INTRODUCTION: Massive allograft bone is the primary source of bone graft material for use in limb salvage procedures after oncological tumor resection. However, allograft bone has been found to incorporate slowly resulting in allograft non-union, fracture, and fatigue failure. As a result, massive allograft bone has a 50% to 75% success rate at 10 years. This project examined the influence of low-intensity pulsed ultrasound on allograft incorporation in an animal model. LIPUS has shown clinical success in accelerating the course of fracture healing by activating mechanotransduction pathways in bone tissue. Increased graft porosity has also been shown to improve the tissue’s osteoconductivity by forming a conduit from the host tissue into the graft. Longitudinal perforations (LAP), as opposed to perforations perpendicular to the long axis of the graft, may more adequately promote creeping substitution of the host’s reparative tissue across the host-graft junctions. The objectives of this research were to evaluate the biological responses of cortical allografts when exposed to LIPUS and LAP, both individually and in combination, in an ovine model compared to the natural course of allograft (negative control) and autograft (positive control) healing.

MATERIALS AND METHODS: Experimental Design: Fifteen animals were assigned to five groups based on intercalary graft type and treatment: +CTL, -CTL, LIPUS, LAP, LIPUS+LAP. +CTL animals (n=3) received a tibial ostectomy with immediate replacement of the resected autologous graft. The –CTL group (n=3) received fresh frozen ovine tibiae. Neither the –CTL nor +CTL groups received LAP or LIPUS treatments. The LIPUS treatment group (n=3), following grafting with fresh frozen ovine tibiae, received LIPUS for 20 minutes/day, 5 days/week, for the duration of the healing period. The LAP treatment group received fresh frozen ovine allografts with 500 µm longitudinal perforations. The LIPUS+LAP treatment group received both LAP and LIPUS treatments. Tetracycline and calcine fluorochrome labels were intravenously administered to all sheep 3 days (tetracycline at 30 mg/kg IV) and 14 days (calcine green at 20 mg/kg IV) prior to euthanasia. All animals were euthanized at four months. Tissue Processing: Following biomechanical testing, transverse cuts were made to isolate the 5 cm allograft plus 2 cm of proximal and 2 cm of distal host bone. The specimens were sectioned in the longitudinal, sagittal plane, creating medial and lateral tibial halves. The medial sections were processed for undecalcified histology and the lateral longitudinal sections were processed using decalcified histology techniques. Histopathology: Tissue sections were stained with hematoxylin-eosin and graded according to a scale developed in our laboratory to stage extent of callus development and callus tissue type, graft vascularity, immune response elicited against the graft, and bone remodeling both in the graft and in the adjacent host (Fig 1). Treatment effects on qualitative rankings were statistically determined using a Wilcoxon or a Kruskal-Wallis test at a significance level of α=0.05. Dynamic Histomorphometry: Medial tibial halves underwent standard undecalcified histological processing and embedding in polymethylmethacrylate. 250 um sections were cut and ground using an Exakt cutting system and imaged using fluorescence microscopy. High-resolution digital images were acquired using ImagePro Plus (MediaCybernetics) and qualitative histomorphometric observations were made with regard to degree of new bone penetration within the longitudinal perforations as well as callus maturation.

Histopathological Scoring System

Host-Graft Bridging: 0↔1
(No bridging ↔ Bridging callus and cortex on two sides)

Callus Tissue Type: 0↔3 (Fibrous/Pseudoarthrosis ↔ Bone)

Host/Graft Direct Interface: 0↔3 (Fibrous/Pseudoarthrosis ↔ Bone)

Callus (% of Cortical Thickness): 0↔5 (None↔200%)

Graft Vascularity: 0↔3 (None↔Prolific)

Host Remodeling Continuum: 0↔6
(No Oc resorption ↔ Extensive Oc resorption/Extensive Ob presence)

Graft Remodeling Continuum: 0↔6
(No Oc resorption ↔ Extensive Oc resorption/Extensive Ob presence)

Inflammatory Cell Presence: 0↔2 (Many↔None)

Live Cells Present in Graft: 0↔1 (No↔Yes)

RESULTS Connectivity scores are presented below in Figure 2. LIPUS and LIPUS+LAP treatments scored higher on average than the -CTL for all of the connectivity categories, though these differences were not significant in any comparison (p>0.05). No improvement in graft remodeling was noted for treatment with LIPUS, LAP or combination therapy and there was extensive osteoclastic scalloping with little new bone formation evident in all grafts except those of the +CTL. In all reconstructions there appeared to be a high degree of both osteoclastic resorption and new bone formation in the host. The newly deposited bone appeared to originate peripherally from the highly active and vascularized periosteal callus. No inflammatory cells were observed adjacent to the grafts in any treatment group, a finding substantiated by three independent investigators. Living cells were documented as osteocytes in all grafts in the lacunae surrounding the new bone laid down peristoeally. Qualitative dynamic histomorphometry revealed new mineralizing osseous tissue had penetrated the length of the perforations in both the LAP and LIPUS+LAP treatment groups (Fig 4).

DISCUSSION: Host graft junctions treated daily with LIPUS for four months indicated extensive periosteal callus formation of a more mature tissue type than untreated reconstructions. Thus improved structural performance of sheep tibiae reconstructed with intercalary allografts appears to be the result of acceleration of the endochondral ossification process within the callus, a direct result of daily exposure to LIPUS and a finding similar to that reported in fresh fractures or non-unions in human and animal clinical studies. The LAP treatment appears to serve as an adequate conduit for reparative tissue and appears to serve as an internal template for allograft revitalization.

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