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Conversion of borate-based glass scaffold to hydroxyapatite in a dilute phosphate solution*

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Abstract

Porous scaffolds of a borate-based glass (composition in mol%: $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $36B_2O_3$, $18SiO_2$, $2P_2O_5$), with interconnected porosity of ~70% and pores of size 200–500 μm, were prepared by a polymer foam replication technique. The degradation of the scaffolds and conversion to a hydroxyapatite-type material in a 0.02 M K_2HPO_4 solution (starting pH = 7.0) at 37 °C were studied by measuring the weight loss of the scaffolds, as well as the pH and the boron concentration of the solution. X-ray diffraction, scanning electronic microscopy and energy dispersive x-ray analysis showed that a hydroxyapatite-type material was formed on the glass surface within 7 days of immersion in the phosphate solution. Cellular response to the scaffolds was assessed using murine MLO-A5 cells, an osteogenic cell line. Scanning electron microscopy showed that the scaffolds supported cell attachment and proliferation during the 6 day incubation. The results indicate that this borate-based glass could provide a promising degradable scaffold material for bone tissue engineering applications.

1. Introduction

Tissue engineering provides an alternative way to regenerate diseased or damaged tissues back to their original state and function. In the bone tissue engineering approach, a porous material or scaffold serves as a substrate for cell attachment, proliferation and differentiation. Ideally, the scaffold should be biocompatible and biodegradable, and have a porous three-dimensional structure with the requisite mechanical properties [1].

Bioactive glasses have attractive properties for bone tissue engineering. They react with the body fluids to form a hydroxyapatite-type surface layer which is responsible for forming a strong bond with bone [2]. Since the discovery of 45S5 glass by Hench [3], most bioactive glass materials have been based on the silicate 45S5 composition. The conversion of silicate bioactive glass to hydroxyapatite (HA) in an aqueous phosphate solution involves a series of reactions, such as ion exchange reactions between the glass and the solution, leading to the formation of a SiO_2-rich layer on the glass surface, followed by the precipitation of an amorphous calcium phosphate layer on the SiO_2-rich layer, which eventually crystallizes to HA [4–6]. The formation of this HA layer is responsible for the strong bonding between the bioactive glass and surrounding bone and soft tissue, so the rate at which the bioactive glass converts to HA provides a criterion for evaluating the *in vitro* bioactivity of a material. There has been growing interest in the use of bioactive glass as scaffolds for bone tissue engineering [7–11]. However, despite their excellent bioactivity, the silicate-based bioactive glasses such

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as 45S5 and 13–93 convert slowly and incompletely to HA, so their degradation *in vitro* is limited [12].

Recent studies showed that the partial or complete replacement of SiO_2 in silicate 45S5 or 13–93 bioactive glass with B_2O_3 resulted in bioactive glass compositions with lower chemical durability, leading to faster and more complete conversion of the glass to HA. These borate-based glasses could be used as potential scaffold materials in bone repair, providing both controllable degradation and bioactivity [13, 14].

With an increase in the degradation rate of the bioactive glass, the rate at which the degradation products are released from the glass will increase, and the mechanism of converting the glass to HA could be different from that for the silicate-based bioactive glass. It is reasonable to assume that the more rapid degradation of the borate-based bioactive glass and its conversion to HA which starts at the surface of the glass will have a marked effect on the ability of the scaffolds to support cell proliferation, and therefore on the potential use of these scaffolds *in vivo*.

In the present study, a borate-based glass scaffold was prepared by a polymer foam replication method and evaluated *in vitro*. The conversion kinetics of the scaffold to HA in an aqueous phosphate solution was evaluated by measuring the weight loss of the scaffolds as a function of time. The degradation of the scaffolds during the conversion process was evaluated by measuring the pH value and boron concentration of the phosphate solution. Structural and compositional changes of the scaffold surface which resulted from the conversion of the glass to HA were studied using scanning electron microscopy (SEM), energy-dispersive x-ray (EDS) analysis and x-ray diffraction (XRD). The response of osteogenic cells to the scaffolds was studied to evaluate the biocompatibility of the scaffolds.

