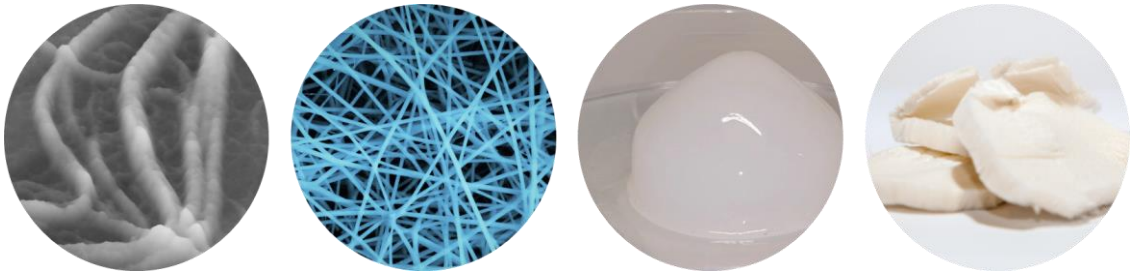


Electrospinning of Collagen

Introduction: Electrospinning is an extremely versatile technique for the production of micro and nanofibers, as a consequence electrospun fibres have been fabricated for a wide range of applications within regenerative medicine including tissue engineering scaffolds and wound dressings. All these applications are seeking to replace or repair tissue architecture and functionality. Thus, collagen which is a major component of tissues is an obvious choice of biomaterial for electrospinning fabrication of such structured medical devices. In this application note we review the electrospinning technique from bench to industrial scale and highlight electrospinning fabrication using collagens to improve applications within regenerative medicine.

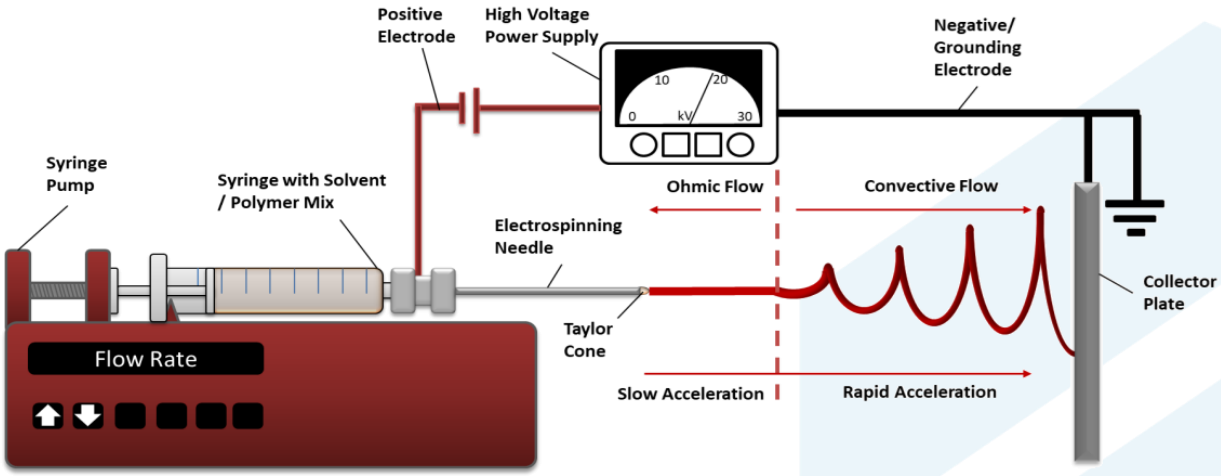


Figure 1 Needle-based electrospinning set up

Standard Electrospinning Set Up: Figure 1 shows the basic electrospinning set up which uses a high voltage power supply to create a large potential difference between a grounded “collector” structure and a polymer solution or melt being delivered at a constant rate through an aperture, such as a blunt end needle. As the voltage is increased the like charges within the polymer fluid directly oppose surface tension, resulting in the normally spherical droplet at the aperture distending into a conical shape. This cone is referred to as the “Taylor” cone, after Sir Geoffrey Taylor who first mathematically modelled the phenomenon. At a critical voltage the electrostatic attractive force between the solution and the collector causes a jet of polymer solution to be expelled from the cone tip towards the grounded collector surface. This jet then undergoes a whipping instability and dries in flight, depositing the fibres on the collector.

