Osteoprogenitor Cells Preserved in the Osteocel® Tissue Product Result in Enhanced Bone Formation

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Introduction: In bone grafting, autograft has been the gold standard for decades because it combines the bone growth abilities inherent in osteoinductive and osteogenic cells with the osteoconductive matrix which the body naturally utilizes for bone growth. The problems associated with autograft such as donor site morbidity, unreliable quality and limited quantity have prompted a search for a viable substitute. Until recently, no off the shelf bone grafting product has provided all three of these vital components due to the inability to easily retain the living cells required for osteogenesis. Osteocel®, an HTC/P allograft product, has been processed in such a way as to selectively deplete immunogenic cells while maintaining a rich source of live multipotential stem cells deliverable via a cancellous bone matrix. The cells in Osteocel® have been classified via Flourescent-activated cell sorting (FACS) analysis by confirming the presence of two cell markers, CD106 and CD155, expressed at high levels by adult stem cells and the absence of CD45, a marker present on all nucleated hematopoietic cells, confirming their stem cell identity and non-hematopoietic lineage.1,2,3 In vitro analysis of these cells demonstrated multipotentiality similar to that demonstrated by Pittenger, et. al, for mesenchymal stem cells.4,5 The animal study described here uses a subcutaneous implant analysis of two versions of Osteocel®, one containing living cells and one in which all cellular components have been killed, to evaluate if the osteoprogenitor cells in Osteocel® play a role the mechanism of bone repair Osteocel® provides.

Methods: Osteocel® was manufactured per standard manufacturing methods from a single cadaveric tissue donor approved for research use. Half of the product was subjected to 5 freeze-thaw cycles (from -80°C to 70°C) over the span of 4 days resulting in test product devoid of living cells and consisting primarily of cancellous matrix. The remaining product was preserved frozen (-80°C) and quality tested to meet specifications for osteoprogenitor cell quantity of ≥50,000 cells/cc and ≥70% cell viability, as required of commercially available Osteocel®. A 1 cm incision was made over the scapulae, bilaterally, of ten athymic nude rats weighing at least 100g each. Pockets were made in the subcutaneous tissue by blunt and sharp dissection into which approximately 300+/-25mg (~0.5cc) of material from each test group was implanted, contralaterally. Animals were sacrificed at 28 days and explants evaluated histopathologically via microscopy after hematoxylin and eosin staining.

Results: Histological evidence of new bone formation was present in the majority of sites implanted with live cell product. Histopathological analysis of explants revealed the presence of osteoblasts in 8 out of 10, new bone in 8 out of 10, and bone marrow in 7 out of 10. In contrast, the test material in which the cellular components were lysed prior to implantation tested positive for bone growth in only one of ten explants. The number of live cell sites exhibiting histological signs of bone formation were statistically significant (P<0.005 by Fischer's Exact Test). These results are summarized in Table 1 (see poster).

Discussion: From this study we conclude that the osteoprogenitor cells present in Osteocel® play a key role in new bone formation due to the significant increase observed in de novo bone formation when product containing live cells is implanted compared to acellular product (Table 1). It is our hypothesis that the role played by the osteoprogenitor cells in Osteocel® is similar to the role the cells play during natural bone repair and these results demonstrate the benefit of live osteoprogenitor cells integrated with their natural matrix as found in Osteocel® compared to acellular bone graft materials. Future studies aim to determine if the osteoprogenitor cells present in Osteocel®, the osteoblasts arising from these cells, or a combination of the two are involved in the mechanism of bone repair provided by Osteocel®.

References:
Pittenger MD & Martin BJ. Experimental Hematology. 28 (2000) 875-84.