Fibrous scaffolds made by electrospinning for tendon repair

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The biomaterials and tissue engineering sector has become a routinely accepted practice for improving people's quality of life – be it contact lenses, hip and knee replacements, or skin grafts. Coupled with an ageing population there is an increasing need for regenerative medicines.

The majority of biomaterial and tissue engineering applications – regardless of their final location in the body – require the fabrication of a construct, with dimensions and structure that can fill the damaged area, promote tissue ingrowth and perform the original tissues role. Constructs, more commonly referred to as scaffolds, often aim to replicate the original tissues ultra-structure.

Scaffolds fabricated via electrospinning are being increasingly used in a wide range of biomaterial applications due to the ability to create nanoscopic fibres with diameters as small as 5 nm [1]. Additionally, the flexibility of this technique allows networks of nanofibres to be created in many different orientations and sizes that are comparable to the ultra-structures of many tissues [2].

An inexpensive and relatively easy set-up, electrospinning uses high voltages to generate internal repulsive forces within a polymer solution, which are then expelled in the form of fine diameter fibres. In brief, a voltage supply is connected to a needle-tipped syringe containing a flowing solution of polymer dissolved in solvent. An earthed, target collector is positioned at a set distance from the needle. Application of a high voltage causes charging of the polymeric solution and formation of a Taylor cone [3]. Once the charge intensity of the solution is sufficient to overcome the surface tension and visco-elastic forces of the Taylor cone, the polymer is expelled as a charged jet [4]. This jet travels across an air-gap whilst simultaneously undergoing solvent evaporation and stretching and thinning of the polymer occurs due to charge repulsion before coming into contact with the earthed collector, at which point the process is complete, charges dissipate and a network of fibres are formed.

The last 8 years of research has been spent focusing on utilising this technique to produce electrospun scaffolds as a potential new therapy for repairing damaged tendons. In order to achieve this, it is important to understand the tissue to be repaired/replaced and the clinical need. Tendons are a type of connective tissue that frequently withstand high tensile loads, which can predispose them to injuries (or tendinopathies), such as, spontaneous rupture or chronic degeneration of the tissue leading to eventual rupture. They are also susceptible to other types of injuries, including lacerations. With a poor healing response, there are a number of surgical interventions available that aim to restore the tendon to its original structure and function; yet there is no clear leader and autografting remains the gold standard therapy for segmental repairs or reconstructions. However, problems can arise as there is no guarantee the patient will have sufficient, usable tendon tissue that can be sourced from a secondary site. Additionally when healthy tissue can be harvested, the patient is left susceptible to an increased risk of infection and prolonged rehabilitation. Predominantly composed of uniaxially aligned collagen fibres that take on a hierarchical structure, electrospinning lends itself as the ideal technique for producing fibrous scaffolds that can imitate the natural tendon tissue [5].

By electrospinning onto the edge of a mandrel rotating at an optimised speed, highly aligned fibres can be collected [6]. As shown in the schematic, these ribbons of aligned fibres can be further manipulated to create 3D bundles of fibrous yarn that are more reminiscent of the tendon hierarchy than the initial 2D electrospun sheet of fibres (Figure 1). In addition to resembling the natural tendon structure, there are several other key factors that should be incorporated when developing synthetic scaffolds for this type (and many other tissues) – the scaffold should (i) be biocompatible and promote an appropriate tissue healing response, (ii) provide sufficient mechanical strength to withstand applied forces and support new tissue formation, and (iii) degrade with time without build-up of toxic by-products and at a rate that allows a smooth load transfer.

