Article ID: 1000-324X(2021)09-1006-07 **DOI:** 10.15541/jim20210030

In vitro Mineralization Property of Borosilicate Bioactive Glass under DC Electric Field

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Abstract: Borosilicate bioactive glass has excellent bioactivity and bone conductivity, but most bioactive glasses exhibit nonlinear degradation and mineralization behavior, with mineralization property declining over time. The direct current (DC) electric field, as an outfield-assisted approach to regulation, can interfere in the ion exchange and diffusion of the glass to modify its property. In this study, a DC electric field is used to intervene *in vitro* mineralization of borosilicate bioactive glass to accelerate the bioactivity in the slower degradation phase. Borosilicate bioactive glass with the composition of $18SiO_2$-$6Na_2O$-$8K_2O$-8MgO-22CaO-$2P_2O_5$-$36B_2O_3$, prepared by the melting method, was immersed in simulated body fluid (SBF). A current in range of 0−90 mA was applied to study the effect of DC electric field on the degradation and *in vitro* mineralization property of borosilicate bioactive glass. The results show that the application of electric field increases the degradation rate and ion release of borosilicate bioactive glass. Compared to the control group (without electric field), the weight loss rate increased by 3%–5% and the dissolution of B and Ca ions increased by 2.3–2.9 and 1.9–2.3 times, respectively. Meanwhile, the electric field assists glass network hydrolysis and surface hydroxylation, accelerating the generation of hydroxyapatite (HA). Analyzing the surface structure of the borosilicate bioactive glass particles, we found that an apatite layer was formed on the surface of the sample exposed to the electric field. Hence, the application of a DC electric field can improve the degradation and *in vitro* mineralization property of bioactive glass, providing a new idea for bone repair effect enhancement.

Key words: borosilicate bioactive glass; DC electric field; mineralization property

Bioactive glasses (BGs) are considered as 3rd generation materials, which exhibit excellent biocompatibility, bioactivity, and osteoinductive activity. BG's degradation products can promote growth factor production, cell proliferation, osteogenic and angiogenic gene expression, and thus enhance bone tissue regeneration[1]. These unique features make BGs one of the most promising artificial bone repair materials. Since their invention by Prof. Larry L. Hench in the early 1970s and their first clinical use in the mid-1980s, various forms of BGs-based bone substitutes have been developed, including porous scaffolds, bone cements, and thin films[2-5]. However, the problems associated with BGs, such as their intrinsic brittleness, poor formability, and decreased fracture resistance, limit their application as bone grafts to treat bone defects. These limitations can be overcome by combining BGs with biopolymers. Such composites have been proven to exhibit improved mechanical and biological property, comparing to property possessed by individual materials[6]. However, most BGs/polymer composites suffer from limited bioactivity and are therefore usually used in combination with osteoinductive supplements[7]. Furthermore, most bioactive glasses exhibit nonlinear degradation and mineralization behavior, in which a high initial dissolution rate followed by significantly slower dissolution is usually observed. In particular, the formation of hydroxyapatite layer on the surface of BGs when exposed to biological fluids hinders further release of the ions from BGs.

It has been widely accepted that the interaction of BG

Received date: 2021-01-15; **Revised date:** 2021-02-08; **Published online:** 2021-03-12
Foundation item: National Key Research and Development Projects (2018YFC1106300); National Natural Science Foundation of China (51972232)
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surfaces with body fluids starts with an ion exchange of alkali and alkaline-earth ions with protons from body fluid, which leads to an increase of pH of the medium, followed by the formation of a silica-rich layer and then by the growth of a calcium-phosphate rich layer on the BG surface[8]. Therefore, it is anticipated that various external stimuli which can accelerate the ion exchange, and enhance the bioactivity of BGs/polymer composites. As a commonly external stimulation, electric field has been widely used to assist ion exchange and diffusion processes to modify the optical, electrical, mechanical, and biological property of glass[9]. Takamure, *et al*[9] applied an assisted electric field of 1500 V to the glass to accelerate the ion exchange between Na and Ag to introduce Ag into sodium-calcium silicate glass. Additionally, electric field application leads to a net negative charge at the glass surface due to the formation of positive ion depletion layers, which facilities the deposition of calcium-phosphate rich layer[10]. Besides, electrical stimulation is predominantly used as a physical therapy modality to promote the repair and regeneration of damaged tissues. Hence, we anticipate that the electric field stimulation would accelerate the degradation and mineralization of the bioactive glasses, particularly in the stages where the degradation rate is slow.

