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Influence of sodium oxide content on bioactive glass properties

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The rate of *in vivo* degradation and level of bioactivity of bioactive glasses are composition dependent [1]. By altering bioactive glass composition, the rate of resorption can be controlled. The network connectivity of a glass can be used to predict various physical properties of the glass including its solubility and, hence, its bioactivity [2]. Glass solubility increases as network connectivity is reduced. Glasses in the soda-lime phosphosilicate system were studied. The initial choice of composition was based on phosphate content and low network connectivity. A systematic substitution of calcium oxide for sodium oxide on a molar basis was made in order to examine the influence of sodium oxide content on the glass properties while keeping the network connectivity constant. The glass transition temperature and the peak crystallization temperature were seen to decrease linearly with increasing sodium oxide content. Thermal expansion coefficient and glass density were also seen to be related to sodium oxide content. Preliminary *in vitro* biocompatibility studies revealed that the glasses of higher sodium oxide content were associated with a cytotoxic response. The measurement of media pH indicated that this cytotoxic effect was due to ion exchange reactions at the glass surface.

1. Introduction

A bioactive glass is one of such composition that it undergoes surface dissolution in a physiological environment [3]. The formation of hydroxyapatite on the glass surface is a direct result of the surface reactions which take place [4] and enables the formation of a chemical bond with bone tissue [5]. The sequence of surface reactions proposed by Hench [6] may be summarized as follows:

1. Exchange of Na^+ for H^+/H_3O^+ at the glass surface → pH increase.
2. Break up of silicate network → silanol groups.
3. Condensation and polymerization of silanols → silica gel layer.
4. Migration of Ca, P to surface → Ca, P-rich film.
5. Crystallization of calcium phosphate → hydroxyapatite.

This mechanism is widely accepted but does have some shortcomings. It is not particularly useful as a predictive tool. Ebisawa *et al*. [1] have produced a bioactive glass with a molecular formula of $CaO{\cdot}SiO_2$. The mechanism for bioactivity proposed by Hench cannot account for the bioactivity of this glass [2].

If silicate glasses are considered to be inorganic polymers of silicon cross-linked by oxygen, concepts such as cross-link density or network connectivity can be applied to describe their structure [7]. The network connectivity of a glass is defined as the average number of additional cross-linking bonds (more than two) for elements other than oxygen that form the network backbone. The calculation of network connectivity of a glass network is based on the relative numbers of network-forming oxide species (those which contribute ''bridging'' or cross-linking oxygen species) and

network-modifying species (those which result in the formation of ''non-bridging'' species) present.

The network connectivity of a glass can be used to predict various physical properties of the glass, including its solubility [2]. The silicate structural units in a glass of low network connectivity are probably of low molecular mass and are capable of going into solution. Consequently, glass solubility increases as network connectivity is reduced. Glasses of low network connectivity are thus potentially bioactive.

Work carried out by Lockyer *et al.* [8] determined the effect of substituting sodium oxide for calcium oxide on some glass properties. This study, along with most studies on bioactive glass systems however, was carried out on a weight per cent basis. This has the effect of masking the composition–property relationships of bioactive glasses [2] as there is no account taken of the degree of disruption of the glass network. Lockyer *et al.* have noted this shortcoming [8] as has Strnad [9]. Network connectivity calculations are carried out using mole fraction or mole per cent values. Mole per cent substitutions are known to have more significance on a structural level.

Fig. 1 is a representation of a highly disrupted glass network with a network connectivity value of 2.00. It can be seen that for every mole of calcium oxide removed from the glass network, one mole of sodium oxide must be added in order to maintain the same number of non-bridging oxygen species and, hence, the same network connectivity value. Carrying out such a substitution on a weight per cent basis produces a change in the relative number of non-bridging oxygen species and bridging oxygen species, with the result being a change in network connectivity.

In order to understand the influence of substituting one network-modifying oxide in favor of another, it is necessary to eliminate any possible interference that could be attributed to changing the value of the network connectivity. This work uses the concepts of network connectivity for the purposes of designing bioactive glass compositions. Sodium oxide content has been shown previously [8, 10] to influence the physical properties of glass systems. This work undertakes a substitution of calcium oxide for sodium oxide on a mole per cent basis in order to determine its influence on glass properties with a view to obtaining simultaneous control of the physical, chemical and biological properties of bioactive glasses.

