In vitro interaction between brushite calcium phosphate cement and osteoclasts

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Introduction

Calcium phosphate ceramics are an alternative to autografts and allograft, which show good results in many clinical applications. The calcium phosphate cements, in the form of either apatite or the more soluble brushite, belong to a class of orthophosphates commonly used for bone grafting (1). Brushite cements have been previously shown to be degradable *in vitro* in phosphate-buffered saline, serum and simulated body fluid (2), and to undergo cell-mediated degradation (3). However, it is also important to consider that biomaterials may have reciprocal effects on formation and activity of cells responsible for bone resorption, osteoclasts. In this study, we investigated the effect of brushite cement on osteoclasts formed from RAW264.7 murine monocytic cells.

Material and methods

Brushite cement: β-TCP was synthesized by heating a mixture of monetite (DCPA; Mallinckrodt Baker, Germany) and calcium carbonate (CC; Merck, Germany) to 1050°C for 24h, followed by quenching to room temperature in a desiccator. The product consisted of pure and highly crystalline β-TCP as verified by X-ray diffraction. The sintered cake was crushed with pestle and mortar and passed through a 355 µm sieve and milled for 1 h. Equimolar amounts of β-TCP and commercially available monocalcium phosphate hydrate (Baker, Germany) were mixed and the liquid component, 0.8 M citric acid solution, was added to the mixture, with a powder/liquid ratio 3.5 g/ml. Cement was set in the form of disks ∅10 mm. *Cell culture:* RAW264.7 cells were seeded into wells with or without the cement at a density of $2.5x10^5$ cells/cm^2. Cells were cultured in DMEM with 10% FBS, 1% antibiotics, for 5 days at 37°C, 5% CO_2. To induce osteoclast differentiation, a pro-resorptive cytokine was added RANKL (50ng/mL). On day 5, cells were fixed using 4% paraformaldehyde, stained for osteoclast marker TRAP, and the numbers of multinucleated, TRAP positive cells were assessed. Osteoclast resorption requires formation of specialized cytoskeletal structure, actin ring. To characterize actin organization in osteoclasts, we used BODIPY 581/591-conjugated phalloidin and DAPI to visualize F-actin and nuclei using fluorescence microscopy.

Results/Discussion

Osteoclast differentiation in the presence or absence of brushite cement was observed only in the presence of RANKL (Figure 1). Since the brushite cement is opaque, we assessed the numbers of osteoclasts formed on the plastic surrounding and underlying the cement and compared to the numbers of osteoclasts formed in the absence of the cement. RANKL induced osteoclast formation to similar extent independent of the presence of cement in the well (Figure 2). These data indicate that the brushite cement does not exhibit detrimental or stimulatory effects of on osteoclast formation.

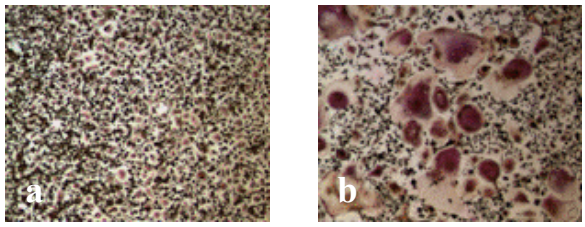

Figure 1: (a) RAW264.7 cells; (b) TRAP+ multinucleated osteoclasts formed from RAW264.7 cells in the presence of cement and RANKL.

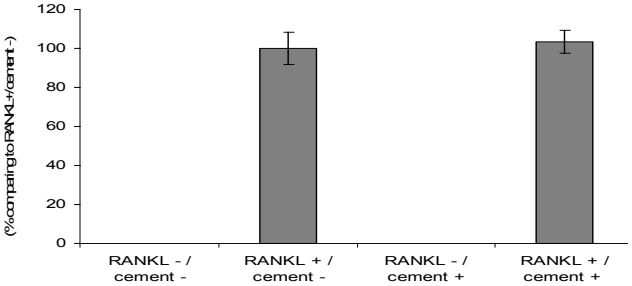

Figure 2: Percentage of TRAP positives cells normalized to positive control (RANKL + / cement -).

To observe osteoclasts formed directly on the cement, we used fluorescent probes and inverted epi-fluorescence microscopy. In the presence of RANKL, osteoclasts formed exhibited an actin ring surrounding numerous nuclei (Fig.3a). Without RANKL, no actin ring formation was observed. These data indicate that cement supports adhesion and formation of functional osteoclasts. The effect of brushite on osteoclast activity may be estimated from the proportion of osteoclasts exhibiting actin ring.

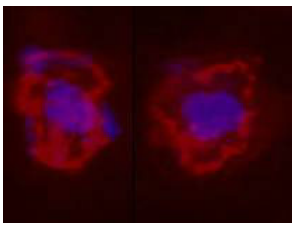

Figure 3: Actin (red) and nuclei (blue) in osteoclasts formed on the brushite cement from RAW264.7 cells cultured with RANKL

It is important to note that osteoclasts are sensitive to pH variations, and generally the osteoclast resorption is stimulated by low pH. Since brushite may significantly reduce pH of the medium, we will investigate if this material may additionally stimulate osteoclast activity.

Conclusions

We have shown that although brushite cannot directly stimulate osteoclastogenesis from RAW264.7 cells, it supports formation of functional osteoclasts *in vitro* in the presence of RANKL. This study describes a useful model to investigate the reciprocal effects of brushite cements and osteoclast *in vitro*.

References

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