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# Pneumatospinning of collagen microfibers from benign solvents

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## Pneumatospinning of collagen microfibers from benign solvents

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Supplementary material for this article is available [online](#)

## Abstract

**Introduction.** Current collagen fiber manufacturing methods for biomedical applications, such as electrospinning and extrusion, have had limited success in clinical translation, partially due to scalability, cost, and complexity challenges. Here we explore an alternative, simplified and scalable collagen fiber formation method, termed ‘pneumatospinning,’ to generate submicron collagen fibers from benign solvents. **Methods and results.** Clinical grade type I atelocollagen from calf corium was electrospun or pneumatospun as sheets of aligned and isotropic fibrous scaffolds. Following crosslinking with genipin, the collagen scaffolds were stable in media for over a month. Pneumatospun collagen samples were characterized using Fourier-transform infrared spectroscopy, circular dichroism, mechanical testing, and scanning electron microscopy showed consistent fiber size and no deleterious chemical changes to the collagen were detected. Pneumatospun collagen had significantly higher tensile strength relative to electrospun collagen, with both processed from acetic acid. Stem cells cultured on pneumatospun collagen showed robust cell attachment and high cytocompatibility. Using DMSO as a solvent, collagen was further co-pneumatospun with poly(D,L-lactide) to produce a blended microfibrillar biomaterial. **Conclusions.** Collagen microfibers are shown for the first time to be formed using pneumatospinning, which can be collected as anisotropic or isotropic fibrous grafts. Pneumatospun collagen can be made with higher output, lower cost and less complexity relative to electrospinning. As a robust and rapid method of collagen microfiber synthesis, this manufacturing method has many applications in medical device manufacturing, including those benefiting from anisotropic microstructures, such as ligament, tendon and nerve repair, or for applying microfibrillar collagen-based coatings to other materials.

## 1. Introduction

Working with biological polymers, such as type I collagen, manufacturing of commercialized medical devices has been largely limited to crude and subtractive (top-down) manufacturing methods, such as lyophilizing, hydrogel formation, allograft or xenograft processing (e.g. decellularization of skin and milling of bone). However, the bottom-up assembly from the near molecular through the micro- and meso-scales to macroscale of collagen-based products have recently gained significant interest. Bottom-up manufacturing allows for more control of the

manufactured material physical characteristics, such as fiber and pore size, mechanical properties, and further offers overall improved consistency relative to many of the top-down approaches currently used, particularly regarding features at the small-micron and nanoscale.

Electrospinning is a well-established bottom-up fiber production method dating back to at least 1900 [1]. Electrospinning of polymers from liquid solutions is a technique that has been used widely in the generation of three-dimensional (3D) fibrous scaffolds for tissue engineering applications [2–8]. This technology allows formation of fibers with diameters ranging

from tens of nanometers to several microns. The small fibers produced by electrospinning provide a large surface-to-volume ratio and an interconnected pore structure with high permeability, both of which are desirable in a biological setting. The electrospinning process is not classically considered an additive manufacturing technique due to the dynamic and often uncontrolled nature of fiber deposition [9]. However, the combination of electrospinning and additive manufacturing approaches has been utilized to achieve fiber collection and deposition in an ordered and controlled fashion [9–13].

Technically, electrospinning is an electrohydrodynamic process wherein a polymer solution, or melt, is pumped from a syringe or otherwise controllably introduced to a static electric field (around 5–100 kV at negligible amperage). Electrostatic repulsions overcome the surface tension of the polymer(s) in solution by applying sufficiently high voltage. Droplets of the solution are stretched in eruptions (Taylor cones) that elongate the liquid jet. The jet becomes a fiber that travels at a high rate and continues to elongate as it moves toward a grounded or oppositely charged collector. The solvent evaporates during transit from the droplet to the collector allowing for collection of a dry fibrous material. Elongation and thinning of the fibers are further driven by bending instabilities caused by the electrostatic repulsions [5, 14]. The magnitude of the fiber elongation and thinning can be controlled to a large extent by altering processing parameters to produce fibers of variable geometries. Engineered collecting surfaces, such as a high-speed rotating drum or wire wheel, spinning disk, or other surfaces can be used to drive the spatial patterning of the produced fibers.

Historically, denaturing and caustic solvents have been used to electrospin collagen into small-micron fibers [15, 16]. Even with more benign solvents, electrospun collagen is still not completely renatured, and the resulting fibers are not typically stable in aqueous solutions, nor do they have the strength or the chemical structure of native collagen fibrils [6, 17–22]. Furthermore, pure collagen electrospinning is typically a slow process, expelling fibers at around  $0.2 \text{ ml h}^{-1}$  with a collagen concentration of only around  $75 \text{ mg ml}^{-1}$  in solution, which is problematic for industrial product development for clinical translation [6, 19–21].

Few alternative methods to microfiber production have been investigated, including solution blowing and centrifugal spinning [23]. In studies by Tutak *et al* microfiber scaffolds were prepared from polymers and compared from both the electrospinning and airbrushing techniques to assess differences in fiber morphology, mechanical properties, and cellular response [24, 25]. Polymer scaffolds that were produced from the simple airbrush were found to be cheaper and safer to manufacture and produced materials at a faster rate. In contrast, the electrospun

scaffolds of the same synthetic polymers were smaller in average fiber diameter and demonstrated superior mechanical properties in this prior work. The polymers generated were also collected as unaligned sheets [24]. Many applications in biomedical engineering, including ligament, tendon and neuronal tissue repair, require fibers to be highly aligned for enhanced biomechanical properties [26].

Here, we test the hypothesis that, if a solvent used to dissolve clinical grade type I collagen can be volatilized using a high-speed compressed air jet, such as by a common airbrush (here termed ‘pneumatospinning’), then type I collagen will polymerize into cyto-compatible microfibers or nanofibers. The process for forming pneumatospun collagen fibers is shown, along with extensive physical and chemical biomaterial and cytocompatibility testing of pneumatospun collagen-based grafts as compared to electrospun collagen. To accelerate the potential translational potential of this work, all studies further use clinical grade collagen, made under current good manufacturing practices and with the feedstock collagen currently used in medical devices approved by the Food and Drug Administration.

