ORIGINAL ARTICLE

Use of Inductive Coupled Plasma Mass Spectroscopy to Analyze the Properties of Ions Released by Bioactive Glasses and Bone Scaffolds

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ABSTRACT

This research discussed the characterization, fabrication and in vitro study of bone scaffolds and bioactive glasses. Bioactive glass is a novel material that dissolves and forms a bond with bone when exposed to body fluids. Bioactive glasses are silicate based, having calcium and phosphorous in equal ratio to those of the natural bone and this is the reason for their high biocompatibility. Traditionally bioactive glasses have been used to restore bone defects. The bioactive bone scaffolds have been discussed on the basis of melt derived bioactive silicate glass compositions. In addition ion release effects of bioactive glasses and scaffolds concerning, osteogenic and angiogenic responses are addressed. In this study the current knowledge on the bone tissue engineering scaffold and their clinical implication has been discussed. Inductive couple plasma mass spectroscopy was carried out to determine the release of zinc, strontium, phosphorous and cobalt within bioactive glass and bone scaffolds over period of time. ICP Vista Pro was used to carry out inductive couple plasma mass spectroscopy. The results show significant amount of release of cobalt,, zinc and strontium over period of time which confirmed the bioactive nature of glasses and justified the properties of ions released by glasses and scaffolds..The diversity of the bioactive glasses and bioactive scaffolds and their complex nature leaves enough space for the studies to be carried on.

Keyword: Inductive Coupled Plasma Mass Spectroscopy, Bioactive glasses, Bone scaffolds

INTRODUCTION

One of the functions of tissue engineering and regenerative medicine is to restore diseased and damaged tissues. For this purpose functional cells in combination of biodegradable scaffolds are used which are made up of engineered biomaterials(Hench et al., 2002)**.**Bone tissue engineering is one of the most interesting clinical applications of bioactive glasses. Bioactive glasses have wide ranging clinical applications which include bone grafts scaffolds and coating materials for dental implants**(**Williams, D. 2004)**.** Bioactive scaffolds could be used as an osteoconductive scaffold and has anosteostimulatory effect (Delben et al., 2009).The first bioactive glass 45S5 was made by Hench. The composition of this glassincludes45S5 wt.% SiO_2 , 24.5 wt.%Na_2O and CaO and 6 wt% P_2O_5,(Brunner et al., 2006).

The current research focuses on the addition and removal of certain ions for making bioactive more suitable for various clinical applications. Cobalt ions act as chemical mimickers of hypoxia and stimulate vascularization in a bone graft substitute in vivo. Hypoxic condition leads the cells to compensate by releasing angiogenic factors which results in new blood vessels formation (Kobayashi et al., 2010) .The presence of phosphate in bioactive glasses is in the form of orthophosphate**(**Elaine Quinlan et al., 2015**).**When the bioactive glasses are exposed to bodily fluid they form a hydroxycarbonate apatite layer **(**Tahmasebi Birgani Z1 et al., 2016**)**. The formation of hydroxycarbonate apatite layer increases the biocompatibility of these bioactive glasses**(**Brendan M et al ., 2014**).**Similarly phosphorous containing molecules are often used in bone tissue engineering to facilitate binding of calcium ions and increase the bone formation **(**Stancu Ietal., 2004), (Dadsetan M. et al ., 2012), (Nuttelman CR et al., 2006**).**

The positive effect of strontium on bone metabolism is widely recognized. It has been noticed that strontium ions inhibits osteoclast activity and it promotes osteoblastic activity and it favors bone formation. Another important significance of strontium is its bactericidal effect at the same concentration that it upregulates the osteoblast activity which has a big advantage **(**Liu X, Zhu S, et al., 2014), (Brauer DS et al., 2013).Zinc improves the ability of bonding of glass with the bone(Tang ZL1 et al., 2001). Zinc

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has another important role for DNA replication and stimulation of protein synthesis. (Li B et al., 2014). Studies have shown that zinc metal has a stimulatory effect on osteoblastic bone formation (Wang JY et al., 1996).

