A COMPARISON OF THE CENTRIFUGAL FORCE SPINNING AND ELECTROSPINNING OF COLLAGEN UNDER DIFFERENT CONDITIONS

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Abstract

Collagen makes up one of the most important parts of the extracellular matrix and connective tissue. Collagen type I accounts for up to 90% of the collagen found in the body and it can be isolated and purified in large quantities and processed into a variety of forms. With respect to the process of the treatment of collagen into the nano-fibrous and submicron-fibrous forms, severe conditions leading to the denaturation of the collagen and thus the gelatin content should be avoided. Centrifugal force spinning and electrospinning processes appear to present promising methods for the spinning of collagen solutions. This paper deals with a comparison of the various methods applied in the processing of collagen, i.e. principally needle and needleless centrifugal spinning technology and electrospinning technology. In addition, the effect of process parameters and collagen solution conditions on the final form of the material was analyzed. Spun layers were prepared based on collagen type I isolated from calf skin. Nano-structured layers were prepared employing the spinning of 4-16 wt% collagen solutions in phosphate buffer saline and ethanol. The layers thus prepared were characterized by means of scanning electron microscopy and Fourier transform infrared spectroscopy.

Keywords: Collagen, electrospinning, centrifugal force spinning, triple-helix

1. INTRODUCTION

Collagen consists of a molecule with a complex hierarchical arrangement which means that even subtle changes in its structure may have unforeseen consequences in terms of its function. In the course of collagen treatment, from isolation to processing into a variety of forms, many of the methods employed represent a compromise between the degree of disruption/modification of its native structure and the conservation of its biological functions to the maximum extent. Moreover, no non-invasive method is yet available for the processing of collagen while fully preserving its native structural properties. Indeed, the question arises as to whether this is even possible. It is, however, possible to apply various spinning methods in the preparation of collagen submicron fibers and nanofibers. Similarly, differing conditions may be applied with respect to their preparation, i.e. different solvent systems [1], solution parameters [2], spinning conditions, types of emitters and collectors etc. Moreover, it is difficult to maintain the delicate balance between the successful dissolution/dispersion of the collagen and the preservation of its structure (in the absence of denaturation) during the preparation process and it is still unclear as to whether the spinning process influences the structure of collagen. To make matters more complicated, natural polymers are difficult to spin due to their variable and high molecular weight, viscosity, rigid chain conformation, etc. Some polymers cannot be spun in a single solvent system; however, it is possible that the addition of other miscible polymers, the functionalization of reactive groups on the surface and the introduction of co-solvents into the electrospinning process will overcome this problem [3]. This paper provides a comparison of needle and needleless centrifugal force spinning and the electrospinning of collagen solutions with different concentrations and with or without the

addition of polyethylene oxide (PEO). In addition, the paper provides an analysis of the effects of process parameters and collagen solution conditions on the final form of the material.

2. MATERIALS AND METHODS

Submicron- and nanofibrous materials were prepared based on collagen (type I, VUP Medical, Czech Republic) and fibrous mats were prepared employing the spinning of 4, 6, 8, 12 and 16 wt% collagen solutions in phosphate buffer saline and ethanol with (ES+P) and without (ES-P) the addition of 8 wt% PEO (Sigma-Aldrich, Germany). Electrospun mats were prepared using a high voltage level of 45 kV (4SPIN, Contipro, Czech Republic). Two methods were employed for the centrifugal force spinning of collagen solutions with 8 wt% PEO, i.e. needle (N) and needleless (NL) spinning using laboratory-made equipment and two different circumferential velocities were applied, namely 15 (N15 and NL15) - 35 $m.s^{-1}$ (N35 and NL35). For the purposes of this study, none of the spun mats were cross-linked following preparation.

