THE EFFECT OF MESENCHYMAL STEM CELLS ON ALLOGRAFTS IN A CANINE INTERCALARY BONE DEFECT

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**Introduction** The treatment of large structural bone defects in orthopaedics after bone tumor resection or in conjunction with osteosynthesis in failed total joint arthroplasty are a clinical challenge with high complication rates. Mesenchymal stem cells are pluripotential and capable of differentiating into cells from a variety of mesenchymal tissues including: fat, muscle, bone and cartilage. We are interested in the use of mesenchymal stem cells with bone allografts to heal large structural bone defects. A process which could upregulate allograft bone resorption and enhance incorporation into the host bone could reduce the potential risk of long-term failure, especially in cases in which relatively large bone defects are bridged. It also would be particularly beneficial if at the same time the strength of the allograft/host complex were not significantly compromised. We investigated in this study the effect of canine mesenchymal stem cells applied to the intramedullary aspect of a segmental intercalary allograft in a canine femoral defect model.

**Methods** Sixteen adult hound-type canines ranging from 24 to 35 Kg (31.4 ± 4.4 (mean ± S.D.)) were used in the study. The allografts were removed from each animal, wrapped in sterile gauze, and frozen at -80°C for at least three days between surgeries. Prior to insertion into the defect, the 4 cm bone segments were debrided of all soft tissues (periosteum removed and the medullary canal completely curetted of marrow) and subsequently rinsed in normal saline. Prior to insertion and fixation in the intramedullary canal an implant consisting of mesenchymal stem cells in the treatment group and nothing in the negative control was inserted into the intramedullary aspect of the allograft. The allografts were fixed as previously described. The protocol was approved by the Institutional Animal Care and Use Committee. Load bearing was measured before surgery and at three, six, and nine weeks postoperatively. During each measurement, a minimum of six successful runs was obtained for each hindlimb. Standard radiographs were taken immediately postoperatively and at two, four, six, eight, ten, and twelve weeks postoperatively to monitor periosteal callus area. Periosteal callus area was measured directly on anteroposterior and mediolateral radiographs using an image analyzer software package (Biqoquant System IV; R & M Biometrics, Inc., Nashville, TN) and a sonic digitizer (Summa Sketch II Plus; Summagraphics, Seymour, CT). Animals were euthanized and femora harvested at postoperative week twelve. The proximal segment of the host bone-allograft construct was used for mechanical testing (Fig. 2). The distal segments, as well as the proximal segments after mechanical testing, were prepared for undecalcified histological analysis. Mineral apposition rate in the cortical bone area was measured using a double labeling technique. Labeled osteon density, the density of fluorochrome-labeled osteons per unit area in a given section, was determined using digitized images of contact microradiographs (Fig. 3). Mechanical testing and histological data were collected in a blinded fashion, with the sample code unknown to the evaluator. Paired data from the control and treatment groups were compared using a paired Student's t-test. Time-reported data, such as gait and callus area measurements, were analyzed with analysis of variance and Tukey's post-hoc test. A difference was considered significant when p values were less than 0.05.

**Results**

#### Table: Maximum torque

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<thead>
<tr>
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<th>Maximum torque (N)</th>
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<tbody>
<tr>
<td>Stem cell</td>
<td>18000</td>
<td></td>
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<tr>
<td>Control</td>
<td>12000</td>
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**Discussion** This study represents an attempt to examine the effects of mesenchymal stem cells on long bone structural allograft healing. The results indicate increase in callus formation and a decrease in fracture at the allograft host junction in the stem cell treated group. However there was a decrease in maximum torque and torsional stiffness in the stem cell treated group which was felt to be due to the weakening of the allograft bone in the demineralization process in the stem cell treated group. Further engineering of the pluripotential mesenchymal stem cell could improve allograft healing and will be investigated in the future.

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**References**


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