**Effect of controlled Local Delivery of Simvastatin/PLGA Carrier on Bone Healing**

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**INTRODUCTION:** Statins, a hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is known to inhibit cholesterol biosynthesis. Currently, many studies indicated that simvastatin stimulate bone formation in vitro by stimulate BMP-2 expression, but the in vivo studies and clinical applications are still controversial. It is because that statins will undergo first pass metabolism by oral administered and do not likely reach sufficient blood concentrations to reliably cause substantial increases in bone formation. In order to avoid first pass metabolism, many repet use local delivery to stimulate bone formation. Accordingly, we hypothesized that local administration of simvastatin carried by biomaterial with control release property may enhance bone healing. The drug can locally releasing continued and maintain the therapeutic concentration in tibia fracture model will improve bone healing quality.

**METHODS:** Simvastatin encapsulated PLGA microspheres were produce by double emulsion (w/o/w) method. The size of PLGA microsphere was measured by laser particle size analyzer and morphology of the PLGA microsphere was observed by scanning electron microscopy (SEM). The releasing profile of simvastatin from simvastatin/PLGA microsphere was measured by using the high performance liquid chromatography (HPLC) to detect the simvastatin concentration and monitor the pH value. The bioactivity of released simvastatin on bone mesenchymal stem cells (BMSCs) was tested by Alizarin Red S Staining. The bone healing efficiency of simvastatin/PLGA was tested on a tibia fracture animal model (osteonecrosis defect). The osteonecrosis defect was performed in mouse tibia by following the standard animal center protocol. We used the 3 mg of simvastatin/PLGA microspheres for treatment dosage. Radiographic analysis was done to evaluate the effect of bone healing. We used the image proplus software to quantify the Hematoxylin and eosin staining (H&E stain) and calculate the number of endothelial cell in the graft bone. Statistical analyses were performed using Student’s t-test, with p values below 0.05 being considered significant.

**RESULTS:** The size and morphological analysis results showed that the size of the synthesized simvastatin/PLGA microspheres were average of 45.17 µm in diameter with smooth surface (Figure1a,b). The encapsulated simvastatin was control released from the PLGA microsphere over two weeks with the therapeutic concentration (0.01uM ~1uM) and the pH was maintained throughout this period at the range of 7.4±0.5 (Figure2a,b). The Alizarin red S staining assay showed that the simvastatin released from PLGA microspheres were potentially enhanced the cell mineralization on BMSCs (Figure3). The H&E stains quantification further confirmed that the simvastatin/PLGA microsphere group was significantly increased than control group (Figure4a). The simvastatin/PLGA microspheres treated groups showed the higher number of endothelial cell per mm² in the graft bone in comparison with control group (Figure4b).

**DISCUSSION:** From these results, we suggest that simvastatin released from PLGA microsphere can enhance cell mineralization and local delivery of control released simvastatin/PLGA can improve bone healing in tibia fracture. In future, we will go to stain the BMP-2 expression by immunohistostchemistry and analyze the BMP-2 mediated molecular mechanisms. Therefore, PLGA microsphere may be a suitable carrier for control released the drug of osteogenic differentiation.