Micro-CT Evaluation of Human Fat Grafts Enriched with Adipose-Derived Stromal Cells in Nude Mice

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Abstract

Background: Despite accumulating evidence demonstrating the utility of adipose-derived stromal cells (ASCs) in regenerative applications, little is known about the existing heterogeneity.(1) This heterogeneity undoubtedly contributes to variability in results, including fat graft resorption rates. The present study evaluated the ability for functionally distinct ASC subpopulations to promote restoration of soft tissue in a murine model using a novel imaging technique.

Methods: To evaluate the ability for FACS to isolate subsets of ASCs with increased adipogenic capacity, a sort was performed based on BMPR-IA (**Figure1**).(2) Lipoaspirate specimens were combined with BMPR-IA^{+} ASCs (n=4). Fat without ASCs were similarly injected subcutaneously into the scalp of ten adult nude mice. Micro-CT was performed at day 3 and then every two weeks. Three-dimensional volume was calculated through cubic-spline interpolation (**Figure2**). At week 12, fat was explanted, and radiographically measured volumes were confirmed. Explanted fat was also sectioned and stained with hematoxylin and eosin (H&E) to confirm viability of fat.

Results: Micro-CT scanning demonstrated progressive volume loss over the time course, with significantly less volume in animals receiving fat alone. Volumetric analysis at the 8-and 12-week time points stabilized, showing an average of 62.2% and 60.9% persistence, respectively. Histological analysis of sectioned specimens at week 12 confirmed the presence of viable injected fat.

Conclusions: Studies have begun to evaluate the ability for ASCs to augment volume retention of injected fat. To this end, we have incorporated an imaging modality to interrogate *in vivo* volume of injected fat following placement into the scalp of immunocompromised mice. In this study, we have demonstrated the ability to employ micro-CT for 3D reconstruction and volumetric analysis of human fat grafts in a mouse model. Importantly, this model provides a platform for subsequent study of fat manipulation and viability as well as soft tissue engineering with adipose-derived stromal cells.

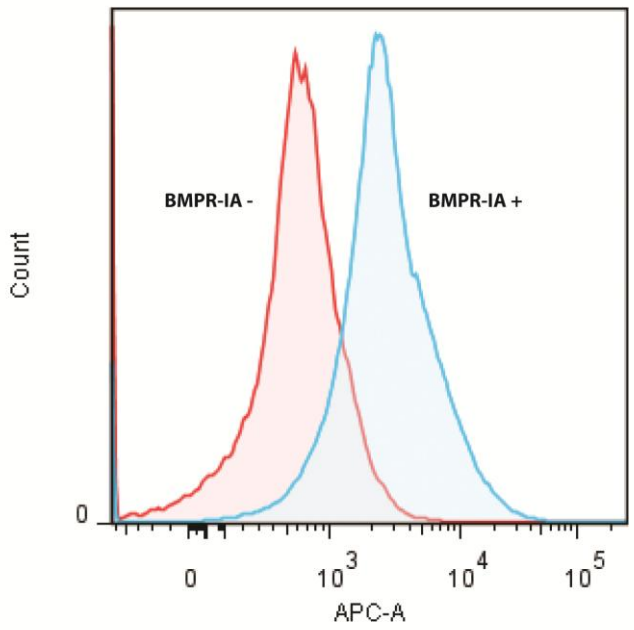

Figure 1. BMPR-IA sort of ASCs. FACS plot of freshly harvested ASCS based on BMPR-IA.

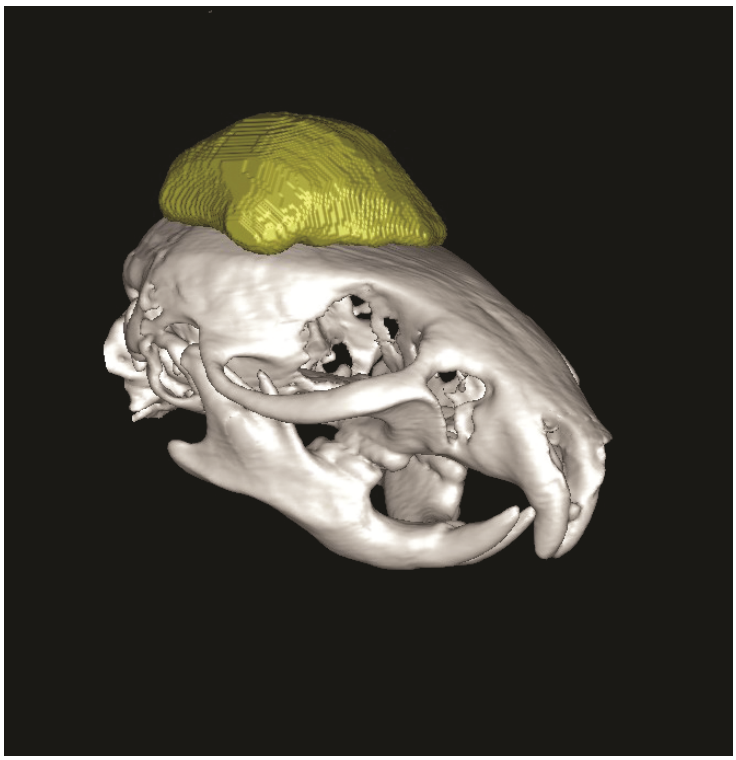

Figure 2. *In vivo* fat grafting. Three-dimensional volume for injected fat was calculated through cubic spline interpolation of user-defined regions of interest.

References

1. Yoshimura, K., Shigeura, T., Matsumoto, D., et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol* 208: 64-76, 2006.

2. Chen, D., Ji, X., Harris, M. A., et al. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J Cell Biol* 142: 295-305, 1998.

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