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Alternating field electrospinning of blended fish gelatin/poly(ε-caprolactone) nanofibers

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Abstract

Blended nanofibrous biomaterials from natural and synthetic sources show promise for better biointegration. This study explores high-yield alternating field electrospinning (AFES) of blended cold-water fish skin gelatin (FGEL) and polycaprolactone (PCL) nanofibrous meshes with up to 30 wt% PCL at 7.8–14.4 g/h fiber productivity, depending on the composition. FGEL/PCL nanofibers reveal smooth surface morphology and 237–313 nm average diameters after thermal crosslinking. FTIR analysis indicated little FGEL/PCL interaction and notable changes in PCL crystallinity in the crosslinked nanofibers. A 14-days *in-vitro* analysis shows good cellular viability and nanofibrous FGEL/PCL mesh stability. Results demonstrate that AFES provides efficient, scalable production of blended FGEL/PCL nanofibrous biomaterials with suitable characteristics.

Keywords: AC Electrospinning, Nanofibers, Fish Gelatin, Polycaprolactone, Biomaterials

1. Introduction

Fish gelatin (FGEL), partly in the form of nanofibers, is gaining significant interest in biomedical applications [1] but its composition and properties differ from those of gelatin from mammal sources [2]. Gelatin blends with other biopolymers have been used to develop new nanofibrous biomaterial combinations with desirable and tunable characteristics [3], however, reports on blended fish gelatin/PCL nanofibrous materials are scarce [4]. Most studies employed direct current (DC) electrospinning to fabricate gelatin-based nanofibrous materials. Sizeable and sustainable fabrication of gelatin-based nanofibers, which is critical for economic feasibility of such biomaterials, remains a challenge. Pristine FGEL and PCL nanofibers were prepared at >10

g/h productivity by using alternating field electrospinning (AFES, a.k.a. AC-electrospinning) [5-7], but there are no reports on AFES of FGEL/PCL nanofibers. This study exploits for the first time the AFES capability for the high throughput manufacturing of blended FGEL/PCL nanofibrous meshes with up to 30 wt% of PCL content.

2. Experimental

2.1. Materials

The materials included polycaprolactone (80,000 MW), cold water fish skin gelatin (both from Sigma-Aldrich), glacial acetic acid (AA, Alfa Aesar), sodium acetate (NaAc, LACHEMA), mouse fibroblasts (NIH/3T3 Swiss Albino, ATCC, CZ), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin/amphotericin B (PSAB) (all from Lonza, CH).

2.2. Precursor Synthesis

A 30 wt% FGEL solution was prepared in 95:5 v/v AA/DI water, whereas 20 wt% PCL solution was prepared in AA only. Stable precursors with 0.9:0.1, 0.8:0.2, and 0.7:0.3 FGEL/PCL mass ratios were made by mixing the FGEL and PCL solutions under magnetic stirring. NaAc (1 wt% with respect to polymer) was added to improve spinnability [6]. The precursors with higher PCL content become foggy due to poor miscibility with FGEL, and quickly lose spinnability due to PCL degradation.

2.3. Precursor Parameters

Viscosity tests at shear rates up to 1000 s^{-1} (HAAKE RotoVisco 1, Thermo Scientific) were performed in a liquid rod-cup configuration. Steady-state conductivity values were recorded using Eutech Instruments CON510 Bench Conductivity meter. Tests were run in triplet (viscosity) and quintet (conductivity).

2.4. Precursor flow rate and process productivity

Precursor flow (R_f, $mL \cdot min^{-1}$) and fiber production (R_p, $g \cdot h^{-1}$) rates were calculated using Equation 1 and 2, respectively.

$$R_f = \frac{V_e}{t} \quad (1)$$

$$R_p = \frac{m_{nf}}{t} \quad (2)$$

where V_e, m_{nf} and t are the precursor volume, fiber mass and time, respectively.

2.5. Nanofibers fabrication

FGEL/PCL precursors were spun using two AFES devices [5-7] (Fig.1a) at 20–35 kV rms AC voltage (either 50 or 60 Hz) at 20–23°C and 35–55% humidity using different spinnerets (Fig.1c) to determine their effect on the process performance. A rotating cylindrical plastic collector was used to form 0.1–2.2 mm-thick nanofibrous meshes (Fig.1d,e).

Meshes were thermally treated between 100 to 160 °C for 4 h using Panasonic Dry Heat Sterilizer MOV-212S-PE to crosslink and sterilize the material. To maintain sterility, cell study samples were prepared and treated within sterile sealed glass petri dishes.