## 2. Material and methods

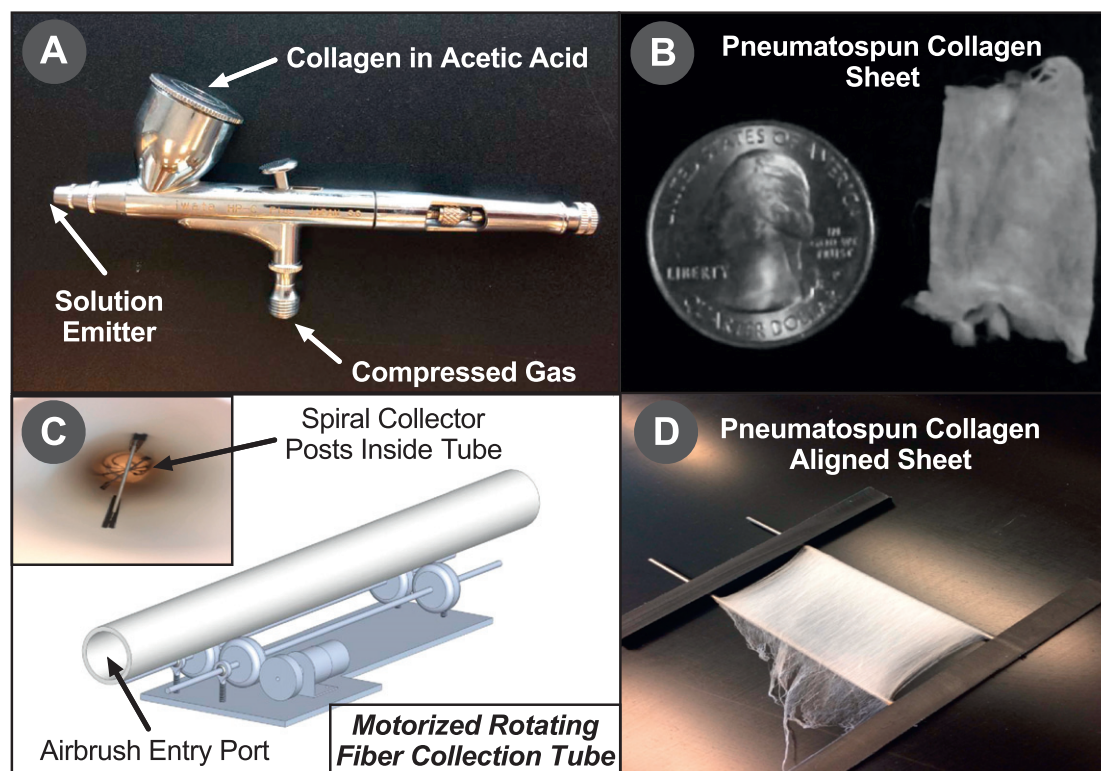
### 2.1. Collagen solution preparation

An Iwata Gravity feed airbrush (Iwata, Japan) was modified to produce pneumatospun collagen fibers (as in figure 1). An air pressure of 60 psi (pounds per square inch) was used, and the inner needle of the airbrush was withdrawn approximately 1 mm from the end of the solution emitter to prevent clogging from the viscous collagen solution. Up to  $500 \text{ mg ml}^{-1}$  of clinical grade type I atelocollagen (Collagen Solutions, San Jose, CA) was dissolved for 2–4 h in 20%–50% acetic acid (Sigma-Aldrich, St. Louise, MO) by shaking. The solutions were then tested for the ability to form fibers by pneumatospinning.

In a separate study, telocollagen (Collagen Solutions, San Jose, CA) was also dissolved with poly-D,L-lactide (Polysciences, Warrington, PA) in dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO), or DMSO and absolute ethanol (Sigma-Aldrich) at  $150 \text{ mg ml}^{-1}$  and collected on a grid. The overall ratio between collagen and poly-D,L-lactide was 30:70, respectively, as empirically determined for optimal fiber production.

### 2.2. Pneumatospun scaffold generation

Collagen fibers were collected on either static grid (2.5 cm squares of a common 50 ml Eppendorf test tube rack) or were sprayed into a custom rotating tube to collect aligned scaffolds. In the custom engineered rotating tube design, several pairs of perpendicular rods were inserted through a tube to catch the passing fibers while the airflow aligned them in the direction of



**Figure 1.** Pneumatspinning apparatuses. (A) An Iwata airbrush is used to pneumatspin clinical grade bovine type I atelocollagen dissolved in acetic acid (40%), as driven by regulated compressed air, with (B) a sheet of around 200  $\mu\text{m}$  thick dry collagen produced after 5 min of spraying. (C) To generate aligned pneumatspun collagen fibers a motorized rotating fiber collector was used, comprised of a tube with a spiral array of spokes inserted (insert in (C) and posts in (D)), which catches the fibers in the air stream to generate anisotropic 3D grafts (D).

**Table 1.** Collagen concentration in acetic acid test matrix for pneumatspinning.

Concentration of collagen in acetic acid	Produced fibers
150 mg ml <sup>-1</sup>	No
200 mg ml <sup>-1</sup>	No
250 mg ml <sup>-1</sup>	Yes
300 mg ml <sup>-1</sup>	Yes
400 mg ml <sup>-1</sup>	Yes
450 mg ml <sup>-1</sup>	Yes
500 mg ml <sup>-1</sup>	No

**Table 2.** Collagen fiber manufacturing rate.

Output	Electrospinning	Pneumatspinning
Deposition rate	0.0625 g h <sup>-1</sup>	2 g h <sup>-1</sup>
Collection efficiency	52%	23%

flow (illustrated in figure 1). Rotating the tube allowed for even fiber collection, with the airbrush held at approximately 45° with respect to the tube inlet. Fibers were collected for 5 min of pneumatspinning and used in subsequent experiments after storage in a desiccator to ensure dryness.

### 2.3. Electrospinning

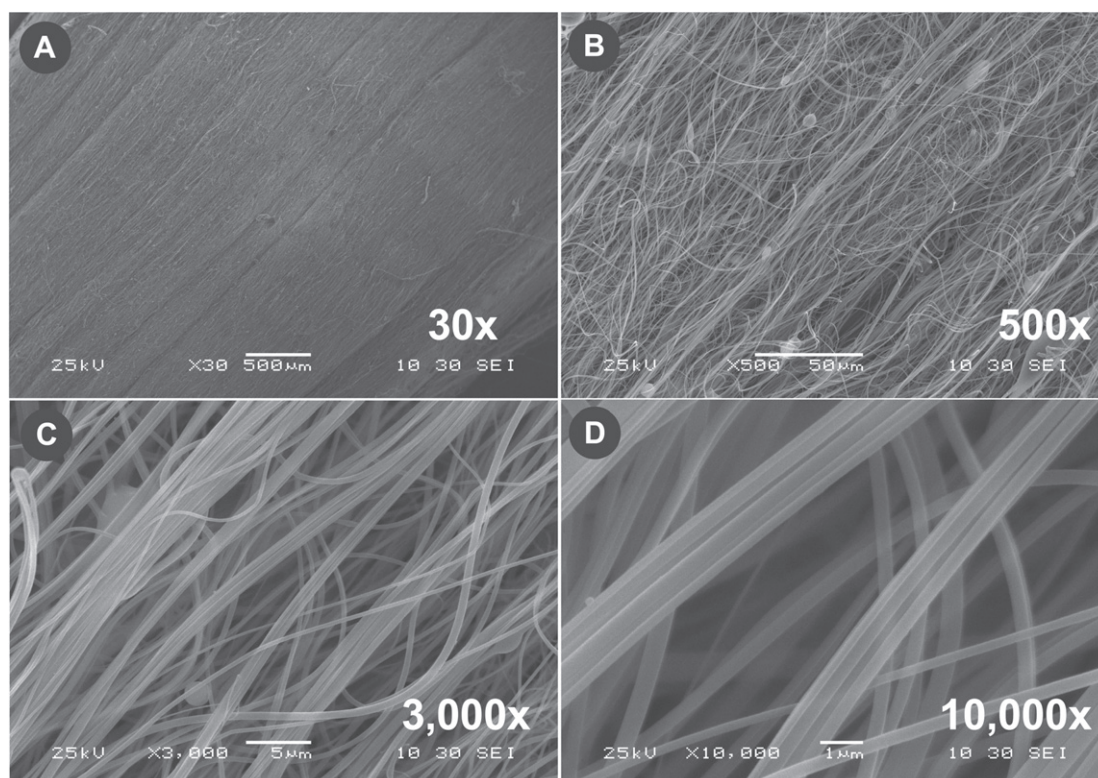
Electrospun scaffolds were collected using a high-speed drum with a surface speed of 10 m s<sup>-1</sup> to

