by $KMnO_4$ prior to electrospinning (O-PCL) (Figure 1). We evaluated the molecular weights of PCL, O-PCL, and different PCL-O materials using gel permeation chromatography (GPC), which revealed that the number-average molecular weight (M_n) of PCL was 8.4 (±0.2) × 10^4 g/mol. After chemical treatment, the M_n of PCL-6, PCL-18, PCL-24, PCL-36, PCL-48, and O-PCL decreased to 7.1 (±0.2) × 10^4 g/mol, 6.5 (±0.2) × 10^4 g/mol, 4.9 (±0.2) × 10^4 g/mol, 3.0 (±0.2) × 10^4 g/mol, 2.4 (±0.3) × 10^4 g/mol, and 2.2 (±0.1) × 10^4 g/mol, respectively (Table 1). The M_n of the PCL-O polymers significantly decreased after the chemical treatment and continued to decline with longer reaction times. We also investigated the use

Table 1. Molecular Weights and Polydispersity Index (PDI) of Poly(ε-caprolactone) (PCL), Chemically Modified PCL Membranes (PCL-O), and Pre-Modified PCL Membranes (O-PCL)

Material	M_n (g/mol)	PDI
PCL	8.4 (±0.2) × 10^4	1.29
PCL-6	7.1 (±0.2) × 10^4	1.33
PCL-18	6.5 (±0.2) × 10^4	1.38
PCL-24	4.9 (±0.2) × 10^4	1.46
PCL-36	3.0 (±0.2) × 10^4	1.59
PCL-48	2.4 (±0.3) × 10^4	1.73
O-PCL	2.2 (±0.1) × 10^4	1.52

https://doi.org/10.1021/acsmaterialsau.3c00027
ACS Mater. Au 2023, 3, 540−547

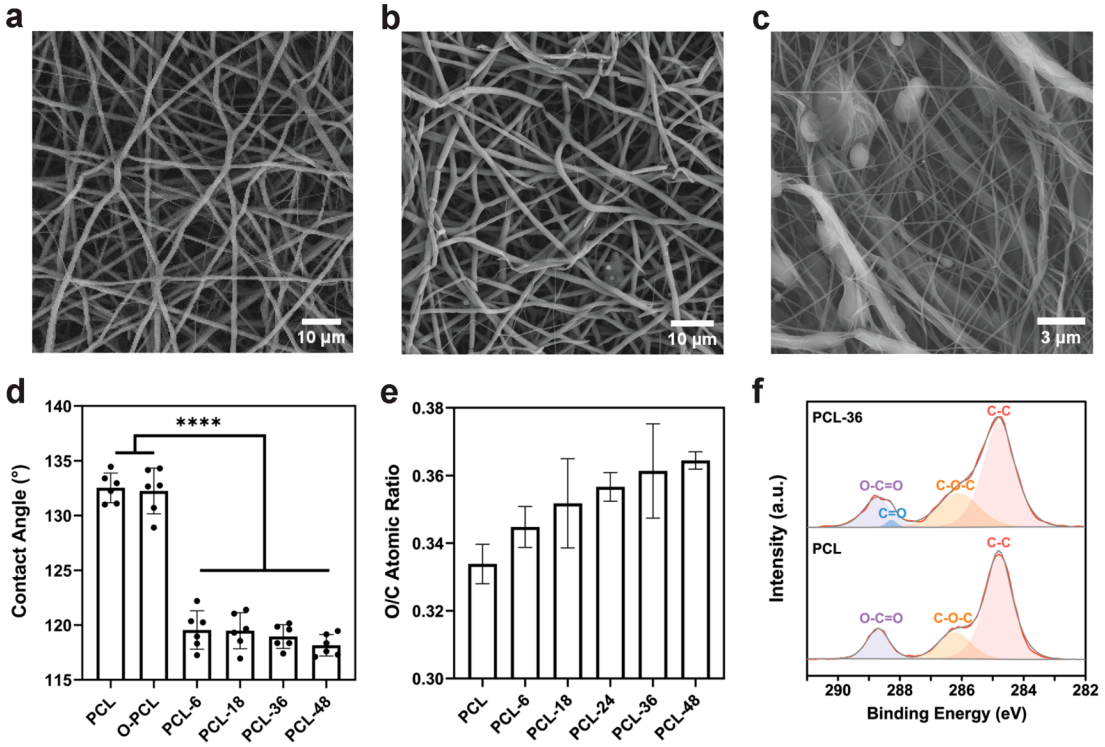


Figure 2. Scanning electron microscopy (SEM) images of (a) poly(ε-caprolactone) (PCL), (b) post-modified PCL (PCL-O) membranes and (c) pre-modified PCL (O-PCL) membranes. Chemical properties of PCL membranes using different approaches. (d) Static contact angles on membranes.****$p < 0.0001$. (e) Oxygen-to-carbon atomic ratio (O/C) of PCL and PCL-O membranes by X-ray photoelectron spectroscopy (XPS) characterization. (f) XPS spectra of carbon 1s peaks with assignments.

of oxygen plasma as an alternative surface modification method for PCL membranes, producing PCL-P. However, we observed that PCL-P underwent shrinkage, partial melting, and increased rigidity after plasma treatment. The unevenness and rigidity of PCL-P render it unsuitable for tissue engineering applications. This result is in contrast with the results of chemical modification with potassium permanganate, which showed no visual difference in membrane appearance before and after treatment.

