Solution-Blown Nanofiber Mats from Fish Sarcoplasmic Protein

S. Sett\textsuperscript{1}, K. Stephansen\textsuperscript{2}, A.L. Yarin\textsuperscript{1}*  

\textsuperscript{1}Department of Mechanical and Industrial Engineering,  
University of Illinois at Chicago,  
842 W. Taylor St., Chicago IL 60607-7022, USA  
\textsuperscript{2}Nano-Bio Research Group, National Food Institute,  
Technical University of Denmark  
Soltofs Plads 227, 2800 Kongens Lyngby, Denmark

Abstract

In the present work, solution-blowing was adopted to form nanofibers from fish sarcoplasmic proteins (FSPs). Nanofiber mats containing different weight ratios (up to 90/10) of FSP in the FSP-nylon 6 blended nanofibers were formed from formic acid solutions, and compared to electrospun fibers made from the same solutions. The nanofiber mats produced by the two methods were characterized in terms of FSP content, fiber diameter distribution, fiber mat porosity, and mass of the fibers collected by the two processes. The mechanical strength of the solution-blown fibers was also measured. Overall, fibers made from the two techniques were similar, but with some exceptions. The fiber diameter of the electrospun fibers was slightly
smaller than those made using solution-blowing, however in both cases the fiber diameter increased with increasing FSP content. Interestingly, for uniform fibers the stretchability of the fibers increased with increasing FSP content, indicated by an increased strain at rupture. Moreover the mechanical tests showed that up to 50% of nylon 6 could be replaced with FSP without compromising the mechanical properties, compared to pure nylon 6 nanofibers. Comparison of the yield showed that the production rate of solution-blowing was increased 30 fold in relation to electrospinning. Overall, this study reveals FSP as an interesting biopolymeric alternative to synthetic polymers, and the introduction of FSP to nylon 6 provides a composite with controlled properties.

1. Introduction

Biopolymers are biodegradable and biocompatible, which make them useful as natural substitutes for traditional synthetic polymers. One of the widely used polymers, polyethylene terephthalate (PET), takes about 40 – 50 years to degrade under normal atmospheric conditions causing severe environmental pollution and toxicity concerns [1]. The extensively used plastic bags made from polyethylene take hundreds of years to degrade under normal atmospheric conditions [2]. Such severe adverse effects on the environment increase the demand for novel biopolymer-derived materials to replace synthetic polymers. Several plant-derived proteins have already been applied to this effect. For instance, soy is used for biodiesel production [3] and soy plastics [4, 5]. Cellulose is also used for biofuel production [6], as well as it is traditionally employed in paper, filter media, and textile industries and in biomedical applications [7-9]. Corn
is also used for fuel production [10], and several plant-derived proteins such as soy protein, zein, lignin, and cellulose acetate have successfully been formed into nanofibers [11-18].

Over the last few years, novel materials made from animal-derived proteins have also been widely explored. Sericin (silk protein), which is a major constituent of silk and widely used in pharmaceuticals and cosmetics [19], was electrospun into nanofiber mats [20-22]. Such nanofibers find their application in the biomedical industry [21, 23]. The close resemblance between serum albumin protein in the human body and bovine serum albumin (BSA) makes the latter ideal to mimic the human protein environment. Electrospun BSA nanofibers are thus widely used in the biomedical industry for wound dressings and as drug carriers [24-26]. Collagen and elastin are two principal proteins found in the extracellular matrices of human tissues, and they have been electrospun especially for wound dressings [27, 28]. Water soluble fish sarcoplasmic proteins (FSP) from the Atlantic cod (Gadus morhua) are largely available from fish, but also from the waste water from the fish industry [29-31]. Moreover, FSP has several health benefits; examples are their ability to improve insulin sensitivity in insulin-resistant subjects [32, 33], and to inhibit dipeptidyl peptidase-4 (DPP-IV) [34] - an enzyme with an essential role in glucose metabolism, and linked to type 2 diabetes. The bioactivity of FSP and its availability in large quantities from both fish and the waste water from the fish industry reveal this material as an interesting biopolymer. FSP was recently electrospun into nanofibers [35], and the potential of using the FSP fibers for drug delivery have been explored [36-38]. In these studies the fibers were produced from electrospinning using 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) as solvent, which can limit the range of applications for the fibers. HFIP has also been used as a solvent for electrospinning proteins such as collagen [28], BSA [39], and silk protein [40]. HFIP is a fluorinated alcohol and is a highly corrosive liquid that causes severe
respiratory problems [41], and accordingly its use should be avoided if possible. In general, solvents used for electrospinning the above-mentioned proteins are often organic which can have severe adverse effect, if residual solvents remain present in the fibers, even in small quantities. In this study formic acid was explored as an alternative solvent to HFIP. Formic acid is a simple carboxylic acid and it is widely used as a preservative and antibacterial agent. Moreover, formic acid is a generally recognized as safe (GRAS) substance, which makes it appealing to use as a solvent.

