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The effect of residual glassy phase in a bioactive glass-ceramic on the formation of its surface apatite layer *in vitro*

P. LI *, Q. YANG, F. ZHANG
Department of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China

T. KOKUBO
Institute for Chemical Research, Kyoto University, Uji, Kyoto-fu 611, Japan

A bioactive glass containing (in wt %) SiO_2 48, P_2O_5 9.5, Na_2O 20 and CaO 22.5 was transformed into a glass-ceramic through a heat treatment. The apatite formation on the surface of this glass-ceramic was examined in a simulated physiological solution. The data from X-ray diffraction, infrared reflection spectroscopy, scanning electron microscopy together with energy-dispersive X-ray analysis and composition imaging of backscattered electrons showed that the formation of the surface apatite layer depends on the relative amount of residual glassy phase in the glass-ceramic. The apatite layer was found to form *in vitro* on its surface if the glass-ceramic contained a residual glassy phase in a relative proportion more than a limiting volume. It lay on a layer rich in silica. However, only, a silica-rich layer was developed within the surface region of the glass-ceramic during the interaction with solution if the glass was almost completely crystallized. It is proposed that the apatite formation on the surface of the glass-ceramic is mainly caused by its residual glass. The residual glass facilitating apatite formation is considered to provide a negatively charged surface developed during its corrosion in the surrounding solution. The negatively charged surface attracts calcium ions and creates a solution within the glass–solution interface that is highly supersaturated with respect to hydroxyapatite. This leads to the formation of apatite on the surface of the glass-ceramic.

1. Introduction

Since Hench *et al.* [1] discovered a group of special glasses based on the 45S5 Bioglass to bond with bone, other glasses and glass-ceramics differing in composition and mechanical properties have been developed to adhere directly to bone tissue [2]. The common characteristic of these bioactive glasses and glass-ceramics is the formation of a biologically active apatite layer which provides the bonding interface [3]. It is concluded that a prerequisite for glasses and glass-ceramics to bond to living bone is the formation of an apatite layer on their surface in the body [4].

Many efforts have been made to understand the formation of apatite on bioactive ceramics, especially on bioactive glasses. Several explanations have been presented [5–8]. However, a full understanding has not yet been reached. We previously proposed that the negative charged surface of bioactive glasses developed during their corrosion in the surrounding solution plays an important role in the formation of the apatite layer [9]. Because of the Coulomb force, calcium ions are attracted by the surface charged negatively and accumulate in the glass–solution interface. The solution located in the interface approaches a supersaturated one with respect to hydroxyapatite as the release of phosphorous ionic species from the glasses proceeds. Therefore, apatite deposits on their surface. The relation of charged surface to the structure of its bulk phase leads to expectation that the formation of the apatite layer is probably affected by the crystallization of glass. As expected, it was confirmed recently that the formation of the apatite layer can be prevented when a bioactive glass is completely crystallized [10].

The surface change of bioactive glass-ceramics *in vivo* and *in vitro* is more complex than that of single-phase bioactive glasses by virtue of their multiphase. Only a few papers [6, 11] involved the effect of crystallization of bioactive glasses on the apatite formation on their surfaces. The effect of the relative proportion of glass matrix to crystal phases on bioactivity are unknown as a whole [6].

It was the objective of the present work to study the effect of glassy residue of a glass-ceramic on apatite

* *Present address:* Dept of Biomaterials, University of Leiden, Building 55, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

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formation *in vitro*. A discussion based on the viewpoint of the charged surface via the structure of its bulk phase is also within the scope of this paper.

2. Experimental

The preparation of a bioactive glass, referred to as II-3BG, whose composition was (in wt %) SiO_2 48, P_2O_5 9.5, Na_2O 20 and CaO 22.5, was described in detail in [12]. A rectangular specimen 15 mm × 10 mm × 3 mm polished with cerium oxide underwent heat treatment to give the bioactive glass-ceramic which is termed II-3BGC. The treatment was carried out in a SiC furnace. The samples were heated at 5 °C min^{-1} to 670 °C and held for nucleation, and then heated further to 750 °C for crystallization. Finally, the samples were cooled to room temperature in the furnace. The processes of nucleation and crystallization lasting for different periods to obtain various amounts of glassy residue are shown in Table I.

