Synthesis and Invitro Characterisation of Lithium Doped Bioactive Glass through Quick Alkali Sol-Gel Method

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Abstract - **The sol–gel bioactive glass (60%- SiO_2 36%- CaO 4% -P_2O_5) was modified by adding Lithium (Li) a biologically relevant therapeutic element. Quick alkali mediated sol-gel method was used for the synthesis. Thus synthesized bioactive glass with the composition of 55 % SiO_2-36% CaO-4% P_2O_5-5% Li_2O (BGLi5) was compared with the reference BGLi0 with a composition of 60%- SiO_2 36%- CaO 4% -P_2O_5. The synthesized bioactive glasses were characterized using Field Emission Scanning Electron Microscope (FESEM), Energy Dispersive X-ray Analysis (EDX), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy. *In-vitro* Surface reactivity of bioactive glasses were also evaluated through the formation of apatite layer over it after immersion in simulated body fluid (SBF) for different time intervals and subsequently confirmed using Raman & XRD patterns. The change in pH was also measured during the immersion period. The antibacterial activity of Li containing glass against *Enterococcus faecalis* was measured by the agar diffusion method. The results indicated that lithium addition reduced the rate of dissolution, thus slowed down the formation of apatite layer, enhanced chemical durability and revealed an excellent antibacterial activity.**

Keywords: lithium; bioactive glass; apatite; quick alkali

I. INTRODUCTION

Recently different kinds of bio ceramics have been developed and used to treat the diseased and damaged bones. These bio ceramics are broadly classified as bio inert (Al_2O_3, ZrO_2), bioactive (bio glass and hydroxyapatite) and biodegradable materials (tri calcium phosphate).[1-3] The field of bioactive ceramics came into prominence after the invention of bioglass in 1970 by Hench et.al with the chemical composition of 45wt% SiO_2, 24.5 wt% Na_2O, 24.5wt % CaO, 6 wt% P_2O_5. Since then numerous modified bioactive glasses have been reported with varying properties. These second generation bioactive material were utilized as bone graft materials or fillers owing to their good bioactivity, osteoconductivity, biodegradability and capacity to inducing bone bonding ability not only to bone, but also to soft tissues [4]. The silica based glasses (bioactive glass) have unique properties viz., rapid rate of surface reaction that leads to direct attachment to the bone through a chemical bond, relatively low softening temperature, ease of compositional design with properties specific to particular clinical applications, excellent controllability over a wide range of chemical properties ,rate of bonding with tissues etc.[5-7]

In general, bioactive glass can be formed by conventional melt quenching method, for mass production, it is considered as simple and reliable method. However, during high temperature process the evaporation of volatile P_2O_5, has limited this method [8]. Sol-gel method appears to be a valuable way for fabricating bioactive glasses [9-10]. This method has great versatility since, it requires low temperature, involving homogenous mixture , provides high surface area, useful for developing wide range of composition up to 90 mol% of Silica and highly bioactive than conventional one [4].

These glasses have ability to form hydroxyl carbonate apatite layer (HCA) on their surface in the living environment and bond to tissues and bone through this apatite layer. Formation of HCA layer is 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; it depends on the chemical composition, texture, density, porosity and structure [11-13]. Hench et.al proposed five interfacial reactions occurred on the surface of bioglass on immersion in physiological solutions such as SBF, Initially, alkali ion leaching and formation of silanols, loss of soluble silica and formation of silanols, polycondensation of silanols to form hydrated silica gel, formation of an amorphous calcium phosphate layer, and crystallization of a carbonated apatite layer [14-15].

Recently, therapeutic stimulatory ion like Mg, Sr, Zn, Ag etc., incorporated bioactive glass were developed in order to improve the surface reactivity, enhance the cellular response and rate of tissue healing process and regeneration. The ionic dissolution products from these bioactive glasses have been known to stimulate osteogenesis, angiogenesis, neovascularization, antibacterial and inflammatory response in both in-vitro and in-vivo conditions [42, 28]. The incorporation of Zn into a bioglass is beneficial for cell attachment and for maintaining the pH of SBF [6]. In vitro bioactivity of Zn doped bioglass enhanced the formation of apatite layer and bone bonding calcite [16]. Increased the thickness of the HA layer with Mg incorporated bioactive glass was reported by vallet-Regi et.al [17]. Silver release from bioactive glass is more responsible for the protection of various surfaces against bacterial infection [18]. The strontium substituted bioactive glass revealed an increase in osteoblast proliferation and ALP activity besides inhibiting TRAP activity [19]. Li et al. demonstrated that the presence of MgO, ZnO, and CuO slowed down the deposition rate of HA [20]. Hence, by varying the chemical nature and the concentration of glass network, new biological important properties were added and the glasses can be tailored to specific clinical applications.