2. Experimental procedure

2.1. Preparation of glass scaffolds

Borate-based glass with the composition of $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $36B_2O_3$, $18SiO_2$, $2\ P_2O_5$ (mol %) was prepared by heating a mixture of Reagent grade $CaCO_3$, Na_2CO_3, $MgCO_3$, K_2CO_3, SiO_2, H_3BO_3 and $CaHPO_4{\bullet}2H_2O$ (Shanghai Chemical Reagent Distribution Co., Shanghai, China) in a Pt crucible for 2 h at 1150 °C to form a melt, and then quenching the melt between cold stainless steel plates to form glass frits. The glass frits were crushed and ground with a hardened steel mortar and pestle, and sieved through a stainless steel 270 mesh sieve to obtain particles of size $<53\ \mu$m.

Porous glass scaffolds with a microstructure similar to that of dry human trabecular bone were prepared using a polymer foam replication technique, as described in detail elsewhere [15]. Briefly, a mixture of 66.1 wt% glass powders, 31.2 wt% ethanol solution and 2.6 wt% ethyl-cellulose was ball milled to produce a homogenous slurry. Polyurethane foams (open porosity ∼50 pores per inch) were immersed in the slurry, in order to infiltrate the foams with the slurry. The particle-coated foams were dried for >24 h in air at room temperature, heated at 1.5 °C min^{-1} to 450 °C to burn off the polymeric foam and then heated for 2 h at 550 °C (heating rate = 2.5 °C min^{-1}) to sinter the particles into a dense, three-dimensional glass network.

2.2. Characterization of the scaffolds

The degradation of the scaffolds and their conversion to HA in a dilute phosphate solution (0.02 M K_2HPO_4) with a starting pH value of 7.0 at 37 °C were evaluated. A fixed ratio of the scaffold mass (1 g) to the volume of the solution (100 ml) was used in the experiments. After immersion for selected times (3 h, 24 h, 72 h, 168 h and 360 h), the scaffolds were removed from the solution, dried for 24 h at 90 °C and weighed. Since the conversion of the glass to HA was accompanied by a weight loss, the results of these weight loss experiments were used to evaluate the conversion kinetics of the scaffold to HA.

The conversion of the borate-based glass to HA is also accompanied by the dissolution of some ions from the glass into the phosphate solution. In these experiments, the pH and the boron concentration of the solution were measured as a function of time to evaluate the degradation of the scaffolds. Following the removal of the scaffolds for the weight loss measurements described above, the phosphate solution was cooled to room temperature and its pH was measured using a pH meter. The boron concentration of the phosphate solution was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optima 2100DV, USA).

The phase composition of the as-prepared scaffolds and the converted scaffolds immersed in the phosphate solution for selected times was determined using x-ray diffraction (XRD; D/max2550; Rigaku, USA). The scaffolds were ground into a powder and analyzed at a scan rate of 1.8° 2θ per minute using CuK_α radiation ($\lambda = 0.154\ 06$ nm). Scanning electron microscopy (SEM; Quanta 200 FEG; FEI Co., USA) was used to observe the microstructure of the as-prepared and converted scaffolds. Energy dispersive x-ray (EDS) analysis in the SEM was used to detect the elements present on the surface of the scaffold, and to determine the Ca/P atomic ratio.

2.3. Cell culture

The response of MLO-A5 cells, a well-characterized post-osteoblastic cell line [16] kindly provided by Professor Lynda F Bonewald, University of Missouri-Kansas City, was used to assess the biocompatibility of the scaffolds. The MLO-A5 cells were cultured in an α-MEM medium supplemented with 5% FCS and 5% newborn calf serum (NCS) plus 100 U ml^{-1} penicillin on a collagen-coated plate. All cell cultures were carried out at 37 °C in a humidified atmosphere of 5% CO_2 with the medium changed every 2 days. Dry heat sterilized scaffolds (10 mm in diameter × 5 mm) were seeded with 50 000 MLO-A5 cells suspended in 40 μl medium, incubated for 4 h to allow cell attachment and transferred to a 24-well culture plate containing 2 ml of complete medium per well. After 2, 4 and 6 days, the scaffolds were removed, washed with warm PBS, fixed and observed using SEM.

