Protein and Gene Profiles of Osteoprogenitor Cells on Allograft Bone

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Statement of Purpose: Autografts possess the three components necessary for bone production: viable osteoblasts, scaffold and growth factors, yet autograft is limited in supply and harvesting is often associated with residual morbidity. Allograft bone is an alternative, but incorporation proceeds more slowly. Osteoinductive growth factors may improve allograft integration, but delivering the necessary growth factors at biologically critical times and doses during osteoprogenitor cell proliferation and differentiation is challenging. We hypothesized that the addition of exogenous osteoprogenitor cells would improve bone formation and that the cells would express a specific, ordered sequence of growth factors when cultured on corticocancellous allograft bone. We tested this hypothesis over a 4-week period using a novel murine model.

Methods: 1.5 mm thick cortico-cancellous allograft bone discs were made from the distal femurs of euthanized 10-week old C57 male mice. Osteoprogenitor cells were harvested from the long bones of other mice, expanded, and plated on the allograft discs. $1x10^6$ cells were seeded on each allograft for 72 hours, at which time osteogenic media was added. The seeded allografts were maintained under osteogenic culture conditions for 4 weeks. The culture media was assayed at 0, 2 and 4 days, and every 4 days subsequent for BMP-2, IGF-1, Osteocalcin, TGF-β, and VEGF-a protein using ELISA.. RT-PCR was used to assay 18S, BMP-2, BMP-7, Ctnn-b1, FGF-b, IGF-1, PDGF-a, PDGF-b, Runx-2, TGF-β, and VEGF-a mRNA levels. SPSS 14.0 was utilized to perform a one-way ANOVA with a Tukey test for each assay ($p < 0.05$). New bone formation of cell-seeded grafts was measured with μCT at 0 and 4 weeks.

Results: The concentration of osteocalcin [Fig. 1] peaked at 4 days at 13 pg/ml, a significant increase over all other time points ($p < 0.05$). IGF-I protein decreased significantly to 575 pg/ml ($p < 0.05$) at 2 days compared to 8-day values [Fig. 2]. VEGF protein increased significantly to 164 pg/ml ($p < 0.05$) at 8 days compared to 0, 24, and 28-day time points [Fig. 3]. TGF-β protein increased significantly to 69 pg/ml ($p <0.05$) at 2 days compared to time points from 8 days to 28 days [Fig. 4]. mRNA patterns did not precede protein expression [Fig. 2-4]. μCT showed a 7.7% increase in bone volume.

Conclusions: Our experiments demonstrate specific time dependent expression patterns for key bone-related proteins when osteoprogenitor cells are cultured on cortico-cancellous allograft bone. Although these expression patterns generally parallel those found when osteoprogenitor cells are cultured alone, differences in the profiles suggest that the allograft bone scaffold is modulating osteoprogenitor cellular activity. Furthermore, the finding that the protein often increases earlier in the time course than mRNA suggests that pre-formed protein is released, or that there is a decoupling between the mRNA and protein expression in these circumstances. Allograft incorporation may potentially be enhanced by adding osteoprogenitor cells, choosing scaffolds with specific physico-chemical properties, or adding specific exogenous growth factors at critical times during osteoprogenitor cell proliferation and differentiation.

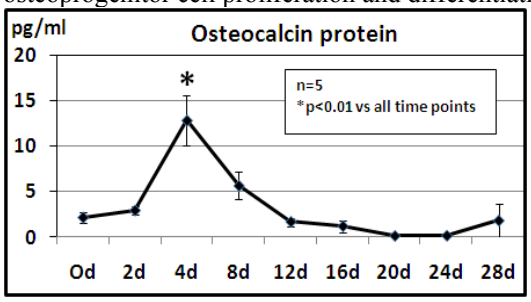

Fig. 1. Osteocalcin protein releasing profile

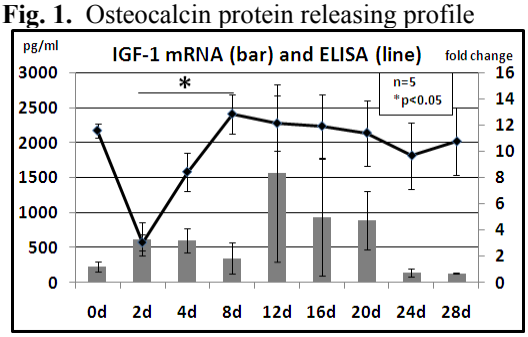

Fig. 2. IGF-I protein and mRNA releasing profiles

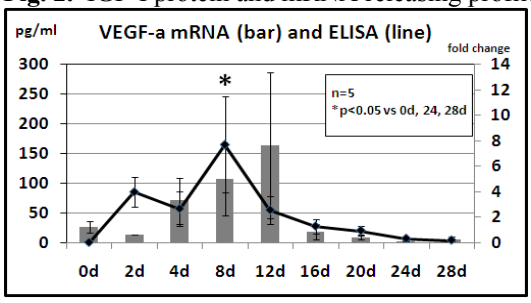

Fig. 3. VEGF protein and mRNA releasing profiles

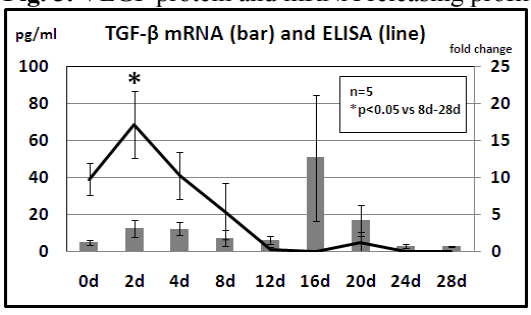

Fig. 4. TGF-β protein and mRNA releasing profiles

Acknowledgements: This research was supported by the Musculoskeletal Transplant Foundation, the Stanford University Medical Scholars Program and the Ellenburg Chair in Surgery.