# Properties of electrospun pollock gelatin/poly(vinyl alcohol) and pollock gelatin/poly(lactic acid) fibers

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## ARTICLE INFO

## ABSTRACT

Pollock gelatin/poly(vinyl alcohol) (PVA) fibers were electrospun using deionized water as the solvent and pollock gelatin/poly(lactic acid) (PLA) fibers were electrospun using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as the solvent. The chemical, thermal, and thermal stability properties were examined for the electrospun samples. The electrospun PVA samples generally had thinner and more uniform fibers than the electrospun PLA samples. For the PVA samples, an increase in total solids content and PVA to gelatin ratio generally resulted in higher average fiber diameter values and wider diameter distributions. Pollock gelatin in both types of electrospun samples remained amorphous. The PVA in electrospun samples had comparable melting temperatures to that of neat PVA, whereas the PLA in electrospun samples had slightly lower melting temperatures than that of neat PLA. Also, the PLA in electrospun samples had crystallization temperatures approximately 30 °C lower than that in neat PLA. This was due to better alignment of PLA chains during electrospinning, which resulted in the chains being more readily crystallized at lower temperatures. In addition, the electrospun PVA samples completely dissolved in water at room temperature after soaking for one day, whereas the electrospun PLA samples remained intact even after soaking for three days.

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## 1. Introduction

Gelatin and gelatin combined with various polymers had been electrospun for tissue engineering [1–18], wound dressing [19–23], and drug release [24] applications. Gelatin is soluble in water so the electrospun fibers are usually cross-linked or gelatin is electrospun with other polymers to reduce solubility in aqueous solutions. Some of the polymers that had been electrospun with gelatin included polycaprolactone (PCL) [1,7–10,19,25], poly(lactic acid) (PLA) [6,12,13,22,26], poly(vinyl alcohol) (PVA) [18,24,27,28], poly(lactide-co-glycolide) [4], poly(L-lactide-co-ε-caprolactone) [11], and polyaniline [3]. The tissue engineering studies had been especially promising, with the electrospun fibers having similar physical dimensions to extracellular matrices. In fact, some studies [1,2,7,10,12,17,22] had shown better growth of cells on electrospun fiber scaffolds containing gelatin than scaffolds without gelatin. For instance, Kim et al. [12] and Yuan et al.

[13] showed that cells grown on bovine gelatin/PLA fibers exhibited greater viability than those grown on just PLA fibers.

Most studies on electrospun gelatin fibers had focused on using gelatin from mammalian sources, such as bovine or porcine gelatin. A major drawback of using mammalian gelatin was that it behaved as a gel at room temperature. One way to overcome this problem involved electrospinning the gelatin samples above the gelation temperature of mammalian gelatin solutions (~35 °C) [14,18,29,30]. However, electrospinning a sample above room temperature required modification of the electrospinning apparatus. To electrospin mammalian gelatin samples at room temperature, various authors had used other solvents besides water. These included organic solvents, such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) [2] or 2,2,2-trifluoroethanol (TFE) [5,26,31,32], or acid solutions, such as those containing formic [15,33] or acetic acid [16,20–23,28,34]. However, the organic solvents were not environmentally friendly and the acidic solutions had been shown to hydrolyze gelatin [15,21,33].

An alternative to using mammalian gelatin in electrospinning involved the use of fish gelatin. The main difference between fish and mammalian gelatin is that fish gelatin has lower concentrations of proline and hydroxyproline. This results in fish gelatin

solutions having lower gelation temperatures. In some cases, such as gelatin extracted from cold-water fish species, fish gelatin solutions had much lower gelation temperatures than mammalian gelatin solutions and remained as a liquid at room temperature [35]. Fish gelatin could then be mixed with other water soluble polymers, such as poly(vinyl alcohol), and electrospun into fibers by using only water as the solvent. Only a few studies had focused on electrospinning fish gelatin samples [36–38]. Songchotikunpan et al. [36] electrospun Nile tilapia gelatin using solutions containing acetic or formic acid. Meanwhile, Kejing et al. [37] electrospun catfish gelatin using solutions containing formic acid as well as catfish gelatin/poly(L-lactide) blends in solutions containing formic acid and dichloromethane. Also, Hofman et al. [38] were not able to produce fibers by electrospinning hoki gelatin using solutions containing acetic acid.

In this study, we electrospun Alaska pollock (a cold-water fish species) gelatin/PVA samples using only deionized water as the solvent as well as Alaska pollock gelatin/PLA samples using HFIP as the solvent. We examined how total solids concentration and polymer to gelatin ratio affected the diameter distribution and appearance of the electrospun fibers. We also characterized the chemical, thermal, and thermal stability properties of the electrospun fibers using Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA), respectively.