MATERIALS AND METHODS

The materials used in the study were ICI16M and QMNR2 glasses and their composition included Silicon dioxide (SiO_2), Calcium carbonate (Ca_2Co_3), Sodium Carbonate (Na_2CO_3), Potassium Carbonate (K_2CO_3), Cobalt Oxide (CoO), Zinc Oxide (ZnO), Magnesium oxide (MgO), Strontium oxide (SrO), Phosphorous pentoxide (P_2O_5). For scaffold preparation sodium alginate was added slowly to the beaker and then afterwards bioactive glass was added in it. After a thorough mix the gel solution was left to cool down. Gel was then poured into plastic bottles and immediately dipped into liquid nitrogen for freezing the scaffold sample.

The ICP was performed on samples of the scaffolds made up of glasses QMNR2 and ICIE16M. It was also performed in the same manner for ICI16M and QMNR2 glasses. The samples were dip into the simulated body fluid in the centrifuge tubes for 12 hours, 24 hours, 120hours and 2 weeks. The samples were kept in an incubator for the desired period of time and after wards the samples were filtered out from the solution with the help of a filter paper and solutions were kept in the centrifuge tubes. These tubes were placed in commercial fridge afterwards at 7˚C until ICP was performed. A parts per million conversion was done on Microsoft Excel. Then a standard solution was made for running ICP in which 2.5ml of sample solution, 2.25ml of H_2O and 0.25ml of Nitric acid (HNO_3) were present. ICP was carried out to determine the release of zinc, strontium, phosphorous and cobalt within glasses and scaffolds over period of time to see formation of apatite. It was also performed in the same manner for ICIE16M and QMNR2 glasses.

RESULTS

Fig. 1 Comparison of cobalt release in scaffolds and glasses. Here S stands for scaffold and G stands for glass.

