

Development of novel 3D porous melt-derived bioactive glass scaffolds

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Declaration

I hereby certify that the work presented in this thesis is the result of my own investigations carried out at Imperial College London during the period from October 2006 to October 2009, except where otherwise stated.

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Abstract

The aim of this thesis is to develop a method to produce melt-derived bioactive glass scaffolds, without the glass crystallizing into a glass-ceramic, while establishing an interconnected pore network suitable for bone tissue regeneration. In order to achieve this, the scaffold must have the ability to closely mimic the porous structure of cancellous bone and its mechanical properties. Two bioactive glasses were used in this project, both are modified from the Bioglass[®] composition: ICIE 16 (49.46% SiO₂, 36.27% CaO, 6.6% Na₂O, 6.6% K₂O and 1.07% P₂O₅, all in mol%) and ICIE 16M (49.46% SiO₂, 27.27% CaO, 6.6% Na₂O, 6.6% K₂O, 3% ZnO, 3% MgO, 3% SrO and 1.07% P₂O₅, all in mol%). Gel-cast foaming produced improved pore networks over alternative methods for producing porous scaffolds. There are many variables in the process. An initial protocol was established and each of the variables assessed systematically. The relationships between each component, the gelling time and the foam body volume were evaluated to develop an optimized protocol for the process. The size of the glass powder was critical in determining the sintering efficiency. A suitable drying and sintering program was also determined to prevent crystallization of the glass and formation of crystal species from by-products of the process. The scaffolds were characterized in terms of the interconnect size, the rate of hydroxycarbonate apatite (HCA) formation in simulated body fluid (SBF) solutions, the ion release rate and the compressive strength. The results showed that ICIE 16M sintered better and was stronger than ICIE 16, however in the bioactivity aspect of view, the rate of HCA formation in SBF was faster for ICIE 16 than ICIE 16M.

To my parents Jianmíng Zhong and Jiánwéi Wu

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List of Abbreviations

3D	Three Dimensional
Ea	Activation Energy
APS	Ammonium Persulfate
BGs	Bioactive Glasses
DSC	Differential Scanning Calorimetry
FDA	Food and Drug Administration
FTIR	Fourier Transmission Infra-red Spectroscopy
HA	Hydroxyapatite
HCA	Hydroxycarbonated apatite
HF	Hydrofluoric acid
ICP	Inductively Coupled Plasma Spectroscopy
ISO	International Standards Organisation
MAE	Mixed Alkali Effect
MIP	Mercury Intrusion Porosimetry
NC	Network Connectivity
RP	Rapid prototyping
SEM	Scanning Electron Microscopy
TCP	Tricalcium Phosphate
TEMED	<i>N,N,N',N'</i> -tetramethylethylene diamine
T _c	Glass Crystallization Temperature
T _g	Glass-transition Temperature
T _p	Peak Crystallization Temperature
XRD	X-ray Diffraction Spectroscopy
SBF	Simulated Body Fluid

1. Introduction

The number of people suffering from pathological bone diseases and non-healing bone fractures are increasing every year. Approximately 500,000 bone grafting procedures are performed each year in United States and over 2.2 million worldwide [1]. Autografts (bone transplanted from a donor site of the patient, e.g. the iliac crest) remain the gold-standard treatment for bone defects and non-healing fractures, however they are very limited in supply and recovery time can be long and painful. Allografts (irradiated cadaveric bone from bone banks) in comparison are more available and at a lower cost, however their mechanical properties can be poor and there is risk of disease transmission [2]. Alternatively implantation is a popular choice, which is the use of prosthetic materials to replace the damaged tissue. In orthopaedics, implants that replace bone are usually metallic; they have a limited lifespan because of problems such as stress shielding (the decrease in bone density due to lack of stress applied to the surrounding bone tissue of the implant as a result of bone remodeling itself according to stress applied, i.e. Wolff's Law) and inflammation (the reaction of the immune system to foreign substances inside the body), which lead to re-implantation and more pain for the patients. Meanwhile, diseases such as osteoporosis (the decrease in bone mineral density and the disappearance of bone tissue due to low hormone level, as a result bone grows more weakly and bone fracture risks significantly increases) are still uncureable, although treatments are available to both prevent and slow down the progress of the disease, for example modifying life style or take certain types of medicine accordingly. The increasing number of patients who suffer from bone injury, the high cost of operation, plus the lack of bone stock in autografts, while

allografting and implantation both had strong side effects; as a result, scientists have focused towards bone-tissue engineering. No one has yet succeeded in regenerating the bone tissue and tissue engineering of bone is a strategy that has not seen clinical translation. Conventional strategies are to build a three-dimensional bone structure mimicking scaffolds for in vitro tissue regeneration, where the cells were seeded onto the scaffold and grown into desired tissue, which was then placed in the targeted site. Scaffolds that can regenerate tissue in-situ while resorbing at the same rate as the new tissue formation and releasing non-cytotoxic and beneficial dissolution products is the ultimate goal for tissue regeneration, which is also the aim of this project

There are many types of materials available to fabricate such scaffolds, including ceramic-polymer composites, bioactive glasses (BGs), hydro-gels, hydroxyapatite coated metals, hybrids and many more. Since the invention of Bioglass[®] (46.1% SiO₂, 26.9% CaO, 24.4% Na₂O and 2.6% P₂O₅, all in mol%) [3], which is the first bioactive material as it would interact with the host tissue, BGs have been thoroughly studied for their excellent ability to establish a close bond to the bone without the formation of fibrous capsulation. In addition, BGs can stimulate osteogenesis, i.e. promote bone growth [4-5].

Glass is an amorphous material, supercooled from its molten state to prevent crystallization occurring, which means it has a random network very similar to that of a liquid. It is homogenous and very stable at room temperature. However given the required amount of energy, the glass can crystallize again and become a ceramic or glass-ceramic.