In recent years, many authors have reported that glass and glass ceramics with Li_2O-SiO_2 system has potential applications in medicine. This type glass ceramics are applied as dental bridges, crowns or veneers due to their special properties like high strength, opalescence, thermal stability and chemical resistance. In general, Pingping Han et al, suggested that the combination of Li with β-TCP bioceramics may be a promising method to enhance bone/ cementum regeneration as Li-β-TCP possesses excellent in-vitro osteogenic and cementogenic stimulation properties by inducing bone/cementum-related gene expression in both hPDLCs and hBMSCs[22]. Lithium has great potential to treat fracture healing and its effectiveness shows that it is best candidate to incorporate in CaP bone substitute as orthopaedic implants [14]. M.Khorami et.al[23] concluded glass reactivity is completely failed in low concentration of Li, improved glass reactivity and better proliferation rate, alkaline phosphates activity of osteoblasts at higher concentration of Li. Interestingly, medical researchers has discovered, lithium acts as antidepressant and has powerful immune stimulating property and it is very effective against a wide range of microbes [24]. Although there are several studies showing that Li ions could regulate the apatite mineralization of bioglass and enhance osteoblast proliferation of hydroxyapatite.

The main objective of this study to synthesis Li incorporated highly reactive bioactive glass (BGLi0) through quick alkali sol-gel method. The bioactivity i.e., the ability of these materials on forming calcium phosphate layer on its surface will assess by immersed in SBF for 15 days period. Anti-bacterial effect of this bioactive glass will also be examined.

II. EXPERIMENTAL

PREPARATION AND CHARACTERISATION:

A. MATERIALS

The chemicals are used for the synthesis and evaluation, Tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) Lithium nitrate , HNO_3,HCl, NH_3, NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$, Na_2SO_4 and tris-(hydroxymethylamino)methane [Tris-buffer, $(CH_2OH)_3CNH_2$], were reagent grade and purchased from Merck Inc.

B. PREPARATION OF BG

Bioactive glass containing SiO_2-CaO-P_2O_5 (BGLi0) and SiO_2-CaO-P_2O_5-Li_2O (BGLi5) (mol %) were synthesized through a quick alkali-mediated sol–gel method as previously reported by Yi-Fan Goh et.al [16]and their composition is depicted in Table-1 The tetraethyl orthosilicate, 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, $Ca(NO_3)_2 \cdot 4H_2O$, $LiNO_3$, allowing 30 min for each reagent to reacts completely. After the final addition, whole mixture was allowed 1 hr stirring 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 rode (like as mechanical stirrer) to prevent the formation of a bulk gel. Finally, each 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. Bioactive Glass Composition (Mol %)

Bioactive glass	SiO_2 (mol %)	CaO (mol %)	P_2O_5 mol %)	Li_2O (mol %)
BGLi0	60	36	4	0
BGLi5	55	36	4	5

C. *CHARACTERISATION*

The morphology and composition of synthesized glasses were investigated using FESEM attached with EDX – Philips 501 Scanning electron microscope. The samples were 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 samples were 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}. Confocal Raman spectroscopy was also used (Raman I-11 Model, manufactured by Nanophoton Corporation, Japan). The wavelength of laser is 532nm, uses YAG Laser as the source. The change in pH was measured throughout the immersion period using ELICO LI 120 pH meter.

D. *INVITRO BIOACTIVITY STUDY*

In-vitro bioactivity study was carried out by immersing the bioactive glass disc samples (1mg/ml) in simulated body fluids solution at 37°C for intervals from 1 to 15 days. The SBF solution at pH 7.4 was prepared according to the kokubo's specification [25]. After immersion period, the discs were removed from the solution, rinsed thrice with acetone and distilled water, dried at room temperature then the samples were preserved for characterization using Raman & XRD in order to verify the apatite forming ability of bioactive glasses.

E. *ANTIBACTERIAL STUDY*

The agar diffusion method was followed for antibacterial susceptibility test. Petriplates were prepared by pouring 10 ml of Mueller Hinton Agar for bacteria and allowed to solidify. These agar plates were inoculated with 0.1 ml of standardized bacterial suspension ($2x10^6$cells/ml) and uniformly spread. A 6 mm well was cut at the centre of the agar plate and the well was filled with 10% bioactive glass in DMSO (BGLi5). The diameter of the inhibition zone around the well was measure for each bacterium after 24 hrs of incubation at 37° C. The well filled with sterile distilled water served as control.

III. RESULTS & DISCUSSION

1. CHARACTERISATION OF AS SYNTHESIZED GLASSES

A. XRD ANALYSIS

The XRD pattern of both the samples BGLi0 and BGLi5 before immersion confirms amorphous [26-31,16] nature of bioactive glass by characteristic broad diffraction bands observed between 2Θ values 20° to 32°, as shown in Fig 4., indicative of internal disorder and glassy nature of these materials. As previously discussed, heat treatment around 700°C is optimized temperature for amorphous nature, crystallization occurs only at temperature from 800°C and above [29].

B. FTIR ANALYSIS

For both glasses, similar peaks were obtained, dotted circle around 900-1200 cm^{-1} was assigned to asymmetric stretching vibrational mode (Si-O-Si) and also assigned to P-O bond, although superimposed by the broad silicate band . [23, 29- 32,] .The band at 801cm^{-1} is assigned to Si-O-Ca vibrational mode and symmetry stretching non-bridging oxygen bonds (Si-O-) (i.e., Si–O- moieties ionically bound to either Li^+ or Ca^{2+} counter-cations) [28-32,34,]. The peak at 1643 cm^{-1}[33] was assigned to O-H stretching group due to residual H_2O absorbed in sol-gel derived bioactive glass. The peak at 1460 cm^{-1}[29, 33] were visible in Ca doped silica corresponds to carbonate absorption band, since carbonates are existing on the surface because of the reaction between atmospheric CO_2 and some available Ca^{2+} surface ions.