Solution blowing of petroleum-derived and biopolymers became an attractive method of forming nanofibers [42-47]. Recently the present group demonstrated for the first time that solution blowing of polymer nanofibers can be scaled up to the industrial level [47]. On the other hand, electrospinning is still the most common method used to form nanofibers from different polymers and proteins. However, electrospinning has a low production rate, which is considered a drawback for some fiber applications. Therefore, an alternative technique, solution-blowing, has been developed to form nanofibers from different proteins and biopolymers, such as soy protein [11-13], zein, lignin, cellulose acetate, sericin, and BSA [14], albeit it has never been used before to form nanofibers from fish sarcoplasmic proteins (FSPs). Solution-blowing is expected to be superior to electrospinning in terms of production rate, making it appealing for materials that are not sensitive to e.g. high pressure.

The aim of this study is in the formation of FSP/nylon 6-based nanofibers using solution blowing, which is the first effort of this kind, as to our knowledge. Also, for the first time we compare the properties of solution-blown FSP nanofibers with those of electrospun FSP nanofibers. FSP is a highly interesting biopolymer, especially due to its availability from the wastewater that can provide a huge source of this material, as well as increase the yield of the
fish as a resource. Moreover, since FSP is a biodegradable biopolymer, the possibility of it as a substitute to petroleum-derived synthetic polymers is highly appealing from an environmentally point of view. A prerequisite to the development of novel FSP nanofibers and their applications is the possibility of large-scale production. Accordingly, in the focus of the present study, for the first time, is the development of solution-blown FSP/nylon 6 nanofibers. In addition, several of our findings described below reveal that this novel technology has potential as an eco-friendly alternative to synthetic polymers.

2. Experimental

2.1 Materials

Nylon 6 pellets, $M_w = 35$ kDa, were obtained from Polysciences. Formic acid, $M_w = 46.03$ Da, was obtained from Sigma Aldrich and used as a solvent for all solutions. Fish sarcoplasmic protein (FSP) was extracted from fresh cod fillets [35] stored at -30°C. The frozen fillets were first defrosted and chopped. They were then centrifuged for 15 min, 18,000 x g, 5 °C (4K15, Sigma Laboratory centrifuges, Germany), and the supernatant was freeze dried [35].

2.2 Solution Preparation

Electrospinning and solution-blowing of different solutions of FSP/nylon 6 were conducted to generate monolithic fibers. Initially 12 % w/w nylon 6 solution was prepared by adding 1.2 g of nylon 6 pellets to 8.8 g of formic acid, and the solution was left for 3 h on a hot plate at 60 °C with constant stirring. Separately, the pure FSP solution with FSP concentration of 175 mg/mL was prepared by adding 1.75 g of FSP to 10 mL of formic acid and stirring for 15
mins at room temperature. After the nylon 6 solution cooled to room temperature, it was mixed with different volumes of the FSP solution to obtain different weight ratios of FSP to nylon 6 in the mixed solution, namely 0/100, 10/90, 25/75, 50/50, 75/25, 90/10 and 100/0. The blended solutions of FSP and nylon 6 had no visible grains of protein, as FSP was completely dissolved in formic acid. These solutions were kept on a stirring plate for 15 mins at room temperature before solution-blowing or electrospinning.

In distinction from the above-mentioned solutions, if the basic FSP solution concentrations were below 175 mg/mL, the blended solutions with the FSP to nylon 6 ratios of 10/90, 25/75, 50/50, 75/25, and 90/10, were insufficiently elastic and nanofibers could not be formed either by electrospinning or by solution-blowing. Electrospinning of such solutions typically led to dripping, whereas solution-blowing led to spraying without any fiber formation. Increasing the FSP concentration above 175 mg/ml caused the blended solutions to dry at the needle tip during solution-blowing or electrospinning. Hence the FSP concentration of 175 mg/mL was found to be the optimum concentration to be used for preparations of blended solutions for solution-blowing and electrospinning.