The fresh surface layer of a monolithic II-3BGC sample was identified structurally by X-ray diffraction (XRD) analysis. The monolithic sample was crushed and powder XRD analysis is done to examine any possible orientation of crystallization on the surface. The content of glassy phase within a fresh surface layer of a sample was determined semiquantitatively by the ratio of the sum of its integrated intensity of all peaks to that of the reference, a sample considered to be completely crystallized.

The synthetic physiological solution was a Tris-hydroxymethylaminomethane and hydrochloric acid buffer solution with pH 7.4 at 37 °C, and is termed TBS. The TBS was free of Ca and P ionic species. Each sample of size mentioned above was placed in 40 ml TBS which was enclosed in a covered Teflon cup. The cup was kept at 40 °C in a water bath. The weight of a specimen decreases during the interaction. This was measured with a balance at certain reaction times. The II-3BGC samples were removed and washed with acetone after their weight became constant in TBS. The reaction time for II-3BG was 10 h and the reacted sample was also washed with acetone after it was removed from the TBS and dried at room temperature. The surface reacted layers of specimens were characterized by XRD, infrared reflection spectroscopy (IRRS), scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDX) and composition imaging of back-scattered electrons. The solution was analysed with atomic adsorption spectroscopy (AAS) and inductive coupled plasma (ICP) for the concentrations of Ca, Na, P and Si.

3. Results

The XRD analysis showed that all II-3BGC specimens were mainly constituted of three phases: sodium calcium silicate ($Na_2CaSi_3O_8$, or N_2CS_3), an apatitic compound with probable formula $Ca_{10}(PO_4)_6(O, (OH)_2)$ and a residual glassy phase. The XRD pattern of a monolithic sample was not different from that of its powder sample. Hence, no favourable orientation of crystallites was presented and crystallization was considered to be uniform in II-3BGC. The relative percentage of glassy phase in II-3BGC was related to the experience of heat treatment. The XRD patterns of II-3BGC1 and II-3BGC4 in Fig. 1 reveal the discernible difference in the strength of their corresponding diffraction peaks. However, no distinction was observed in the XRD patterns of those specimens which underwent nucleation and crystallization for 5 h or more. These data are not presented here. Hence, II-3BGC which experienced a nucleation stage of 5 h followed by another 5 h crystallization was considered to be a completely crystallized material. It is treated as a reference for semiquantitative determination of the content of the residual glassy phase. The relative content of glassy phase in various specimens are given in Table I.

These data are approximate, but they do satisfy the object of this work. At one extreme is the interaction of bioactive glass II-3BG with TBS. It was proven earlier that an apatite layer forms on the surface of this glass at the very beginning of the reaction, long before the saturation of the solution with apatite [12]. The data concerning the surface change of II-3BG after interaction with TBS for 10 h are shown in Figs 2, 3 and 5, below. It is clear that apatite forms on the silica-rich layer which was developed first during the interaction. A similar Ca and P film is observed on the surfaces of glass-ceramics II-3BGC1 and II-3BGC2 in Fig. 2. The structure of the film was characterized by XRD. The results are shown in Fig. 3. The indistinct peaks of apatite become stronger than those of fresh samples in Fig. 1, whereas the peaks corresponding to sodium calcium silicate (N_2CS_3) are weakened. This

TABLE I

	Heat treatment[a]		Glassy phase (wt %)	Reaction time (h)	Concentration of TBS (p.p.m.)				Surface layer
	t_N (min)	t_C (min)			Ca	P	Si	Na	
II-3BG			100	10	20	4	15	23	Apatite
II-3BGC1	0	0	38	126	47	6	62	85	Apatite
II-3BGC2	15	15	11	101	44	5	62	84	Apatite
II-3BGC3	60	60	5	140	37	5	56	70	SiO_2-rich
II-3BGC4	180	180	< 1	154	36	5	52	70	SiO_2-rich

[a] t_N Time for nucleation and t_C time for crystalization.

