In Vitro Evaluation the Influence of Glass-Ceramic Degradation Products on Osteoblast Cells.

Israa K. Sabree
Babylon University/College of Materials
israaks@yahoo.com

J.E. Gough
The University of Manshester/School of Materials
J.Gough@manchester.ac.uk

B. Derby
The University of Manshester/School of Materials
Brian.Derby@manchester.ac.uk

Abstract

Regenerative medicine focuses on using biomaterials as three-dimensional (3D) porous scaffolds, specifically designed to mimic the nature of host tissue and hence to promote cell growth and tissue regeneration. 3D bioactive glass-ceramic scaffolds are one of the most frequently studied types of scaffolds for bone tissue engineering because of their excellent bioactivity and potential for stimulating osteogenesis and angiogenesis. For such purposes, porous 3D 70%SiO_2-30%CaO bioactive glass-ceramic scaffolds with three different pore sizes and identical porosity are used in present study to investigate *In vitro*, the effect of pore size on the degradation rate of scaffold which is achieved through examining changes in the composition of the immersion solution(SBF, simulated body fluid), and to investigate the action of released ions from the bioactive glass-ceramic scaffold during soaking process on osteoblast cells

The results confirmed that all three scaffolds behaved in a similar manner and the ions release from the three scaffolds were of comparable concentration, which may be attributable to the identical porosity for all the scaffolds in addition to the using static immersion which delays ions diffusion. The pH of culture media increased from 7.6 to 8.2 after one day soaking. The optical microscopy images demonstrated that high ion concentration (Si, Ca, P) in the culture medium could have a negative effect on the cells and induce cell death, while low concentration of ionic dissolution products induces osteoblast proliferation in dilute culture medium.

Key words: Bioactive glass-ceramic, Tissue regeneration, Bioglass scaffold, Bioceramic degradation, Bone scaffold.

الخلاصة

يركز الطب التجديدي على استخدام المواد الحيوية ثلاثية الأبعاد (3D) كالسقالات المسامية، والمصممة خصيصا لتحاكي طبيعة الأنسجة المضيفة وبالتالي إلى تعزيز نمو الخلايا وتجديد الأنسجة. السقالات المصنوعة من السيراميك والزجاج النشط بيولوجيا هي واحدة من أكثر أنواع السقالات المستخدمة لدراسة هندسة النسيج العظمي بسبب النشاط الحيوي الممتاز والقدرة على تحفيز تكون العظم والأوعية الدموية. لهذا الغرض ، استخدمت سقالات سيراميك مسامية مصنوعة من الزجاج السيراميكي النشط بيولوجيا نوع 70%SiO_2-30%CaO مع ثلاثة قياسات مختلفة للمسام مع الحفاظ على المسامية متطابقة وذلك لدراسة تأثير حجم المسام على معدل تحلل السقالة وذلك من خلال مقارنة التغير في مكونات محلول الغمر(وسط زراعة الخلايا) وتاثير تركيز الايونات الذائبة على الرقم الهيدروجيني للوسط، وكذلك التحقيق من تاثير الايونات الذائبة من السقالات السيراميكية أثناء عملية النقع على الخلايا العظمية

أكدت النتائج أن السقالات الثلاثة لها سلوك متماثل من حيث التحلل وتقارب تركيز الايونات في محاليل الغمر ،الذي يمكن أن يعزى إلى ان جميع السقالات تمتلك نفس المسامية والسبب الاخر هو استخدام اسلوب الغمر الثابت مما يؤددي الى بطئ انتشار الايونات من داخل السقالة الى وسط الغمر. أظهرت صور المجهر الضوئي أن التراكيز العالية لايونات (Si, Ca, P) يمكن أن يكون لها تأثير سلبي على الخلايا وإحداث موت الخلايا، في حين أن التراكيزالقليلة من هذه الايونات تحفز خلايا العظم على التكاثر والنضوج.

الكلمات المفتاحية: سيراميك–زجاج حياتي، بناء النسيج الحي، سقالة زجاج حياتي، تحلل السيراميك الحياتي، سقالة العظام.

Introduction

Scaffold is an essential component for tissue engineering strategy. Both the scaffold material and its architectural design (pore size,porosity and interconnectivity) play a significant role in governing tissue regeneration and defining the ultimate shape of newly grown soft or hard tissue (Hollister, 2005;Liu and Czernuszka, 2007).