2.6. FTIR analysis

FTIR spectroscopy was performed using Bruker Vertex 70 FT-IR Spectrometer in transmission mode at 2 cm^{-1} resolution.

2.7. Scanning electron microscopy imaging

A scanning electron microscope (SEM, Tescan, VEGA3 SB) was employed at 20 kV accelerating voltage and SEM images were analyzed by ImageJ. Samples were sputter-coated with Au-Pd (Denton Vacuum Desk II) to prevent charging.

2.8. Cellular studies

In vitro studies were conducted for 14 days using 3T3 mouse fibroblasts in concentration $1.0 \cdot 10^4$/well in 24-well plates maintained in DMEM+10% (v/v) FBS+1% (v/v) PSAB. Cells were cultured in an incubator (37°C, 5% CO_2). Medium was exchanged 3×/week, and the second passage was used for following experiments. Crosslinked/sterilized samples were cut to fit 24-well plates. Cell counting kit-8 (CCK8, Dojindo) assessed viability following Dojindo manufacturer procedure methods respective to cell plating density. Tecan SPARK® Multimode Microplate Reader (450 nm absorbance) analyzed samples and exported files for manual statistical analysis.

3. Results and Discussion

3.1. AFES of FGEL/PCL nanofibers

AC voltage (28–30 kV rms), temperature (22 °C) and humidity (>30%) were the best for AFES of all tested precursors despite the variations in viscosity and electrical conductivity (Table 1). The fiber productivity increased proportionally to the spinneret diameter, with 25-mm spinneret produced ×2 the fiber mass than with 12.5-mm spinneret, and fibrous flow remained consistent in appearance and efficacy in all cases (Fig.1c). The precursor composition dependent variations in the flow rate and fiber productivity, as well as in fiber diameter are ascribed to the opposing trends in precursor viscosity and electrical conductivity. The poorer FGEL/PCL miscibility with increased PCL concentration [8] also led to some drop in viscosity of 0.7:0.3 FGEL/PCL precursor. A combined effect of strong decrease in the electrical conductivity, moderate increase in viscosity and, probably, polarizability of FGEL and PCL molecules resulted in the changes in total electric force acting on the precursor and generated liquid jets, which led to the observed flow rates and fiber productivities. The average diameters of blended FGEL/PCL nanofibers correlated with

precursor viscosity. Larger diameter of pristine FGEL nanofibers is probably due to a lower total electric force that led to smaller elongation of the propagating liquid precursor jets in AFES, whereas opposite behavior was observed for pristine PCL nanofibers.

3.2. FGEL/PCL nanofibers analysis

3.2.1. Fiber diameter and morphology

SEM imaging showed that FGEL/PCL nanofibrous meshes were similar to those of pristine FGEL in terms of the fiber uniformity, smooth surface morphology, and random orientation (Fig.2a-d). The average FGEL/PCL fiber diameter varied from 237 to 313 nm, which is about half of that in FGEL fibers. No significant difference in fiber morphology and diameter was noted between as spun and crosslinked samples.

3.2.2. Molecular structure

FTIR spectra of 100 °C crosslinked meshes and as spun PCL are shown in Fig.2e. Amide-I band (1652 cm^{-1}) of gelatin slightly narrows with the increasing PCL content in blended fibers. The 1193 cm^{-1} peak of PCL becomes more pronounced in the heat-treated FGEL/PCL nanofibers, while the intensity of 1193/1160 cm^{-1} band reduces with respect to 1723 cm^{-1} peak, likely because of changes in crystalline domains' orientation due to melting and recrystallization of PCL fraction. Fig.2f-i shows PCL 1723 cm^{-1} band fitting with three Gaussians centered at 1733 cm^{-1} (vibrations in amorphous phase), 1720 cm^{-1} (vibrations in crystalline phase), and 1705–1710 cm^{-1} (CH bonding). PCL crystallinity X_c was determined as $X_c = I_{1720} \times 100\% / (I_{1720} + \gamma I_{1733})$, where I_{1720} and I_{1733} are the intensities of corresponding Gaussians, and the absorption coefficient $\gamma = 1.46 \pm 0.03$ [9]. The calculation revealed 49.2, 31.3, 46.2, and 45.9% (all ±1.2%) crystallinity for PCL and 0.9:0.1, 0.8:0.2, and 0.7:0.3 FGEL/PCL blends, respectively. Crystalline phase formation could be hindered when PCL is better miscible with gelatin in 0.9:0.1 FGEL/PCL material. The crosslinking at 160 °C increases PCL crystallinity by 2–3%. The PCL crystallinity in FGEL/PCL nanofibers is likely due to PCL chains aggregation in precursors at higher PCL content. Such aggregates can crystallize easily in the heat-treated FGEL/PCL material.

