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Dynamic loading of electrospun yarns guides mesenchymal stem cells towards a tendon lineage

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ABSTRACT

Alternative strategies are required when autograft tissue is not sufficient or available to reconstruct damaged tendons. Electrospun fibre yarns could provide such an alternative. This study investigates the seeding of human mesenchymal stem cells (hMSC) on electrospun yarns and their response when subjected to dynamic tensile loading. Cell seeded yarns sustained 3600 cycles per day for 21 days. Loaded yarns demonstrated a thickened cell layer around the scaffold's exterior compared to statically cultured yarns, which would suggest an increased rate of cell proliferation and/or matrix deposition, whilst maintaining a predominant uniaxial cell orientation. Tensile properties of cell-seeded yarns increased with time compared to acellular yarns. Loaded scaffolds demonstrated an up-regulation in several key tendon genes, including collagen Type I. This study demonstrates the support of hMSCs on electrospun yarns and their differentiation towards a tendon lineage when mechanically stimulated.

1. Introduction

It has long been known that cells respond to mechanical stimuli and that this directly affects their local tissue physiology (Wang and Thampatty, 2006). This is also the case for *in vitro* studies, where mesenchymal stem cells (MSCs) have been driven towards a vascular smooth muscle cell phenotype following their cyclical stretching on flexible, silicone membranes causing an increased expression of smooth muscle contractile markers (Park et al., 2004); and repeated compressive loading of MSCs resulting in raised levels of aggrecan and glycosaminoglycans, indicative of their differentiation towards a chondrocyte/cartilaginous phenotype (Mauck et al., 2007). Similar studies have been performed using tendon fibroblasts and MSCs in order to stimulate production of tendon-like tissue *in vitro* for tissue engineering applications (Garvin et al., 2003; Cao et al., 2006; Nirmalanandhan et al., 2007; Issa et al., 2011; Teh et al., 2013).

Tendons are a type of connective tissue, capable of withstanding high tensile loads to enable movement. They are susceptible to injury caused by wear and tear or spontaneous rupture. A segmental repair or reconstruction of the tissue may be required depending on the type of injury incurred. In cases like this, surgeons will graft autologous tissue taken from a secondary site. However, problems can arise when the patient does not have sufficient and/or adequate tissue to harvest as a graft. This lack of usable tissue has driven researchers in the biomaterials and tissue engineering field to develop alternative replacement graft strategies.

In an innovative study, Garvin et al. (2003) fabricated bioartificial tendons using tendon fibroblasts (sourced directly from avian flexor tendons) suspended in collagen type I gels that were subjected to cyclical loading using a Flexcell Tissue Train system. A loading regime of 1 h per day at 1% strain and frequency 1 Hz, was sufficient to generate changes in gene expression levels. Where a number of key

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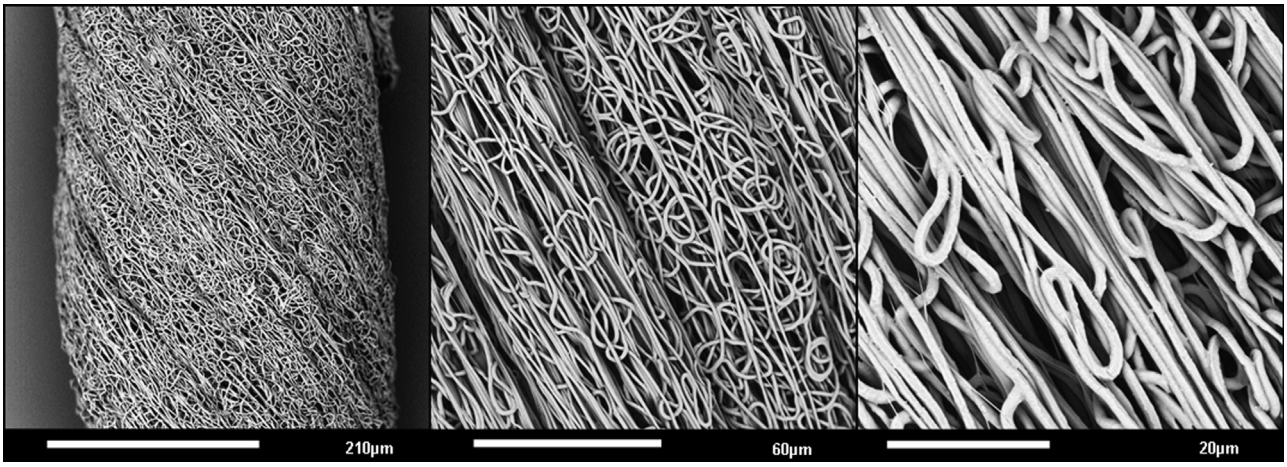