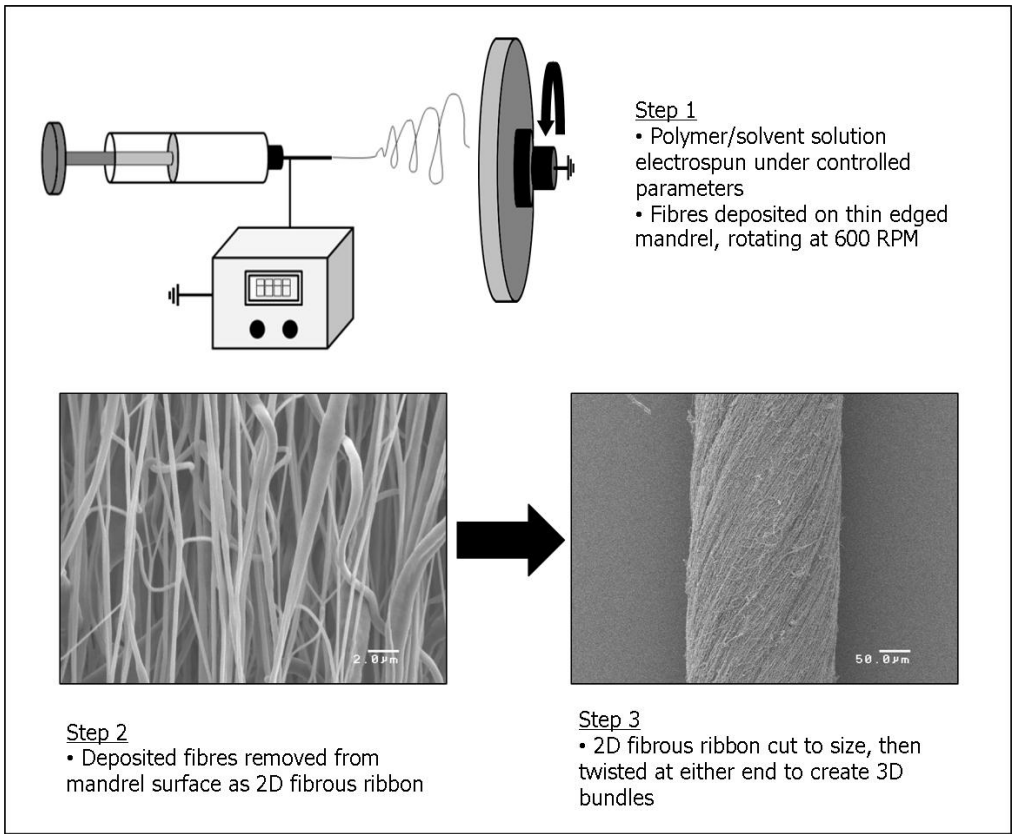

Figure 1 – Electrospinning set-up and process for fabricating 2D fibrous ribbons and formation of 3D yarns.

Initially a range of polymers and solvents were trialled and it was possible to create yarns from each combination [7]. However, their tensile properties varied significantly: use of poly(lactic-*co*-glycolic) acid ($PLGA_{85:15}$) resulted in greatest stiffness, but did not have a correspondingly high tensile strength. Similarly use of acetone as a solvent resulted in both low stiffness and strength. Poly(ε-caprolactone) (PCL) dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was deemed to be the optimal polymer/solvent combination for this research, providing Young's Modulus and tensile strength of 68 MPa and 42 MPa, respectively.

In vitro characterisation of PCL/HFIP yarns were found to support the adhesion and proliferation of both equine tenocytes and human mesenchymal stem cells (hMSC) (Figure 2a-d). In addition, the surface topography of the scaffold influenced cell behaviour causing cells to align parallel to the underlying fibres – similar to their natural morphology within tendon tissue [6]. Analysis of gene expression for hMSC-seeded yarns subjected to a tensile, sinusoidal loading regime for one hour each day for 21 days, suggested their differentiation towards a tendon-lineage as an up-regulation was observed in several key genes, including; collagen type I and III, tenascin-C, elastin and fibronectin [8] (Figure 2e).