2.3 Solution-blowing

Monolithic nanofibers from blends of FSP/nylon 6 were formed using the solution-blowing technique. The experimental setup and method are described in detail elsewhere [11-14]. In brief, the solutions were contained in a 5 mL syringe and supplied to a 16G needle using a syringe pump. The flow rate varied between 7 – 10 ml/h. For pure nylon 6 solution, the flow rate was set at 7 ml/h. The effective viscosity of the solutions decreased with increasing FSP content in the blend, and the solution flow rate had to be increased for continuous nanofiber
The needle was surrounded by a concentric annular nozzle which issued a high-speed air stream supplied at a pressure of 60 psi. The tip of the needle from which the solution was issued was surrounded by this turbulent air jet. Therefore, the solution jet was stretched and bent due to the aerodynamically-driven bending instability, leading to formation of monolithic nanofibers. Solution-blowing of all solutions was conducted at room temperature and the relative humidity was 25-35%. Solution-blowing was also attempted at higher temperatures by preheating the solution-blowing chamber prior to solution-blowing. However this led to drying of the solution at the needle exit, and also resulted in more entangled fibers in the laydown. A wire-mesh collector was used as a nanofiber collector. To facilitate collection on the mesh, the latter was connected to a vacuum pump. Suction provided by the vacuum pump caused nanofiber deposition on the mesh. The distance between the needle tip and the collector was maintained at 15 cm. For each sample, nanofibers were collected for 5 min.

2.4 Electrosprning

A standard electrosprning setup was used to form FSP/nylon 6 monolithic nanofibers. The electrosprning setup is described in detail elsewhere [48-50]. In brief, electrosprning of the solutions was conducted using a 20G needle with the outer diameter of 0.908 mm and the inner diameter of 0.603 mm. The solutions were supplied by a 5 mL syringe connected to a syringe pump with the flow rate of 0.2 ml/h. A positive voltage of 15 – 20 kV was applied to the needle tip, and the distance between the tip of the needle and the aluminum foil used as grounded collector was 8 cm. The collector plate, covered with the aluminum foil, had dimensions of 10 cm × 10 cm. Electrosprning was conducted at room temperature and the relative humidity was 40% – 45%. For comparison of the fiber diameter distribution, porosity, and mass of collected
fibers using solution-blowing and electrospinning, fibers were collected for 5 min by both methods.

The mass of the nanofibers formed by electrospinning in 5 min was very small (of the order of 1 mg), for which reason the weight difference of the aluminum foil before and after electrospinning could not be used as a reliable estimate of the production rate, since it inevitably gave erroneous values. However, for all ratios of the blended FSP/nylon 6 solutions, nanofibers were deposited only around the center of the collector. Hence, it was safe to assume that all fibers formed by electrospinning were collected, and their mass accumulated in 5 min could be calculated from the flow rate of the solution.

2.5 Optical Characterization

The morphology and fiber structure of the solution-blown and electrospun fibers were studied using scanning electron microscopy (SEM). The SEM imaging and energy dispersive X-ray spectroscopy (EDX) analysis were conducted using Hitachi S-3000 N (Hitachi, Japan). The size distribution of the fiber diameters was found by processing the SEM images using the Olympus MicroSuite™ FIVE imaging software [51, 52]. For each sample, fiber diameters were measured from 10 different SEM images and the distribution histogram of the fiber diameters plotted.

2.6 Porosity Calculation

The porosity of fiber samples was assessed by analyzing the SEM images using Adobe Photoshop and MATLAB. The topmost layer of the nanofibers in SEM image (Figure 1a) was isolated using Photoshop by adjusting the brightness and contrast so that only the top layer fibers
in focus could be recognized (Figure 1b). Using MATLAB, this processed image was then converted to a gray scale image, with the fibers being black and the pores being white (Figure 1c). The porosity was calculated from the number of white pixels in the image. For each sample, 10 different SEM images were taken at the same magnification to obtain the porosity value.

