Development of Zirconia Substituted 1393 Bioactive Glass for Orthopaedic Application

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ABSTRACT

Zirconia was used as a bone substitute, since it has excellent mechanical properties. It is also good bioinert to bone tissue. In this study, we report on zirconia substituted 1393 bioactive glass. In novel melt-derived “1393” Bioactive glass (53 SiO₂, 6Na₂O, 12 K₂O, 5MgO, 20CaO, and 4 P₂O₅ wt %) with CaO was doped by ZrO₂ for and were melted at 1400°C in alumina crucibles in electric furnace with air as furnace atmosphere. Bioactivity of these samples were analysed by put in the SBF for different time periods. Hydroxy Carbonate Apatite layer was developed and identified by FTIR, XRD and SEM. Chemical durability was also determined by weight loss method. Density of the samples were determined and found to increase significantly with increasing amount of (0-2 wt %) zirconia, reason is due to replacement of lighter element of CaO has been replaced by heavier element ZrO₂. Finally, this investigation clearly concluded that ZrO₂ substituted bioactive glass would be potential biomaterials for biomedical applications.

Keywords: Bio-active glasses, Simulated body fluid, XRD, SEM, FTIR, pH, Chemical durability, Density.

INTRODUCTION

Ceramics which are used for filling and rebuilding of non-healing bone defects and for healing of damaged or diseased parts of the muscular-skeletal system is termed as bio ceramics¹. Bioinert (alumina, zirconia), bioactive (hydroxyapatite, glass, and glass-ceramics), resorbable (tricalcium phosphate) or porous for tissue in growth (hydroxyapatite-coated metals, alumina) may be used in the bio ceramic composites². Several types of bioactive glasses are developed over past years. Bioactive glasses (BG) have been known for their bioactive properties and their aptitude to form a strong bond to bone by formation of hydroxyapatite surface layer³. Bioactive glasses (BGs) such as “45S5 Bio-glass” (45 SiO₂, 24.5CaO, 24.5Na₂O, 6 P₂O₅ wt %) and “1393” (53 SiO₂, 6Na₂O, 12 K₂O, 5 MgO, 20 CaO, and 4 P₂O₅ wt %) compositions have been widely used for bone tissue engineering applications²⁻⁴. Hench et al., examined the in vitro bonding mechanism with synthetic material.
because of the chemical reactions taking place over glass surface. These chemical reactions strongly help the implants to bond with the bone tissues; hence one can replace the diseased or damaged part of the human bone\(^5\). Many glass compositions have been developed by doping therapeutically active ions such as strontium, zinc, magnesium, fluoride and cobalt in silicate glass system have been discovered in the past\(^6\)-\(^9\). Several researches are going on for preparation and characterization of glasses and glass-ceramics, doped with some ions such as Zr, Li, Fe, Ti, K, Zn, Sr and Mg because of their unique effect on differentiation, osteoblastic cell proliferation and thus bone mineralization\(^10\)-\(^15\). The ZrO\(_2\) substituted is widely used as a substrate in hard tissue applications due to its excellent strength and fracture toughness\(^16\). It was discovered from the reaction product obtained after heating gems by the German chemist Martin Heinrich Klaproth in 1789\(^17\). Several research articles have suggested that zirconia has good chemical and dimensional stability, mechanical strength and toughness and it is also biologically inert\(^18\). Many study have shown that the compressive strength of ZrO\(_2\) is relatively higher than porous HA and ZA20 (20 wt. % Al\(_2\)O\(_3\) added TZP) ceramics. In vitro evaluation has also shown that ZrO\(_2\) is not cytotoxic\(^19\)-\(^21\). Vyas et al.,\(^22\)-\(^27\) in an earlier investigation had also shown that the addition of cobalt oxide and nickel oxide up to 0.2 wt% in 45S5 glass (BG) and glass-ceramic (BGC) has resulted an increase physico-mechanical properties and bioactivity of their samples due to formation Co-O-Si and Ni-O-Si bonds. Tripathi et al.,\(^28\) have also found the characterization of Li\(_2\)O, CaO, Al\(_2\)O\(_3\), P\(_2\)O\(_5\), SiO\(_2\) glasses as bioactive material and all the elastic moduli values were establish to increase with the increasing in Al\(_2\)O\(_3\)/Li\(_2\)O ratio.