To demonstrate the hypothesis, the *in vitro* degradation, mineralization, and ion release behavior of the melt-derived borosilicate bioactive glass particles were investigated under an external DC electric field with a lower current. As a comparatively new bioactive glass class, borate-based bioactive glasses have received considerable interest due to their controlled and complete degradation behavior[11]. Notably, boron (B) plays an essential role in angiogenesis. Therefore, the controlled and localized release of B ions from BGs can provide a promising therapeutic alternative for regenerative medicine of vascularized tissues[12-14]. Our results indicated that the application of an electric field provides an additional driving force to promote the ion exchange reaction between the glass and SBF, degradation of the glass, and nucleation of apatite, and thereby enhancing the bioactivity of the bioactive glass. Consequently, the present study provides a viable strategy for tailoring the ion release behavior and enhancing bioactivity to match specific requirements.

1 Materials and methods

1.1 Materials

Boric acid (H_3BO_3), calcium carbonate ($CaCO_3$), silicon dioxide (SiO_2), sodium carbonate anhydrous (Na_2CO_3), potassium carbonate (K_2CO_3), magnesoum carbonate hydroxide ($(MgCO_3)_4{\cdot}Mg(OH)_2{\cdot}5H_2O$), ammonium dihydrogen phosphate ($NH_4H_2PO_4$) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All chemical reagents were chemical grade.

1.2 Preparation of borosilicate glass particles

A borosilicate bioactive glass composition was selected as $18SiO_2$-$6Na_2O$-$8K_2O$-8MgO-22CaO- $2P_2O_5$-$36B_2O_3$[15]. This component has better bioactivity, and ensures certain biological safety, which has a better application prospect[16-18].

Glass samples were prepared by melting reagent grade chemicals in a Pt crucible at 1150 ℃ for 1 h, and the melt was poured onto a cold stainless steel plate. The obtained glass was crushed and sieved to give particles sizes in the range of 0.15–3.00 mm.

1.3 *In vitro* mineralization of borosilicate bioactive glass

Simulated body fluid (SBF) with ion concentration close to that of human plasma was configured according to the method described by Kokubo[19] (σ_{SBF}=20 $S{\cdot}m^{-1}$, at 37 ℃). The borosilicate glass particles were immersed in SBF with a solid/liquid ratio of 0.02 $g{\cdot}mL^{-1}$ at 37 ℃, where the glass particles were placed uniformly at the bottom of the container holding the SBF. Two platinum electrodes of 20 mm×15 mm were immersed in the SBF. A DC adjustable power supply (PS-6402M) was used to allow the current to flow through the solution. Controlling the currents and loading time, 30, 60, and 90 mA were applied respectively for 1 h per day, up to 14 d, where the voltages were 3–7 V. The sample without an electric field was defined as the control group. The experimental setup is shown in Fig. 1, where the two platinum electrodes are spaced 35 mm apart.

