Nano-grafts for Anterior Cruciate Ligament Reconstruction

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Statement of Purpose: A clinical need exists for better anterior cruciate ligament (ACL) graft materials because current graft materials suffer from poor performance and frequent failure to remodel. Existing materials lack the necessary cellular integration and slow degradation rate of successful tissue scaffolds. The present study investigated the development of new ACL xenograft scaffolds conjugated with gold and hydroxyapatite nanoparticles. Gold nanoparticles (AuNP) have been shown to enhance cell attachment and proliferation, encourage cellular remodeling, and prevent infection [1]. Hydroxyapatite nanoparticles (HANP) attached to the ends of the graft are proposed to help create a better ligament-bone attachment site to create a more stable reconstruction.

Porcine diaphragms were harvested, decellularized, crosslinked with nanoparticles, sterilized, then characterized and tested for biocompatibility and cell proliferation. The goal of this research is to create an improved ACL graft designed to promote remodeling; thereby reducing revision surgeries as well as patient pain and hospital costs.

Methods: Porcine diaphragm tendons were obtained from University of Missouri School of Medicine (Columbia, MO) and decellularized by a protocol developed by Deeken et al. [2]. Nanoparticles were attached by using EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiiamide hydrochloride), a zero-length crosslinker. AuNP of 20nm and 100nm diameter and HANP of <200nm and of <40nm were used. HANP concentrations added to scaffolds ranged from 0.001%-10% w/v in solution. Scaffolds were sterilized in 0.1% v/v paracetic acid with 1.0M NaCl.

An FEI Quanta^{TM} 600 FEG scanning electron microscope (SEM) (FEI Company, Hillsboro, OR), a Hitachi S4700 scanning electron microscope, a JEOL JEM-1400 transmission electron microscope (TEM), and energy-dispersive X-ray spectroscopy (EDS) were used to confirm attachment and to characterize scaffolds and nanoparticles.

L-929 mouse fibroblast cells were used for all cell culture assays. A 3 day WST-1 assay was used to evaluate biocompatibility. A 3, 7, and 10 day Quant-iT$^{\mathbf{TM}}$ PicoGreen® dsDNA assay (Molecular Probes, Inc. Eugene, OR) was used to evaluate cell proliferation.

A TA Instruments differential scanning calorimeter (DSC) was used to investigate thermal stability of scaffolds.

Results: Successful attachment of nanoparticles was confirmed with electron microscopy and EDS (Figure 1). TEM results showed that the HANP of <200nm diameter were spherical but not of uniform size. The HANP of <40nm had rough edges and appeared plate-like but were of a fairly uniform size.

The denaturation temperature and heat of enthalpy determined from the DSC showed that attachment of nanoparticles and sterilization of the scaffold did not have a negative effect on the thermal properties of the scaffolds. The denaturation temperatures and heat of enthalpies were not significantly different between groups of sterilized and non-sterilized samples and between samples with increasing concentrations of HANP.

The dsDNA assay showed increasing fibroblast growth at 3, 7, and 10 days with no significant difference between scaffolds with and without HANP. The WST-1 assays showed biocompatibility of scaffolds at all concentrations of nanoparticles. Spherical HANP of <200nm showed decreased biocompatibility as compared with plate-like HANP of <40nm.

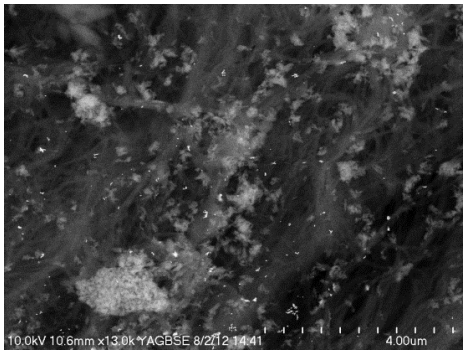

Figure 1. SEM image of porcine diaphragm scaffold crosslinked with 20nm AuNP and <40nm HANP.

The highest cell viability was achieved by attaching scaffolds crosslinked with a 1% w/v solution of HANP.

Conclusions: Gold and hydroxyapatite nano-grafts were synthesized and characterized. Neither AuNP nor HANP were toxic to fibroblast cells when attached to a decellularized porcine tendon. Scaffolds with plate-like HANP may have increased biocompatibility compared to scaffolds with spherical HANP. This may be due to the disproportionate size distribution of the <200nm spherical nanoparticles which contained mostly nanoparticles several times smaller than 200nm. These small nanoparticles can be internalized by cells and cause cell death. A higher surface energy of plate-like HANP may also lead to increased cellular attachment. The biocompatibility results of this study show promising preliminary results to utilize gold and hydroxyapatite nano-grafts for ACL repair. Future studies will include a non-functional *in vivo* biocompatibility test to determine the ability of the nano-graft to enhance cellularity and remodeling.

References:

1. (Deeken CR. J Biomed Mater Res B Appl Biomater. 2011. 96B: 351-359).
2. (Deeken CR. J Biomed Mater Res B Appl Biomater. 2011. 93:199-206).

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