BGs can be produced by two methods: traditional melt-casting and sol-gel processing. Whilst sol-gel derived BGs can be easily made into 3D porous foams with very high porosity, interconnectivity and surface area; conventional BGs cannot. For example, Bioglass[®] has a very small processing window, which means its glass transition temperature T_g (glasses are supercooled from their molten state before nucleation could take place and the molecular arrangements are disordered with adequate cohesion between the molecules to maintain a solid state. T_g is the energy barrier significantly below the melting temperature T_m that the glass has to overcome in order to become less rigid and less viscous so it can flow slightly) and crystallization temperature T_c (where the amorphous molecular structure becomes well ordered and thermodynamically stable) are very close together [6-7]. The narrow sintering window causes the glass to crystallize shortly after heated beyond T_g . Once the crystallization takes place, the bioactivity of Bioglass[®] drops extensively [6, 8-9] while the degradation behavior becomes unpredictable [10].

However a scaffold can only be made from powder by going through sintering, sufficient amount of fluid flow is the key to compose the structure. As a result, Bioglass[®] can only be used in monolith or powder form and contribute in coating of other scaffolds or implants. Perioglas[®], Novabone[®] by NovaBone Products LLC. US; NovaMin[®] by Sultan Healthcare, US and Vitoss[™] and Vitoss[™] BA by Orthovita Inc. US are the only commercial products [11-13]. Later researches have shown that this crystallization in Bioglass[®] would retard the formation of hydroxycarbonate apatite (HCA) layer [14-19] and would therefore significantly reduce the bioactivity.

In order to solve this problem, the composition needs to be modified to provide a better sintering window (the gap between T_g and T_c) so the glass particles will have time to sinter together without crystallizing. The wider the sintering window, the better the sinterability of the glass. It is well studied that the bioactivity can be controlled by varying the composition of the BG [20-22], new sinterable melt-derived BGs were produced whilst maintaining the bioactivity of the Bioglass[®]. The variation in the composition has led to the difference in the glass properties and hence affected the performance of the scaffold in terms of the viscosity during sintering, the processing window, the foam structure (which determines the pore size and interconnect size), the dissolution rate and the HCA deposition rate.

This thesis is mainly focused on achieving a porous 3D interconnected structure from a melt-derived bioactive glass. A background to the bone tissue engineering and the history of biomaterials is discussed in Section 2.1 and 2.2; followed by a detailed assessment of bioactive glasses in Section 2.3. Section 2.4 reviews the requirement of an ideal bone scaffold and the range of scaffold materials. The review will end in Section 2.5 with the history and development of different types of techniques that were used to produce porous ceramic foams, in particular the gel-casting technique. The development and characterization of gel-cast melt-derived bioactive glass scaffolds is illustrated in Chapters 3 – 6; the experimental techniques and parameters involved will be described in Section 2 of each chapter, followed by a comprehensive discussion of the results in Section 3. Conclusions are drawn in Chapter 7 with a suggestion for future work.

2. Literature review

2.1 Human Bone

Bone is a vital part of the human body, it supports the body weight, enables us to move, and protects the other organs from external damage. Bone also stores important minerals such as calcium and sodium inside the body, and produces red and white blood cells. The bone structure illustrated in Figure 2.1 is incredibly complicated, but essentially bone is a nano-composite that consists of collagen fibre with carbonated hydroxyapatite crystals. This combination allows the bone to be both strong in compression, tough and lightweight, while fulfilling its other functions [2, 23-24]. The density and composition of bone varies with gender, age and whether it was pathologically affected [25].

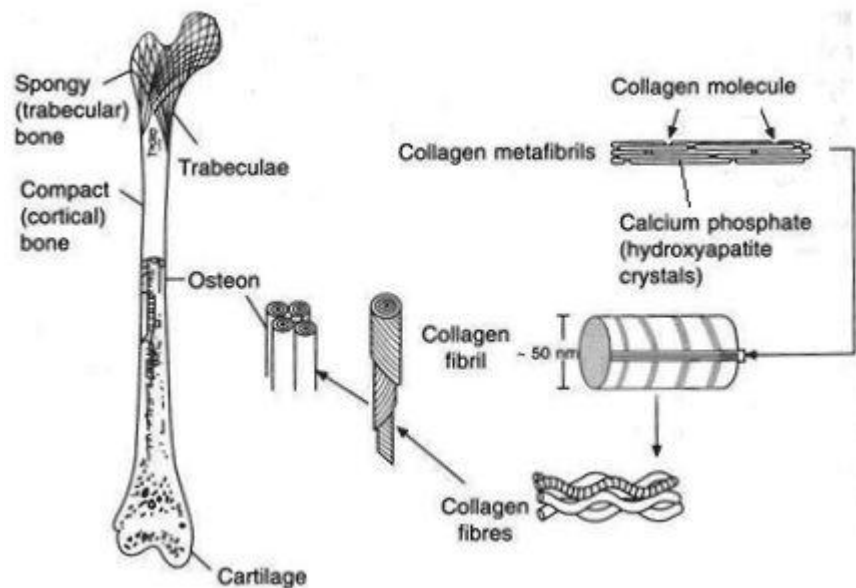


Figure 2.1 Schematic diagram of bone structure [26]

There are two types of bone, trabecular and compact, as shown in Figure 2.2. The outer layer is called the compact bone, which contributes 80% of the weight of a human skeleton and is therefore very dense and hard. It is formed by multiple stacked layers of osteon, which is the fundamental bone unit. It consists of coiled up collagen fibers with HCA crystals in-between the ends and is hollow centered to allow the blood vessels to go through. The second part of osseous tissue is known as the trabecular bone, which fills the inner cavity of long bones. It has lower density and strength compared to the compact bone, but is very high in surface area [27]. The external layer of trabecular bone contains red bone marrow and most of the arteries and veins of bone organs are also found in here. It is this area where the bone scaffolds aim to repair. The mechanical properties are summarized in Table 2.1.

