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Effect of boron oxide addition on structural, thermal, *in vitro* bioactivity and antibacterial properties of bioactive glasses in the base *S53P4* composition

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ABSTRACT

The present work explores the potential of B_2O_3 substituted bioactive glasses towards improvement in surface apatite formation along with better cell proliferation and improved antibacterial properties. While the base glass was selected from SiO_2-Na_2O-CaO-P_2O_5 system (commercially available *S53P4* glass), four B_2O_3 modified glass compositions were formulated by replacing SiO_2 in the base glass composition by B_2O_3 with 25, 50, 75 and 100%. To understand the amorphous nature of the glasses as well as the structural changes in the glasses, X-ray diffraction (XRD) analysis and Fourier transform infrared (FTIR) spectroscopy were performed, respectively. The thermal behaviour of the glasses were analysed using differential scanning calorimetry (DSC) and an improvement in the glass stability factor (ΔT), was observed at optimized B_2O_3 concentration. The apatite formation on the surfaces of glasses after bioactivity study for various time periods was analysed using XRD and FTIR. A gradual increase in the pH value and ion concentration (*e.g.* Ca^{2+}, B^{3+}) in simulated body fluid (SBF) was observed along with faster apatite formation with gradual increase in B_2O_3 substitution. The *in vitro* cell proliferation studies using MTT assay demonstrate that the cell proliferation was better on the glasses (BG1B, BG2B and BG3B) compared to base glass (BG0B). Antibacterial study was performed using broth microdilution method against *Escherichia coli (E. coli)* bacteria exploring the antibacterial effects of these glasses from which B_2O_3 substituted glasses were observed to exhibit improved antibacterial properties compared to base glass (BG0B).

1. Introduction

Bioactive glasses are clinically used for treatments related to bone fractures, bone diseases and also dental applications due to their ability of bone tissue regeneration [1,2]. The first developed bioactive glass was *45S5* (Bioglass®) based on SiO_2-Na_2O-CaO-P_2O_5 system and it is the most clinically used glass for bone repair and regenerative applications [1]. Other commercially developed bioactive glasses like *13–93* [3] and S53P4 (BonAlive®) [4] are also silicate based bioactive glass systems and have shown their suitability for bone regeneration and dental repairs. Besides silicate based bioactive glasses, borate based glasses have shown faster dissolution rates and faster apatite formation on surface in comparison to silicate based glasses [5]. Such type of glasses have an added advantage of tailoring the rate of apatite formation to match with the host bone-tissue metabolism resulting in gradual replacement of bone due to controlled dissolution of the glasses [2]. More specifically, borate glasses have shown many advantages such as faster dissolution of ions, faster surface apatite formation, soft tissue repair, wound healing applications and blood vessel formation by release of vascular growth factors [2,6,7]. Recently, the effect of borate addition in bioactive glasses on their bioactivity and dissolution, *in vitro* cell interactions and *in vivo* studies was reported in a comprehensive review on borate based bioactive glasses by Balasubramanian et al. indicating their importance for hard and soft tissue engineering applications [8]. It is important to mention that boron plays an important role in bone health and its trace level presence has been previously reported by many research groups in healthy human bones [9–12].

Bioactive glasses being widely used for bone repairs can be improved by imparting antibacterial properties in them which can be used for treatment of hospital acquired infections (nosocomial infections) and treatment of osteomyelitis. In 1852, Edouard Chassaignac, a French surgeon first described osteomyelitis as a bone disease that occurs in the form of a bone inflammation mainly from infectious microorganisms [13] and is commonly caused by *Staphyllococcus aureus (S. aureus)*

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bacteria [14]. The current treatment methods involve removal of infected part surgically followed by antibiotic treatment at the infected site while no additional support matrix is provided for bone regeneration [15]. To heal the osteomyelitis infected bone, bioactive glasses exhibiting antibacterial properties along with their bone regenerative abilities could be used for treatments [15].

The antibacterial activity in bioactive glasses were exhibited either by the local physiological changes caused by the bioactive glasses after implantation or by doping trace quantities of elements having antibacterial properties which get periodically released in a controlled rate at the implant site. Another approach to impart antibacterial effect in bioactive glass could be achieved by loading antibiotics in glass which gets released at the implant site as the bioactive glass converts into bone mineral [15]. The bioactive glasses like S53P4 exhibited antibacterial properties from the pH increase in the surrounding medium taking place due to exchange of alkali (Na^+) and alkaline earth (Ca^{2+}) ions from the glass with H^+ ions from the surrounding medium [16,17]. Antibacterial bioactive glasses have also been developed by incorporating antibacterial effect exhibiting ions like silver (Ag^+), cerium (Ce^{3+}), copper (Cu^+), strontium (Sr^{2+}) and zinc (Zn^{2+}) into the glass system [18].

Borate buffered solutions have shown antibacterial properties as borate ions possess in-built antibacterial capabilities [19,20]. Previously, borate glasses were used as antibiotic drug delivery systems for the treatment of osteomyelitis due to controlled degradation resulting in controlled release of drugs at the implant site [21–24]. Borate bioactive glasses added with silver ions have shown bacterial inhibition against *S. aureus* and *E. coli* bacteria [25]. Recently, antibacterial effects of some borate glasses were studied and inhibition against various Gram-positive and Gram-negative bacterial species was demonstrated where borate ions were playing major part in exhibiting antibacterial effects [26].