generate aligned fibers. Type I atelocollagen was dissolved at 250 mg ml<sup>-1</sup> in 40% acetic for 2–4 h with gentle rocking. The collagen solution was pumped at 0.2–0.5 ml h<sup>-1</sup> using a syringe pump (NE-4000 Programmable, New Era Pump Systems, Farmingdale, NY) through a 2 inch long blunt tip 20G needle. The distance from the needle tip to the collector (grounded wires spaced 25 mm apart for ‘air gap’ electrospinning) was 10 cm and the needle was charged to +18 kV.

### 2.4. Crosslinking

A protocol by Mekhail *et al* was followed to crosslink both electrospun and pneumatspun aligned type I collagen [27]. A 0.03 M solution of genipin (Sigma-Aldrich) in 97% Ethanol (Sigma-Aldrich) was used to crosslink pneumatspun and electrospun scaffolds. Pneumatspun samples were given a short, even twist to improve fiber packing for crosslinking. During crosslinking, samples were clamped to hold tension on the fibers and prevent shrinkage and folding upon itself during the 7 d and incubation period at 37 °C. The genipin-ethanol bath was checked every day to ensure the graft remained covered in crosslinking solution. Genipin crosslinked scaffolds were washed 20 times with phosphate-buffered saline (PBS) (Cellgro, Manassas, VA) to wash away residual, unreacted genipin, producing deep blue scaffolds.





**Figure 2.** SEM of aligned pneumatospun collagen. (A)–(D) Scanning electron microscopy at 30 $\times$  (A), 500 $\times$  (B), 3000 $\times$  (C) and 10 000 $\times$  (D) revealed the generation of individual fibers assembled by the pneumatospinning of large sheets of anisotropic collagen microfibrils.

## 2.5. Mechanical testing

The material properties of hydrated electrospun and pneumatospun (around 1.5 cm diameter  $\times$  4 cm long) scaffolds were tested under uniaxial tensile testing using an MTS Criterion, Model 42 (Eden Prairie, MN). All mechanical testing was performed at room temperature. Scaffold diameter and thickness were measured with precision digital calipers and recorded to calculate cross sectional area. Samples were hydrated in Dulbecco's Modification of Eagle's Medium (DMEM) (Fisher Scientific, Hampton, NH) for 1 h and then loaded on the MTS machine with six samples ( $n = 6$ ) in each group.

## 2.6. Scanning electron microscopy (SEM)

The structure of uncrosslinked pneumatospun and electrospun scaffolds was analyzed by SEM. Genipin crosslinked pneumatospun scaffolds were also assessed after 30 d soaking un-tensioned in DMEM at 37  $^{\circ}$ C. Fiber formation, dimensions and matrix alignment was assessed with the Orientation J feature of ImageJ software (NIH, Bethesda, MD). SEM imaging was performed at Jefferson Labs (Newport News, VA) using a JEOL JSM-6060 LV microscope (JEOL Ltd, Tokyo, Japan) with a 20 kV beam intensity.

## 2.7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

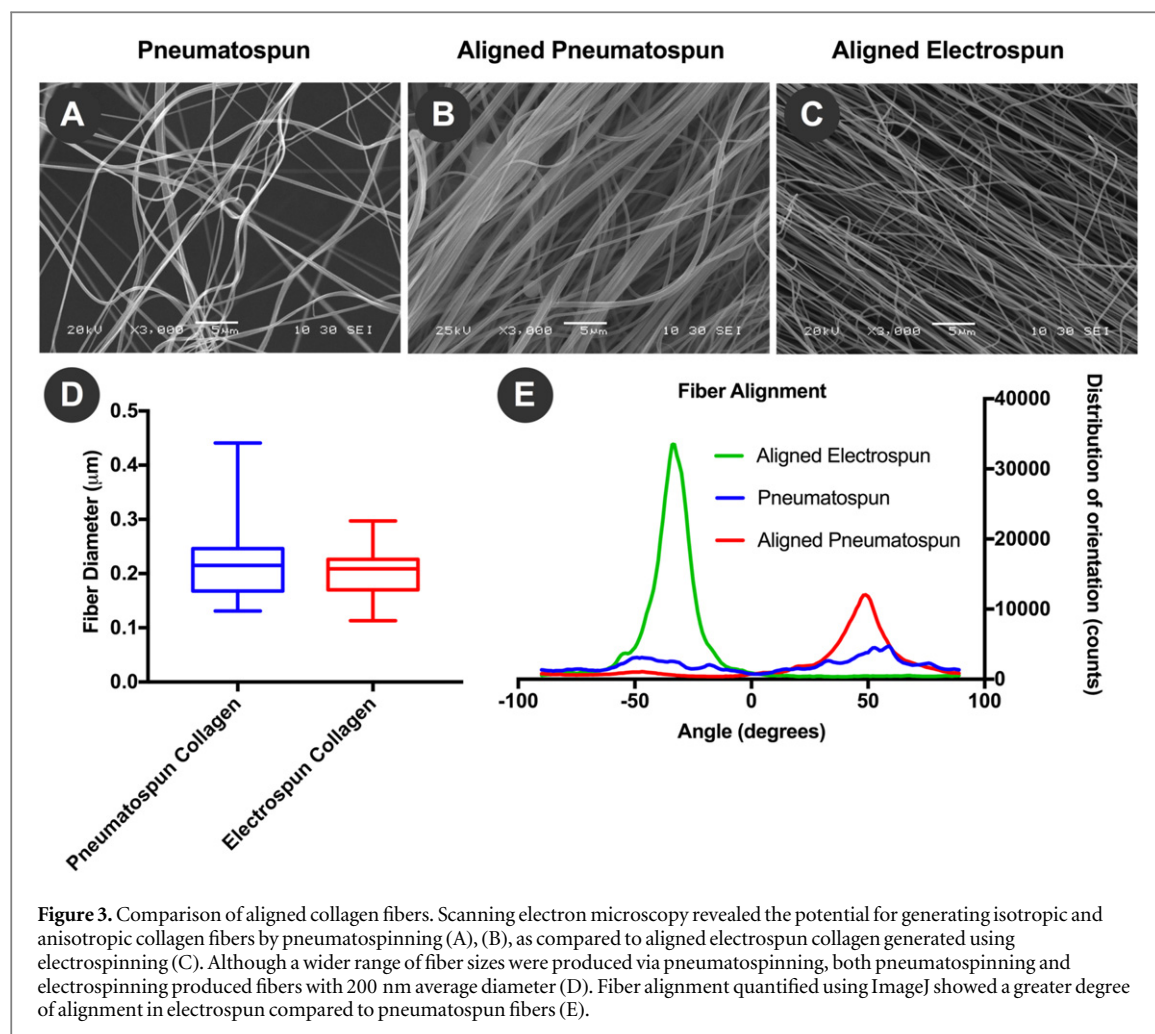
SDS-PAGE was used to compare the collagen feed-stock (Collagen Solutions), pneumatospun and electrospun collagen grafts (uncrosslinked, both dissolved in acetic acid). Gradient gels (3%–8%) (Invitrogen, Carlsbad, CA) were run at 150 kV on a Xcell SureLock (Invitrogen) gel apparatus. Gels were stained with SimplyBlue<sup>TM</sup> gel stain (Invitrogen) and then rinsed with deionized water. The gel was then imaged under white light to view the protein bands.