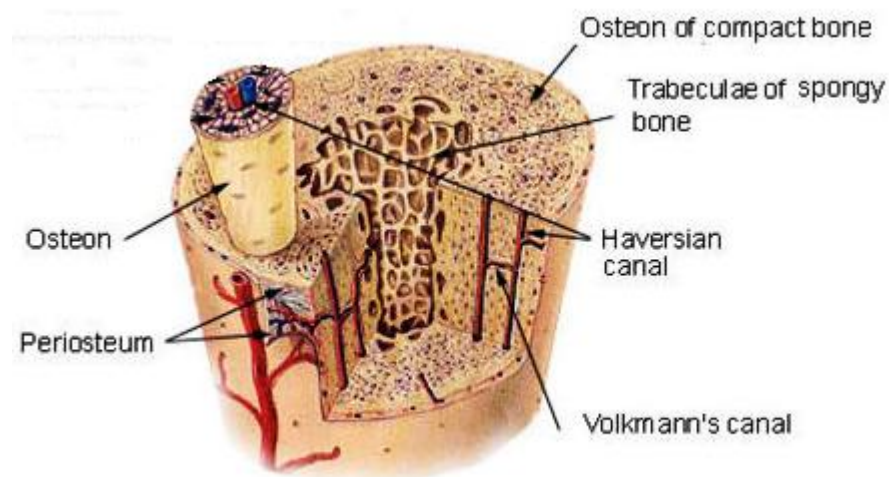


Figure 2.2 **Graphic illustration of the two different types of bone [28]**

Table 2.1 The mechanical properties of compact and trabecular bone [26]

Property	Compact Bone	Trabecular Bone
Compressive strength (MPa)	100-230	2-12
Flexural, tensile strength (MPa)	50-150	10-20
Strain to failure (%)	1-3	5-7
Young's (tensile) modulus (GPa)	7-30	0.5-0.05
Fracture toughness (K_{IC})	2-12	n/a

Bone has the ability to heal itself but only to a certain extent, for damage caused by pathological diseases or pathological fractures, bone cease to repair itself; biomaterials are hence developed in order to help restore the continuity in the bone structure.

2.2 Bone tissue engineering

Tissue engineering aims to develop biological substitutes to maintain, restore or improve a certain type of tissue or organ. For bone tissue engineering, there are 2 major techniques: *in vitro* and *in-situ (in vivo)* tissue regeneration. By the means of their names, the *in vitro* method is to harvest cells and culture them onto a scaffold where the target tissue is grown to matured state before implantation, while the *in vivo* method consists of implanting only the scaffold to the defected site, as the cells grow and attach themselves to the structure, the scaffold will resorb at the same rate as the new bone grows. [29]

There are limitations to both methods; the *in vitro* method is preferred in the cases of older patients as their bone tissue regeneration rate is slower than the

younger patients or at the presence of large areas of defects [30], whereas the *in vivo* method requires the scaffold to be mechanical identical to the host bone. Both scaffolds should resemble the bone structure as closely as possible in order to fulfill their duties. In general, the ultimate goal is to create a scaffold that regenerates bone tissue *in vivo* as the *in vitro* method requires significant amount of waiting time before the implantation.

There is a wide selection of candidates for the scaffolding material and a number of requirements must be met for an ideal scaffold, which will be discussed in the following sections.

2.3 Biomaterials for bone tissue engineering

The use of synthetic materials as a way to replace damaged tissues has been studied and practiced for a long time. All materials that are implanted into the body elicit a response from their host tissue [31]. There are 4 types of possible responses as shown in the table:

Table 2.2 Consequences of Implant-Tissue Interactions [31]

Implant-Tissue Reaction	Consequences
Toxic	Tissue dies
Biologically nearly inert	Tissue forms a non-adherent fibrous capsule around the implant
Bioactive	Tissue forms an interfacial bond with the implant
Dissolution of the implant	Tissue replaces the implant

For the first implants, the goal was to find an ideal biomaterial that would evoke minimum body responses to the implantation of the material. The most important criteria were precise fixation of the implant and adhesion in the biomaterial-tissue system [29, 32]. This had led to the research of first generation of biomaterials, which included the use of stainless-steel, cobalt-chrome alloys, titanium alloys and polymers with very large molecular weights. They were termed bio-inert materials. However all bio-inert implants were found to be encapsulated by a non-adherent fibrous tissue as a result of protective mechanism mainly by macrophages and/or foreign-body giant cells of the host tissue [33]. The thickness of the fibrous capsule might vary but it would generally isolate the implant from the tissue so that the implant-bone interface was poor, leading to micro-motion of the implant and loss of bone. All metal orthopaedic implants have a limited lifespan due to wear particles and a modulus mismatch between the implant and the bone (stress shielding) causing bone loss, which eventually leads to loosening of the implant. As life expectancy is continually increasing, the survival time of bioinert implants is not enough for many applications. The focus has since shifted from the replacement of tissues to the regeneration of tissues, in order to satisfy the growing need for very long term orthopaedic repair [34].

Second generation of biomaterials were developed, which were bioactive and biocompatible, i.e. they could establish a chemical bond to the host tissue and would not evoke any harmful responses from the body when implanted. A great example is the synthetic hydroxyapatite (HA), which is the closest synthetic form to the mineral part of bone tissue and because of this feature it is the most

widely applied bioactive implant material. While HA does not elicit immunological reactions, its dissolution rate and ion exchange rate is very low and hence its ability to form a hydroxycarbonated apatite (HCA) layer is weak [30]. The formation of the HCA layer marks whether the material is able to bond to the bone. A similar material to HA is tricalcium phosphates (TCPs), which are again very popular as a bioactive implant material, however they have similar problems to that of HA and they are very unpredictable in terms of degradation rate [35].

