Efficacy of Mesenchymal Stem Cell Sheets to Enhance Structural Allograft Healing of Critical Bone Defects

Teng Long, Ph.D., Matthew Hilton, Ph.D., Hani A. Awad, PhD, Edward M. Schwarz, PhD, Yufeng Dong. 1

1Department of Orthopaedics, Shanghai 9th hospital, Shanghai, China, 2University of Rochester, Rochester, NY, USA.

Disclosures:

Introduction: Limb salvage procedures following massive segmental bone loss due to traumatic extremism injuries is a major challenge to the field of orthopaedics. Critical bone defect surgeries like these require large devitalized segmental allograft transplantation to replace missing host bone segments, however significant problems often arise due to the impaired ability of the devitalized allograft to incorporate into the host bone. One of the most exciting strategies to promote and enhance allograft incorporation and critical bone defect healing involves the use of the patient’s own bone marrow derived mesenchymal stem/progenitor cells (MSCs). This approach entails isolating MSCs from the patient, expanding them in culture, and seeding the cells on a devitalized allograft prior to transplantation. Following transplantation the MSCs are exposed to endogenous factors within the injured and healing region that promote their osteogenic differentiation, resulting in increased bone formation and enhanced osteointegration of the allograft. While this approach has demonstrated some success, problems remain in most cases including: inefficient MSC attachment to allograft via culturing techniques, uneven MSC distribution across the graft, as well as weak adhesion of MSCs to the graft resulting in cell detachment in vivo. Since cell sheet technology utilizing temperature-responsive culture dishes has been applied to tissue engineering for several years to regenerate multiple damaged tissues except bone [1], we set out to demonstrate that MSC sheets, generated in temperature-responsive culture plates, will significantly enhance critical segmented bone defect healing when wrapped onto devitalized allografts of a femoral allograft mouse model [2].

Methods: Mouse MSCs were isolated from 6-week-old C57BL/6J mice using a mouse bone marrow stem cell isolation kit (Stem Cell Technologies, Inc.) Flow cytometry analysis was performed using stem cell marker CD105-FITC, CD29-PE and Sca1-APC antibodies after cell isolation and expansion of total MSCs for 2 passages. Maintenance of “stemness” for MSC in cell sheet was determined by flow cytometric analysis and real-time RT-PCR before and after generating cell sheets to determine the effect of short-term sheet culture on the percentage of MSCs retaining the full complement of their initial cell surface markers, as well as the expression of stem cell regulatory factors: Oct4, Sox2, Nanog. Following MSC expansion procedures, an adequate number of the total MSCs were passaged to thermo-responsive culture plates to form cell sheets in 24 hours. Four mm long devitalized allografts wrapped with cell sheets were transplanted into a mouse model of a femoral critical segmented bone defect. Allograft alone and allograft with direct seeding of MSCs were used as controls. On days 14, 28, and 42 following transplantation we harvested femurs from sets of mice (n=24) for use in X-ray, micro-CT, and histological analyses. We also assessed MSC incorporation, bone regeneration, and the allograft osteointegration process. Biomechanical torsion testing was performed at 6 weeks to assess strength and integrity of the healing bones from each experimental and control group.

Results: Flow cytometry data showed that at least 63% of freshly isolated total MSCs were CD105 positive, 82% were CD29 positive, and 76% were Sca1 positive, indicating that our MSC preparation contains enriched cells expressing MSC surface markers, and therefore could be used for in vivo bone tissue regeneration. Real-time qPCR for Sox2, Oct4, Nanog, and CyclinD1 and flow cytometry for CD105 in cells before (80% confluent) and after cell sheet formation (24 hours) showed no change in the gene expression and only mild changes in the number of CD105 positive MSCs (Fig.1), suggesting that short-term (24 hours) MSC culturing in cell sheets does not significantly change the MSC phenotype. To test their in vivo efficacy, MSC sheets were wrapped around allografts and transplanted into 4-mm femoral defects. These grafted femurs were compared to MSCs directly seeded onto allografts and allografts without cells. Evaluations consisted of: 1) autoradiography, microCT, histology, and biomechanical testing at 4- and 6-weeks. Our findings demonstrate that MSC sheets induce prolonged cartilage formation at the graft-host junction at 4-weeks, and enhanced bone callus formation, as well as, remarkable graft-host integration by 6-weeks. Moreover, a large periosteal callus was only observed on the surface of allografts with MSC-sheets (Fig. 2), which partially mimics autograft healing. Finally, biomechanical tests showed a significant increase in the biomechanical properties of the MSC-sheets group as compared to the other groups. These results demonstrate that MSC sheets exhibit enhanced osteogenicity as compared to traditionally seeding MSCs during allograft bone defect repair.

Discussion: Our data illustrate the remarkable ability of MSC sheets to function as a pseudo-periosteum and enhance bone callus formation during allograft healing and osseosintegration in the mouse model of massive femoral defect reconstruction. These data further suggest that MSC sheets may be a potential clinical treatment to promote osteoblastic bone formation and functional healing in settings of critical sized defects, and possibly non-unions. These results should motivate further exploration into the identification of appropriate MSC populations for generating cell sheets in order functional parameters of bone healing.
similar to that of autograft healing.

**Significance:** These data suggest that our MSC sheet technology may provide a means for the functional revitalization of devitalized allografts in the field of bone tissue repair/regeneration.

**Acknowledgments:** This work was supported by research grant from the Airlift Research Foundation (108648) and the NIH/NIAMS R21 Grant AR063803 to YD and the NIH/NIAMS P30 Grant AR061307 to ES, HA, and MJH

**References:**

---

**Figure 1.** Short-term cell sheet culture does not significantly change the MSC phenotype and this MSC sheet could be easily detached for transplantation: (A and B) CD105 subpopulation in MSCs before and after forming cell sheets. (C) Comparison of stem cell marker gene transcript changes before and after formation of MSC sheet. (D) MSC sheet was easily detached from dish after 24 hours of culture. (E) MSC sheet was transplanted to mouse femur bone defect model combined with allograft.

**Figure 2.** MSC sheet enhances devitalized allograft osseointegration with the host bone, and induces extensive trabeculated bone callus formation on the periosteal of the allograft at 4 and 6 weeks post-surgery. Representative micro CT volumetric rendering of the grafted femurs coated with MSC (B, E, H, K) and MSC sheets (C, F, I, L) at 4 and 6 weeks (e-f). Allograft alone was used as control (A, D, G, J). Quantification of BV/TV in newly formed callus (M). (* p < 0.01 compared with allograft alone; # p<0.05 compared with allograft + MSC).