The conversion of borosilicate glasses to hydroxyapatite (HA) in the SBF is accompanied by a decrease in the mass of the glass. After each immersion, the samples

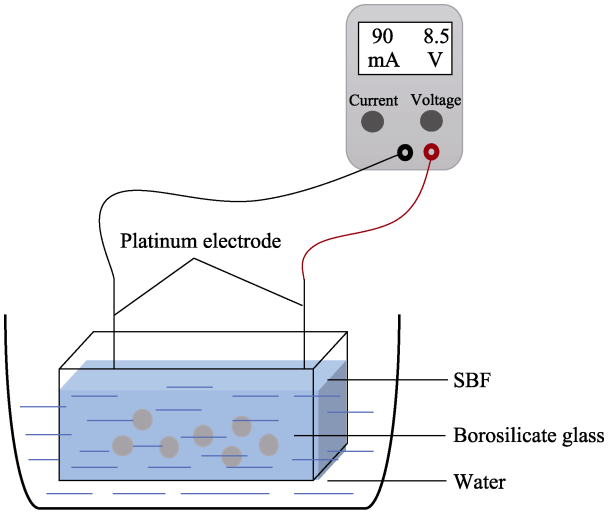

Fig. 1　Scheme of DC electric field

were removed from SBF, washed with deionized water, and dried at 90 ℃. The weight loss (α) was calculated according to Eq. (1),

$$\alpha = \frac{W_0 - W}{W_0} \times 100\% \quad (1)$$

where W is the mass of the collected sample and W_0 is the initial mass of the glass particles. The weight loss was measured after removing the sample from the SBF, washing, and drying with deionized water in order to keep the precision.

The concentrations of the ions released from the borosilicate bioactive glass particles during degradation were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Optima 2100DV, USA). The structure of glass was determined by Fourier Transform Infrared Spectrometer (FTIR, EQUINOX 55) and X-ray photoelectron spectroscope (XPS, Thermo ESCALAB 250XI). The formation of calcium-phosphate rich layer on glass surfaces was determined by X-ray diffraction (XRD, Smartlab9, Rigaku Co., Japan) and a field emission scanning electron microscope (FESEM, Quanta200FEG).

2 Results and discussion

2.1 Effect of DC field on the degradation rate of borosilicate glass

During the immersion in SBF, the borosilicate bioactive glass particles were attacked by the surrounding environment, and the network-modifying ions were leached out from the glass, which led to structural loosening. B and Si ions, the network formers, were continuously leached out as well. As a result, the glass particles gradually degraded, accompanied by weight loss. The weight loss of the glass particles increased apparently with time (Fig. 2(a)), indicating that the borosilicate glass continuously degraded in SBF during 3 d of immersion. Furthermore, the application of DC field promoted the degradation of the glass particles. The total weight loss increased by 3%–5% compared to the case without the electric field. The weight loss of the glass particles reached a maximum when a current of 60 mA was applied, and further increase of the applied current to 90 mA led to a slight decrease of the weight loss. The increased release of Ca^{2+} caused faster precipitation of the reactive layer, in turn hindering further release of ions from the glass particles.

The ions released from the glass particles were quantified by ICP-OES (Fig. 2(b)). As expected, the dissolution rate of B and Ca ions is substantially enhanced when various magnitudes of current flew through the

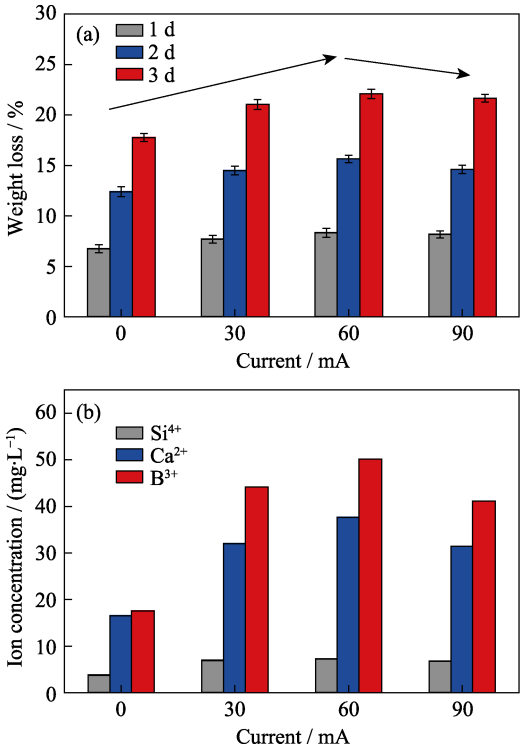

Fig. 2 Weight loss (a) and comparison of cumulative ion concentration (b) of borosilicate bioactive glass immersed in SBF with different currents applied for 3 d at 37 ℃