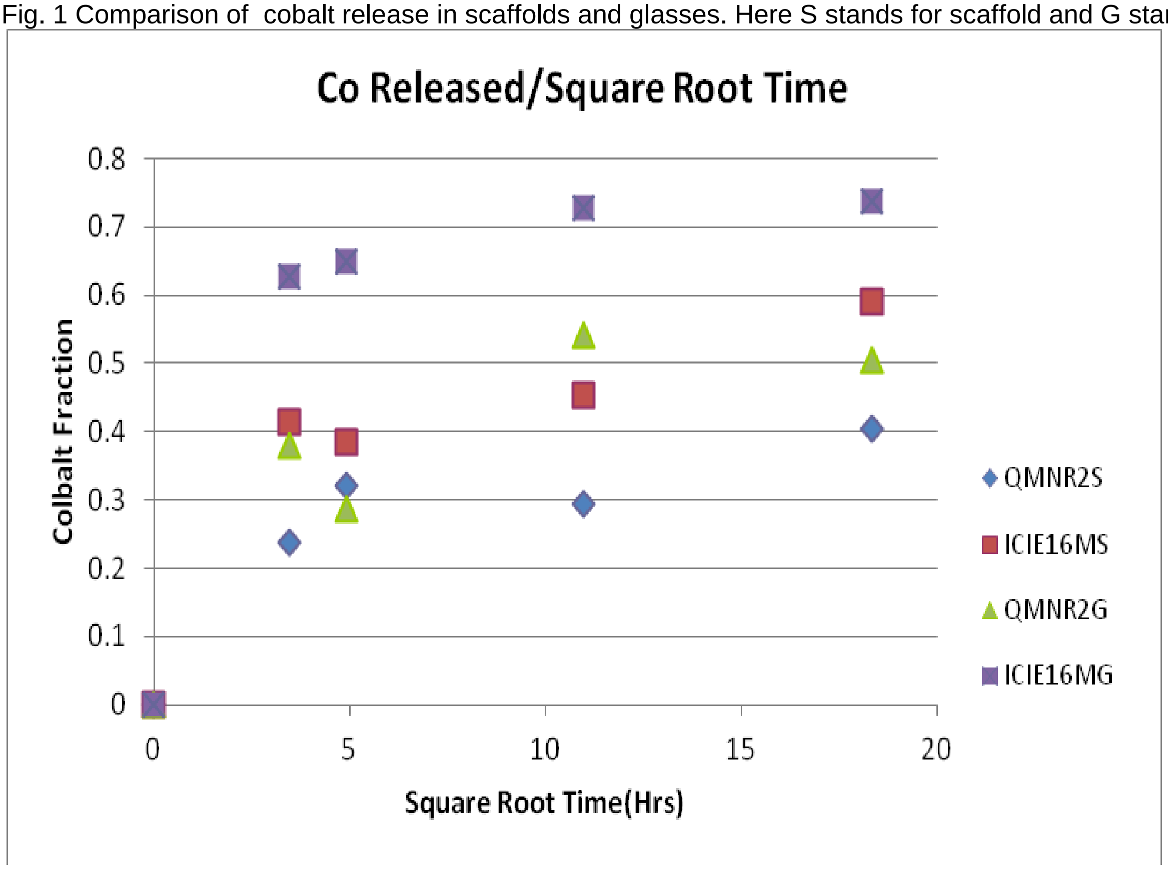


Fig. 2 Comparison of phosphorus release in scaffolds and glasses. Here S stands for scaffold and G stands for glass.

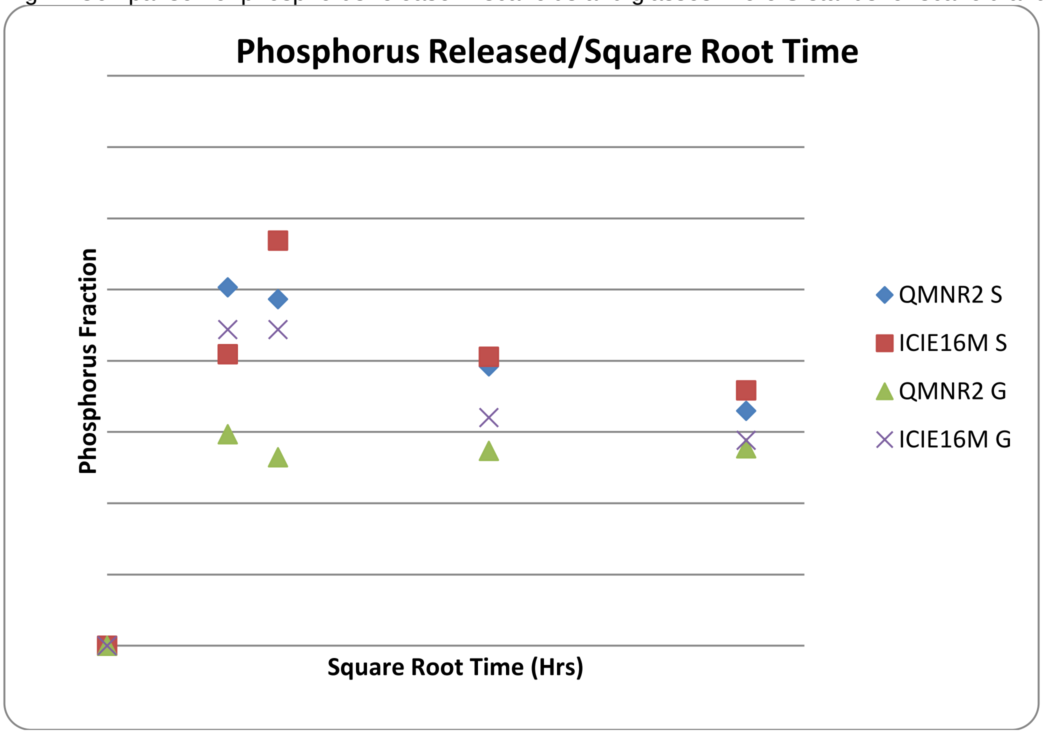


Fig 3 Comparison of strontium release in scaffolds and glasses. Here S stands for scaffold and G stands for glass.