Third generation of biomaterials are those ones that encourage cell attachment, differentiation and growth whilst the implant itself would gradually dissolve away at the same rate as the tissue grows, leaving the defect site replaced by the natural tissue [36], for example the BGs. The development of porous scaffolds from bioactive and resorable materials with sufficient mechanical properties would be a large step towards successful bone regeneration.

2.4 Bioactive glasses

There is a large difference in the bonding rate of the vast variety of bioactive material to the host tissue, i.e. the bioactivity, so that they are further classified into two categories. Bioactive glasses have been shown to bond to bone and soft tissue as well as to stimulate osteoblast differentiation and cell growth, i.e. they are osteopductive, a good example is the Bioglass[®] [4-5]; Synthetic HA [37] and TCP are only osteoconductive, which means that they encourage cell attachment and growth along their surfaces from the bone-implant surface [38-39].

As aforementioned, the glass has to have <59 mol% of SiO₂ in its composition in order to be bioactive [40]; within that limit, there are only a few composition that are highly bioactive, which lies within the centre region of the schematic diagram shown in Figure 2.3. Region B at the top of the diagram represents the compositions of the glass that will act as an inert material due to high network connectivity values and form fibrous capsule at the implant site, whereas region C represented the ones that degrade in 10-30 days and the compositions in region D will not form glasses [41].

Phosphorus (P) plays an interesting role in BGs, it is not necessary to include it in the composition in purpose of HCA formation but the inclusion of phosphorus in a small amount will increase HCA crystallization rate, due to phosphorus accelerate glass degradation when released, resulting a higher degree of supersaturation in the body fluid, and faster crystallization rate of the HCA layer [42]. In addition, presence of P balances pH level around the implant site, if the pH is too high bone bonding mechanism will not occur at the tissue-implant interface [43]. P-free BGs forms a non-uniform HCA layer along its surface that penetrates through the silica layer, result in a weaker bone-bonding strength compared to the P-containing BGs [40, 44].

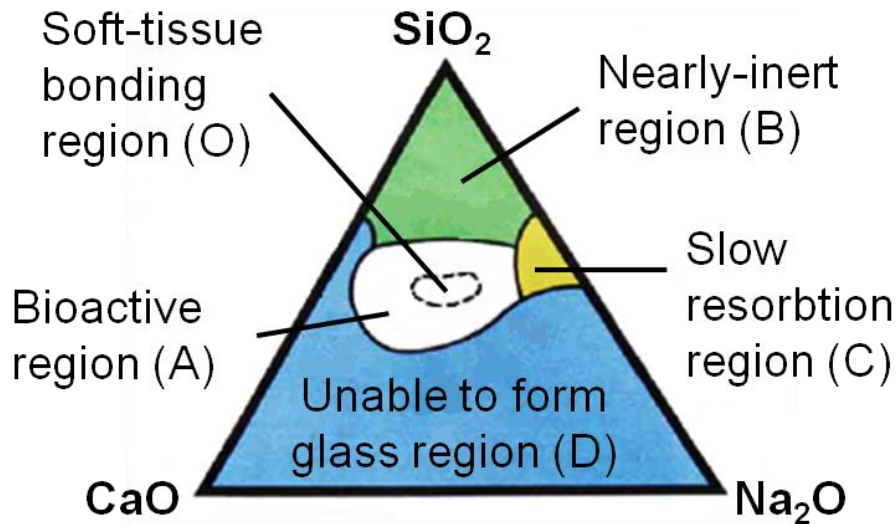


Figure 2.3 Diagram in wt% illustrating the compositional dependence of bioactivity for glasses related to 45S5 [45]

Even the slightest alteration in the glass composition could evoke a radical change in the dissolution rate and mechanism [46-47]. In order to expand the sintering window, various ingredients are added into the original composition. Brink *et al.* found that bioactive glasses in the system $\text{Na}_2\text{O-K}_2\text{O-MgO-CaO-B}_2\text{O}_3\text{-P}_2\text{O}_5\text{-SiO}_2$ have a large working range [48], and borosilica bioactive glasses was proved to be bioactive as well as biodegradable with a controllable degradation rate by varying the boron content in the glass composition. Borate as the major degradation product is known to be cytotoxic in a static culture environment [49], although the environment is dynamic in vivo and the toxicity is more likely to be alleviated, it is still a major concern. A desire to develop safe and processable melt-derived bioactive glass is unavoidable. Hill *et al.* and his research group carried out extensive studies on the role of different oxides contributed, termed as the Mixed Alkali Effect (MAE) [50] and invented the ICIE 16 & ICIE 16M glasses, which are chosen to

be applied in this project [37, 51] to the sinterability (Table 2.3) and bioactivity of the modified Bioglass[®] compositions.

Alkali oxides consist of Group 1 elements except for hydrogen (Li, Na, K, Rb, Cs and Fr) and the MAE refers to the non-linear change of ionic related properties of a glass (e.g. electric conductivity, viscosity and ionic diffusion) by the substitution of one alkali oxide for another. The non-linear relationship is due to decreased mobility of the individual ions, which is caused by the presence of a second ion. Changes in other properties such as molar volume, density and thermal expansion coefficient remain linear. The MAE is commonly observed in silicate, phosphate, borate, halide and germanate glasses. [50, 52-53] Incorporating Na⁺ and K⁺ into BG composition would reduce ion diffusion rate but increase chemical durability than single alkali, as well as allow glass formation at lower temperatures glasses, all because of the ion migration rates were restricted in mixed alkali glasses [37]. This enables the glass properties to be tailored to suit specific applications, e.g. controlled degradation rate without compromising too much bioactivity.

