In Vivo Study of a Novel AuNP-Tissue Scaffold

S.A. Grant[1], C.R. Deeken[3], D.A. Grant[1], S.L. Bachman[2], and B.J. Ramshaw[4]
[1]Dept of Biological Engineering and [2]Dept of Surgery, University of Missouri; [3]Dept of Surgery, Washington University;
[4]Halifax Health, Daytona Beach, FL.

Statement of Purpose: The extracellular matrix (ECM) of a variety of human and animal tissues have been utilized for a number of soft tissue applications including vascular grafts, hernia repair, dermal grafts, and tendon, ligament, cartilage, and urological reconstruction. While these biologic scaffolds are expected to demonstrate superior tissue integration, there is very little evidence documenting the properties and behavior of these materials *in vivo*. The type of tissue, species of origin, and the unique processing procedures may affect their properties, cellular infiltration, remodeling and thus their efficacy. This *in vivo* study was performed to investigate four biologic scaffolds: two commercially available biologics (a moderately crosslinked mesh and a non-crosslinked mesh) and two novel porcine diaphragm biological scaffolds with and with/out the incorporation of gold nanoparticles. The meshes were implanted into a porcine model and evaluated over the course of one, three, and six months.

Methods: The following four biologic tissue scaffold materials were implanted into the abdominal walls of fifteen female, Landrace pigs.

1. "Non-crosslinked" (Surgisis-Cook Biotech Incorporated, West Lafayette, IN) scaffolds: This scaffold material was comprised of several layers of non-crosslinked porcine small intestine submucosa. The product was used as received.
2. "Slightly crosslinked" (AuNP-crosslinked) scaffolds: This scaffold material was comprised of one layer of porcine diaphragm tissue that was crosslinked with mercaptoethylamine (MEA)-functionalized gold nanoparticles (AuNP) in combination with EDC and NHS. This was our novel scaffold. Details on the fabrication can be found elsewhere [1].
3. "Slightly crosslinked" (MD-crosslinked) scaffolds: This scaffold material was comprised of one layer of porcine diaphragm tissue that was crosslinked using the chemical crosslinkers 1-ethyl-3-[3-dimethyl aminopropyl] carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Details on the fabrication can be found elsewhere [1].
4. "Moderately crosslinked" (Permacol, Covidien, New Haven, CT) scaffolds: This scaffold material was comprised of one layer of hexamethylene diisocyanate crosslinked porcine dermis. The product was used as received.

The abdominal wall of each pig was divided into four regions separated from each other by at least one inch on each side. A 16cm^2 piece of each of the four types of scaffolds was placed into these quadrants. Five pigs were sacrificed at each of the three time points (one, three, and six months). At the time of sacrifice, full-thickness sections of the abdominal wall, including all four scaffold sites and 1cm of surrounding tissue, were harvested from each animal and preserved in 10% neutral, buffered formalin and then subjected to histological analysis. The four types of implanted scaffolds were examined and compared for the presence of multinucleated giant cells (MNGC), connective tissue organization, and periphery neovascularization and center neovascularization. They were scored according to a semi-quantitative scale (0 to 3) found in the literature [2].

Results: MNGC: None of the scaffolds evaluated displayed a marked presence of multinucleated giant cells at the 1, 3, and 6 month time points.

Connective Tissue Organization: Connective tissue organization was not examined at the 1 month time period, only at the 3 and 6 month time period. Permacol did not display any connective tissue organization and scored 0 at 3 and 6 months. Surgisis scored an average of 1.6 and 2 for the 3 and 6 months time periods respectively. MD scaffolds and AuNP scaffolds scored an average of 2 for both the 3 and 6 month time periods. There were significant differences in connective tissue between the Permacol scaffolds and the Surgisis scaffolds ($p<0.001$), and between the Permacol and the MD scaffolds ($p<0.001$), and the AuNP scaffolds ($p<0.001$) respectively.

Neovascularization: For all time points, the scaffolds displayed higher neovascularization at the periphery than at the center. A notable exception was the AuNP scaffolds at 6 months which displayed a higher center vascularization than periphery. At 6 months, the center vascularization was significantly different between Permacol and AuNP scaffolds ($p<0.01$). As shown in Figure 1, the AuNP-tissue scaffold displayed connective tissue (CT) and neovascularization (BV). The increase in center vascularization could be attributed to the gold nanoparticles enhancing cellular attachment and ingrowth.

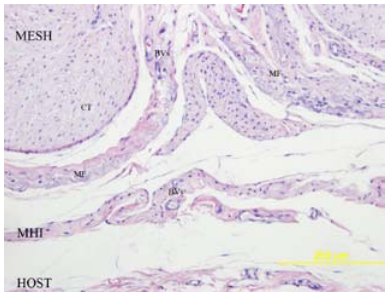

Figure 1. AuNP scaffold after 6 months (H&E stain, 20x).

Conclusions:
The moderately crosslinked scaffolds did not promote cellular integration, while the non-crosslinked scaffolds quickly delaminated. Both diaphragm scaffolds, with and without AuNPs, showed good cellular integration with the AuNP showing slightly better integration.

References:

1. Deeken, CR, Accepted by JBMR: Part B – Applied Biomaterials, 10-2010.
2. Valentin JE, Bone Joint Surg Am 2006;88:2673-2686.