**Keynote Lecture (KL)**

**Stimulation of new bone formation by local delivery of magnesium ions**

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**INTRODUCTION**

Magnesium ions can affect many cellular functions including the transport of potassium and calcium ions, whilst it also modulates signal transduction, energy metabolism and cell proliferation. It was recently reported that the presence of magnesium in the bone system is beneficial to bone growth and play a key role in bone remodeling and skeletal development. In the present study, new bone substitutes containing surface treated magnesium micro-particles have been specifically designed in order to deliver proper amount of magnesium ions for stimulating in vivo bone formation.

**METHODS**

The bone substitutes were prepared by incorporating 9% TMSPM-treated Mg granules (i.e. 45µm & 150µm) into biodegradable polymer, polycaprolactone (PCL). The TMSPM silane-coupling agent treatment was used to protect the Mg particles from rapid degradation. Compression test was performed to study the mechanical properties of the bone substitute by using the MTS machine. A 7-day stimulated body fluid (SBF) immersion test was conducted to test their bioactivity. The surface composition was checked by energy dispersive x-ray spectroscopy (EDS) after immersion. The cytocompatibility and osteogenic differentiation properties of the bone substitutes were studied by MTT, ALP assays and qRT-PCR with the use of MC3T3-E1 pre-osteoblasts and human mesenchymal stem cells. Finally, the in vivo response of the bone substitutes was evaluated by using rat model of 2 months. Micro-CT was used to monitor the volume change of bone formation. Pure PCL was used as the control.

**RESULTS AND DISCUSSION**

The results of in vitro experiments demonstrated that the release of magnesium ions at 50-100 ppm/day could enhance the expression of early bone markers including alkaline phosphatase (ALP), osteopontin (OPN), runt-related transcription factor 2 (Runx2), collagen type I (Col1A1) and also the late bone marker, osteocalcin (OCN) as compared to the control (without additional Mg ions). Furthermore, magnesium ions were also able to induce ERK1/2 activation at post-culture 48 hours. With the use of the ERK1/2 inhibitor, the effect of magnesium ions on osteogenesis was attenuated. All these results suggested that specific amount of magnesium ions is participating in the bone formation process. Additionally, the effect of magnesium ions to osteoclastogenesis was studied as well. Our results proposed that same amount of magnesium ions could down-regulate the osteoclastogenic markers including macrophage colony-stimulating factor (c-fms), dendritic cell-specific transmembrane protein (DC-STAMP), interleukin 1 beta (IL-1) and receptor activator of nuclear factor-kappa B (RANK), indicating that the released magnesium ions was able to suppress osteoclasts differentiation. The volume of newly formed bone in the Mg-contained sample was found to be 80% higher than the control in rat animal model. However, bone resorption was found in the sample with high concentration of magnesium ions. Hopefully, hydrogen gas accumulation was not found in both treated and untreated samples. The compressive modulus of new bone substitutes generally maintained during the test. Although the literatures have proposed the growth and healing of bone with the presence of magnesium ions, our results can successfully identify the particular concentration of Mg ions applied to stimulate bone formation and its mechanism.
CONCLUSIONS
The present results demonstrate that the concept of the use of magnesium ions to stimulate bone formation in vivo is promising.

ACKNOWLEDGEMENTS This project is financially supported by RGC GRF (#718913), NSFC (#31370957) and the funding from Shenzhen Key Laboratory of Innovative Technology for Orthopaedic Trauma.