The dotted circle around 3500 cm^{-1}[33] assigned to H bonded O-H group , due to molecular water coordinated at room temperature on bioactive glasses, Ca present in 58S bioactive glass has a tendency to induce the stronger and larger water uptakes.

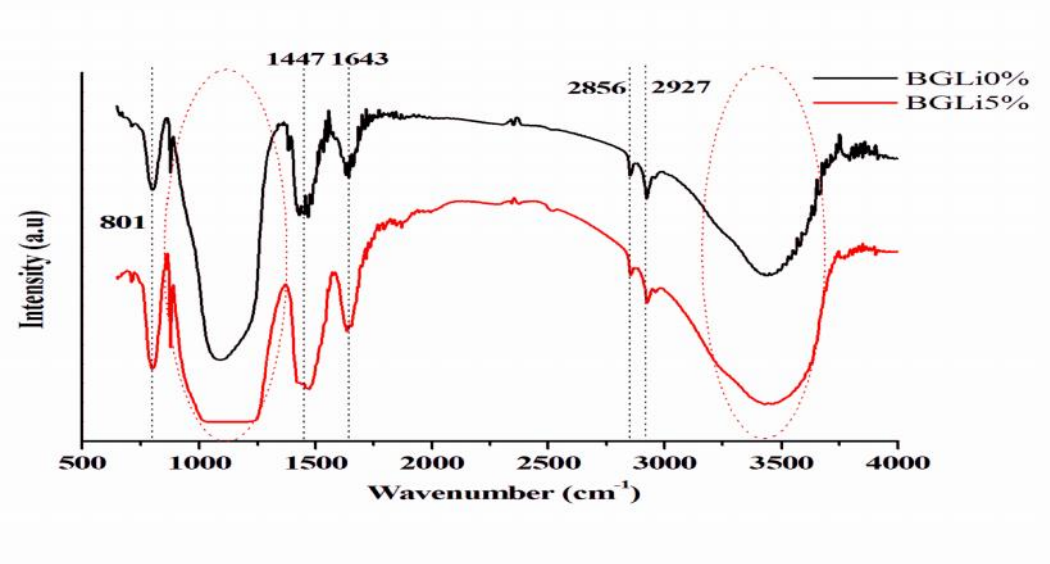

Fig: 1 FTIR Spectrum of as synthesized samples

But the intensity of bands is reduced in case of lithium containing bioactive glass (BGLi5) due to the non-homogeneous distribution of Ca and the presence of multiple phases. Inhomogeneity is caused by the migration of cations by capillary diffusion during drying process. [34]

C. RAMAN SPECTRA

Raman spectrum of both bioactive glasses thus synthesized showed well defined vibrational bands. Both BGLi0 & BGLi5 revealed similar peak at 1050, 780, 580 cm^{-1} as shown in Fig 2. Based on the literature reports it is suggested that the peak at 1050 cm^{-1} may be ascribed to the presence of one of the constituents used to form bioactive glass or it may be associated with the bending mode of Si-O-Si. The broad & strong peak at 1050 cm^{-1}[37,6,38,33] assigned to the stretching vibration bands associated to Si-O-Si bond in silica tetrahedral with the different number of non-bridging oxygen (NBO) involving two dimensional structures $Si_2O_5^{2-}$. In case of BGLi5,this particular peak is considered as the vibrational indicator of the strong asymmetry bonded oxygen band of SiO^-Li^+ since this also acts as a strong modifier which is likely to increase the Si-O-NBO vibrations and the stretching vibrations[38].

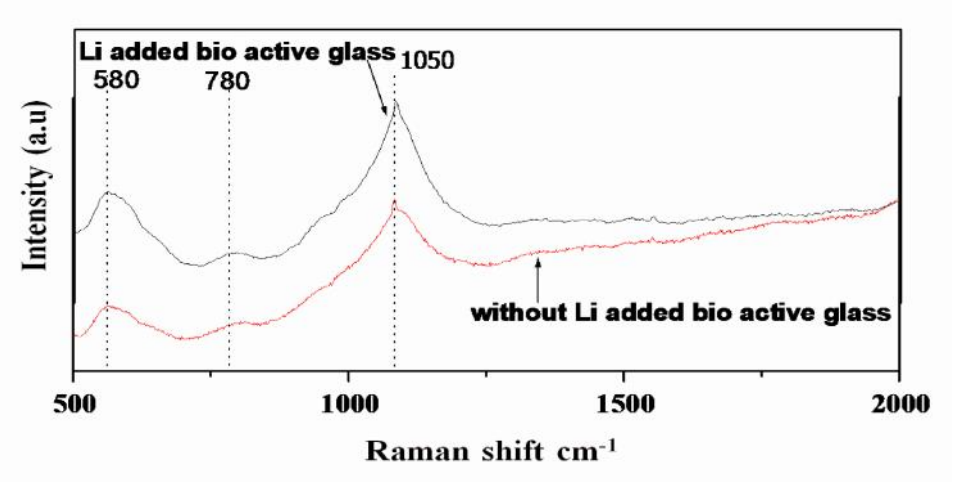

Fig: 2 Raman Spectra for as synthesized samples

The peak at 580 cm^{-1} corresponds to the symmetric stretching of the P-O bond and O-P-O bending of orthophosphate PO_4^{3-} unit. [6]. Bond bending vibration band appeared at 780cm^{-1} shows the oxygen atoms move to and from the two adjacent Si atoms in the Si-O-Si plane [38]. From above observations, prepared glasses are tends to be in agreement with the nominal composition of bioactive glasses. In case of BGLi5, as discussed earlier[39],the Raman shifts are more sensitive to the composition changes and the addition of alkali metal ions to the silica network, as such, peak shifting and intensity variations are usually noticed because of the increase or decrease of the local symmetry of the silica network.

