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Bioactivity of Strontium Doped Boro phosphate Disordered Glass

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Abstract: Bioactive glasses with composition $\text{Na}_2\text{O}-\text{Li}_2\text{O}-\text{SrO}-\text{CaO}-\text{B}_2\text{O}_3-\text{P}_2\text{O}_5$ and $\text{Na}_2\text{O}-\text{Li}_2\text{Cl}-\text{SrO}-\text{CaO}-\text{B}_2\text{O}_3-\text{P}_2\text{O}_5$ were prepared by the traditional melt-quench method. Bioactive glasses are able to bond to bone by forming hydroxyl carbonate apatite layer on their surfaces. The *in vitro* bioactivity test was conducted on the prepared glass samples by using simulated body fluid (SBF), which confirms the formation of hydroxyl carbonate apatite layer on the glass samples. X-ray diffraction (XRD) characterization showed the amorphous nature of glasses. For surface morphology the prepared glasses have been characterized by Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) confirmed the formation of hydroxyl carbonate apatite layer formation.

Keywords: Bioactive Glass; Strontium; Simulated body fluid; Borophosphate glass; *in vitro*;

I. INTRODUCTION

The field of biomaterials is playing very important role for mankind. Now a day's implants prepared with bioactive materials are in great demand for medical applications. Because of increased life span, accidents and sports there is a great need to replace and repair human body parts. Biomaterials may be bio inert, bioactive and bioresorbable [2]. Specially, bioactive materials are capable to bond with both hard and soft tissues [1]. Hence at the interface of bioactive implant and body tissue bioactive materials are biocompatible means they are capable to bond with both hard and soft tissues. The National Institute of Health Consensus Development Conference defined a bioactive material as "any substance or combination of substances, synthetic or natural in origin, which can be used for any period of time, or as a whole or as a part of a system which treats, augments, or replaces any tissue, organ or function of the body"[2,3]. After the great invention of first bioactive glass with composition $24.5\text{Na}_2\text{O}-24.5\text{CaO}-6\text{P}_2\text{O}_5-45\text{SiO}_2$ in wt% by Hench and his colleagues in 1969 many researchers are focussing on the field of bioactive materials to prepare new glasses or glass ceramics with new composition to enhance the properties of existing glasses [4]. Due to their biocompatibility and bioactivity bioactive glasses are used as implant materials to repair or replace deceased or damaged human body parts [5, 6].

When the bioactive glasses are in contact with body fluids there is a formation of rich hydroxyl carbonate apatite layer on their surface [7,8]. The formation of hydroxyl carbonate apatite layer is the main prerequisite for the bonding of bioactive glass to living tissue and is an indication of bioactivity nature of bioactive glass [9]. Hydroxyl apatite layer is a crystalline form of calcium phosphate similar to the mineral phase present in bone and of define composition $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ [10]. Hench and Anderson successfully explained the reactions occurred on the surface of bioactive glass when immersed in simulated body fluid. They are leaching and formation of silanols, dissolution of the glass network, silica gel polymerization, and then formation of calcium phosphate rich layer on the surface [3, 4, 14, 15]. This layer finally crystallized to a hydroxyl carbonate apatite later. In general, bone bonding ability of bioactive material is evaluated by examining the formation of hydroxyl carbonate apatite layer on the surface of glass when treated in simulated body fluid (SBF) [11, 12, 13]. In this present paper *in vitro* studies of bioactivity of the prepared glass samples were reported.

In search of new bioactive glass ceramics three glass samples with composition $\text{Na}_2\text{O}-\text{Li}_2\text{O}-\text{SrO}-\text{CaO}-\text{B}_2\text{O}_3-\text{P}_2\text{O}_5$, $\text{Na}_2\text{O}-\text{Li}_2\text{O}-\text{SrO}-\text{CdO}-\text{B}_2\text{O}_3-\text{P}_2\text{O}_5$ and $\text{Na}_2\text{O}-\text{Li}_2\text{Cl}-\text{SrO}-\text{CaO}-\text{B}_2\text{O}_3-\text{P}_2\text{O}_5$ were prepared by popular melt quench method. The formation of hydroxyl carbonate apatite layer on the surface of synthesized glass samples was investigated by using Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) which confirmed the bioactivity nature of glass samples.

II. MATERIALS AND EXPERIMENTAL PROCEDURES

A. Glass synthesis

Bioactive glasses with composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$, $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ and $8\text{Na}_2\text{O}-8\text{Li}_2\text{Cl}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ in Wt% were prepared by conventional melt quench method. The analytical grade

chemicals were weighed in appropriate amount and mixed then melted in a porcelain crucible for 1 hour at 1000°C in an electrical muffle furnace. After melting the glasses were rapidly quenched.