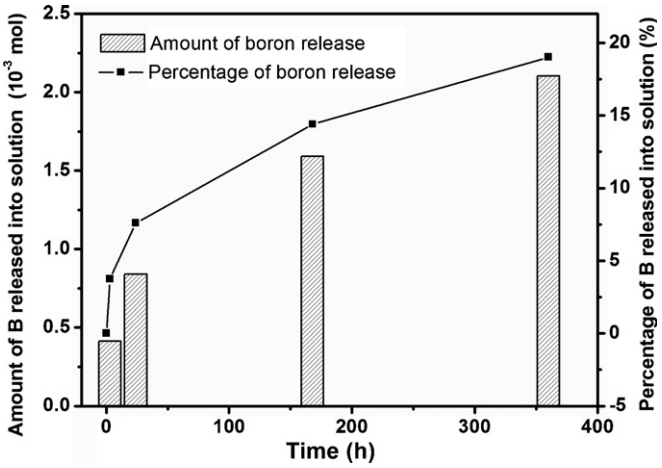

Figure 1. Effect of immersion time in the 0.02 M K_2HPO_4 solution on the degradation of borate-based bioactive glass scaffold. The actual amount of boron released from the scaffold into the solution, and the amount normalized to the boron content of the starting glass scaffold are plotted.

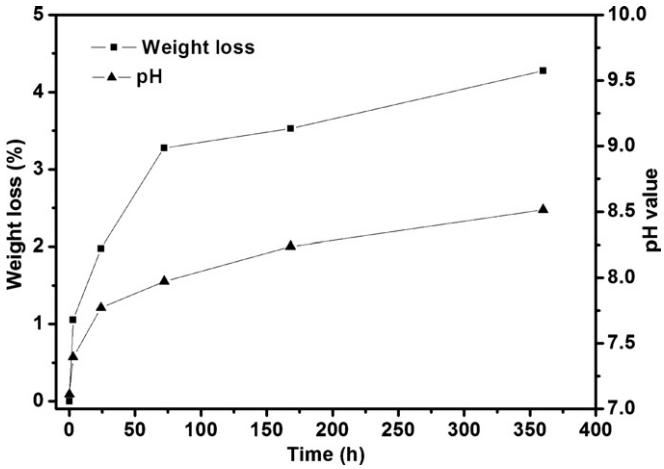

Figure 2. Weight loss of the borate-based glass scaffold and the pH value of the phosphate solution as a function of immersion time of the scaffold in the solution.

3. Results and discussion

3.1. Degradation of scaffolds and conversion to HA in a dilute phosphate solution

Figure 1 shows the amount of boron released into the solution after various immersion times of the borate-based glass scaffold in the 0.02 M K_2HPO_4 solution. Both the actual accumulated amount of released boron (mol) and the boron amount normalized to the total amount of boron in the as-prepared glass scaffold are plotted. The boron concentration increased more rapidly during the first 24 h of immersion, after which the concentration increased more slowly with increasing immersion time. At the end of the experiments (immersion time = 360 h), ∼20% of the boron content of the as-prepared glass had dissolved into the solution.

Because of its high B_2O_3 to SiO_2 content, the glass used in this work was composed mainly of a borate network, which is expected to be less durable than a silicate glass network. It is expected that the degradation of this borate-based glass would be controlled by the breakage of the borate network [17]. During the degradation process, diffusion of the borate ions from the glass into the phosphate solution is expected to be rapid, so the amount of boron in the solution can be used to monitor the degradation rate of the scaffold.