Table 2.3 The role of different ions in melt-derive silicate BG and their effects on the sinterability of the glass

Ions	Role	Effect on sinterability
Si^{4+}	Network Former	Forms the SiO_2 network which is the essential structure of glass
P_5^+	Network Former	Forms a P_2O_5 network (phase separation in SiO_2)
Na^+	Network Modifier	Reduces viscosity and T_g due to disruption of the glass network, reduces T_c but not significantly
Mg^{2+}	Network Intermediate	Effectively increases sintering window width [54] but also reduces viscosity and solubility with increasing content [55]
Zn^{2+}	Network Intermediate	Increases sintering window width
Sr^{2+}	Network Modifier	Decreases both T_g and T_c without changing the window width
K^+	Network Modifier	Reduces viscosity and T_g due to disruption of the glass network
Ca^{2+}	Network Modifier	Reduces viscosity, T_g and make glass less soluble in water

SiO_2 forms the bulk of the glass network and is therefore a network former, it is also a major contributor to the bone mineralization and gene activation activities [34]. According to Zacharisen's random network theory, there are 3 types of oxides participating in the glass formation system [56] (Figure 2.4). Network formers such as Si, P and B, form bridging oxygen (BO) bonds, which are the type of bond that is shared between an oxygen atom and any two network former molecules, i.e. the O in Si-O-Si, P-O-Si, P-O-P or B-O-B is a BO. They generally have a coordination number of 3 or 4. A network modifier forms non-bridging oxygen (NBO) bond, which is the bond between any other oxygen

that is not shared by the two tetrahedral but the surrounding ions, this results in a weakened glass network, they have a coordination number of 6 or less. A network intermediate with coordination number of 6 or 8 can function either as a network former (but on their own they cannot form a glass network) or as a modifier depending upon the glass compositions involved [57]. In general, the role of the cations depends on the size of the ion, the coordination number and the strength of the bond it forms with oxygen.

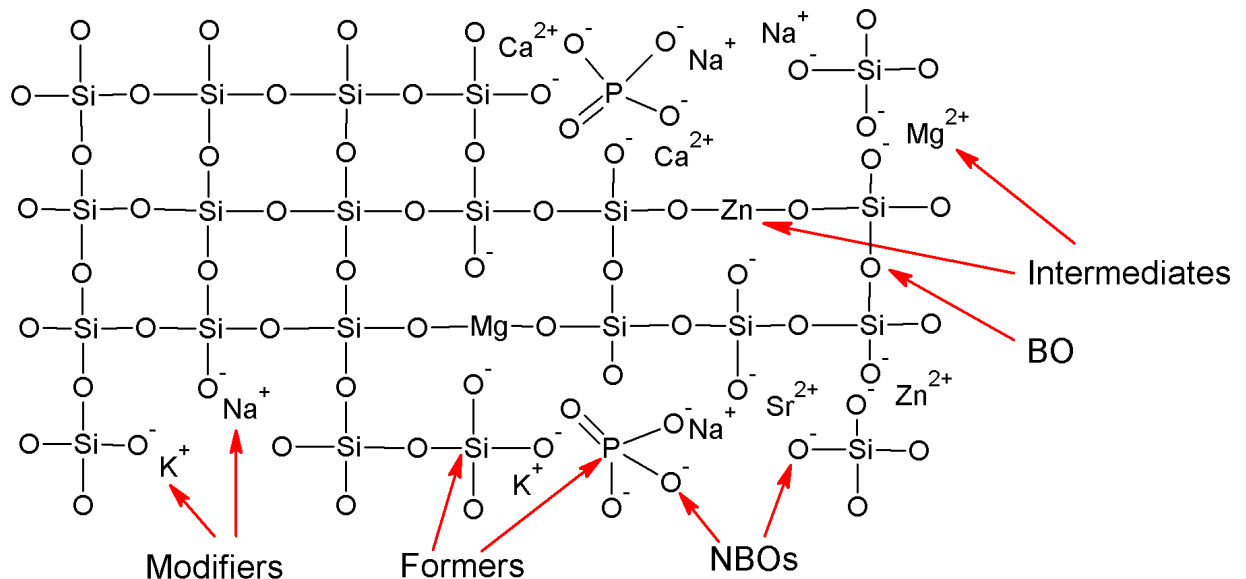


Figure 2.4 Schematic diagram of the soda-lime phosphosilicate glass structure showing BOs and NBOs as well as network formers, intermediates and modifiers

The number of NBOs in a glass is important because it determines the network connectivity (NC) and the crosslink density of the glass, which are the average number of bonds that link each repeat unit in the network and the average number of additional crosslinking bonds (above two) for elements other than oxygen which form the network backbone, respectively. The more the NBOs,

the less BOs, the smaller the network connectivity and the more loosely packed the glass structure, which gives faster dissolution rate and the glass is hence more bioactive. NC is closely associated with the bioactivity of the glass, the lower the crosslink density, the lower the T_g and the more ‘broken’ the glass network, i.e. the faster the reactivity and solubility of the glass [37]. A crosslink density of 0 is equivalent to a network connectivity of 2, therefore the closer to 2 is the network connectivity, the better the glass is in terms of sinterability and bioactivity [58]. The value of NC can be calculated using Equation 2.1:

$$\text{Network Connectivity (NC)} = 2 + \frac{\text{Number of BOs} - \text{Number of NBOs}}{\text{Total Number of bridging species}}$$

(2.1)

For a pure silicate glass, the network connectivity is 4 as each silica is covalently bonded to 4 oxygens, i.e. 4 BOs; but for a 50% SiO_2 – 50% Na_2O glass the NC is 2, as there are now only 2 BOs as the other 2 are each bonded to 2 Na^+ ions via ionic bonding.

