Discrepancies In Cellular Composition And Gene Expression In Long Acellular Allografts

Deng Pan, BS, Ellen Larson, BS, Anja Fuch, PhD, Sally Jo, BS, Xueping Ee, MD, Katherine Santosa, MD, Alison Snyder-Warwick, MD, Susan Mackinnon, MD, Matthew Wood, PhD

Washington University in St Louis, Saint Louis, MO, USA

PURPOSE: Acellular nerve grafts (ANAs) represent clinical alternatives to nerve autografting. However, as ANAs increase in size and length, these alternatives exhibit reduced capability to facilitate axon regeneration across the graft. Understanding why this deficiency develops is critical to designing autograft alternatives.

METHODS: Rat sciatic nerves were transected and repair with either short (2 cm) or long (4 cm) ANAs. Grafts were analyzed after 2, 4 and 8 weeks in vivo with histology, lectin perfusion, gene expression, histomorphometry, and immunohistochemistry. Results are presented as means with a student’s two-tailed t-test to assess statistical significance (p<0.05).

RESULTS: Histomorphometry assessed the extent of axon regeneration across 2 cm and 4 cm ANAs. The number of myelinated axons that crossed the graft mediated by 2 cm ANAs were significantly greater than 4 cm ANAs (6449 vs 44 myelinated axons) by 8 weeks. Considering early gene expression, analysis of a select panel of inflammatory and angiogenetic genes showed that at 2 weeks, long ANAs experienced reduced VEGFa, IL-4, and IL-10 gene expression, while having an elevated iNOS gene expression. Considering angiogenesis as VEGFα was decreased in long compared to short ANAs, at 2 weeks, total blood vessel length (indicated by RECA-1+ immunohistochemistry) of long ANAs were one third the value of short ANAs at 2 weeks, and only reached half the RECA-1+ values by 4 weeks. Considering functional blood vessels, lectin intravenous perfusion also showed similar reduction of blood vessels in long ANAs compared to short ANAs at 4 weeks. To further determine the impact of the altered inflammatory profile between long and short ANAs, we considered leukocyte populations within ANAs. While macrophage composition was similar between the long and short ANAs at 2 weeks (38% vs 45%), there was a reduction in the proportion of macrophages at 4 weeks between long and short ANAs (10.6% vs 21.9%). Additionally, while T cells represented a small proportion of cells within short and long grafts at 2 weeks (4.2% vs 0.9%), their relative proportion became significant by 4 weeks with stark differences between short and long ANAs (16.3% vs 4.3%).

CONCLUSIONS: Long ANAs mediated significantly reduced axonal regeneration in rat model of sciatic repair. Gene expression studies demonstrated a different inflammatory environment within the long ANAs compared to short ANAs, with elevated inflammatory gene (iNOS) and reduced anti-inflammatory genes (IL-4, IL-10). VEGF, important for angiogenesis, was also reduced. Histologically, we observed reduced angiogenesis in long ANAs, as demonstrated by reduction of total blood vessel length, at 2 weeks and 4 weeks, which was confirmed by intravenous infusion of fluorescently labeled lectin at 4 weeks. Finally, T cell accumulation is reduced in long ANAs. Previous studies have suggested that T cells may play a role in mediating regeneration through tissue engineered scaffolds. Therefore, our future studies will assess these associations to determine whether a causal relationship is present.

D. Pan: None. E. Larson: None. A. Fuch: None. S. Jo: None. X. Ee: None. K. Santosa: None. A. Snyder-Warwick: None. S. Mackinnon: None. M. Wood: None.

A Patient Specific Tissue Engineered Biomimetic Platform For High Throughput Analysis Of Breast Cancer Therapeutic Options

Yoshiko Toyoda, BA, Karel-Bart Celie, BA, Justin Buro, BA, Alexandra Lin, BA, Jonathan Xu, BA, Andrew Abadeer, MEng, Julia Jin, BS, John Morgan, PhD, Kristy A. Brown, PhD, Jason A. Spector, MD
**Weill Cornell Medical College, New York, NY, USA**

**PURPOSE:** Breast cancer (BC) research has suffered from a lack of model systems that can recapitulate the complex 3D microenvironment that exists within the tumor. This has limited the translation of many promising pre-clinical therapies into clinical application. The tumor microenvironment significantly influences BC cell phenotype and similarly the microenvironment is modulated by the tumor cells themselves. In the breast, adipose stromal cells (ASCs) and mature adipocytes are thought to affect BC cells via paracrine signaling as well as through direct metabolic effects; immune cells and fibroblasts may promote desmoplasia; stiffer extracellular matrices are thought to enhance cancer aggression. Herein, we present an organotypic model of BC, with the full complement of breast adipose, vascular, and epithelial cells, functional epithelial ducts, and vascular channels within a biocompatible and tunable collagen construct. We tune the mechanical and cellular properties of this system to study their influence on tumor progression and vascular remodeling.

**METHODS:** Discarded tissue was acquired from patients undergoing breast reductions and abdominoplasties and digested to retrieve adipocytes and stromal cells. Adipocytes and stromal cells were encapsulated into type I collagen and injected into a polydimethylsiloxane base which was fabricated using positive molds constructed by additive manufacturing (3D printing). Three 1mm diameter lumens were formed and seeded with fluorescently labeled vascular cells, MDAMB 231 (BC cell line), and epithelial cells, to mimic the vasculature, ductal carcinoma, and healthy breast ducts, respectively. The adipocytes were also fluorescently labeled with the lipid dye boron-dipyrromethene (BODIPY 493/503 and BC cell invasion into the collagen-stromal bulk was analyzed with confocal microscopy.

**RESULTS:** Over 40 adipose tissue specimens were collected and included in a tissue biobank. The collagen hydrogel bulk demonstrated a dense culture of mature adipocytes containing fluorescent lipid droplets neighbored by stromal cells, mimicking the native architecture of breast adipose tissue. The fluorescent tags allowed for observation of each of the 3 luminal structures. Metastatic potential was evaluated by assessing BC cell invasion from the lumen into stromal cell-containing hydrogels. Within the ductal carcinoma lumen, BC cells formed a mass of cells and fluorescently tagged cells were observed leaving the original lumen into the breast stromal bulk collagen. In the vascular lumen, green fluorescent endothelial cells formed a single layer along the lumen. Vascular remodeling was analyzed by endothelial cell lining disruption.

**CONCLUSION:** We have established a novel 3D biomimetic tissue engineered platform to study the BC microenvironment, utilizing primary cells derived from specific patients’ tissues. Our high fidelity biomimetic platform provides the means for more accurate interrogation of the complex interplay within the BC tumor microenvironment and will allow for high-throughput diagnostic and therapeutic analyses in a patient-specific manner.

Y. Toyoda: None. K. Celie: None. J. Buro: None. A. Lin: None. J. Xu: None. A. Abadeer: None. J. Jin: None. J. Morgan: None. K.A. Brown: None. J.A. Spector: None.