The surface morphology of the PCL, PCL-O, and O-PCL membranes were investigated using scanning electron microscopy (SEM). As observed in the SEM images, PCL-O membranes were able to maintain their original fibrous structure after chemical treatment, and no significant differences were seen when comparing them to untreated PCL membranes. As shown in Figure 2a and 2b, the porous structures of the PCL and PCL-O membranes were formed by fibers with diameters of approximately 800 nm, which provide support and guidance for cell growth, proliferation, and differentiation on the membranes. This morphology is well suited for tissue engineering and other biomedical applications.[33,36−38] However, the O-PCL membranes showed distinct morphological changes (Figure 2c), with the presence of beads with diameters ranging from 2 to 5 μm, and decreased fiber diameter to ca. 150 nm. These changes are due to the decreased molecular weight of the polymer chains after chemical treatment and prior to electrospinning, which reduces their ability to have sufficient entanglement to produce continuous fibers; instead, beads were formed.[41−43] Thus, the permanganate treatment makes the modified PCL less electrospinnable.

X-ray photoelectron spectroscopy (XPS) was employed to investigate the changes in the surface chemistry of PCL membranes after treatment. The increase in the oxygen 1s peak indicated the oxidation of PCL during treatment. Quantitative analysis of the XPS spectrum revealed changes in the carbon and oxygen atomic ratios in different membranes. The oxygen-to-carbon ratio increased from 1:3 in PCL to 1.03:3 in PCL-6, 1.07:3 in PCL-24, 1.08:3 in PCL-36, and finally reached 1.09:3 in PCL-48, with the oxygen ratio increasing as the reaction duration increased (Figure 2e). The increase in oxygen content provided direct evidence of oxidation and indicated the introduction of hydroxyl, carbonyl, and/or carboxyl groups in PCL-O membranes. The C 1s XPS spectra showed that, after modification, the C−C peak area decreased, and the C−O−C (C−O−H) peak area increased. Additionally, a new C═O peak appeared in the modified PCL. The PCL initially has 66.7% alkyl carbon, and this number decreased by 7% in PCL-36 (Figure 2f). The reduction was accompanied by increases in the C−O−C/C−O−H and C═O peaks, indicating the introduction of hydroxyl and carbonyl groups to the alkyl carbon in the PCL backbones.

Surface hydrophilicity is an important factor that influences cell adhesion and proliferation on biomaterials. In this study, water contact angle measurements were carried out to investigate the surface hydrophilicity of PCL membranes before and after each modification step. The results showed significant differences in contact angles between the unmodified and modified PCL membranes, except for the O-PCL membranes (Figure 2d, Figure S5). The contact angle decreased from 133 ± 1.3° on PCL membranes to 130 ± 1.3°, 120 ± 1.8°, 120 ± 1.6°, 119 ± 1.1°, and 118 ± 1.0°, for acid-treated PCL, PCL-6, PCL-18, PCL-24, PCL-48 respectively,

https://doi.org/10.1021/acsmaterialsau.3c00027
ACS Mater. Au 2023, 3, 540−547

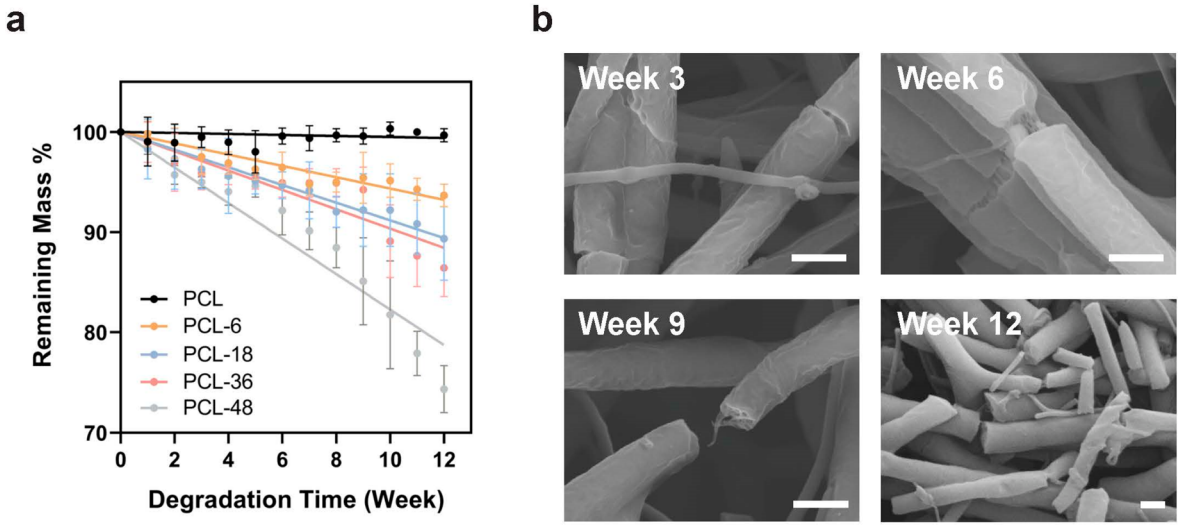


Figure 3. Accelerated degradation studies performed in 1% H_2O_2 in simulated body fluid (SBF) at 37 °C. (a) Degradation profiles of post-modified poly(ε-caprolactone) (PCL-O) reported in % remaining mass. (b) Scanning electron microscopy (SEM) images of crack development in PCL-48 membranes at degradation week 3, week 6, week 9, and week 12. Scale bars = 1 μm.

