Effect of controlled released PTH (1-34) encapsulated PLGA microspheres on treatment of early osteoarthritis in rat

INTRODUCTION:
Parathyroid hormone (PTH) is an 84 amino acid polypeptide that acts as the most important regulator of calcium homeostasis in the human body through its direct action on bone and kidney. Previously we reported that PTH(1-34) inhibits terminal differentiation of articular chondrocytes and in turn suppresses the progression of osteoarthritis (OA). However, the treatment needs the injection of PTH every 3 days over the treatment period. We hypothesized that encapsulation of PTH(1-34) in PLGA microspheres with controlled release property may prolong the injection durations and can be used to treat OA with less injection frequency. Here in we studied the effect of released PTH(1-34) from PLGA(65:35) encapsulated PTH(1-34) (PLGA/PTH) microspheres on the papain-induced OA in the rat knee joints. In vitro study, we studied the microspheres morphology, PTH(1-34) encapsulation efficiency, release profile, bioactivity. In vivo study, we evaluated the changes of localized glycosaminoglycan (GAG), Type II collagen, and Type X collagen in the articular cartilage of rat knee.

METHODS:
The PLGA (65:35)/PTH(1-34) microspheres were fabricated by the w/w double emulsion technique. The surface morphology and size of the PLGA microspheres were evaluated by the scanning electron microscopy (SEM) and particle size analysis, respectively. The PTH (1-34) release kinetics, the encapsulation efficiency and concentration of PTH(1-34) were calculated using the ELSA kit. The bioactivity of released PTH(1-34) were measured by calculating the expression of cAMP from released PTH(1-34) treated MC3T3-E1 cells using ELISA kit. At every indicated time interval, cells were collected for further experimental analysis. In vivo study, OA was induced in the right knees of rats in the OA and OA+PTH groups with intra-articular injections of 20μl of 4% papain and 20μl of 0.03 M cystein. The injections were given with a 26-gauge needle via the patellar tendon on days 1, 4, and 7 of the experiment. In the OA+/PTH, and OA+/PTH/PLGA groups, after OA-induction, the right knees were injected intra-articularly with 40μl of 10nM PTH(1-34) every three days and 0.4 mg of PTH/PLGA microspheres at 1st and 15th day, respectively until sacrifice. In the PTH group, the same PTH(1-34) treatment was performed but without OA-induction. The rats were sacrificed with an overdose of CO2-inhaled at the same time point at 5 weeks. We evaluated the changes of localized glycosaminoglycan (GAG), Type II collagen, and Type X collagen in the articular cartilage of rat knees by histochemical analysis. For quantifying of histological analysis we used the Image-Pro plus 5.0 software. Statistical analyses were performed using one-way analysis of variance (ANOVA), and multiple comparisons were performed by Scheffe’s method. A p<0.05 was considered significant.

RESULTS:
The SEM observation shows that the surface of the PLGA microspheres was smooth, and the size of the microspheres was in the range of 51-127 μm. The PLGA/PTH microspheres shows 62.7 % encapsulation efficiency and sustain released the PTH(1-34) for 19 days with the concentration range 5-100 nM covering the expected therapeutic schedule of PTH(1-34). However, the concentration range 5-100 nM of PTH(1-34) was not significantly higher than that of the OA group (t=1.4) (Fig. 2). After 5 weeks of treatment, histological analysis on immunolocalized type II Collagen (stained brown) showed that the brown:total ratio in the OA+PTH/PLGA and OA+/PTH(1-34) group were significantly higher than that in the OA group (P<0.01) (Fig. 3). Immunolocalized type X collagen (stained brown) was predominantly found in articular chondrocytes from the OA group, but less positive stained cells were found in cartilages of OA+PTH, and OA+/PTH/PLGA groups after 5 weeks of treatment (Fig. 4).

DISCUSSION:
The fabricated PTH encapsulated PLGA microsphere released the therapeutic concentration of PTH(1-34) for 19 days with bioactivity. In the papain-induced rat model of OA, our results showed that intra-articular treatment of PLGA/PTH (once in 15 days) for 5 weeks significantly suppressed the loss of GAG and Col II that occurred in OA cartilage and those no significantly different from the normal cartilage. Furthermore, treatment with PLGA/PTH for 5 weeks appeared to suppress the level of Col X caused by OA-induction. The in-vivo studies illustrated that the intra-articular injection (once in 15 days) of PLGA/PTH controlled local delivery system can reverse the papain induced OA in rat knee. Therefore, the PTH(1-34) encapsulated PLGA microsphere may be a potential carrier for PTH(1-34) delivery system to treat early OA.