In the present work, the effect of gradual addition of B_2O_3 replacing SiO_2 in commercial *S53P4* glass composition on the apatite forming ability, cell proliferation and antibacterial properties has been explored and discussed. Moreover, thermal stabilities of these boron oxide modified glasses were evaluated to understand their suitability for sintering and scaffold processing.

2. Materials and methods

2.1. Glass synthesis using melt-annealing technique

Conventional melt-annealing technique was used for the synthesis of the glasses which were chosen from the system SiO_2-Na_2O-CaO-P_2O_5-B_2O_3. Table 1 shows the composition of glasses (in mol%) where base glass is denoted as BG0B and four B_2O_3 substituted glasses denoted as BG1B, BG2B, BG3B and BG4B, respectively. BG1B, BG2B, BG3B and BG4B glasses have B_2O_3 replacing total SiO_2 with 25%, 50%, 75% and 100%, respectively in base glass composition.

The glasses in the present study were prepared from various raw materials like SiO_2 (99.8%, Sipur A1 Bremtheler Quartz-itwerk), Na_2CO_3 (99.5%, Merck, India), $CaCO_3$ (99%, Sigma Aldrich, Japan), $CaHPO_4.2H_2O$ (98%, Sigma Aldrich, Germany) and H_3BO_3 (99.9999%, Merck, Germany). Each batch was prepared for synthesis of 100 g glass and the required reagents were carefully weighed and mixed thoroughly. The base glass (BG0B) batch was melted at 1450 °C and the melting temperature was gradually reduced with progressive B_2O_3 substitution up to 1050 °C for BG4B glass. During batch melting, a fused silica rod was used to stir the melt manually twice to obtain homogeneity. After visually inspecting for bubble free melt, the melt was casted on a stainless steel to form a glass block and annealed. The annealed glass block was cut into samples having dimensions of 10 mm × 10 mm × 2 mm followed by grinding and polishing. Few bulk glass pieces were ground to obtain glass powder which was sieved through 45 μm mesh. The below 45 μm powder were collected and characterised using X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy along with thermal (onset of glass transition temperature, glass thermal stability factor), physical (density) and mechanical (Vickers micro-hardness, elastic modulus) properties. The powder and bulk samples were characterised for their bioactivity using simulated body fluid (SBF) at *in vitro* conditions followed by ion release studies and pH measurements on the SBF supernatant (from the studies on bulk glass samples). After SBF immersion of glass samples, apatite formation on the surface of the glasses was studied. Cell proliferation was performed on the bulk glass samples while the antibacterial studies were performed on powder samples.

Table 1
Composition of base glass and borate substituted glasses in mol%.

	BG0B	BG1B	BG2B	BG3B	BG4B
SiO_2	53.85	40.3875	26.925	13.4625	0
Na_2O	22.65	22.65	22.65	22.65	22.65
CaO	21.77	21.77	21.77	21.77	21.77
P_2O_5	1.72	1.72	1.72	1.72	1.72
B_2O_3	0	13.4625	26.925	40.3875	53.85

2.2. X-ray diffraction (XRD)

The precursor glass powder and glass powder subjected to SBF immersion were characterised using X-ray Diffractometer (Ultima IV, Rigaku) which uses Ni-filtered CuK_α radiation having wavelength 1.5418 Å.

2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR transmission spectra of precursor glass powder and powder after *in vitro* bioactivity studies were recorded using FTIR/FIR spectrophotometer (Frontier IRL 1280119; Waltham, MA, USA) in mid-infrared (MIR) (4000–400 cm^{-1}) range. The glass powder dispersed inside KBr pellet were formed by mixing KBr powder with the samples (ratio 100:1) and analysed in transmission mode.

2.4. Differential scanning calorimetry (DSC)

The onset of glass transition temperature (T_g), crystallisation onset temperature (T_x), crystallisation peak temperature (T_p) and thermal stability factor ($\Delta T = T_x - T_g$) of the glasses were analysed using the DSC at a heating rate of 10 K/min using simultaneous thermal analyzer (model: STA 449F3, Netzsch GmbH, Selb, Germany). DSC analysis was done on around 20 g of each powder sample placed on Pt−Ir crucibles up to 1200 °C. The error values were the standard deviation in the intercept of extrapolated lines for obtaining T_g and T_x values from which the error values of thermal stability factor (ΔT) were obtained. The error values of T_p are the standard deviation in the highest point in the crystallisation peak after peak profiling.

2.5. Density, micro-hardness and elastic modulus measurements

Archimedes' buoyancy principle was used for density (ρ) value estimations of glass samples by measuring the dry weight and suspended weight of each sample. The measurements were carried out on Mettler-Tolledo analytical balance (Model: AB-204-S/FACT, Mettler Toledo, Greifensee, Switzerland) equipped with density measurement setup with double distilled water as immersion medium. The error values were the standard deviation in calculated density from the dry and suspended weights measured in triplicates.

The micro-hardness values of all bulk glass samples were measured using Vickers micro-hardness tester (Clemex CMT, Longeuli, Canada) with 50 g load on the indenter with a holding time of 10s. The average value from six measured micro-hardness values was reported along