The degradation of the borate-based glass in the phosphate solution is also accompanied by the release of the glass modifiers, such as Na, K, Ca and Mg, and the conversion of the glass to a hydroxyapatite-type material [11, 13]. Because of the high solubility of sodium and potassium phosphates, the Na^+ and K^+ ions dissolved from the glass should remain as soluble ions in the phosphate solution. On the other hand, hydroxyapatite, $Ca_5(PO_4)_3(OH)$, the most stable calcium phosphate phase in aqueous solution at pH >4–4.5, has a low-solubility product at room temperature ($K_{SP} = 2.35 \times 10^{-59}$) [18], so the Ca^{2+} ions are incorporated in the precipitated hydroxyapatite-type material [13]. The concentration of Mg in the solution was not measured in these experiments. However, $Mg_3(PO_4)_2$ has a low solubility product ($K_{SP} = 1.0 \times 10^{-25}$) [19], and Mg^{2+} is isovalent with Ca^{2+}. Therefore, the Mg^{2+} ions could precipitate on the glass surface as a separate $Mg_3(PO_4)_2$ phase, or incorporated into the hydroxyapatite-type material as a solid solution. Previous work on the conversion of silicate 13–93 glass, with the composition of $53SiO_2$-$6Na_2O$-$12K_2O$-5MgO-20CaO-$4P_2O_5$ wt.%, showed the formation of a hydroxyapatite-type phase, with no evidence for the formation of a separate $Mg_3(PO_4)_2$ phase [7]. It is therefore likely that the Mg^{2+} ions are incorporated into an Mg-substituted hydroxyapatite phase.

In the degradation of the borate-based glass and its conversion to a hydroxyapatite-type material in the phosphate solution, borate ions are released into the solution, while phosphate ions are consumed from the solution. Since boric acid is weaker than phosphoric acid, this process coupled with the release of the alkali ions (Na^+ and K^+) from the glass leads to an increase in the pH of the solution. Figure 2 shows that the increase in the pH value of the phosphate solution versus immersion time followed a trend similar to that for the degradation of the scaffolds, as measured by the release of boron into the solution.

Since the degradation of the borate-based glass scaffolds and the conversion to form a hydroxyapatite-type material are controlled by essentially the same reactions, the conversion kinetics of the scaffold should show a trend similar to those for the boron release and the pH of the phosphate solution. The data for the weight loss of the scaffold versus immersion time (figure 2) show that this was indeed so. Figure 2 also shows that for an immersion time of 360 h, the weight loss is only ∼4%, although ∼20% of the boron present in the starting glass had dissolved into the solution (figure 1). The concentration of Si^{4+} in the solution was not measured. However, assuming that 20 wt% of the glass was converted to Mg-substituted hydroxyapatite, and that no SiO_2 in the glass was dissolved into the solution, the calculated weight loss is ∼7.5%. This calculated weight loss is not vastly different from the observed value.

