

IN VITRO DEGRADATION OF PSEUDOWOLLASTONITE AND IN VITRO CYTOTOXICITY EVALUATION

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INTRODUCTION: Bioactive materials including a chain silicate minerals as pseudowollastonite (CaSiO₃) (psW) have demonstrated the formation of hydroxyapatite-like layer on their surface both *in vivo* and *in vitro*^{1,2,3}. This ceramic material appears to offer therapeutic potential in situation requiring bone augmentation or replacement^{4,5}. In previous study, we have demonstrated that psW can released substances able to induce an impact on cellular viability with a kinetic reaction. Indeed, a decrease of cellular viability was observed in the early phase of psW degradation and a better cells viability in function of psW degradation time⁶ (Fig.1). Our hypothesis is that the interaction between Silicate and Calcium, released by psW, can play an important role on cell metabolism. The aim of this work consists in the study of calcium and silicate effects on cellular viability.

METHODS: Cytotoxic assays on silicate and calcium:

Human osteosarcoma cell lines (SaOS-2; ATCC: HTB-85) were incubated with medium [DMEM/HAM-F12 (50% v/v), 10% (v/v) fetal bovine serum, 100 u/ml penicillin, 100µg/ml streptomycin) supplemented with different concentrations of calcium (+ 50%, +100%, +150%) and silicate (sodium silicate solution; 1.6 and 6.25 mM). At 24 and 48 hours after incubation, cells were incubated for 30 min in DMEM/HAM-F12 (no serum) containing 8 µmole/L 2',7'-bis-carboxyethyl)-5-

carboxyfluorescein acetoxymethylester (BCECF-AM, Molecular Probes), an esterified dye that, when internalized by living cells, is hydrolyzed by cellular esterases to a membrane-impermeable fluorescent species. The cells were then lysed by incubation with 1% Triton X-100, and the released dye collected in the supernatant for quantification by spectrofluorimetry (485 nm excitation and 535 nm emission).

Silicon and calcium released of psW pellets: PsW pellets were incubated in conditioning medium at different concentrations (10, 15, 50 and 100 mg/ml) during 1,2,3,7 and 9 days. Silicon and

calcium released were performed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

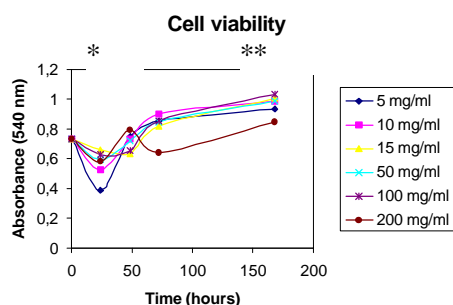


Fig. 1. Cell viability: Relative MTT formazan formation by SaOS-2 cells incubated, during 24 hours, with psW extracts (24, 48, 72, 168 hours of extraction) (n=8) (*: p<0.005; **: p<0.05).

RESULTS: No significant modification of cellular viability was observed for SaOS-2 cells incubated with medium supplemented with different concentrations of calcium (at 24 and 48 hours) (Fig.2). However, a significant decrease of cell viability was observed for cells exposed to silicate solutions (Fig.2,3). ICP-AES has confirmed that psW samples, incubated in medium, released at day 1 and day 2, a major quantity of silicon compared with sample incubated during 3,7 and 9 days.

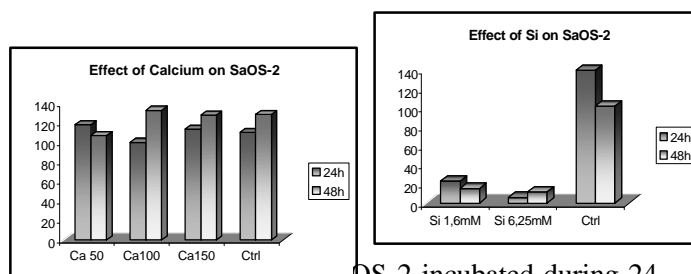


Fig. 2. Cell viability: SaOS-2 incubated during 24 and 48 hours with medium supplemented with calcium (left) and silicon (right).

DISCUSSION & CONCLUSIONS: No cytotoxic effect was demonstrated for psW. However, we

have observed few changes in cell viability or stress for cell exposed to psW extract in the early phase of extraction (24 hours). This phenomenon could be correlated with an increase of silicon released by psW which can initiate a cellular toxicity. Further studies will be necessary to obtain a better understanding of psW degradation which will be primordial to obtain a better interaction between cell and bioactive ceramic.

REFERENCES: ¹ Siriphannon P et al (2000) *J Biomed Mater Res* **52**: 30-9.² De Aza PN et al (1999) *J Dent* **27**: 107-13.³ Nishio K et al (2001) *J Biomed Mater Res* **55**: 164-76.⁴ Mousa WF et al (2000) *Biomaterials* **21**: 2137-46.⁵ Fujita H et al (2000) *J Bone Joint Surg Br* **82**: 614-18.⁶ Dufrane D et al (2001) *European Cells and Materials* **1** (supplement 2): 64.