3.3. Cellular Response

The cells attachment and proliferation on crosslinked nanofibrous pristine FGEL (Fig.3a) occurred similarly to control. CCK8 assays showed cell viability with FGEL nanofibers similar to positive control (Fig.3b). However, FGEL nanofibrous substrate dissolved completely after 7 days in culture medium. Cell proliferated more gradually on 0.8:0.2 and 0.7:0.3 FGEL/PCL nanofibrous substrates (Fig.3c,d) due to slower dissolution of FGEL fraction. They attained elongated shape due to a better substrate stability and less fiber swelling (Fig.3c, inset), which promoted cell growth along the nanofiber bundles. Visualization of entire cell bodies via PI fluorescent images reveal good cell coverage after 14 days on both 0.8:0.2 and 0.7:0.3 FGEL/PCL substrates (Fig.3d,e). Cellular actin (green) and nuclei (blue) stained DAPI-Phal images (Fig.3f,g) showed a web-like green fluorescence background from nonspecific binding to FGEL fraction in FGEL/PCL nanofibers (Fig.3f, inset) indicating that the substrate remains almost intact.

4. Conclusions

Successful facile, scalable AFES production of blended FGEL/PCL nanofibrous materials exhibiting cellular response suitable for biomaterials has been achieved. Limited miscibility and interaction of FGEL and PCL in the precursors and in blended nanofibers can be of interest for future studies on fabrication of blended polymeric biomaterials with tailored molecular structure and properties.

CRediT authorship contribution statement

Hannah Lacy: Conceptualization, Writing – original draft. Věra Jenčová: Methodology, Investigation, David Lukáš: Data curation; Andrei Stanishevsky: Formal Analysis, Writing – review & editing.

Competing Interests

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.

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References

1. H.W. Kwak, H. Woo, I.C. Kim, K.H. Lee, Fish gelatin nanofibers prevent drug crystallization and enable ultrafast delivery, RSC Adv. 7 (2017) 40411-40417.
2. A.M. Soliman, S.L. Teoh, S. Das, Fish Gelatin: Current Nutritional, Medicinal, Tissue Repair Applications, and as a Carrier of Drug Delivery, Curr. Pharm. Des. 28 (2022) 1019-1030.
3. Â. Semitela, A. F. Girão, C. Fernandes, G. Ramalho, I. Bdikin, A. Completo, P. A. Marques, Electrospinning of bioactive polycaprolactone-gelatin nanofibres with increased pore size for cartilage tissue engineering applications. J. Biomater. Appl., 35 (2020) 471-484.
4. V. Perez-Puyana, P. Wieringa, Y. Yuste, F. de la Portilla, A. Guererro, A. Romero, L. Moroni, Fabrication of hybrid scaffolds obtained from combinations of PCL with gelatin or collagen via electrospinning for skeletal muscle tissue engineering. J. Biomed. Mater. Res. A 109 (2021) 1600-1612.
5. A. Kennell, M. MacEwen, M. Armstrong, T. Nicola, B. Halloran, N. Ambalavanan, A. Stanishevsky, Fish skin gelatin nanofibrous scaffolds spun using alternating field

electrospinning and *in-vitro* tested with tdTomato mice fibroblasts. Mater. Today Comm. 31 (2022) 103417.
6. C. Lawson, A. Stanishevsky, M. Sivan, P. Pokorny, D. Lukáš, Rapid fabrication of poly(ε-caprolactone) nanofibers using needleless alternating current electrospinning. J. Appl. Polym. Sci. 133 (2016) 43232.
7. M. Sivan, D. Madheswaran, S. Hauzerova, V. Novotny, V. Hedvicakova, V. Jencova, E. K. Kostakova, M. Schindler, D. Lukas. AC electrospinning: Impact of high voltage and solvent on the electrospinnability and productivity of polycaprolactone electrospun nanofibrous scaffolds. Mater. Today Chem. 26 (2022) 101025.
8. D. Kolbuk, P. Sajkiewicz, P. Denis, E. Choinska, Investigations of polycaprolactone/gelatin blends in terms of their miscibility. Bul. Polish Acad. Sci-Tech. 61 (2013) 629–632.
9. Y. He, Y. Inoue, Novel FTIR method for determining the crystallinity of poly(ε-caprolactone), Polym. Int. 49 (2000) 623-626.