Figure 1. Image processing of the SEM images of FSP/nylon 6 (50/50) solution-blown fibers. (a) The original SEM image, (b) top layer of the fiber mat separated using Photoshop, and (c) further processed Matlab image used for porosity calculation. The scale bars correspond to 10 μm.

2.6 Tensile Tests of Nanofiber Mats

A 100 N capacity mechanical testing machine (Model 5942, Instron, Massachusetts, US) was used to conduct tensile tests of the solution-blown nanofiber mats, according to the approach of the present group used elsewhere [11, 14]. Rectangular pieces approximately 5 cm long and 2 cm wide were cut from the solution-blown fiber mats and their two ends (in the longest direction) clamped by the top and bottom pneumatic grips of the Instron machine. The thickness of the samples was in the 0.1 – 0.15 mm range. For the tensile tests, the upper end of the sample was stretched at a constant rate of 0.1 mm/min, while the lower end was kept fixed at its initial position [11]. The stretching was conducted till sample breakage. Each measurement was carried out 10 times and the mean ± SD was reported. Electrospinning of the blended solutions of
FSP/nylon 6 did not generate mats appropriate for tensile tests. Therefore, these tests have not been conducted with electrospun fiber mats.

2.7 Statistics

Statistical analysis was performed using GraphPad (GraphPad, La Jolla, CA, USA). Unpaired Student’s t test was used for assessment of statistically significant differences.

3. Results and Discussion

3.1 Solution-Blown Monolithic FSP/nylon 6 Nanofibers

Monolithic nylon 6 nanofibers obtained by solution-blowing are shown in Figure 2. The fiber distribution was uniform along the entire collection area and the fibers were smooth without any beads. Monolithic fibers obtained from different blends of FSP and nylon 6, from 10/90 % w/w FSP/nylon 6 to 90/10 % w/w FSP/nylon 6, are shown in Figure 3. Uniform continuous fibers were obtained by solution-blowing for 10/90 – 75/25 % w/w FSP/nylon 6.

Figure 2. (a) Overall view of solution-blown nylon 6 nanofiber mat, and (b) a zoomed-in view. The scale bars correspond to 10 μm.
solutions. On further increasing the FSP weight percentage to 90%, the fiber distribution in the laydown became highly non-uniform, with fiber strips surrounded by areas with only few fibers. However, the morphology of these isolated fibers was still similar to those obtained at lower FSP contents (Figure 3e). The FSP solution alone was also blown but no fibers were formed and only spraying was observed.
Figure 3. Solution-blown FSP/nylon 6 fibers with dry weight ratios of (a) 10/90, (b) 25/75, (c) 50/50, (d) 75/25, and (e) 90/10. Each panel shows the overall fiber distribution on top and the zoomed-in images on the bottom. The scale bars correspond to 10 μm.

3.2 Electrospun Monolithic FSP/nylon 6 Nanofibers

Different blends of FSP/nylon 6 were electrospun to compare the resulting nanofiber mats with those formed by solution-blowing. For a proper comparison the same solutions were used in both cases. The SEM images of the electrospun fibers are shown in Figure 4. The collected fibers were uniform and beadless. Notably, both solution-blowing and electrospinning were able to form nanofibers from the 90/10 % w/w FSP/nylon 6 solution. As described in the experimental section, for all solutions, the fibers were collected directly beneath the needle and covered quite a small area with the radius in the 2 – 3 cm range. Electrospinning of pure FSP solution resulted in spraying and there was no fiber formation.

Figure 4. Electrospun FSP/nylon 6 fibers with the dry weight ratios of (a) 0/100, (b) 10/90, (c) 25/75, (d) 50/50, (e) 75/25, and (f) 90/10. The scale bar corresponds to 10 μm.
3.3 FSP Content in Solution-blown and Electrospun Nanofibers

Energy dispersive X-ray spectroscopy (EDX) was used to identify the unique atomic markers for FSP, to differentiate it from nylon 6 in the solution-blown and electrospun monolithic nanofibers. Figure 5a shows the EDX spectra of a cast film of FSP solution, where phosphorus (P), sulfur (S), chlorine (Cl), and potassium (K) peaks are clearly distinct, with P and K being in higher quantities than sulfur and chlorine. Hence, P, S, Cl, and K can be considered to be unique markers of FSP. Figures 5b and 5c show the EDX spectra of solution-blown or electrospun nanofibers obtained from 50/50 blend solution of FSP/nylon 6, respectively. The P, S, Cl, and K peaks are distinctly visible for both types of nanofibers.