In the current investigation an endeavour has been made to study the construct of zirconia doping in silica-based bioactive glass and to verify its bioactivity and other characteristics.

**MATERIAL AND METHODS**

### Preparation of glass

For the source of SiO\(_2\),\(^15\) Na\(_2\)O, CaO, K\(_2\)O, P\(_2\)O\(_5\) and MgO, Fine grained quartz, anhydrous sodium carbonate, anhydrous calcium carbonate, Potassium Carbonate, ammonium dihydrogen orthophosphate and Magnesium Carbonate respectively were used\(^4\). The ZrO\(_2\) is available as it is and was added for preparation of bioactive glasses. All the batch materials were of analytical grade chemicals and were used without further purification. The weighed batches were mixed properly with the help of mortar and pestle. Before mixing the mortar and pestle were cleaned thoroughly and allowed to dry properly. After thorough mixing of batch materials were kept in alumina crucible and placed in an electric furnace. The furnace temperature was set to 1400°C and the duration to reach 1400°C was set to 3 h and after reaching 140°C the steady state was maintained for more 3 hours. After melting the prepared bioactive glass samples were poured into moulds and were directly transferred to a regulated muffle furnace at the temperature of 500°C for annealing. The muffle furnace was left to cool to room temperature after 1 h Annealing is done to remove the internal stress after the glasses are formed.

**Simulated Body Fluid (SBF)**

Kokubo and his teams developed Simulated Body Fluid (SBF) which has similar inorganic ion concentrations to those of human extracellular fluid\(^22\),\(^29\)-\(^31\). The ion concentrations of SBF are given on Table. 2.

**Procedure**

1. The HCl available in the laboratory is of 11.6 N. So it was diluted to 1 N-HCl by the following formula.

\[
N_1V_1=N_2V_2
\]

Where \(N_1, N_2\) = Normality of 1\(^{st}\) and 2\(^{nd}\) solution respectively

\(V_1, V_2\) = Volume of 1\(^{st}\) and 2\(^{nd}\) solution respectively

We have taken 100 ml distilled water and we need to find out how much HCl of 11.6N is required to be mixed with it to get 1N-HCl solution.

\[
11.6 \times V_1 = 1 \times 100
\]

\[
=> V_1 = 8.62 \text{ ml}
\]

So 8.62 ml of 11.6N HCl was mixed with 100ml of distilled water to get 1N-HCl.

2. 700 ml of distilled water is poured into magnetic stirrer.
3. The 1 to 5 materials mentioned in the table 2 were added one by one.
4. The 1N-HCl was added to the above mixture of about 20 ml and pH was maintained to be 2.0/2.5.
5. Then the material numbered 7-8 were added one by one slowly.
6. Finally the tris-buffer was added very slowly. Then HCl was added and the pH was adjusted to be within 7.2/7.25.

Thus, SBF solution was prepared which was used for evaluation of bioactivity of artificial materials in vitro.

Characterization
Using the XRD, crystalline phases present in before and after SBF treated glass samples were identified. The samples were ground to 75μm and subjected to XRD using RIGAKU-MiniX II diffractometer of range between 20° and 80°. Fourier trans-form infrared (FT-IR) spectrometer (VARIAN scimitar 1000, USA) range was used to investigate the functional groups present in bio-glass samples in the range of 4000–400 cm\(^{-1}\). The surface morphology of bioactive glass samples was analysed by SEM (Inspect S50, FEI) before and after SBF behaviour.

In vitro bioactivity test
In vitro test was performed to examine the bioactivity of all samples by immersing 0.5 g of each samples in 50 ml of SBF solution in a plastic box and incubated at 37.5°C in a static condition for 2, 5, 7, 15 and 31 days. pH was also measured using Universal Biomicroprocessor pH meter calibrated with buffer solutions of pH 4.00 and 7.00 at room temperature. pH values have been recorded during above given time periods. The surface morphology of bioactive glass samples was analysed by SEM (Inspect S50, FEI) before and after SBF behaviour.