The most challenging factor for the success of a given tissue engineering strategy in the bone regeneration field is the appropriate design of the scaffold. The design influences not only the mechanical properties but also the attachment and penetration of cells and mass transport through the scaffold. The scaffold must also have sufficient architectural characteristics i.e. porosity, pore size, interconnectivity and surface area which influence the degradation rate and bioactivity (Dias *et al*., 2012;Liu and Czernuszka, 2007), The degradation rate should be at the same rate as new matrix deposition, with non-toxic degradation products, fast degradation will reduce mechanical stability of scaffold (Jones *et. al*., 2007; Rahaman *et. al.*, 2011).

Many researchers have demonstrated that the bioactivity of porous bioceramics is influenced by the construct surface area, which is controlled by porosity, pore size distribution and interconnectivity. Increasing the surface area enhances material resorbability and the osteoinductive potential (El-Ghannam, 2013)

Highly porous scaffolds give a high surface area, which favors cell attachment and growth by facilitating the access of nutrients and waste transport. The surface area/volume ratio of a porous scaffold depends on its relative density and the average diameter of the pores (Yang *et. al*., 2001). The mean pore size of a scaffold plays an important role in controlling cell adhesion, migration, tissue formation, nutrient and gas diffusion as well as waste removal (Lien *et. al*., 2009). In the initial work of Hulbert *et. al*. (1970) the minimum requirement of pore size for bone scaffold was first defined as 100 μm. However, many later studies proved that better osteogenesis and tissue vascularization occur with constructs having a pore size > 300 μm (Loh *et. al*.,2013; Karageorgiou and Kaplan, 2005). Micropores and porosity are considered to be important factors that affect the degradation rate of a bioceramic scaffold after implantation. Increasing the number of micropores and porosity leads to a faster degradation rate because bioceramic materials degrade by a solution/dissolution process that occurs easily on the boundary of the open micropores (Lin *et al*., 2009). For this reason scaffolds fabricated from high degradation rate bioceramic materials should not have high porosities (> 90%) as the rapid degradation of the scaffold will compromise its mechanical and structural integrity before substitution by newly formed bone tissue (Karageorgiou and Kaplan, 2005)

The surface reactivity of the scaffold and the consequent releasing ions into the culture medium lead to the bioactive process and the bone-bonding property of the glass scaffold (Jones, 2013).

The aim of this study is investigating the effect of varying internal architectural designs of porous scaffolds on the degradation rate, pH and releasing ions during immersion in the culture media .In addition investigate the action of released ions from the bioactive glass-ceramic scaffold during soaking process on osteoblast cells.

Exparemental Work

1- Three dimensional (3D) glass-ceramic scaffolds

Ready- made three porous glass-ceramic scaffolds that have been prepared in previous work (Sabree *et.al.*, 2015) using Gel-casting method followed by sintering at 1200°C were used . The Three porous scaffolds were designed using computer aided design to have identical porosity (42.6%) and different pore size (300,400, 600)μm as shown in figure 1 in order to determine the effect of pore size on the concentration of releasing ions from the scaffolds during soaking in culture media. To maintain a constant level of porosity, the dimensions of the struts in each scaffold was double the pore size, as shown in table (1).

Scaffold Porosity

Porosity is defined as the void space in a porous solid (Grant *et. al*.,2006). It is a morphological property independent of the material. Pores are necessary for bone

tissue formation because they allow the migration and proliferation of cells and nutrients transport as well as the proper vascularization of an implant. In addition, a porous surface improves the mechanical attachment between the implant biomaterial and the surrounding natural bone, providing greater mechanical stability at the interface (Ramay and Zhang, 2003).

The density and porosity of three different samples for each type of the scaffolds were measured using the buoyancy method. This is based on the Archimedes' principle, which states that the volume of an immersed object is equal to the volume of liquid which is displaced (Grant *et. al*., 2006). Sample dimensions (w, l, d) were measured and weighed in air (Wdry). The apparent, bulk densities and porosities of the samples were calculated according to ASTM C-373 using the following equations (Callcut *et. al*, 2002; Grant *et. al*., 2006):

$$\text{Apparent density} = \left(\frac{W_{dry}}{W_{sat} - W_{sub}}\right) \rho H_2O \qquad 1$$

$$\text{True porosity} = 1 - \frac{\text{Apparent density}}{\text{Real density}} \qquad 2$$

Where: W_{dry} = the weight of dry sample.