D. FESEM & EDAX ANALYSIS

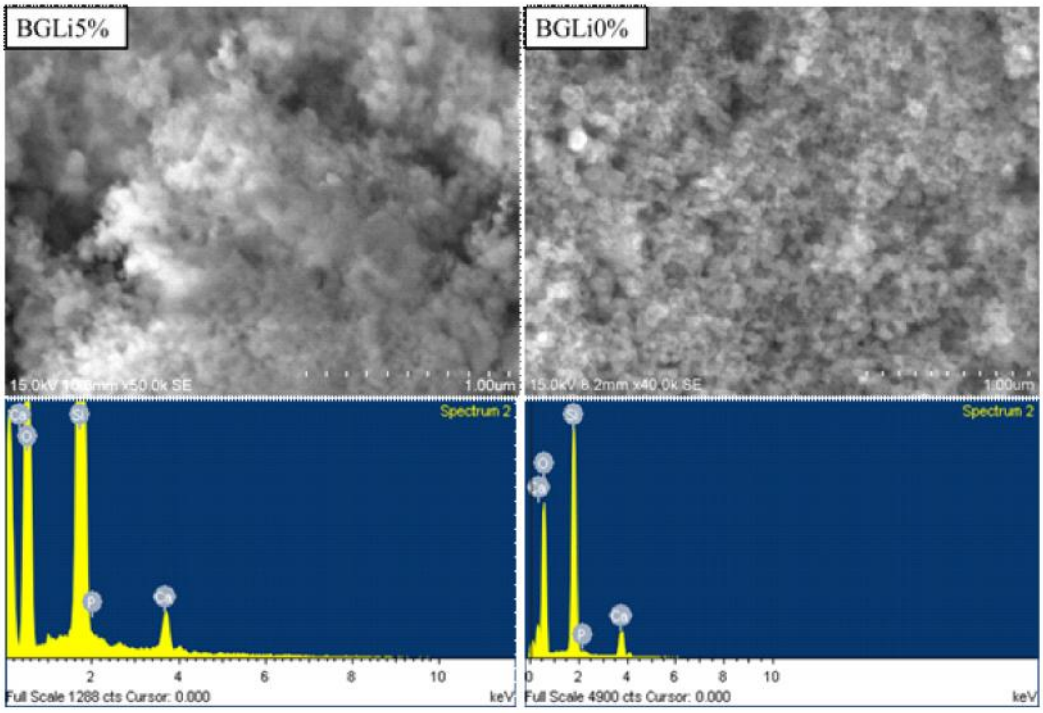

Fig: 3 FESEM & EDAX Patterns for as synthesized samples

The synthesized BG was subjected to FESEM & EDX in order to sketch the morphology, size and elemental percentage. The observed images of both glass surfaces revealed a heterogeneous surface with random sized particles ranging from Nano size to micron size. As per, EDX Si peak is more predominant & thereafter Ca, P are also observed as nominal composition of the BG.

2. INVITRO CHARACTERIZATION OF BIOACTIVITY

A. XRD ANALYSIS

In order to verify the presence of new apatite crystalline phases on the glass surfaces, XRD analysis were performed for the glasses (BGLi0 & BGLi5) after immersion in SBF for 1,8,15 days are shown in Fig 4. The apatite layer formation was confirmed by two characteristics peaks located at 2Θ = 26°, 32° corresponds to (002) (211) according to the standard JCPDS card no (09-0432) [28, 29, 31].

The obtained results demonstrated that in case of without Li (BGLi0) after immersion in SBF, the formation of apatite layer gradually increases as immersion time increases by observing the diffraction peak intensity of (211) planes followed by the growth and appearance of (002) plane . Another apatite peak at 2 Θ =28° (102) plane [31] was also developed after 8^{th} day of immersion. Here broadening of peaks was attributed to the partial crystallization of apatite phase formation. After 15 days of immersion, all peaks may become more evidenced.

In case of lithium doped bioactive glass (BGLi5) after immersion in SBF as shown, Initially, spectrum obtained was poorly resolved and gave no clear evidence for the presence of any crystalline phase, diffraction peaks were clearly observed only after 15th day of immersion period, until it shows some characteristic amorphous phase, suggesting the slow ability of this glass to form apatite layer. This shows the ability of the bioactive glass acting on nucleation and slowdown the bone formation in the body condition. The time taken for the formation of apatite layer is quite complex phenomenon and dependant on the composition of the bioglass.

