Assessment of a Decellularized Porcine Diaphragm and Gold Nanomaterial Composite as a Tissue Scaffold for Wound Healing

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Statement of Purpose: Impaired healing responses in chronic wounds have been attributed to mechanisms including reduced growth factor production, reduced vascularization, and abnormal free radical levels [1]. Decellularized tissues such as allografts or xenografts have been used to provide the wound bed with an extracellular matrix in which to have cells grow. Various nanostructures have been generated and have shown good cell interaction. Gold nanoparticles (AuNPs), for example, encourage fibroblast adhesion and proliferation while serving as free radical scavengers [2, 3].

The purpose of this study was to assess a decellularized porcine diaphragm and gold nanomaterial composite as a potential tissue scaffold for wound healing. It was hypothesized that the gold nanomaterial scaffolds, which incorporated gold nanorods (AuNRs) or AuNPs, would result in a biocompatible tissue scaffold capable of promoting fibroblast attachment and proliferation while reducing the number of free radicals.

Methods: The central tendon portions of porcine diaphragms were collected after euthanization of swine at the University of Missouri School of Medicine (Columbia, MO). They were decellularized by a 1% (v/v) tributyl phosphate solution wash. AuNRs and AuNPs, at various concentrations, were crosslinked to 1 cm discs of decellularized diaphragm using a zero-length crosslinker, 1-ethyl-3-[3-dimethyl aminopropyl] carbodiimide hydrochloride (EDC). These were sterilized in a sodium chloride solution with 0.1% (v/v) peracetic acid.

Scaffold Morphology: Scanning electron microscopy (SEM) was used to evaluate the scaffold morphology as well as confirm the presence of the gold nanomaterials.

Biocompatibility: Cell Proliferation Reagent WST-1 (Roche Diagnostics Corporation, Indianapolis, IN) was used to assess the percent viability of cells on gold nanomaterial scaffolds relative to Decellularized scaffolds. L-929 fibroblasts were cultured with the gold nanomaterial scaffolds for 3 days before adding the WST-1 reagent. Absorbance readings, which correspond to cell activity, were collected after a 4 hour incubation.

Cell Attachment/Morphology: L-929 fibroblasts were cultured with the gold nanomaterial scaffolds for 3, 7, and 14 days at which point the cells were labeled with the LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Invitrogen Corporation, Carlsbad, CA). Confocal microscopy was then used to image the 3-D scaffolds and evaluate cell attachment and cell morphology.

Cell Proliferation: Scaffold enhancement of L-929 proliferation over 3, 7, and 14 days was assessed using Quant-iT PicoGreen dsDNA Reagent (Invitrogen Corporation, Carlsbad, CA). Increased fluorescence indicated increased dsDNA content and cell proliferation.

Free Radical Scavenging: Ability of the gold nanomaterial scaffolds to reduce the number of free radicals generated was measured using the OxiSelect ROS Assay Kit (Cell Biolabs, Inc., San Diego, CA). Increased fluorescence signified an increase in free radical concentration.

Results: Scaffold Morphology: SEM images depicted gold nanomaterials dispersed throughout the scaffolds which had maintained their natural, highly porous microstructure throughout the crosslinking process.

Biocompatibility: The WST-1 assay showed Crosslinked, AuNP-1X, and AuNP-4X to be statistically indifferent ($p>0.05$) from Decellularized while 1X AuNR was the only group statistically different ($p<0.05$) from Crosslinked. (n=8)

Cell Attachment/Morphology: Confocal microscopy images depicted an increase in cell attachment with increasing culture time. Proper L-929 cell morphology was also apparent.

Cell Proliferation: Figure 1 depicts dsDNA content for scaffolds after 3, 7, and 14 days in culture. AuNR-1/2X demonstrated a higher ($p<0.05$) dsDNA content at Day 3. AuNR-1X and AuNR-4X showed increases ($p<0.01$) in dsDNA content from Day 3 to Day 7 and Day 7 to Day 14, respectively. No differences ($p>0.05$) were found relative to Decellularized at Day 7 or Day 14. (n=4)

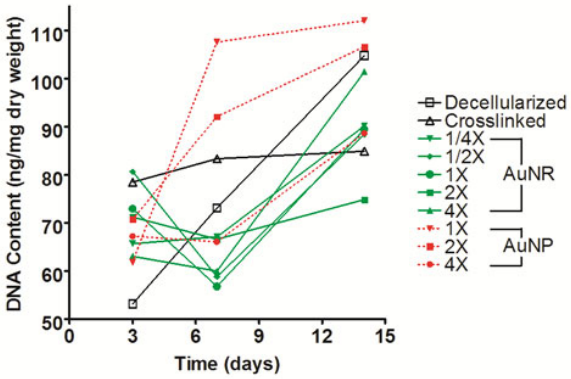

Figure 1. Results of the dsDNA assay.

Free Radical Scavenging: Free radical levels were higher for AuNP-2X ($p<0.01$) and AuNP-4x ($p<0.001$) when compared to Decellularized. All other groups were statistically indifferent ($p>0.05$) from Decellularized. Moreover, AuNP-4X was statistically greater ($p<0.05$) than AuNP-2X. (n=4)

Conclusions: The gold nanomaterial scaffolds used demonstrated good biocompatibility, cell attachment and morphology, and enhanced cell proliferation with free radical generation being dependent on particle shape and concentration. Thus, the scaffolds have the potential to enhance wound healing rates while modifying the atypical free radical levels found in some chronic wounds.

References: **1.** Fonder MA. J Am Acad Dermatol. 2008;58:185-206. **2.** Huang C-L. Thin Solid Films. 2009;517:5386-9. **3.** Zhang Z. J Am Chem Soc. 2003;125:7959-63.