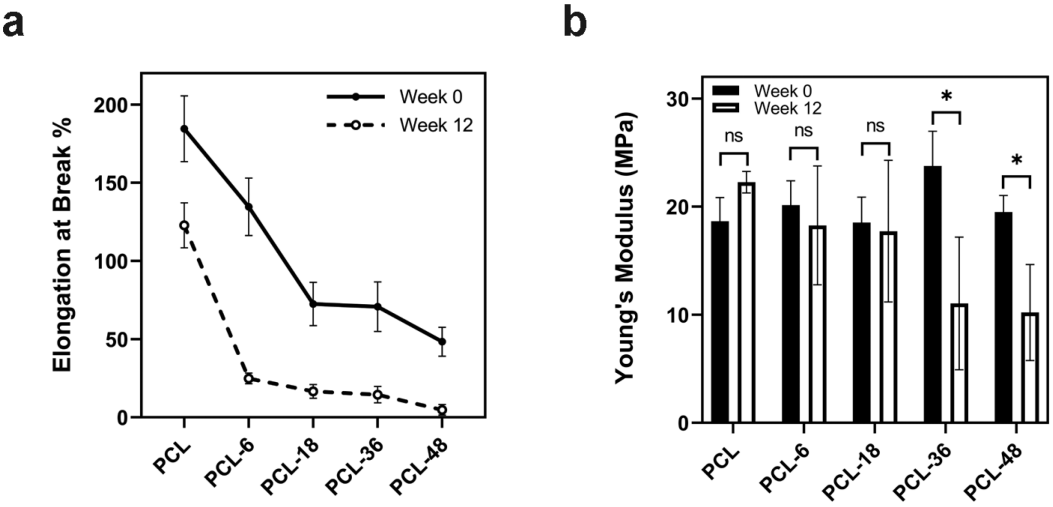


Figure 4. Mechanical characterization of poly(ε-caprolactone) (PCL) and post-modified PCL (PCL-O) on week 0 and week 12 of degradation: (a) Elongation at break. (b) Young's modulus. *p < 0.05.

indicating increased surface hydrophilicity after treatments. These results are consistent with the XPS analysis, which indicated the introduction of hydrophilic hydroxyl and carbonyl groups to the PCL backbone. We note that PCL is a semicrystalline polymer, and the $KMnO_4$ treatment can partially dissolve the amorphous regions of the PCL, resulting in rough surfaces on the PCL membrane. In contrast, O-PCL had similar contact angles to PCL. This result can be attributed to the surface roughness created by the presence of beaded fibers and the lower abundance of hydrophilic functional groups on the surface, as compared to PCL-O. After redissolution, the polymer chains are reoriented, and most of the modified hydrophilic groups are buried *inside* the electrospun fibers. As a result, the concentrations of hydrophilic groups on the surfaces of the O-PCL membranes are diluted, making the contact angles similar to those of PCL. Due to the rough surface and poor hydrophobicity of O-PCL, we eliminated this group from further studies. Overall, the contact angle measurements provide evidence that the chemical modification of PCL can significantly increase the hydrophilicity of the PCL membranes, which is beneficial for cell adhesion and tissue engineering applications.

The degradation of PCL and PCL-O membranes was investigated in simulated body fluid (SBF), which closely mimics the pH and ion concentrations of human blood plasma. To accelerate the degradation process, 1% hydrogen peroxide solution was added to the SBF. Over the 12 weeks of degradation, the weight of the untreated PCL membranes remained stable, while the modified PCL membranes showed increased degradation rates compared to untreated PCL membranes. Additionally, the longer treatment led to higher degradation rates, with PCL-48 losing more than 25% of its initial mass in 12 weeks (Figure 3a). This study demonstrates that the degradation rate of PCL can be significantly accelerated by chemical treatment, and the rate can be adjusted based on the length of the reaction. The degradation occurs at ester linkages on the PCL backbone through random hydrolysis. The hydrolytic reaction that occurred during the chemical modification step acts as a predegradation procedure. In addition, the oxidation reaction during the treatment can

https://doi.org/10.1021/acsmaterialsau.3c00027
ACS Mater. Au 2023, 3, 540−547

also accelerate the degradation process. Electron-withdrawing groups, such as hydroxyl and carbonyl attached to the α-carbon, make esters electron-deficient and more easily hydrolyzed. Scanning electron microscopy was also used to monitor the changes in nanofiber morphology every 3 weeks (Figure S1). The process of degradation was confirmed in the form of crack developments in the PCL-48 fibers (Figure 3b), with flaws appearing on smooth fibers at week 3, flaws enlarging and cracks developing at week 6, and fibers breaking at week 9, ultimately leading to fragmentation and disintegration of the membrane by week 12, at which point the degradation study was halted.

