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ELECTROSPINNING COLLAGEN AND ELASTIN FOR TISSUE ENGINEERING SMALL DIAMETER BLOOD VESSELS

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Summary

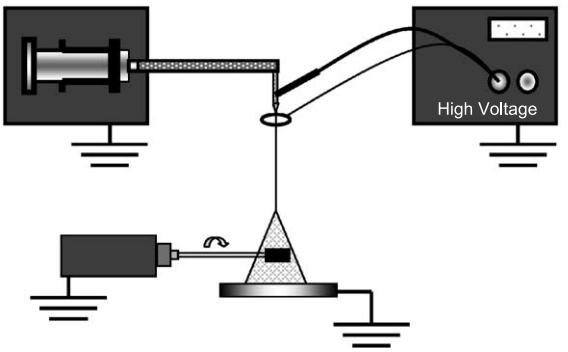
Nonwoven fibrous matrices of collagen and elastin have been prepared by means of electrospinning. Variations in the morphology of the scaffolds have been evaluated as a function of the ratio of the two proteins. Scaffolds having an architecture resembling the media of natural blood vessels have been obtained. Crosslinking has been used as a valuable method to improve the stability of such matrices. The obtained nonwoven matrices can be used for tissue engineering applications.

Introduction

Tissue engineering of small diameter (<6 mm) blood vessels is regarded as an excellent opportunity to overcome the problems and poor performance of synthetic artificial blood vessels. Our approach comprises the design and development of a biodegradable porous three-layered tubular scaffold. Electrospinning is used to reach this aim. In this technique, a polymer solution is subjected to an electric field that permits the formation of a fibre from a charged jet. An advantage of this technique is that fibres having a diameter in the range of a few hundred nanometres can be produced. This permits to obtain scaffolds with a large surface area and a high porosity, two essential characteristics for cell culturing.

Experimental methods

Solutions of collagen/elastin/PEO (C:E:P) are prepared in hydrochloric acid, containing NaCl and are then charged in a syringe connected to a syringe pump in a horizontal mount. The solution is directed to a capillary blunt needle tip through a silicone tube. An electronic potential is applied to the needle by an electrode connected to a high-voltage supply. Fibres are collected either on a rotating mandrel placed between the capillary tip and a grounded aluminium plate or on the grounded aluminium plate itself. A charged steel ring is placed perpendicular to the jet at the end of the capillary tip to stabilize the jet and to direct it downwards (Fig. 1).



Results and discussion

Solutions of C:E:P having different weight ratios of collagen and elastin have been prepared in order to study the influence of spinning conditions on the morphology of the resulting meshes. Fibres are formed when the repulsive force produced by the mutual charge repulsion in the drops at the tip of the spinneret, exceeds the surface tension [2]. Addition of PEO is necessary to produce fibres, while the presence of NaCl ensures a better conductivity of the solution. In this study, the effect of the concentration of elastin is evaluated since it is known that solution viscosity and surface tension of the solution are the main factors determining the formation of electrospun fibres [1]. Under otherwise similar conditions, a decrease in elastin concentration causes an increase in surface tension. As a consequence, the voltage needed to produce a fibre increases as well (data not shown). A decrease in elastin also causes an increase in viscosity, as can be seen from the rheometry properties of different solutions (Fig. 2). Both phenomena can be explained by the higher hydrophobicity of elastin compared to collagen. Increasing the concentration of the former protein leads to an increase in hydrophobic elements both at the surface of the solution and in the bulk, thus causing the observed decrease in viscosity and surface tension.

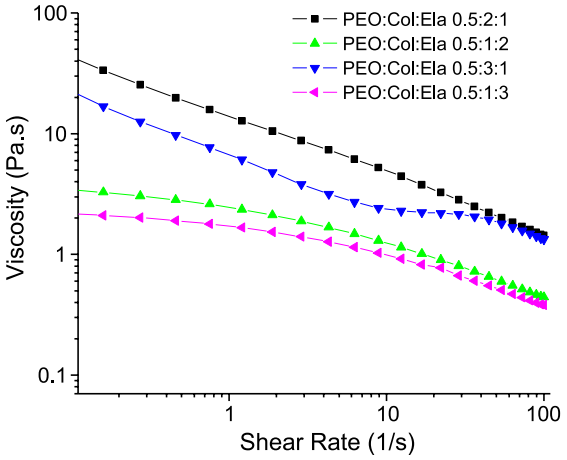


Fig. 2. Rheometry properties of different solutions.

As expected, variations in the composition of the spun solution influence the morphology of the obtained fibre. In particular, a higher viscosity favours the formation of flat fibres [3]. This is verified by spinning at exactly the same conditions three different solutions having a different collagen:elastin ratio; a higher ratio ensures the formation of completely circular fibres while a lower one is responsible for the production of completely flat fibres (Fig. 3a, b, c).