![Figure 5](image)

**Figure 5.** EDX spectra of (a) FSP film, (b) solution-blown 50/50 FSP/nylon 6 nanofibers, and (c) electrospun 50/50 FSP/nylon 6 nanofibers.

**Table 1.** Element content in different samples of FSP/nylon 6 monolithic nanofibers. The characters S and E in the second column corresponds to solution-blown and electrospun nanofibers, respectively. Mean values, n = 8.

<table>
<thead>
<tr>
<th>FSP/Nylon (% w/w)</th>
<th>C (%)</th>
<th>O (%)</th>
<th>P (%)</th>
<th>S (%)</th>
<th>Cl (%)</th>
<th>K (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSP film</td>
<td>59.4</td>
<td>25.9</td>
<td>4.2</td>
<td>1.8</td>
<td>1.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>
Table 1 lists the elemental composition established by the EDX analysis of solution-blower and electrospun nanofibers obtained from different FSP/nylon 6 solutions. As expected, an increase in the weight ratio of FSP in the nanofibers (from 0/100 to 90/10) results in an increase in the weight percentages of P, S, Cl, and K. It should be emphasized that these results show that the FSP/nylon 6 fibers contain FSP in the same proportion as in the blended solution (data not shown), indicating that the solutions were homogeneous without the presence of any undissolved protein.

### 3.4 Fiber Diameter Distribution
The size distribution of the fiber diameters for the FSP/nylon 6 fibers obtained by solution-blowing and electrospinning are shown in Figure 6, while the corresponding mean values and SD are listed in Table 2. For a specific FSP/nylon 6 ratio, the mean diameter of the electrospun fibers was smaller than the solution-blown fibers. Also, the solution-blown fibers had a wider size distribution of the fiber diameters as compared to the corresponding electrospun fibers.

Figure 6. Fiber diameter distribution for (1) 0/100, (2) 10/90, (3) 25/75, (4) 50/50, (5) 75/25, and (6) 90/10 % w/w FSP/nylon 6 blended monolithic nanofibers. The suffix S in the panel notation corresponds to the solution-blown nanofibers, while E corresponds to electrospun fibers.
The average diameter of pure nylon 6 nanofibers was 213 nm for solution-blowing and 180 nm for electrospinning. Upon blending nylon 6 with FSP and thereby increasing the FSP content in the fibers, the average fiber diameter increased. For solution-blown fibers the average fiber diameter increased from 213 nm (for 0/100 % w/w FSP/nylon 6) to 407 nm (for 90/10 % w/w FSP/nylon 6). Also, the size distribution of the fiber diameters increased with increasing FSP ratio as seen in Figure 6 and in Table 2, being more pronounced when the FSP content was above 50 % w/w. The average fiber diameter for the FSP/nylon 6 blended fibers were similar to those obtained by solution-blowing of the other blended biopolymer solutions, such as soy protein/nylon 6 [13], lignin/nylon 6, sericin/nylon 6, and zein/nylon 6 [14]. On the contrary, the fiber diameter of the electrospun fibers had relatively small standard deviation. In all the cases, except for 90/10 FSP/nylon 6 nanofibers, 50% or more of the nanofibers were in the same diameter range as seen in Figure 6. Even for 90/10 FSP/nylon 6 electrospun fibers the standard deviation was less than 50 nm. The average diameter increased from 180 nm for pure nylon 6 fibers to 344 nm for electrospun 90/10 FSP/nylon 6 fibers. These diameters were of the same
order as those for electrospun biopolymer blend nanofibers reported elsewhere [53]. Moreover, the fiber diameters of electrospun FSP/nylon 6 fibers were similar to those of pure FSP electrospun nanofibers [35]; at low FSP concentrations the fiber diameters were in the 100 – 700 nm range and increased with increasing FSP concentration. However, for pure electrospun FSP fibers, wide size distributions and irregularities were observed in previous studies [35], which were not found for fibers obtained from FSP/nylon 6 blends in the present work. It should be emphasized that all fibers obtained in this study, either by solution-blowing or electrospinning, were in the submicron range.