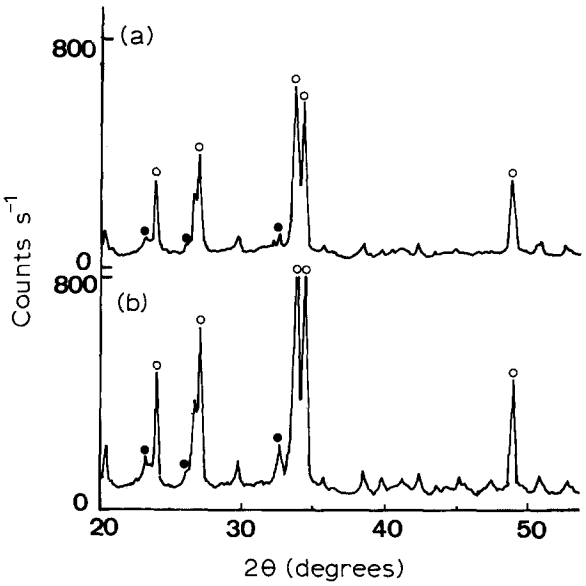

Figure 1 XRD patterns (CuK_α radiation) of surface layers of (a) II-3BGC1 and (b) II-3BGC4 before their interaction with TBS. (○) N_2CS_3 and (●) apatite.

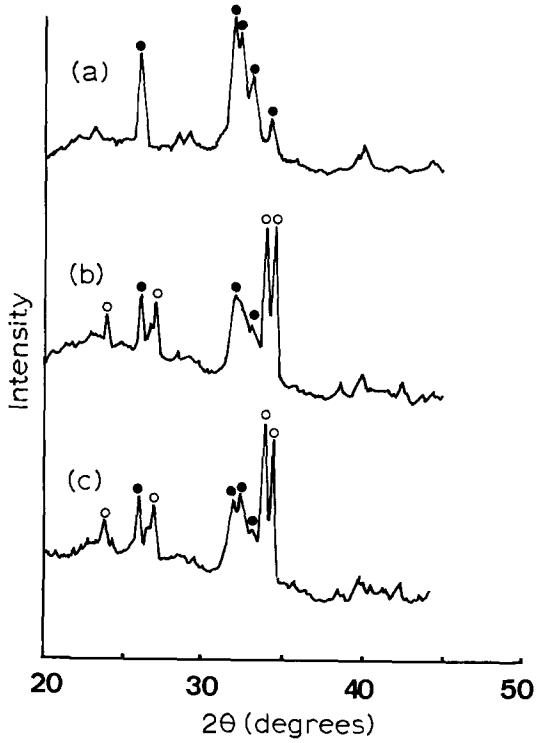

Figure 3 XRD patterns (CuK_α radiation) of surface layer of (a) II-3BG, (b) II-3BGC1 and (c) II-3BGC2 after their interaction with TBS for 10 h, 126 h and 101 h respectively. (○) N_2CS_3 and (●) apatite.

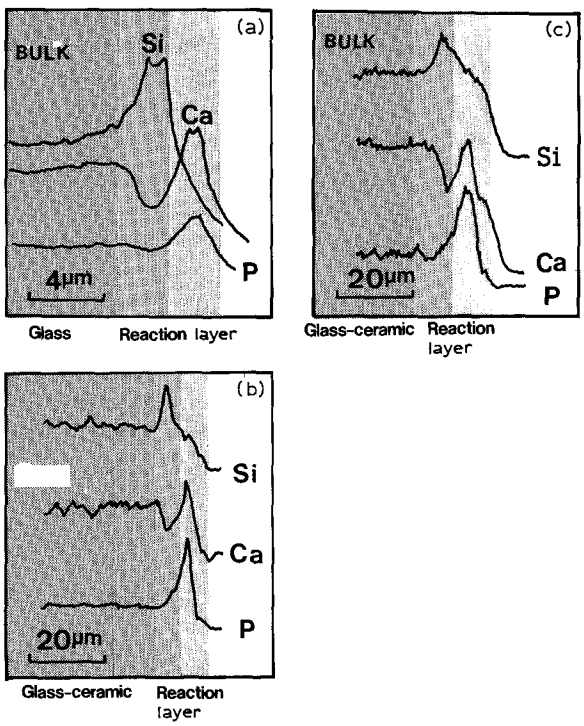

Figure 2 Composition profile in depth of reaction layer of (a) II-3BG, (b) II-3BGC1 and (c) II-3BGC2 after their interaction with TBS for 10 h, 126 h and 101 h, respectively, obtained by SEM-EDX.