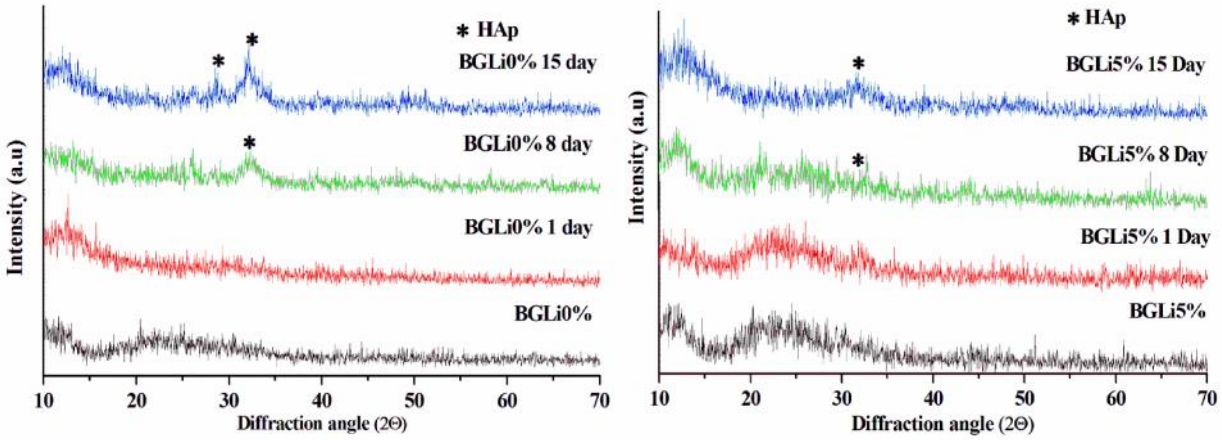

Fig: 4 XRD Patterns for samples before & after immersion in SBF

M.Khorami et.al [23] studied the effect of lithium instead of sodium in the bioglass 45S5. In this case of lithium added to 58S bioactive glass, Li_2O was replaced for SiO2 in Mol %. Li+ has a strong attraction for bonding to oxygens & tends to contract the free spaces in the silicate network. However, Li+ has lowest ionic radius and atomic weight and greatest field strength, thus the glass with more compacted structure is expected when Li2o is added. Rapid ion exchanges, surface hydrated silica layer formation, silanol groups are specific sites for apatite nucleation are responsible for *in-vitro* apatite formation. In lithium containing glasses, exchange of ions is hindered, due to lower diffusion coefficient [40] of Li+, this obstruction activity may be due to the function of Li ion concentration. It was also confirmed by M.Khorami et.al using XRD and quantitative ICP data (i.e., the presence of Li in 45S5 glass composition is responsible for a drastic reduction in the overall leaching activity in case of 3 & 7 wt% of Li). This kinetically reduces the rate of glass dissolution & improves chemical durability.

B. RAMAN SPECTRA:

The *in-vitro* activities of both BGLi0 and BGLi5 in SBF for the different days of immersion were analyzed with Raman spectra and are shown in the Fig 5. The formation of HCA has been recognized by some of its typical Raman features. Initially, both the glasses has the strong and symmetric peak centered at 1086 cm^{-1}after one day of immersion [36,37,39] is due to the stretching vibration mode of CO_3 group incorporated by the exposure of the atmosphere carbon dioxide and also the reports are suggested the asymmetric stretching vibration of $PO4^{3-}$ groups. It was correlated that the spectrum is similar to Raman spectra of pure component, the calcite, which is discussed earlier by Regina K.H et al. It can be concluded that initially calcite formation was formed, while immersion time increases the peak intensity at 960cm^{-1} for apatite is progressively increased and the band at 1086cm^{-1} gradually decreased (i.e., strong increase of HCA phase at the expenses of the calcite one), until it cease to be observed after 15 days of immersion, in both cases of BGLi0 and BGLi5. Many researchers explained the formation of HCA layer of the sample similar to bioglass & has been assigned the peak at 960cm^{-1}[35-37, 39] confirmed for the mode of apatite (HCA).

The formation of the apatite layer in both cases showed a difference in the peak intensity, which corresponds to the formation propensity of the bioglass in the presence and absence of the lithium. The formation of the apatite growth is much faster in the BGLi0 when compared with BGLi5. The initiation and growth of the apatite layer is influenced by the lithium doping as suggested by earlier reports. In case of BGLi5, the peak intensity at 960 cm^{-1} is less intense, until day 15 when compared toBGLi0