## 2.8. Fourier-transform infrared spectroscopy (FTIR)

FTIR (Platinum ATR, Bruker, Billerica, MA) was used to assess the presence of Type I collagen, as determined by the three major amide bonds characteristic at 1235, 1560, and 1650  $\text{cm}^{-1}$  wavelengths. Electrospun and pneumatospun scaffolds were compared to the starting material by assessing peak displacement and sample purity with the Essential FTIR bioinformatics software (Operant, Madison, WI).

## 2.9. Circular dichroism

A far UV CD (J-815, JASCO, Easton, MD) was taken to compare the CD spectra of pneumatospun scaffolds with electrospun and starting material. CD spectra was obtained using a cuvette path length of 0.1 cm.



Samples were dissolved in 50 mM acetic acid at a concentration of  $0.5 \text{ mg ml}^{-1}$  for analysis.

## 2.10. Cell viability on pneumatospun collagen scaffolds

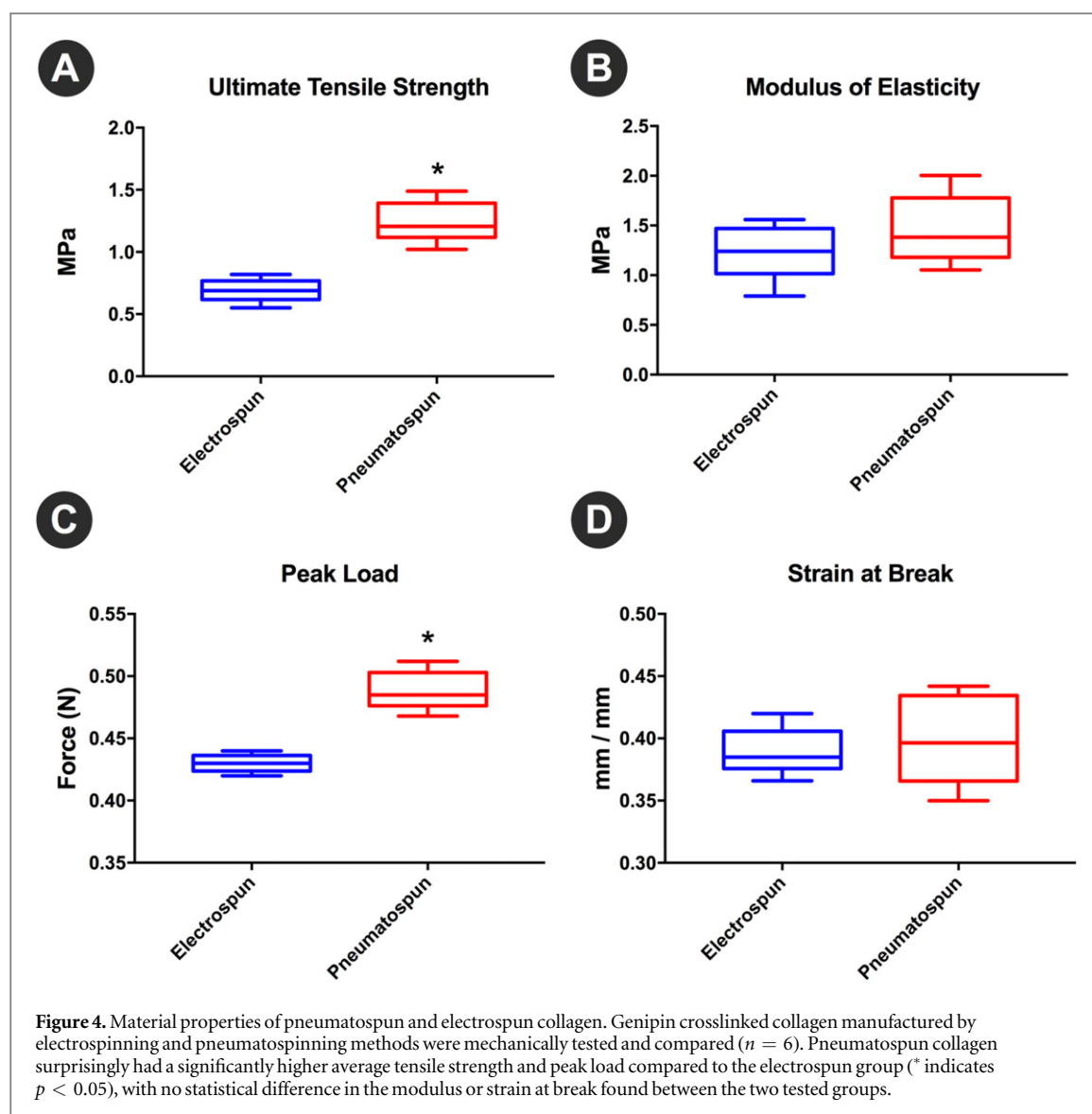
Tissue culture 96 well-plates were coated with  $200 \mu\text{l}$  of 7% poly(2-hydroxyethyl methacrylate) (PHEMA) (Sigma-Aldrich) to prevent cells from attaching to cell culture vessels. Six millimeter diameter scaffold disks from genipin crosslinked pneumatospun and electrospun collagen were cut from the scaffold sheets using a tissue biopsy punch. The samples were then disinfected by soaking in 70% isopropanol for 30 min, followed by three ten-minute washes in PBS. One scaffold disk was used per well. Human adipose-derived stem cells (ASCs) (ZenBio, Research Triangle Park, NC) were mixed and suspended in DMEM. These cells were seeded at a density of  $5 \times 10^4$  cells/well on both the electrospun and pneumatospun scaffolds. Cell viability on collagen-coated wells of a 96-well plate was used as a positive control. Wells were assessed after days 1, 4, and 7 using alamarBlue™ (BioRad, Hercules, CA) viability assay.