W_{sub} = the weight of the submerged sample after degassing.

W_{sat} = the weight of saturated sample.

ρH_2O = the density of water.

w is the width of sample; *l* is the length of sample; *d* is the depth of sample.

2- In Vitro Dissolution Characteristics of Scaffolds

The three types of scaffolds used in this study were soaked in culture medium and incubated at 37°C with 95% humidity and 5% CO_2 at static conditions for 6 days. The composition of the culture medium was: Dulbecco's Modified Eagles Medium (DMEM) Low Glucose (1g/l), L-Glutamine (PAA, The Cell Culture Company, UK) supplemented with 10% FBS, antibiotics (100U/ml penicillin, 100 mg/ml streptomycin), 50μM ascorbic acid including a source of phosphate in its inorganic salts. The medium was changed every two days. The culture medium was analyzed every day using Inductively Coupled Plasma Spectroscopy (ICP-AES) to determine the corresponding concentration profiles for Si, Ca and P, as a function of soaking time, for all the scaffolds. The results reported correspond to the average concentrations calculated from two samples of medium.

3- pH Measurement during Incubation in the Culture Medium

In previous studies, it was found that the initial *in vitro* dissolution of the bioactive glass caused an increase in the pH of the culture medium. The three scaffold types were soaked in culture medium and incubated at 37°C with 95% humidity and 5% CO_2 for 6 days. During that period, the pH of the culture medium was measured every day with a pH-meter (Fisherbrand Hydrus 300, U.S.A.). pH measurements were carried out in triplicate.

4- The Response of Osteoblasts to the Culture Media

Observing osteoblast behavior in the presence of ionic products from the dissolution of bioglass-ceramic scaffolds is a suitable experimental method for evaluating scaffold biocompatibility. Si and Ca ions dissolving from a bioactive glass have been shown to promote regeneration of new tissue by influencing cell division, production of growth factors and extracellular matrix proteins (Jallot *et. al*., 2010). In order to observe the effect of ions released from the scaffold during the incubation period on cell viability, three types of culture medium were prepared: normal culture

medium before scaffold incubation, without any ionic products, conditioned medium (prepared by incubating a scaffold in 1 ml normal culture medium for 24 hours) and dilute medium (conditioned medium diluted with normal medium1:1).Human osteoblasts (HOBs) (Promocell C-12720) at a density of 1 x 10^5 cells/ml were cultured in a 24 well plate using the three medium types, then incubated at the standard conditions of 37°C with 95% humidity and 5% CO_2. Cells responses were observed using Optical microscopy images over 1, 7 and 14 days' culture period.

Results and Discussion

1 - Scaffold Bioactivity and Dissolution Results

The result of soaking the scaffolds in the culture medium is relevant to the evaluation of the biocompatibility of the scaffolds and the formation of an HCA-like layer under standard cell culture conditions *in vitro*. Figure (2) shows the dissolution profile of each scaffold as a function of soaking time in the culture medium. Recent research has shown that silicon ions released from bioactive glasses activate gene expression that enhances osteoblast cell proliferation and differentiation and up-regulates bone growth (Xynos *et. al*., 2000; Xynos *et. al*., 2000). Figure (2) reveals a rapid increase of the Si ion concentration in the culture medium after a one day soaking period for the three scaffold types followed by little reduction in the Si release rate on the second and third day. The increase on day four is due to the changing of the culture medium, after which the solution neared saturation and equilibrium during the fifth and sixth days, which is consistent with the results of Jones *et. al*. (2007) who found that the Si concentration stays approximately constant after 2 days soaking for 70%SiO_2-30%CaO glass powder. The above behaviour describes the second and third stage of hydroxyapatite formation on the bioactive glass surface (release of soluble silica to the solution in form of $Si(OH)_4$ and repolymerization), which agrees with the results given in many papers (Marques *et. al*., 2009; Coleman *et. al*., 2007; Saravanapavan *et. al*., 2003). It is clear that Si ion release from the three scaffolds was of comparable concentration because there was no significant difference in the results, which may be attributable to the identical porosity for all the scaffolds studied.

