Evaluation of Amniotic Derived Membrane Biomaterial as an Adjunct for Repair of Critical Sized Bone Defects

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Introduction: Autogenous bone graft is the gold standard in reconstruction of bone defects. Unfortunately, the use of autogenous bone graft is problematic because of limited availability of bone as well as donor site morbidity. The objective of this study is to evaluate a novel biomaterial that could be used as an alternative to autogenous bone graft. The biomaterial evaluated was amniotic membrane, which is rich in growth factors and mesenchymal stem cells (MSCs). We have previously shown amniotic membrane to improve the biomechanical strength of rat Achilles tendons.

Methods: Twenty-one adult male Sprague-Dawley Rats were implanted with the biomaterial using the rat critical size femoral gap model. A HMW polyethylene fracture fixation plate was attached to the femur. Next, an 8mm transverse mid diaphyseal bone segment was removed. After creation of the critical size femoral gap animals were randomized to one of the following groups: 1. Group 1 (Control): gap left empty and received no treatment. 2. Group 2 (Experimental): the gap was filled with commercially available bone graft. 3. Group 3 (Experimental): the gap was filled with bone graft + Nucel amniotic tissue preparation. The animals were sacrificed at six weeks post operatively and femurs harvested. Fracture healing was analyzed using histology and radiography.

Results: The surgery was well tolerated with one animal per group lost to follow-up due to failure of hardware. The control, empty group exhibited little bone fill upon imaging. In contrast both experimental groups demonstrated excellent conduction of new bone formation across the critical size gap. Histological analysis of the control samples showed a partially organized network of tissue containing a mixture of cells including marrow elements as well as inflammatory cells. There was little evidence of bone filling although some woven bone was detected at the defect margins. Group 2 bone graft samples showed improved osteoconduction with partial bridging of the osteotomy site. There remained a small layer of cartilage within the middle of the callus with woven bone surrounding the fragments of bone graft. Group 3 showed near complete bridging of the defect gap with pronounced periosteal woven bone formation observed. Quantitative histology demonstrated that group 1 empty controls had an average of 11.1% new bone formation in the defect site. Group 2 showed a new bone formation rate of 37.8%. Group 3 achieved the highest new bone formation rate of 49.2%. The results of the one-way ANOVA showed there was a significant difference between the 3 groups (p < 0.001). A post hoc Tukey’s HSD test showed that there was a significant difference between groups 3 and 1 (p < 0.001). However, there was only a marginal difference between groups 2 and 3 (p = 0.062).

Fig 1: Radiographs of critical size femoral gap defects in the rat.
Control group 1 empty defect. Note the lack of any significant bone present;
Group 2, Bone graft (BG) alone, new bone present but not bridging;
Group 3 bone graft (BG) plus addition of amniotic membrane allograft with robust bone formation and complete bridging of defect gap.

Fig 2: The highlighted regions of the radiographs represent the corresponding histology in figure 3.

Fig 3:
Photomicrograph of group 1 Control defect: prominent gap present with no new bone formation. Mallory’s trichrome
Photomicrograph of group 2 Bone graft alone: bone conduction is present adjacent the bone graft granules. Mallory’s trichrome
Photomicrograph of group 3 Bone graft plus amniotic membrane allograft: note robust new bone formation throughout the defect site. Mallory’s trichrome

Fig 4: Histological results using image analysis defining new bone formation among the groups.

Discussion: The clinical experience of treatment of osseous bone defects with autografts has had mixed results. While many studies have shown good to excellent results, several trials have reported mixed outcomes using autologous bone grafting. Rates of union in a trial of comminuted forearm fracture were equivocal in those treated with or without autograft. Patients treated for tibial non unions with autograft, while reporting a union rate of 85%, still had significant deformity. The primary limitation of autografts is morbidity associated with donor harvest site. The most common complication is pain at the donor site, which can be as high as 50% and lasting as long as one year. These findings have increased the need for new materials for bone graft. Our results demonstrated strong radiographic differences among groups although it did not demonstrate a significant histological difference between bone graft and bone graft plus the addition of amniotic allograft tissue. However, the trend showed a very
close to statistical significant result. We attribute this finding to several factors including the small n enrolled and the surgical dropouts from the groups leading to a loss of statistical power. Other factors may be extravasation of the material from the defect site leading to loss of critical concentration of active material.

**Significance:** The study demonstrates that amniotic membrane products have the potential to provide bridging of bone defects. The ability to fix large bone defects without harvesting autogenous bone would provide a significant improvement in patient care.

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**References:**