The incorporation of the ions, which when released is not only non-cytotoxic but beneficial to the body, another reason for incorporating them into the composition. Network modifiers such as Na^+ , K^+ and Ca^{2+} ions lower the NC by disrupting Si network and promoting formation of non-bridging oxygen bonds [48]. Osteoporosis is caused by the inactivity of osteoblasts (cells that form and build bone tissue) and the activity of osteoclasts (cells that remove and resorb bone tissue), Sr^{2+} promotes osteoblast activities and inhibits osteoclast activities, while acting as a network modifier [59]. Mg^{2+} ions control the dissolution rate [60] and if the content is >7 mol% they slow down the rate of HCA layer

formation, they also increase the thickness of HCA layer [61], whereas Zn^{2+} ions control the reaction between the glass-ceramic and SBF and enhance bone regeneration [62]. The bioactivity is not only influenced by the calcium content and the NC values, it is also directly related to phosphorus content in the glass [63] as it contributes to the formation of HCA layer. An increase in P_2O_5 content increases the NC but increases the bioactivity at the same time, this intriguing phenomena was discovered by O'Donnell *et al.* [64]. The enhanced apatite formation is due to the presence of orthophosphate in the silica network, which leads to continuous phosphate leaching at the contact with the SBF and the surface area increases rapidly. Increase in P_2O_5 also increases the T_c of the glass [63-64] whilst weakens the compressive strength of the glass [63, 65].

Of all the present bioactive materials, only handfuls are highly bioactive materials including certain compositions of BGs, which have similar compositions to Bioglass[®]. They bond to bone much quicker than bioceramic materials as they are amorphous. The bone bonding mechanism [45], also known as the Hench mechanism, is a 11-stage reaction, based on soda-lime-silica glass corrosion. Dissolution starts with rapid ion exchange of the network modifiers such as Na^+ or K^+ with the ions in the surrounding solution, such as H^+ or H_3O^+ . The breakdown of the silica network, the breaking of Si-O-Si bonds when the local pH increases above 9, results in the detachment of soluble silica from the bulk glass in the form of $\text{Si}(\text{OH})_4$, leaves the surface rich with silanol (Si-OH) groups, which eventually forms an alkaline depleted layer rich in SiO_2 . The Ca^{2+} ions will then migrate from inside of the glass into the solution, and as it passes through the silica-rich layer it is met by PO_4^{3-} ions

in the solution, which results in the formation of a CaO-P₂O₅-rich film on top of the silica-rich layer, followed by the growth of the film as the calcium, phosphates, hydroxyls and carbonates from the solution are incorporated into the system. The crystallized form of this film is the HCA layer. The differentiation of stem cells is activated by the adsorption and desorption of biological growth factors taking place in the HCA layer, which is followed by the macrophages activities that clear the defect and allowing in-growth of new cells. Progenitor cells such as mesenchymal stem cells attach themselves onto the implant surface, after which the cells are differentiated into osteoblasts; the osteoblasts then spawn an extracellular matrix to form bone and the inorganic part of the matrix is later crystallized, forming a composite structure with bone cells distributed within. The dissolution of a random amorphous network begins much earlier than that of a crystalline ceramic therefore the HCA forms more quickly on the glass than on synthetic HA [26]. The ability of bioactive glasses to accelerate cell growth has been studied and attributed to the release of soluble silica and calcium from these glasses, which would affect intracellular mechanisms and boost bone regeneration by interacting with the genes. This ability is known as the osteoinductivity [39]. The bone regeneration can be elevated by the dissolution products in two ways, either they enhance the differentiation of stem cells into osteogenic cells or they improve the relevant gene transcription of osteogenic cells such as osteoblasts [4-5].

The BGs can be made in two ways, conventional melt-quenching or sol-gel derived. The conventional way involves the mixing of oxide powders and heating up to over 1000 °C for melting, which is followed by quenching in water at room temperature. The sol-gel process on the other hand is conducted at room temperature; the process begins with hydrolysis of alkoxide precursors, which is followed by polycondensation of the colloids which harden into a gel. It can take several days to complete the reaction, unless catalysts are introduced to speed up the process [66]. Both types of glass have advantages and disadvantages depending on the application.

2.4.1 Sol-gel derived BGs

The sol-gel method is a relatively new way to produce glasses with very high levels of chemical homogeneity and surface area as it is nano-porous. Its potential was noted and discussed by Hench and West at 1990 [66] and the processing protocol to produce a foam using this type of glass was developed by Sepulveda and Jones [67] 10 years later.

For sol-gel glasses, a solution that is made of water and nitric acid (HNO_3), and tetraethyl orthosilicate (TEOS), then calcium nitrate (CaNO_3) is added for producing glass containing calcium, such as 70S and 58S. The compositions are given in Table 2.4. Polycondensation of (-Si-OH) groups continues after hydrolysis is complete, beginning the formation of the silicate (-Si-O-Si-) network. With the increase in the network connectivity and viscosity a gel is eventually formed [34].

Table 2.4 Composition of typical Sol-gel BGs to prepare 250ml of sol

	58S	70S	100S
SiO₂ (mol%)	58	70	100
CaO (mol%)	38	30	0
P₂O₅ (mol%)	4	0	0

Sol-gel-derived BGs are mesoporous, which means the surface of the glass is covered with nano-scaled pores, allowing them a much greater surface area than melt-derived BGs. The increase in surface area leads to the increase in the number of nucleation sites for HCA layer formation, and the dissolution rate of sol-gel BGs is hence much faster than melt-derived BGs of similar composition. This means that sol-gel BGs are more bioactive than melt-derived BGs. As a matter of fact, because of its high bioactivity, the only ingredients that are absolutely essential to include in the composition of sol-gel glasses are SiO₂ and CaO, whereas in the melt-derived BGs more ingredients need to be introduced to increase the sinterability [26, 68]. For melt-derived BGs the mol% of SiO₂ must be less than 60 (preferably less than 55 [69]) otherwise they will lose their bioactivity whereas in sol-gel BGs this limit is pushed to 90 mol%. The original limited choice of glass compositions that exhibit high osteoproduktive bioactivity is also widened [69].