Density measurement
The density of samples was measured by Archimedes principle at room temperature. For immersing the samples into water thin copper wire was used. The density was determined from the following equation:

\[
\text{Density} = \frac{\text{Ma}}{\text{Ma} - \text{Mw}} \times 0.988
\]

Ma Mass in air and Mw is the Mass in water.

The measurements were carried out in triplicate.

Weight loss measurement
The solubility of bioactive glasses was finding by measuring weight loss in SBF at 37.5°C in the incubator. With the help of 500 grit polishing papers the samples were polished. Then they were washed in acetone for a minute and were placed in small plastic containers which contain SBF. On various days, the samples were taken out and with the help of tissue excess moisture was removed. Then the samples are weighted. The percentage changes in weight loss were directly correlated to glass corrosion or solubility in SBF. The amount of weight loss was calculated using the following equation:

\[
\text{Weight loss (mg/g)} = \frac{W_i - W_f}{W_i}
\]

Where \(W_i\) is the initial weight of the specimen and \(W_f\) the weight on different days.

RESULT AND DISCUSSION

FTIR Absorbance Spectrometric Investigation of bioactive glass
Figure 1 shows the FT-IR absorbance spectra of glass samples before put into SBF solution. From Fig.1 it was trend that G-1 and G-2 are showing almost same peak i.e. at wave number 1338.54384 cm\(^{-1}\) respectively. Another peak at 1031 and 945 cm\(^{-1}\) are shown by G-1 and G-2. From the infrared absorption band table we can see that these peaks are referring to -O-Si- bond and -Si-P- bond\(^{15}\). The rest three glasses are showing peaks in the range 1562, 1556 and 1558 , 1338  and 945, 943, 947 cm\(^{-1}\). The 1338 cm\(^{-1}\) wave number corresponds to P=O bond\(^{32,38}\). The wave number 1556 cm\(^{-1}\) corresponds to –Si-P- bond\(^{39}\). The FTIR spectra peaks of G-2, G-3, G-4 and G-5 samples
have the same behaviour of G-1 with small change in the peaks intensities as shown in the Fig.1. The glasses doped with Zirconia are not showing remarkable changes in the FTIR absorption spectral characteristics.

**In vitro bioactivity by FTIR Absorbance Spectrometric**

The FT-IR absorbance spectral bands of the glasses before and after immersion in SBF for different days such as 2, 5, 7, 15, and 31 days were shown in Fig. 2, 6. The increased in the intensity of absorption band is due to increase in molecular concentration of species on the surface of the glasses after soaking into SBF with time. The FTIR analysis of G-1 before and after drenched into SBF solution for 2, 5, 7, 15 and 31 days were presented in Fig. 2. The new bands were establish to come out after soaking into 2 days in SBF solution and the corresponding wave numbers are 3745, 1558, 738 cm\(^{-1}\). The FTIR absorbance spectral band at 738 corresponds to Si-O-Si symmetric stretch of tetrahedral non-bridging oxygen atoms. The major bands at about 1558 are credited to Si-P bond. On 15th day and 31st day peaks are found at 3184 cm\(^{-1}\) which corresponds to –OH group (associated). The 1296 cm\(^{-1}\) wave number corresponds to P=O bond. The band corresponding to wave number 3745 cm\(^{-1}\) belongs to hydroxyl group (O-H stretch (free)). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl apatite (HA) layer.

Figure. 3 depict it the FT-IR analysis of G-2 both before, after drenched into SBF solution for 2, 5, 7, 15 and 31 days. On 5th days of soaking the prominent wave numbers are obtained at 3136, 1552, 1338, 1028 cm\(^{-1}\). The 1552 corresponds to Si-P bond. Here a new peak was obtained corresponding to 1338 cm\(^{-1}\) wave number which is associated with Zr-OH bonding. This is because bio glass is doped with zirconia. The band corresponding to wave number 1028 cm\(^{-1}\) is Si-O-Si stretching bond. The bands at 652 cm\(^{-1}\) correspond to (carbonate) C-O stretching mode. The wave number 3136 cm\(^{-1}\) is 31 days corresponds to OH group (associated). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer.

