Crosslinked, Biomimetic Fibrous Scaffolds for Ligament Tissue Engineering

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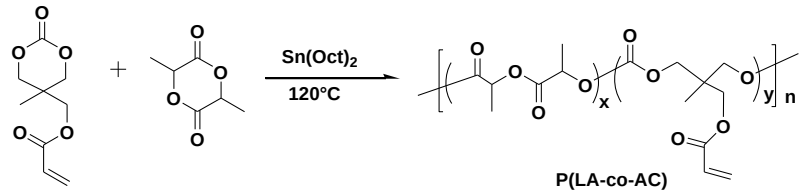
Introduction:

Electrospun biodegradable polymer fibers have been widely used as scaffolding for many tissue engineering applications due to their biomimetic nano-fiber diameters with high surface-to-volume ratio and their porous 3D inter-connected structures. In the past, electrospun biomaterial scaffolds have been successfully used in nerve and cardiovascular tissue engineering, but have not had much success with the anterior cruciate ligament (ACL). The ACL is one of the most frequently injured ligaments of the knee due to the high stresses these ligaments encounter during physical activities. Some reasons for the limited success of an electrospun ligament scaffold for generating effective ACL tissue are partly due to the difficulty in mimicking the complex extracellular architecture (crimp structure) of the ACL and/or the mismatched mechanical properties of the fibers. Thus, it still remains a challenge to prepare a biomimetic and stable polymer fibrous scaffold with high modulus to support ligament development *in vitro* and *in vivo*. Herein, we developed a novel *in situ* forming fibrous scaffold, which closely mimics the crimp structure of the native ACL tissue.

Materials and Methods:

Synthesis of poly(L-lactide-co-acryloyl carbonate) (P(LLA-AC)copolymers:

The AC monomer was prepared as described in [1]. P(LLA-AC) copolymers were synthesized as shown in Scheme 1.



Scheme 1. Synthesis of P(LLA-AC).

Preparation of stable and crimped scaffolds:

The crimped fibrous scaffolds were prepared by electrospinning P(LLA-AC) copolymers dissolved in DCM/DMF(3:1) solution within a 1 kV/cm field, and collecting the fibres with a rotating mandrel. After removing from the mandrel the aligned scaffolds were induced to form crimp structures by immersing into hot water above their T_g.

Cell culture and cell morphology:

Cell studies were performed using NIH 3T3 fibroblasts. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS Gemini, Calabasas, CA) and 1% antibiotic in a 37 ℃ and 5% CO_2 incubator. Discs (8 mm dia.) were cut from

the fibrous scaffolds and placed in a 24 well culture plate. Cells were seeded onto P(LLA-AC) scaffolds at 2.5 x 10^4 cells/scaffold and maintained in 2 mL of medium. Medium was replaced after 2 days. Cells were analyzed using the MTT assay and SEM analysis at 24h, 48h and 72h after seeding.

Results:

P(LLA-AC) copolymers were readily prepared by bulk ring-opening copolymerization of LLA and AC at 110 ℃ in the presence of stannous octoate as catalyst. The composition of the copolymers was easily controlled by the feed molar of the comonomers. The aligned P(LLA-AC) fibrous scaffolds could be induced to crimp by post heat-treatment of the fibers above their T_g. Crosslinked and crimped scaffolds prepared with P(LLA-AC) containing 5% AC showed high modulus (120 MPa) and were resistant to cyclic mechanical stretching (Fig 1A-C). The crimp parameters were similar to those of human ACL (wavelength 45-60 μm) and could be readily controlled by post-treatment conditions. 3T3 fibroblast cells adhered to and proliferated on the fibres, and grew along the direction of fiber alignment (Fig 1D).

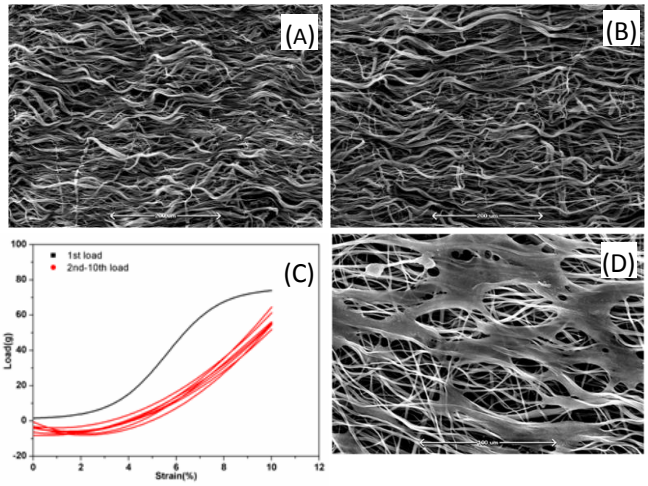


Fig 1.SEM pictures of P(LLA-AC)-5% crimped fibers before(A) and after (B) 40 times 10% stretching; (C) The cyclic mechanical stretching test for P(LLA-AC) fiber with 10% stretching; (D)SEM pictures show 3T3 cells attach and proliferate on crosslinked fibers after 72h seeding.

Discussion:

The crimp structure was stabilized by photo-crosslinking the polymer fibers. The crimped and photo-crosslinked P(LLA-AC) fibre scaffolds retained their initial stress-strain response, maintaining a consistent toe region after the 10 loading cycles. This was not possible with the previously used biodegradable polymers (e.g. PLLA)[2]. Moreover, the crosslinked biomimetic scaffolds possess a modulus within the range of that of native ACL and are ready for further conjugation with bioactive molecules (e.g. RGD peptide).The generated scaffolds with recoverable crimp-like structure upon crimp unfolding mimic the biomechanical response of crimped collagen fibers and possess potential as a scaffold for ligament tissue engineering.

Acknowledgements:
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References:

1. Chen, W.; Yang, H. C.; Wang, R.; Meng, F. H.; Wei, W. X.; Zhong, Z. Y. *Macromolecules* 2010, 43, 201-207.
2. Surrao, D.C.; Waldman, S.D.; Amsden, B.G. *Acta Biomaterialia* 2012, 8, 3997-4006.