SBF, comparing to the control group. Besides, the current of 60 mA exhibited the most pronounced enhancement effect, and in this case the cumulative amount of B and Ca ions released increased ~3 and 2.3 times, respectively. While released Si ions was relatively low, its cumulative released amount still increased by two times. It appears to be evident that the external electric field acts as an additional driving force to assist the concentration gradient-driven ion exchange of alkali and alkaline-earth ions from the glass with the proton ions from the SBF, and thus accelerates the degradation and ion release of the borosilicate bioactive glass.

2.2 Effect of DC field on *in vitro* mineralization of the borosilicate glass particles

The effect of electric field on the *in vitro* mineralization behavior of the borosilicate bioactive glass was assessed by immersion in SBF and evaluation of the HA forming ability. The phase composition of the mineralized products was analyzed by XRD after 14 d of immersion in SBF. As shown in Fig. 3, the sample without application of electric field presents a typical amorphous diffraction pattern, presumably because only small amount of reaction products was formed. Upon application of electric field for 1 h per day, the diffraction peaks corresponding to standard HA $Ca_{10}(PO_4)_6(OH)_2$ (JCPDS 72-1243) were clearly detected, suggesting electric field apparently promoted the conversion of glass to

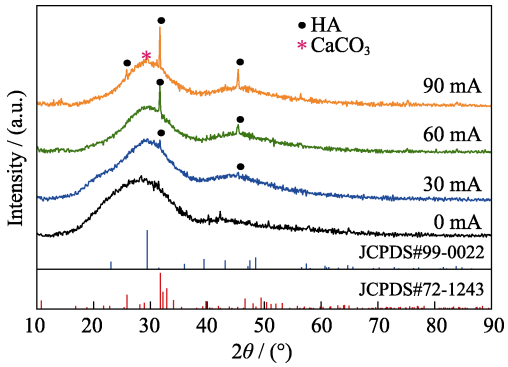

Fig. 3　XRD patterns of bioactive glass immersed in SBF with an applied electric field for 14 d

HA. The HA diffraction peak intensity rose with applied current increasing from 30 to 90 mA, indicating a more pronounced enhancement effect. *In vitro* formation of HA on the glass surface is usually taken as an indicator for *in vivo* bioactivity. Accordingly, the application of external electric field has a positive effect on the bioactivity of the bioactive glass. Moreover, it is noticeable that under the current of 90 mA, a small amount of $CaCO_3$ crystals (JCPDS 99-0022) was also formed during the mineralization process. This formation is the result of the reaction between Ca^{2+} released from the glass and CO_2 dissolved into SBF.

Fig. 4 shows the surface morphologies of the glass particles after immersion in SBF for 3 d. As shown in Fig. 4(a1), abounding cracks were formed on the surfaces of the glass particles, indicating that the glass surface has been progressively corroded. A higher magnification SEM image (Fig. 4(a2)) shows the surface of the sample is covered by agglomerated tiny particles. The corresponding EDS spectrum (Fig. 4(a3)) confirmed that the Ca/P of the precipitations was 1.13, suggesting the formation of calcium deficient apatite precipitates. Upon exposure to electric fields, more severe corrosion occurred.