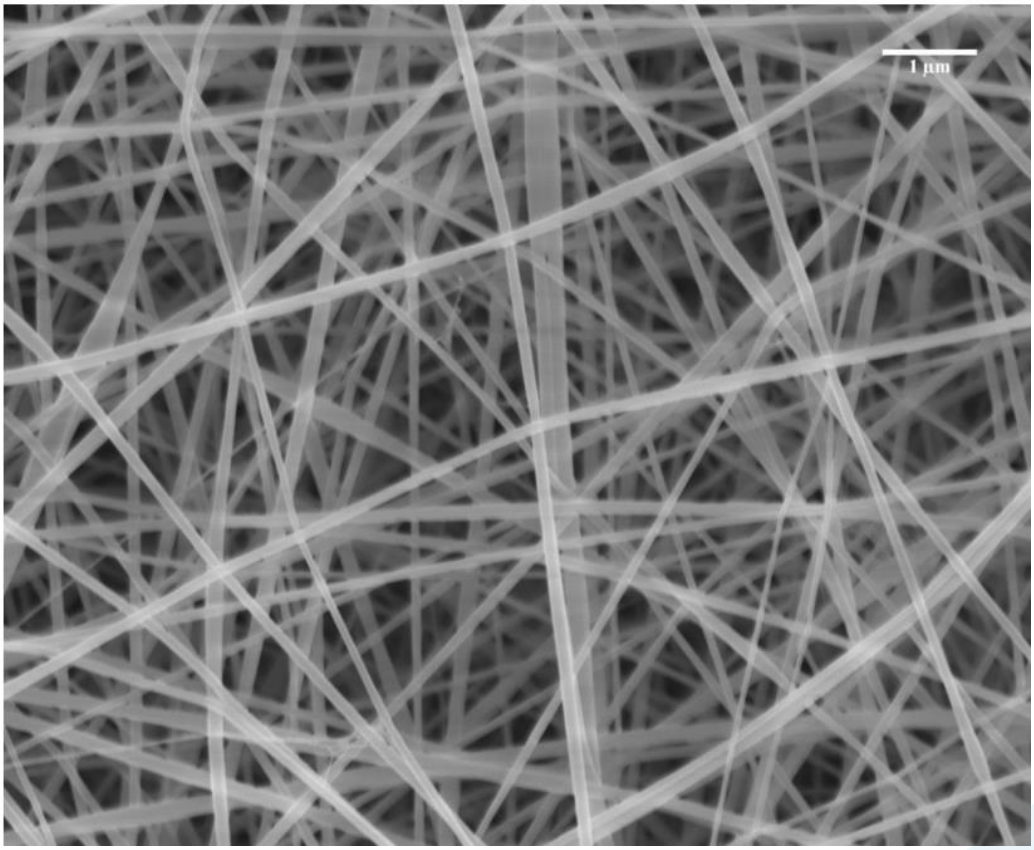


Figure 2 SEM micrograph of scale up of acid soluble collagen (ASC) electrospun from a solution of 10% ASC in 100% HFP. Scale Bar = 1 µm

Electrospinning Collagen: The electrospinning of collagen is subject to much discussion as many believe that the actual process of electrospinning denatures the collagen structure as aggressive solvents and high shear environments are required. Denaturing the collagen during the electrospinning process results in loss of collagen functionality in the final scaffold product leading to opinion that the process may as well started with gelatine rather than using more expensive collagen. Thus, research into collagen electrospinning has been under pressure in recent years due to the findings that the primary solvents used to electrospin collagen, namely 1,1,1,3,3,3 Hexafluoro-2-Propanol(HFP) and 2,2,2-Trifluoroethanol (TFE) have been shown to denature collagen when dissolved into either of these solvents. There have been efforts since to try and electrospin collagen using benign solvent mixtures, however many of these are unable to replicate the successes in fibre morphology and homogeneity that collagen electrospun out of HFP and TSE produced (Figure 2). ProColl have overcome the issues associated with electrospinning collagen by using the single chain form of the molecule which is water soluble.

