MESENCHYMAL STEM CELL AUGMENTATION UPON ALLOGRAFT INCORPORATION IN DIABETIC RODENTS
Sharonda Meade1, Vikrant Azad1, Yee-Shaun Lee2, Eric A. Breitbart1, Sloane Yeh1, Loay Al-Zube1, Treena Arinzeh2, Sheldon S. Lin1
1Department of Orthopedics, UMDNJ- New Jersey Medical School and Graduate School of Biomedical Sciences, Newark, NJ; 2Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ
breitber@umdnj.edu

Introduction: More than one million orthopaedic operations are performed in the United States each year for reconstructive surgery, trauma, or abnormal skeletal defects. Often large amounts of autologous or alternative large bulk allograft are needed in the surgical procedures to achieve the reconstructive goals. One major issue affecting both large autograft and allograft incorporation is the presence of systemic disease like diabetes mellitus (DM). Exhaustive research has documented that DM is related to complications in nearly every organ system. Despite the paucity of literature on the impact of DM on allograft incorporation and fracture healing, there are a few clinical series that demonstrate a significant deleterious effect of this systemic disease [1-3]. Several approaches are utilized to elicit the formation of bone within defects and to promote their healing. One concept is based upon an ex vivo expansion of multipotent mesenchymal stem cells (MSCs) that are loaded into a carrier system [4, 5]. The purpose of this study is to analyze the effect of MSC augmentation upon allograft incorporation in a critical-sized defect in DM rats.

Materials and Methods: General Health: Blood glucose (BG) and urine were monitored for glycosuria and hyperglycemia. Once BG became greater than 250 mg/dl, the rats were treated with a subcutaneous insulin releasing implant (LIN-PLANT®) to control the increase in BG. Surgical Procedure: A 5mm critical size defect was created in the right femur of 48 male DM BB Wistar rats. The femur was stabilized using a custom-fabricated polymer bone plate, which was held in place with 4 stainless steel screws and cerclage wires. Implant Materials: DM/Allograft(All)+DBM: In one group, the defect was filled with a bone allograft that was produced by removing 5mm of bone from a normal non-DM donor rat filled with approximately 0.05mm³ of demineralized bone matrix (DBM), DM/All+DBM/MSCs: In a second group, the defect was filled with All + DBM, loaded with 10 x 10⁶ MSCs/mL[4]. Serial Radiographs: All animals were examined using microradiography every two weeks. Histology: An equal number of animals from each group were sacrificed at 4 and 8 weeks post-surgery. After the femurs were extracted and cleaned of extraneous soft tissue, the samples were fixed, dehydrated, embedded in PMMA and processed for undecalcified histology. Histomorphometry: The area of total new bone, new defect bone, endosteal and periosteal bone using Image-ProPlus. Data Analysis: Results were analyzed using an ANOVA at each time point (Sigma Stat).

Results: The average BG and weight levels throughout the study are shown in Table 1.

<table>
<thead>
<tr>
<th>Elevation</th>
<th>Weight Gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM/Al+DBM</td>
<td>9.5±1.9 (12)</td>
</tr>
<tr>
<td>DM/All+DBM/MSCs</td>
<td>7.6±1.2 (12)</td>
</tr>
</tbody>
</table>

Table 1. Average BG and weight gain in DM/Al+DBM vs. DM/All+DBM/MSCs throughout study

Discussion: Studies using autologous and allogeneic MSCs for healing bone defects have demonstrated favorable results. The potential advantage of this strategy consists of a decreased need for massive cellular proliferation and osteoblast progenitor cell chemotaxis in the defect as well as development of appropriate signaling for early bone formation at the graft site [5], wherein, this cellular activity and signaling may be impaired in defects due to a systemic disease like DM [6]. Our results support the concept of using MSCs in combination with allograft to treat a critically-sized defect in a DM animal model. Bone formation and bony union at the host bone-allograft interface were enhanced with the use of MSCs as compared to allograft alone. To date, this is the first study to demonstrate the effect of MSCs on bone healing and allograft incorporation in a DM model.