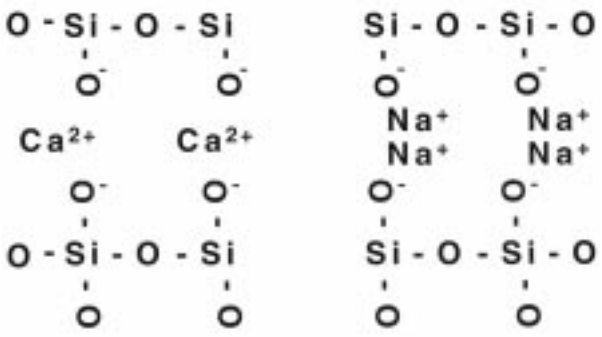

Figure 1 Glass structure representation.

2. Materials and methods

2.1. Glass production

A series of five glasses in the soda-lime phosphosilicate system of the general formula $3SiO_2 \cdot 0 \cdot 07P_2O_5 \cdot (3\text{-}X)CaO \cdot XNa_2O$ was produced. A systematic substitution of CaO for Na_2O on a molar basis was made and all of the glasses had a network connectivity of 2.04. The compositions were based on Ebisawa *et al.*'s [1] $CaO \cdot SiO_2$ composition. A low phosphate content was chosen in order to keep the network connectivity low, (phosphate results in an increase in network connectivity). Appropriate amounts of reagent-grade SiO_2, P_2O_5, $CaCO_3$ and Na_2CO_3 were mixed on a ball mill for 1 h followed by firing in a furnace for one hour at temperatures between 1350 and 1570 °C (depending on the composition) (Table I). The glasses were quenched in a vat of water and were shown to be amorphous using X-ray diffraction (XRD).

2.2. Physical property analysis

Differential scanning calorimetry (DSC) was carried out on 50 mg samples of the glasses between 25 and 1200 °C at a ramp rate of 10 °C min^{-1}. Three particle sizes were analyzed: frit (> 150 μm), coarse (< 150 μm, > 45 μm) and fine (< 45 μm). Dilatometry was carried out on cast bars of the glasses between 30 °C and their appropriate glass transition temperatures at a rate of 5 °C min^{-1} in order to determine their thermal expansion coefficients (TEC). Glass density was determined for samples of cast glass and the value of glass oxygen density was calculated using these measurements according to Ray [11].

2.3. Cell culture sample preparation

The glasses were sintered at temperatures between their glass transition temperatures and the onset of crystallization such that the samples produced were amorphous. This was checked using XRD. Approximately 0.3 g of coarse powder (< 150 μm, > 45 μm) was compressed in a die at a pressure of 100 MPa. The discs produced were heated to an experimentally determined temperature at a

TABLE I Glass data

Glass	Value of *X*	Network connectivity	Firing temperature (°C)	Sintering temperature (°C)
LG208	1.6	2.04	1350	520
LG211	1.2	2.04	1375	550
LG214	0.8	2.04	1400	654
LG223	0.4	2.04	1470	736
LG224	0	2.04	1570	820

ramp rate of 50 °C min^{-1}. The samples were held at this temperature for 30 min and cooled to room temperature over a period of 30 min to overcome the effects of thermal shock. The discs produced were of 8 mm diameter and 2 mm thickness.

2.4. Cell culture

The sintered discs were sterilized by dry heat (12 h at 200 °C). The samples were placed in 24-well cell culture dishes and inoculated with rat osteosarcoma cells (ROS 17/2.8, Merck Inc.) at a seeding density of 1.25×10^4 cells ml^{-1} with a total well volume of 2 ml. The growth medium consisted of Ham's F12 with the addition of 10% fetal calf serum by volume. The cell culture plates were incubated at 37 °C with a 5% CO_2 atmosphere. The cell culture medium was changed on day 3. Control samples consisted of ROS cells grown on tissue culture plastic.

Six samples of each glass were set aside for scanning electron microscopy (SEM). The influence of including a medium ''pre-wash'' step in the sample preparation was examined using these samples. Two samples of each glass were inoculated at day 1 while two were inoculated at day 5 following a ''pre-wash'' in cell culture medium. The remaining two were not inoculated with cells but were soaked in cell culture medium for the duration of the experiment. Medium changes were carried out on day 3 and day 5. Control samples consisted of ROS cells grown on tissue culture plastic discs.

The biological response to the sintered glass samples was observed with time using an inverted light microscope. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out on six samples of each glass on day 5 of the experiment and the absorbance at 570 nm compared to that of the control wells. A total protein determination was carried out on six samples of each glass using the Lowry method and the results compared to the control wells at day 5. The samples were observed under the SEM at day 5 (day 8 in the case of the samples inoculated on day 5 and the samples which were not inoculated). The pH of the removed medium was measured for all the samples at days 3 and 5 and compared to the pH of the medium removed from the control wells.

