Development of bioactive glass-ceramics

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Abstract

A bioactive glass-ceramic with rhenanite, $NaCaPO_4$, was developed in the SiO_2 - Na_2O - CaO - P_2O_5 – F system. Crystallization and microstructure were determined by room temperature X-ray diffraction (RT-XRD) and scanning electron microscopy (SEM) methods. The surface properties of the monolithic material and its surface reactivity were investigated after immersion in simulated body fluid (SBF) for 1 to 10 days. The layer that formed was identified as hydroxyapatite (HA) using surface-sensitive analytical methods. Ion exchange processes of Ca^{2+} and phosphate ions were determined. HA formed on the surface of the samples within 48 hours. An open-porous scaffold was prepared using the polymer sponge method. Based on the main results of the investigation, we conclude that rhenanite glass-ceramics possess very good bioactive properties.

Introduction

Glasses, glass-ceramics as well as crystalline compounds such as calcium phosphate (hydroxyapatite or tricalcium phosphate) are used as materials for the replacement of living bones or to heal bone defects [1,2]. One special compound is $NaCaPO_4$, also known as rhenanite, which also demonstrates good bioactive properties. In some cases, its reaction behaviour even exceeds the reactivity of TCP [3,4]. Rhenanite also plays an important role in the phase formation process of apatite glass-ceramics (solid state reaction)[5].

The objective of this investigation was to crystallize rhenanite in a silicate glassy matrix, which results in a glass-ceramic material with good surface active properties, such as high reaction behaviour in SBF and good osteoconductivity.

Experimental

Approximately 40 glasses of the SiO_2 - Na_2O – CaO - P_2O_5 - F system were prepared and investigated - one special glass with 49.9 wt% SiO_2, 22.4 Na_2O, 12.4 CaO, 14.4 P_2O_5, 0.3 F, 0.6 impurities (K_2O, Al_2O_3) was studied. The crystalline phases were analyzed by RT-XRD (Bruker AXS D505) using pulverized samples (< 90 µm). To analyze the microstructure, the fracture surfaces of the solid samples were etched and examined by means of SEM (LEO DSM 962).

In order to investigate the surface reactivity of the glass-ceramic, solid samples were immersed in revised and conventional simulated body fluid (R-SBF and C-SBF) [6]. Cylindrical, polished glass-ceramic specimen were used. 25 ml of SBF solution were prepared per sample. Three different SBF test methods were used: test 1 (R-SBF, 1 to 30 days), test2 (R-SBF, 2 to 10 days and liquid refreshment every 2 days); test3 (C-SBF, 2 to 10 days and liquid refreshment every 2 days). In test 2 and test 3, dynamic conditions were simulated by changing the SBF-solution. The formation of the surface layer was examined by surface-sensitive analytical methods, i.e. scanning electron microscopy (SEM) combined with energy dispersed spectroscopy (EDS), while the corresponding ion exchange processes were detected by ICP-OES (TJA IRIS/AP DUO 532ICP020) and the SiO_2–content determined by AAS (Perkin Elmer 510).

The open-porous scaffold of this glass-ceramic material was prepared by using the polymer sponge method according to Ramay and Zhang [7]. Its surface reactivity was tested with R-SBF for 10 days.

The *in-vitro* investigations were carried out with osteosarcoma cells (SAOS-2).The samples were pre-treated in the same way as for the SBF tests. Vitality, proliferation and the content of intercellular, osteoblastic marker were determined.

Results and discussion

Phase analysis and microstructure

When the glass melt was cooled down, the selected composition crystallized sponteanously. As the main crystalline phase rhenanite, $NaCaPO_4$, was identified (ICDD 33-1229). An Na_2O surplus in the base glass composition prefers rhenanite as the single crystalline phase. The presence of 0.3 wt% F also promoted crystallization. Figure 1 shows the spherulitic crystals of the chosen sample. The crystals have a size of approximately 5 – 20 µm. It is obvious to characterize the

glass-ceramic as a material with a high crystalline content and a low residual glassy matrix phase.

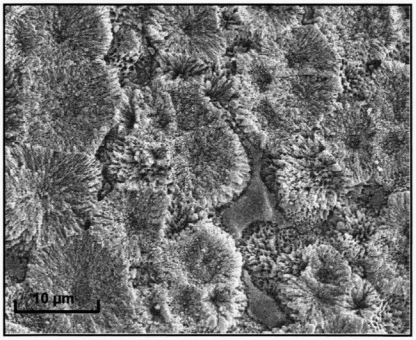

Fig.1 Microstructure of a glass-ceramic with spherulitic rhenanite crystals (fracture surface, 5% HF:15 % HNO_3 = 1:1, 10 s).

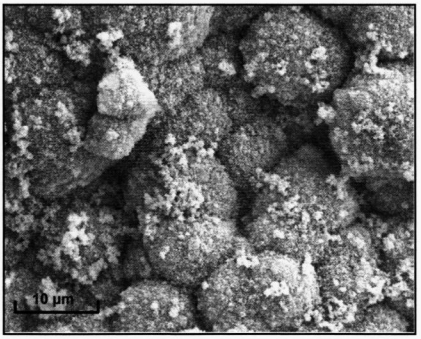

Fig.2 Calcium phosphate layer after immersion in R-SBF for 30 days.

Reaction behaviour of the glass-ceramic

As early as within the first 48 hours, the growth of a crystalline phosphate layer on the sample surface was observed during all immersion tests with SBF. The white layer covering the sample, which formed within 10 days, firmly adhered to the surface. The layer thickness increased with longer immersion times. Figure 2 shows the typical morphology of the ball-shaped crystal aggregates. The samples of test 2 and test 3 showed a similar reactive behaviour and morphology.