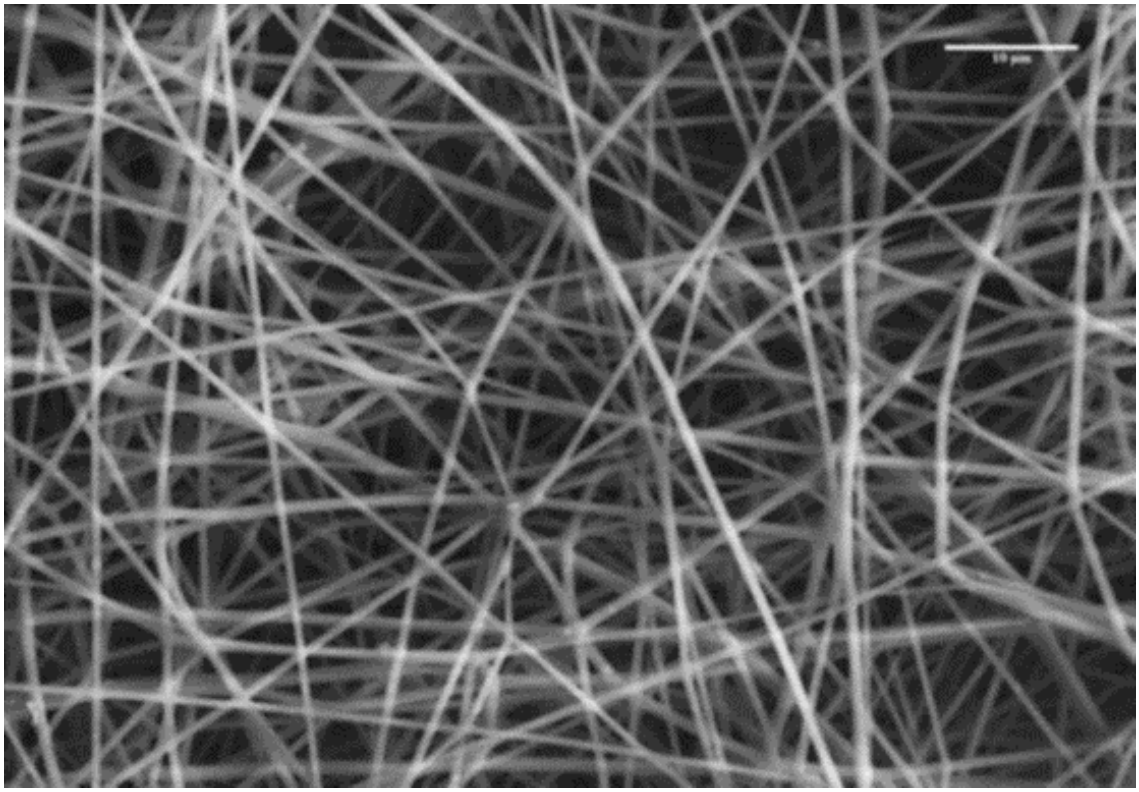
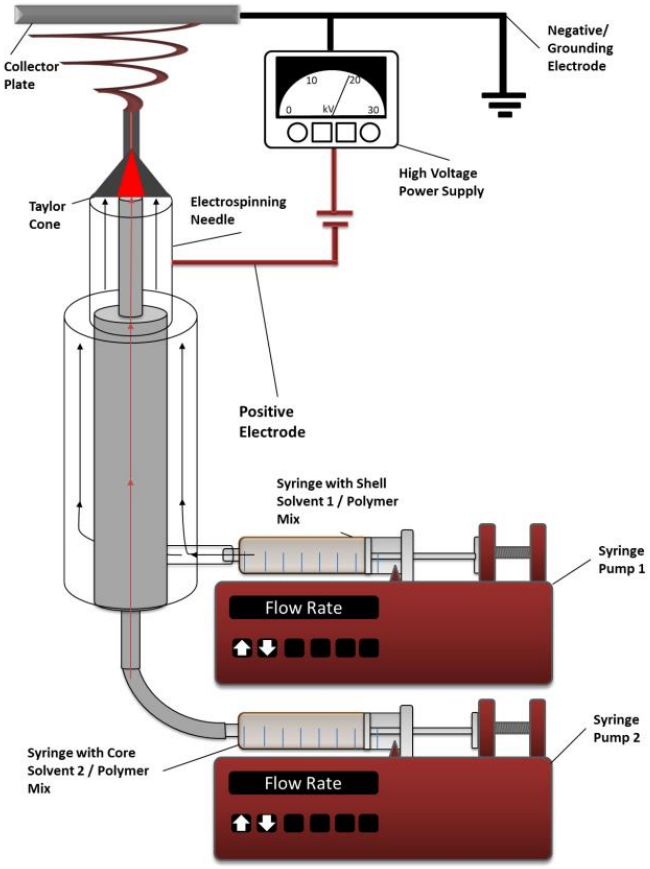


Figure 3 SEM Micrograph of needle electrospun Single Alpha Chain Collagen fibres. Solution composition was 25% Collagen (w/v) in PBS. Fibre Diameter 646nm ±121nm. Scale Bar = 10µm.