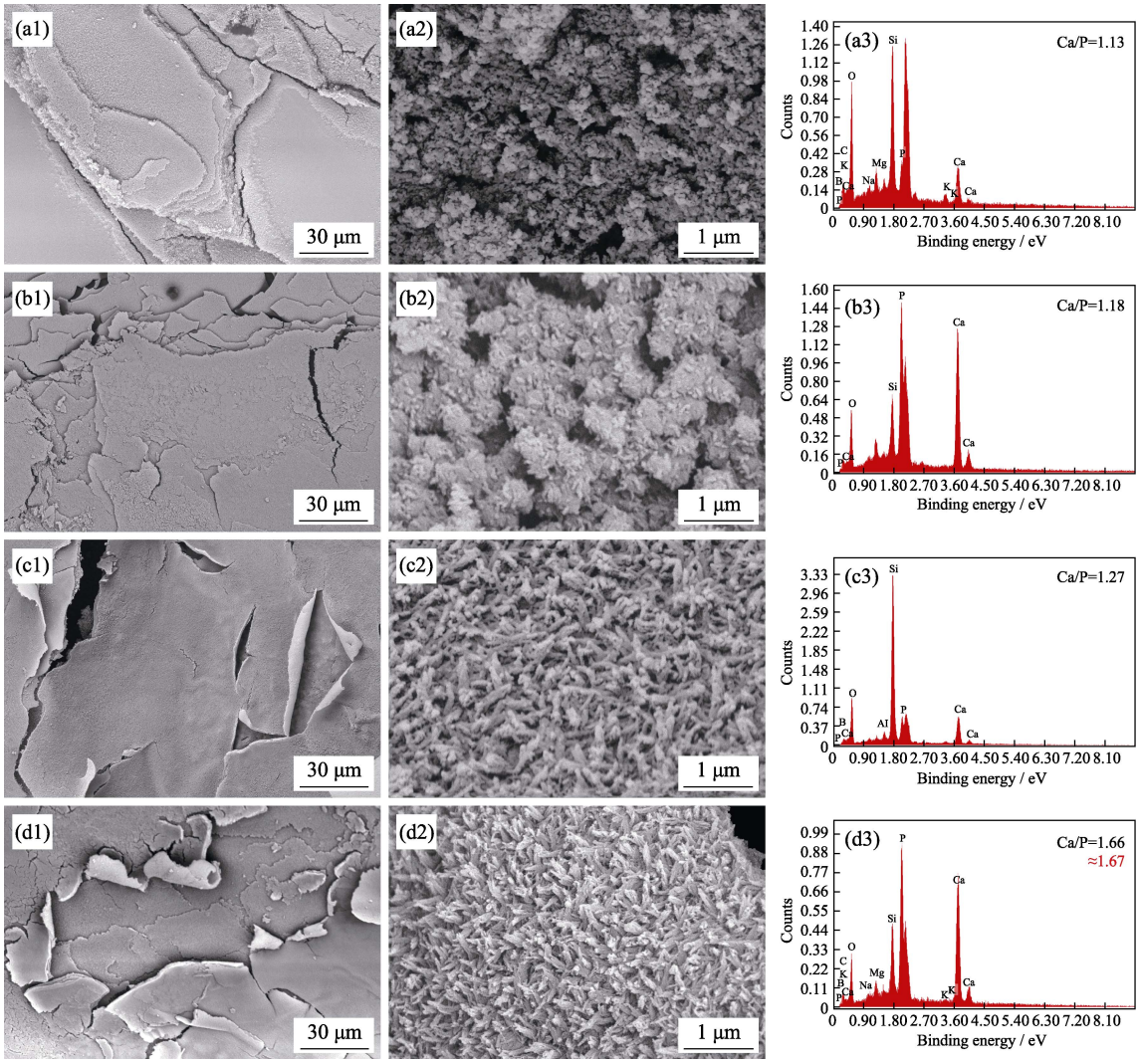

Fig. 4　Microscopic morphologies of borosilicate bioactive glass immersed in SBF for 3 d after applying electric field and EDS analysis of the formed HA