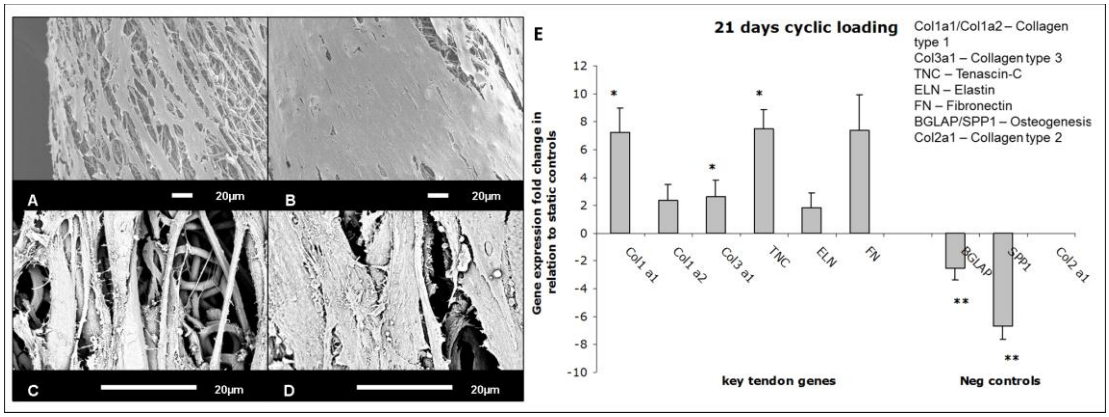

*Figure 2 - Scanning Electron Micrographs of poly(ε-caprolactone) electrospun fibres with equine tenocytes after 1 week (A) and 2 weeks (B), and human Mesenchymal stem cells after 1 week (C) and 2 weeks (D); (E) Gene expression markers for hMSC-seeded scaffolds (n=4) subjected to cyclic loading compared to hMSC-seeded scaffolds held under static conditions. (Data represented as mean ± st. deviation; T-test - ** p<0.01, * p<0.001)*

In vivo assessment of electrospun yarns implanted into critical sized defects within the flexor digitorum profundus tendons of mice was performed over a six-week time frame (Figure 3). Haematoxylin and Eosin (H&E) staining after three days showed the outer perimeter of the PCL graft to be completely surrounded by a thick layer of cells. By three weeks, cells had migrated through to the scaffold core, which is essential for integration and the long-term success of the implant. Yet by six weeks, the PCL material appeared to have undergone complete degradation and a prominent cell response was observed. Comparison to autograft similarly demonstrated a high cell presence at six weeks, which could be due to the tissue remodelling. Tendons are known to be slow-healing tissues and as such, degradation of the PCL at this rate is too fast. To overcome this, we are in the process of repeating these animal studies using a different grade of PCL and our initial observations demonstrate this material to still be present and intact at six months.

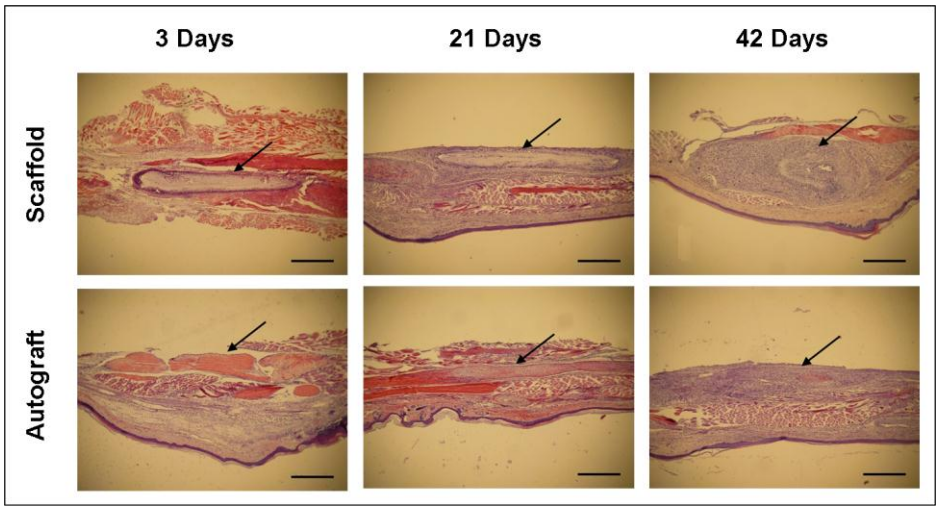

Figure 3 - H&E light microscopy images of poly(ε-caprolactone) scaffolds and autograft tissue within murine tendons at 3 days, 3 weeks and 6 weeks. Arrows indicate position of graft; scale bar = 500 µm.

Currently funded by the MRC-DPFS, the project team is continuing to make significant progress in the development of this scaffold, and is focusing on up-scaling this prototype as a device for clinical translation and use in patients with damaged tendons.

Acknowledgements

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