Improved Electrospinning of Collagen: ProColl have electrospun single chain collagens in standard cell culture buffers such as Phosphate Buffered Saline (PBS), to form fibres using both needle and needleless (scale-up, see below) forms of electrospinning. The single chain collagen scaffold maintained its structure and function after electrospinning we optimised the process based on a 25% (w/v) solution of single alpha chain collagen in PBS. Figure 3 shows the resultant collagen fibrous scaffold with nanofibres that mimic the extracellular matrix of tissues. We confirmed that the molecular structure was maintained during electrospinning by dissolving the collagen scaffold in PBS and applying SDS-page gel electrophoresis to demonstrate the presence of full chain length collagen. The biological activity of the single chain collagen was confirmed using cell culture assays that showed an up regulation of IL 6 and IL 8; known cellular response to single chain collagen. For needleless electrospinning we used Elmarco's Nanospider™ free liquid surface electrospinning setup to achieve a higher volume of scaffold manufacture



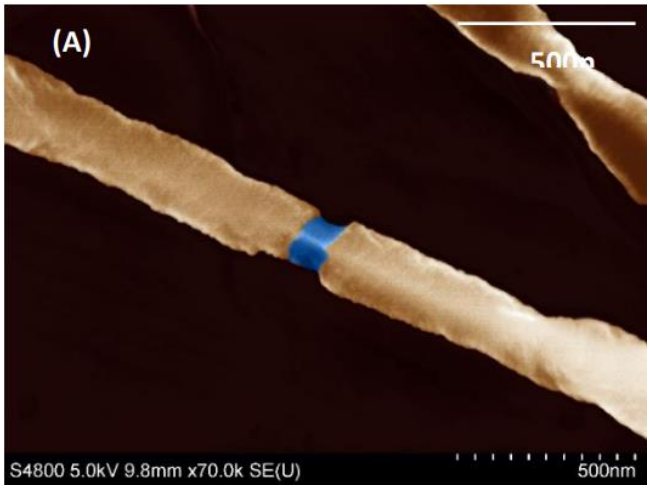


Figure 4 Schematic representation of the co-axial electrospinning system for formation of core/sheath fibres. SEM image shows coaxial nanofibre electrospun from PEO/Collagen, the blue rendering reveals the coaxial structure

Coaxial Electrospinning: An interesting technique which provides greater functionality to the fabricated fibres is coaxial electrospinning, which produces fibres comprised of two or more distinct layers, arranged around a common central longitudinal axis, similar to the insulation around an electrical cable. An example of such a collagen fibre can be seen above in Figure 4. The co axial system is nearly identical to the standard electrospinning set up with the only change being applied to the capillary or needle through which the electrospinning solution is delivered. A schematic diagram of the coaxial electrospinning system set up can be seen in Figure 4.

By delivering two distinct materials to the inner and outer portions of the capillary separately a compound Taylor cone forms from the two materials. From this point the electrospinning process is remarkably similar, the compound Taylor cone erupts at a critical voltage to form a compound jet which undergoes a whipping instability, removing the solvent and producing nanofibres with a core-sheath architecture of the two materials. Figure 4 shows coaxial fibres of PEO with a core of collagen produced in the ProColl laboratories.

Scale Up with Needleless Electrospinning: A major concern with conventional electrospinning setups is poor volume through put and the tendency of the electrospun solution to dry within the needle or aperture, causing blockage. The latter problem is so pervasive in the electrospinning technique that certain solute/solvent combinations are termed un-spinnable and ruled out entirely. In terms of scale up, the single-jet electrospinning methodology is very limited, usually operating in 0.1-1g/hr of collected fibre weight. To counter this, it is possible to design an electrospinning system to spin directly from a reservoir without the use of an aperture, this is known as the “needleless electrospinning” method.