### 3.5 Porosity of FSP/nylon 6 Fiber Mats

The porosity of the fiber mats was calculated from the SEM images as described in the experimental section. The porosity values for different blends of FSP/nylon 6 are shown in Figure 7 and listed in Table 3.
Figure 7. Porosity for solution-blown and electrospun FSP/nylon 6 fiber mats. In the histogram, the bars with continuous lines correspond to solution-blown fibers (left) and those with dots correspond to electrospun fibers (right).

Table 3. Porosity of solution-blown and electrospun FSP/nylon 6 nanofiber mats. Mean ± SD are reported, n = 8.

<table>
<thead>
<tr>
<th>FSP/Nylon (% w/w)</th>
<th>Solution-blown</th>
<th>Electrospun</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/100</td>
<td>0.48 ± 0.04</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>10/90</td>
<td>0.50 ± 0.04</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>25/75</td>
<td>0.54 ± 0.05</td>
<td>0.49 ± 0.05</td>
</tr>
<tr>
<td>50/50</td>
<td>0.55 ± 0.07</td>
<td>0.53 ± 0.04</td>
</tr>
<tr>
<td>75/25</td>
<td>0.56 ± 0.08</td>
<td>0.54 ± 0.07</td>
</tr>
<tr>
<td>90/10</td>
<td>0.62 ± 0.11</td>
<td>0.58 ± 0.09</td>
</tr>
</tbody>
</table>

For pure nylon 6 fiber mats, the porosity was 0.48 and 0.45 for the solution-blown and electrospun fiber mats, respectively. The porosity of the solution-blown and electrospun fiber mats increased with increasing FSP content. For a specific FSP/nylon 6 ratio, the solution-blown fiber mats possessed higher porosity than the corresponding electrospun fiber mats. Unlike electrospinning, where there is a strong electric force pulling the fibers to the collector, solution-blown fibers do not experience such pulling force to the collector after stretching by the high pressure air stream (even with suction applied). Hence solution-blown fiber mats are fluffier and more porous than their equivalent electrospun fibers.

The porosity of the 90/10 FSP/nylon 6 fiber mat was significantly higher than for the others. This can be explained as follows. The fibers obtained from the 90/10 FSP/nylon 6 blended solution were entangled in bundle-like structures and the fiber distribution on the collector was not uniform, with some areas with multiple fibers and other areas with no fibers.
The bundling effect was higher for solution-blown fibers compared to the electrospun fiber, resulting in an increased porosity of the solution-blown 90/10 FSP/nylon 6 fiber mats (0.61), compared to 0.58 of their electrospun counterparts.

3.6 Collection Rate of Solution-blown and Electrospun Nanofibers

For all solutions, monolithic FSP/nylon 6 nanofibers were electrospun at a solution flow rate of 0.2 ml/h. As described in the experimental section, the mass of the electrospun fibers collected for 5 min was negligibly small, for which reason the amount of collected fibers was calculated from the solution flow rate rather than by weighing the collected fibers. The concentration of the basic nylon 6 solution (12 % w/w corresponds to 166 mg/ml) was less than that of the basic FSP solution (175 mg/ml), and accordingly, increasing weight ratios of FSP in the blended solutions led to an increased mass of nanofibers formed per unit time. For pure nylon 6, 2.43 mg of nanofibers were formed in 5 min, whereas for 90/10 FSP/nylon 6 solution, 2.86 mg of nanofibers were formed. The amount of nanofibers formed in 5 min by electrospinning for different blend ratios of FSP/nylon 6 are listed in Table 4.

Solution-blowing, on the other hand, is a much faster fiber forming process. Indeed, 5 min of solution-blowing of FSP/nylon 6 resulted in thick nanofiber mats on the collector, which could be easily peeled of and weighed. The average mass of the nanofiber mats measured in 5 min of solution-blowing were 78±4 mg, 81±7 mg, 82±6 mg, 87±10 mg, 90±11 mg, and 94±9 mg for FSP/nylon 6 weight ratios of 0/100, 10/90, 25/75, 50/50, 75/25, and 90/10, respectively. The high speed air jet used for the solution-blowing caused some of the fibers to be deposited outside the collector, leading to decrease in yield. Accordingly, as evident from Table 4, the amount of nanofiber calculated from the solution flow rates was always higher than the actual masses of
solution-blown nanofiber collected. Comparing the rates of fiber formation for the two processes, it can be concluded that solution-blowing generates nanofibers 30 times faster than electrospinning.