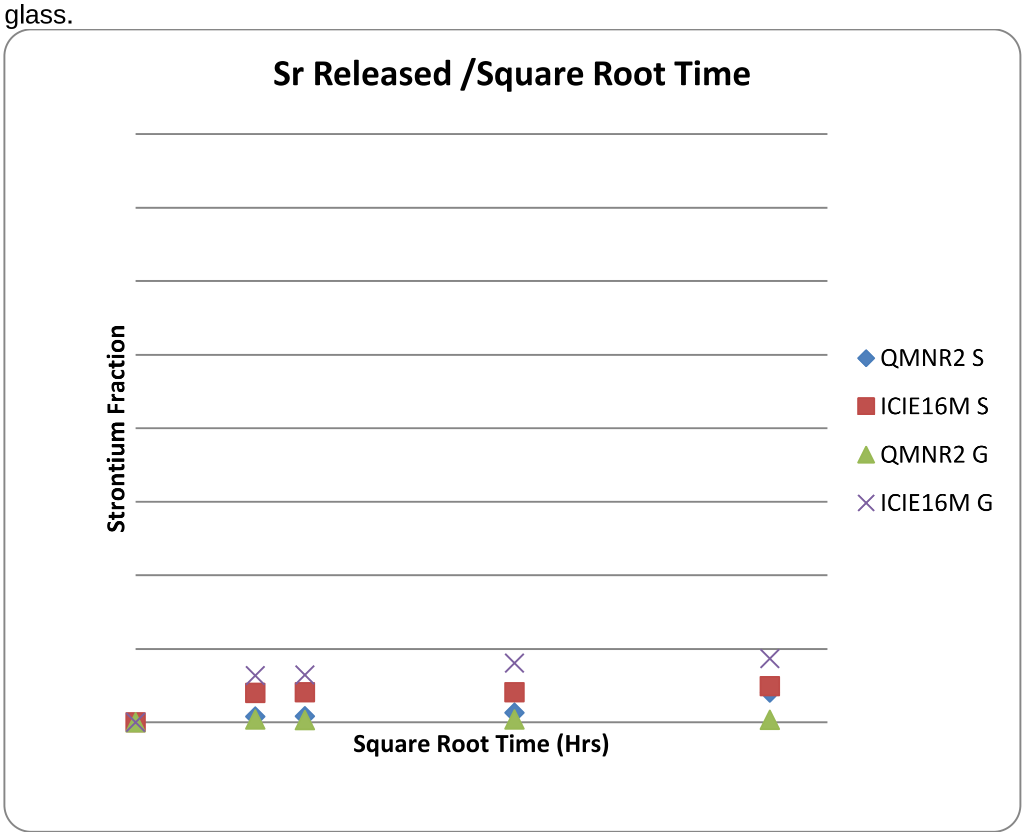
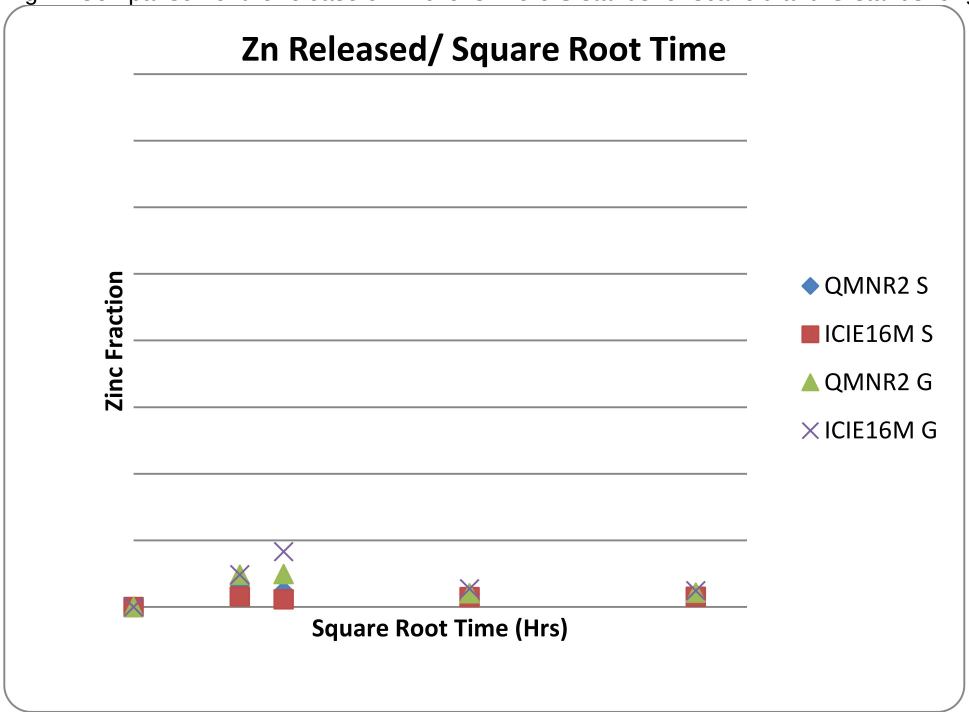