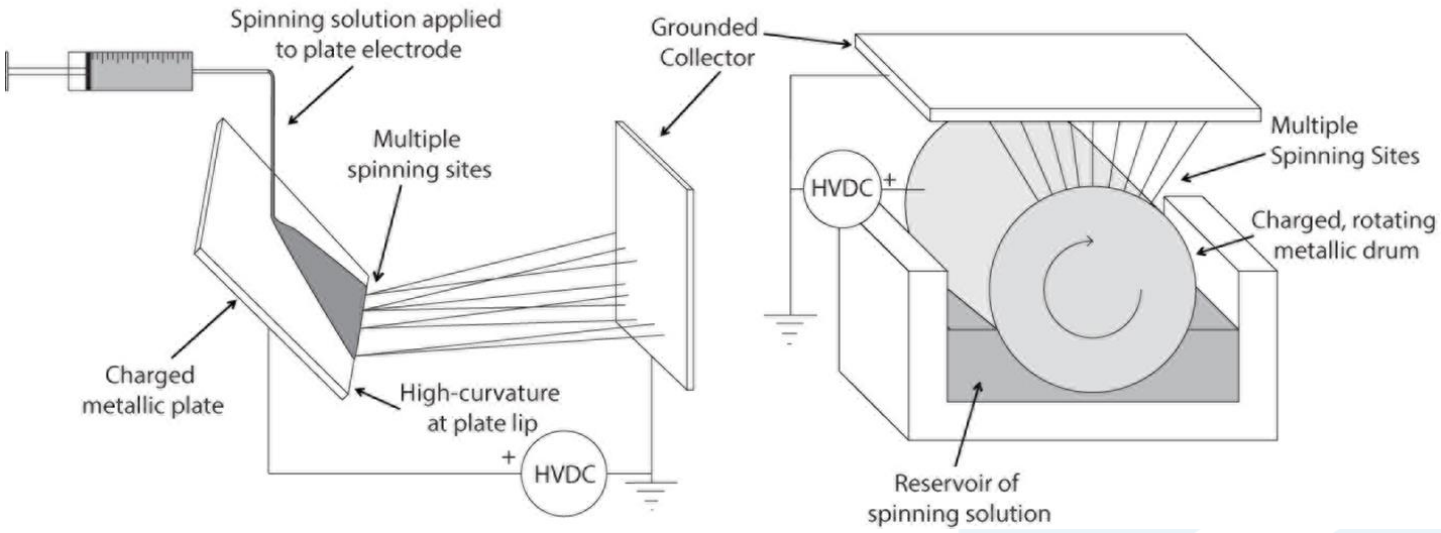
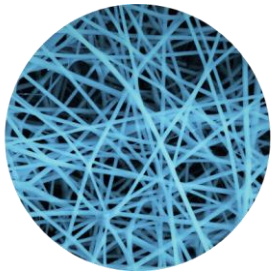


Figure 5 (a) Plate and (b) Rotating-drum variations of the needleless electrospinning to achieve scale up

There are two primary variations on the needleless electrospinning method, the first uses a structure such as an inclined surface or plate cone, and a rotating cylinder or other geometric object to carry the electrospinning solution into proximity of the grounded collector and provide an area of high curvature. Schematics of both plate-edge and drum electrospinning are illustrated in Figure 5. ProColl has used the rotating drum needleless electrospinning system to fabricate collagen nanofibrous scaffolds for tissue engineering scaffolds and wound healing.

Conclusion: Electrospinning is an extremely useful and versatile technique for the production of fibrous structures. The architecture of electrospun materials enables them to be used as analogues when modelling, engineering and repairing tissues. Electrospun structures are often used for drug delivery as the distribution of material as nanofibers within a structure means that the surface area to volume ratio is high and thus more efficient for interfacial transfer of material and biochemical signals into a tissue system. This is supported further by the ease at which bioactive materials can be incorporated into the electrospinning process for production of fibrous materials delivering the functionality of the bioactive. As collagen exists as fibres within tissues, electrospinning is the ideal way to deliver both the mechanical and biochemical functionality of collagen in regenerative medicine applications.

If you wish to know more about the process of electrospinning, please see our publications.

- Electrospun Collagen Nanofiber Membranes for Regenerative Medicine Widdowson, Jonathan P. Hilal, Nidal. Wright, Chris J.(2019). In Nanofiber Membranes for Medical, Environmental, and Energy Applications.(pp. 57-87).NY:CRC press.

- Electrospun Antimicrobial Membranes-Functionality and Morphology Mortimer, Chris J.,James,Sean.,Hilal, Nidal.& Wright, Chris J. (2019).In Nanofiber Membranes for Medical, Environmental, and Energy Applications.(pp. 17-37).NY:CRC Press

- Electrospinning of Functional Nanofibers for Regenerative Medicine-From Bench to Commercial Scale Mortimer, Widdowson and Wright (2018) in Nanofibers-Advanced Preparation and Emerging Applications In Tech Open ISBN 978-953-51-5815-8 https://www.intechopen.com/chapters/59853

If you wish to use the electrospinning technique to create collagen structures for your applications, please don't hesitate to contact us. ProColl engineers would be happy to advise you on the set up of equipment and the fabrication process. ProColl recommends its single alpha chain products for electrospinning of collagen; available as recombinant and bovine sources at www.procoll.co.uk.