Figure (3) shows the release profile of calcium from the three scaffolds. The concentration of calcium ions increased within the first three soaking days, which indicates the exchange of Ca2+ cations with H+ from the culture medium. On the fourth day there was a reduction in Ca concentration due to changing the media, whereas it increased again on the following two days.

In contrast, figure(4)shows depletion of phosphate ions from the culture medium and this reduction in concentration continued throughout the six soaking days, which means that the scaffold surface absorbs phosphate from the surrounding media to form the HCA layer. The rate of phosphate ion removal from the culture medium was relatively slow, which affected the formation of a bone-like apatite layer. This is in agreement with the results of Lacroix et. al.(2013),Jallot et. al.(2010) and Coleman et. al.(2007) for phosphorus-free glass.

The crystallized structure of the sintered scaffolds probably reduces the dissolution rate and the absorption of phosphate ions from the culture medium (Jones et. al.,2013).

It is clear that all three scaffolds behaved in a similar manner: ion exchange took place first, followed by silica network breakdown and dissolution, then repolymerization and finally deposition of amorphous calcium phosphate. This agrees with the general behaviour of bioactive glass- ceramic reported in previous research (Jones et. al.,2010;Mozafari et. al.,2010;Saravanapavan et. al.,2003) but with different dissolution rates according to the glass type and conditions.

Previous works have studied the effects of dissolution products from bioactive glass in a culture medium on cell behaviour and osteogenesis. There has been agreement that a critical concentration of Si and Ca ions provides signals to the cells to stimulate osteoblast maturation and extracellular matrix production. Xynos et. al. (2000) showed that 16.6 μg/ml Si and 88.35 μg/ml Ca released from melt-derived bioglass activated genes. Saravanapavan et. al.(2003) demonstrated that after 1 day of soaking 70%SiO2–30%CaO bioactive glass powder in SBF, the Si concentration was approximately 60 mg/l. Gough et. al. (2004) demonstrated that a diluted conditioned culture medium with 47 ± 7.8 μg/ml Si ions would stimulate mineralization and nodule formation by osteoblast cells. In the present work, the Si concentration reached 54.3 ± 0.53 μg/ml for the three scaffold types after 3 days soaking which is comparable to the active concentration used by both Saravanapavan and Gough.

2-The pH Value of the Culture Medium

The surface reactions of the bioactive glass in the culture medium are believed to cause an increase in the concentration of cations and media alkalization leading to an increase in pH due to exchanges between Ca2+ and H+ in the medium (Silver et. al., 2001;Miguel et. al., 2010; Mozafari et. al., 2010). Figure (5) shows a rapid increase in pH from 7.6 to more than 8.2 at the earliest period of soaking time for the three scaffold types. ICP data confirmed that the exchange of Ca2+/H+ ions is responsible for the increase in the pH. After two days there is a slight decrease in pH until it reaches a plateau value at days 5 and 6. This has also been found in other studies (Gough et. al., 2004; Miguel et. al., 2010; Lacroix et.al., 2013).

The convergence of the pH value for the three scaffold types after soaking except for the first day was confirmed by ICP and it is found to be comparable ion concentrations released from the three scaffold types because they have the same porosity. Jones et. al.(2007) found that when a 70%SiO2 – 30%CaO foam scaffold with 91% porosity was soaked in the culture medium, the pH changed from 7.72 to 9.95, a significant increase in pH predicated to high scaffold porosity. Cerruti et. al. (2005) stated that for bioglass 45S5, at a pH higher than 8, calcium carbonate was precipitated on the scaffold surface more than HCA and at pH 8 a 3D silica network structure is formed.

3- Osteoblast Morphology in Conditioned media

The optical microscopy images for the osteoblasts in the three types of media over 14 days shown in figure (6).The high ion concentration in the conditioned medium could be having a negative effect on the cells and inducing cell death. Gough et. al. (2004) and Varanasi et. al.(2009) stated that the initial high concentration of ions released from bioactive glass inhibits cell viability and proliferation, which agrees with the results presented here.

A significant increase in cell metabolic activity in the dilute medium at 1, 7 and 14 days indicates that reducing the ion concentration released from the scaffold leads to a good cell response. Gough *et. al*. (2004) found that a low concentration of ionic dissolution products promotes mineralized tissue formation.