To evaluate the mechanical properties of PCL membranes and their changes after degradation, mechanical tests were conducted (Figure 4). Unmodified PCL and all PCL-O electrospun membranes had Young's moduli of ca. 20 MPa. The Young's moduli were not significantly altered by the $KMnO_4$ treatment, which only modified the surfaces of the PCL, targeting the amorphous region before affecting the crystalline region that determines the stiffness of PCL.[39,40] As a result, the chemical modification did not change the crystallinity, nor did it break the electrospun fibers or interrupt their entanglements within the membranes. Therefore, Young's moduli remained stable before and after the reaction, and there was no significant difference between the control and modified PCL membranes. After chemical treatment, elongation at the break of membranes decreased as reaction time increased, indicating that the modification influenced the amorphous regions in the membranes, reducing their extendibility. In addition to causing differences of elongation at the break between groups, varied amounts of affected amorphous areas also result in differences in degradability between PCL membranes treated for different lengths of time. After 12 weeks of degradation, Young's moduli of PCL-36 and PCL-48 significantly decreased to ca. 10 MPa due to polymer dissolution, the detachment of cross-linked fibers, and the breakage of scaffolds. After degradation, membrane elongation at the break also decreased, due to the further weakened amorphous regions and the failure of the electrospun fibers.

The biofunctionality and cytocompatibility of the unmodified and treated membranes were tested by performing in vitro cell studies. Dental pulp stem cells (DPSCs) were cultured on both PCL and PCL-O membranes for 7 days (Figure 5). Confocal images taken on day 1, day 3, and day 7 showed that PCL-O membranes had higher cell viability and better cell adhesion compared to PCL membranes. We attribute the increased cell adhesion to the extra hydroxyl, carbonyl, and carboxyl groups introduced by the chemical modification process. Furthermore, the degree of cell adhesion was observed to increase with the duration of the chemical treatment. The lowest numbers of cells were found on untreated PCL membranes, and cells had rounded morphologies on PCL. However, the numbers of cells increased on PCL-18 and PCL-24 membranes, and cells started to elongate on the latter. The PCL-36 samples showed even more cell adhesion, with cells proliferating and spreading out. The PCL-48 samples had the highest cell confluency and presented the greatest cell adhesion, indicating that the modification procedure enhances the cell adhesion, proliferation, and biofunctional features of PCL and can accelerate tissue regeneration.

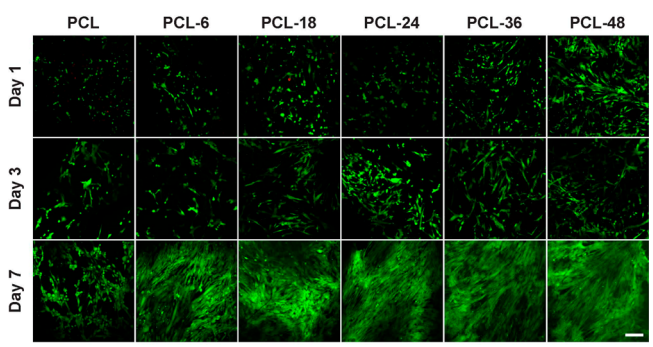


Figure 5. Cell viability of dental pulp stem cells (DPSC) on poly(ε-caprolactone) (PCL) and postmodified PCL (PCL-O) membranes on day 1, day 3, and day 7. Cells were stained with Calcein-AM (green) and ethidium homodimer-1 (red). Scale bar = 100 μm.

CONCLUSIONS AND PROSPECTS

Here, we introduced a method to alter the degradability, mechanical properties, and biofunctionality of electrospun PCL membranes through a one-step, straightforward chemical modification procedure. Our findings demonstrate that chemical modification expands the capabilities of electrospun PCL membranes, with PCL-O membranes showing increased degradability, sustained stiffness, higher biocompatibility, and better cell adhesion. The modification of PCL has broadened its potential application in tissue engineering, including fast-resorbable implant materials. In preliminary studies using a periodontal membrane model, modified PCL has shown promise as a suitable material for periodontium regeneration.[30] Moreover, the use of $KMnO_4$ treatment as an efficient method can be scaled up to treat larger membranes and surfaces. Additionally, the proposed method offers a new solution to the problem of poor electrospinnability of low molecular weight polymers by first creating high molecular weight electrospun membranes and subsequently degrading them controllably.

While this study focused on evaluating properties relevant to tissue engineering applications, further investigations should be conducted to explore additional properties such as crystallinity, porosity, and thermal characteristics of the materials and in vivo studies to evaluate long-term cell adhesion and tissue integration. By monitoring the molecular weight and crystallinity through GPC and X-ray diffraction analyses during the degradation process, mathematical models can be developed to study the degradation mechanism and to predict degradation time. Moreover, in vivo studies should be conducted in order to determine the degradation rate of these membranes when placed in more complex and realistic biological environments. We note that although the degradation rate of PCL-48 was increased compared to its unmodified counterpart, limitations remain for short-term biomedical applications that require still higher degradation rates. Further research aimed at understanding the degradation mechanisms can enable the controlled design of PCL-O with faster degradation rates to broaden its application range.

MATERIALS AND METHODS

Materials

Cell culture and staining reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Poly(ε-caprolactone) (PCL, Mw = 80,000 g/mol), hexafluoroisopropanol (HFIP), potassium permanganate, citric acid, ethanol, tetrahydrofuran (THF), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Periodontal ligament stem cells (PDLSC)

https://doi.org/10.1021/acsmaterialsau.3c00027
ACS Mater. Au 2023, 3, 540−547

and dental pulp stem cells (DPSC) were generously provided by Dr. Bo Yu's lab at University of California, Los Angeles (UCLA). All procedures were approved by the Ethics and Institutional Review Board.

