Microencapsulated TGFβ1 Released from Hollow Titanium Implants Enhances In Vivo Bone Ingrowth

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Statement of Purpose: Titanium implants restore skeletal function lost due to disease, congenital disorders, and trauma. Delivery of growth factors including TGFβ1 plays a critical role in the bone ingrowth process (Puleo and Nanci 1999). Microencapsulation in poly-lactic-co-glycolide (PLGA) microspheres allows for this controlled delivery of TGFβ1, while retaining its biologic activity (Clark et al. 2005A). **This study aimed to determine if microencapsulated TGF-β1 increases bone ingrowth into macroporous titanium implants *in vivo*.** For this purpose, we have fabricated a novel hollow implant so that growth factors and/or cells can be delivered from the hollow core. Our present *in vivo* findings indicate that controlled release of TGF-β1 from these implants enhanced bone ingrowth in proximal humerus.

Methods: PLGA (50:50) microspheres were fabricated using the oil-water-oil double emulsion technique per our prior methods (Moioli et al. 2005) (**Fig. 1A**). Previous work has shown controlled release of TGFβ1 from these microspheres up to 4 wks (**Fig. 1B**). *In vitro* experiments were performed to examine migration and proliferation of osteoprogenitor cells under controlled delivery of TGFβ1. Calculating from the release profile of TGFβ1 (**Fig. 1B**), PLGA microspheres releasing 0.1 ng/ml or 1 ng/ml TGFβ1 or PBS (blank control) were packed into hollow titanium implants, placed onto a confluent monolayer of osteoprogenitors, and incubated up to 4 wks. For *in vivo* experiments, custom-made macroporous hollow implants (**Fig. 1F**) were fabricated and packed with TGFβ1 microspheres encapsulating 50 ng (≈ 1 ng/ml concentration, **Fig. 1B**) of growth factor. These implants were placed bilaterally into the humeri of skeletally mature New Zealand white rabbits and allowed to heal for 4 wks. Fluorescent labels, xylenol orange and calcein blue, were injected to label new bone formation. After harvest, the samples were embedded, trimmed with a diamond saw, and analyzed by visual examination and fluorescent microscopy.

Results / Discussion: After 4 wk *in vivo* implantation, titanium implants showed visible integration with host bone (**Fig. 1G**). Visual examination revealed increased bone ingrowth into implants releasing TGFβ1 (n=4) (**Fig. 2B**) as compared to the control without TGFβ1 delivery (n=4) (**Fig. 2A**). Fluorescence microscopy of new bone formation verified these results and showed more new bone formation within the macropores of implants releasing TGFβ1 (**Fig. 2F**) as compared to the control (**Fig. 2E**). Fluorescence also revealed closer contact between new bone and the titanium implant in the samples releasing TGFβ1 (**Fig. 2D**) as compared to the control (**Fig. 2C**). Supporting *in vitro* data showed increased migration and proliferation of osteoprogenitor cells into titanium implants releasing TGFβ1 (**Fig. 1D**) as compared to control implants without release of TGFβ1 (**Fig. 1C**), after fluorescent visualization of nuclear staining. Fluorescent microscopy was verified using a metabolic assay to quantify the number of osteoprogenitor cells that had migrated from the monolayer into the gelatin sponge, and showed significantly higher cell number in implants releasing TGFβ1 compared to controls without TGFβ1 delivery (**Fig. 1E,** n=6, $p < 0.05$).

Conclusions: Modulation of cellular processes critical to bone ingrowth could improve both short and long term titanium implant efficacy. The current study showed microencapsulation of TGFβ1 in PLGA and delivery up to 4 wks, increasing osteoprogenitor cell migration and proliferation. Furthermore, TGFβ1 may enhance *in vivo* bone ingrowth into macroporous implants as evidenced by fluorescence microscopy. Quantification of several static and dynamic histomorphometrical parameters is currently underway using our previous methods (Clark et al. 2005B). TGF-β1 release from hollow titanium implants could potentially be used clinically to accelerate bone ingrowth and improve implant efficacy.

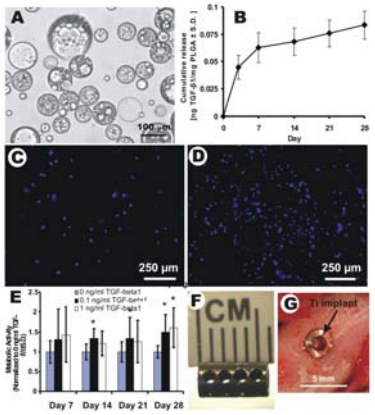

Fig. 1: PLGA microspheres (mean dia: 64±16 μm) were fabricated (**A**) and released TGFβ1 up to 28 days (**B**). When packed into hollow titanium implants and placed on monolayer of osteoprogenitor cells, increased migration of cells into implants releasing TGFβ1 (**D**) as compared to controls without TGFβ1 delivery (**C**) was observed. Quantification of cell number showed significant increases in number of cells at 4 wks under TGFβ1 delivery as compared to controls (n=6, *: $p < 0.05$) (**E**). Macroporous hollow titanium implants were custom made measuring 2.8×6 mm^2 (dia×length) with 1 mm pores (**F**), bilaterally placed in the humeri of skeletally mature rabbits, and allowed to integrate for 4 wks (**G**).

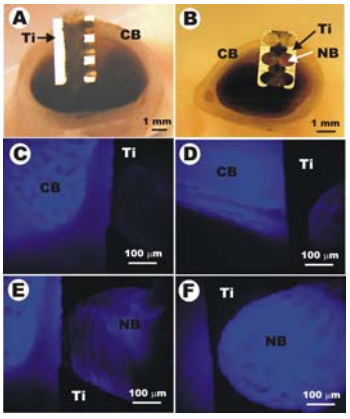

Fig 2: Gross examination showed greater new bone (NB) formation in the macropores of titanium implants (Ti) releasing TGFβ1 (**B**) as compared to controls with no TGFβ1 delivery (**A**). Fluorescence microscopy revealed closer cortical bone (CB) contact with TGFβ1 delivery (**D**) as compared to control (**C**) as well as greater new bone (NB) formation inside the macropores (**F**: TGFβ1 delivery; **E**: control without TGFβ1 delivery).

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References: (**1**) Clark et al. Trans Orthop Res Soc 2005A (Abstract). (**2**) Clark et al. J Appl Physiol. 2005B: 1922-9. (**3**) Moioli et al. Tissue Eng 2005 (in press). (**4**) Puleo D, Nanci A. Biomaterials 1999: 2311-21.