Table 4. Nanofiber masses formed using solution-blowing and electrospinning of different FSP/nylon 6 solutions for 5 min.

<table>
<thead>
<tr>
<th>FSP/Nylon (% w/w)</th>
<th>Calculated nanofiber mass formed by electrospinning (mg)</th>
<th>Calculated nanofiber mass of fibers formed by solution-blowing (mg)</th>
<th>Measured nanofiber mass of fibers formed by solution-blowing (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/100</td>
<td>2.43</td>
<td>84.69</td>
<td>78</td>
</tr>
<tr>
<td>10/90</td>
<td>2.47</td>
<td>86.12</td>
<td>81</td>
</tr>
<tr>
<td>25/75</td>
<td>2.53</td>
<td>88.39</td>
<td>82</td>
</tr>
<tr>
<td>50/50</td>
<td>2.65</td>
<td>92.67</td>
<td>87</td>
</tr>
<tr>
<td>75/25</td>
<td>2.78</td>
<td>97.05</td>
<td>90</td>
</tr>
<tr>
<td>90/10</td>
<td>2.86</td>
<td>100</td>
<td>94</td>
</tr>
</tbody>
</table>

3.7 Tensile Tests of Solution-blown Nanofibers

Stress-strain dependence for a 50/50 FSP/nylon 6 solution-blown nanofiber mat is shown in Figure 8. Initially, the stress depended on the strain linearly, obeying Hooke’s law. At higher strains, the dependence became non-linear indicating a shift from elasticity to plasticity. The rupture stress and strain for the 50/50 FSP/nylon 6 sample was in the range of $\sigma_{xx,\text{rupture}} = 0.15$-0.85 MPa and $\varepsilon_{\text{rupture}} = 12\%-17\%$.
Figure 8. (a) Experimental data for tensile stress-strain curve for a solution-blown nanofiber mat of 50/50 FSP/nylon 6. (b) The same experimental data fitted with the phenomenological model of eqn. (1). The red line corresponds to the theoretical prediction and the blue line to the experimental data.
**Figure 9.** (a) Rupture stress $\sigma_{xx,\text{rupture}}$, (b) rupture strain $\varepsilon_{\text{rupture}}$ (%), (c) Young’s modulus E, and (d) yield stress Y for different samples of solution-blown FSP/nylon 6 nanofibers. Mean ± SD, n = 10, ns: no significant difference.

Similar results were obtained for solution-blown monolithic fibers of the other blend ratios of FSP/nylon 6. The rupture stress and strain for the different samples are shown in Figure 9a and 9b, respectively, and listed in Table 5. Interestingly, there was no significant difference in the rupture stress between pure nylon 6 nanofibers and nanofibers where up to 50 % of nylon 6 were replaced with FSP. Increasing the FSP ratio further caused a decrease in the rupture stress. However, the presence of protein in the monolithic fibers provided stretchability, as indicated by the increasing strain at rupture of samples with increasing FSP ratio (Fig. 9b). Both the rupture stress and strain were significantly lower for the 90/10 % w/w FSP/nylon 6 samples compared to the rest. This is most likely because the 90/10 % w/w FSP/nylon 6 nanofiber mats were not uniform.

The phenomenological equation for the uniaxial stretching of a strip in the elastic and plastic zone is given by [11]

$$\sigma_{xx} = Y \tanh \left( \frac{E \varepsilon}{Y} \right)$$

where E is the Young’s modulus, Y is the yield stress, $\sigma_{xx}$ is the tensile stress, and $\varepsilon$ is the tensile strain.

The experimental stress-strain curves in the elastic and plastic zone were fitted to eqn. (1) as shown in Figure 8b, and the values of Young’s modulus E and the yield stress Y were thus determined. The values of E and Y for different blends of FSP/nylon 6 in monolithic fibers are depicted in Figures 9c and 9d, respectively. Young’s moduli and the yield stresses for all the...
samples were in the 5 – 11 MPa and 0.2 – 0.7 MPa ranges, respectively, except for the 90/10 % w/w FSP/nylon 6 samples for reasons described above. These values were in reasonable agreement with those for other biopolymer solution-blown fibers [11, 14]. Similar to the rupture stress, there was no significant difference between pure nylon 6 nanofibers and nanofibers where up to 50% of nylon 6 were replaced with FSP (25% for the yield stress). All together these results indicate that 50% of nylon 6 can be replaced with FSP without compromising the mechanical properties of the nanofibers, making FSP an interesting substitute material for decreasing the consumption of synthetic polymers like nylon 6.