Figure. 4 depicted the FT-IR analysis of G-3 before and after drenched it into SBF for 2, 5, 7, 15 and 31 days. On 5th days of soaking, the prominent wave numbers are obtained at wave number 3745, 3136, 2553, 2158, 1558, 1338, 1016, 652 cm\(^{-1}\) respectively. The peak obtained at 1338

### Table 1: Composition of base and doped glass

| Glass sample \ Composition | SiO\(_2\) (wt %) | Na\(_2\)O (wt %) | K\(_2\)O (wt %) | P\(_2\)O\(_5\) (wt %) | MgO (wt %) | CaO (wt %) | ZrO\(_2\) (wt %) |
|---------------------------|----------------|----------------|----------------|----------------|-----------|-----------|----------------|
| G-1                       | 53             | 6              | 12             | 4              | 5         | 20.0      | 0.0           |
| G-2                       | 53             | 6              | 12             | 4              | 5         | 19.5      | 0.5           |
| G-3                       | 53             | 6              | 12             | 4              | 5         | 19.0      | 1.0           |
| G-4                       | 53             | 6              | 12             | 4              | 5         | 18.5      | 1.5           |
| G-5                       | 53             | 6              | 12             | 4              | 5         | 18.0      | 2.0           |

### Table 2: Ion concentration (m M/liter) of SBF

| Ions | Na\(^+\) | K\(^+\) | Mg\(^{2+}\) | Ca\(^{2+}\) | HCO\(_3\) | HPO\(_4\)\(^{2-}\) | SO\(_4\)\(^{2-}\) | Cl\(^-\) |
|------|---------|--------|------------|------------|----------|----------------|----------------|--------|
| SBF  | 142.0   | 5.0    | 1.5        | 2.5        | 4.2      | 1.0            | 0.5            | 147.8  |
| WBC  | 142.0   | 5.0    | 1.5        | 2.5        | 27.0     | 1.0            | 0.5            | 103.0  |
is corresponds to Zr-OH bonding\textsuperscript{44,45}. This is because bio glass is doped with zirconia. The band corresponding to wave number 1016 cm\textsuperscript{-1} is Si-O-Si stretching bond\textsuperscript{15}. The wave number of 1558 cm\textsuperscript{-1} is linked with Si-P bond. The bands at 652 and 2553 cm\textsuperscript{-1} are correspond to (carbonate) C-O stretching mode. The wave number at 3136 and 3745 cm\textsuperscript{-1} are corresponds to OH group (associated) OH group (Free) respectively. This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer\textsuperscript{43}.

Figure. 5 depicted the FTIR analysis of G-4 before and after drenched it into SBF solution for 2, 5, 7, 15 and 31 days. On 15\textsuperscript{th} days of soaking, the prominent wave numbers obtained are at 3348, 1629, 1558, 1398, 1037, and 628 cm\textsuperscript{-1}. The band corresponding to wave number 1037 is Si-O-Si stretching bond\textsuperscript{15}. Here the peak obtained at 1398 cm\textsuperscript{-1} wave number is corresponds to Zr-OH bonding\textsuperscript{44,45}. This is because bio glass is doped with zirconia. The wave number of 1558 cm\textsuperscript{-1} is associated with Si-P bond. The bands at 628 cm\textsuperscript{-1} corresponds to (carbonate) C-O stretching mode. The wave number at 3136 cm\textsuperscript{-1} corresponds to OH group (associated). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer\textsuperscript{43}.

