Surface Reactivity of Quick Alkali Mediated Bivalent Metal Ions (Zn-Mg-Sr) Doped Bioactive Glass

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Abstract- **Bioactive glass with frame of SiO_2-CaO-P_2O_5-ZnO-MgO-SrO was prepared using quick alkali mediated sol-gel process. The functional group analysis, morphology, composition and phase analysis of this glass were done by using FTIR, FESEM, EDX and XRD respectively. The bioactive nature of the sample was analyzed by immersion in Phosphate Buffer Saline (PBS) for a period of 7 days and characterized using FESEM and EDX. The surface reactivity results revealed a change in morphology. This could be attributed to the stimulating effect of the dopants in the faster mineralization and producing bone like morphology.**
Keywords- bioactive glass; therapeutic; PBS.

I. INTRODUCTION

Bioactive glasses (BG) have been extensively investigated as bone graft materials or fillers in bone regeneration applications owing to their good bioactivity, osteoconductivity, biodegradability and their ability to bond to bone or living tissues without forming fibrous tissues [1]. Formation of hydroxyl carbonate apatite (HCA) layer is a prerequisite for the bonding of bioactive glasses to induce the bone formation and to promote the contact between the implant and the host tissue. This biologically active HCA layer is chemically similar with the mineral phase of natural bone. The bioactivity of the BG further depends on the chemical composition, texture, density, porosity and structure [2, 3]. In recent years, inorganic metal ions like zinc, magnesium, strontium, silver, cobalt, copper, have emerged as potential therapeutic agents with the ability to enhance the bone formation due to their stimulating effects on osteogenesis, as well as angiogenesis [4]. Recently, these three bivalent ($Zn^{2+,}$ Mg^{2+} and Sr^{2+}) metal ions have created quite high interests in the biomedical field due their physiological role. The Zn^{2+} and Sr^{2+} enhance osteogenesis by influencing both osteoblastic and osteoclastic processes. Thus stimulates bone formation process and occlusive bone resorption process respectively. The Mg^{2+} is related to angiogenesis and is an essential element and the tenth most abundant element in the human body. About 65% of total body magnesium is contained in bone and teeth. It directly stimulates the proliferation of osteoblasts with an effect comparable to that of insulin, a well-known growth factor for osteoblasts [5].

Numerous reports are available on synthesis and in-vitro behaviour of bioactive glasses with ensured amounts of specific therapeutic ions by melting and sol–gel methods. Oki et al [6] reported that the Zn-containing glass exhibited a good ability to induce the deposition of hydroxyapatite (HA) in SBF. Fatemeh Baghbani et al [7] have developed both Zn, Mg containing bioactive glass and revealed that these ions promote varying chemical durability, antibacterial activity and bioactivity as a function of composition of BG. Balamurugan et al. [8] reported that the CaO-SiO_2-P_2O_5-MgO glass system showed significant enhancement in bioactivity within few days of immersion in SBF solution. Strontium doped bioactive glass induced biological stimulation in osteoblastic cells with the advantages of the sol-gel process, such as increased porosity and bioactivity as reported by J Isaac *et al*. [9]. Y. rezaei et al showed both Sr and Mg doped bioactive glass has shown significant enhancement in bioactivity within few days of immersion in SBF solution [10].

However, introducing these three (Zn^{2+}, Mg^{2+} and Sr^{2+}) bivalent metal ions doped bioactive glass and their combined role on properties and characteristics BG have not been reported in the literature. Hence, the present work is aimed to investigate the combined effect of these bivalent ions on nature of the bioactive glass and its surface reactivity on the apatite formation (bioactivity). The bioactive glass in the system of SiO_2-CaO-P_2O_5-ZnO-MgO-SrO was synthesized by using the quick alkali mediated sol-gel process and the in-vitro bioactivity of the synthesized sample was investigated by immersing in PBS for 7 days period.