Although sol-gel glasses were thought to be homogeneously structured throughout, Lin *et al.* has recently discovered otherwise [70]. His work stated that the 70S30C glasses traditional sol-gel technique were in fact heterogeneous, with a transparent inner core surrounded by an opaque outer shell caused by the accumulation of calcium nitrate along the surface during the drying stage of the sol-gel process. The calcium-rich outer layer was significantly different to the original glass with reduced Si content, much smaller surface area and larger nano-pore size. However, the homogeneity could be improved by using a Teflon mould as it was highly hydrophobic, which effectively prevented calcium deposition on the surface and allowed more time for calcium diffusion and distribution; low drying rate ($0.1\text{ }^{\circ}\text{Cmin}^{-1}$) would also increase the homogeneity.

The extremely fast dissolution rate of sol-gel glasses was always a concern, especially in cases that slower and steadier degradation was required, the solution is to use melt-derive BGs.

2.4.2 Melt-derived BGs

Hench *et al.* invented the first ever bioactive material - the Bioglass[®] 45S5 in 1969 [71], which was a milestone in the history of biomaterials for it integrated with the host tissue. Sol-gel BGs are more bioactive than the melt-derived BGs, however the fast dissolution rate of sol-gel BGs and the even faster calcium ion release rate means their ability to bond to bone is weaker. Bioglass[®] still remains to date, the material that bonds best to the host tissue, and because of its superior properties, many attempts have been made since its invention to produce 3D porous scaffolds. The only way to produce such scaffold is to sinter

the particles, which is problematic for Bioglass[®] due to its narrow sintering window. Glasses have then been developed to overcome such problem, which are derived from Bioglass[®] 45S5 with alternation in the composition to expand the sintering window but to maintain the bioactivity at the same time, for example 13-93 invented by Brink *et al.* [72] and ICIE 16/ICIE 16M by Elgayar and Hill *et al.* [37]. These glasses and their properties will be examined in greater details in Chapter 3.

2.4.3 Summary

There are two types of bioactive glasses, sol-gel and melt-derived. Sol-gel BGs are mesoporous, simple in composition, fast in degradation; whereas melt-derived BGs have complex composition systems, include network formers, modifiers and intermediates, all playing a different role in affecting the glass behavior in terms of sinterability, bioactivity and thermal and mechanical properties. NC is determined by the percentage of BOs in the glass network. A NC value of 2 gives the most broken network, i.e. fastest reactivity and degradation rate. If the NC is less than 2, i.e. there are more network modifiers than network formers, the glass is thermally unstable and devitrifies easily when heated beyond T_g. Its dissolution rate is rapid, which leads to cell death and fibrous encapsulation [73]. Novel approach in the field is to produce scaffolds that are made of bioactive glasses.

2.5 Bone Scaffolds and potential scaffold materials

According to Jones *et al.* [34], the ideal scaffold should mimic the extracellular matrix of the bone tissue both structurally and mechanically. In order to do so it needs to act like a template in three dimensions for cell to attach and grow on, as well as fulfill the following requirements at the same time:

- 1) Exhibit a surface that promotes cell adhesion, adsorption of biological metabolites.
- 2) Influence the genes in the bone generating cells to enable efficient cell differentiation and proliferation.
- 3) Absorb at the same rate as the tissue is repaired, with degradation products that are non-toxic and are able to be excreted by the body easily, for example via the respiratory or urinary systems.
- 4) Be made from a processing technique that can produce irregular and complex shapes to match that of the defect in the bone of the patient.
- 5) Has the potential to be commercially producible to the required International Standards Organisation (ISO) or Food and Drug Administration (FDA) standards.

There is a vast choice of potential scaffold materials, varying from metals to ceramics to polymers and composites. Titanium and cobalt-chrome alloys are the most popular, which are bioinert materials, but there is a great disadvantage considering the formation of fibrous capsule. Despite this, the metals are extremely high strength in compression and can withstand cyclic loading. However they also have a high Young's modulus, which will evoke stress

shielding after it was implanted, as the result of bone responding to environmental stimulation. The outcome is severe, the surrounding bone tissue will gradually disappear due to remodeling by the osteoclast activities, exposing the implant; and because metals are not biodegradable, the implant will have to be taken out and revision surgery required. The brittleness of the ceramic nature limits their applicability in bone tissue regeneration as they are easy to fracture during stress. Polymers on the other hand are more flexible than ceramics.

Most of the synthetic polymers used in the field are either biologically stable or resorbable and some are even osteoconductive [74]. Common FDA approved biodegradable polymers are polyesters of poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(lactic acid-co-glycolic acid) (PLGA). Scaffolds made from synthetic and natural polymers have been investigated extensively for bone repair for three reasons [26, 75]:

- 1) It is easier to process polymers in the shape of a 3D scaffold with a pore morphology suitable for tissue engineering applications than it is ceramics and glasses
- 2) The high tensile properties and high toughness of polymers matches with the requirements of bone in load bearing situations, and the mechanical properties of polymers can be controlled very easily by changing the molecular weight (chain length) of the polymer
- 3) Bioresorbable polymers have been used successfully as dissolving sutures for many years

The techniques used to produce porous networks in these polymers are fiber bonding or weaving, solvent casting, particulate salt leaching, phase separation, gas foaming, freeze drying and extrusion. Polymer solutions can also be foamed to produce an open pore structure by using blowing agents, gas injection, supercritical fluid gassing or freeze-drying [26].