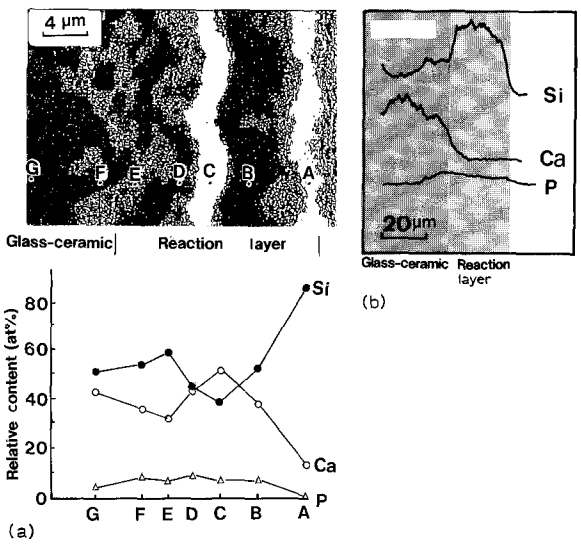

Figure 4 (a) In-depth composition image of backscattered electrons for reaction layer of II-3BGC3 after its reaction with TBS for 140 h (top) and element composition of points indicated in the top image, given by EDX analysis (bottom). (b) In-depth compositional profile of surface layer of II-3BGC4 after its reaction with TBS for 154 h, obtained by SEM-EDX.

indicates that the apatite forms on the surfaces of II-3BGC1 and II-3BGC2. In contrast, no apatite is shown in Fig. 4 to form on the surfaces of II-3BGC3 and II-3BGC4 under the same reaction conditions for II-3BG, II-3BGC1 and II-3BGC2. Only a silica-rich layer forms on the surface of II-3BGC3 and II-3BGC4. This result is further supported by the IRRS observation shown in Fig. 5. No innate bands of apatite and other calcium phosphorous compounds appear in the spectra. Only strong peaks contributed to the vibration of Si–O–Si are shown. The concentrations of Na, Ca, Si and P in the reacted solution measured by AAS and ICP are presented in Table I. Although the dissolution of the materials in TBS decreases with the transformation of their glassy phase to crystallite, there is no direct relationship between their solubility and the apatite formation. The concentrations of Ca and P in TBS were only 20 and 4 p.p.m., respectively, when apatite formed on the surface of II-3BG. These are much lower than those in TBS reacted with II-3BGC3 and II-3BGC4. The latter two materials cannot induce apatite formation on their surfaces.

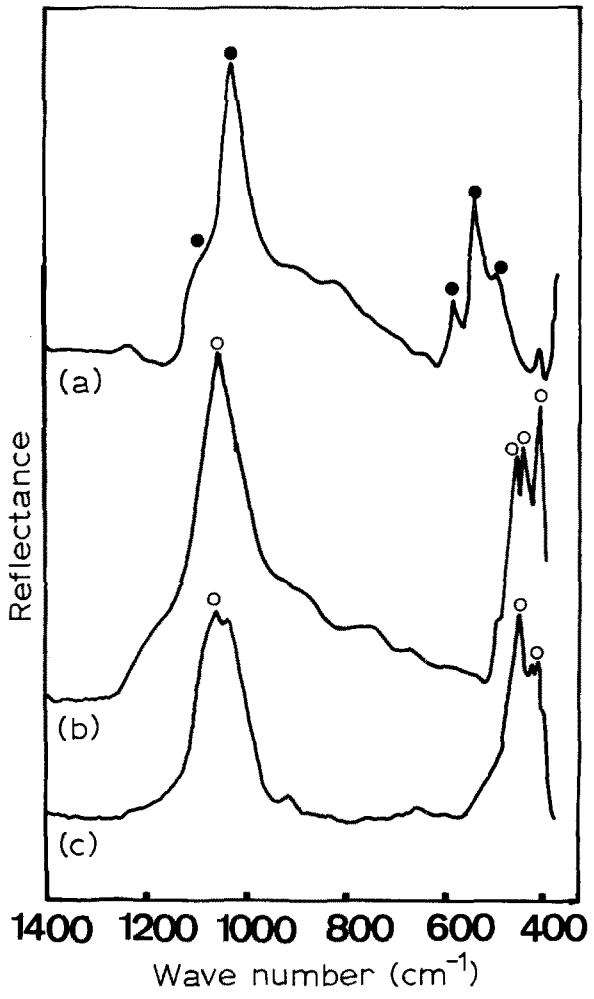

Figure 5 IRRS spectra of surface layers of (a) II-3BG, (b) II-3BGC3 and (c) II-3BGC4 after their interaction with TBS for 10 h, 140 h and 154 h, respectively. (○) Si–O–Si and (●) PO_4^{3-} (hydroxyapatite).