Conclusions:

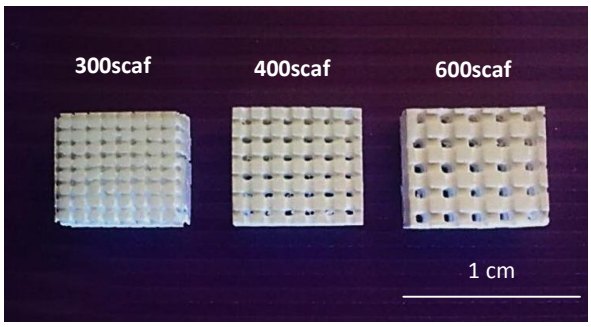
1- The crystallized structure of the scaffolds resulting from high sintering temperature (1200 °C) probably reduces and delay the dissolution of scaffolds

2- All three scaffolds behaved in a similar manner during soaking; that can be attributed to the identical porosity for all the scaffolds studied and to the use of static soaking.

3- The scaffolds degradation leads to a rapid increase in pH of culture medium that may have effect on osteoblast viability.

4- High concentration of Si and Ca ions leads to cell death while critical concentration provides signals to the cells to stimulate osteoblast maturation and extracellular matrix production.

Top view of the scaffolds



Side view of the scaffolds

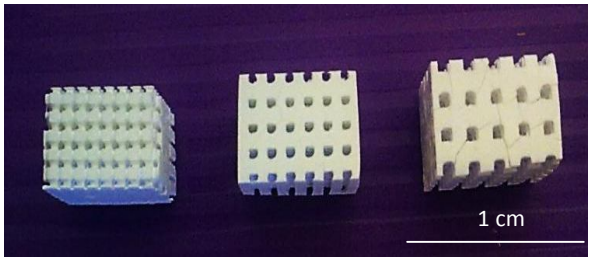


Fig. 1 Photographic images showing the difference in the internal architecture of the three types of scaffolds.

Table 1 The internal architecture dimensions using CAD for the three types of scaffolds.

Overall surface area (m^2)	Porosity%	Strut size(µm)	Pore size(µm)	Scaffold name
$1.102x10^{-3}$	42.6	600	300	300scaf
$0.82x10^{-3}$	42.6	800	400	400scaf
$0.55x10^{-3}$	42.6	1200	600	600scaf

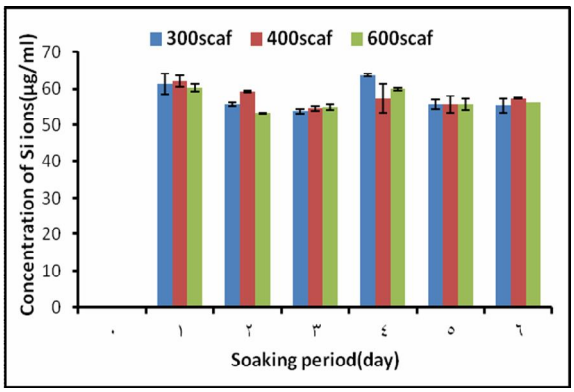


Fig. 2 Dissolution profile of Si in the culture medium as a function of soaking time for all the scaffolds using (ICP-AES). (Two different culture media were used for each scaffold).

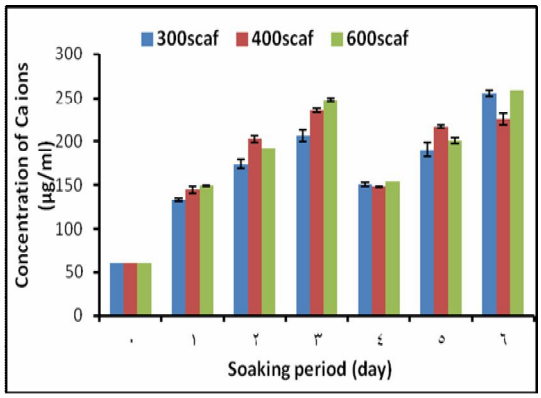


Fig. 3 Dissolution profile of Ca in the culture medium as a function of soaking time for all the scaffolds using(ICP-AES) . (Two different culture media were used for each scaffold)