An attempt was made to determine the composition of the layer by means of EDS analysis. The glass-ceramic sample of test 1 (immersed in R-SBF for 30 days, see Fig.2) showed a content of cations of 22.1 calcium at.%; 13.0 phosphorus; 1.9 sodium, 1.0 silicon; 1.7 magnesium; 60.0 oxygen. The Ca/P atomic ratio was 1.7, which is close to that of hydroxyapatite (1.67). Therefore, the composition of the layer indicates the presence of HA. In this context, it is interesting to note the loss of silicate structure units (just 1.0 at.% Si) in the detected layer. Thus the layer has a chemical composition that differs from that of the bulk material.

The changes of the ion concentration in Ca^{2+} and phosphate, determined as P^{5+} by inductively coupled plasma-optical emission spectroscopy (ICP-OES), are graphically represented for test1 (see Fig.3). The calcium ion concentration in the

SBF solution increased over the first 3 days of immersion due to leaching of Ca^{2+} ions from the glass-ceramic surface. After the 3rd day, the Ca^{2+} and phosphate ion concentration in the SBF decreased, which, according to Juhasz et al. [8], indicates an enrichment of the layer on the surface of the glass-ceramic with calcium and phosphate ions.

The concentration of silicates, determined as Si^{4+} in the SBF, decreased constantly to 42 mM (test2, 10 days) or to 60 mM, respectively (test3, 10 days). The forthcoming degradation process, which the silicate network of glass-ceramic will undergo, is recognisable.

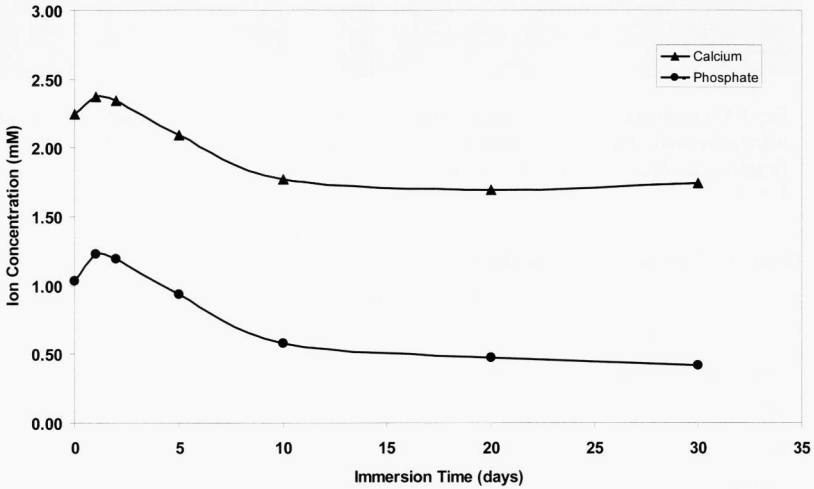

Fig.3 Ca^{2+} and phosphate ions exchange (determined as P^{5+}) of test1.

The reactivity of the generated open-porous scaffold was investigated according to the conditions of test 2 (immersion time 10 days). Figure 5 and 6 show the micro-structure before and after immersion. The open porosity with pores of approximately 150 – 300 µm was maintained after immersion. The surface reactivity is remarkably increased in comparison with the bulk material.

With the help of in-vitro investigations involving human bone cells, the bioactive and osteoconductive properties of the material were characterized in detail. The glass-ceramic material demonstrated good cell adherence and proliferation. After 21 days of storage in the cell medium, 94 % of the cells remained vital. The number of cells

exponentially increased on the sample surface over time. A cell density of $9*10^5$ cells per cm^2 was reached, which indicates a multi-layer growth process. To prove cell differentiation and/or the formation of a true cell phenotype, the content of intercellular, osteoblastic marker, alkaline phosphatase (ALP), was determined. After three weeks, the content was 0.82 units/10^6 cells.

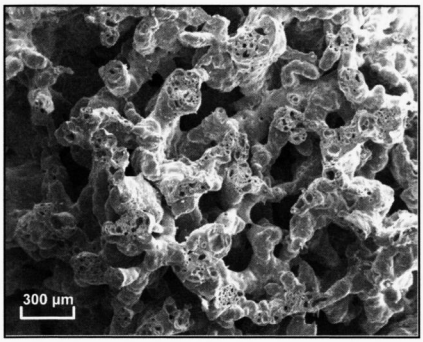

Fig. 5 Microstructure of the open-porous scaffold before immersion in SBF.

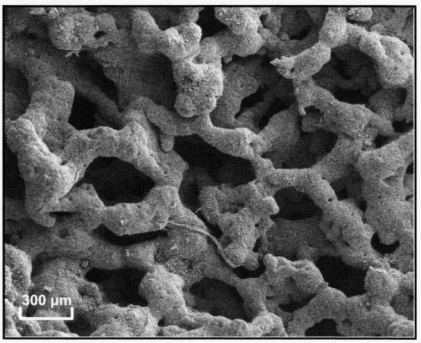

Fig 6. Microstructure of the open-porous scaffold after immersion in R-SBF for 10 days.

Conclusions

A glass-ceramic material based on a silicate glassy matrix and a crystalline phosphate phase (rhenanite) was crystallized and characterized. In SBF, the rhenanite monolithic glass-ceramic can precipitate a calcium phosphate layer within 48 hours, whose composition is similar to that of HA. By using a ceramic suspension, an open-porous, highly reactive scaffold was generated. First in-vitro investigations with human bone cells showed adherence, proliferation and the beginning of a differentiation of cells. Thus we conclude that the special combination of a crystalline phase and residual glassy matrix, in conjunction with the typical microstructure and surface reactivity, results in good bioactive properties.

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