In fact, it was confirmed previously that apatite forms on the surface of II-3BG in the first 5 h of its immersion in TBS when the concentrations of Ca and P were only 10 and 3 p.p.m., respectively. Therefore, it can be concluded that the apatite formation on the surfaces of II-3 glass-ceramics depends on their glassy content, and this cannot be simply attributed to the change in the dissolution of materials caused by their crystallization.

4. Discussion

We examined the behaviour of a bioactive glass in TBS and proposed early that the electrical double layer developed during the interaction of the glass with the solution plays a key role in the formation of surface apatite [9, 12]. The surfaces of bioactive glasses are charged negatively when their ionic exchange with the surrounding solution proceeds. This negatively charged surface attracts Ca ions and causes their accumulation within the glass–solution interface. It was deduced that the degree of accumulation of Ca ions is directly proportional to the magnitude of the surface negative potentials. The solution in the vicinity of a negatively charged surface becomes supersaturated with respect to hydroxyapatite with the sequential liberation of Ca and P ionic species from the glasses, and apatite deposits on their surfaces. Fig. 6 shows schematically the apatite formation on the surface of a bioactive glass.

It was determined that sodium calcium silicate (N_2CS_3) is constructed with separated anionic group $Si_2O_7^{6-}$ or $Si_3O_9^{6-}$ [14]. These groups are small in size and easily liberated into the solution. The release of anionic groups leaves a positive charge on the surface of N_2CS_3. This counteracts the negative charge generated by the leaching of Na^+ and Ca^{2+} into the solution during the dissolution of N_2CS_3. On the other hand, the leaching of Na^+ and Ca^{2+} could be hampered by the crystallization. This is supported by data in Table I, showing that the Na and Ca concentrations in TBS decrease with the increase of relative crystallite content, but the silica concentration is basically unchanged. Hence, N_2CS_3 possesses a higher point of zero charge (p.z.c.) and lower magnitude of surface potential than the glass with the same formula. This suppresses the apatite formation on the surface of N_2CS_3.

The published data on the p.z.c. of hydroxyapatite ranging from about pH 7 to 8 [15–17] support the assumption that the surface potential of apatite in TBS is not high enough to create the interfacial solution with supersaturated degree required for new apatite to deposit on its surface. Therefore, only the residual glassy phase of the glass-ceramic can promote the apatite formation due to its negatively charged surface.

Kokubo and co-workers confirmed that the apatite formation on the surface of their A–W bioactive glass-ceramic containing apatite (A), wollastonite (W) and 28 wt % glassy matrix [4] cannot be attributed to apatite phase within A–W. The apatite formation was totally suppressed with the addition of small amount of Al_2O_3 and TiO_2 in the A–W mother glass which could totally enter into the residual glassy matrix during subsequent heat treatment of A–W glass [4, 18]. This indicates that wollastonite cannot induce apatite formation either. In other words, the genesis of apatite comes from the glassy matrix. Similar results were reported, showing that ion exchange of glassy phase in the glass-ceramic ceravital with surrounding tissue leads to its bioactivity [19, 20].

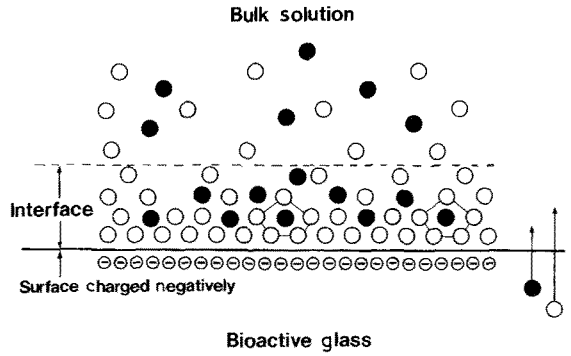

Figure 6 Schematic diagram of apatite formation on the surface of a bioactive glass caused by its negatively charged surface which attracts calcium ions within the interface: (○) Ca^{2+} and (●) P ionic species.

5. Conclusion

The formation of an apatite layer on the surface of glass-ceramic II-3BGC in a simulated physiological solution is dependent on the relative content of its residual glassy phase. It can be completely inhibited when the relative percentage of glassy phase is 5 wt % or less. Sodium calcium silicate and apatite existing in the glass-ceramic cannot stimulate the formation of new apatite. The residual glassy phase facilitating apatite formation probably provides a negatively charged surface which builds the solution within the solid–solution interface to a supersaturated level high enough for apatite to form on the surface of glass-ceramic.

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