Electrospinning

The PCL was dissolved in HFIP to achieve a 10% w/v solution. The PCL membranes were electrospun using an electrospinning instrument (NE100, Inovenso, Cambridge, MA, USA) with a constant infusion rate of 5 mL/h at 20 kV. Each fabrication used 1 mL of PCL solution and created a membrane with a thickness of ca. 0.05 mm. The membranes were washed with ethanol and Milli-Q water, then stored under vacuum to remove residual solvent.

Chemical Modification

The PCL membranes were immersed in 0.2 M potassium permanganate solution with 1 M sulfuric acid for 6, 18, 24, 36, and 48 h. Modified membranes were washed with 0.1 M citric acid solution overnight and then rinsed with Milli-Q water to remove the residual solvent. The O-PCL membranes were electrospun from PCL that were pretreated with potassium permanganate for 48 h. The obtained membranes were then cut into the desired sizes and subjected to different tests. In order to test the absence of trace amounts of chemicals on the surfaces, we conducted XPS analyses on PCL and PCL-O membranes. The XPS spectra revealed the absence of S, K, and Mn peaks, indicating the potential removal of residual sulfuric acid and potassium permanganate from the near-surface region within a depth of ca. 10 nm. To test the complete removal of these residuals beyond the probed depth of XPS, we performed additional cell viability tests, which were also consistent their absence. Prior to performing the cell studies, membranes were sterilized using 75% ethanol, then dried with nitrogen gas, and finally exposed to ultraviolet light for 30 min. The PCL electrospun membranes were treated with oxygen plasma for 1 min using a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY, USA) to produce PCL-P.

Degradation Study

The degradation behavior of unmodified PCL, O-PCL, PCL-6, PCL-18, PCL24, PCL-36, and PCL-48 membranes was studied in simulated body fluid (SBF) plus 1% H_2O_2 at 37 °C. PCL membranes were cut into 0.5 cm × 2.0 cm ribbons, weighed, and placed into the well of Petri dishes containing the degradation solution. Samples including fragmentations were filtered out weekly, rinsed with water, dried overnight, and weighed. Fresh SBF and H_2O_2 solutions were replaced on a weekly basis, and samples were placed in a fresh solution after each weighing step. The degradation rate was determined by subtracting the initial and weekly weights for samples in each treatment group.

Scanning Electron Microscopy

The morphology of membranes was imaged using SEM (Supra 40VP, Zeiss) at 10 kV with an SE2 detector. Samples from different treatment groups were first mounted on SEM stubs using carbon tape and then sputter coated with approximately 8 nm of gold before imaging.

Gel Permeation Chromatography

The molecular weights of the PCL polymers before and after the chemical modification steps were measured by GPC (LC-2020C 3D, Shimadzu, Kyoto, Japan; Dawn Heleos-II and Optilab T-rEX, Wyatt Technology, Santa Barbara, CA, USA) at room temperature. Tetrahydrofuran was used as an eluent in GPC analysis. Samples were dissolved in THF to make 2 mg/mL solutions. For each measurement, 50 μL of the solution was injected.

Water Contact Angle Measurements

Water contact angles on samples were measured using a contact angle goniometer (FTA1000, First Ten Angstroms, Portsmouth, VA, USA). Samples from different treatment groups were cut into 0.5 cm × 2.0 cm ribbons. Their contact angles were measured using a 20 μL water droplet at room temperature.

X-ray Photoelectron Spectroscopy Measurements

X-ray photoelectron spectroscopy (XPS) was performed (AXIS Ultra DLD, Kratos Analytical Inc., Chestnut Ridge, NY, USA) with a monochromatic Al Kα X-ray source in ultrahigh vacuum to access the surface chemical properties of the PCL membranes before and after the modification steps. Survey spectra were obtained at 12 kV, 10 mA, and 160 eV, while high-resolution spectra were scanned using a 20 eV pass energy.

Mechanical Testing

Electrospun membranes were cut into 0.5 cm × 2.0 cm rectangles and subjected for mechanical testing (Figure S2). Tensile tests were performed on an Instron universal testing system (68CS, Norwood, MA, USA) to determine the tensile stress, which was given by force/cross-section area, and the tensile strain, which was defined as the change in length relative to the initial sample length. Young's modulus was calculated through the slope of the linear part of the stress−strain curve.

In Vitro Cell Studies

Culture media used for DPSCs and DPLSCs were alpha minimal essential medium (α-MEM) containing 15% fetal bovine serum (FBS), 1% GlutaMAX (100×), and 1% Penicillin-Streptomycin (100×). Cells were cultured in an incubator at 37 °C and 5% CO_2 with saturating humidity. Cell viability assay was conducted using LIVE/DEAD Kit for mammalian cells. Live cells were stained with calcein-AM and dead cells were indicated by ethidium homodimer-1. Adherent cells on membranes were incubated in staining solution for 30 min at room temperature and washed with 1× PBS three times. For immunofluorescence staining (Figure S3), DPLSCs were fixed with formaldehyde and blocked using BSA solution. Filamentous actin (F-actin) was labeled with Alexa Fluor 488 phalloidin, and cell nuclei were stained with Hoechst 33342. Fluorescent images of cells on membranes were taken using confocal laser scanning microscopy (SP8-STED/FLIM/FCS, Leica).

Statistical Analyses

Data are presented as mean values ± standard deviation. Contact angles were statistically analyzed using a one-way analysis of variance (ANOVA), and Young's moduli were analyzed using the Student's t-test with a significance level of $p = 0.05$: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmaterialsau.3c00027.