Bioactivity shown by X-ray diffractometer
Figure. 6 depicted the FTIR analysis of G-5 before and after drenched it into SBF solution for 2, 5, 7, 15 and 31 days. The G-3 sample has a peak at wave number 1028 cm\textsuperscript{-1} which corresponds to Si-O-Si symmetric stretching. On 15\textsuperscript{th} day of soaking, the prominent wave numbers obtained are at wave number 3290, 1629, 1558, 1037, and 628 cm\textsuperscript{-1} respectively. The band corresponding to wave number 1037 is Si-O-Si stretching bond\textsuperscript{15}. Here the peak obtained at 1398 cm\textsuperscript{-1} wave number is corresponding to Zr-OH bonding\textsuperscript{44,45}. This is because bio glass is doped with zirconia. The wave number of 1629 cm\textsuperscript{-1} is associated with Si-P bond. The wave number of 1558 cm\textsuperscript{-1} is associated with P=O bond. The bands at 628 cm\textsuperscript{-1} corresponds to (carbonate) C-O stretching mode. The wave number at 3290 corresponds to OH group (associated). This long duration of the sample soaked into SBF shows small decrease in the intensities of the bands which is the result of formation of hydroxyl carbonate apatite (HA) layer\textsuperscript{43}.

Figure. 7-11 shows the XRD of the glass samples (G-1, G-2, G-3, G-4 and G-5) before and
after putting in SBF for 15 days. The bioactive glasses have shown amorphous nature as there is no sharp peak in the XRD before immersion into SBF solution. The graphs which are drawn after soaking into 15 days in SBF solutions showed broad peaks within the range of 30° to 35°.

Figure 7.11 Contains the XRD pattern of Hydroxy Carbonated Apatite (HCA) powder to compare formation of HA layer on the surface of the SBF treated glass samples. The XRD graphs of the SBF treated samples have clearly shown the formation of crystalline HA on their surfaces after putting in SBF for 15 days. From Fig. 7.11 it was observed with increase in Zirconia content the number of peaks becoming more in numbers and broad. The XRD peak located at around 32° corresponds to Hydroxyapatite \([\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2]\) and the diffraction peaks were matched with the standard PDF#: 74056546.

Here it is identified that HA layer is getting formed when zirconia doped 1393 bioactive glasses were immersed into SBF solutions for varying period. The diffraction pattern of all the bioactive glasses showed the Hydroxy Carbonate Apatite crystalline phases. With increase in zirconia content the peaks which belong to HA were getting sharper.

**pH performance in Simulated body fluid**

Variation in pH values of bioactive glass after drenched into simulated body fluid (SBF) for 2, 5, 7, 15 and 31 days are presented in Fig. 12. It was observed that up to 7 days all glass samples are showing increase in pH values almost linearly from pH value 7.3. The G-4 bioactive glass is showing maximum pH value on 7th day i.e. 9.5. Due to the addition of zirconia there are variations in the pH values for different samples. The G-4 bioactive glass is showing maximum pH at the end of 31st day. The G-4 which consists of 1.5 wt% of Zirconia is showing maximum pH value. It was found that in all cases the pH value is decreasing after 7 days and attended a constant value up to 31st days. The increase in pH values of bioactive glass in SBF is due to release of \(\text{Ca}^{2+}\) and \(\text{Na}^+\) ions from the sample surface22, 23, 25, 26. The sample number G-4 with higher zirconia content was found to show maximum pH value may be due to high rate of dissolution as compared to base sample G-1. The incorporation of Zirconia into 1393 glass resulted in an increase in the pH of SBF. Their high
degradation rate leads to a higher pH value and this agrees to the condition of formation of HA like layer on the surface of the samples with more crystallinity.

**Weight loss measurement**

Figure 13 shows the % weight losses of glasses are presented in Fig. 13. It was observed that the base glass which is un-doped is showing less weight loss after soaking into SBF solutions. In all cases up to 7 days the weight loss is increasing proportionately with time and after that weight loss is almost constant for respective samples. From the pH graph the inference is already drawn that the pH is increasing due to release of Ca\(^{2+}\) and Na\(^{+}\) ion into SBF solution that means weight loss in G-4 glass is more which is exactly shown by the weight loss graph. It was found from that up to 7 days the weight loss in G-3, G-4 and G-5 glasses are almost same. That means the glasses with higher content of zirconia are possessing higher rate of dissolution, so % weight loss is more.
Density Measurement

