Effect of Bone Marrow upon Orthopedic Biocomposite in Allograft incorporation

HYPOTHESIS:
Bone marrow stem cell play a critical role in the incorporation of allograft, specifically after plated onto Orthopedic Biocomposite used to replicate "artificial periosseum". This concept of "artificial periosseum" replicates the critical role that normal periosseum plays in bone healing process.

PROJECT DESCRIPTION (Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)

Over one million reconstructive surgery, trauma, or abnormal skeletal defect operations are performed in the United States annually. To achieve reconstructive goals, large amounts of autologous or alternative large bulk allograft are needed in the surgical procedure. Allograft bone material and alternatives such as synthetic grafts lack the necessary cellular and other biological components for bony union and healing to occur. A novel approach to overcome these shortcomings would be to apply an artificial periosseum as an adjunct to the allograft treatment that provides osteogenic cells as well as bioactivity to the site to stimulate bone repair. Recently, the natural periosseum has been identified as a critical component for new bone formation in graft incorporation. To our knowledge, no studies have examined the use of an artificial periosseum that incorporates tissue regenerative strategies. The artificial periosseum will consist of a mechanically flexible, composite matrix with bioactive properties that can be used to augment allograft incorporation similar to the natural periosseum or can be used alone without the allograft. The matrix can also be incorporated with bone marrow cells. The bone marrow can be harvested from the patient and loaded onto the matrix a few hours prior to insertion, as an intra-operative point-of-care technology.

The artificial periosseum consists of a bioactive ceramic-polymer composite, having nano to microscale fiber morphologies with a uniform dispersion of nanoceramics to improve cellular attachment, infiltration and bioactivity. The nanoceramics are composed of an optimum ratio of hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic, which has been identified by the PI's group to accelerate stem cell induced bone tissue formation both \textit{in vitro} and \textit{in vivo}. Findings to date have shown that the artificial periosseum promotes the osteogenic activity of human bone marrow derived stem cells. The goal of this study is to investigate the use of the composite matrix as an adjunct to allograft treatment in large segmental bone defects.

The hypothesis to test is whether the composite matrix in combination with whole bone marrow will enhance allograft incorporation. The following specific aims will be investigated:
Aim 1: To evaluate the design of the composite matrix in supporting osteogenic activity and bone tissue formation of bone marrow-derived stem cells(completed by Dr Arinze).

Aim 2. To investigate the efficacy of the composite matrix with autologous bone marrow as an adjunct in a femoral segmental bone defect. The effectiveness of allograft bone combined with the composite matrix and bone marrow will be characterized in a rat segmental bone defect model. Level of bony union, bone tissue formation, and vascularization will be evaluated in the defect and compared to the gold standard of allograft bone alone. Histological and mechanical analyses will be performed.

The milestones are to identify an appropriate matrix design that supports the greatest amount of bone tissue formation (Year 1) and to evaluate its use in a clinically relevant model, a critically-sized segmental bone defect (Year 2).

Study Design and Overview of Methods
A rat, femoral segmental defect will be treated with the composite with whole bone marrow (BM) in combination with allograft. This model is well established in the co-investigator’s laboratory\cite{4, 5}. This model was chosen because it is a load-bearing critical sized matrix defect (Figure 4).
Summer Student Research Program
Project Description

which results in a non-union if left untreated. Furthermore, quantification of new bone formation by radiographical and histological measurements can easily be performed. The defect size is 5 mm which has been successfully used in the past, yielding a non-union in untreated animals as shown in our group and as others and is a suitable size to adequately place the hardware and grafts. Male BB Wistar rats approximately 80 days old will be utilized for this experiment. No restrictions on weight bearing or activity are made post-surgery. All research protocols used in this study are approved by the University of Medicine and Dentistry of New Jersey (UMDNJ) Institutional Animal Care and Use Committee. Defects will be treated with six experimental groups (Table 1), created unilaterally in the right femur. Limbs will be harvested at 8 weeks post-implantation for histological analyses (n=6 per group) and mechanical testing (n=8 per group). Therefore, the number of animals needed for this study = (n=6 histo. per group x 6 groups x 1 time point) + (n=8 mechanical test per group x 6 groups x 1 time point) for unilateral surgery = 84 animals. An additional 10% is included for experimental loss. Therefore, the total number of animals is 92 for the study. The time point of 8 weeks and sample size was determined based on previous studies examining the use of allografts in this model.

