Comparison of Osteogenesis of an Early Lineage Stem Cell to Bone Marrow Aspirate and Pure Mesenchymal Stem Cells within a Demineralized Bone Scaffold in an Athymic Rat Model

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Purpose: Previous in-vitro testing of early lineage adult (ELA) stem cells demonstrated superior bone marker expression compared to pure mesenchymal stem cells (MSCs), indicative of cell maturation and mineralization. The purpose of the current study was to evaluate the osteogenic capability of an ELA stem cell compared to pure MSCs in an athymic rat muscle pouch model.

Methods: Nine athymic mature Sprague Dawley rats were randomized to three treatment groups (n=3/group). Each treatment group utilized 0.3cc of the same demineralized bone (DB) scaffold as a carrier matrix. Cellular component of the study groups were one of the following: human bone marrow aspirate (BMA) (1.5 million cells / implantation), human MSCs identified by CD-105 surface marker (1.5 million cells/implantation), and human ELA stem cells (1.5 million cells/implantation) (PureGen™, Alphatec Spine, Carlsbad, CA). Implantations were done bilaterally in the biceps femoris muscle (6 limbs/group).

Radiographs were taken at 2, 4 and 6 weeks and reviewed by a blinded third party radiography specialist for radiodensity evaluation (Syncare, San Francisco, CA). Density was ranked 0-3 with 0 representing no calcification present, 1 representing hypodense bone relative to the proximal femur in each limb, 2 representing isodense bone and 3 representing hyperdense bone. Radiographic scores at the six week time point were analyzed with a one-way ANOVA (p< 0.05) comparing the ELA group to both BMA and MSC groups. Tissue samples were allocated to: one of five sections for H&E stain, 3 sections for immunohistochemistry (IHC) and one section for human class I MHC for bone producing markers and human class I HMC markers.

Results: None of the implantation sites demonstrated bone formation at the 2 week time point (all scores = 0). All animals showed bone growth at 4 weeks (BMA = 0.67±0.52, MSC = 0.42±0.49, ELA =1±0). All animals showed bone growth at six weeks (BMA = 1.08±0.2, MSC = 0.67±0.68, ELA =1.58±0.38) (Figure 1). All of the DB scaffolds with bone marrow aspirate (6/6) and ELA cells (6/6) were visible at 6 weeks, but only half (3/6) of the DB scaffolds with MSC's were visible at six weeks. The six week data showed statistically greater radiographic bone formation for the ELA group as compared to either BMA or MSC groups (p< 0.017).

In examination of the histology, osteocalcin, osteopontin, and alkaline phosphatase indicated that osteogenesis was occurring in all animals with the DB scaffold. B2M staining indicated that viable ELA stem cells were present in all implants.

Conclusion: Improved bone formation was found when using an early lineage adult (ELA) stem cell as compared to either BMA or MSC with a demineralized bone scaffold in an athymic rat muscle pouch model. Histologic analyses found this to be consistent with bone formation. Based on the encouraging results seen with ELA stem cells, subsequent studies in in-vivo spinal fusion models are being pursued.
Figure 1: Radiographic data at two weeks (left column) and six weeks (right column) for the BMA group (top row), MSC group (middle row) and ELA group (bottom row).

[Graph 1]