The Evaluation of Gold and Hydroxyapatite Nano-grafts in a Green Fluorescent Protein Porcine Model

Sarah Smith[1], Richard White, M.D.[1,2], David Grant[1], Sheila Grant, Ph.D.[1]
[1]Department of Bioengineering, University of Missouri, [2]Department of Orthopaedic Surgery, University of Missouri

Statement of Purpose: An increasing number of novel soft tissue repair materials are being developed for musculoskeletal applications. This increase arises from the poor performance of existing materials due to the inability to effectively induce *in vivo* cellular integration and tissue remodeling. With an increased demand for superior repair materials there is also a need for additional methods to evaluate soft tissue integration and remodeling. When using heterogeneous extracellular matrix (ECM) derived scaffolds for soft tissue repair, current histological methods of *in vivo* evaluation can fail to provide a clear distinction between host collagen and implanted scaffolds making it difficult to assess newly synthesized host collagen as part of host tissue integration and remodeling. In this study we investigate the use of green fluorescent protein (GFP) expressing swine and fluorescence imaging as a novel method to observe host tissue integration and remodeling into a novel graft material. Graft materials were implanted into GFP swine and evaluated using histology and confocal imaging. It is hypothesized that fluorescent host tissue can be observed migrating into non-fluorescent scaffolds. The purpose of this study is to both evaluate scaffolds conjugated with nanoparticles for host tissue integration and biocompatibility as well as to assess green fluorescent protein (GFP) expressing swine as a new animal model to evaluate soft tissue repair materials using different fluorescence imaging preparation techniques.

Methods: The scaffolds used in this study comprised of decellularized human anterior tibialis tendons conjugated with 100nm gold nanoparticles (AuNP) and <40nm plate-like hydroxyapatite nanoparticles (nano-HAp) using zero-length crosslinker, EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiiamide hydrochloride). Grafts were sterilized in 0.1% v/v paracetic acid in 1M NaCl. Three allograft samples were implanted into each of twelve green-fluorescent protein (GFP) expressing pigs. Each set contained samples without nanoparticles, with AuNP, and with both AuNP and nano-HAp. Four pigs were sacrificed at 1, 3, and 6 months and samples were explanted and either immediately placed in formalin or frozen on dry ice. Explanted samples were prepared for H&E histology and scored for cellular infiltration, vascularity, multi-nucleated giant cells (MNGC), fibrous encapsulation, scaffold degradation and connective tissue organization. Samples were prepared for confocal microscopy using five different methods including formalin fixation and paraffin embedding, vapor fixation, freshly prepared paraformaldehyde (PFA) fixation, and fresh frozen tissue. Confocal images were taken on a Zeiss LSM 510 META confocal microscope (Oberkochen, Germany). Confocal images were evaluated to observe host tissue integration and remodeling by the distinction between host tissue and scaffold without interfering autofluorescence.

Results: Histological scoring indicates biocompatibility and remodeling of the scaffolds with and without nanoparticles at 1, 3, and 6 months. Increased cellular infiltration, vascularity and connective tissue organization from 1 to 6 months was evident with little to no MNGC response. No significant differences in histological scoring were seen between scaffolds with and without nanoparticles. All methods of confocal microscopy preparation techniques exhibited autofluorescence of scaffold tissue except for fresh frozen tissue preparation. Confocal microscope images using fresh frozen tissue display host tissue integration into scaffolds although non-specificity of GFP does not allow for quantification of integration.

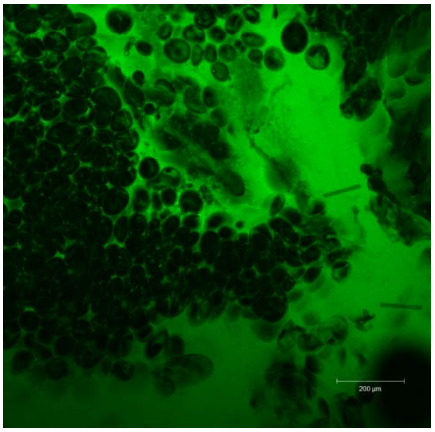

Figure 1. Confocal miscroscopy images of explanted allografts 1 month after implantation.

Conclusions: The nanoparticle-scaffolds are biocompatible and promote integration although there were no significant differences in the histology and confocal images as compared to the scaffolds without nanoparticles. By using fresh frozen tissue as a preparation method, autofluorescence is not present and desired features are distinguishable. Host tissue integration appears to be evident in explanted samples prepared by fresh frozen tissue and is the best option as an imaging preparation technique. Although quantification of integration cannot be achieved due to limitations concerning GFP expression, confocal images do allow for spatial observation of host tissue migration into the scaffolds at different depths of penetration. It is concluded that the use of GFP expressing swine can aid in visualizing the scaffold/host interface and host cell/tissue migration. Thus there are potential benefits for the use of GFP expressing swine for visualization of scaffolds implanted into soft tissue.

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