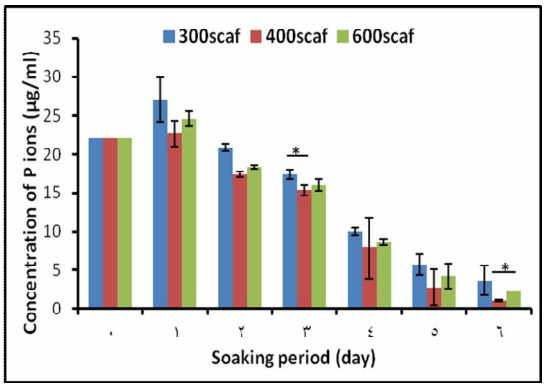


Fig. 4 Dissolution profile of phosphate in the culture medium as a function of soaking time for all the scaffolds using (ICP-AES). (Two different culture media were used for each scaffold) (*p ≤ 0.05, **p ≤ 0.01, ***p ≤0.001).

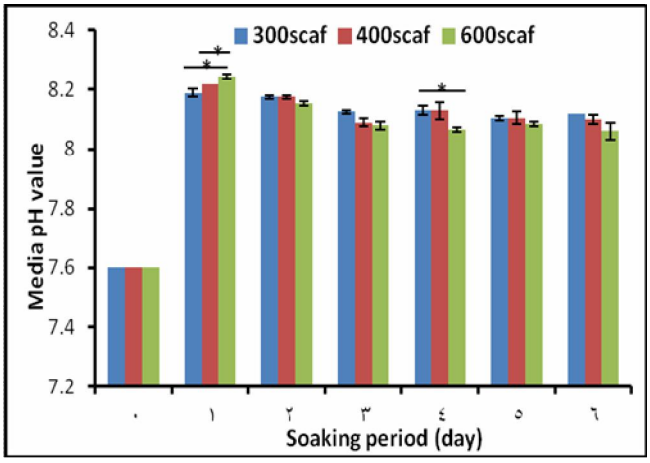


Fig. 5 pH variation induced by dissolving the scaffold in the culture medium at different soaking times. (Using two different culture media for each value)