II. EXPERIMENTAL

PREPARATION AND CHARACTERISATION:

A. MATERIALS

The chemicals used for the synthesis and evaluation are reagent grade (Merck Inc)Tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), calcium nitrate tetra hydrate ($Ca(NO_3)_2 \cdot 4H_2O$), Zinc nitrate ($ZnNO_3$), Magnesium nitrate ($MgNO_3$), Strontium nitrate (SrNO3), HNO_3,HCl, NH_3, Na_2HPO_4, NaCl, KCl**,** and KH_2PO_4 **.**

B. PREPARATION OF BIOACTIVE GLASS

Bioactive glass containing SiO_2-CaO-P_2O_5-ZnO-MgO-SrO (mol %) were synthesized through a quick alkali-mediated sol–gel method and their composition is depicted in Table-1. The tetraethyl ortho-silicate, distilled water (1:2) and 2M nitric acid (as a hydrolysis catalyst to adjust the pH at 2), were successively added and the mixture was allowed to react for 1 hr under continuous stirring for the acid hydrolysis of TEOS. This was followed by the addition of TEP, $Ca(NO_3)_2 \cdot 4H_2O$, $ZnNO_3$, $MgNO_3$, $SrNO_3$, allowing 30 min for each reagent to react completely. After the final addition, whole mixture was stirred for 1 hr to obtain a clear sol. Excess Ammonia (2M) solution (a gelation catalyst) was added into the sol in an ultrasonic water bath until gelation results. The mixture was then agitated with glass rod (like as mechanical stirrer) to prevent the formation of a bulk gel. Finally, prepared gel was dried at 75°C for 2 days in an air oven, followed by sintering at constant heating rate of 10 °C min^{-1} in muffle furnace up to 700°C for 2hrs.

Table 1. Composition of Bioactive glass

Bioactive glass	SiO_2 (mol %)	CaO (mol %)	P_2O_5 (mol %)	ZnO (mol %)	MgO (mol %)	SrO (mol %)
Modified 58S	55	36	4	2	2	1

C. *CHARACTERISATION*

The morphology and composition of synthesized glass was investigated using FESEM attached with EDX – Philips 501 Scanning electron microscope. The sample was coated with a thin layer of gold by Edwards sputter coater S150B instrument, due to the non-conductive nature of the sample. XRD analysis for phase composition was done using GE-X-ray diffraction –XRD 3003 TT applying Cu Kα radiation at 50 kV voltages and 100 mA current. Functional groups were ascertained using Fourier –transform infrared spectroscopy (FTIR- Agilent CARRY 630). The sample was prepared for FT-IR by mixing synthesized BG with KBr to make a pellet. The spectrum was recorded from the range of 650 to 4000 cm^{-1}.

C. INVITRO BIOACTIVITY STUDY

In-vitro bioactivity study was carried out by immersing the bioactive glass disc sample (1mg/ml) in PBS solution at 37°C for intervals from 1 to 7 days. The PBS solution at pH 7.4 was prepared. After immersion period, the disc was removed from the solution, rinsed thrice with acetone and distilled water, dried at room temperature. The disc was further subjected to FESEM & EDX investigation to know the apatite forming ability of bioactive glass.

III.RESULTS & DISCUSSION

A. XRD ANALYSIS:

The XRD pattern of the as synthesized bioactive glass was shown in fig 1. The characteristic weak diffraction band observed at $2\Theta = 32.34°$ corresponding to calcium silicate (Ca_2SiO_4), which is in accordance with the JCPDS no 29-0369 [11]. Generally, as discussed earlier, heat treatment around 700°C is optimized temperature for amorphous nature, crystallization occurs only at temperature from 800°C and above. However the presence of this broad peak ascribed to the partial crystallization, which took place during the calcinations process at

700°C. Hence, the amorphous nature of bioactive glass indicating of internal disorder and glassy nature of this material. [12]