Fig. 4 Comparison of the release of zinc ions. Here S stands for scaffold and G stands for glass.



DISCUSSION

The pattern of cobalt release as shown in Figure 1 is showing controlled release of Co ions over the period of time in both glasses and scaffolds. The release of cobalt ions could be very helpful in forming bone and provides a stimulus for the formation of new blood vessels,(Mark W et al., 2008), (Chengtie et al., 2012). This phenomenon would be achieved by mimicking hypoxia in situ and this is achieved by the activation of hypoxia inducing factor -1α (Maria Goretti et al., 2012). The release of ions of cobalt increase with the passage of time. These results are comparable to the work done by (Saad M et al., 2017).

Figure 2 shows release of phosphorus. It is evident from the data that the amount of phosphorus release is decreasing with the passage of time in glasses and scaffolds. These results indicate that phosphorus might being used for the formation of calcium phosphate and later for the buildup of hydroxyapatite. These results are comparable to the results shown by (Xinkun Shen et al., 2014)

The ICIE16M scaffold and glass show an increasing trend of the release of strontium with the period of time as shown in Figure 3. The strontium release for QMNR2 glass remains constant while for the scaffold it increases slightly with the passage of time. Important significance of strontium is its bactericidal effect at the same concentration that it upregulates the osteoblast activity which has a big advantage in bone formation (Brauer DS et al., 2013).

If we follow the trend of zinc in all four of them we would notice that in start zinc is releasing, with the passage of time then the amount of zinc decreases as shown in Figure 4. One of the possibilities could be that the zinc might be entering glass network and making glass more stable (Liu X et al., 2014). Another important feature of zinc is to promote the growth of osteoblasts and results in the formation of the bone. (Liu X et al., 2014)

CONCLUSION

Bioactive glasses have many uses in medicine and dentistry. Altering the composition of the bioactive glass by adding different ions, changes its suitability for certain clinical procedures. The use of inductive couple plasma mass spectroscopy in this study determined the release of ions from the bioactive glasses and scaffolds over certain period of time.The release of ions confirmed the bioactive nature of bioactive glasses and bioactive scaffolds.The diversity of the bioactive glasses and bioactive scaffolds and their complex nature leaves enough space for the studies to be carried on.

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