Scanning electron microscopy images of polycaprolactone (PCL) and postoxidized PCL membranes; immunofluorescence of periodontal ligament stem cells (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Maryam Badv − *Department of Biomedical Engineering, Schulich School of Engineering, University of Calgary, Calgary, Alberta T2N 1N4, Canada;* orcid.org/0000-0003-2226-3533; Email: maryam.badv@ucalgary.ca

Alireza Moshaverinia − *California NanoSystems Institute (CNSI) and Weintraub Center for Reconstructive Biotechnology, Section of Prosthodontics, School of Dentistry, University of California, Los Angeles, Los Angeles, California 90095, United States;* orcid.org/0000-0002-1542-6202; Email: amoshaverinia@dentistry.ucla.edu

Paul S. Weiss − *Department of Chemistry and Biochemistry and Department of Bioengineering, University of California,*

Los Angeles, Los Angeles, California 90095, United States; Department of Materials Science and Engineering, University of California Los Angeles, Los Angeles, California 90095, United States; California NanoSystems Institute (CNSI), University of California, Los Angeles, Los Angeles, California 90095, United States; orcid.org/0000-0001-5527-6248; Email: psw@cnsi.ucla.edu

Authors

Jun Shen − *Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90095, United States; California NanoSystems Institute (CNSI), University of California, Los Angeles, Los Angeles, California 90095, United States*

Weihao Yuan − *Weintraub Center for Reconstructive Biotechnology, Section of Prosthodontics, School of Dentistry, University of California, Los Angeles, Los Angeles, California 90095, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmaterialsau.3c00027

Author Contributions

CRediT: **Jun Shen** conceptualization (lead), data curation (lead), formal analysis (lead), investigation (lead), methodology (lead), writing-original draft (lead), writing-review & editing (equal); **Weihao Yuan** conceptualization (supporting), investigation (supporting), writing-review & editing (supporting); **Maryam Badv** conceptualization (lead), data curation (equal), formal analysis (equal), investigation (lead), methodology (lead), validation (equal), visualization (equal), writing-original draft (lead), writing-review & editing (equal); **Alireza Moshaverinia** conceptualization (equal), funding acquisition (equal), methodology (equal), project administration (lead), resources (lead), supervision (lead), writing-review & editing (supporting); **Paul S. Weiss** conceptualization (equal), funding acquisition (lead), investigation (supporting), methodology (equal), project administration (lead), supervision (lead), writing-original draft (equal), writing-review & editing (lead).

Notes

The authors declare the following competing financial interest(s): A.M. and P.S.W. have patents related to this work. The other authors declare no competing financial interests.

■ ACKNOWLEDGMENTS

The authors thank the UCLA Technology Development Group Innovation Fund for support of this work. M.B. acknowledges a Natural Sciences and Engineering Research Council of Canada (NSERC) postdoctoral fellowship. A.M. acknowledges support from the National Institutes of Health National Institute of Dental and Craniofacial Research (grant #DE029876).

■ REFERENCES

(1) Madike, L. N.; Pillay, M.; Popat, K. C. Antithrombogenic Properties of *Tulbaghia violacea*−Loaded Polycaprolactone Nanofibers. *J. Bioact. Compat. Polym.* **2020**, *35*, 102−116.

(2) Formas, K.; Kurowska, A.; Janusz, J.; Szczygieł, P.; Rajzer, I. Injection Molding Process Simulation of Polycaprolactone Sticks for Further 3D Printing of Medical Implants. *Materials* **2022**, *15*, 7295−7308.

(3) Lykins, W. R.; Bernards, D. A.; Schlesinger, E. B.; Wisniewski, K.; Desai, T. A. Tuning Polycaprolactone Degradation for Long Acting Implantables. *Polymer* **2022**, *262*, 125473−125480.

(4) Wiggenhauser, P. S.; Balmayor, E. R.; Rotter, N.; Schantz, J. T. *In Vivo* Evaluation of a Regenerative Approach to Nasal Dorsum Augmentation with a Polycaprolactone-Based Implant. *Eur. J. Med. Res.* **2019**, *24*, 6.

(5) Waterkotte, T.; He, X.; Wanasathop, A.; Li, S. K.; Park, Y. C. Long-Term Antibody Release Polycaprolactone Capsule and the Release Kinetics in Natural and Accelerated Degradation. *ACS Biomater. Sci. Eng.* **2022**, *8*, 4428−4438.

(6) Zhu, B.; Chen, Y.; Lu, W.; Zhang, Q.; Gao, S.; Sun, L.; Chen, S.; Hu, R. A Biodegradable Long-Term Contraceptive Implant with Steady Levonorgestrel Release Based on PLGA Microspheres Embedded in PCL-Coated Implant. *J. Drug. Delivery Sci. Technol.* **2022**, *67*, 102955.

(7) Kim, J.; Kudisch, M.; da Silva, N. R. K.; Asada, H.; Aya-Shibuya, E.; Bloomer, M. M.; Mudumba, S.; Bhisitkul, R. B.; Desai, T. A. Long-Term Intraocular Pressure Reduction with Intracameral Polycaprolactone Glaucoma Devices That Deliver a Novel Anti-Glaucoma Agent. *J. Controlled Release* **2018**, *269*, 45−51.