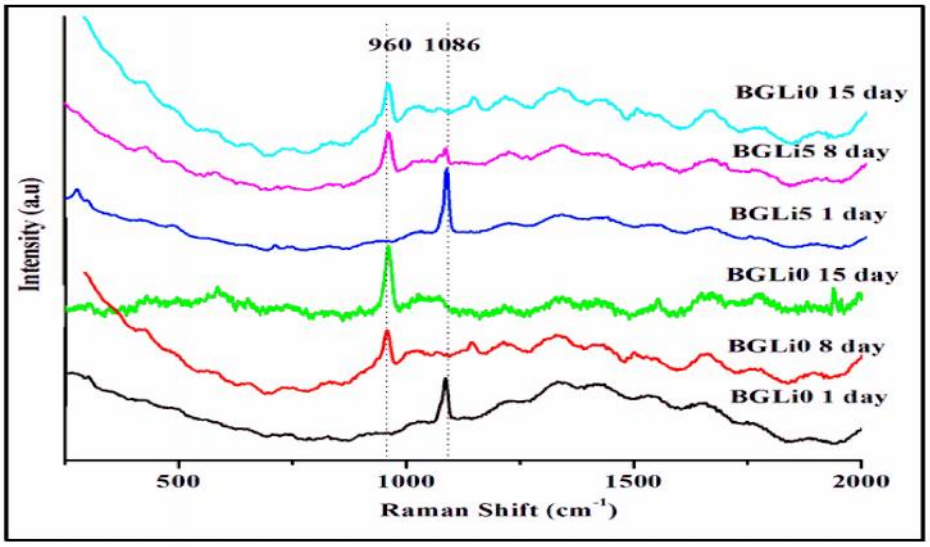

Fig: 5 Raman Spectra for samples after immersion in SBF for 15 days

Corroboration of results obtained with XRD & Raman patterns of both glasses it evident that the formation of apatite layer on their surface after immersion in SBF solution is slow with lithium incorporated bioactive glass (BGLi5). Initially Ca release from both the glasses associated with atmospheric CO_2, thus forming Calcite formation which is evidenced from Raman data. As immersion time increases, apatite forms at the expenses of calcite. Lithium containing glasses reduced the rate of dissolution of glass, thus formation of apatite layer is slowed down and is significant only around 15 days of immersion. This slow rate of dissolution is also beneficial when bioactive glasses are used in combination of liquid and solid systems such as in the preparation of tissue engineering constructs and injectable systems. [9]

C. CHANGE IN pH MEASUREMENT

The samples were immersed in SBF solution and the pH was measured throughout the immersion period of 15 days. The results indicated a steady state increase with increasing immersion time. During dissolution process, releases of Ca and Si ions which are responsible for the increase in pH. BGLi0, showed a higher pH variation from 7.4 to 7.92, hence, it is obvious from the results obtained from XRD & Raman that during dissolution process, an easy and very quick interchange takes place between Ca^{2+} ions of glass and H_3O^+ ions of the solution. In BGLi5, pH variation is from 7.4 to 7.8 which indicate that the exchange of ions (release of Si and Ca ions) takes place to a very limited extent probably due to the presence of alkali metal ion in the silica network. Therefore, the leaching of soluble silicon is an indication of the resorptive ability of the BG. As discussed earlier[23], lithium doped glass has high density, lower diffusion coefficient, strong attraction towards oxygen makes a glass more compacted structure, that hinder the exchange of ions quickly and leads to higher chemical durability of the glass.

3. ANTIBACTERIAL TEST:

Lithium containing bioactive glass has considerable antibacterial activity against Enterococcus faecalis (as shown in Fig 6). It is a gram positive bacteria, belongs to the Group D streptococcus system. It can cause life threatening infections in humans, especially in the nosocomial environment. It has been frequently found in root canal treated teeth ranging from 30% to 90% of the cases. E.Faecalis is more likely to entertain in root canal treated teeth than primary infections. As it can be seen, the inhibitory zone of the bacteria is transparent and their mean zone of inhibition is around 6 ±_0.2mm. Many therapeutic metals like Ag, Zn, Cu, has efficient antibacterial activity as reported earlier by S.Jaiswal et.al.[18] According to the obtained results, it can be concluded that lithium containing bioactive glass also has great potential to be used as antibacterial agents in dental applications.

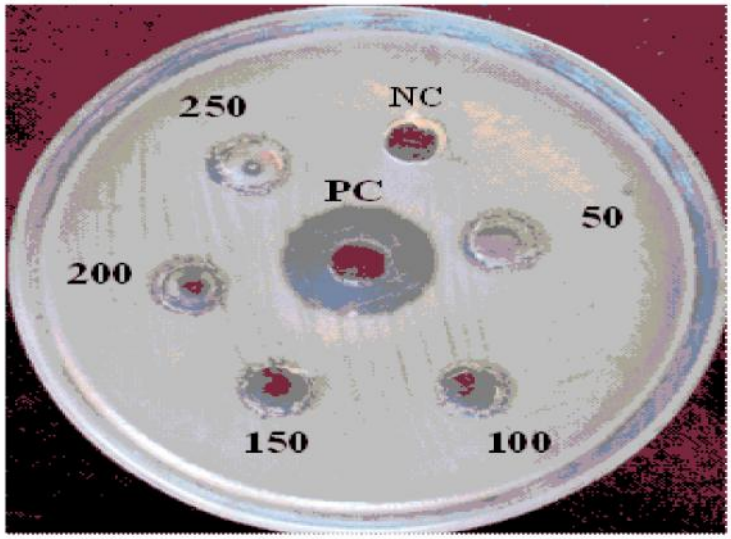

Fig: 6 Antibacterial study of sample (BGLi5) against *Enterococcus faecalis*

IV. CONCLUSION

Bioactive glasses with Li containing composition (55% -SiO2 36%-Cao 4%-P2O5 5%-Li2O) have been synthesized through quick alkali sol-gel process and their ability to stimulate apatite formation after immersion in SBF was studied. From Raman & XRD analysis, BGLi5 has apatite formation after 15 days of immersion, but it is very limited when compared to BGLi0. Rather Li addition enhanced chemical durability and antibacterial activity against Enterococcus faecalis. It is observed that like any other stimulatory ions, Li also has ability to influence apatite formation but at higher immersion time due to slow dissolution rate. Indeed, it proved its ability as excellent antibacterial agent, indicating its potential not only as biocompatible bioactive material but also as a candidate for the treatment of root canal treated tooth infections in dental applications.