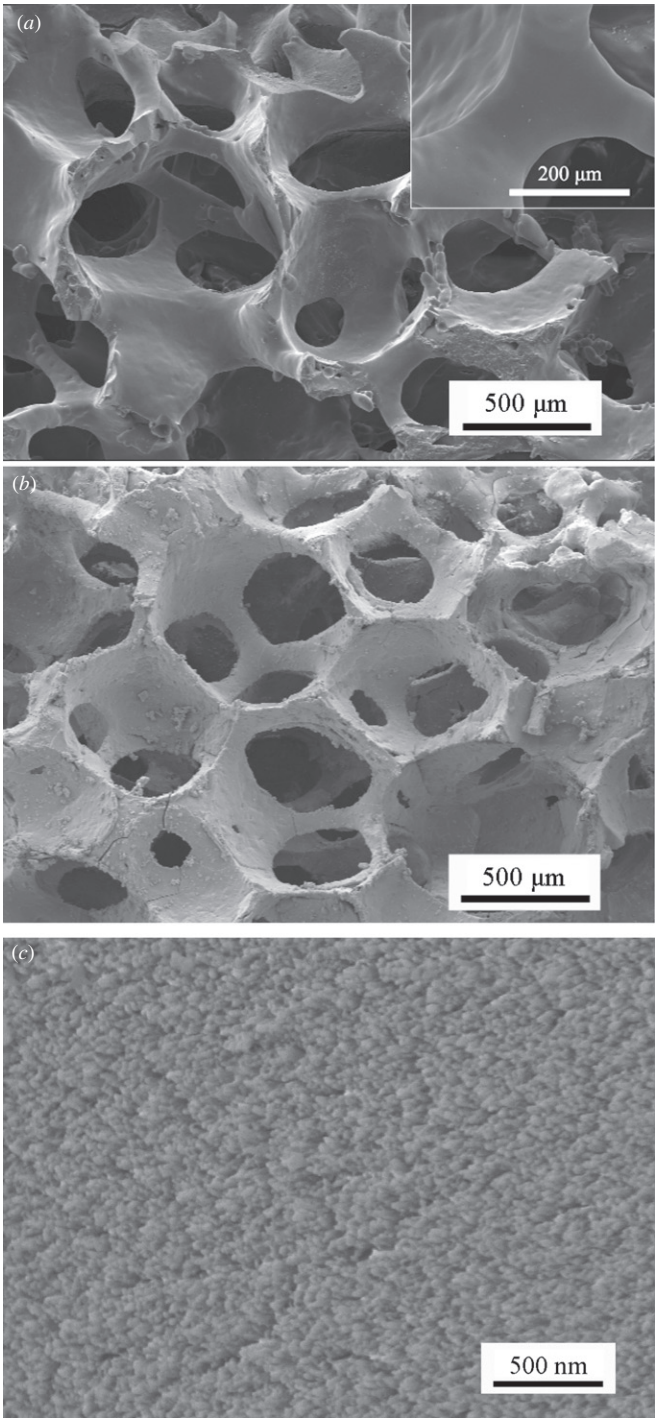

Figure 3. SEM images of (*a*) the as-prepared glass scaffold, and (*b*), (*c*) the scaffold after immersion in the aqueous phosphate solution for 360 h.

3.2. Microstructure and phase composition of converted scaffolds

SEM images of the as-prepared bioactive glass scaffolds and the scaffolds after immersion for 360 h in the phosphate solution are shown in figure 3. The as-prepared scaffolds (figure 3(*a*)) had a porosity of $= 70 \pm 3\%$, as measured by the Archimedes method, and pores of size 200–500 μm, as determined by SEM observations. These pore characteristics are considered to be favorable for supporting tissue ingrowth [20]. A higher magnification image of the as-prepared scaffold (figure 3(*a*), inset) showed that the solid struts of the scaffold were dense, with a fairly smooth surface typical of a glass. Immersion of the scaffolds in the phosphate solution resulted in the formation of a rougher surface of the solid network (figure 3(*b*)), which on observation at higher magnification, consisted of a nano-sized particulate structure (figure 3(*c*)).

EDS analysis of the surface of the as-prepared borate-based glass scaffold showed a Ca/P atomic ratio equal to 5.4, a value which was close to that (5.5) calculated from the nominal

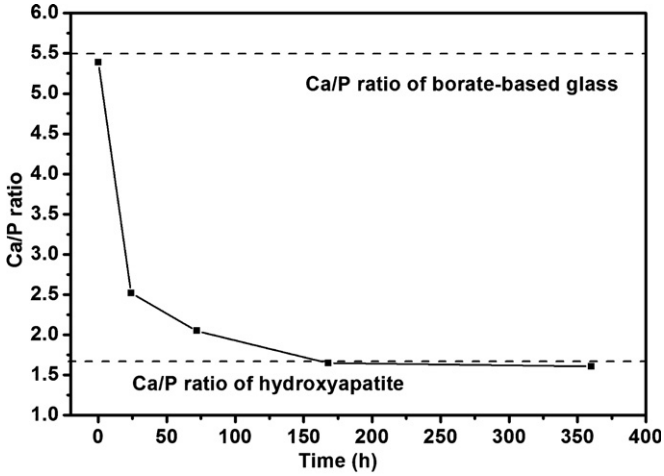

Figure 4. The calcium to phosphorus (Ca/P) atomic ratio for the conversion product formed on the surface of the borate-based bioactive glass scaffold as a function of immersion time of the scaffold in the phosphate solution.

