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Structural, physico-mechanical and in-vitro bioactivity studies on SiO_2–CaO–P_2O_5–SrO–Al_2O_3 bioactive glasses

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ABSTRACT

Strontium based bioactive glasses have shown a better biocompatibility than calcia based bioactive glasses. In this report, we have shown that the bioactivity is found to be even more when we incorporate Al_2O_3 upto 1.5 mol % in SiO_2–CaO–P_2O_5–SrO bioactive glass. We have studied the structural, physico-mechanical and bioactive properties in these glasses with varying alumina concentration from 0.5 to 2.5 mol%. The bioactivity of the glasses is evaluated by in vitro test in simulated body fluid (SBF). The formation of hydroxy carbonated apatite layer (HCA) on the surface of glasses after immersion in SBF is identified by the XRD, FTIR and SEM. The substitution of Al_2O_3 for SrO in these glasses demonstrates a significant enhancement in compressive strength and elastic modulus. However cytotoxicity and cell viability assessed using human osteosarcoma U2-OS cell lines show the growth of the cells without causing any significant loss of viability and cell death upto 1.5 mol% addition of Al_2O_3. Osteosarcoma cells grow on the surface of bioglasses which make them biocompatible and fit for use in clinical trials.

1. Introduction

Bioactive glasses are of immense interest for use as bone grafts [1], implant coatings [2,3], bone cement [4] and even in dentifrices [5]. The first bioactive glass known as 45S5 Bioglass® has been developed by Hench [6] having composition 46.1 SiO_2–2.6 P_2O_5–24.4 Na_2O–26.9 CaO (mol%). It has been shown that in 45S5 Bioglass® SrO can be substituted for CaO due to similar ionic radii of Ca^{2+} (0.94 Å) and Sr^{2+} (1.16 Å) [7] to improve bioactivity and other physico-mechanical properties. The small difference in size allows the substitution of the strontium for calcium ions not only in glass composition but does not develop any strain in the crystal lattice [8]. However, it is important to emphasize that due to twice the atomic weight of Sr than that of Ca, it finds more useful to substitute SrO for CaO on a molar basis than the weight basis [9,10]. In addition, it is expected that SrO substituted bioactive glasses may offer an excellent opportunity in delivering therapeutic strontium (Sr^{2+}) ions [11,12,13] in human body. Sr^{2+} ions have been shown to stimulate osteoblastic bone formation and to inhibit osteoclastic bone resorption both in vitro and in vivo [14,15,16]. Indeed strontium ranelate (Protelos®) is a drug approved for treatment and prevention of osteoporosis [17,18] which is used as a commercial anti osteoporotic oral drug that has been proven to reduce the incidence of fractures in osteoporotic patients [19,20] whilst Sr-containing bioactive glasses are shown to combine the known bone regenerative properties of bioactive glasses with the anabolic and anti-catabolic effects of Sr^{2+} cations in vitro [21]. In addition, Strontium is an important source of interest in recent years because of its effect on bone cells. However, the amount of strontium in bone is typically only 3.5% of its calcium content [22]. It is preferentially found in new bones rather than old and more in cancellous than cortical bones. Both in vitro and in vivo studies have demonstrated stimulatory effects of Sr on osteoblasts and an inhibitory effect on osteoclasts, associated with an increase in bone density and resistance [14,15,23].

Moreover, these bioactive glasses possess low mechanical strength which may be a hindrance for repairing defects especially in load-bearing bones. Hench developed the 45S5 Bioglass® having wt% composition (45 SiO_2-24.5Na_2O-24.5CaO-6P_2O_5) containing calcium and phosphate in the proportion identical to natural bone as an implant material which is being clinically used worldwide [6]. After implantation in bone tissue, they found that their material resisted removal from

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its implant site and was found to be bonded with bone by forming a strong chemical bond which was termed as bioactive glass. In general, the bioactivity of the sample is associated with the ability of hydroxy carbonate apatite [Ca_{10} $(PO_4)_6$ $(CO_3)_x$ $(OH)_{2-2x}$, where $0 \leq x \leq 2$] layer formation on their surface in SBF under physiological conditions. Bioactive glass and glass ceramics were also earlier investigated extensively because of their high bioactivity and ability to form an apatite layer on the surface of the substrate upon immersion in body fluids [24,25].The HCA is a critical species having bone like structure which is responsible for bonding the bioactive implants interfacially with hard as well as the soft tissues in vivo [26]. Wilson et al. [27] mentioned that in animal studies it had been shown that bone and soft tissue connections were restored to a level close to that of normal teeth when 45S5 Bioglass® was used. It was mentioned earlier that bioactive glasses having different compositions were able to bond to soft tissues and bone as implant materials which had got their variable reactivity, speed of bonding and ability of the bone to provide mechanical strength. They have been successfully applied clinically as solids as well as particulates which might be combined with natural and synthetic materials.

Hench presented the idea of a safe bonding amongst bone and synthetic materials practically due to the chemical reactions taking place over the glass surface. These chemical reactions strongly helped bioactive glasses to form interfacial bond with the bone, thus replacing the damaged and the diseased bone. For this characteristics quality bioactive glass was regarded as one of the exceptionally suitable biomaterial for orthopaedic and dental applications [28]. Clinical applications of bioactive glasses had been earlier reviewed by different workers [6,29–31]. The application of 45S5 Bioglass in endosseous ridge maintenance [32] as well as middle ear replacement and its success in repair of periodontal defects [6,33] had been found to be successful.

