Three Dimensional Multiphase Biomimetic Scaffold for Tissue Engineering Muscle-Tendon Junctions

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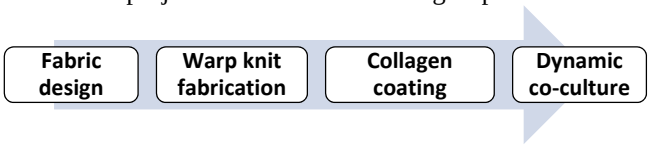
Introduction: While considerable success has been reported for tissue engineering single cell lines, there is a growing demand for co-culturing a combination of cell lines on a gradient scaffold that will regenerate a multiple tissue junction [1,2]. The goal of our study is to develop and evaluate 3D multiphase tissue engineering scaffolds that mimic the anatomical, mechanical, and physiological characteristics of the muscle, tendon and interfacial components in native muscle-tendon junction (MTJ) tissue. The project includes the following steps:



Materials and Methods: Polyethylene terephthalate (PET) was melt spun into 150 denier multifilament yarns with a 12.5μm diameter for each filament. The ideal structure was selected from ProCAD, a 3D knitting design simulation software. The latest warp knitting technology was applied to fabricate the design of 3D prototype scaffolds which have three distinct and contiguous regions to mimic the characteristics of the muscle, tendon and the interface of the MTJ (Figure 1). The physical and mechanical properties can be modified during the warp knitting process. Four different three phase scaffold structures were fabricated and examined for their structural characteristics as well as their physical and mechanical properties. Type I collagen was coated on the scaffold fabrics to activate the surface. Murine skeletal muscle cells and dermal fibroblasts were cultured simultaneously on the scaffold in a dynamic stretching bioreactor. The cell viability and phenotype are being examined by qPCR and immunohistochemistry for MTJ specific markers such as paxillin and vinculin [2].

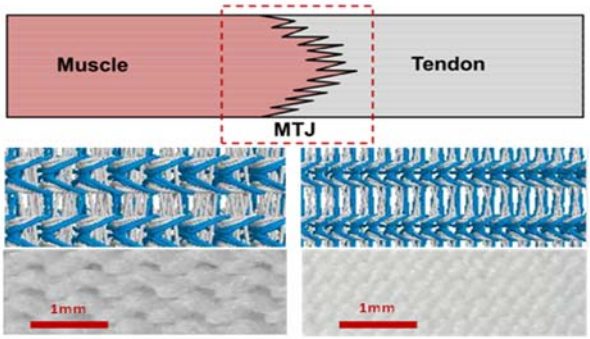


Figure 1. Design concept, simulation and optical images of the multiphase scaffold prototypes.

Results: The overall porosity, thickness, fabric density, compression and recovery properties have been tested using standard test methods. The ultimate tensile properties including the tensile strength, breaking strain and initial Young's modulus measured between 10%-30% strain have been performed under dry-conditions (Figure 2). The fabric scaffold structures have been shown to have superior tensile strength and elongation than normal human tissue in both the muscle and tendon regions. Preliminary biocompatibility studies using an MTT assay, scanning electron microscopy (SEM) and laser scanning confocal microscopy (LSCM) after 14-days of culture with human dermal fibroblasts (HDF) under static conditions have indicated good cell attachment to the scaffold surface and proliferation through the thickness of the construct (>2mm) (Figure 3). The scaffold was co-cultured with murine skeletal muscle cells and dermal fibroblasts simultaneously in a cyclic stretching bioreactor with constant strain for up to two weeks.

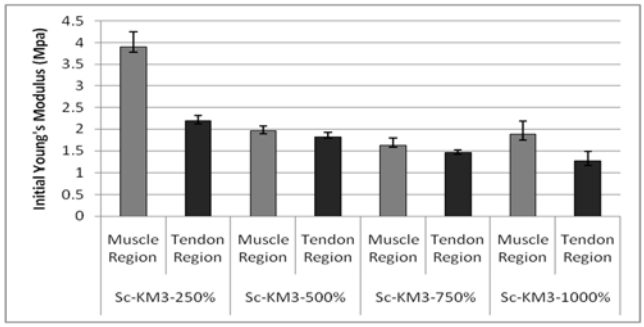


Figure 2. Initial Young's modulus of four prototypes.

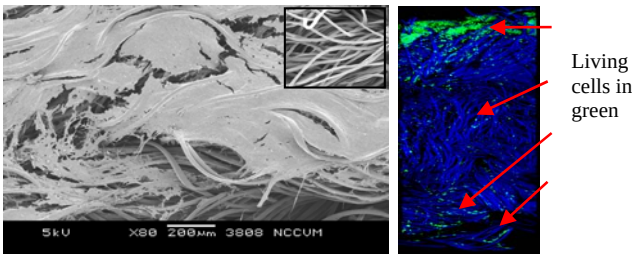


Figure 3 SEM (left) and LSCM (right) images, showing cell migration after 14 days of static culture across the surface (left) and through the thickness (right).

Conclusions and Future Work:
A series of 3D PET prototype biocompatible multiphase knitted structures with a contiguous porosity gradient have been successfully designed and fabricated. They provide appropriate structural and mechanical properties for the different regions of the MTJ. Future work will involve qPCR and immunohistochemistry tests on the engineered muscle-tendon junction.

Reference:
[1] Chung S, King MW. Design concepts and strategies for tissue engineering scaffolds. *Biotech Appl Biochem.* 2011:58(6):423-438.
[2] Ladd MR, Lee SJ, *et al.* Co-electrospun dual scaffolding for muscle-tendon junction tissue engineering. *Biomaterials.* 2011:32(6):1549-1559.