The prepared layers were characterized by means of both scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy and the samples were characterized using scanning electron microscopy (QUANTA 450, FEI, USA and TESCAN Vega 3SB Easy Probe, Czech Republic). Further, image analysis was employed in order to characterize the fiber size distribution of the spun samples from SEM pictures using NIS Elements software (LIM s.r.o., Czech Republic) or ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016). Fourier-transform infrared spectroscopy (FTIR) was applied so as to evaluate the extent of the disruption/modification of the collagen native structure; a Protégé 460 E.S.P. infrared spectrometer (Thermo Nicolet Instruments, USA) equipped with an ATR device (GladiATR, PIKE Technologies, USA) with a diamond crystal was used for this purpose. All the spectra were recorded in absorption mode at a resolution of 4 cm^{-1} and 128 scans. The areas of the bands (integral absorbencies) were determined using OMNIC 7 software. Collagen lyophilisate (type I, VUP Medical, Czech Republic) served as a positive control. Statistically significant differences were investigated principally by means of nonparametric methods (STATGRAPHICS Centurion XV, StatPoint, USA) due to the problematic nature of the verification of the normality of the assessed data (Shapiro-Wilk test) or the violation of homoscedasticity (Leven's tests); the Kruskal-Wallis test was used for this purpose and the Mann-Whitney W test was used for the conducting of post hoc analysis. All the variance analyses were performed at a 95% confidence level (p values <0.05 were considered to be significant).

3. RESULTS AND DISSCUSION

The electrospinning process was optimized by means of changing the concentration of the collagen solution. **Figures 1** and **2** provide representative SEM images of electrospun collagen from solutions with different concentrations.

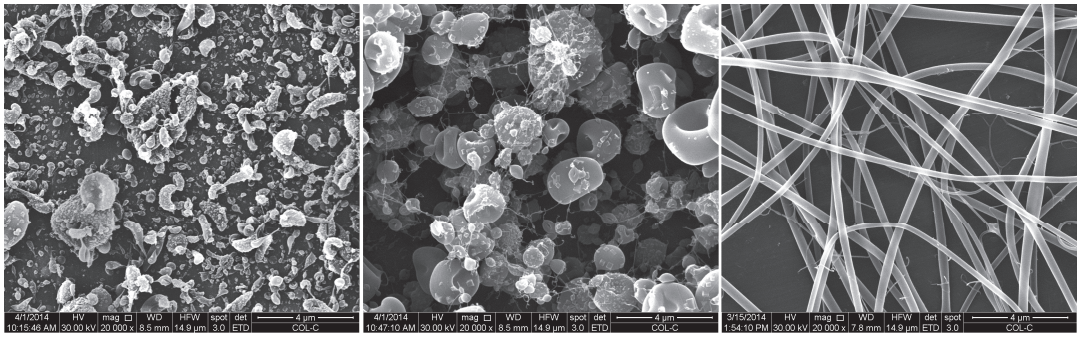

Figure 1 Representative SEM images of samples electrospun from collagen solutions (without PEO) with concentrations of (from left) 4, 8 and 16 wt% (mag. 20,000x)

In the case of solutions with no PEO, fibers were formed only from a solution with a concentration of 16 wt% (**Figure 1**). Following the addition of PEO, fibers were formed from collagen solutions with a lower concentration of 8 wt% (**Figure 2**).

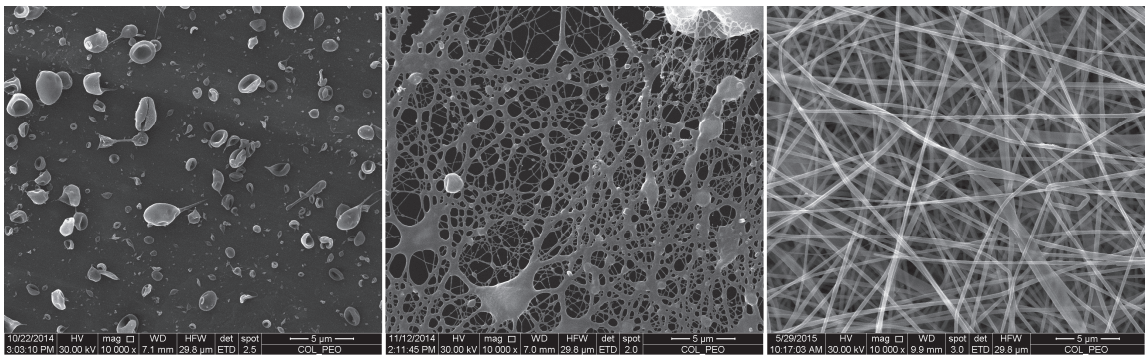

Figure 2 Representative SEM images of samples electrospun from collagen solutions (with PEO) with concentrations of (from left) 4, 6 and 8 wt% (mag. 10,000x)