## 2.11. Cell morphology analyses

After 7 d of culture, the cell-seeded electrospun and pneumatospun scaffolds were fixed and stained to assess cell morphology and attachment. One set of the samples ( $n = 2$ ) was fixed in 4% paraformaldehyde (Thermo Fischer Scientific, Hampton, NH) and stained for nuclei and actin filaments using DAPI (Vector Laboratories, Burlingame, CA) and Alexa Fluor® 594 phalloidin (Thermo Fischer Scientific), respectively. These stained samples were imaged using confocal microscopy (ZEISS Axio Observer Z1 Inverted Motorized Microscope, Oberkochen, Germany). A second set of samples ( $n = 2$ ) was fixed in 2% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA) and stained with osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA) to image cell morphology on the collagen scaffolds.

## 2.12. Statistical analyses

One-way analysis of variance followed by the post-hoc Tukey's multiple comparison test was used to assess any difference in fiber alignment between groups. Two-way analysis of variance followed by the post-hoc Tukey's multiple comparison test was used to assess the differences in cell viability between groups and over time. An unpaired *t*-test was used to assess any



differences in mechanical strength between the electrospun and pneumatospun collagen scaffolds. *A priori*,  $p$  values  $< 0.05$  were defined as significant. All tests were performed using GraphPad Prism 7, and all parameters are expressed as mean  $\pm$  standard error of the mean (S.E.M.).

### 3. Results

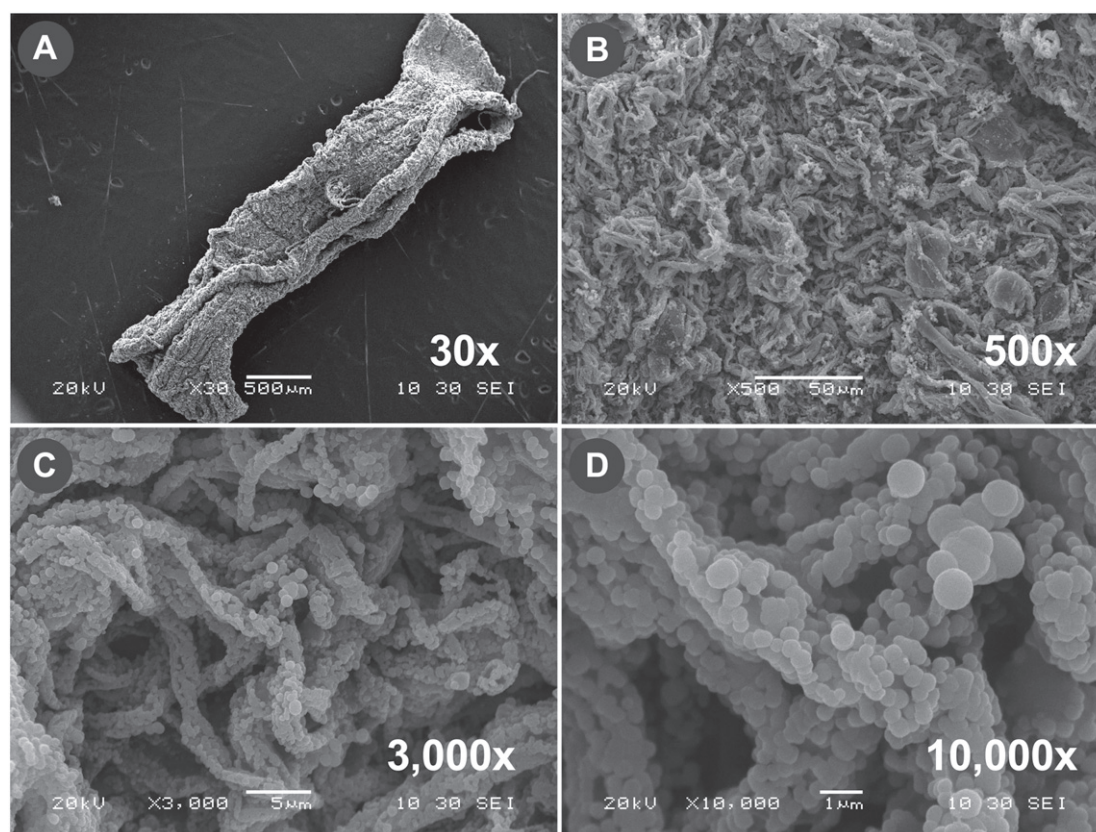
#### 3.1. Pneumatospinning of pure collagen fibers

Pneumatospinning from acetic acid solutions with 200–250 mg ml<sup>-1</sup> of type I atelocollagen yielded few fibers. Solutions with 45%–50% collagen produced fibers, yet the solutions were poorly solubilized, very viscous, and resulted in discontinuous fiber production during pneumatospinning (table 1). The 40% collagen in 40% acetic acid was found to optimally produce a continuous spray of collagen fibers and generate robust sheets of collagen with a static collector that was densely coated with fibers by 5 min of spraying (figure 1(B)), thus these parameters were

used for all subsequent experiments. Pneumatospun scaffolds were collected at around a 32× increased rate relative to electrospinning from acetic acid (table 2). Faster deposition rates of pneumatospun fibers were achievable at higher air pressures but with decreased collection efficiency.

Collagen fibers pneumatospun in the rotating tube collection apparatus designed to impart a higher degree of anisotropy to the collected fibers showed a generally aligned array of fibers under SEM (figure 2). Orientation J (ImageJ plugin) quantified the overall significant improvement in fiber alignment in pneumatospinning in the rotating collection tube relative to collecting pneumatospun collagen on a static grid (figure 3). However, electrospun collagen microfibrous scaffolds consistently demonstrated a significantly higher degree of alignment ( $p < 0.05$ ) than that of the pneumatospun scaffolds (figure 3). The average fiber diameter of pneumatospun relative to electrospun collagen from acetic acid was  $0.224 \pm 0.051 \mu\text{m}$ , whereas electrospun collagen had





**Figure 5.** Stability of pneumatospun collagen in media. Pneumatospun aligned collagen was crosslinked with genipin and incubated (without tension) for 30 d in DMEM at 37 °C, showing the resulting fiber morphology at 30× (A), 500× (B), 3000× (C) and 10 000× (D). The fibrous structure of these scaffolds noted prior to genipin crosslinking (figure 3) is altered to bead-like strands of crosslinked, condensed collagen, with loss of alignment imparted by grafts being hydrated and left loose (not under tension) over the 30 d of incubation.

an average fiber diameter of  $0.201 \pm 0.047 \mu\text{m}$  (figure 3).