B. Preparation of Simulated Body Fluid

Simulated body fluid (SBF) ion concentrations approximately equal to that of human blood plasma. Hence simulated body fluid has been used to test the in vitro bioactivity of prepared glass samples. Simulated body fluid (SBF) was prepared by the method proposed by Kokubo et al. [13]. For in vitro bioactivity test simulated body fluid was prepared. The reagent grade chemicals NaCl, NaHCO_3 , KCl, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , Na_2SO_4 were mixed in appropriate portions in an ion exchanged distilled water. The solution is then buffered at pH of 7.4 with suitable amount of tris-hydroxymethyl amino methane and hydrochloric acid [13]. For in vitro bioactivity test the prepared samples were immersed in simulated body fluid (SBF) for 7 days. The temperature of the incubation chamber was maintained at 36.5°C . The samples were taken into plastic containers and immersed in simulated body fluid and were placed in incubation chamber for 7 days. After 7 days the samples were taken from the incubator and cleaned gently with 100% ethanol and then with distilled water. The samples were left to dry at room temperature in a desiccator. The dried samples were analysed by using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) unit attached to the SEM.

III. RESULTS AND DISCUSSION

The X-ray diffraction (XRD) pattern of prepared glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ before soaking in SBF is shown in fig 1, which confirms the amorphous nature of glass sample. XRD pattern of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ after soaking in simulated body fluid for 7 days are as shown in fig 2. In order to obtain X-ray Diffraction patterns the glass samples were characterized by Philips X'pert diffractometer ($\text{Cu K}\alpha$ radiation) in the 2θ range $5-80^{\circ}$ with step size of 0.02° operating at 40kV, 30mA. The XRD results for the glass samples $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ and $8\text{Na}_2\text{O}-8\text{Li}_2\text{Cl}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ after immersion in SBF are shown in fig 3 and fig 4. New peaks can be observed at 2θ in the range $25-30^{\circ}$ assigned to crystalline phase of hydroxyl carbonate apatite layer on the surface of the glasses.

Fig. 1 XRD spectrum of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

Fig. 2 XRD spectrum of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

Fig. 3 XRD spectrum of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

Fig. 4 XRD spectrum of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{Cl}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

The Scanning Electron Microscopy (SEM) was used to investigate surface morphology of glass samples. The Energy Dispersive X-ray Spectroscopy (EDS) analysis at an accelerating voltage of 15kV was carried out to determine the presence of elements on the prepared glass sample surface. The micrograph provides visual evidence of the formation of an apatite layer on the surface of a glass samples. Fig 5 Shows the SEM micrograph of bioactive glass sample of composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$. Fig 6 and fig 7 shows the SEM micrograph of bioactive glass samples of composition $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ and $8\text{Na}_2\text{O}-8\text{LiCl}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$.

Fig. 5 SEM micrograph of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

Fig. 6 SEM micrograph of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

Fig. 7 SEM micrograph of glass sample $8\text{Na}_2\text{O}-8\text{LiCl}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

The EDS analysis of synthesized glass samples reveals the formation of a hydroxyl apatite layer on the surface of glass samples after immersion in SBF for 7 days. The precipitates on the surface of the sample are made up of calcium and phosphorous are shown in fig 8, fig 9 and fig 10. The micro analysis of the precipitates reveals the presence of small quantities of Na, P, Ca, C, Sr are shown in EDS. It may be concluded that the surface layer forms crystalline phase of hydroxyl apatite layer.

Fig. 8 EDS data of glass sample $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$

Fig. 9 EDS data of glass sample 8Na₂O-8Li₂O-8SrO-20CdO-50B₂O₃-6P₂O₅

Fig. 10 EDS data of glass sample 8Na₂O-8LiCl-8SrO-20CaO-50B₂O₃-6P₂O₅

After immersion of glass samples in simulated body fluid the changes in pH was determined with pH meter. The glasses showed a increase in pH upon immersion in SBF shown in fig11. Initially the pH increased with time for all 3 glasses and there was a slight reduction in pH at 300 - 400 minutes in SBF. The Ca/P molar ratio was determined by EDS analysis on the surface of the glass samples after 7 days ranges between 1.50 – 1.58.

Fig. 11 pH changes in glass samples

IV. CONCLUSIONS

The in vitro bioactivity of the three glass samples $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$, $8\text{Na}_2\text{O}-8\text{Li}_2\text{O}-8\text{SrO}-20\text{CdO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ and $8\text{Na}_2\text{O}-8\text{Li}_2\text{Cl}-8\text{SrO}-20\text{CaO}-50\text{B}_2\text{O}_3-6\text{P}_2\text{O}_5$ were reported. The amorphous nature of the glass analysed by XRD before soaking in SBF. After soaking in SBF the XRD pattern of glasses confirms the crystalline phase of hydroxyl apatite layer on their surface. The hydroxyl apatite forming ability of strontium has been investigated. The surface morphology was examined by using SEM micrograph and EDS data. SEM and EDS data revealed the biocompatibility of the prepared glasses. The change in pH of the fluid shows the bioactivity of the glasses. The Ca/P ratio values are in good agreement with biological apatite.

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