Fig. 1 – Scanning electron micrographs demonstrating the structure and surface topography of the electrospun yarn without cells at increasing magnifications.

tendon genes, such as collagen Types I, III and XII, fibronectin and tenascin, were expressed at levels similar to those within natural avian flexor tendon tissue. After stimulation for 7 days, the mechanical strength of the bioartificial tendons was almost three times stronger compared to the non-loaded control group, but remained significantly weaker than the native tendon. Cao et al. (2006) conducted a study whereby tendon fibroblasts were seeded onto unwoven polyglycolic acid (PGA) fibres that were mounted on a U-shaped spring for six weeks. In the control group, cell-seeded fibres were cultured strain-free, and in the test group the spring had a constant strain applied. Their results showed it was possible to generate tendon tissue and that the tissue structure matured and strengthened significantly over time when a constant strain was applied. However, continuous strain affected the morphology of the formed tissue as the collagen fibres appeared compacted when compared to natural tendon tissue and the authors surmised that applying a constant tension was not appropriate for this type of tissue engineering, which was aiming to replicate the natural physiology. More recently, Teh et al. (2013) investigated the synergistic effects of mechanically stimulating aligned silk fibroin hybrid scaffolds seeded with MSCs. A loading pattern of 12 h per day, frequency 0.1 Hz and 5% translational strain and 90° rotational strain were applied for 11 days. Their findings determined tenogenesis to be enhanced for scaffolds held under dynamic culture conditions as gene expression levels, including collagen Type I, tenascin-C and tendomodulin, were up-regulated compared to static controls. In terms of mechanical properties, the loaded scaffolds were similarly found to possess greater tensile strength than their static cultured counterparts.

A common opinion in biomaterials and tissue engineering is to produce scaffolds that mimic the architecture of the natural tissue as closely as possible (Ma et al., 2005; Vasita and Katti, 2006). As such, electrospinning has become a widely used technique to easily produce fibrous scaffolds with structures reminiscent of a tissue's extracellular matrix (Wang et al., 2013). Capable of supporting a wide range of cell types, electrospun scaffolds have been investigated for the repair and regeneration of bone (Yang et al., 2013), nerves (Koh et al., 2010), bladder (Stankus et al., 2008), amongst many others. Electrospun scaffolds that possess a parallel arrangement of fibres are currently being researched for the repair of damaged tendons (Bosworth et al., 2013). In this case, three-dimensional electrospun fibrous yarns – a continuous strand of twisted fibres – were found to be superior scaffolds compared to the more common two-dimensional sheets of aligned fibres for this particular tissue type. This study builds on this previous research and investigates the effects of cyclically loading electrospun yarn (Fig. 1) cultured with and without hMSCs, in order to determine if the yarns provide a suitable topography and structure to support hMSCs and transfer the mechanical stimulus to the cells to instigate a change in phenotype.

2. Materials and methods

2.1. *Electrospinning of scaffolds*

A 10%w/v solution of poly(ε-caprolactone) (PCL; Purac Purasorb PC12 (M_w 120,000 g/mol) dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP; Sigma) was electrospun using parameters: voltage – 20 kV, flow-rate – 1 ml/h, distance to collector – 200 mm, spinning time – 15 min. Fibres were collected on the edge of a mandrel (Ø 120 mm, width 3 mm) rotating at 600 RPM, and removed as a single fibrous ribbon.

Electrospun yarns were fabricated by cutting the fibrous ribbon into 50 mm lengths and then briefly submerging in distilled water. The strips were then manually twisted along their lengths to create yarns of electrospun fibres (Ø ~200 μm) (as previously described in Bosworth et al., 2013).

2.2. *Sterilisation of electrospun yarns*

Yarns were sterilised in increasing concentrations of ethanol (VWR) in distilled water (50, 70, 90, 100%v/v; 24 h per concentration), and then washed twice in Phosphate Buffered Saline solution (PBS) (Invitrogen; 12 h per wash). Following washing with PBS, yarns were submerged in mesenchymal stem cell culture medium with supplement mix (PromoCell) and 1% antibiotic (penicillin/streptomycin; Gibco).