(a1, a2, a3) Without electric field; (b1, b2, b3) 30 mA; (c1, c2, c3) 60 mA; (d1, d2, d3) 90 mA

Comparison of Fig. 4(b2-d2) revealed that larger applied current resulted in more compact HA deposition, and the crystals changed from granular, spherical to bone-like needle-shaped morphology. The relatively dense apatite surface layer acts as a diffusion barrier leading to decreased ion release, as evidenced in Fig. 2(b). Meanwhile, with the growth of applied currents from 30 to 90 mA, the Ca/P of the mineralized products generally increased to 1.66, which is quite close to that of stoichiometric HA. This evidences that crystallization process is enhanced due to the influence of electric field stimulation. The results above highlight that the application of DC field promote the degradation and mineralization of the borosilicate bioactive glass.

In the body fluid and SBF environments, Si–O–M and B–O–M (where M is modifying ion) in borosilicate glass undergo bond breaking, alkali and alkaline-earth ions are rapidly dissolved. The negatively-charged surface can link to hydrogen ions in the SBF, making the surface hydroxylation. Subsequently, the deprotonation of the OH group leads to a negatively-charged glass surface, which attracts Ca^{2+} dissolved from the glass. Ca^{2+} combines with PO_4^{3-} in the SBF to form a HA layer afterwards[8,20].

Samples were analyzed by FT-IR spectra after 1 d of immersion in SBF to study the structural change of borosilicate glass at the early stage of mineralization. During this period of immersion, several magnitudes of electric current were applied continuously for 15 h. As shown in Fig. 5, for the original glass, the IR band around 715 cm^{-1} is assigned to bending vibration of B–O–B linkages, and the broad band around 1400 cm^{-1} corresponds to asymmetric stretching vibration of B–O bonds of trigonal [BO_3] units, respectively. The broad band at wavenumbers range of 800–1200 cm^{-1} is attributed to a combination of vibration modes from Si–O–Si asymmetric stretching, B–O stretching vibration in BO_4 units, B–O–M (where M is modifying ions) as well as B–O–Si and B–O–B linkages[21-22]. Compared to the original glass, the mineralized samples present identical spectral features. However, a broad band in the range of 3200–3700 cm^{-1} associated to the OH stretching vibration and an H–O bending band at 1620 cm^{-1} are obvious. The presence of the hydroxyl groups and water molecules on the glass surface is explicit.

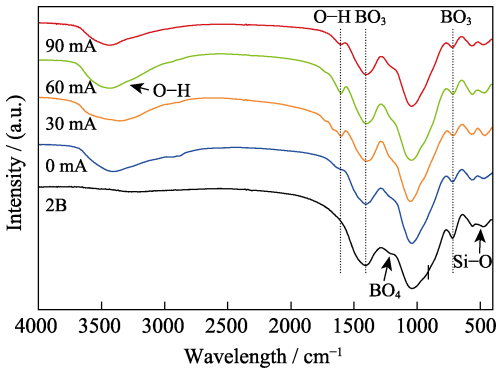

Fig. 5 FT-IR spectra of bioactive glass immersed in SBF with an applied electric field

Meanwhile, Fig. 5 showed that the broad band in the 800–1200 cm^{-1} range sharpened and shifted to higher wavenumber. Especially, a shoulder at 920 cm^{-1}, corresponding to Si–O and B–O vibration, disappeared after the immersion in SBF. The change is indicative of metal cations connected to non-bridging oxygen atoms (nbO) being released (ion exchange) and OH groups forming[22]. The changes in these bands were more pronounced in the samples exposed to electric field. Thus, the electric field assisted the ion exchange reaction between the glass and SBF and hydrolysis of the glass network.