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Table 1. Characteristics of FGEL/PCL precursors, process, and nanofibers

Composition	Conductivity (μS/cm)	Viscosity (mPa·s)	Flow rate (mL/min)	Fiber productivity (g/h)	Average fiber Ø (nm)
FGEL	629.75±5.2	325±5	0.600	10.80	534±308
FGEL/PCL=0.9:0.1	382.75±43.4	344±15	0.462	7.81	237±63
FGEL/PCL=0.8:0.2	365±9.6	372±15	0.598	9.72	313±141
FGEL/PCL=0.7:0.3	230.25±8.5	358±8	0.910	14.36	277±84
PCL	6.53±0.13	600±40	1.577	18.94	268±144

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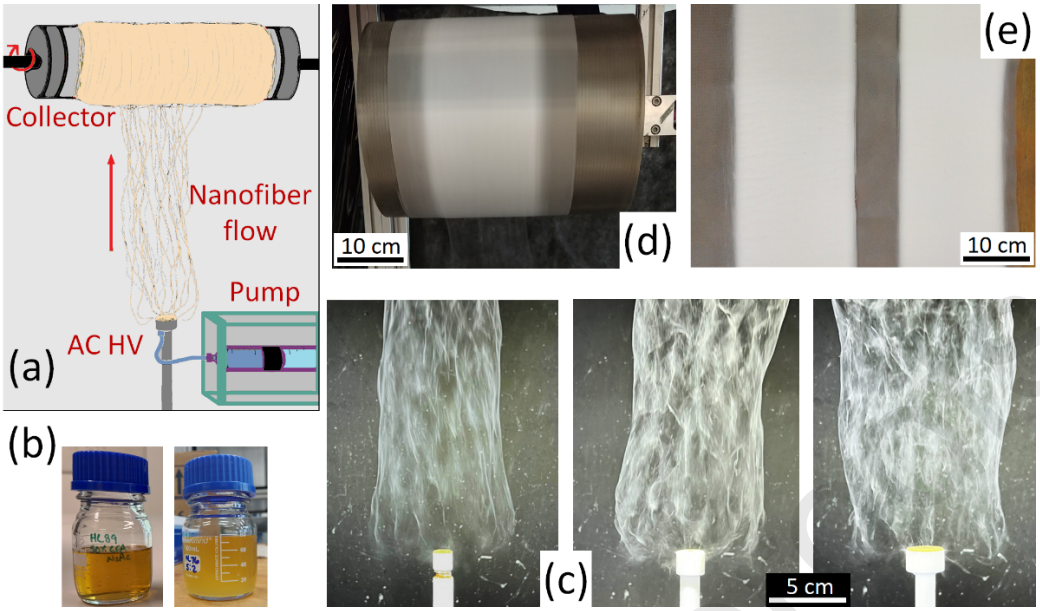

Figure 1. (a) Simplified AFES schematic, (b) FGEL and 0.8:0.2 FGEL/PCL precursors, (c) fibrous flow with 12.5, 19 and 25 mm spinneret diameters, and (d,e) collected FGEL/PCL nanofibrous meshes (~20 cm width).