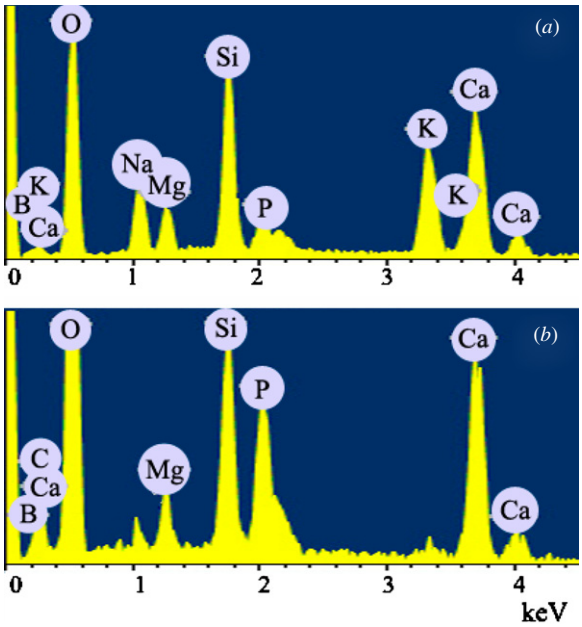

Figure 5. Energy dispersive x-ray (EDS) spectra of the surface of (*a*) the as-prepared borate-based bioactive glass scaffold, and (*b*) the scaffold after immersion for 168 h in the phosphate solution.
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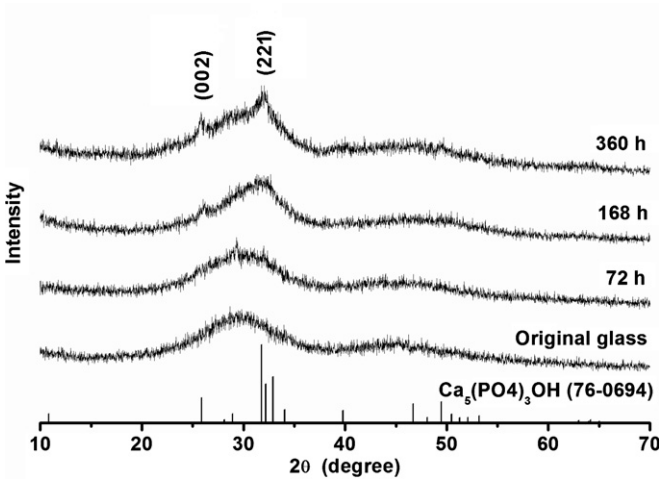

Figure 6. X-ray diffraction patterns of the as-prepared borate-based bioactive glass scaffold and the scaffold after immersion in the phosphate solution for the times shown.

composition of the starting glass. With increasing immersion time in the phosphate solution, the Ca/P ratio of the scaffold surface became smaller (figure 4), and after 168 h, it became nearly constant, with a value approximately equal to that of HA (1.67).

Figure 5 showed the EDS spectra of the surface of the as-prepared glass scaffold and the scaffold immersed for 168 h in the phosphate solution. The spectrum of the as-prepared glass (figure 5(*a*)) showed all the metallic and metalloid elements known to be present in the starting glass composition. The height of the P peak was much lower than that of the Ca peak. After immersion for 168 h (figure 5(*b*)), the height of the P peak increased markedly relative to the Ca peak, indicating that the Ca/P ratio was lower than the value for the as-prepared glass scaffold. Magnesium peaks were also found in the spectrum, which indicated the presence of Mg in the converted layer on the scaffold surface, or in the underlying unconverted layer. The absence of measurable potassium and sodium peaks in the EDS spectrum of the converted scaffold indicated that these elements had dissolved in the solution.