To further clarify the variation of the chemical composition at initial immersion stage, the surface of the glass particles immersed in SBF for 1 d was analyzed by XPS, and results expressed in atomic percentage are shown in Table 1 and Fig. 6. Because of the low sensitivity of XPS to K, only Ca, P, Mg, Na, Si, and B were analyzed compositionally. Ca and P atomic percentage on the glass surface appear to be significantly higher than the calculated values from original glass, especially for the sample exposed to electric field. Such a high Ca/P ratio illustrates the formation of Ca-enriched phosphate precipitation. This is probably because the negatively-charged glass surface by deprotonation of the OH groups, attracted Ca^{2+} and Mg^{2+} in the SBF. The cations on the surface will further attract HPO_4^{2-} and PO_4^{3-}

Table 1 Elemental atomic composition of the surface of bioactive glass particles/at%

	Ca	P	Mg	Na	Si	B
0 mA	16.55	8.79	7.98	5.36	26.65	34.67
60 mA	21.10	10.66	5.87	8.69	9.18	44.50
Original glass	16.18	2.94	5.88	8.82	13.24	52.94

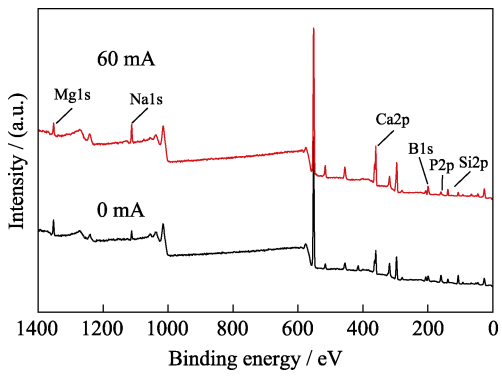

Fig. 6 XPS survey spectra of bioactive glass surface

in SBF to form apatite[23]. The increased release of Ca under the assistance of electric field generated thicker apatite deposition on the glass surface.

Table 1 and Si2p spectra (Fig. 7(a)) present that an extremely weak Si signal was observed on the surface of the sample exposed to electric field. The weak signal was expected to come from the silica-riched gel layer or unreacted glass beneath the apatite layer. Hence, an apatite layer was formed on the glass surface. Additionally, for the samples exposed to electric field, O1s spectra (Fig. 7(b)) present a slight shift in peak position towards lower binding energy of 531.6 eV, which agreed well with the P–O bond of HA. Fig. 7(c) shows the strength of B1s peak increased, but symmetry decreased. This phenomenon can be related to changes in the proportion of different species, while possibly also the borate part of the network breaks down and partly dissolves.