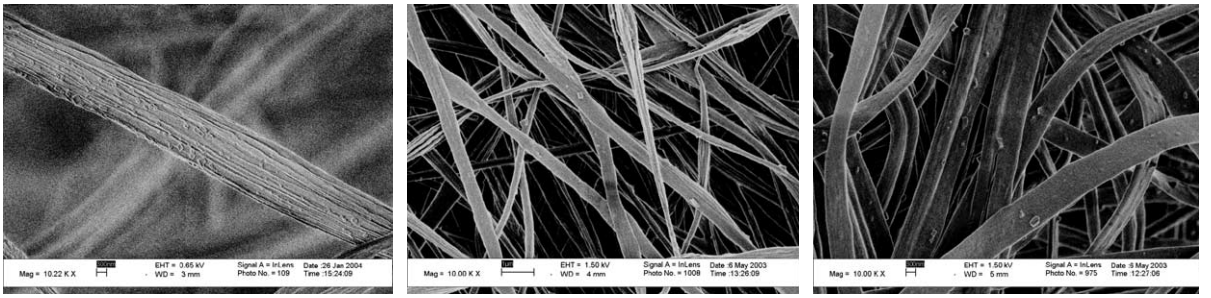


Fig. 3. SEM images of the fibres obtained from solutions having different compositions: (a) C:E=1:1; (b) C:E=1:2; (c) C:E=1:3. All the fibres have been spun at 22 kV and collected at 20 cm distance. Magnification is 10 kV. Scale bars are 300 nm.

Moreover, fibres containing a higher percentage of elastin have bigger diameters (Fig. 4a, b, c).

The obtained nonwoven meshes have been crosslinked with a soluble carbodiimide in order to prevent solubilisation in aqueous environment and to improve the mechanical performance. Crosslinking efficiency is determined by measuring the amount of free amino

groups left after the reaction, since this process occurs by formation of peptide-like bonds between the carboxylic groups of aspartic or glutamic acid residues and the free amine residues of lysine and hydroxylysine groups. A higher thermal stability is also observed as a result of crosslinking. For example, after crosslinking, only 26% amino groups are left free in a C:E=1:1 sample, while its denaturation temperature increases from ~46 to 79 °C. Moreover, PEO is washed out from the sample during the crosslinking procedure, as verified by differential scanning calorimetry and solid state CP-MAS ^{13}C NMR. Crosslinking does not affect the morphology of the obtained scaffolds.

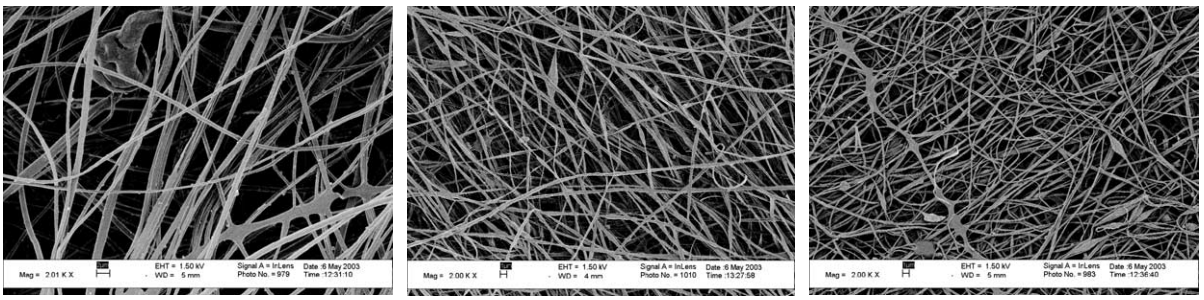


Fig. 4. A higher content in elastin leads to fibres having bigger diameters: (a) C:E=1:3, diameter=600 nm; (b) C:E=1:2, diameter=330 nm; (c) C:E=2:1, diameter=220 nm. All the fibres have been spun at 22 kV and collected at 20 cm distance. SEM images are taken at 2 k× magnification; scale bars are 1 μm.

Conclusions

Three-dimensional, porous, nanoscale fibre-based collagen/elastin matrices have been produced by means of electrospinning. Unwoven meshes have been successfully crosslinked by means of a water-soluble carbodiimide thus improving the stability of the scaffolds. By varying the collagen:elastin ratio, structures having a wide variety of diameters can be obtained. This will influence also their mechanical properties since they depend on the dimensions and assembly of the fibres. Thanks to these characteristics, such scaffolds could be used in different tissue engineering applications, like vascular grafts, as well as in drug delivery applications.

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