XRD patterns of the as-prepared glass scaffold and the scaffolds after immersion for various times in the phosphate solution are shown in figure 6. The pattern of the as-prepared glass showed a broad band typical of an amorphous material, and little change was observed after immersion for 72 h. However, after immersion for 168 h, a broad peak centered around the (2 2 1) reflection for a reference hydroxyapatite (JCPDS 76-0694) was observed, and a smaller peak centered at the (0 0 2) reflection began to protrude above the background. These peaks became more distinguishable in the pattern for an immersion time of 360 h. The low intensity of the peaks above the background, coupled with the broadness of the peaks, might indicate only limited conversion of the glass scaffold to a (crystalline) hydroxyapatite material or the formation of a poorly crystallized hydroxyapatite-type material.

3.3. Cell attachment and proliferation on scaffolds

Figure 7 shows SEM images of the morphology of MLO-A5 cells grown on the surface of the borate-based glass scaffolds for 2, 4 and 6 days. The cells were well spread at all three culture intervals, with numerous projections, and they appeared to be well attached to the surface of the scaffolds. The number of cells on the scaffold increased as a function of culture time, indicating the ability of the scaffolds to support cell proliferation.

Based on the degradation of the scaffolds in the K_2HPO_4 solution (figures 1 and 2), the fastest degradation of the scaffolds and release of ions into the culture media is expected to occur at early culture times (1–2 days). Cellular response to the high concentration of degradation products, such as borate ions, would therefore be most important at early incubation times. These results indicate that the cells can survive the degradation process, and that the scaffolds provide a favorable substrate for supporting the attachment and proliferation of osteogenic cells.

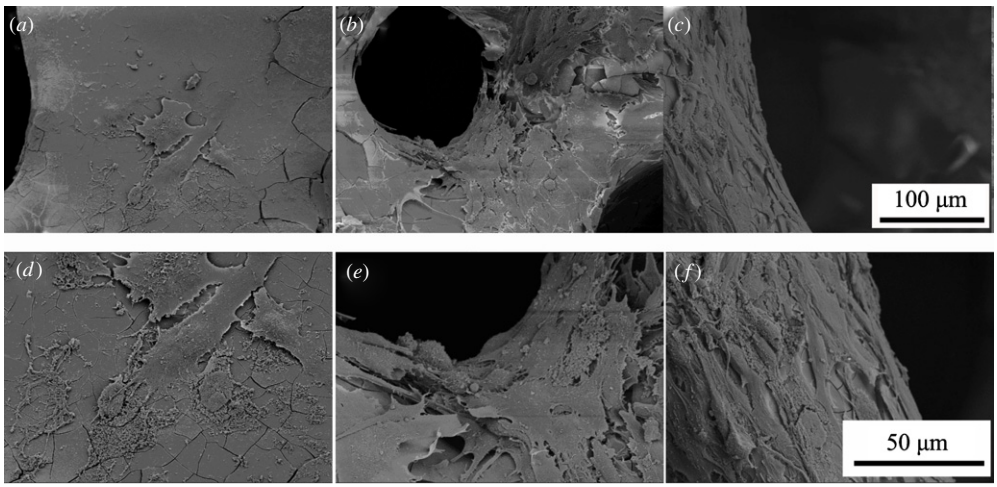

Figure 7. SEM images of borate-based bioactive glass scaffolds seeded with MLO-A5 cells and cultured for (*a*), (*d*) 2 days; (*b*), (*e*) 4 days and (*c*), (*f*) 6 days.

4. Conclusions

Borate-based bioactive glass scaffolds (porosity ≈ 70%; pore size = 200–500 μm), with a microstructure similar to that of dry human trabecular bone, were prepared using a polymer foam replication method and evaluated *in vitro*. Immersion of the scaffolds in the 0.02 M K_2HPO_4 solution resulted in the degradation of the scaffolds and conversion to a hydroxyapatite-type material. The amount of boron released from the scaffold into the solution increased rapidly during the first 24 h, reaching a value equal to ∼20% of the boron content of the starting glass scaffold after an immersion time of 360 h. The conversion reaction in the phosphate solution resulted in the formation of an amorphous calcium phosphate material on the surface of the scaffolds, which started to crystallize to a hydroxyapatite-type material within an immersion time of 7 days. The borate-based bioactive glass scaffolds provided a favorable substrate for the attachment and proliferation of osteogenic MLO-A5 cells.

Acknowledgments

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