Self Crimping Electrospun Fibres as Scaffolds for Connective Tissue

Denver C. Surrao[1,3], James W. Hyami[1,3], Stephen D. Waldman[1,2,3] and Brian G. Amsden[1,3].
[1]Department of Chemical Engineering, [2]Department of Mechanical and Materials Engineering, and [3]Human Mobility Research Centre, Queen's University, Kingston, ON, Canada.

Introduction: Collagen fibres in connective tissues have a waveform pattern called crimp. The crimp unfolds with the application of load giving rise to the characteristic non-linear mechanical behaviour of soft connective tissues. This behaviour has been suggested to provide shock absorption due to the creation of a compliant toe-region of the load-deformation curve. In tissue engineering, is important to replicate this crimp pattern in scaffolds designed for connective tissues to impart functional properties. Therefore, the aim of this study was to induce and characterize crimp in electrospun polymeric fibres used for tissue engineering scaffold development.

Methods:

Electrospinning: Poly(D,L-lactide-*co*-ε-caprolactone) (PDLLACL) (80:20 mol:mol) with a Mn of 50 kDa was used in this study. The PDLLACL was dissolved in a 75:25 volume ratio solution of dichloromethane: dimethylformamide to form a 35 w/v% solution. The polymer was electrospun onto a wire mandrel rotating at 1000 rpm as follows: flow rate 0.03 mL/minute, air gap 13.5 cm, and a 2 kV/cm positive electric field. ***Fibre Characterization:*** The diameter of the fibres was determined from Brightfield images taken randomly along the length of the fibre (n=10). The images were analyzed with SigmaScan Pro 5 to determine the diameter of the fibres. ***Surface Modification:*** The fibres were based etched with sodium hydroxide (0.5 M) for 30 minutes, followed by washing and drying overnight. Fibre surface morphology following base etching was characterized by scanning electron microscopy (SEM). ***Mechanical Characterization of Fibres:*** Fibres were tested in uniaxial tension at 37°C using a Mach-1™ micromechanical tester (n=10). The fibres were tested to failure at a rate of 1 % strain/s. Tensile stress in the fibres (force normalized to the total cross sectional area of the fibre mat) was plotted as a function of the applied strain (deformation normalized to the gauge length of the sample). ***Degradation Studies:*** Electrospun fibres (n=6, for each time point) were placed in a phosphate buffered saline (PBS) solution at 37 ^{o}C. At specific time points (week 1, 2, 3 and 4) the thermal and crimp properties of the electrospun fibres were assessed. ***Cell Culture:*** Primary fibroblasts were isolated from the central ligament of the metacarpal joint of 12-18 month-old calves, under sterile conditions. The isolated fibroblasts were expanded in T-flasks till P3. Base etched PDLLACL fibre mats (n=6, for each time point) were seeded with 50 µL of media containing $2x10^5$ cells/mL. The fibres were sterilized using UV light and soaking in 70% ethanol for 30 minutes respectively. The media was changed every 2-3 days with a total culture period of 4 weeks. ***Cell Viability and Attachment:*** 1X PBS containing 2 µM calcein AM and 4 µM ethidium homodimer 1 was used to stain the cells, for 30 minutes. The stained cells were imaged on the fibres using an Olympus Fluoview FV1000 confocal microscope with the dye appropriate filter sets.

Results: Continuous and defect-free fibres were collected on a rotating wire mandrel via electrospinning. Upon release from the mandrel, the fibres crimped to amplitudes and wavelengths similar to those of native collagen (Fig. 1). The crimp structure was characterized with an amplitude and wavelength of 10 µm (±1) and 55 µm (±3) respectively. Fibre crimp is hypothesized to result from stresses induced due to winding onto the wire mandrel during electrospinning being relieved by polymer relaxation after removal from the mandrel. Crimp only occurred when the polymer was plasticized with residual DMF. The average diameter, modulus and yield strain of the fibres were determined to be 0.93 ± 0.02 µm, 170 ± 61.8 kPa and 3.7 ± 0.2 kPa respectively.

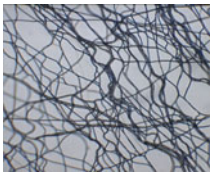

Fig 1: Crimped Fibres (scale 200 X)

Fig 2: Fibre surface following base etching (scale 2µm)

Following base etching, the surface of the fibres was rough (Fig. 2). The crimp resulted in the presence of a characteristic toe region when the fibre mats were subjected to uniaxial tension (Fig. 3).

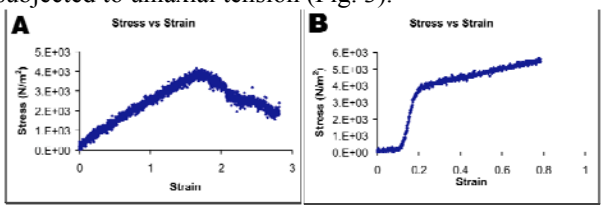

Fig. 3: Performance of fibre mats subjected to uniaxial tension: A) uncrimped and B) crimped.

During *in vitro* degradation, the glass transition temperature (31 ± 0.05 ^{o}C) and strain required to unfold crimp (0.28 ± 0.02 mm/mm) remained unchanged. Furthermore, primary fibroblasts attached, proliferated and aligned along the length of the base etched fibres.

Conclusions: The combination of electrospinning to generate micron-sized fibres with tension imparted during winding onto a wire mandrel yielded a process capable of generating crimped microfibers. Crimping of the polymer fibres occurred if the polymer used was allowed to relax following removal from the collection mandrel. The crimped fibres possessed a toe region characteristic of collagen subjected to uniaxial tension. This process has the potential for generating effective scaffolds for engineering connective tissue.