• Local Lovastatin Injection Enhances Bone Regeneration Using Biodegradable Polyurethane Scaffolds

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Introduction

Autologous bone is the ideal graft material for use in reconstructive orthopaedic surgery. However, its harvesting is closely associated with donor site morbidity, and limited availability. Thus, synthetic or biological material substitutes have been increasingly studied for bone tissue repair. We have developed biodegradable polyurethane (PUR) scaffolds, which can support cell attachment/growth and degrade to nontoxic products [1]. Statins (HMG-CoA reductase inhibitors) are drugs widely prescribed to reduce cholesterol levels and the risk of cardiovascular events. It has been shown that statins promote osteogenesis by upregulating BMP2 [2] and local injection of lovastatin (LV) accelerates fracture repair [3].

In this study, we examined the effect of local LV injection on bone regeneration using PUR scaffolds in two rat models: 1) a femoral plug defect model and 2) a critical-sized segmental defect model.

Materials and Methods

Porous PUR scaffolds were synthesized by one-shot gas foaming of hexamethylene diisocyanate trimer and a hardener consisting of a polyester triol, water, catalyst, stabilizer, and pore opener, using previously reported techniques (Fig. 1A) [4]. LV-loaded PEG microparticles (LV-MP; Surmodics Pharmaceuticals) 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).

1. Rat distal femur plug defect model: All procedures performed in this study 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.

Male Sprague-Dawley rats (8 weeks of age) were used for this study. A monocortical plug bone defect (3mm) was created in the distal region of the femur diaphysis, and a cylindrical PUR scaffolds (3×5 mm) were implanted into the defect. The experimental groups consisted of PEG microparticles (LV-MP) injected to the defect site either with a) vehicle alone, b) 25 µg of LV (LV 25) or 100 µg of LV loaded micro-particles (LV 100) (n=6). After four 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). The data were analyzed by one-way ANOVA.

2. Rat femur critical-sized segmental defect model: We further examined the effect of LV-MP injection on bone regeneration using a critical-sized segmental defect model fixed by threaded K-wire and external fixator. A segmental defect (6mm) was created in the middle diaphysis of the femur, and a cylindrical PUR scaffold (4×6 mm) was implanted into the defect. Then vehicle or LV-MP (LV 100) (n=10) was injected to the defect site at the surgery and 2 weeks post-surgery. The bone defect healing was assessed by X-rays bi-weekly using a Faxitron cabinet X-ray system (40kV at 8 s exposure time). The data were analyzed by unpaired t-test.

Results

1. Rat distal femur plug defect model: At week 4, µCT analysis showed substantial new bone formation within the PUR scaffold in LV-treated groups (Fig. 2). The most extensive bone ingrowth was observed in LV100. 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 100 was significantly greater (p<0.05) than that of vehicle-treated.

2. Rat critical-sized segmental defect model: X-rays at 4 weeks post-surgery showed acceleration of healing by LV treatment (Fig. 4). New bone formation area in LV100 group was significantly higher (p<0.05) than that of vehicle-treated.

Discussion

The local injection of 100 µg of LV loaded PEG micro-particle enhanced the healing of bony defects both in a plug defect and a more challenging critical-sized segmental defect, without any adverse events such as severe inflammation. Since injection of LV can be performed several times if needed, it may be very beneficial to achieve acceleration of healing in challenging defects. Further experiments will be required to confirm the effect of LV injection on bone tissue repair. However, the presented study showed the possibility that this therapeutic approach is safe and reliable for clinical bone reconstruction surgery.