**Local Lovastatin Injection Enhances Bone Regeneration Using Biodegradable Polyurethane Scaffolds**

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**Statement of Purpose:** While autologous bone may be the ideal graft material for use in reconstructive orthopaedic surgery, its harvesting often causes donor site morbidity and has limited availability. Thus, synthetic or biological material substitutes have been increasingly studied for bone tissue repair. We have developed injectable biodegradable polyurethane (PUR) scaffolds with high porosity (>90%) that support cell migration and proliferation, and degrade to non-cytotoxic products [1]. Lovastatin (LV) has been shown to promote osteogenesis by upregulating BMP2, and local delivery of LV could optimize its efficacy in vivo [2]. In this study, we investigated the effect of local LV injection on bone regeneration using PUR scaffolds in a critical-sized segmental defect model.

**Methods**: Porous PUR scaffolds were synthesized by one-shot gas foaming of lysine triisocyanate and a hardener consisting of a polyester triol, water, catalyst, stabilizer, and pore opener, using previously reported techniques (Fig. 1A) [1]. LV-loaded PEG microparticles (LV-MP: Surmodics Pharmaceuticals) suspended in PBS were prepared for local injection (Fig. 1B). The *in vitro* release profile of LV from PEG micro-particles (in PBS) was measured by HPLC (Fig. 1C).

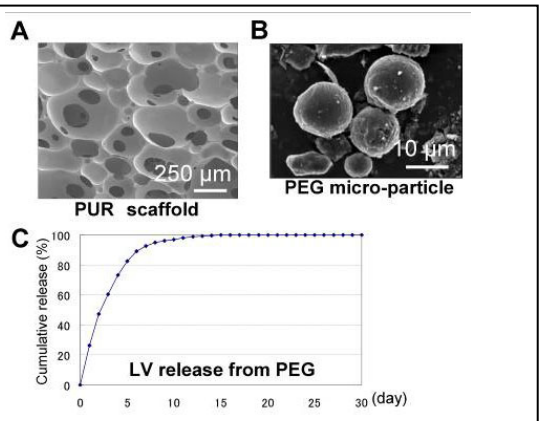


Fig 1. SEM images of (A) a porous PUR scaffold & (B) PEG microparticles. (C) *In vitro* release LV

Male Sprague-Dawley rats (8 weeks of age) were used for this study, and all procedures were approved by the Institutional Animal Care and Use Committee and conform to the National Institutes of Health (NIH) guide for the care and use of laboratory animals. A segmental defect (6 mm) was created in the middle diaphysis of the femur and fixed by threaded K-wires and an external fixation device. A cylindrical PUR scaffold (4×6 mm) was fitted into the defect, followed by injection at the defect site of 100 mg LV-MP or the equivalent with no LV (control). These injections were repeated 2 weeks post-surgery. Healing was assessed by bi-weekly x-rays using a Faxitron at 40kV tube voltage and 8 s exposure time. At 8 weeks post-implantation, the rats were sacrificed and the femurs removed and fixed in 10% phosphate-buffered formalin. Quantitative 3D analysis of bone formation in the scaffolds was performed using a µCT40 (SCANCO Medical), at a voxel size of 24 mm. The X-ray source settings were 55 kVp and 145 mA with an integration time of 300 ms. Utilizing the Scanco evaluation software, we evaluated the amount of bone formation in the scaffold. Then rat bones were then decalcified with 10% ethylenediaminetetraacetic acid (EDTA Invitrogen), dehydrated, embedded in paraffin, and sectioned at 5-µm thickness. The sagittal slice sections were stained with hematoxylin and eosin (H&E).

**Results:** X-rays at 4 & 8 weeks (Fig. 2) post-surgery showed acceleration of healing by LV treatment. µCT analysis showed substantial new bone formation within the PUR scaffold in LV-treated groups. Quantitative µCT analysis demonstrated significant increase ($P<0.05$) in the volume and density of newly formed bone of LV100 compared to control. Histological analysis corroborated the results observed by µCT where local LV injection enhanced new bone formation in PUR scaffolds (Fig. 3), The quantitative histomorphometric analysis revealed that the area of newly formed bone in LV-treated defects was significantly greater ($p<0.05$) than that of vehicle-treated.

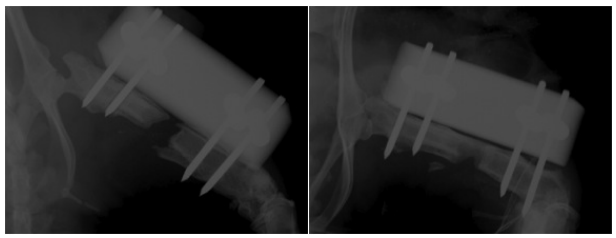

Fig 2. X-rays of segmental defects at 8 weeks: Control (left) & LV treated (right).

**Conclusions:** These biodegradable PUR scaffolds coupled with local injection of LV-loaded PEG microparticles were previously shown to promote healing of a plug defect model. In this study they also demonstrated enhanced healing of a segmental defect – a more challenging model. With >90% porosity, cells readily infiltrated the scaffolds and produced new tissue. Since injection of LV can be performed several times if needed, it may be beneficial to achieve acceleration of healing in challenging defects. This study demonstrates the possibility that this therapeutic approach is safe and reliable for clinical bone reconstruction surgery.

**References:** [1] Hafeman A, et al. Pharm Res 2008. [2] Mundy G, et al. Science 1999.

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