Table 5. Rupture stress $\sigma_{xx,\text{rupture}}$, failure strain $\varepsilon_{\text{rupture}}$, average Young’s modulus $E$, and yield stress $Y$ for different samples of solution-blown FSP/nylon 6 nanofibers. Mean ± SD are reported, n = 10.

<table>
<thead>
<tr>
<th>FSP/Nylon (% w/w)</th>
<th>max. strain at rupture (%)</th>
<th>max. stress at rupture (MPa)</th>
<th>avg. Young’s modulus E (MPa)</th>
<th>avg. yield stress Y (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/100</td>
<td>6.68 ± 1.15</td>
<td>0.61 ± 0.02</td>
<td>8.89 ± 0.75</td>
<td>0.63 ± 0.02</td>
</tr>
<tr>
<td>10/90</td>
<td>8.18 ± 1.53</td>
<td>0.59 ± 0.03</td>
<td>8.71 ± 0.88</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>25/75</td>
<td>10.76 ± 1.91</td>
<td>0.58 ± 0.05</td>
<td>8.41 ± 0.46</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>50/50</td>
<td>14.60 ± 2.14</td>
<td>0.47 ± 0.05</td>
<td>7.91 ± 0.67</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td>75/25</td>
<td>17.94 ± 1.60</td>
<td>0.40 ± 0.02</td>
<td>7.07 ± 0.77</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>90/10</td>
<td>9.433 ± 3.14</td>
<td>0.31 ± 0.04</td>
<td>5.06 ± 0.84</td>
<td>0.33 ± 0.05</td>
</tr>
</tbody>
</table>

4. Conclusions

In this work, we have formed, for the first time, novel solution-blown nanofibers from fish sarcoplasmic protein (FSP), an animal-derived protein. The bioactivity and health benefits of FSP, which includes improving insulin sensitivity in insulin-resistant subjects and the inhibition of DPP-IV enzyme which has an essential role in glucose metabolism, make FSP an attractive
biopolymer. Due to the abundant availability of FSP from fish, as well as from wastewater from the fish industry, FSP is an inexpensive biopolymer which can potentially replace synthetic polymers. For the first time, we have formed solution-blown nanofibers from blends of FSP and nylon 6 in formic acid, the latter being a simple and safe solvent as compared to generally used organic corrosive solvents like HFIP – a solvent often used for fiber generation from proteins. We have shown that nanofibers containing FSP can be formed on a mass scale using solution blowing technique, which produces fibers 30 times faster than traditional electrospinning, making such nanofibers industrially viable and relevant. Namely, we have generated nanofibers from blends of FSP/nylon 6 by solution blowing, characterized their mechanical properties and compared them to electrospun FSP/nylon 6 nanofibers. Using EDX, we found the unique marker elements of FSP which we then used to measure FSP content in the solution-blown FSP/nylon 6 nanofibers. We showed that the solution-blown nanofiber mats are fluffier and more porous than their electrospun counterparts, making the solution-blown nanofibers preferrable for such industrial applications as tissue scaffolds, chemical sensors, filtration, etc. We also showed for the first time, that up to 50 % w/w of synthetic polymer nylon 6 can be replaced by biopolymer FSP without compromising the mechanical properties of the nanofibers. In fact, such substitution leads to enhanced strechability of the nanofibers. In brief, we formed solution-blown nanofibers using the industrially-scalable technique from a new type of biopolymer (FSP), making them an interesting substitute eco-friendly material for synthetic polymers.

5. Acknowledgments
The authors gratefully acknowledge Alex Kolbasov’s help in measurements of porosity of nanofiber mats using their SEM images, and the Danish Strategic Research Council (DSF-10-93456, FENAMI Project) are acknowledged for financial support.

6. References


16. Z. Ma, M. Kotaki and S. Ramakrishna. Electrospun cellulose nanofiber as affinity membrane. *Journal of Membrane Science*, 2005, **265**(1), 115-123.