Figure 14 shows the density of ZrO$_2$ doped 1393 glass. It is observed that the density of un-doped sample is found to be 2.44 gm/cm$^3$ and gradually it is increasing up to G-5 which is the highest doped glass ceramic (2.0 wt% Zirconia). The increase in density is due to replacement of CaO with ZrO$_2$ which is attributed due to the replacement of a light element (density of CaO– 3.35 g/cm$^3$) with a heavier one (ZrO$_2$ – 5.68 g/cm$^3$). The rate of increment of density is not same in all the cases as the density of G-3 is increased slightly whereas the rate of increment of density from G-3 to G-4 is more. The total range of density is found to be from 2.44 g/cm$^3$ to 2.495 g/cm$^3$. The density of bioactive glass is directly proportional to the solidity of the configuration\textsuperscript{22, 23,25,26}. Here G-4 and G-5 glass are showing approximately same density which means these materials are suitable for making scaffolds for bone tissue engineering\textsuperscript{37}.

Surface morphology by SEM

Figure 15 and Fig. 16 show the surface morphology of base glass (G-1) and the bioactive glass samples (G-2, G-3, G-4 and G-5) by their SEM images before and after the put in the SBF for 15 days at 37.5°C. The reflections in the SEM images are showing the glassy surfaces. The SEM images of samples before and after put in SBF shown uniform polycrystalline particle on the surfaces of the glass. These developed crystals on the surface of the glasses are assumed to be HCA\textsuperscript{48}. It is observed that for increasing ZrO$_2$ content, the crystalline phase formed is getting uniform shapes on the glass surfaces and for G-5 the hydroxyl carbonate apatite formed is having needle like structure. It can be concluded that the zirconia doped 1393 glasses can also generate HA like structure on their surfaces after immersion in to SBF solution. Previously it was already found out from the XRD results and FTIR spectrometry that, the HA layer is getting formed after immersion of glass into SBF for 15 days.

CONCLUSION

The XRD analysis showed the amorphous nature of the glass and FTIR absorbance spectra, pH behaviour; XRD and SEM images show the formation of HCA layer on the surface bioactive glasses after putting in SBF. Densities of substituted bioactive glasses are increased with increasing concentration of ZrO$_2$ while their Chemical durability decreased.
So it can be concluded from the experimental work that all the ZrO$_2$ substituted bioactive glass have shown improved properties. Among all the samples the G-4 glass is the best one as it has shown high pH value which suggests formation of HCA layer. It can be observed from the FTIR diagram of G-4 that all the bonds are showing prominent peaks and SEM images are also showing impressive results of HA layer formation. The prepared bioactive glasses can be used as for bone tissue engineering applications.