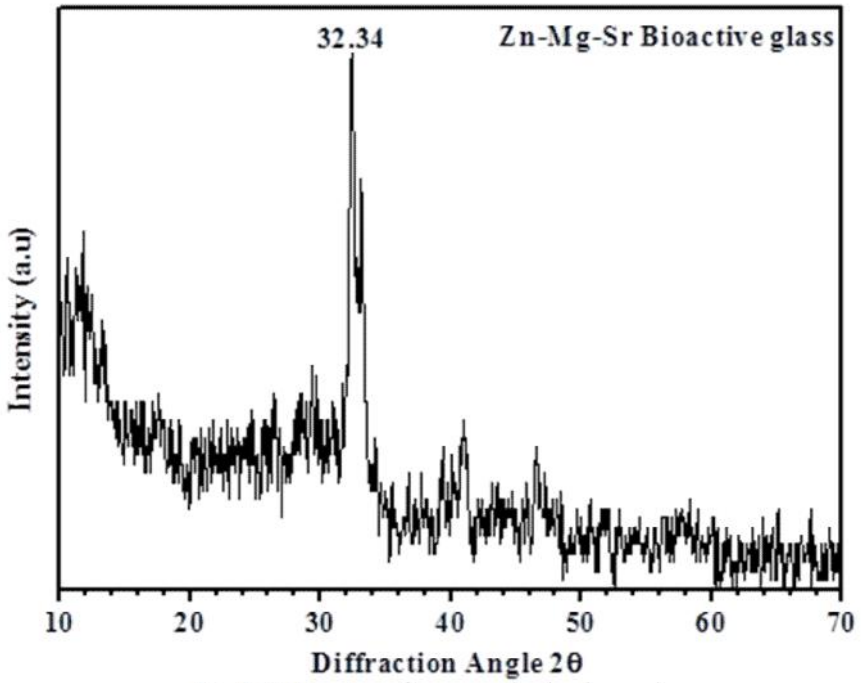

Fig.1 XRD pattern for as synthesized sample

FTIR ANALYSIS:

Fig 2 shows the FTIR spectra of the as synthesized sample. The sample exhibited the band around 900-1100 cm^{-1} corresponding to the vibrational mode of asymmetric stretching of Si-O-Si and also assigned to P-O bond. Both this peak superimposed to a broad silicate band. The band at 915 cm^{-1} assigned to the Si-O-Ca vibrational mode. The band at 1460 cm^{-1} shows the carbonate absorption band. The band at 1640 cm^{-1} was assigned to O-H stretching group due to residual H_2O absorbed in sol-gel derived bioactive glass. The band at 3453 cm^{-1} exhibited the H-bonded O-H group. The band at 2921cm^{-1} and 2855cm^{-1} shows (υ(O-H) of $H_2PO_4^-$). Hence, the synthesized bioactive glass was in good agreement with the results obtained in previously reported literature. [6, 7, 12-16]

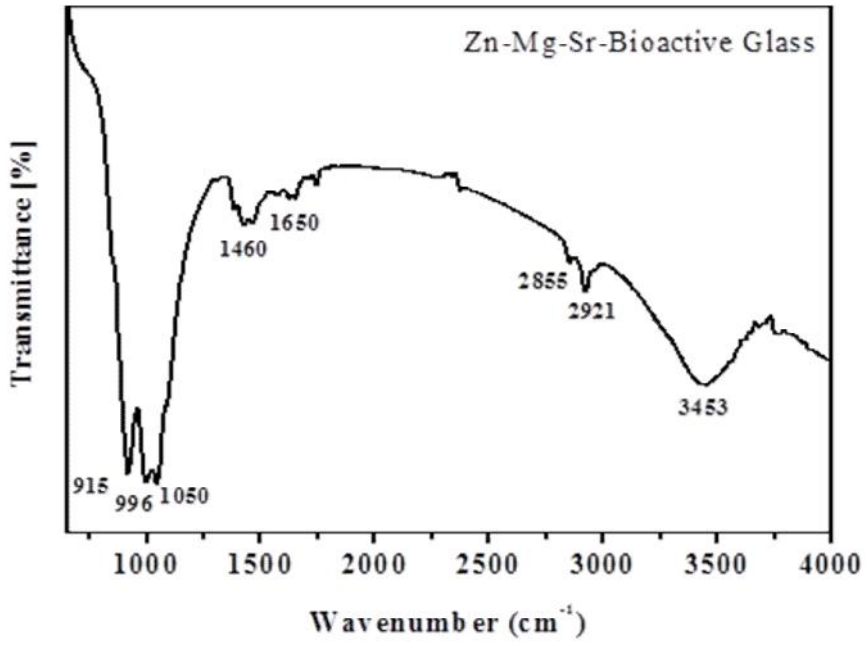

Fig 2: FTIR spectrum of as synthesized sample

FESEM & EDX ANALYSIS:

