Spatial Engineering of Soft Tissue Within Osseous Defect Using Microencapsulated Transforming Growth Factor-β3

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Statement of Purpose: Bone tissue engineering focuses on promoting osteogenesis. However, inhibition of ectopic osteogenesis and maintenance of soft tissues is necessary in skeletal tissue engineering, such as the engineering of tendons, ligaments, fascia, and cranial sutures (Hong and Mao, 2004; Harris et al., 2004). Several clinical models such as craniosynostosis, osteopetrosis, and osteosarcoma are characterized by unwarranted osteogenesis and clearly demand our improved understanding of inhibition of osteogenesis. Ossification of heart valves and arteries also demand soft tissue preservation. **The present study investigates whether controlled release of TGFβ3 using PLGA microspheres inhibits osteogenesis in a calvarial model useful for tissue engineering and clinical applications.**

Methods: ***PLGA Microsphere Fabrication***. Microspheres were fabricated from PLGA (50:50) by double-emulsion technique as previously described (Moioli et al., 2005). ***Calvarial model and implantation of engineered construct.*** Full-thickness calvarial defects (5×5 mm^2) were created in Sprague Dawley rats using a dental bur (**Fig 1E**). Collagen sponges containing TGFβ3 or placebo (empty) microspheres were implanted into the defects for 4 wks (**Fig 1F**). Our previous studies have shown that TGFβ3 released from PLGA microspheres at 1ng/ml inhibits osteogenic differentiation of mesenchymal stem cells *in vitro* (Moioli et al., 2005). Consequently, using the measured TGFβ3 release kinetics (**Fig 2D**), the amount of PLGA microspheres required for a continuous release of 1ng/ml TGFβ3 over the 4wk period was calculated and corresponding microspheres were added to collagen sponge using light vacuum (**Fig 2C**).

Results/Discussion: Histology and x-ray imaging of calvarial defect after 4wks implantation of tissue-engineered construct indicates inhibition of bone healing of the defects filled with implants containing TGFβ3 loaded microspheres (**Fig 2,3**). H&E staining of calvarial cross-sections showed fibrous tissue preservation in the TGFβ3 positive group (**Fig 3B**). Calvarial defects filled with collagen sponge loaded with placebo microspheres resulted in bone healing after 4 wks implantation (**Fig 3A**). The surgically created calvarial defect promoted no spontaneous healing (**Fig 2E**). Sustained release of TGFβ3 from PLGA microspheres was detected up to 35 days *in vitro* (**Fig. 1D**). SEM of PLGA microspheres encapsulating TGFβ3 showed smooth spherical surface and average diameter of 108±62 μm (**Fig 1A,B**).

Conclusions: The present study demonstrates a novel approach for inhibiting osteogenesis and engineering soft tissue within an osseous environment using controlled release of TGFβ3. The surgically created calvarial defect heals upon the delivery of collagen sponges loaded with placebo microspheres, but remain fibrous in the TGFβ3 microsphere loaded implants. Given the frequency of occurrence of ectopic osteogenesis in several clinically relevant models, including ligaments, tendons, and cranial sutures, this approach presents potential for clinical applications. By including active inhibition of osteogenesis, the present engineered construct may improve current tissue engineering approaches that include an osseous/soft-tissue interface by controlling osteogenesis. TGFβ3 can be released long-term as demonstrated in the PLGA microsphere system, and presents the potential to modulate skeletal development.

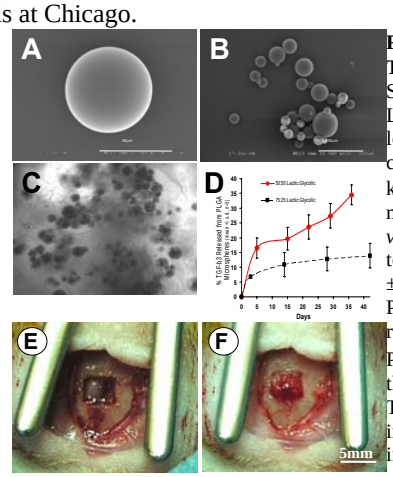

Fig. 1. PLGA microspheres and TGFβ3 release kinetics. A,B. SEM image of microspheres. **C.** Light image of calvarial implant loaded with microspheres in collagen sponge. **D.** Release kinetics of TGFβ3 from PLGA microspheres up to 35 days *in vitro* expressed as percentage of total encapsulation (n=3, mean ±s.d.). A ratio of 50:50 PLA:PGA showed more rapid release rate than 75:25 PLA:PGA. **E**. 5×5mm^2 full thickness calvarial defect. **B.** Tissue-engineered construct implanted over the defect to inhibit re-ossification.

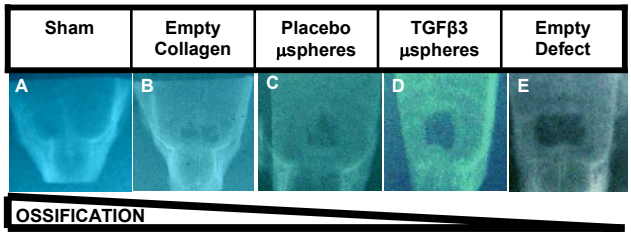

Fig.2. Representative x-ray image of calvarial defect after 4wks implantation. A. Sham control with no defect creation. **B**. Full thickness defect filled with empty collagen sponge**. C**. Defect filled with collagen sponge loaded with placebo (empty) microspheres. **D**. Defect implanted with TGFβ3 loaded microspheres in collagen sponge. **E**. Defect left untreated. The greatest amount of ossification was observed in sham controls (left) and decreased to a minimum in TGFβ3 loaded and empty defect groups (right). TGFβ3 loaded microspheres in collagen sponge inhibited osteogenic healing of full thickness calvarial defect.

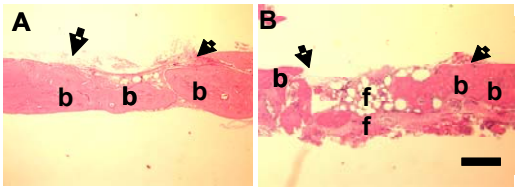

Fig. 3. *In vivo* inhibition of osteogenesis in calvarial model. A. Osteogenic healing of calvarial defect after 4wks when implanted with placebo microsphere loaded construct. **B.** Inhibition of osteogenesis in calvarial defect by TGFβ3 loaded microspheres loaded implant (4wks). Arrows point to edge of cranial defect. b=bone, f=fibrous tissue. Scale bar = 1mm. H&E stain.

References: **1**. Hong L. and Mao JJ. J Dent Res. 2004; 83(10):751-6. **2**. Harris MT. J Orthop Res. 2004;22(5):998-1003. **3.** Moioli EK. Tissue Eng. 2005; In Press.