It is agreed that the bioactive glasses are not so strong to be used in load bearing applications but previously attempts were made to resolve this problem by coating the mechanically tough substrates with a bioactive glasses. The glass coating was found to protect the substrate from chemical corrosion and degradation as well as the adjacent tissues from the corrosion products. The bioactive glass coating with high mechanical integrity had been also found to provide the interfacial attachment to the bone with bioactive fixation by chemical reactivity [6]. Although, it has low fracture toughness and also it suffers with mechanical weakness due to its amorphous nature, that is why the improvement in its mechanical strength is essentially needed in order to ensure its applications in load bearing conditions as well as long-term stability. In recent years several attempts were made to tailor the degradation rate and to improve the mechanical strength of bioactive glasses by changing their chemical composition with incorporation of various other metal oxides like MgO, ZnO, B_2O_3, Al_2O_3, etc. Amongst several oxides, Al_2O_3 is expected to improve the long-time stability of the implants needed for bone defect repairing [34] and to control the degradation rate. Alumina has been found to increase the chemical durability of the glass tremendously more than any other oxides. Tripathi et al. [35] had studied the in vitro bioactivity and structural characterization of SiO_2-CaO-P_2O_5-K_2O-Al_2O_3 glass by replacing K_2O with Al_2O_3 (0 to 2.5 mol%) and reported that an increase in Al_2O_3/K_2O ratio has resulted an enhancement in the physico-mechanical properties of the glass appreciably. At higher concentration alumina has entered as AlO_4 tetrahedra into SiO_4 tetrahedral network resulting an enchantment in physico-mechanical properties, whereas addition of alumina in lower concentration as AlO_6 octahedra has resulted an increase in the bioactivity of the glasses. The SrO and Al_2O_3 both play the role of stabilizers but SrO forms weak ionic bonds (Si—O—Sr) whereas Al_2O_3 forms strong covalent bonds (Si—O—Al) in silicate glass structure which compresses the structure of the glass more. So, in the present studies Al_2O_3 was substituted for SrO in order to improve the mechanical properties of the bioactive glasses along with its bioactivity which was expected to bridge a gap effectively in earlier researches in the area of bioactive glasses. Since bioactive glasses are the biodegradable materials in vivo under the influence of physiological fluids as such dissolution of large particles of S53P4 bioactive glass on prolonged duration after 14 years as reported by Lindfors et al. [36] was a normal process. However, the authors had found that S53P4 Bioglass was a potential material of choice in benign bone tumor surgery both in children and adults. They also found that these bioactive glasses can be used in cavities under high-loading bearing conditions safely as bone substitute with good long-term results. The studies on the influence of aluminum on the crystallization and bioactivity of $NaCaPO_4$–SiO_2 system by Sitarz et al. [41] have indicated that addition of Al^{3+} ion in appropriate proportion increases the mechanical resistance of the bioglasses as well as influences homogeneity of the glass texture. However it has been shown that the addition of Al_2O_3 in large quantities in bioactive glasses is not desirable because of its carcinogenicity and adverse impact on the bioactivity of the glass [42,43]. Therefore it is important to study the effect of Al_2O_3 on in vitro bioactivity in simulated body fluid (SBF), physico-chemical, mechanical properties as well as in vitro cell culture of the glasses. So a base glass system SiO_2–P_2O_5–CaO–SrO was designed and the concentration of SrO was varied with molar addition of Al_2O_3 from 0to 2.5%. It is expected that the bioactivity and physico-mechanical properties would be improved significantly with increasing the concentration of Al_2O_3 in the base bioactive glass. The idea for assessment of in vitro bioactivity in simulated body fluid (SBF), physico-chemical, mechanical properties as well as in vitro cell culture of the glasses has been also undertaken herewith. Furthermore, an in vitro cell culture studies, such as cell viability and cytotoxicity have been extensively investigated for better biocompatibility and it has been found that there is an improvement in the bioactivity of the glasses due to incremental addition of Al_2O_3 in the base system. Interestingly, it can also be seen that all the samples are tolerant to white blood cells (WBC) and RBC causing no significant loss of viability or hemolysis.

2. Materials and methods

2.1. Sample preparation

Glasses were prepared in five different compositions by taking the starting materials as reagent grade fine-grained quartz (SiO_2), analytical reagent grade calcium carbonate ($CaCO_3$), ammonium dihydrogen phosphate ($(NH_4)H_2PO_4$), strontium carbonate ($SrCO_3$) in the required stoichiometric ratio in mol% (Table 1). All the chemicals were of AR grade and were purchased from LobaChemie, Mumbai, India. The required amount of analytical reagent grade Al_2O_3 was added in each batch for the partial substitution of SrO. The raw materials for different glasses were properly weighed. The mixing of different batches was done for 30 min and then after that, the raw batches were melted in a 100 ml platinum-2% rhodium crucible for 4 h kept in a globar electric furnace at 1400 °C in air atmosphere. The temperature of the furnace was controlled with in ± 10 °C by an automatic temperature indicator-cum-controller. Further, the glass melt was taken out of the furnace, poured on an aluminum sheet in a rectangular mold and transferred immediately to an annealing furnace. The glasses were properly annealed at 500 °C for 1 h and cooled slowly to room temperature with a controlled rate of cooling inside the muffle furnace to remove the

Table 1
Mol% compositions of the bioactive glasses.

Sl. no.	SAMPLE	SiO_2	CaO	P_2O_5	SrO	Al_2O_3	Al_2O_3/SrO ratio
1.	Sr-1	42	34	6	18	0	0.00
2.	Sr-2	42	34	6	17.5	0.5	0.028
3.	Sr-3	42	34	6	17	1.0	0.058
4.	Sr-4	42	34	6	16.5	1.5	0.090
5.	Sr-5	42	34	6	15.5	2.5	0.161