3. Results and discussion

3.1. Physical properties

The glass transition temperature was seen to decrease with increasing Na_2O content as shown in Fig. 2. This strongly linear relationship has been seen previously [8, 10]. A linear relationship was also observed between the peak crystallization temperature and Na_2O content. (Fig. 3). An excellent correlation is obtained between the TEC and Na_2O content in Fig. 4. The oxygen density in the glass network was also observed to vary linearly with Na_2O content (Fig. 5).

Properties such as glass transition temperature and peak crystallization temperature are critical for the sintering of glasses. A large window between these temperatures is required to ensure that the glass sinters without crystallizing. Crystallization of a bioactive glass inhibits its bioactive properties as the presence of crystalline phases gives rise to increased resistance to the ion exchange reactions which are seen to occur in physiological environments [12]. The crystallization of a glass also interferes with the correlation of its network connectivity with its physical and bioactive properties.

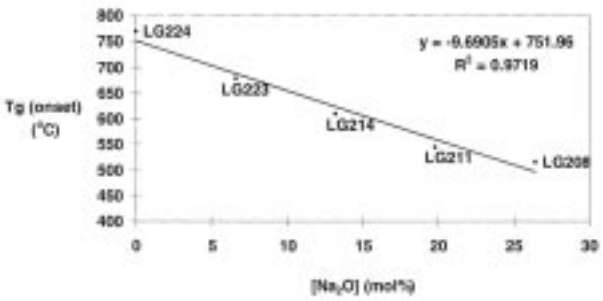

Figure 2 Influence of sodium oxide content on glass transition temperature onset.

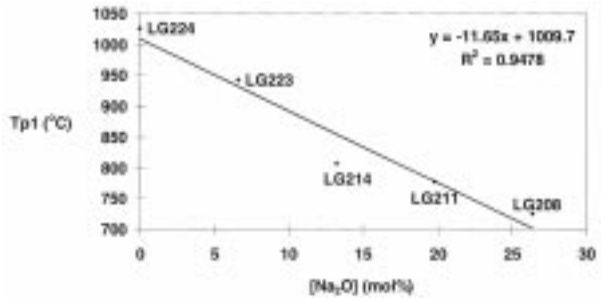

Figure 3 Influence of sodium oxide content on the peak crystallization temperature.

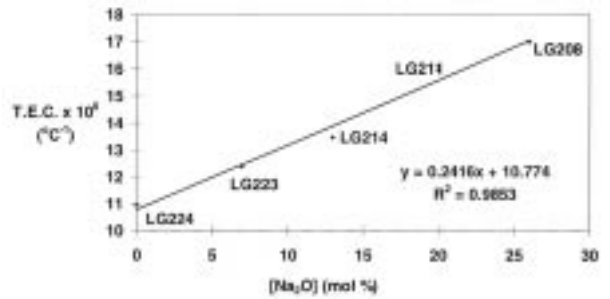

Figure 4 Influence of sodium oxide content on thermal expansion coefficient.

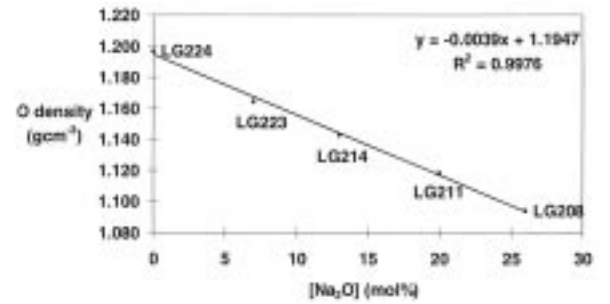

Figure 5 Influence of sodium oxide content on the oxygen density in the glass.

One of the potential applications for bioactive glasses is as a coating on metal prosthesis. In order to prevent interfacial separation of a glass and its metallic substrate, it is necessary to match the TEC of the glass and the metal. The TEC data obtained in this work (Fig. 4) indicate that it is possible to design bioactive glasses with a particular TEC without eliminating the bioactivity. In this way, it should be possible to produce a bioactive glass with an appropriate TEC for use as a coating on metallic orthopaedic implants such as Ti (TEC $= 8 \times 10^6\,^{\circ}C^{-1}$), Ti_6Al_4V (TEC $= 8 \times 10^6\,^{\circ}C^{-1}$) and Vitallium (TEC $= 12 \times 10^6\,^{\circ}C^{-1}$).

