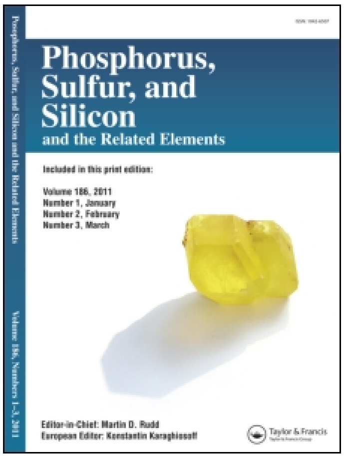

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Generation of Active Oxygen Species on Reaction Between Ceramics in $CaO-SiO_2-P_2O_5$ System and Polymorphonuclear Cells

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GENERATION OF ACTIVE OXYGEN SPECIES ON REACTION BETWEEN CERAMICS IN $CaO-SiO_2-P_2O_5$ SYSTEM AND POLYMORPHONUCLEAR CELLS

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Abstract
The glass powders in the system of $(100\text{-}x)Ca(PO_3)_2$ $xSiO_2$ were injected intraperitoneally into C57BL/6 mice. The mortality gave a maximum at the composition of x = 30. This result is discussed in terms of generation of superoxide or active oxygen species induced when the materials are contact with polymorphonuclear cells.

INTRODUCTION

Bioceramics are expected to be used in a living body more often in the near future[1]. However, a long term toxicity of ceramic materials in a living body has not been cleared yet. The purpose of this paper is to review briefly our works on toxicity of silica-containing calcium phosphate glass powders when injected intraperitoneally into mice and to present some new data on the generation of active oxygen species when various ceramics such as calcium phosphate glasses and hydroxyapatite are,in vitro, contact with polymorphonuclear cells. It is very interesting and important that glass powders at around x = 30 (mol %)in the system of $(100\text{-}x)Ca(PO_3)_2\text{-}xSiO_2$ exhibit a maximum mortality, although the glasses at x = 0 and x = 100 are nontoxic (mortality = 0), respectively[2]; on the other hand, the glasses at x = 30 are found to exhibit a maximum amount of the generation of the active oxygen species. It is supposed that there exists a significant correlation between the toxicity and the generation of the active oxygen species.

Experimental

Glasses in the system of $CaO-SiO_2-P_2O_5$ were prepared in a Pt-crucible by a conventional melt-quenching technique using a mixture of raw materials such as H_3PO_4, $Ca(H_2PO_4)H_2O$, $CaCO_3$ and SiO_2. The powders of approximately 5 μm diameter were used for the present toxicity test. The protein contamination of the glass powders was checked by the direct Ninhydrin reaction and Bradford's method using Coomassie Brilliant Blue[3]. The six- to eight-week-old male C57BL/6 mice used in this study were acclimated in an animal care facility at Gunma University School of Medicine for one week prior to use. Ten animals were given an intraperitoneal doses of the glass powders (5000 mg/kg body wt) suspended in 1 ml of normal saline. All animals were studied simultaneously and were dosed from the same single glass stock suspensions to determine the mortality.

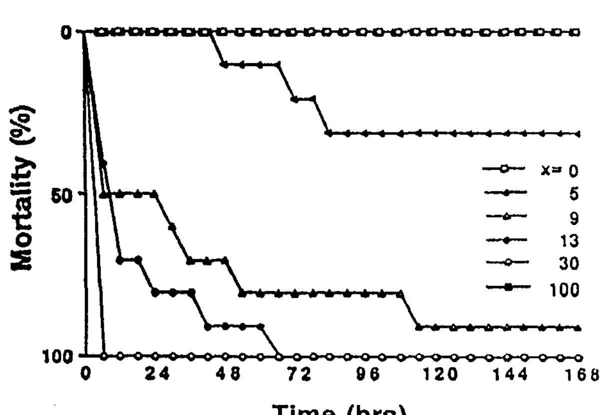

Fig.1. Plots of mortality vs injecting time (After Nagase et al, Ref.2)

The generation of superoxide, one of active oxygen species, was measured by reduction of ferricytochrome C (type III horse heart)[4]. Synthetic monosodium urate crystals(MSU) and the chemotactic peptide f-Met-Leu-Phe(FMLP) were used as positive controls.