In the case of needle and needleless centrifugal force spinning, the production process was optimized by means of changing both the concentration of the collagen solution and the circumferential velocities. Fibers were formed from collagen solutions with concentrations higher than 12 wt% following the application of both methods (see **Figure 3**). Droplets present within the centrifugal spun layers and the broad range of fiber diameters were caused by the Rayleigh instability of the polymer jet and the velocity of the evaporating solvent [4].

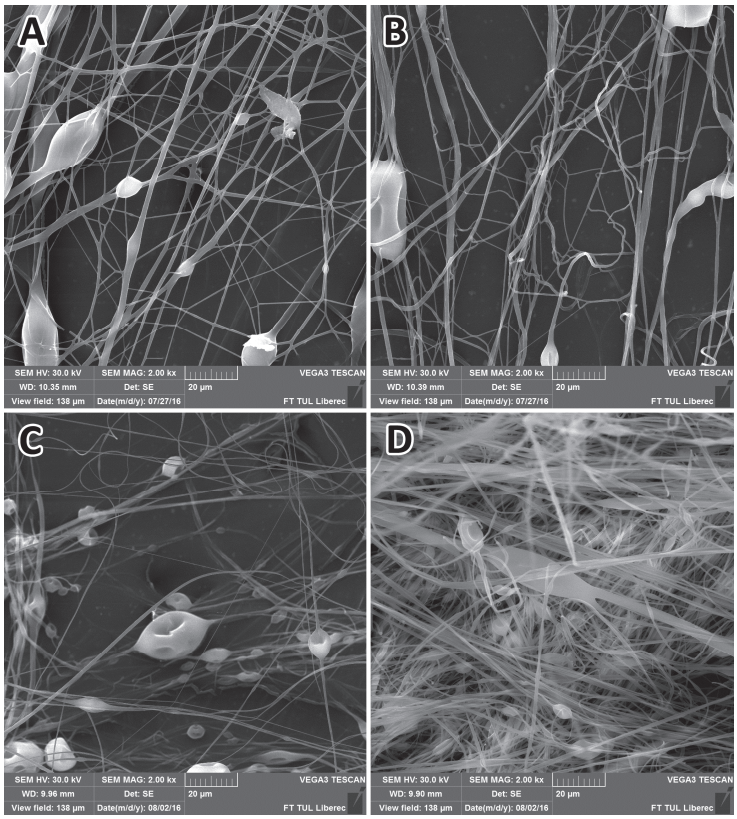

Figure 3 Representative SEM images of samples prepared employing the needleless (A, B) and needle (C, D) centrifugal force spinning of the collagen solutions (with PEO) with a concentration of 12 wt% and circumferential velocities of 15 $m.s^{-1}$ (A, C) and 35 $m.s^{-1}$ (B, D) (mag. 2,000x)

Nevertheless, the main complication issuing from the centrifugal force spinning of the solutions consisted of the rate of production; consequently, only the 12 and 16 wt% collagen solution samples spun at velocities of 15 m.s^{-1} and 35 m.s^{-1} were subjected to further analysis. **Figure 4** illustrates the effect of solution concentration and the applied technology on the diameter of the fibers.

