INTRODUCTION: The thrombin-related peptide, TP508 is a synthetic 23-amino acid peptide, which represents the receptor-binding domain of thrombin (1). Thrombin is responsible for initiating many of the cellular events involved in soft-tissue and hard-tissue repair. TP508 mimics thrombin by interacting with specific thrombin receptors on cells involved in tissue repair without affecting the blood clotting activity of natural thrombin (2). We have previously reported that a single injection of TP508 accelerated fracture healing in a closed diaphyseal fracture model (3). The purpose of this study was to evaluate if TP508, formulated in biodegradable time-release microparticles, could enhance healing of segmental defects. To determine if TP508 has utility in larger hard tissue lesions, we used a rabbit ulna segmental defect model.

METHODS: The rabbit ulnar segmental defect model (4) was employed in these studies using New Zealand white rabbits (2.5-3.5 kg, n=24). Bilateral defects were created in the ulna of each forelimb by removing 1.5 cm of midshaft diaphyseal bone with an oscillating saw. TP508 was formulated in 20μm PLGA (83:15) porous microspheres (courtesy of Dr. Antonios Mikos, Rice University). The left limb defect sites were filled with 30 mg of control PLGA microspheres. The right limb defect sites were filled with 30 mg of a mixture of control and TP508-containing microspheres to give total TP508 doses of 100 or 200 ug/defect site. Rabbits were radiographed at 3, 5, 7 and 9 weeks, with the animals sacrificed at 9 weeks. Ulna from 8 rabbits were carefully dissected and wrapped in saline soaked gauze for mechanical testing. Mechanical testing was performed on a Minibionix Model 858. The remaining ulnas were fixed in buffered formalin for histological evaluation (in progress). Bone regenerates from 6 defect sites were also analyzed by synchrotron x-ray tomography (synXTM), with the samples scanned at Stanford Synchrotron Radiation Lab at 23μm resolution. Statistical analysis of mechanical test data was performed by ANOVA using Bonneferronni’s criteria. The contralateral control limbs were pooled together and a comparison was made with the respective TP508 treated groups. Statistical significance was defined as p < 0.05.

RESULTS: Radiographic evaluation showed various amounts of bone regeneration in the defect sites in both control and TP508 treated limbs. Regeneration in the control limbs was limited in most cases to bone formation along the osteoconductive periosteal surface of the adjacent radius, which acts as a splint in this model. In contrast, bone regeneration was observed across the defect site as shown in Figures 1 and 2. SynXTM images indicated the regenerated bone had normal morphology and mineralization.

DISCUSSION: These results indicate that the controlled release of the thrombin-related peptide, TP508, can stimulate the healing of critical size segmental defects in the rabbit ulna model. These results complement previous work we conducted on the effect of TP508 as an injectable stimulator of fracture repair. The radiographic and mechanical testing results indicate a dose response with the best effect observed in the 200ug group. Additional studies with alternative formulations and doses are underway to confirm the dose response and determine the most effective concentration and release kinetics of TP508. In conclusion, these data indicate that TP508, formulated in a controlled release vehicle, may be useful in clinical repair of segmental defects and other orthopaedic indications.

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