### 3.2. Stability and mechanical properties of pneumatospun collagen

Pneumatospun collagen was not stable in aqueous media without crosslinking, forming a tacky, gel-like film when hydrated, in contrast to complete dissolution of uncrosslinked electrospun fibers placed in aqueous media. Genipin was chosen to crosslink the electrospun and pneumatospun samples because it has an established low toxicity profile and is used in clinically approved medical devices. Despite lesser alignment of the fibers relative to electrospun collagen scaffolds (figure 3(E)), pneumatospun collagen crosslinked with genipin (tested hydrated for 1 h in DMEM) were significantly stronger, at  $1.23 \text{ MPa} \pm 0.11$ , compared to electrospun scaffolds (figure 4(A)). There was no significant difference in modulus of elasticity or strain between the groups (pneumatospun collagen at  $1.45 \text{ MPa} \pm 0.34$ ) (figures 4(B), (D)). Pneumatospun collagen scaffolds crosslinked with genipin remained intact for at least 30 d unrestrained and submerged in DMEM at 37 °C (figure 5) was morphologically altered with an apparent constriction and

coiling of the fibers into spheres on the previously fibrous graft.

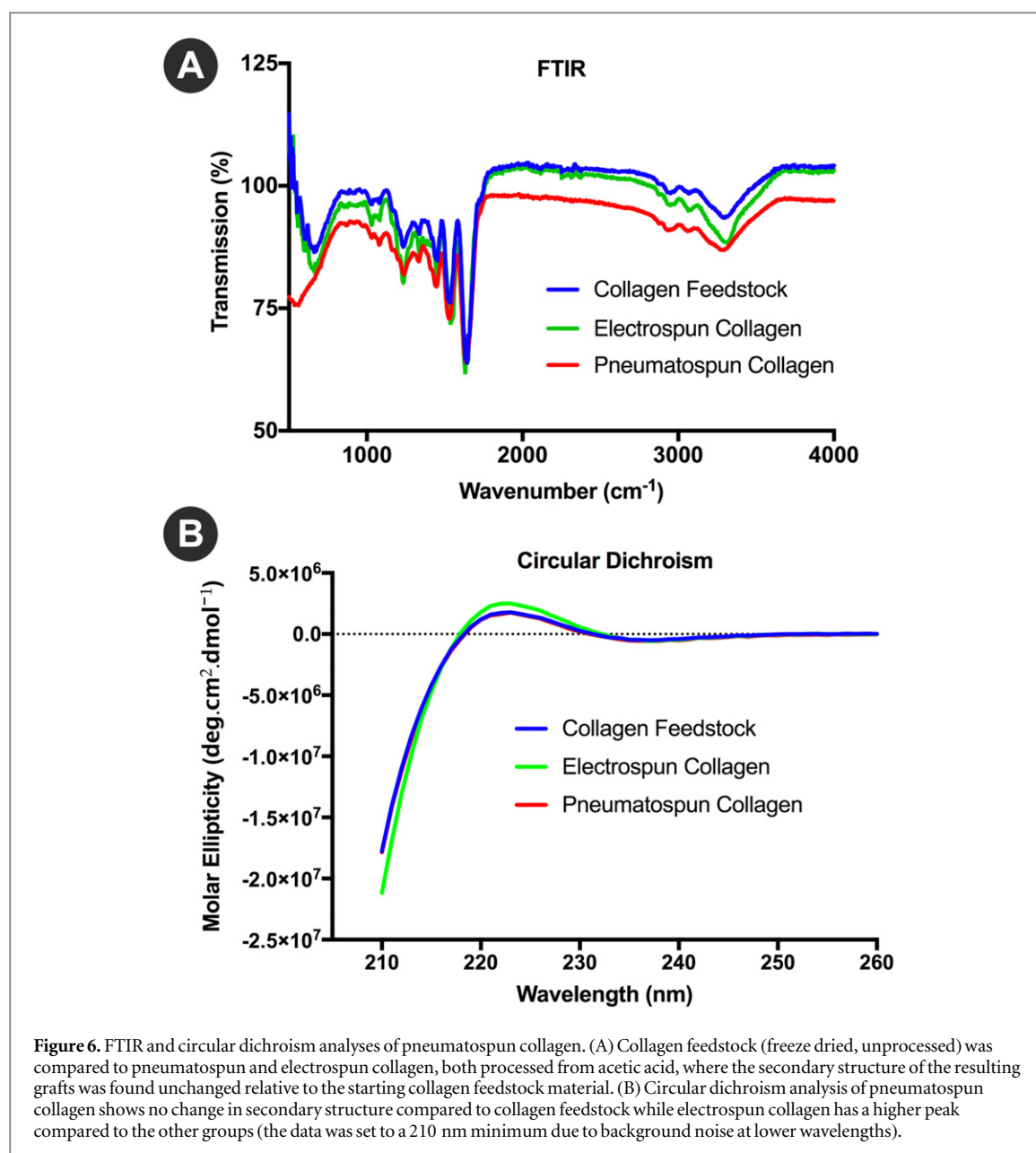
### 3.3. Chemical and structural characterization of electrospun and pneumatospun collagen

FTIR analyses (figure 6(A)) between the collagen feedstock (unprocessed, freeze dried), electrospun and pneumatospun collagen showed no shifts in the carboxyl and three amide bonds that are characteristic of type I collagen, suggesting integrity of the primary and secondary structure. Circular dichroism analyses showed comparatively little change in pneumatospun collagen relative to the feedstock collagen (figure 6(B)), suggesting the pneumatospinning process does not denature the protein, preserving the native triple helical structure. SDS-PAGE further confirmed the presence of alpha, beta and gamma chains of collagen present in unprocessed, pneumatospun and electrospun collagen fibers (figure S1 is available online at [stacks.iop.org/BF/10/045004/mmedia](http://stacks.iop.org/BF/10/045004/mmedia)).

### 3.4. Viability of ASCs on electrospun and pneumatospun collagen scaffolds

An alamarBlue™ assay was performed to assess the metabolic activity of ASCs grown on pneumatospun





and electrospun scaffolds, with metabolic activity quantified as increasing over time (figure 7(A)), indicative of cellular proliferation and viability over 14 d in culture. Confocal imaging of ASCs grown for two weeks on pneumatospun and electrospun collagen crosslinked with genipin reveal that cells were present throughout the matrices (figures 7(B), (C)). A confluent layer of cells was found atop both groups by SEM (figures 7(D), (E)), collectively indicating strong cell attachment and cytocompatibility for pneumatospun collagen.