Results and Discussion

The results of mortality test of glass powders in the system of (100 - x)$Ca(PO_3)_2$ xSiO_2 show that the mortality goes through a maximum of 100 % at glass compositions at around x = 30, while it is zero % for pure silica glass and pure $Ca(PO_3)_2$ glass, respectively (Fig.1). The LD_{50} value for the powders at x = 30 after intraperitoneal administration was 750 mg/kg body wt.[5] The amount of SiO_2 dissolved out of the glasses when immersed into water at 37 °C gives a maximum at the glass composition of x = 30, as shown in Fig.2.

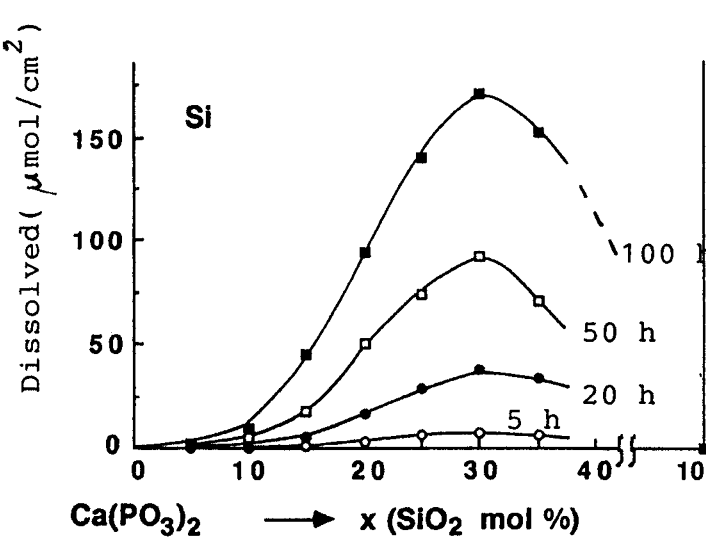

Fig.2. Dependence of dissolving silica on the glass composition (37 °C).

Superoxide production induced when the materials are contact with polymorphonuclear cells was studied. Fig. 3 indicates that the superoxide production depends on the amount of SiO_2 of the glasses; it goes through a maximum at x = 30, and then it decreases. It is noted that the glass composition at x = 30 gives a maximum of mortality, a maximum of the dissolving SiO_2 in water, and a maximum of the superoxide generation.

Generation of active oxygen species including superoxide induced by the contact with polymorphonuclear cells are also reported for the ceramic materials such as hydroxyapatite and alumina, and discussed in terms of the particle size and the preparation conditions of the materials.

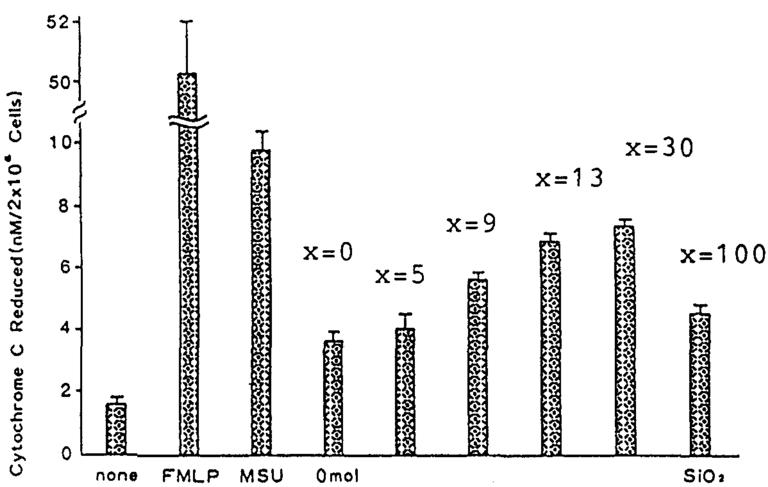

Fig.3. Generation of superoxide when the materials are contact with polymorphonuclear cells.

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