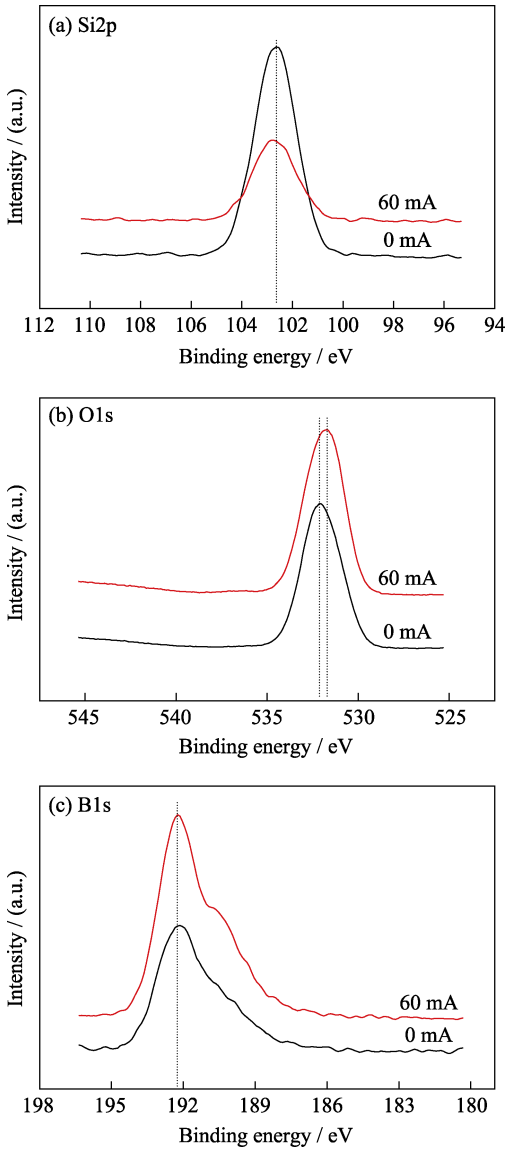

Fig. 7　XPS spectra of bioactive glass surface

As mentioned in the previously work, borosilicate glasses are composed of borate and silicate network formers, consisting of structural units such as BO_3, BO_4 and SiO_4. BO_4 and SiO_4 are linked preferentially *via* BO_3[24]. Because of its threefold coordination number, BO_3 obtains a high dissolution when immersed in the SBF. Accordingly, depolymerization of the glass network and subsequent precipitation of apatite layer occurred. The application of an electric field provides an additionally driving force to promote the ion exchange reaction between the glass and SBF, degradation of the glass, and nucleation of apatite, and thereby enhancing the bioactivity of the bioactive glass.

3　Conclusions

Electric field, as an outfield-assisted method, can assist ion exchange and diffusion in glass. In this paper, a DC electric field was applied to borosilicate bioactive glass to analyze the effect of the electric field on the degradation and *in vitro* mineralization property. DC electric field can increase the degradation rate of borosilicate bioactive glass, while ion dissloution can be increased significantly. In particular, the increase of boron ions can improve the bioactivity of borosilicate bioactive glasses under the premise that the mineralization property of bioactive glasses would gradually slow down with time. Meanwhile, DC electric field also can accelerate the conversion of HA, and assist glass network hydrolysis and surface hydroxylation. As an outfield-assisted approach to regulation, DC electric field can improve the mineralization performance of borosilicate bioactive glass and enhance the bioactivity. It is expected to act as an *in vitro* modulation to accelerate the degradation of bioactive glass and HA conversion to enhance bone repair performance.

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硼硅酸盐生物活性玻璃在直流电场下的体外矿化性能

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摘 要: 硼硅酸盐生物活性玻璃具有良好的生物活性和骨传导性, 但大多数生物活性玻璃表现出非线性降解和矿化行为, 矿化性能会随着时间而减缓。电场作为一种外场辅助调节的方法, 能够干预玻璃的离子交换和扩散。本研究利用直流电场干预硼硅酸盐生物活性玻璃的体外矿化, 加快降解较慢阶段中硼硅酸盐生物玻璃的生物活性。将熔融法制备的成分为 $18SiO_2$-$6Na_2O$-$8K_2O$-8MgO-22CaO-$2P_2O_5$-$36B_2O_3$ 的硼硅酸盐生物活性玻璃浸泡在 SBF 生理模拟液中, 施加 0~90 mA 的电流, 研究直流电场对硼硅酸盐生物玻璃降解及体外矿化性能的影响。研究结果表明, 施加电场不仅可以提高硼硅酸盐生物活性玻璃的降解率和离子释放量, 而且有利于玻璃网络水解和表面羟基化, 加速羟基磷灰石的生成。其中失重率比对照组提高了 3%~5%, 硼和钙的离子释放量分别较对照组提高了 2.3~2.9 倍和 1.9~2.3 倍。对硼硅酸盐生物活性玻璃表面结构分析得出, 暴露在电场下的样品表面生成了磷灰石层。应用直流电场可以提高生物活性玻璃的降解及体外矿化性能, 为提升骨修复效果提供了一种新思路。

关 键 词: 硼硅酸盐生物活性玻璃; 直流电场; 矿化性能

中图分类号: TQ171 **文献标志码**: A