Electrospinning Collagen Nanofibres: Retaining Native Structure and Avoiding Aggressive Solvents.

Key words: Type I collagen, animal free, electrospinning, scaffolds.

Introduction

Collagens are by far the main protein constituent of the extracellular matrix (ECM), the natural scaffolding that houses and supports cells within tissues of the body. Tissues have a fibrous structure, and electrospinning is uniquely placed as a technology that can produce scaffolds that mimic the ECM when engineering tissue and repairing wounds (Figure 1). Consequently, there has been a lot of research on developing electrospinning methods that use collagen to create scaffolds. Unfortunately, a serious issue has been the denaturing of the collagen by the chemical and physical environment during the electrospinning process (1). In this white paper, we describe how ProColl have solved this problem and created scaffolds that maintain the native structure of the collagen, and thus its essential bioactivity, while also improving the potential for regenerative medicine applications, by removing the need for aggressive solvents. For a more detailed review of electrospinning read our book chapters (2,3,4).

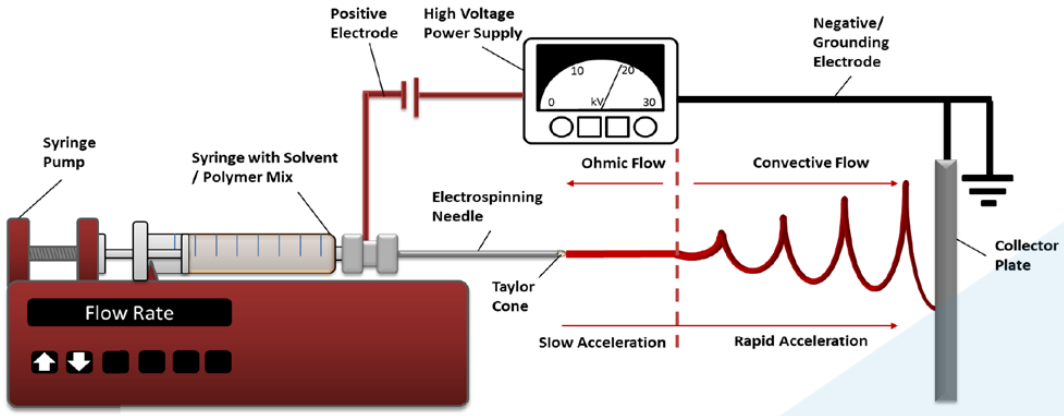

Figure 1 *Basic electrospinning setup, where a polymer solution is placed within a syringe pump at the desired flow rate. A high voltage power supply is connected to the needle, producing an electric field of repulsion. With control of parameters, such as concentration and polymer choice, this leads to the ejection of solution from the positive source to ground, which collects dried fibres on a conductive collector plate.*

The Constraints of Electrospinning Collagen

In many cases, tissue scaffolds and medical devices manufactured from biological materials promise better clinical outcomes, providing bio-functionality is preserved. Unfortunately, the very properties (native structure) that have made collagen a key material for regenerative medicine are often lost during processes such as electrospinning (1). This problem has been shown to be the choice of solvent, such as the prevalent use of hexafluoroisopropanol (HFP) (5). A further issue is collagen has traditionally been solubilised in acetic acid in order to achieve concentrations suitable for application; hence the name, acid soluble collagen. Another issue that compromises the application of collagen is attributed to poor processing where collagens are left in close proximity to collagenases (enzymes), as both are found in the animal tissues the collagen is extracted from. These enzymes break down collagen. This favours electrospinning of the material, but each modification reduces the nativity and bio-functionality of the final electrospun structure. ProColl are able to extract and purify single chain collagen at a scale previously unachieved, with a high degree of purity. These single chain collagens are highly soluble in benign physiological buffers, such as PBS and cell culture media, removing the need for acid and/or aggressive solvents when fabricating medical devices. This means that we maintain a more native structure and therefore increased bio-functionality when electrospinning. ProColl's synthetic biology collagens are also able to create animal free single chain collagens that can be electrospun in physiological buffers.

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Electrospinning Single Chain Collagen

We have electrospun single chain collagens in standard cell culture buffers such as Phosphate Buffered Saline (PBS), to form nanofibres using both needle and needleless (scale-up) forms of electrospinning. The single chain collagen scaffold maintained its structure and function after electrospinning. Figure 1 above shows the basic set up of a needle-based system and we optimised the process based on a 25% (w/v) solution of single alpha chain collagen in PBS. Figure 2 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. If you require more specific details of the electrospinning operational parameters, please contact ProColl directly. This work has demonstrated that for the electrospinning of native collagen scaffolds with functionality the choice of the starting material is paramount and single alpha chain collagen supplied by ProColl is ideal.

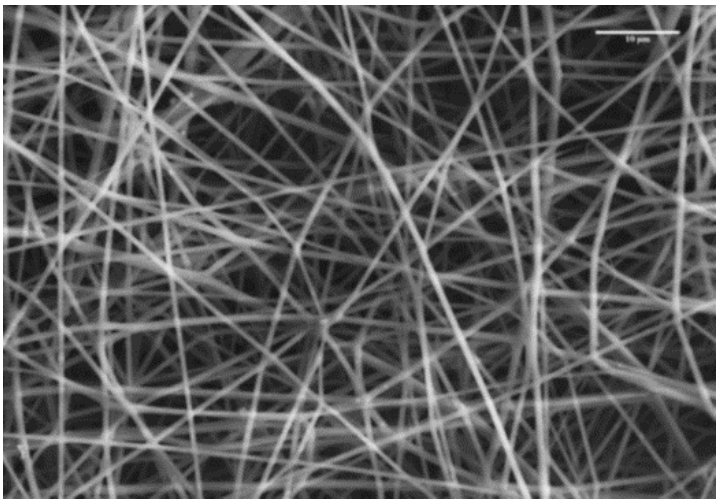

Figure 2 *SEM Micrograph of needle electrospun Single Chain Collagen fibres. Solution composition was 25% Collagen (w/v) in PBS. Fibre Diameter 646nm ±121nm. Scale Bar = 10µm.*

References

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If you are interested in purchasing ProColl's range of collagen products, or joining our growing list of distribution and OEM partners, please use the contact form on our website (www.procoll.co.uk) and one of the team will be in touch. ProColl's collagen is also available direct for both research & bulk orders.

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