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Reference:

1. Larry L. Hench **"**Bioceramics: From Concept to Clinic" J.Am.Ceram.Soc., Vol.74 [7] pp.1487 – 510 .1991
2. Maria Vallet-Regí "Ceramics for medical applications" J. Chem. Soc., Dalton Trans, pp.97–108., 2001
3. Hench LL. "Bioactive glasses and glass-ceramics": A perspective. In: Handbook of Bioactive Ceramics, Vol. 1. Boca Raton: CRC;. pp 7–23 and 73–87 1990.
4. Julian R. Jones "Review of bioactive glass: From Hench to hybrids" Acta Materialia Inc. Published by Elsevier Ltd pp.1742-7061/2012.
5. L. L. Hench, O. H. Andersson, in "Advanced Series in Ceramics" (Eds: L. L. Hench, J. Wilson), Scientific Publishing Co. Ltd, Singapore **1993 .**
6. Anbalagan Balamurugan , Gerard Balossier , Sanjeevi Kannan , Jean Michel , Avito H.S. Rebelo , Jose M.F. Ferreira " Development and in vitro characterization of sol–gel derived CaO–P2O5–SiO2–ZnO bioglass" pp.1742-7061/ 2006
7. Larry L. Hench, Niksa Roki, Michael B. Fenn Bioactive glasses:" Importance of structure and properties in bone Regeneration" Published by Elsevier B.V. pp. 0022-2860/ 2014
8. Zhang Y, Santos JD. "Crystallization and microstructure analysis of calcium phosphate-based glass ceramics for biomedical applications". J Non-Cryst Solids Vol.272, pp.14–21, 2000;.
9. Sepulveda P, Jones JR, Hench LL." Characterization of melt derived 45S5 and sol gel derived 58 S bioactive glasses". J Biomed Mater Res;Vol. 58:pp.734–40. 2001
10. Li, R.; Clark, A.E.; Hench, L.L. An investigation of bioactive glass powders by sol-gel processing. J. Appl. Biomater. Vol. 2(4), pp.231–239. **1991**
11. Melek Erol1 Ayse Özyuguran1 Özge Çelebican1" Synthesis, Characterization, and In Vitro Bioactivity of Sol-Gel-Derived Zn, Mg, and Zn-Mg Co-Doped Bioactive Glasses Chem". Eng. Technol., Vol. 33, No. 7, pp.1066–1074 **2010**