Table 1: Experimental groups for segmental defect model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Defect Treatment</th>
<th>Composite Matrix</th>
<th>BM</th>
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<tbody>
<tr>
<td>A</td>
<td>Bulk Allograft</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>Bulk Allograft</td>
<td>Yes</td>
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</tr>
<tr>
<td>C</td>
<td>Bulk Allograft</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
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<td>No</td>
<td>No</td>
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<tr>
<td>E</td>
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<td>No</td>
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<tr>
<td>F</td>
<td>Unfilled</td>
<td>Yes</td>
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</table>

Animal Model and Experimental Groups
92 male BB Wistar rats will be used (including 10% for experimental loss). A femoral segmental defect will be created unilaterally, according to previously published protocols. Defects will be treated with six experimental groups (Table 1). They include the bulk allograft alone, which is the gold standard, and bulk allograft + composite matrix with or without BM. The composite matrix will also be evaluated alone as a possible graft for treating the defect. Therefore, additional experimental groups include the unfilled defect (negative control), defect treated with the composite matrix with or without BM. For insertion in the defect site, the composite matrix will be wrapped around the allograft and/or the defect to replicate the periosseum. At the time of surgery, bone marrow will be harvested from the contralateral, unoperated femur using an 18-gauge needle attached to a syringe containing saline and heparin, as previously described, and will be loaded onto the matrix using a vacuum technique, according to published protocols.

Radiographic, Histological and Mechanical Analyses
Post-operative radiographs will verify implant placement and serial radiographs will be obtained every 2 weeks post-surgery to assure continued fixation. Histological analyses will include immunohistochemical PECAM-1 detection of new blood vessels formed within the defect using previously described methods, and routine histological staining for bone and cartilage. Level of bony union will be determined as well as histomorphometric analyses to quantify the number of blood vessels and the area of bone and cartilage within the defect, as previously described. Torsional testing will be performed of the limbs using previously published protocols of the PT and others. Structural characteristics of the bone, such as stiffness and torsional strength, will be determined. Comparisons will be made with age appropriate intact limbs.

Statistical Analyses
A power analysis was performed for determining the number of animals needed to achieve statistical significance for quantitative analyses of histomorphometry and mechanical testing. In order to achieve a power of 0.80, an n of 6 and an n of 8 were calculated as the minimum number of samples to test to achieve statistical differences between groups for histomorphometry and mechanical testing, respectively. One way ANOVAs will be performed to test for statistical differences between groups for p<0.05. The Tukey-Kramer Method, p <0.05, will be used to perform multiple comparisons between groups.

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Project Description

IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?
Yes □ or No □
(IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME)

Couler Grant

THIS PROJECT IS: □Clinical □Laboratory □ Behavioral □ Other

THIS PROJECT IS CANCER-RELATED □NA
Please explain Cancer relevance

THIS PROJECT IS HEART, LUNG & BLOOD-RELATED □NA
Please explain Heart, Lung, Blood relevance

THIS PROJECT EMPLOYS RADIOISOTOPES □NA

THIS PROJECT INVOLVES THE USE OF ANIMALS □
PENDING □ APPROVED x□ IACUC PROTOCOL #

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS □NA
PENDING □ APPROVED □ IRB PROTOCOL # M

THIS PROJECT IS SUITABLE FOR:
UNDERGRADUATE STUDENTS □ ENTERING FRESHMAN □
SOPHMORES □ ALL STUDENTS □

THIS PROJECT IS WORK-STUDY: Yes □ or No □

THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR FOR INTERESTED VOLUNTEERS?: Yes □ or No □

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?
Small animal surgery, bone model and outcome parameters of bone healing