Porous Titanium Implant for Soft-tissue In-growth and Biological fixation

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INTRODUCTION: Tendon and ligamentous injuries amount to 17 million of the musculoskeletal injuries that occur each year in the United States.[1] Surgical intervention requires soft-tissue re-attachment, whether to bone tissue, engineered graft, or metal prosthesis. Several approaches to attach metal implants to ligamentous tissue have been explored, including engineered pores or spikes to allow anchoring of sutures and fastening of soft tissue to the implant surface.[2,3] However, soft-tissue re-attachment remains a major clinical challenge, as explored methods generate stress concentrations, inhibit angiogenesis, and achieve only 15% of original healthy tendon-to-bone attachment strength. Weakened functional characteristics of the soft-tissue consequently leads to re-tears/ tissue rupture and increases risk for requiring secondary surgery.[2,3] The ultimate goal of this work is to design, fabricate, and validate titanium implants with lattice-like pores for flexor tendon repair surgery. Implants should stimulate tendon cell migration/recruitment into pores, encourage long-term attachment, facilitate tendon cell infiltration/ingrowth, and integrate with the host-tissue for adequate biological fixation overtime (inducing biological anchorage through tissue in-growth). Suture should provide stability initially, but porous implant itself should promote integration with host tissue during late stage of healing without initiating scarring mechanisms or adverse reaction to implant material. Thus, **the objective** of this study is to determine the optimal manufactured titanium implant pore size and geometry for promoting fibroblast infiltration, viability, and proliferation. **It is hypothesized** that ligament fibroblasts will migrate onto titanium implants, and populate engineered pore geometries. Upon attachment, **we anticipate** that the cells will proliferate, produce a tendon-like matrix, and that smaller pore sizes will be optimal for achieving these outcomes. Cell viability, migration, and matrix deposition was assessed by Live/Dead Confocal Imaging and Scanning Electron Microscopy.

METHODS: *Implant Manufacturing:* Four titanium implant designs were manufactured with a smooth surface finish free of burrs and edges (Ti4Al6V alloy PER ASTM F136; Diameter: 600, 900μm; Geometry: Circle, Square; Fig. 1A). Implants were autoclaved to sterilize and placed in 24 well non-tissue culture treated plates. *Cell Culture:* Primary ligament fibroblasts were harvested from the anterior cruciate ligaments of juvenile bovine tibiofemoral joints obtained from a local abattoir (bACL Fb; Green Village Packing Company, Green Village, NJ). Fibroblasts went through cryopreservation and culture expansion to passage 3 before seeding onto coverslip discs (diameter = 13 mm) at a seeding density of $6x10^4$ cells/cm^2. Fibroblasts were allowed to attach to coverslips and grow for 3 days with one media exchange (fully supplemented (F/S) DMEM). Cell-seeded coverslips were placed on top of titanium plates and allowed to interact before timepoint collection (Day 3 & Day 10, Fig. 1B) with cells seeded onto tissue culture plastic serving as a Monolayer control. Cell migration and attachment onto plates was assessed at each timepoint via Live/Dead assay followed by confocal imaging (Olympus Fluoview FV100 Confocal Microscope; Fig. 2A), and scanning electron microscopy (SEM; Fig. 2C). Cell number was also assessed by fluorescence quantification of Live/Dead staining intensity normalized to standard pixel area (ImageJ, NIH; Fig. 2B). *Statistical Analysis*: Multiway ANOVA and the Tukey-Kramer post-hoc test was used for all pair-wise comparisons ($p < 0.05$, JMP-IN).

RESULTS/DISCUSSION: The fibroblast cell population migrates onto titanium implants and survives by Day 10 *in vitro* culture (Fig. 2A). Fibroblasts proliferate over time for the smaller pore size, demonstrated by the significant increase in Live/Dead fluorescent intensity for the 600 μm pores overtime regardless of pore geometry. While, 900 μm square pores seem to attract the most initial cell homing by Day 3, homed cells did not survive or proliferate by day 10 on this implant design (Fig. 2AB). Matrix deposition occurs as early as Day 3 in culture, with the most matrix observed for the 600 μm circle implant designs and in the corners of square pores (Fig. 2C). Interestingly, geometry seems to play an exclusive role for larger pore size at earlier time points, with no appreciable difference in cell number between geometries at the 900 μm pore size by day 10. Although the number of cells remains significantly lower than Monolayer controls at these early timepoints, we anticipate that pre-soaking implants with fetal bovine serum and longer culture times will mimic more physiologically relevant environment and lead to improved cell infiltration in future studies. Overall, 600 μm circular porous implants are optimal for bACL Fb attachment and integration.

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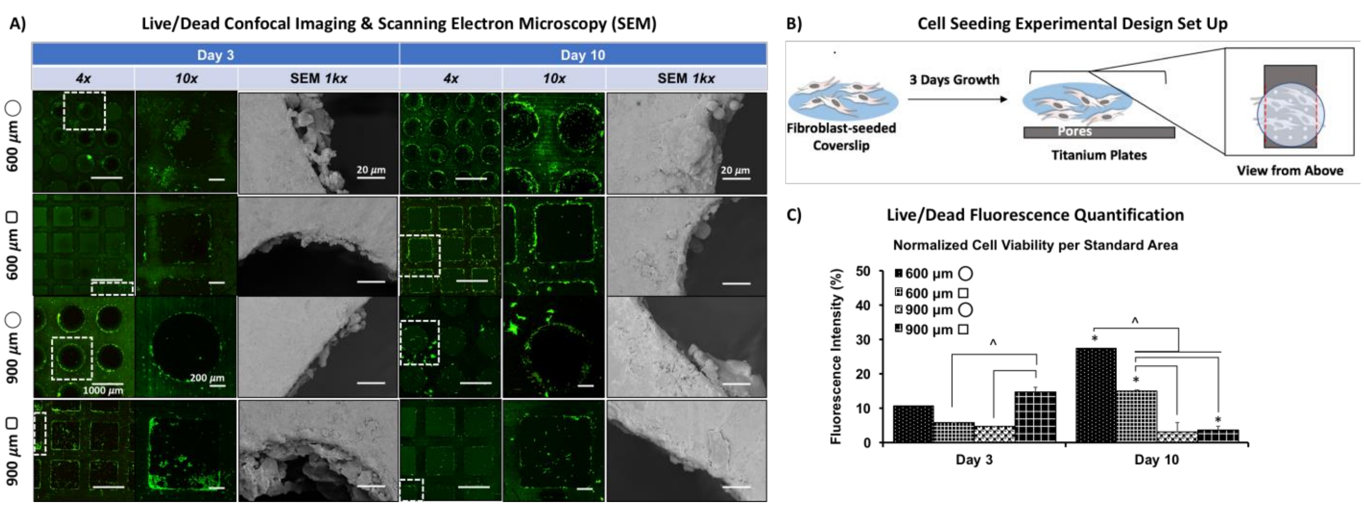

Figure 1. A) Ligament cell migration onto porous titanium implants. Live/Dead confocal imaging and scanning electron microscopy demonstrate that cells migrate onto titanium implants, attach, begin to produce matrix as early as Day 3, and survive by Day 10; proliferation and matrix deposition vary according to pore size and geometry. **B) Cell Seeding Experimental Design Set Up. C) Live/Dead Fluorescence Quantification.**