(8) Ahmad Wsoo, M.; Izwan Abd Razak, S.; Shahir, S.; Ahmed Abdullah Al-Moalemi, H.; Rafiq Abdul Kadir, M.; Hasraf Mat Nayan, N. Development of Prolonged Drug Delivery System Using Electrospun Cellulose Acetate/Polycaprolactone Nanofibers: Future Subcutaneous Implantation. *Polym. Adv. Technol.* **2021**, *32*, 3664−3678.

(9) Miszuk, J. M.; Xu, T.; Yao, Q.; Fang, F.; Childs, J. D.; Hong, Z.; Tao, J.; Fong, H.; Sun, H. Functionalization of PCL-3D Electrospun Nanofibrous Scaffolds for Improved BMP2-Induced Bone Formation. *Appl. Mater. Today* **2018**, *10*, 194−202.

(10) Domingos, M.; Intranuovo, F.; Gloria, A.; Gristina, R.; Ambrosio, L.; Bártolo, P. J.; Favia, P. Improved Osteoblast Cell Affinity on Plasma-Modified 3-D Extruded PCL Scaffolds. *Acta. Biomater.* **2013**, *9*, 5997−6005.

(11) Rajan, R. K.; Chandran, S.; Sreelatha, H. V.; John, A.; Parameswaran, R. Pamidronate-Encapsulated Electrospun Polycaprolactone-Based Composite Scaffolds for Osteoporotic Bone Defect Repair. *ACS Appl. Bio. Mater.* **2020**, *3*, 1924−1933.

(12) Xing, Y.; Gu, Y.; Guo, L.; Guo, J.; Xu, Z.; Xiao, Y.; Fang, Z.; Wang, C.; Feng, Z. G.; Wang, Z. Gelatin Coating Promotes *in Situ* Endothelialization of Electrospun Polycaprolactone Vascular Grafts. *J. Biomater. Sci. Polym. Ed.* **2021**, *32*, 1161−1181.

(13) Duan, H. Y.; Ye, L.; Wu, X.; Guan, Q.; Yang, X. F.; Han, F.; Liang, N.; Wang, Z. F.; Wang, Z. G. The *in Vivo* Characterization of Electrospun Heparin-Bonded Polycaprolactone in Small-Diameter Vascular Reconstruction. *Vascular* **2015**, *23*, 358−365.

(14) Tran, N.; Le, A.; Ho, M.; Dang, N.; Thi Thanh, H. H.; Truong, L.; Huynh, D. P.; Hiep, N. T. Polyurethane/Polycaprolactone Membrane Grafted with Conjugated Linoleic Acid for Artificial Vascular Graft Application. *Sci. Technol. Adv. Mater.* **2020**, *21*, 56−66.

(15) Pillay, V.; Seedat, A.; Choonara, Y. E.; Du Toit, L. C.; Kumar, P.; Ndesendo, V. M. K. A Review of Polymeric Refabrication Techniques to Modify Polymer Properties for Biomedical and Drug Delivery Applications. *AAPS PharmSciTechnol.* **2013**, *14*, 692−711.

(16) Desai, S. M.; Singh, R. P. Surface Modification of Polyethylene. *Adv. Polym. Sci.* **2004**, *169*, 231−293.

(17) Dakshinamoorthy, D.; Peruch, F. Block and Random Copolymerization of ε-Caprolactone, *L*-, and *Rac*-Lactide Using Titanium Complex Derived from Aminodiol Ligand. *J. Polym. Sci. A. Polym. Chem.* **2012**, *50*, 2161−2171.

(18) Lopez de Dicastillo, C.; Garrido, L.; Velasquez, E.; Rojas, A.; Gavara, R. Designing Biodegradable and Active Multilayer System by Assembling an Electrospun Polycaprolactone Mat Containing Quercetin and Nanocellulose between Polylactic Acid Films. *Polymers* **2021**, *13*, 1288−1300.

(19) Aminatun; Huriah, R.; Hikmawati, D.; Hadi, S.; Amrillah, T.; Abdullah, C. A. C. Nanofiber Scaffold Based on Polylactic Acid-

