Characterization and In vivo Studies of a Novel Bio-nanocomposite as a Tissue Scaffold

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Statement of Purpose: Extracellular matrix (ECM) component of human or animal tissues have been utilized to recapitulate tissue at the site of a defect. Tissue regeneration allows optimal restoration of tissues to their original structural and functional state. While the benefit of utilizing these "bioscaffolds" include biological recognition to facilitate cellular adhesion and proliferation, bioscaffolds unfortunately have been plagued with inherent problems such as heterogeneity, unsatisfactory mechanical properties, slow integration, and poor stability which can affect cellular response and new tissue synthesis, thus affecting its clinical utility [1].

To leverage the benefits of natural tissue while reducing their inherent problems, we have been integrating gold nanoparticles (AuNPs) with acellular tissue. We have investigated morphological changes, physicochemical changes, and biocompatibility of the bioscaffold-nanomaterial composite (bio-nanocomposites) via SEM, uniaxial tensile tests, collagenase assay, and *in vivo* rat model studies. We have hypothesized that the bio-nanocomposites will result in a scaffold with flexible meso/micro/nanoscale architectures and significantly improved properties which will optimize cellular regenerative responses at tissue defect sites.

Methods: Central diaphragm tendons were decellularized and stored in phosphate buffered saline (PBS) until needed. A 6cm x 6cm section of the decellularized matrix was combined with 0.5 mL of functionalized AuNP solution and 50mL crosslinking solution and stirred via an orbital shaker table for 24 hours. The crosslinking solution consisted of a 50:50 (v/v) solution of acetone and PBS (pH 7.5) with a final concentration of 5 mM NHS (N-Hydroxysulfosuccinimide) and 2 mM 1-ethyl-3-[3-dimethyl aminopropyl] carbodiimide hydrochloride (EDC), a non-toxic, zero-length crosslinker. After stirring, the bio-nanocomposite was rinsed for 48 hours in PBS and stored in ethanol at 4°C until testing.

Results: Figure 1 displays a SEM micrograph of a sectioned bio-nanocomposite. The figure shows that the AuNPs have attached to the collagen matrix and also have penetrated through the structure.

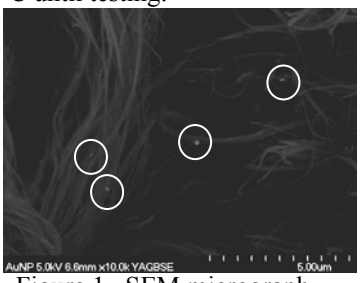

Figure 1. SEM micrograph of bioscaffold.

Tensile testing A standard uniaxial tensile test was performed on ten pieces of tissue. An Instron 5864 Universal Testing Machine was utilized to strain the specimens at a rate of 0.2mm/s until failure. The results of the uniaxial tensile test are depicted in Figure 2 in which the mean tensile stress at yield is plotted ± the standard error. Natural, decellularized porcine diaphragm tendon possessed a tensile strength of 7.8 ±0.5MPa. AuNP tissues possess a significantly higher tensile strength at yield than decellularized tissue ($p<0.001$).

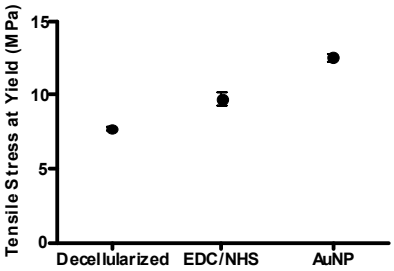

Figure 2. Tensile stress at yield.

However, the AuNP tissue did not possess a significant higher yield than just the crosslinked tissues (EDC/NHS) ($p>0.05$).

Collagenase testing: A collagenase assay was performed according to the accepted method [2]. The μg of hydroxyproline released from each sample after digestion by collagenase was calculated based on the standard hydroxyproline curve. This value was divided by the original mass of the tissue to yield the μg of hydroxyproline released per mg of original tissue. AuNP bioscaffolds showed a significant improvement in their resistance to degradation by the collagenase enzyme compared to natural, uncrosslinked tissue ($p<0.001$) and tissue crosslinked with EDC ($p<0.001$). (n=3)

Rat studies: Forty-five male, Sprague-Dawley rats were utilized to test the biocompatibility of the bionanocomposites. Three time points (7, 21, and 97 days) were utilized with 15 rats per time point. Blunt dissection of subcutaneous tissues created a pocket for placement of the scaffolds. Standard H&E histology was performed. For the AuNP-bionanocomposite, the results showed that at 7 days there was acute inflammation, mild chronic inflammation, blood vessel and fibroblast proliferation, and early replacement of scaffold with new collagen. At 21 days there was moderate chronic inflammation, no acute inflammation. At 97 days there was very little tissue reaction and it was difficult to separate scaffold from new collagen. See Figure 3.

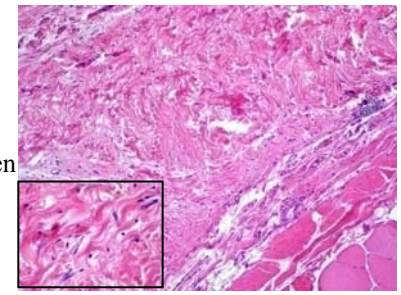

Figure 3. Disorganized mesh with new collagen deposition (100x). Insert: New collagen deposition by fibroblasts (800x)

Conclusions: The bionanocomposites demonstrated good mechanical strength, increased resistance to collagenase degradation, and excellent biocompatibility. Future studies include functional *in vivo* studies for hernia repair applications.

References: 1. Curtis A, et al. Eur. Cell Mat. 2005; 9:50-57. **2.** Duan X., Sheardown H, J Biomed Mater Res A. 2005 75(3):510-8.