Development of Sustainable and Controllable Simvastatin-Releasing Device Based on PLGA Microspheres/Carbonate Apatite Cement Composite Scaffold for Useful Osteoporosis Treatment

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Purpose
Osteoporosis is a progressive and debilitating metabolic bone disease characterized by low bone mass and structural deterioration leading to increased bone fragility. Most conditions leading to osteoporosis are associated with increased osteoclastic bone resorption by osteoclast and the inability of the bone formation by osteoblast process to keep up with the resorption process. Osteoporotic bones have been observed to have lower carbonate (CO3) concentrations. Simvastatin (SMV) which is a classic antihyperlipidemic drug has been remarkably attracted because it was reported that SMV has bone regeneration effect, and thereby, SMV has been expected as the compound to supplement osteoporosis therapy. In this study, we aimed for the development of SMV-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres embedded in self-setting carbonated apatite (CAP) cement for efficient osteoporosis therapy.

Methods
SMV-loaded PLGA microspheres (MSP) were prepared by O/W emulsion technique. The self-setting CAP cements were fabricated using the cement powder consisted of tetracalcium phosphate (TTCP), dicalcium phosphate dehydrate (DCPD) and NaHCO3. Furthermore, to analyze the effect of the method of addition 5 wt% carbonated hydroxyapatite (CHA) as seed crystals on the setting reaction of CAP cement. CHAP was prepared by DCPD hydrolysis for 24 hours in Na2CO3 solution at 95°C. CHAP was analyzed by X-ray diffraction. Two CAP cements, which were containing 0 wt% CHAP or 40 wt% CHAP, were characterized by X-ray diffraction and FT-IR analysis. The SMV release from MSP, SMV/CAP cement (with or without CHAP) composite scaffolds and MSP/CAP (with or without CHAP) cement composite scaffolds in simulated body fluid solution or acetate buffer were determined by UV spectrometer. Cell proliferation was determined using WST-8 assay. MSP, SMV/CAP cement composite scaffolds and MSP/CAP cement composite scaffolds were put into 96 well plate. MC3T3-E1 cells were seeded at 1.0 × 104 cells/well and cultured for 1, 3, 7, 14 days. In addition, alkaline phosphatase activity was evaluated after 7, 14, 21 days post-culture. To further test in vivo, the bone defect region experimentally prepared by surgery on SD rats' calvaria was filled with SMV-loaded PLGA microspheres, CAP cement containing SMV or MSP. After 4 weeks, the bone formation was evaluated by X-ray computed tomography (X-CT).

Results
The X-ray diffraction patterns of the CHAP showed that the peaks become broadened with increasing carbonate content. MSP and MSP/CAP cement composite scaffold exhibited sustainable release profile for 4 weeks without initial burst release. In contrast, SMV/CAP cement composite scaffold released SMV with initial burst within 5 days. These results mean that CAP cement containing MSP can continuously release SMV until the bone regeneration. WST-8 assays demonstrated that both SMV/CAP cement composite scaffold and MSP/CAP cement composite scaffolds promoted MC3T3-E1 cell proliferation. The results revealed that MSP/CAP cement composite scaffold was biocompatible and osteogenic in vitro. The animal experiment was also demonstrated. The rat treated with CAP cement containing MSP showed good outcome in the point of bone formation by the analysis of X-CT.

Conclusion
The results showed that our device could sustainably release SMV and the drug release was controllable. In vitro and in vivo experiment suggested that the MSP/CAP cement composite scaffold fulfilled the basic requirements of bone tissue regeneration scaffold and possessed application potentials in osteoporosis treatment.

Graphical Abstract

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