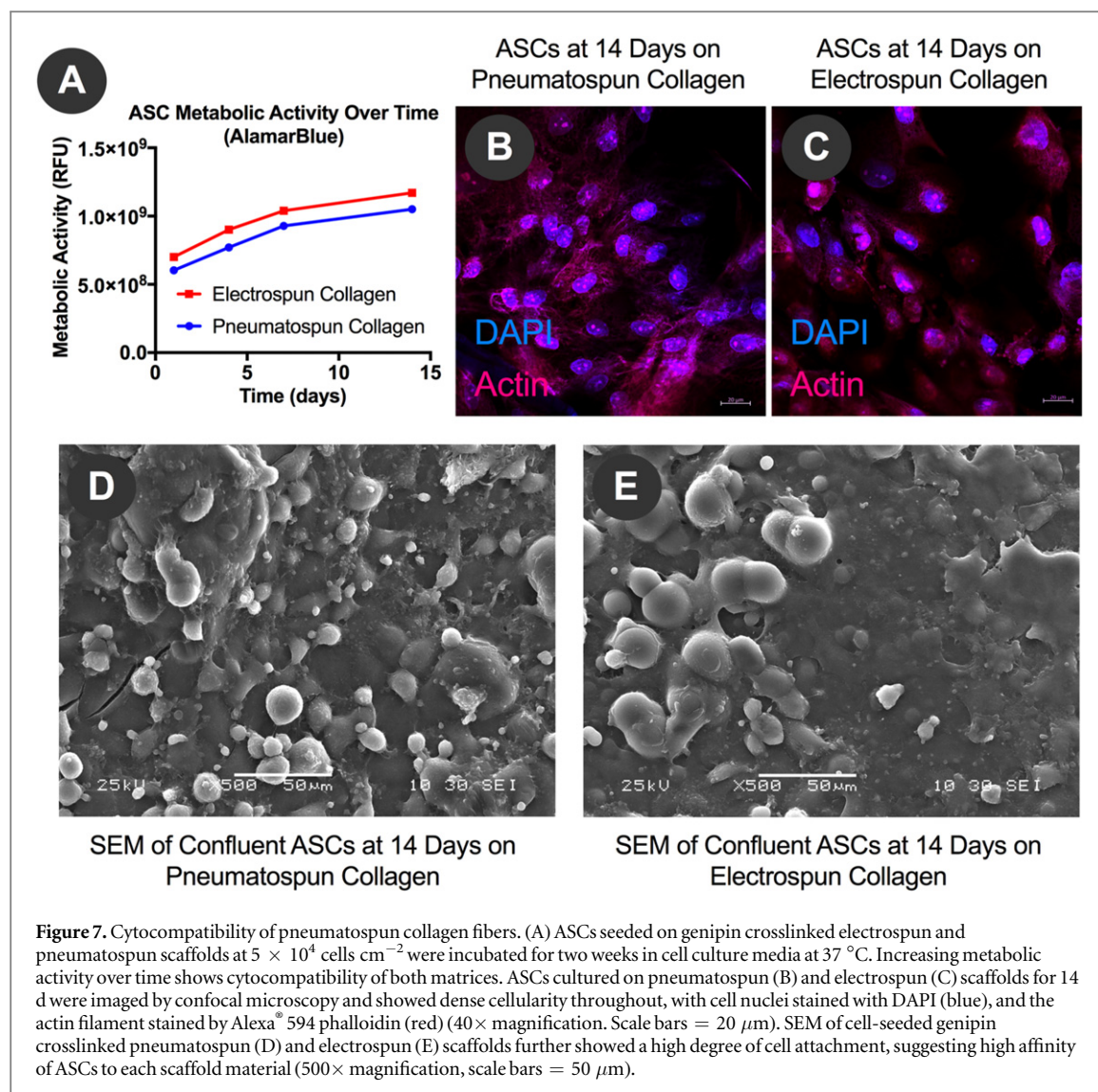
### 3.5. Pneumatospinning collagen and PDLLA blended scaffolds from DMSO

To assess if collagen could be combined with a biopolymer to produce a blended biomaterial via pneumatospinning, collagen was dissolved in a DMSO-based solvent system along with PDLLA in a

30:70 ratio. While pneumatospinning of solely collagen from DMSO was not achieved at the tested concentration, PDLLA alone and collagen: PDLLA copolymer blends were able to form produce scaffolds by pneumatospinning from DMSO alone and from DMSO: ethanol co-solvent system (table 3 and figures 8(A) and (B)). FTIR analyses of the pneumatospun collagen: PDLLA confirmed presence of both biomaterials in the collected scaffold (figure 8(C)).

## 4. Discussion

These studies show an original method of collagen microfiber assembly using high velocity air (pneumatospinning) to generate anisotropic and isotropic scaffolds or collagen coatings on other devices. The defect-free production rate is currently  $2 \text{ g h}^{-1}$  relative to  $0.0625 \text{ g h}^{-1}$  with electrospinning of collagen as



**Table 3.** Solvent compatibility for PDLLA: collagen pneumatospinning from DMSO.

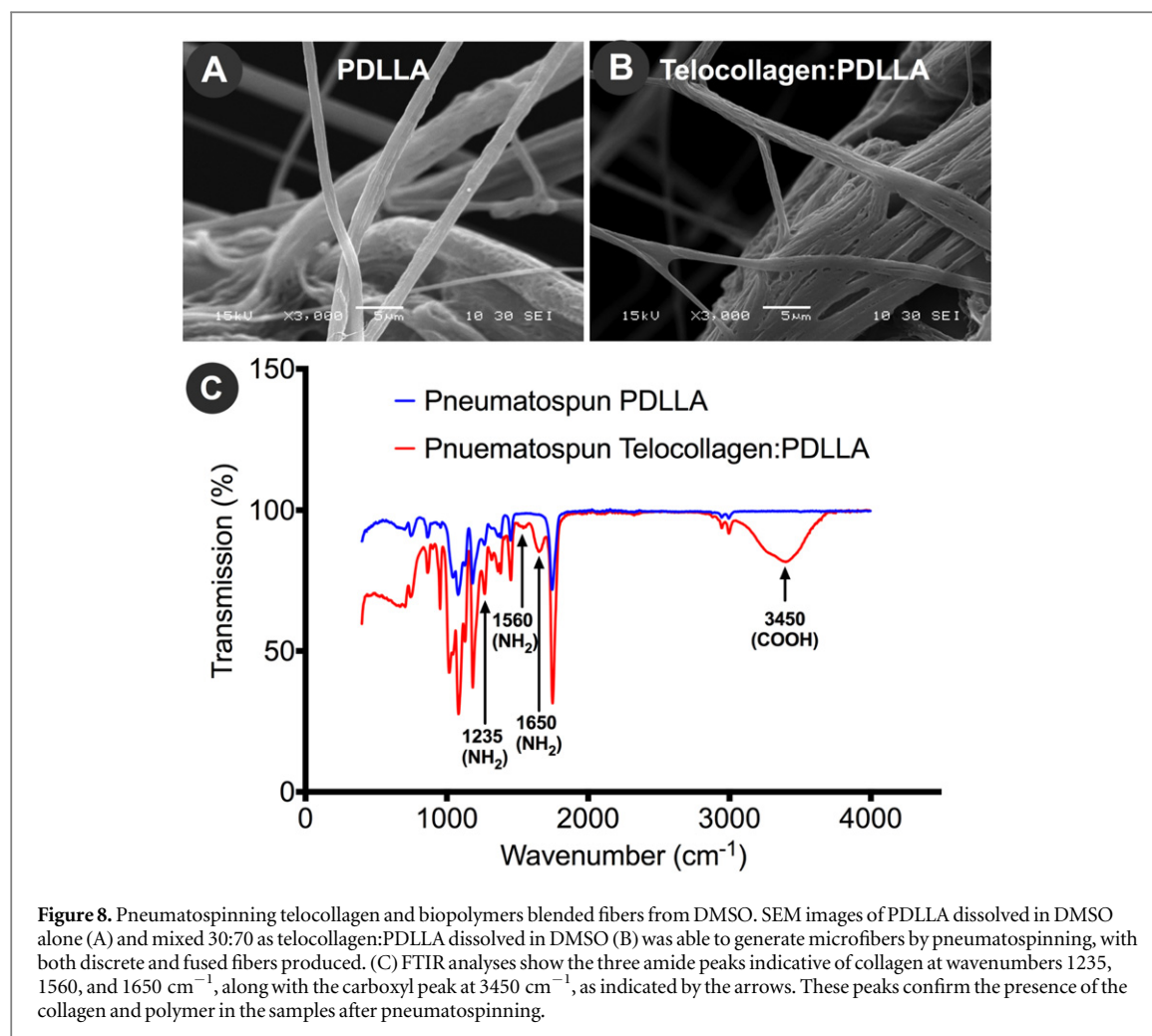
Solvent and ratio	Telocollagen only	PDLLA only	Telocollagen and PDLLA
DMSO (100%)	No	Yes	Yes
DMSO:EtOH (80:20)	No	Yes	Yes
DMSO:EtOH (65:35)	No	Yes	Yes