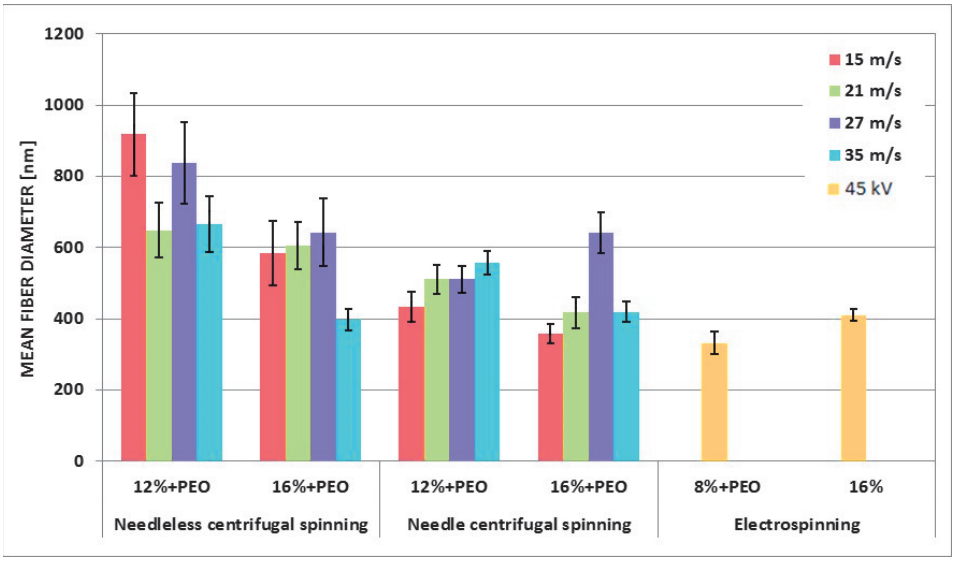

Figure 4 Graph illustrating the effect of solution concentration and the applied technology on the diameter of the fibers

Figure 4 shows that there was no significant dependence between fiber diameter and circumferential velocity. It is possible to observe that the fibers produced by means of needle centrifugal spinning were thinner than the needleless spun fibers and that higher concentration collagen solutions led to a smaller fiber diameter. Electrospun collagen solutions provided both a narrow fiber diameter distribution and the finest fibers.

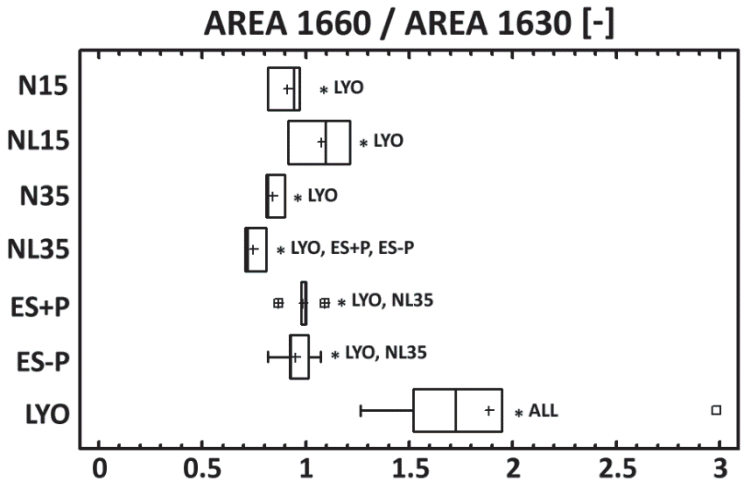

Figure 5 Comparison of the ratio of the integral absorbances of materials prepared using different spinning technologies. N15 and N35 - needle centrifugal spinning with a concentration of 12 wt% and circumferential velocities of 15 m.s^{-1} and 35 m.s^{-1}; NL15 and NL35 - needleless centrifugal spinning with a concentration of 12 wt% and circumferential velocities of 15 m.s^{-1} and 35 m.s^{-1}; ES+P and ES-P - electrospun from collagen solutions with a collagen concentration of 16 wt% (with and without PEO) and their comparison with lyophillized collagen (LYO). * denotes statistically significant differences (Mann-Whitney, 0.05)

The amide I region of the native collagen spectrum (~1650 cm^{-1}) could be deconvolved into three distinct bands with maxima at ~1660, ~1640 and ~1630 cm^{-1} [5]. Following denaturation, the bands did not shift appreciably in terms of position; however, the relative intensities of the 1660 and 1630 cm^{-1} bands shifted from >1 to <1, in other words, the component positioned at around 1630 cm^{-1} increased and the component positioned at around 1660 cm^{-1} decreased in intensity. The ~1660 cm^{-1} band was assigned to the triple helix with contributions from the α-helix and β-turns [5], while the ~1630 cm^{-1} band was assigned to imide residues (and partly to the β-sheet) [6].

