In Vivo Response to Tissue Engineered Injectable Devices for Breast Reconstruction

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Statement of Purpose: In 2005, more than 57,000 breast reconstruction procedures were performed for breast cancer patients who underwent surgical treatment [1]. Our research is directed towards the development of a minimally-invasive device for breast tissue reconstruction that uses biodegradable, injectable microcarrier beads and a hydrogel delivery medium to stimulate regeneration of host adipose cells and fill soft-tissue voids in the breast. Histological analysis of bovine mammary tissue has shown more structural similarities to that of humans than the mammary tissue of mice and rats. Thus, an *in vivo* biocompatibility study using dairy cattle was conducted to evaluate the efficacy of these injectable devices.

Methods: CultiSpher-S Gelatin microcarriers, purchased in dehydrated form (Percell Biolytica, Åstorp, Sweden), were hydrated under sterile conditions in calcium-free and magnesium-free phosphate buffered saline. Poly-L-lactide (PL) beads were fabricated using an oil-in-water (o/w) emulsification process [2].

Subcutaneous adipose tissue samples were excised from the left tailhead region of 3 Holstein heifers. Preadipocyte cells were isolated from the tissue using a systematically modified enzymatic digestion procedure [3]. Cells were cultured on the beads at an initial seeding density of $5x10^6$ cells/ml beads for 7 days. Cell viability, cellular activity and characteristic gene expression were evaluated using specific assays (results not shown).

Composite devices were formed using six combinations of implants, including:

- Alginate carrier + 2 bead types + cells
- Alginate carrier + 2 bead types – cells
- Alginate carrier
- Saline

A 3-row x 4-column rectangular grid pattern was shaved onto the left and right sides of each of the 3 heifers. Implants were injected intradermally at the lower left corner of each square (0.5ml beads/injection). Implanted samples were biopsied following 11 and 27 days of implantation. Tissue samples were fixed in 10% neutral buffered formalin. Paraffin-embedded samples were cut into 5µm thick sections and stained using routine Hematoxylin and Eosin (H&E) and Masson's Trichrome (MT). An adapted semi-quantitative rating system was used to assess capsule formation at the interface of the implant material, inflammatory response based on the abundance or lack of inflammatory cells within the samples, tissue ingrowth within the implant material, and the development of new adipose tissue [4].

Results/Discussion: Figures A and B represent H&E and MT, respectively, stained sections of the normal bovine control skin tissue samples, which received saline injections. Following 27 days of implantation, gelatin (Figure C) and PL (not shown here) beads were still apparent within the tissue samples as evidenced by the clearly rounded structures within the sample. At higher magnification (Figure D), degradation of the beads is observed by the scalloped edges. Each type of implant, regardless of bead type, or cellular content, was generally surrounded by a thin capsule consisting of dense fibrous tissue with putative fibroblasts and inflammatory cells (Figures E and F). Additionally, no new adipose tissue formation was observed in any of the samples.

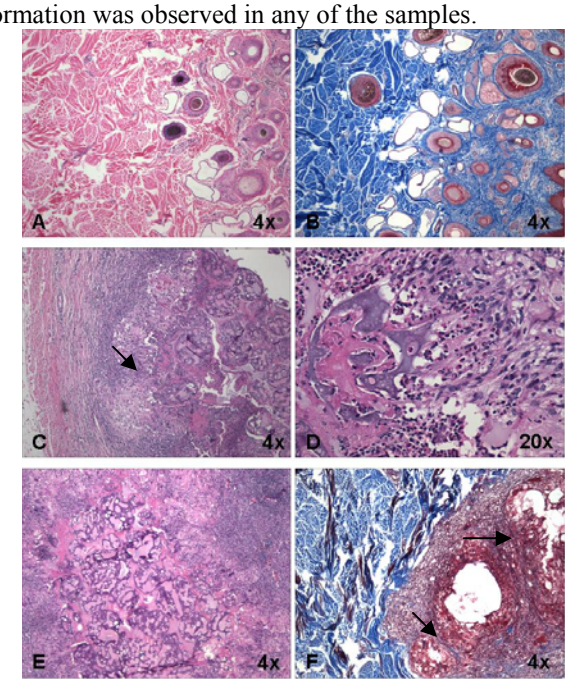

Figure 1: Representative Histological Sections
(Magnification represents objective magnification.)

Conclusions: Based on the qualitative and quantitative histological analyses, the overall goal of inducing adipose tissue formation was not accomplished with this study. However, it was shown that cellular injectable tissue engineered devices that incorporated gelatin and PL beads were biocompatible when used in bovine models and that the injectable devices allowed cellular infiltration post implantation. Further assessment of cell isolation and culturing techniques are required to address the issue of long term tissue bulking and maintenance.

References:

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Acknowledgements: Many thanks to Linda Jenkins, HT for technical assistance. This work was supported by the National Science Foundation, PECASE (BES 0093805).