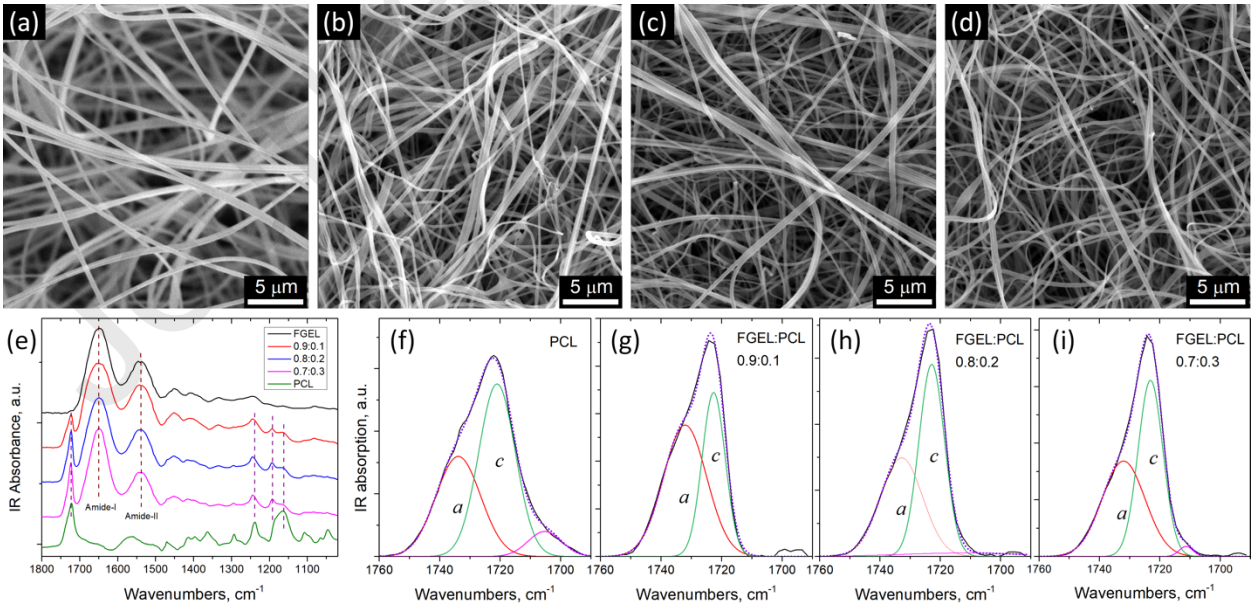

Figure 2. SEM images of (a) pristine FGEL, (b-d) 0.9:0.1, 0.8:0.2 and 0.7:0.3 FGEL/PCL nanofibers; (e) FTIR spectra of as-spun PCL and crosslinked FGEL and FGEL/PCL nanofibers, (f-i) fittings of PCL IR absorption band at 1723 cm^{-1}.

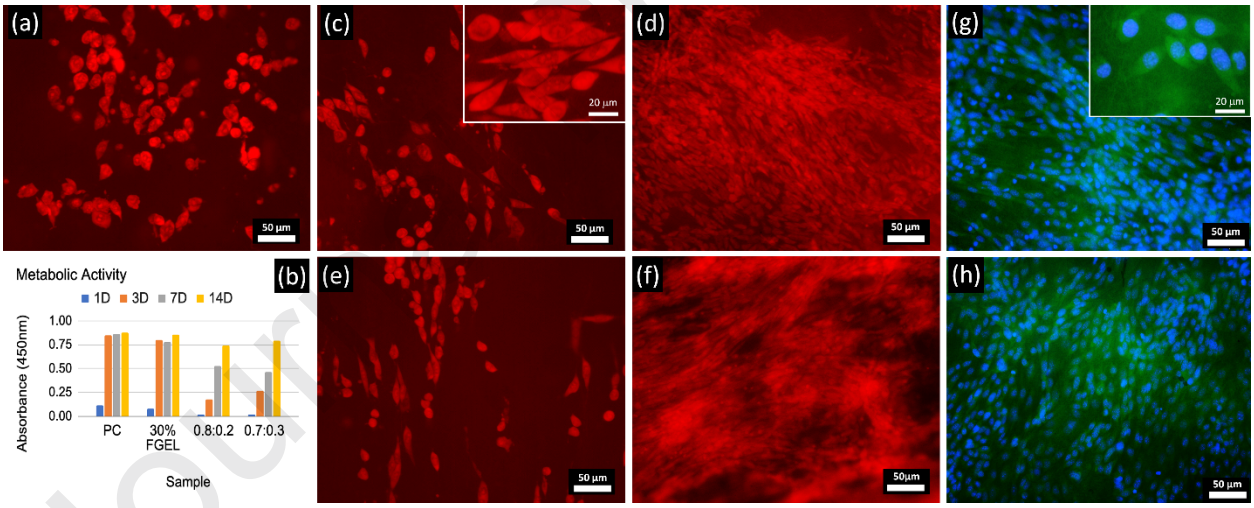

Figure 3. Representative PI (a, c-f) and DAPI/Phal (g,h) fluorescence images of 3T3 cells on (a) FGEL, 1 day, (c,d,g) 0.8:0.2 and (e,f,h) 0.7:0.3 FGEL/PCL nanofibers after 3 (c,e) and 14 (d,f-h) days; (b) metabolic activity over 14 days.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered

as potential competing interests:

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Alternating current (AC) electrospinning of poorly miscible biopolymers reported;

Several grams per hour productivity achieved for fish gelatin and PCL blended nanofibers;

PCL content in fish gelatin nanofibers up to 30 wt% is reached;

Promising cell viability data for blended fish gelatin/PCL nanofibrous scaffolds obtained.

CRediT authorship contribution statement

Hannah Lacy: Conceptualization, Writing – original draft. Věra Jenčová: Methodology, Investigation, David Lukáš: Data curation; Andrei Stanishevsky: Formal Analysis, Writing – review & editing.

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