Growth Factor Incorporated Collagen Threads as Suturable Therapeutic for Tendon Repair

Mousa Younesi[1], Baris Ozgur Donmez[1], Ozan Akkus[1,2]

[1]Department of Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland 44106, US.

[2]Department of Orthopedics, Case Western Reserve University, Cleveland 44106, US.

Introduction: Ruptures and lacerations of tendons occur due to trauma and accidents in a large body of patients. [1]. Sutures are the mainstay in stabilizing such injuries. However, limited cellularity and blood supply in tendons protract the healing process. Incorporation of growth factors to sutures to accelerate the healing has been attempted before [2]. These strategies involve a synthetic polymer suture (such as nylon) coated with collagen and growth factors. Synthetic polymer was needed because collagen lacks suturability. Electrocompaction is a recently emerging method for making collagen threads which have robustness that is converging that of the natural tendon [3]. In this study, we modified such high strength pure collagen threads for sustained delivery of platelet derived growth factor (PDGF-BB). The effect of delivery on tendon-derived cells were also investigated.

Materials and Methods: *Growth factor conjugation and release profile*: Electrochemically aligned collagen threads (ELAC) were fabricated by an automated device at a diameter of about 0.1 mm [4]. Threads were crosslinked in genipin (0.63% in 90% ethanol). Heparin was chosen as a biomolecule for affinity based retention and delivery of the growth factor [5]. Heparin was conjugated on threads with EDC-NHS chemistry [3]. Heparin was crosslinked on threads at concentrations of 1, 3 and 10 mg/mL. Amount of conjugated heparin was determined by dimethyl-methylene blue assay (DMMB). 100 ng of PDGF-BB in 50 µL of carrier solution was loaded on 3 cm of collagen threads. The effect of heparin conjugation on release profile was assessed over 14 days by ELISA (Human PDGF-BB Quantikine ELISA Kit, R&D systems). *Cell Response:* Effect of growth factor that is released from the threads on cell metabolic activity and cell proliferation was investigated with alamar blue assay and DNA measurement, respectively, on day 7. Cells derived from chicken tendons were seeded on collagen gel, ELAC or heparinized ELAC with growth factor. 100,000 cells were cultured on each sample (N = 6/group). Culture medium was αMEM + 10% FBS +1% pen/strep.

Results: EDC-NHS chemistry was successful in conjugating heparin to the threads in a range of 0.01 to 0.08 mg/cm^2 per unit thread area (Fig. 1). Crosslinking of the heparin on ELAC prolonged the release of PDGF-BB from ELAC threads (Fig. 2). In the absence of heparin, 80% of the growth factor was delivered by 24 hours. At high level of heparination, it took two weeks for the same amount to be delivered (Fig. 2). Cell proliferation and metabolic activity was increased when collagen gel vs. threads were compared. More notable increase was observed in growth factor conjugated group. Cells on ELAC with PDGF-BB had higher metabolic activities (45%, 93%) and proliferation rates (37%, 52%) compared to ELAC without GF and random collagen, respectively (Fig. 3a&b).

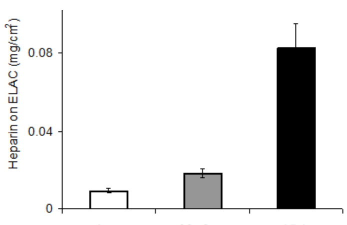

Figure 1. The amount of heparin crosslinked on ELAC threads could be controlled over a large range.

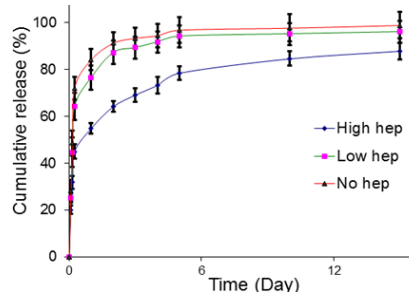

Figure 2. PDGF-BB release was prolonged by heparin conjugation.

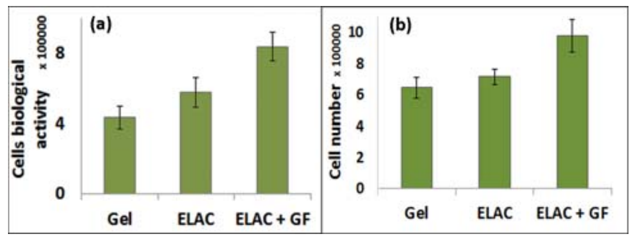

Figure3. PDGF-BB had positive effects on a) cellular metabolic activity and b) proliferation.

Conclusions: The remarkable mechanical robustness of ELAC threads enable them to be sutured through tendon (Fig 4). Such robustness is now synergized with affinity bonded GF delivery to accelerate the healing process. Currently the work is in progress for investigating the effect of this growth factor delivery strategy on expression of tendon-related markers (tenomodulin, collagen I, COMP, scleraxis, etc.) and the type and amount of extracellular matrix made by cells. Following in vitro optimization of PDGF delivery, the bioactive ELAC suture will be investigated in vivo using a chicken model.

Figure 4. Histological section (H&E stain) of a chicken tendon that is showing collagen sutures (A, B, C).

[1] D. L. Butler, et al., Nat Protoc 2010;5:849-63.

[2] A. D. Mazzocca, et al., J Shoulder Elbow Surg., 2007;16: S222-30.

[3] J. A. Uquillas et al., Journal of the Mechanical behavior of Behavior of Biomedical Materials, 15,2012,17 6-189

[4] M. Younesi AI, Vipuil Kishore, James M. Anderson, Ozan Akkus. Advanced Functional Materials. 2014.

[5] J. Lee, et al. Biomaterials 33 (2012) 6709-6720.