Fig. 15. SEM images of glass before putting into SBF solution

Fig. 16. SEM images of glass after putting into SBF solution
REFERENCES

1. Sergey, V. D.; Calcium Orthophosphates as Bio-ceramics: State of the Art., J. Function Biomaterial. 2010, 1, 22–107.
2. Hench, L.L.; Bio-ceramics, J. Am. Ceram. Soc. 1998, 74 (7), 1487-1510.
3. Gerhardt, L.C.; Boccaccini, A.R.; Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering, Materials 2010, 3, 3867-3910.
4. Hoppe, A.; Jokic B.; Janackovic D.; Cobalt-Releasing 1393 Bioactive Glass-Derived Scaûolds for Bone Tissue Engineering Applications, ACS Appl. Mater. Interfaces 2014, 6, 2865-2877.
5. Ylanen, H. O.; "Bioactive glasses materials properties and applications", Wood head PublishingLimited, Biomaterials, 2011, 6, 1-288.
6. Heness, G.; Ben-Nissan, B.; "Innovative bio-ceramics", Mater Forum, 2004, 27, 104-114.
7. Watts, S. J.; O’Donnell, M. D.; Law, R. V.; Hill, R. G.; "Influence of magnesia on the structure and properties of bioactive glasses", J. Non-Cryst. Solid, 2010, 356, 517-524.
8. Brauer, D. S.; Karpukhina, N.; O’Donnell M. D.; Law, R. V.; Hill, R. G.; "Fluoride-containing bio-active glasses: Effect of glass design and structure on degradation pH and apatite formation in simulated body fluid", Acta-Biomater., 2010, 6, 3275-3282.
9. Fredholm, Y. C.; Karpukhina, N.; Law, R. V.; Hill, R. G.; "Strontium containing bioactive glasses glass structure and physical properties", J. Non-Cryst. Solids, 2010, 356., 2546-2551.
10. Fatma, H.; ElBatal; Amany ;ElKheshen; Materials Chemistry and Physics, 2008, 110, 352-362.
11. O’Donnell, M. D.; Candelrioglu, P. L.; Miller C.A.; Gentleman, E.; Stevens, M. M.; J. Mater. Chem., 2010, 20, 8934-8941.
12. Balamurugan, A.; Rebelo, A.H.; Lemos, A.F.; Rocha, J.H.; Ventura, J.M.; Ferreira, J.M.; Dent Mater., 2008, 24, 1374-1380.
13. Oki, A.; Parveen, B.; Hossain, S.; Adeniji, S.; Donahue, H.; J. Biomed Mater Res A. 2004, 69, 216-221.
14. Saboori, A.; Sheikh, M.; Moztarzadeh, F.; Rabiee, M.; Hesaraki, S.; Tahriri, M.; AdvAppl Ceram. 2009, 108,155-161.
15. Vikash Kumar Vyas; Arepalli Sampath Kumar; HimanshuTripathi; Singh, S.P.; Ram Pyare; Effect of Cr2O3 Addition on the Bioactivity and Physico-mechanical Properties of 45S5 Bioactive Glass and Glass-Ceramic, International Journal of Engineering Research & Technology (IJERT) 2014, 3, 1-15.
16. Hulbert, S.F.; The use of alumina and zirconia in surgical implants.In: Hench, L.L.; Wilson J; editors. Advanced series in ceramics, 1: An introduction to bioceramics. London: World Scientiûc; 1993.
17. Julian Jones, R.; New trends in bioactive scaffolds: The importance of nanostructure, Journal of the European ceramic society, 2009, 29(7), 1275-1281.
18. Miao, X.; Hu, Y.; Liu, J.; Huang, X.; "Hydroxyapatite coating on porous zirconia", Materials Science and Engineering: 2007, 27(2), 257-261.
19. Sarkar, R.; Banerjee, G.; Ceramic based biomedical implants. Inter. Ceramic, 2010, 2, 98–102.
20. Zhang, S.; Hydroxyapatite Coatings for Biomedical Applications, Advances in Materials Science and Engineering, C.RC Press, 2013, 9., 469.
21. Ducheyne, P.; Hench, L.L.; Kagan, I.; Martens, A.; Bursens, A.; Muller, J.C.; Effect of hydroxyapatite impregnations on skeletal bonding of porous coated implants. J. Biomed. Mater. Res.,1980, 14, 225–237.
22. Vyas.V.K. Kumar S.A.; Prasad S.; Singh S.P.; Pyare R.; "Bioactivity and mechanical behavior of cobalt oxide-doped bioactive glass", Bull. Mater. Sci., Indian Academy of Sciences 2015, 38(4), 957–964.
23. Vyas.V.K. Kumar S.A.; Prasad S.; Mohammad E.; Singh S.P.; Pyare R.; "Preparation and Characterization of Cobalt Oxide Doped 45S5 Bioactive Glass-Ceramics", Innovations in Corrosion and Materials Science, 2015, 5, 86-92.
24. Vyas.V.K. Kumar S.A.; Singh S.P.; Pyare R.; "Effect of Cobalt Oxide Substitution on Mechanica Behaviour and Elastic
Properties of Bioactive Glass and Glass-Ceramics,” Trans. Ind. Ceram. Soc., 2016, 75, 1, 1-8.

25. Vyas.V.K. Kumar S.A.; Singh S.P.; Pyare R.; “Effect of nickel oxide substitution on bioactivity and mechanical properties of bioactive glass,” Bull. Mater. Sci., 2016, 39, 5, 1355–1361.