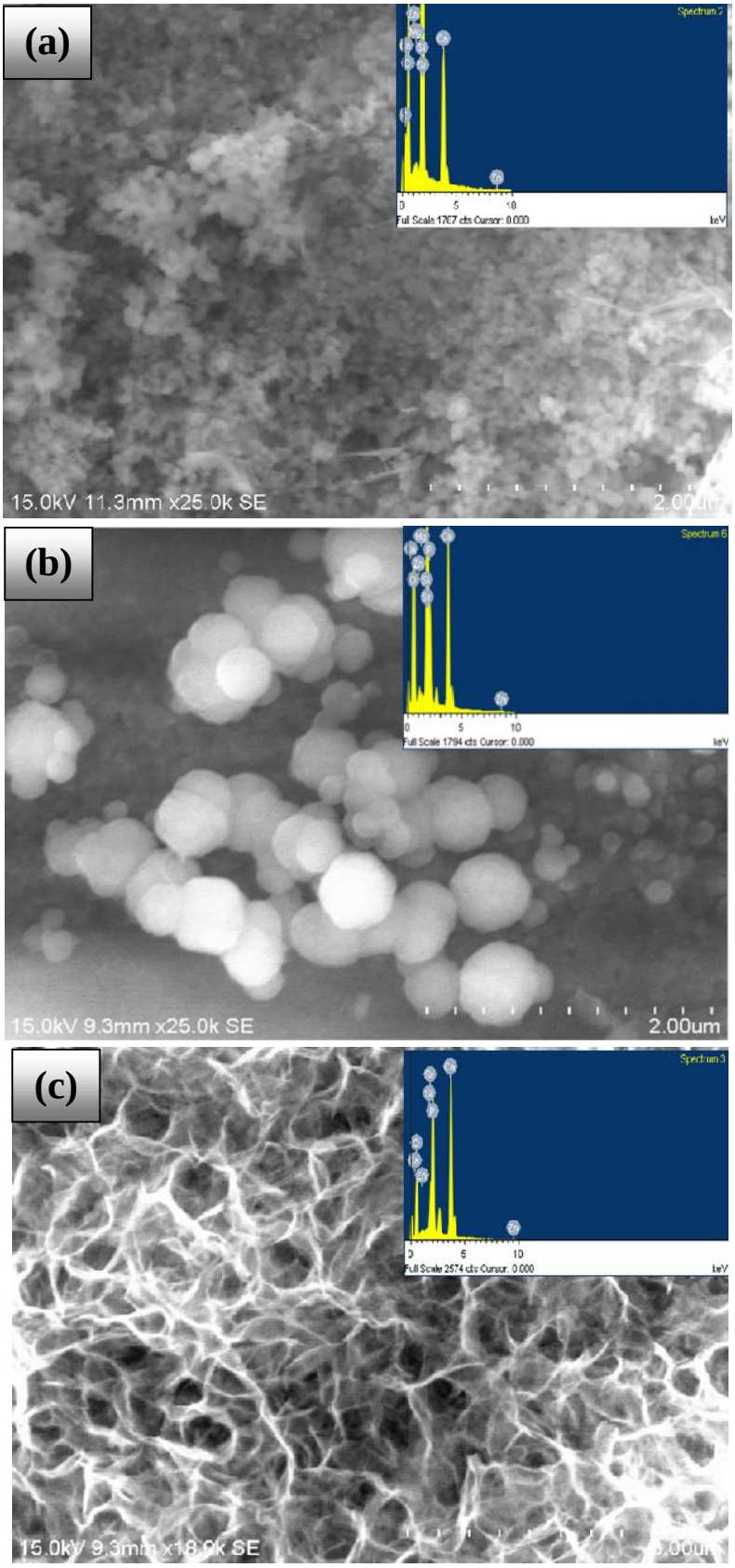

Fig. 3 FESEM AND EDX images (a) Before immersion (b) after immersion for 1 day (c) for 7 days

Fig 3 shows FESEM & EDX patterns of the bioactive glass surface before and after immersion for 1 & 7 days in PBS. Before immersion, heterogeneous surfaces consisting of random sized spherical particles were seen with nominal composition of bioactive glass. After immersion, initially apatite crystals germinated in just 1 day of immersion. As seen in fig 3b, these smooth spheres referred as crystallization nuclei. After 7 days, smooth spheres turned into honeycombed like morphology as seen in fig 3c, similarly, from EDX, variation in composition intensities of calcium, phosphorus was increased as immersion time increases.

IV.CONCLUSION

From the results obtained, it can be concluded that, change in morphology on surface of the glass was clearly noticeable before and after immersion in PBS. The obtained honeycombed like morphology which is similar to bone morphology. Hence, it can be confirmed that doping of combined bivalent metal ions stimulated the mineralization faster and producing the desired characteristics to the bioactive glass i.e., bone bonding and biodegradability.

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