Investigation of Mesenchymal Stem Cell Phenotype and Function in an Allograft Cellular Bone Matrix

Brown LS, Darmoc MM, Clineff TD
Stryker Corp., Malvern, PA

Statement of Purpose: Mesenchymal stem cells (MSCs) have generated a substantial amount of attention from researchers for their potential for use as cell-based therapies. MSCs are capable of differentiating into a number of different cell types; and cells which have retained their MSC identity are commonly identified by the CD 166 and CD 105 surface markers[1]. Osteocel® Plus (NuVasive, Inc., San Diego, CA), Trinity™ Matrix (Blackstone Medical, Inc., Springfield, MA), and Trinity Evolution™ (Orthofix, Curaçao, Netherlands Antilles) are commercially available products which contain MSCs in a bone matrix, providing a scaffold and progenitor cells to heal bony defects. These products are allograft cellular bone matrices which are processed so that immunogenic cells are depleted and the viability of osteogenic cells is retained. The aim of this study was to investigate the number and type of cells contained within the Osteocel and Trinity bone matrices. A common *in vivo* assay to evaluate the ability of bone graft substitutes to form new bone in an ectopic site was also conducted.

Methods: Samples of Trinity and Osteocel were stored at -80°C and thawed according to product instructions.

Collagenase digestion, cell culture, and flow cytometry analysis were carried out by Progenitor Cell Therapy, LLC (Hackensack, NJ). After thawing, 1 cc of Trinity Matrix was incubated in collagenase solution at 37°C under continuous agitation. After 7 hours, the digestion was placed in 2 flasks. After 2 days of culture, the media was removed, centrifuged, and resuspended in α-MEM supplemented with 10% FBS and 2 mM glutamine. Colonies were counted at first appearance after 17 days. Cells were detached and analyzed for expression of either CD 166 or CD 105 surface markers using flow cytometry. Isotype controls and CD 166 and CD 105 antibodies were used to identify cells positively expressing these antigens.

Histology embedding, sectioning, and staining were performed by Premier Laboratory, LLC (Longmont, CO). Trinity Matrix and Osteocel Plus sections were stained by H&E and CD166. Positively stained cells were quantified by the use of color segmentation software.

Surgery and in-life procedures for *in vivo* osteoinductivity evaluations were conducted by Ibex Preclinical Research, Inc. (Logan, UT). Histology preparation and histopathological assessment was conducted by Histion, LLC (Everett, WA). A 0.2 cc volume of Trinity Evolution or 40 mg human demineralized bone matrix (DBM) control was subcutaneously implanted into adult athymic nude rats.

Results: The number of colony forming units-fibroblastic (CFU-F) after 17 days of culture was 38 colonies for Flask 1 and 28 colonies for Flask 2. Each CFU-F forms from a single cell, therefore less than 100 total cells could be collected from 1 cc of Trinity Matrix. As shown by flow cytometry, greater than 99% of the viable cells expressed both CD 166 and CD 105 surface markers.

By H&E stain, typical bone morphology was observed with the presence of nucleated cells encapsulated within bone. Table 1 displays the total and CD 166+ cell counts. Less than 5% of total cells stained positively for CD 166.

Table 1. *In vitro* cell quantification by color segmentation.

	H&E stained	CD 166 stained	Percentage CD 166+
Trinity Matrix	396,000	5,430	1.37%
Osteocel Plus	248,000	12,200	4.92%

Histological scoring showed no statistical differences between the Trinity Evolution and DBM control groups for new bone elements including calcified new bone, chondrocytes, osteoblasts, bone marrow, osteoids, and cartilage at 14 and 28 days. As shown in Table 2, Trinity Evolution was slightly osteoinductive in the *in vivo* assay.

Table 2. Average histological score on a 0-5 scale for the *in vivo* osteoinductivity assay.

	Time Point	Osteoblasts	New Bone	New Bone Elements
DBM powder	14 d	1.0 ± 0.13	0.75 ± 0.16	1.0 ± 0.13
	28 d	1.0 ± 0.13	0.75 ± 0.13	1.0 ± 0.18
Trinity Evolution	14 d	0.25 ± 0.13	0.5 ± 0.13	0.5 ± 0.15
	28 d	0.75 ± 0.13	0.5 ± 0.13	1.0 ± 0.13

Conclusions: Mesenchymal stem cells have the potential to differentiate into a number of cell types; therefore, the ability to deliver MSCs to local defect sites for healing is of clinical interest. This study investigated several cellular allograft products and found that cells contained within these matrices may exhibit phenotypes beyond only MSCs. Immunohistochemical staining demonstrated that only approximately 1-5% of cells within the allograft bone positively express CD 166. While flow cytometry demonstrated that a high percentage of cells positively expressed CD 166 and CD 105, very few could be released using enzymatic digestion. These results combined with the lack of difference in the *in vivo* bone formation observed between the cellular allograft matrix and the demineralized bone matrix suggest that the encapsulated cells may not contribute to new bone formation. A possible reason for this is that following implantation, the viability of the cells may decrease. It is known that bone cells are unable to survive if located more than 100 μm from a vessel[2]. Studies have also shown that osteogenic cells had a significant decrease in viability from 80% at t = 0 to 20% five days following allogenic implantation in a scaffold carrier[3]. As a result, it is possible that cells other than those very close to the surface of the graft die and render the cellular allograft very similar to DBM. Additional studies are necessary to fully understand the impact of cell entrapment within the allograft matrix on the efficacy of new bone formation.

References: [1]Bobis S. Folia Histochem Cytobiol 2006; 44: 215-230. [2]Haiying Y. Biomat 2009; 30: 508-517. [3]Emans PJ. Tissue Eng 2006; 12; 1699-1709.