26. Vyas.V.K.; Kumar S.A.; Singh S.P.; Pyare R.; Ali A.; Prasad S.; Md. Ershad; Srivastava P.; Mallick S.P., “Assessment of nickel oxide substituted bioactive glass-ceramic on in vitro bioactivity and mechanical properties,” Boletín de la Sociedad Española de Cerámica y Vidrio, 2016, 69, 12.

27. Vyas.V.K. Kumar S.A.; Singh S.P.; Pyare R; “Destructive and non-destructive behavior of nickel oxide doped bioactive glass and glass-ceramic”, J Aust Ceram Soc, 2017, DOI 10.1007/s41779-017-0110-2.

28. Tripathi H.; Kumar A. S.; Singh S. P.; “Preparation and characterization of Li2O–CaO–Al2O3–P2O5–SiO2 glasses as bioactive material,” J. Non-Cryst. Solids, 2002, 62 (4), 600–612.

29. Montagne, L.; Palavitz G.; Mairesse G.; Phys. Chem. Glasses, 1996, 37(5), 206.

30. Brown, R.K.; Tallant, D.R.; Myers, S.T.; Phifer C.C.; J. Non-Cryst. Solids, 1995, 45, 191.

31. Corbridge, D.E.; J. Appl. Chem., 1956, 6, 456.

32. Garoig-Lagrange, C.; Ouchetto M.; Elouadi, B.; Can. J. Chem. 1985, (63), 1436.

33. Gabelica-Robert, M.; Tarte, P.; J. Mol. Struct., 1982, (79), 251.

34. Salim, M.A.; Khattak, G.D.; Sakhatow Hussain, M.; J. Non-Cryst. 1995, 185, 101.

35. Chahine, A.; Et-tabirou, M.; Pascal, J.L.; FTIR and Raman spectra of the Na2O–CaO–Al2O3–P2O5 glasses, Materials Letters, 2004, 58, 2776–2780.

36. Abel, E. W.; Sabherwal I. H.; Reactions of the silicon–phosphorus bond. Part I. Insertions in trimethylislyldi phenyl phosphate, J. Chem. Soc. A, 1968, 1105-1108.

37. Rehman, I.; Karsh, M.; Hench, L.L.; Bonùeld, W.; Analysis of apatite layers on glass-ceramic particulate using FTIR and FT-Raman spectroscopy, J. Biomed. Mater. Res., 2000, 50 (2), 97–100.

38. Fligueiras, M.R.; LaTorre, G.; Hench, L.L.; Solution effects on the surface reactions of three bioactive glass compositions, J. Biomed. Mater. Res., 1993, 27(12), 1485–1493.

39. Nayak, J. P.; Kumar, S.; J. Bera, “Sol-gel synthesis of bio-glass ceramics using rice husk ash as a source for silica and its characterization”, J. Non-Cryst. Solids, 2010, 356, 1447-1451.

40. Arepalli S. K.; Tripathi, H.; Vyas, V. K.; Jain, S.; Suman, S.K.; Ram Pyare; Singh, S.P.; “Influence of barium substitution on bioactivity, thermal and physico-mechanical properties of bioactive glass”, Material Science and Engineering (C), 2015, 49, 549-559.

41. Jayakumar, S.; Ananthapadmanabhan, P.V.; Perumal, K.; Thiagarajan, T.K.; Mishra, S.C.; Su L.T.; Tok, A.I.Y.;J Guo, “Characterization of nano-crystalline ZrO2 synthesized via reactive plasma processing”, Materials Science & Engineering B, 2011, 12, 894-899.

42. Sarkar, D.; Mohapatra, D.; Ray, S.; Bhattacharyya S.; Adak, S.; Mitra, N.; “Synthesis and characterization of sol-gel derived ZrO2 doped Al2O3 nanopowder”, Ceramic international, 2007, 33(7), 1275-1282.

43. ElBatal H.; Azooz, M.; Khalil, E.M.; Soltan Monem A.; Hamdy, Y.; Characterization of some bioglass-ceramics, Mater. Chem. Phys., 2003, 80(3), 599-609.