BMP-2 Modified Fiber Meshes to Facilitate Bone Integration for Ligament Reconstruction

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Statement of Purpose: Due to its limited vascularity and interarticular location, the anterior cruciate ligament (ACL) often requires surgical procedures to repair tears and ruptures. While the bone-patellar tendon-bone autograft is considered the gold standard, less invasive reconstruction options exist. Use of the hamstring or quadriceps tendon involves placement of the soft tissue graft within the bone tunnel, and relies on integration of the hard and soft tissues to avoid mechanical instability and graft pullout [1]. These insights suggest that engineered tissues for ACL repair must possess proximal and distal zones to facilitate bony integration with the femur and tibia, respectively. With the ultimate goal of developing a high tensile strength fibrous scaffold for ligament regeneration, this work focuses on the modification of the electrospun fiber meshes to facilitate bone integration. To this end we have recently examined the direct conjugation of morphogenic protein (BMP)-2 to electrospun polycaprolactone (PCL) fibers. We show that this approach facilitates osteoblastic differentiation of mesenchymal stem cells (MSCs) *in vitro*. As a follow-on study, we have recently fabricated BMP-2 presenting chitosan microparticles with the express goal of further enhancing bone integration.

Methods: Polycaprolactone (PCL) alone or with 0.5 or 1 wt% heparin (Hep0.5 and Hep1.0, respectively) were dissolved in tetrafluoroethanol to give 8 wt% solutions. Solutions were electrospun onto a slowly rotating mandrel and collected onto glass coverslips. Resultant fiber meshes were modified with BMP-2 through adsorption by incubating with 250 ng/ml BMP-2 solutions or by covalent conjugation by first activating the fiber meshes with EDC/NHS prior to incubation with BMP-2. Separately, chitosan microparticles were fabricated by the coacervation method [2] by adding 2 wt% chitosan in 2% acetic acid dropwise to a stirred 1 wt% sodium tripolyphosphate solution, and BMP-2 was immobilized to microparticles by EDC/NHS chemistry. BMP-2 was quantified with ELISA and values normalized per unit mass. BMP-2-conjugated fiber meshes or BMP-2-conjugated microparticles on fiber meshes were prepared for cell viability and proliferation studies. Briefly, 4×10^4 MSCs were seeded onto surfaces and cultured for up to 28 days with cells on tissue culture polystyrene serving as the control. Viability was analyzed with Live/Dead cell staining and proliferation by DNA quantification using Quanti-iT PicoGreen kit. Alkaline phosphate (ALP) activity was determined using *p*-nitrophenyl phosphate as a colorimetric substrate and normalized to total cell number. Extracellular mineral deposition was determined by Alizarin red staining, and gene expression of bone markers (e.g., collagen-I, Runx2, osteopontin and osteocalcin) by quantitative real-time polymerase chain reaction.

Results: BMP-2 concentration on the fiber surfaces ranged from 0.5 to 5 ng/cm^2 for both immobilization techniques (Figure 1). The highest BMP-2 was measured for covalent attachment to fiber meshes containing 1% heparin due to the introduction of carboxylic acid groups on heparin that facilitate conjugation [3]. Cell studies on BMP-2 displaying Hep1.0 fiber meshes show that cells attached and remained viable both with and without BMP-2 (Figure 2a). Osteoblastic differentiation studies indicate enhanced ALP activity and mineral deposition (Figure 2b). Studies involving BMP-2-presenting chitosan microparticles are currently ongoing.

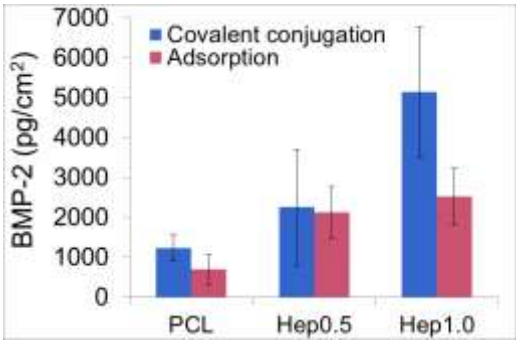

Figure 1. Concentration of BMP-2 by adsorption and covalent conjugation as measured by ELISA. Data presented as mean ± standard deviation (n=3).

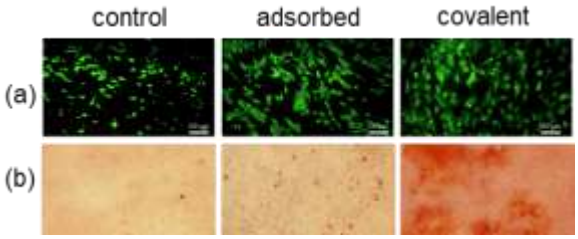

Figure 2. a) Live/Dead staining of MSCs on Hep1.0 meshes after 7 days. b) Alizarin red staining for mineral deposits after 28 days on Hep1.0 meshes.

Conclusions: BMP-2 modified fiber meshes that could ultimately facilitate bone integration in vivo were prepared and BMP-2 concentration depended on the conjugation technique and the fiber chemistry. Resultant, fiber meshes supported viability and proliferation of MSCs. Preliminary studies confirmed the osteogenic effect of BMP-2 on MSCs while ongoing studies will determine if incorporation of additional BMP-2 via chitosan microparticles can further enhance the osteogenicity of the fiber meshes

References: [1] Steiner ME *et al*, *Am J Sports Med.* 1994; 22:240-247. [2] Unagolla JM *et al. Carbohydrate Polym.* 2018; 199:426-436. [3] Gandhi NS *et al*, *Biochim Biophys Acta* 2012; 1824(12):1374-1381.