The quantitative band-fitting analysis of the amide I area (expressed as a ratio of the areas of the 1660 and 1630 bands) of the materials prepared using different spinning technologies and their comparison with lyophillized collagen is summarised in **Figure 5**. Lyophillized collagen (LYO) demonstrated the highest 1660/1630 ratio value and demonstrated statistically significant differences compared to the materials prepared using other spinning technologies, which suggests that LYO contained a high proportion of the component related to the triple helical structure. The NL35 material embodied no further statistically significant differences compared to the materials prepared by means of electrospinning; it contained the lowest proportion of the component related to the triple helical structure (~1660 cm^{-1}) and the highest area contributed by the 1630 component related to imide residues. The other materials were mutually comparable.

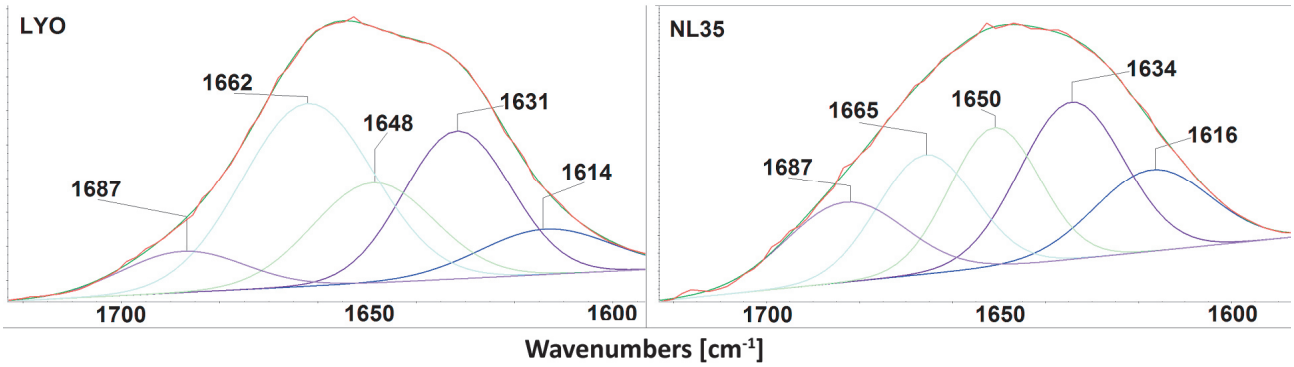

Figure 6 Comparison of the deconvoluted ATR-FTIR spectra of lyophilized collagen (LYO) and the NL35 material

Figure 6 provides examples of the deconvoluted ATR-FTIR spectra of the LYO and NL35 materials. The spectra also contained other components corresponding to other structural states. The component at ~1615 cm^{-1} is related to gelatin, the band at ~1650 cm^{-1} corresponds to random coils and the component at ~1690 cm^{-1} can be attributed to helices of aggregated collagen-like peptide [6]. As is apparent from **Figure 6**, the LYO material contains a proportionally large area related to the triple helical structure (~1660 cm^{-1}) compared to the other structural states (~1615 cm^{-1}, ~1650 cm^{-1} and ~1690 cm^{-1}) whereas the NL35 sample exhibits the opposite behavior. The further processing of lyophillized collagen (dissolution and spinning) was found to lead to spectral changes in the amide I region which corresponded to changes in the secondary structure of the collagen.

4. CONCLUSION

The paper presents an analysis of the effect of process technology, process parameters and collagen solution conditions on the final form and structure of collagen. The processes concerning both the isolation of collagen to its dissolution and from solution to the artificially-produced fibrous form are complex. Solvent systems are capable of breaking down most of the hydrogen bonds within the adjacent collagen molecules; on the other hand, certain inter/intra molecular bonds within the collagen must be maintained so as to stabilize its triple helix. It is clear therefore that it is difficult to maintain the balance between the successful processing of

collagen and the preservation of its structure without denaturation. Our results indicate that centrifugal force spinning and electrospinning lead to the preparation of collagen fibers with very similar properties but that, in general, spinning processes (or the preparation of collagen solutions) influence the structure of the collagen, which was illustrated by the occurrence of spectral changes corresponding to changes in the structure of the collagen. The denaturation rate of collagen and the potential for the improvement of the partially damaged native structure of collagen (e.g. by cross-linking) remain to be specified.

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