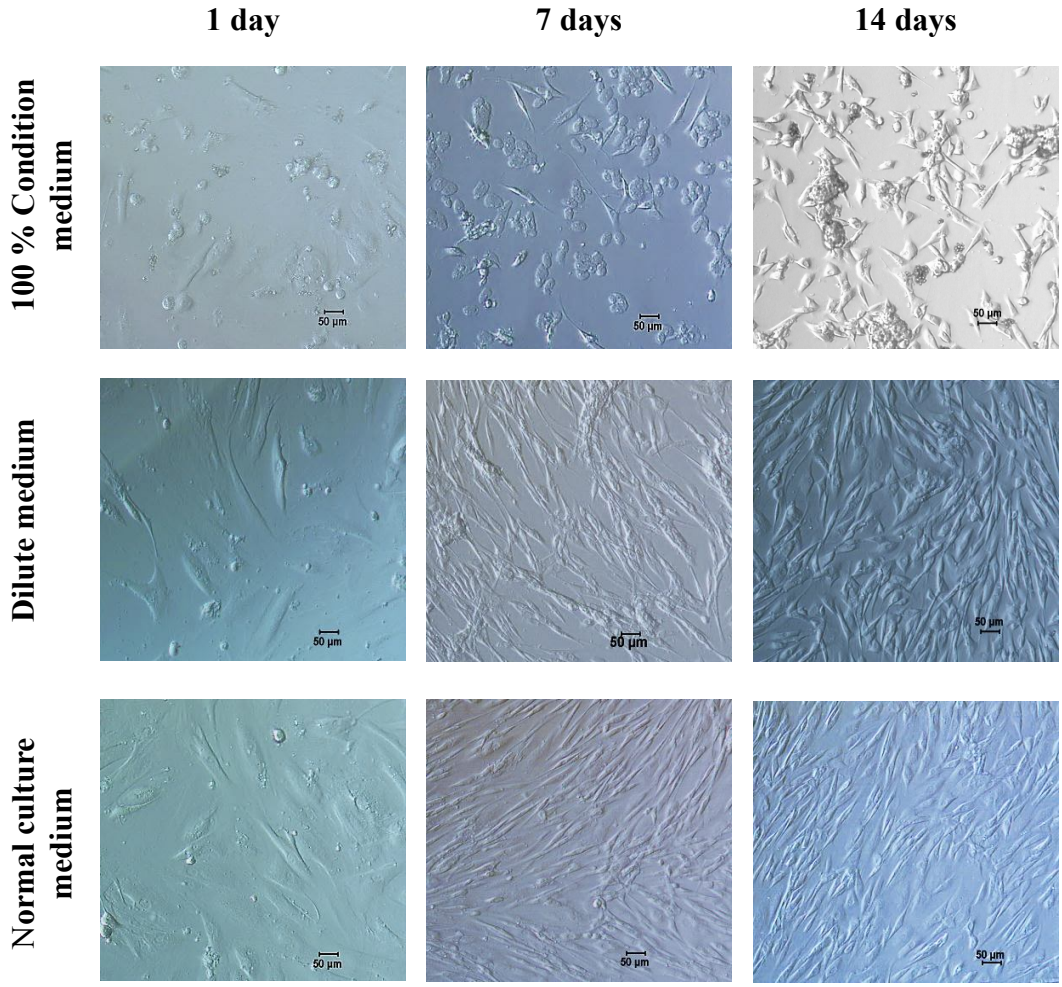


Fig. 6 Optical microscopy images illustrating the effect of ion concentration in culture media on the osteoblast response over 14 days. (Scale bar 50µm).

References

Callcut S. and Knowles J.C.,Correlation between structure and compressive strength in reticulated glass-reinforced hydroxyapatite foam, J. Materials Science: materials in Medicine, 13, 485-489, 2002.

Cerruti M.,Greenspan D.,and Powers K.,Effect of pH and ionic strength on the reactivity of bioglass 45S5, Biomaterials, 26, 1665-1674, 2005.

Coleman N.J., Bellantone M.,Nicholson J.W. and Mendham A.P.,Textural and structural properties of bioactive glasses in the system CaO-SiO2, Ceramics-Silikaty,1,1-8,2007.

Dias M.R., Fernandes P.R., Guedes J.M., and Hollister S.J., Permeability analysis of scaffolds for bone tissue engineering, J. Biomechanics, Vol. 45, 938-944, 2012

El-Ghannam A.,Hart A.,White D.,and Cunningham L., Mechanical properties and cytotoxicity of a resorbable bioactive implant prepared by rapid prototyping technique, J. Biomedical Materials Research part A, Vol. 101 A, 10, 2013.

Gough J.E.,Jones J.R.,and Hench L.L.,Nodule formation and mineralisation of human primary osteoblasts cultured on a porous bioactive glass scaffold, Biomaterials, 25, 2039-2046, 2004.

Grant P., Vaz C., Tomlins P. and Vadgama P., Physical characterisation of a polycaprolactione tissue scaffold. In: Blitz J. and Gunko V., Surface chemistry in biomedical and environmental science, 215-228, 2006.

Hollister S.J., Porous scaffold design for tissue engineering, Nature Materials, Vol. 4, 518-524, 2005.

Hulbert S.F., Young F.A., Mathews R.S., Klawitter J.J., Talbert C.D., and Stelling F.H.,Potential of ceramic materials as permanently implantable skeletal prostheses, J. Biomedical Materials Research, Part A, 4, 433-456, 1970.

Jallot E., Lao J., John L., Soulie J., Moretto P.and Nedelec J.M., Imaging Physicochemical reactions occurring at the pore surface in binary bioactive glass foams by micro ion beam analysis, Applied materials and interfaces, Vol. 2, 6, 1737-1742, 2010.

Jones J., Review of bioactive glass: from Hench to hybirds, Acta Biomaterialia, 9, 4457-4486, 2013.

Jones J.R., Lin S., Yue S., Lee P.D., Hanna J.V., Smith M.E., and Newport R.J., Bioactive glass scaffolds for bone regeneration and their hierarchical characterisation, J. Engineering in Medicine, Vol. 224, Part H, 2010.

Jones J.R., Tsigkou O., Coates E.E., Stevens M.M., Polak J.M., and Hench L.L., Extracellular matrix formation and mineralization on a phosphate-free porous bioactive glass scaffold using primary human osteoblast(HOB) cells, Biomaterials, 28, 1653-1663, 2007.

