STIMULATION OF IN VITRO MINERALIZATION ON STRONTIUM-CONTAINING HYDROXYPATITE BIOACTIVE BONE CEMENT

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Introduction
Osseointegration is the procedure by which mature bone is deposited directly on implant materials without any intervening soft or fibrous tissue. For clinically successful implants, osseointegration is necessary to ensure association between the bone and implant surfaces. Poly(methyl methacrylate) (PMMA) bone cement has been used for over 40 years but is lack of osseointegration. Strontium (Sr) was found to stimulate bone formation and inhibit bone resorption. It has recently been used to treat osteoporosis. Strontium-containing hydroxyapatite (Sr-HA) bioactive bone cement was designed to have stimulation on mineralization and promoted osseointegration. The purpose of this study is to test whether incorporating strontium into hydroxyapatite will increase in vitro mineralization, i.e. achieve osseointegration.

Materials and methods
Preparation of bone cement specimens
Sr-HA bone cement was composed mainly of strontium-containing hydroxyapatite filler (40 wt %) and bisphenol A diglycidylether dimethacrylate (BIS-GMA) based resin (60 wt %). HA bone cement, using HA to replace Sr-HA, was used for comparison. Bone cement was molded into discs of 13 mm in diameter and 2 mm in thickness. The discs were then polished mechanically to 0.1 μm.

Osteoblast attachment
Human osteoblast cell-line (SaOS-2) (American Type Culture Collection (ATCC), Manassas, USA) was used in the in vitro experiments. For attachment evaluation, SaOS-2 cells were plated at a concentration of 1 x 10^5 cells/well on the bone cement specimens. The cells were cultured in DMEM containing 10 % (v/v) FBS for 2 hours, 4 hours, and 1 day, respectively (n = 5 for each time point). The adherent cells were counted using a haemocytometer.

Osteoblast proliferation
For proliferation evaluation, SaOS-2 cells were plated at a concentration of 3 x 10^3 cells/well on the bone cement specimens. After 1 day, the unattached cells were removed and adherent cells were continued to culture for 3, 5 and 7 days, respectively (n = 5 for each time point). The adherent cells were counted using a haemocytometer.

In vitro bone-like nodule formation
SaOS-2 cells were seeded at a concentration of 1 x 10^5 cells/well on the bone cement specimens and cultured for 14 and 21 days, respectively (n = 5 for each day). After confluency, the cultures were supplemented daily with freshly prepared ascorbic acid (0.25 mM) and β-glycerophosphate (3 mM). After removed the medium and washed with PBS, the cells were then fixed with 10 % neutral buffered formalin for 10 minutes and then washed with PBS again. The specimens were stained with 2 % (w/v) Alizarin red S (pH 4.2) for 5 minutes. The newly formed bone-like nodules stained orange red, and the specimens viewed under a light stereomicroscope equipped with a digital camera. The specimens were evaluated using an image analyzer (Image-Pro Plus, Ver 5.0, Media Cybernetics). The amount of bone-like nodules formation was calculated as a percentage of the bone cement disc area.

Statistical analysis
All the measurements were expressed as a mean value ± standard deviation (S.D.). The significant differences between bone cements were analyzed by the Student’s t-test, with p-value ≤ 0.05 being significant.

Results
Both Sr-HA and HA bone cements allowed osteoblast attachment and proliferation. The result of osteoblast attachment is shown in Figure 1. There was a significant increase in the number of osteoblasts attached onto the Sr-HA bone cement than HA bone cement after 4 hours. The number continued to increase from 4 hours to 24 hours. Figure 2 shows the result of osteoblast proliferation. More osteoblasts were measured adhered on the Sr-HA bone cement than the HA bone cement in all the time points. There was a significant increase in the number of osteoblasts adhered on both Sr-HA and HA bone cements from 1 to 7 days.

Discussion
In the present study, the Sr-HA bone cement showed a significant higher osteoblast attachment property than the HA bone cement. This resulted in an increased number of osteoblasts attached on the Sr-HA bone cement after proliferation. This indicates that strontium, in the form of strontium-containing hydroxyapatite, can increase the bioactivity of hydroxyapatite, resulted in stimulation of osteoblasts attachment and proliferation. The Sr-HA bone cement was confirmed to be bioactive and biocompatible.

The bone-like nodule formation was observed on the Sr-HA bone cement and HA bone cement after 2 weeks. More bone-like nodules were formed on the Sr-HA bone cement than the HA bone cement, which indicates strontium can stimulate in vitro bone mineralization.

Conclusion
In summary, compare to hydroxyapatite (HA) bone cement, the strontium-containing hydroxyapatite (Sr-HA) bone cement showed the better in vitro human osteoblasts (SaOS-2) attachment and bone mineralization.

References