12. Thi Thi Nge, Junji Sugiyama "Surface functional group dependent apatite formation on bacterial cellulose microfibrils network in a simulated body fluid"
13. Zhongkui Hong, Rui L. Reis, Joa˜o F. Mano," Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles"
14. P. Habibovic a, J.E. Barralet" Bioinorganics and biomaterials: Bone repair" Published by Elsevier Ltd. on behalf of Acta Materialia Inc pp.1742-7061/2011
15. Takadama H et al." Mechanism of biomineralization of apatite on a sodium silicate glass: TEM–EDX study in vitro". Chem Mater;Vol.13 pp.1108–13. 2001
16. Yi-Fan Goh , Ammar Z. Alshemary , Muhammad Akram , Mohammed Rafiq Abdul Kadir , Rafaqat Hussain , "In vitro study of nano-sized zinc doped bioactive glass". pp. 0254-0584/2012
17. M. Vallet-Regi, C. V. Ragel, A. J. Salinas, Eur. J. Inorg. Chem., Vol.6, pp.1029.2003
18. Swarna Jaiswala,b, Patrick McHaleb, Brendan Duffya "Preparation and rapid analysis of antibacterial silver, copper and zinc doped sol–gel surfaces" pp.0927-7765/ © 2012
19. Eileen Gentleman a,b, Yann C. Fredholma, Gavin Jell a,b, Nasrin Lotfibakhshaiesh a,b, Matthew D. O'Donnell c, Robert G. Hill d, Molly M. Stevens The effects of strontium-substituted bioactive glasses on osteoblasts and osteoclasts in vitro pp.0142-9612/ 2011
20. Xia Li,a Xiupeng Wang,b Dannong Hec and Jianlin Shi" Synthesis and characterization of mesoporous CaO–MO–SiO2–P2O5 (M =Mg, Zn, Cu) bioactive glasses/composites" J. Mater. Chem., Vol. 18, pp. 4103–4109 | 4103 2008
21. Susmita Bose, Gary Fielding, Solaiman Tarafder, and Amit Bandyopadhyay "Understanding of dopant-induced osteogenesis and angiogenesis in calcium phosphate ceramics"pp. 0167-7799/ , Vol. 31, No. 10 October 2013
22. Pingping Han, Mengchi Xu, Jiang Chang, Nishant Chakravorty, Chengtie Wu and Yin Xiao " Lithium release from β-tricalcium phosphate inducing cementogenic and osteogenic differentiation of both hPDLCs" The Royal Society of Chemistry 2014
23. Mina Khorami , Saeed Hesaraki , Aliasghar Behnamghader , Hamid Nazarian , Sara Shahrabi "In vitro bioactivity and biocompatibility of lithium substituted 45S5 bioglass" Materials science and Engineering C Vol.31, 1584 -1592,2011
24. By Julian Lieb, M.D. "Stimulating Immune Function with Lithium. Medical Hypotheses" Vol.69, pp 8-11 2007
25. Kokubo T, Takadama H." How useful is SBF in predicting in vivo bone bioactivity?" Biomaterials ; Vol.27 pp:2907–15.2006
26. A. Moorthi1, S. Saravanan, N. Srinivasan, N. C. Partridge, J. Zhu, L. Qin, and N. Selvamurugan " Synthesis, Characterization and Biological Action of Nano-Bioglass Ceramic Particles for Bone Formation" Journal of Biomaterials and Tissue Engineering Vol. 2, 197–205, 2012
27. A. Balamurugan, G. Balossier, J. Michel, S. Kannan, H. Benhayoune, A. H. S. Rebelo, J. M. F. Ferreira "Sol Gel Derived SiO2-CaO-MgO-P2O5 Bioglass System—Preparation and In Vitro Characterization".Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.30827
28. Yashar Rezaei , Fathollah Moztarzadeh , Sima Shahabi & Mohammadreza Tahriri (2014) "Synthesis, Characterization, and In Vitro Bioactivity of Sol-Gel-Derived SiO2–CaO–P2O5–MgO-SrO Bioactive Glass", Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, Vol. 44:5,pp. 692-701, 2014 "
29. Gisela M Luz and Jo˜ao F Mano "Preparation and characterization of bioactive glass nanoparticles prepared by sol–gel for biomedical applications" NanotechnologyVol. **22** (2011) 494014 (11pp)
30. Hamidreza Pirayesh† and John A. Nychka "sol–gel synthesis of bioactive glass-ceramic 45s5 and its in vitro dissolution and mineralization behaviour" "J. Am. Ceram. Soc.,Vol. 96 [5] pp. 1643–1650 (2013)
31. Fatemeh baghbani, Fathollah moztarzadeh, Leila hajibaki and Masoud mozafari "Synthesis, characterization and evaluation of bioactivity and antibacterial activity of quinary glass system (SiO2–CaO–P2O5–MgO–ZnO): In vitro study" Bull. Mater. Sci., Vol. 36, No. 7, pp. 1339–1346. December 2013
32. Aderemi Oki, Bushra Parveen, Safiyyah Hossain, S. Adeniji, Henry Donahue " Preparation and in vitro bioactivity of zinc containing sol-gel–derived bioglass materials" DOI: 10.1002/jbm.a.20070
33. Marta Giulia Cerruti "Characterization of bioactive glasses. Effect of the immersion in solutions that simulate body fluids".
34. Jipin Zhong, David C." Greenspan Processing and Properties of Sol–Gel Bioactive Glasses" USBiomaterials Corporation, Alachua, FL 32615
35. I. Notinghera,, A.R. Boccaccinia, J. Jonesa, V. Maquetb, L.L. Hencha "Application of Raman microspectroscopy to the .characterisation of bioactive materials"Vol. 03 pp.1044-5803/ 2003
36. Francesca Bonino, Alessandro Damin, Valentina Aina, Marta Miola, Enrica Verne, Oana Bretcanu, Silvia Bordiga, Adriano Zecchina and Claudio Morterra "In situ Raman study to monitor bioactive glasses reactivity" J. Raman Spectrosc; Vol. **39**: pp.260–264. 2008 .
37. D. Bellucci, G. Bolelli, V. Cannillo, A. Cattini, A. Sola "In situ Raman spectroscopy investigation of bioactive glass reactivity: Simulated body fluid solution vs TRIS-buffered solution"pp. 1044-5803/ 2011
38. Lucia Marsich, Loredana Moimas, Valter Sergo and Chiara Schmid "Raman spectroscopic study of bioactive silica-based glasses: The role of the alkali/alkali earth ratio on the Non-Bridging Oxygen / Bridging Oxygen (NBO/BO) ratio Spectroscopy"Vol 23, pp. 227–232 227 2009 .
39. Regina K. H. Seah, Marc Garland, Joachim S. C. Loo, and Effendi Widjaja "Use of Raman Microscopy and Multivariate Data Analysis to Observe the Biomimetic Growth of Carbonated Hydroxyapatite on Bioactive Glass**"**Anal. Chem. **,** Vol. 81, pp. 1442–1449, 2009
40. M. Tylkowski, D.S. Brauer Mixed alkali effects in Bioglass® 45S5 Journal of no-crystalline solids volume: 376, pg 175-181. 2013
41. Judith A. Juhasz , Serena M. Best "Bioactive ceramics: processing, structures and properties" J Mater Sci Vol. 47:pp.610–624.2012
42. Silver, I.A.; Deas, J.; Erecinska, M. "Interactions of bioactive glasses with osteoblasts *in vitro*: effects of 45S5 Bioglass, and 58S and 77S bioactive glasses on metabolism, intracellular ion concentrations and cell viability". Vol.*22*(*2*), pp.175–185.2001