dissolved in acetic acid and has the potential to increase to at least  $8 \text{ g h}^{-1}$  using this approach along with higher efficiency fiber collecting device engineering. Pneumatospinning is thus a markedly more scalable approach to collagen microfiber generation with less specialized and less expensive equipment required for collagen fiber production.

We show that a novel rotating internal post fiber collector (figure 1) can readily and reproducibly generate aligned collagen fibers (figure 2). Interestingly, despite less apparent fiber alignment compared to electrospun collagen (figure 3), the tensile strength of genipin crosslinked pneumatospun collagen was significantly and reproducibly stronger than electrospun scaffolds (figure 4). This may be related to an

improvement in the molecular assembly of the collagen in the pneumatospinning process relative to electrospinning, as indicated by our circular dichroism results (figure 6), or to the slight difference in fiber diameter between the two methods. Electrospinning, conversely, offers more control of both collagen microfiber diameter and for controlling the ordering of the fibers in 2D. Electrospinning, however, has limited ability to produce thick 3D materials due to electrical insulation of the collector, typically limited to a millimeter or less in a single layer. This limitation is not present with collagen pneumatospinning with theoretically unlimited thicknesses possible.

Following genipin crosslinking, the pneumatospun materials are shown to be stable in culture



**Figure 8.** Pneumatspinning telocollagen and biopolymers blended fibers from DMSO. SEM images of PDLLA dissolved in DMSO alone (A) and mixed 30:70 as telocollagen:PDLLA dissolved in DMSO (B) was able to generate microfibers by pneumatspinning, with both discrete and fused fibers produced. (C) FTIR analyses show the three amide peaks indicative of collagen at wavenumbers 1235, 1560, and 1650  $\text{cm}^{-1}$ , along with the carboxyl peak at 3450  $\text{cm}^{-1}$ , as indicated by the arrows. These peaks confirm the presence of the collagen and polymer in the samples after pneumatspinning.

conditions for a month, yet with an apparent alteration of the fiber morphology and overall graft topography to a condensed and coiled appearance (figure 5). This may be partially related to the constriction of material over time as the grafts were not held under tension, along with the crosslinking driving this apparent fiber constriction and coiling. Other crosslinkers, such as glyceraldehyde and glutaraldehyde, did not exhibit this change in morphology (unpublished data), suggesting a genipin-related effect on pneumatspun collagen. Despite morphological changes to the material, cell metabolic activity and related cell viability was high on pneumatspun fibers though at least 2 weeks of culture (figure 7).

The ability to blend collagen with a biopolymer, such as poly-D,L-lactic acid (figure 8), presents further potential applications of the collagen pneumatspinning approach. Synthetic polymer addition presents a means to modulate overall graft material properties such as strength, fiber size, stability and degradation characteristics, while reducing overall graft cost for possibly little loss in cytocompatibility, as suggested by electrospun collagen-biopolymer blend work [3, 28, 29].

In this work we further advance manufacturability and safety of collagen microfiber production.

Common electrospinning solvents for collagen and biopolymers, such as hexafluoroisopropanol, chloroform, tetrahydrofuran, trifluoroacetic acid and other organic solvents have been shown to be reactive with collagen, altering the primary, secondary and tertiary protein structure, and adversely affecting material properties. Pneumatspinning pure collagen from acetic acid, or collagen and PDLLA from DMSO, present solvents that are less reactive, less costly, and better suited to production of medical devices due to being classified as low risk solvents per USP<467>.

These successful proof of concept studies present accessible and effective methods for generating collagen-based fibers for a host of basic and applied research investigations, ranging from cell culture studies to potential therapeutic applications for wound repair and other uses, such as medical device coatings. Currently this technology is limited to relative low efficiency (20%–30% collagen collection with simple collection apparatuses) and limited potential for control of the fiber deposition in a precise additive manufacturing schema, although more advanced air handling and collection assemblies may improve this limitation in our future studies. With further research and development, pneumatspinning of collagen may be a useful bedside therapeutic strategy for many



indications, ranging from applied skin burn dressings to soft and hard tissue void fillers.

## 5. Conclusion and significance

Type I collagen can be formed as submicron, near-nanoscale fibers by pneumatospinning, with the resulting fibers similar in size and strength to collagen fibers electrospun from acetic acid. This is the first report of collagen being manufactured as a fibrous scaffold using pneumatospinning, both as a non-woven mesh or aligned fibers, as well as collagen alone or collagen blended with a biopolymer. Pneumatospun collagen is further found to rapidly produce cytocompatible collagen-based scaffolds. The pneumatospinning process has additional manufacturing benefits relative to conventional methods of collagen microfiber production in reduced cost, complexity, and hazards, along with dramatically higher production rate. Therefore, pneumatospinning of collagen is a potent manufacturing method for producing collagen scaffolds for both basic research and with diverse clinical applications, including medical device manufacturing, for potential bedside and field wound treatments, and other applications.

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