Adipose-Derived Purified Stem Cells Heal Critical-Size Mouse Calvarial Defects

INTRODUCTION:
Autologous bone grafts are commonly used to treat skeletal defects. However, the use of autograft bone is limited by donor site morbidities, lack of an abundant supply, and complications from extended operating time. Additionally, bone marrow stem cells (BMSCs) show reduced osteogenic potential as patients age, thus limiting the usefulness of autograft bone in the elderly. Currently, there is a pressing clinical need for a readily available and plentiful source of osteocompetent cells that circumvents these problems. Our research group and others are interested in evaluating the effectiveness of the adipose-tissue derived stem cell populations (and specifically their purified constituents) as a potential bone graft substitute.

Unlike BMSCs, the stromal vascular fraction (SVF) of adipose tissue is more readily available and accessible with standard liposuction procedures. However, the heterogeneity of this population, which includes non-viable cells, non-stem cells and osteogenic differentiation-inhibiting endothelial cells, reduces its effectiveness as a bone graft substitute. Therefore, a more purified population of adipose-derived stem cells would be highly advantageous. Our research group has designed and implemented a FACS protocol which allows for isolation of a mesenchymal stem cell (MSC) like population termed perivascular stem cells (PSCs). PSCs, isolated from adipose tissue and other organs, are found as pericytes around capillaries and microvessels and in the tunica adventitia of larger veins and arteries. Human PSCs (hPSCs) have the potential to differentiate into fat, muscle, cartilage, and bone, but have the additional benefit of secreting various growth factors in high levels. Furthermore, hPSCs can be isolated from fat tissue in significant numbers without the need for in vitro expansion – thus reducing the time from isolation to application. This also reduces the risk of infection and genetic instability introduced by cell culture. We hypothesized that purified hPSCs would more rapidly heal bone in comparison to their unpurified human stromal vascular fraction (hSVF) equivalent.

METHODS:
Purification of PSCs and Scaffold Preparation. Liposapirate was digested per protocol (RPMI, 3.5% BSA, 10μg/mL DNAase, 1mg/mL collagenase II) and trypan blue staining was used to count the number of viable, resultant hSVF cells. A portion of the hSVF was then used to isolate hPSCs using FACS (fluorescence activated cell sorting). Cells were stained with anti-CD146, FITC (1:50), anti-CD34 APC (1:50), and anti-CD45 APC (1:50). Microvessel pericytes and adventitial cells, which comprise the hPSC population, were isolated from the hSVF of adipose tissue by fluorescence activated cell sorting (FACS). Then, 2.5*10^5 cells from a single-patient derived hPSC population, or single-patient derived, age- and sex-matched hSVF population was loaded onto a custom made, disc-shaped, hydroxyapatite-coated (HA) PLGA scaffold designed to fit exactly into the calvarial defect site (3mm diameter). N=8 implants per treatment group. Surgical Procedure. All surgical procedures were consistent with the regulations put forth by the UCLA Chancellor’s Animal Research Committee. A dental drill was used to create a unilateral 3mm critical defect in the parietal bone of 24 SCID mice, split evenly between treatment groups. Importantly, the dura mater underlying the calvarial defect was left intact—found to be necessary for proper defect healing. Method of Analysis At the zero, two, four, six and eight week post-operative, live microCT was performed in order to determine the level of calvarial defect healing. Each scan was reconstructed in AMIDE and analyzed in CS5 Adobe Photoshop in order to determine healing overtime. A one-tailed t-test was performed to determine significance between groups. At eight weeks, the mice were sacrificed and their calvariae were harvested for further histological analysis.

RESULTS:
Live microCT scans offered both visual and quantitative means of tracking defect healing overtime. Visually, as demonstrated in Figure 1A, the calvarial defect size for the hSVF-treated group at week six appeared larger in size than in the hPSC-treated groups at the corresponding time-point. This was confirmed by quantitative analysis, showing a significant increase in healing of hPSC-treated groups in comparison to the hSVF-treated groups at all time-points, as presented in Figure 1B. Notably, control defects (either an empty HA-PLGA scaffold or no scaffold) were performed which showed little to no healing in groups without an HA-PLGA scaffold and minimal healing (<41%) in HA-PLGA scaffold implantation without cells. Histological analysis confirmed an increase in bone formation among PSC treated defects (data not shown).

DISCUSSION:
Human PSCs significantly increase orthotopic healing of mouse critical-size calvarial defects. hPSCs are FACS sorted which ensures purity of stem cell content and reliability of the cell population constituents in comparison to the more heterogenous hSVF. These studies extend our research group’s previous findings showing that hPSCs are capable of osteogenic differentiation both in vitro and in an ectopic in vivo environment. Future studies will examine increasing hPSC osteogenic efficiency by combining them with osteoinductive cytokines to heal skeletal defects in a faster timeframe.

SIGNIFICANCE:
The ability of purified hPSCs to treat skeletal defects demonstrates its potential as an autologous bone graft substitute. Purified hPSCs are a more effective adipose derived cell source than traditionally derived hSVF, suggesting that hPSCs are a more appropriate choice for future tissue engineering applications.

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