Karageorgiou V., and Kaplan D., Porosity of 3D biomaterial scaffolds and osteogenesis, Biomaterials, Vol. 26, 5474-5491, 2005.

Lacroix J., Jallot E., Nedelec J.M., and Lao J., Influence of glass macroporosity on the bioactive process, J. Physical chemistry B, 117, 510-517, 2013.

Lien S.M., Ko L.Y., and Huang T.J., Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering, Acta Biomaterialia, 5, 670-679, 2009.

Lin L., Zhang H., Zhao L., Hu Q., and Fang M., Design and preparation of bone tissue engineering scaffolds with porous controllable structure, J. Wuhan University of Technology- Materials Science ed., 24, 2, 174-180, 2009.

Liu C. Z. and Czernuszka J.T., Development of biodegradable scaffolds for tissue engineering :a perspective on emerging technology, Materials Science and Technology, Vol. 23 , 4, 379-391, 2007.

Loh Q.L., Eng B., and Choong C., Three-Dimensional scaffolds for tissue engineering applications: role of porosity and pore size, Tissue Engineering: part B, Vol.19, 6, 2013.

Marques A.C., Almeida R.,Thiema A., Wang S., Falk M.M., and Jain H., Sol-gel-derived glass scaffold with high pore interconnectivity and enhanced bioactivity, J. Materials Research, Vol. 24, 12, 2009.

Miguel B.S., Kriauciunas R., Tosatti S., Ehrbar M., Ghayor C., Textor M., and Weber F., Enhanced osteoblastic activity and bone regeneration using surface modified porous bioactive glass scaffolds, J. biomedical materials research part A, Vol. 94A, 4, 1023-1033, 2010.

Mozafari M., Rabiee M., Azami M., and Maleknia S., Biomimetic formation of apatite on the surface of porous gelatine/bioactive glass nanocomposite scaffolds, Applied surface science, 257, 1740-1749, 2010.

Rahaman M.N., Day D.E., Bal B. S., Fu Q., Jung S.B., Bonewald L.F., and Tomsia A., Bioactive glass in tissue engineering, Acta Biomaterialia., 7, 6, 2355-2373, 2011.

Ramay H.R., and Zhang M., Preparation of porous hydroxyapatite scaffolds by combination of the gel- casting and polymer sponge methods, Biomaterials, Vol. 24, 19, 3293-3302, 2003.

Sabree I., Gough J.E., and Derby B., Mechanical properties of porous ceramic scaffolds: Influence of internal dimensions, Vol 41, 8425-8432, 2015.

Saravanapavan P., Jones J.R., Russell S. P., and Hench L.L., Bioactivity of gel-glass powders in the CaO-SiO2 system: A comparison with ternary (CaO-P2O5-SiO2) and quaternary glasses (SiO2-CaO-P2O5-Na2O), J. Biomedical Materials Research, Vol. 66A, 1, 110-119, 2003.

Silver I.A., Deas J., and 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, Biomaterials, Vol. 22, 2, 175-185, 2001.

Varanasi V.G., Saiz E., Loomer P.M., Ancheta B., Uritani N., Ho S.P., Tomsia A.P., Marshall S.J., and Marshall G.W., Enhanced osteocalcin expression by osteoblast-like cells (MC3T3-E1) exposed to bioactive coating glass(SiO2-CaO-P2O5-MgO-K3O-Na2O system) ions, Acta biomaterialia, 5, 3536-3547, 2009.

Xynos I.D., Hukkanen M.V.J., Batten J.J., Buttery L.D., Hench L.L., and Polak J.M., Bioglass 45S5 stimulates osteoblast turnover and enhances bone formation in vitro: Implication and applications for bone tissue engineering, Calcified Tissue International, 67, 321-329, 2000.

Xyons I.D., Edgar A.J., Buttery L.D., Hench L.L., and Polak J.M., Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor ll mRNA expression and protein synthesis, Biochemical and Biophysical Research Communications, Vol. 276, 2, 461-465, 2000.

Yang S., Leong K., Du Z., and Chua C-K., The design of scaffolds for use in tissue engineering. Part I: Traditional factors, Tissue Engineering, Vol. 7, 6, 2001.