## 2. Experimental

### 2.1. Sample preparation and electrospinning procedure

Solutions for electrospinning were made from Alaska pollock (*Theragra chalcogramma*) gelatin mixed with either poly(lactic acid) (Polylactide resin 3001D) from NatureWorks LLC (Blair, NE) or poly(vinyl alcohol) (97% hydrolyzed, MW = 50,000–85,000) from Sigma–Aldrich (St. Louis, MO). Gelatin was extracted from the skins of Alaska pollock obtained from a fish processing plant in Alaska. The extraction procedures were detailed elsewhere [39]. For solutions containing pollock gelatin and PLA, PLA was first mixed with 1,1,1,3,3,3-hexafluoro-2-propanol (99+%, Sigma–Aldrich, St. Louis, MO) at room temperature (23 °C) using a stir bar for 1 h. Pollock gelatin was then added and mixed for an additional 1.5 h. For solutions containing pollock gelatin and PVA, PVA was first mixed with deionized water at 80 °C with a stir bar for 2 h. Pollock gelatin was then added and mixed at 60 °C for 3 more hours. In some samples containing higher concentrations of gelatin, a Tekmar (Mason, OH) SDT Tissumizer homogenizer was used to help completely dissolve the solutes.

The total solids concentrations used in PLA solutions were 6%, 9%, and 12% (w/w). The 6% and 12% (w/w) total solids solutions contained a PLA to pollock gelatin ratio of 1:1. On the other hand, the 9% (w/w) total solids solution contained PLA to gelatin ratios of 1:1, 2:1, and 3:1. Meanwhile, the total solids concentrations used in PVA solutions were 12%, 15%, and 18% (w/w). The 12% total solids solution contained a PVA to gelatin ratio of 1:1. The 15% (w/w) total solids solution contained PVA to gelatin ratios of 1:1, 2:1, and 3:1. The 18% (w/w) total solids solution contained PVA to gelatin ratios of 2:1 and 3:1. Each electrospun sample was referred to as total solids concentration, type of polymer, and polymer to gelatin ratio. For example, a 9 PLA 1:1 sample contained 9% (w/w) total solids, PLA, and had a PLA to gelatin ratio of 1:1.

The electrospinning apparatus consisted of a Glassman (High Bridge, NJ) High Voltage FC Series power supply, a 5 mL Becton–Dickinson (Franklin Lakes, NJ) syringe with a 19 gauge needle placed in a KD Scientific (Holliston, MA) 780101 model syringe pump, and a rotating drum collector. The syringe pump was set to 3.0 μL/min. For the PLA and PVA solutions, the voltages were set to 27.0 kV and 24.0 kV, respectively. The rotating steel drum was covered with aluminum foil and was attached to an IKA Labortechnik (Wilmington, NC) RW20 motor set to a rotation speed of 600 rpm. The distance between the needle tip and the drum was set at 11.5 cm. The electrospinning apparatus was operated in an enclosed chamber maintained at a relative humidity of 60–80% using a saturated KCl (Fisher, Philadelphia, PA) solution and sprayed deionized water. Each electrospun sample was produced from solutions electrospun for 4 h. Media Cybernetics (Bethesda, MD) Image Pro 6.3 software was used to characterize the fiber diameters.

The properties of the gelatin component in electrospun samples were compared to those of bulk pollock gelatin films. These films were prepared by first dissolving 5% (w/w) pollock gelatin in deionized water by mixing with a stir bar at 60 °C for 1 h. The solution was then allowed to cool at room temperature for 50 min before being cast on a Mylar sheet placed over a glass plate. The gelatin film was formed after drying overnight at room temperature.

### 2.2. Fourier transform infrared spectroscopy

A Perkin Elmer (Waltham, MA) 2000 Fourier Transform Infrared (FTIR) Spectrometer was used to characterize chemical changes of electrospun samples. The samples were placed in a DuraSamplIR attenuated total reflectance attachment (ASI SensIR Technology, Danbury, CT). Each IR spectrum contained an average of 50 scans over a 10 min period with a resolution of 4 cm$^{-1}$.

### 2.3. Differential scanning calorimetry

A TA Instruments (New Castle, DE) differential scanning calorimeter (DSC) 2910 was used to measure the thermal properties of the electrospun samples. The samples were conditioned in a 50% relative humidity chamber for at least 48 h prior to each test. The chamber was maintained at these conditions by using a saturated solution of calcium nitrate tetrahydrate (Fisher Scientific, Philadelphia, PA), $Ca(NO_3)_2 \cdot 4H_2O$, in deionized water. The DSC sample amount used was $5.0 \pm 0.2$ mg and each sample was heated from 0 °C to 240 °C at a rate of 5 °C/min. The sample chamber was purged with nitrogen gas at a flow rate of 75 cm$^3$/min.

### 2.4. Thermogravimetric analysis

A TA Instruments (New Castle, DE) thermogravimetric analyzer (TGA) 2950 was used to characterize the thermal stability of the electrospun samples. The samples were conditioned in a 50% relative humidity chamber for at least 48 h prior to each test. Each 8–11 mg sample was heated from 30 °C to 800 °C at a rate of 10 °C/min. The sample chamber was purged with nitrogen gas at a flow rate of 40 cm$^3$/min.

### 2.5. Scanning electron microscopy

A Hitachi (Pleasanton, CA) S-4700 scanning electron microscope (SEM) was used to observe the samples. The voltage was set to 2.0 kV or 10.0 kV and the current was set to 10 μA. A Denton (Moorestown, NJ) Desk II Sputter Coater was used to apply a gold/palladium coating to electrospun samples that were affixed to stubs. The samples were sputter coated for 45 s with the discharge current set at 20–30 mA. The vacuum chamber was lowered to a pressure of less than 100 mTorr.