The density of oxygen in the glass network reflects the degree of packing of the atoms in the network. The substitution of CaO for Na_2O does not affect the network connectivity of the glass, but it does have a marked influence on the degree of packing of the atoms. As Na_2O content increases, the glass network is expanded resulting in a reduction of both glass density and glass oxygen density. This effect of expanding the network indicates that Na_2O is a more effective network disrupting species than CaO. This effect is also obvious in the reduction of the glass transition temperature as Na_2O content is increased. The temperature of the glass transition is a reflection of the degree of network disruption of a glass or cross-link density in the case of organic polymers [13].

The results of this study indicate that it is possible to engineer the properties of bioactive glasses simply by altering the glass composition. By varying the level of disruption in a bioactive glass network, the production of a glass with desirable physical properties should become feasible. This may be achieved through careful manipulation of the relative molar quantities of alkaline earth and alkali oxides present.

3.2. Cell culture

The preliminary *in vitro* biocompatibility study revealed that the biocompatibility of the glass samples was composition dependent. A cytotoxic response was observed in the case of the glasses with higher Na_2O content. The results obtained for the MTT assay (Fig. 6) and total protein determination (Fig. 7) show that glasses of lower Na_2O content were comparable to the controls and can be considered biocompatible. There is a large amount of scatter in some of the data (especially the data obtained from the MTT assay) which was thought to be due to interference by the material and the elevated pH of the medium.

The measurement of medium pH indicated that the cytotoxic response observed was due to an increase in medium pH resulting from ion exchange reactions which are known to occur at the surface of bioactive glasses in physiological environments. Sodium ions in the glass network are exchanged for protons in the external medium [6]. This accounts for the relationship observed between the medium pH and the Na_2O content of the glass samples (Fig. 8).

The glass samples which were subjected to a ''pre-wash'' in cell culture medium indicate a reduced pH effect. This implies that a ''pre-wash'' should be employed in the preparation of bioactive glasses of high Na_2O content for *in vitro* biocompatibility determinations. Such preconditioning has been described previously [12, 14].

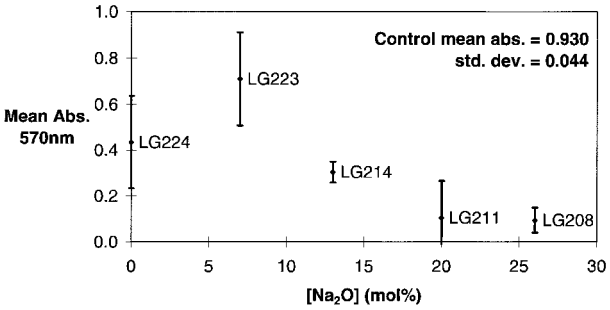

Figure 6 Influence of sodium oxide content on the results of the MTT assay.

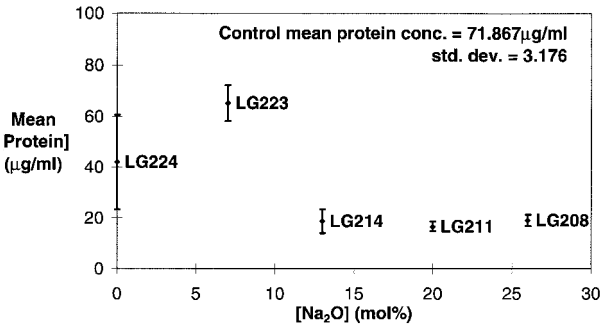

Figure 7 Influence of sodium oxide content on the results of the total protein assay.

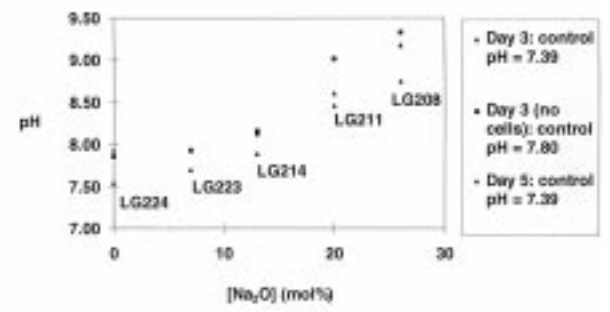

Figure 8 Influence of sodium oxide content on cell culture medium pH.

4. Conclusions

This work shows that it is possible to design bioactive glasses based on the concepts of network connectivity. Important physical properties of bioactive glasses such as their glass transition temperatures and their thermal expansion coefficients can be engineered by altering the glass composition. By altering the composition in such a way that the network connectivity is maintained at a value close to 2.00, it should be possible to produce a glass with useful physical properties without compromising its bioactivity.

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