Polycaprolactone for Anterior Cruciate Ligament Injury. *Polymers* **2022**, *14*, 2983−995.
(20) Rani, S.; Gupta, U. Synthesis, Morphology, and Rheological Evaluation of HPMA (*N*-2-Hydroxypropyl Methacrylamide)-PCL (Polycaprolactone) Conjugates. *ACS Omega* **2021**, *6*, 29788−29803.
(21) Dodero, A.; Alloisio, M.; Castellano, M.; Vicini, S. Multilayer Alginate-Polycaprolactone Electrospun Membranes as Skin Wound Patches with Drug Delivery Abilities. *ACS Appl. Mater. Interfaces* **2020**, *12*, 31162−31171.
(22) Echeverria Molina, M. I.; Chen, C. A.; Martinez, J.; Tran, P.; Komvopoulos, K. Novel Electrospun Polycaprolactone/Calcium Alginate Scaffolds for Skin Tissue Engineering. *Materials* **2023**, *16*, 136−153.
(23) Averous, L.; Moro, L.; Dole, P.; Fringant, C. Properties of Thermoplastic Blends: Starch−Polycaprolactone. *Polymer* **2000**, *41*, 4157−4167.
(24) Koenig, M. F.; Huang, S. J. Biodegradable Blends and Composites of Polycaprolactone and Starch Derivatives. *Polymer* **1995**, *36*, 1877−1882.
(25) Fereshteh, Z.; Fathi, M.; Bagri, A.; Boccaccini, A. R. Preparation and Characterization of Aligned Porous PCL/Zein Scaffolds as Drug Delivery Systems *via* Improved Unidirectional Freeze-Drying Method. *Mater. Sci. Eng., C* **2016**, *68*, 613−622.
(26) Gupta, D.; Singh, A. K.; Kar, N.; Dravid, A.; Bellare, J. Modelling and Optimization of NaOH-Etched 3-D Printed PCL for Enhanced Cellular Attachment and Growth with Minimal Loss of Mechanical Strength. *Mater. Sci. Eng., C* **2019**, *98*, 602−611.
(27) Singh, N. L.; Qureshi, A.; Mukherjee, S.; Rakshit, A. K.; Tripathi, A.; Avasthi, D. K. Surface Modification of Polymeric Blends by Nitrogen Plasma Implantation. *Surf. Coat. Technol.* **2007**, *201*, 8278−8281.
(28) Martins, A.; Pinho, E. D.; Faria, S.; Pashkuleva, I.; Marques, A. P.; Reis, R. L.; Neves, N. M. Surface Modification of Electrospun Polycaprolactone Nanofiber Meshes by Plasma Treatment to Enhance Biological Performance. *Small* **2009**, *5*, 1195−1206.
(29) Zhu, Y.; Gao, C.; Liu, X.; Shen, J. Surface Modification of Polycaprolactone Membrane via Aminolysis and Biomacromolecule Immobilization for Promoting Cytocompatibility of Human Endothelial Cells. *Biomacromolecules* **2002**, *3*, 1312−1319.
(30) Hasani-Sadrabadi, M. M.; Sarrion, P.; Nakatsuka, N.; Young, T. D.; Taghdiri, N.; Ansari, S.; Aghaloo, T.; Li, S.; Khademhosseini, A.; Weiss, P. S.; Moshaverinia, A. Hierarchically Patterned Polydopamine-Containing Membranes for Periodontal Tissue Engineering. *ACS Nano* **2019**, *13*, 3830−3838.
(31) Koski, A.; Yim, K.; Shivkumar, S. Effect of Molecular Weight on Fibrous PVA Produced by Electrospinning. *Mater. Lett.* **2004**, *58*, 493−497.
(32) Suwantong, O. Biomedical Applications of Electrospun Polycaprolactone Fiber Mats. *Polym. Adv. Technol.* **2016**, *27*, 1264−1273.
(33) Greiner, A.; Wendorff, J. H. Electrospinning: A Fascinating Method for the Preparation of Ultrathin Fibers. *Angew. Chem., Int. Ed.* **2007**, *46*, 5670−5703.
(34) Venugopal, J.; Zhang, Y. Z.; Ramakrishna, S. Electrospun Nanofibres: Biomedical Applications. *Proc. Inst. Mech. Eng.* **2004**, *218*, 35−45.
(35) Sabino, M. A. Oxidation of Polycaprolactone to Induce Compatibility with Other Degradable Polyesters. *Polym. Degrad. Stab.* **2007**, *92*, 986−996.
(36) Agarwal, S.; Wendorff, J. H.; Greiner, A. Use of Electrospinning Technique for Biomedical Applications. *Polymer* **2008**, *49*, 5603−5621.
(37) Rahmati, M.; Mills, D. K.; Urbanska, A. M.; Saeb, M. R.; Venugopal, J. R.; Ramakrishna, S.; Mozafari, M. Electrospinning for Tissue Engineering Applications. *Prog. Mater. Sci.* **2021**, *117*, 100721.
(38) Bhardwaj, N.; Kundu, S. C. Electrospinning: A Fascinating Fiber Fabrication Technique. *Biotechnol. Adv.* **2010**, *28*, 325−347.
(39) Shahin, M. M.; Olley, R. H.; Blissett, M. J. Refinement of Etching Techniques to Reveal Lamellar Profiles in Polyethylene Banded Spherulites. *J. Polym. Sci. B Polym. Phys.* **1999**, *37*, 2279−2286.
(40) Abisha, M.; Priya, R. K.; Arunachalam, K. P.; Avudaiappan, S.; Saavedra Flores, E. I.; Parra, P. F. Biodegradable Green Composites: Effects of Potassium Permanganate (KMnO4) Treatment on Thermal, Mechanical, and Morphological Behavior of Butea Parviflora (BP) Fibers. *Polymers* **2023**, *15*, 2197−2212.
(41) Pirzada, T.; Farias, B. V.; Chu, H. M. A.; Khan, S. A. Fabrication of Guar-Only Electrospun Nanofibers by Exploiting a High- and Low-Molecular Weight Blend. *ACS Omega* **2019**, *4*, 10767−10774.
(42) Subrahmanya, T. M.; Arshad, A. B.; Lin, P. T.; Widakdo, J.; Makari, H. K.; Austria, H. F. M.; Hu, C.; Lai, J.; Hung, W. A Review of Recent Progress in Polymeric Electrospun Nanofiber Membranes in Addressing Safe Water Global Issues. *RSC Adv.* **2021**, *11*, 9638−9663.
(43) Fong, H.; Chun, I.; Reneker, D. H. Beaded Nanofibers Formed during Electrospinning. *Polymer* **1999**, *40*, 4585−4592.

https://doi.org/10.1021/acsmaterialsau.3c00027
ACS Mater. Au 2023, 3, 540−547