Resorbable polymeric scaffolds have been recently developed with porous structures similar to the trabecular bone, but the major disadvantage of these kind of scaffolds were that the polymers were not osteoproduktive and could not bond to bone, moreover polymers have a much lower Young's modulus than bone and therefore could not be applied directly in load bearing applications [34]. There were also concerns established over the acidic degradation products of such scaffolds. As a result, researchers have shifted their interests into polymer-ceramic composites instead, because overall bone is such composite in nano-scale.

Orfice *et al.* proved that bioactive polymer composites with modified surfaces, such as the one investigated in his work, could potentially be osteoproduktive materials [32]. He suggested that polymer composites, which consisted of a bioactive phase, glass-ceramic or ceramic introduced in a high mechanical performance polymeric matrix, had potentially the capability of combining bioactive behavior with adequate mechanical properties, the combination osteoconductivity and the stiffness of the material. This was supported by the work of Maquet *et al.* [76], who discovered the addition of increasing amounts of Bioglass[®] into the polymer foams decreased the pore volume, and improved

the mechanical properties of the scaffold.

Despite the similarities of polymer-ceramic composite scaffolds to the bone, there are several drawbacks [77-78]. First of all as shown in Figure 2.5, the mechanical properties are not as high as expected, in fact it is lower than the pure bioactive ceramics scaffolds. This is due to the lack of interface bonding strength between the polymer and the ceramic phase. Secondly the dissolution rate is different between the two phases, biodegradable polymers usually resorb much slower at first than the inorganic phase in the composite structure, but once the degradation of polymer starts, it is a self-catalytic and rapid process that will lead to the loss of the supporting matrix and failure of the scaffold.

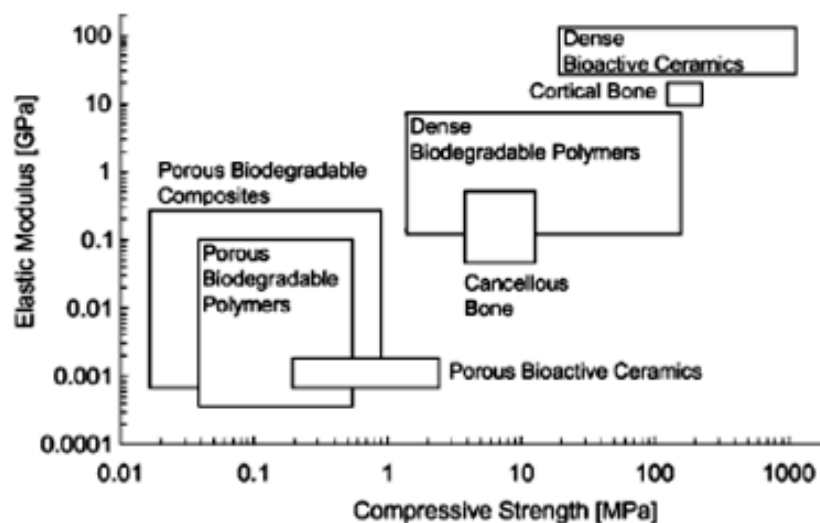


Figure 2.5 Elastic modules VS compressive strength of different kinds of scaffolds and monoliths [77]

2.5.1 Summary

Many materials can be made into porous scaffold; however none can fully fulfill the requirements of an ideal bone scaffold. Metals are bio-inert, and exert stress shielding on the surrounding tissue; ceramics are brittle; polymers lack osteoconductivity in general and will possibly produce an acidic environment through degradation; polymer-ceramic composites have good toughness but are quite weak in compression and the difference in dissolution rate in-between the two phases are always a concern. Therefore current commercial artificial bone grafts are generally bioactive ceramics, usually calcium phosphate based. Porous BG foams would potentially give enhanced biological response and good control of degradation. If a bioactive glass is used that can be sintered without crystallizing, methods used to produce ceramic scaffolds can be applied to glasses.

2.6 Production techniques of porous bioceramic foams

As reviewed in Section 2.4, with the modified composition of Bioglass[®] it can be made into porous foams. There are a number of ways to produce porous ceramic foams, such as modified freeze casting and slip casting in the early stage; later the burning out of a polymeric sponge impregnated with ceramic slurry, the replication of polymer foams by impregnation and the gel-cast foaming process; the most recent approaches are the sol-gel foaming process and rapid prototyping. It is important to choose a suitable method.

2.6.1 Freeze casting

Freeze casting is a simple technique to produce porous complex-shaped ceramics [79]. A slurry of ceramic powder and water is frozen (solidification), encouraging ice dendrites formation. The dendrites push the powders into the inter-dendritic space. The structure is then freeze dried under vacuum conditions, with the ice removed by sublimation to avoid the drying stresses and shrinkage that might lead to cracks and warping during normal drying. After drying, the pre-formed ceramic powder with pores in the size and shape of the dendrites is sintered so that the powder densifies into solid walls around the large pores, replicating the pure ice dendrites that were formed during the freezing process [79-81].

The pore structure and mechanical properties of the freeze cast foams are solely depended on the cooling rate and temperature [82]. Faster cooling rate results in higher compressive strength and smaller pore size. The lower the cooling temperature, the less interconnected the pore network; rapid cooling may also cause aligned, elongated pores [79-80]. Furthermore, the pore size is dependent on the slurry concentration and the powder size, which also influences the porosity, Yoon *et al.* successfully produced porous alumina foams with interconnect size of $>50\text{ }\mu\text{m}$, porosity of 82% and mechanical strength at 11 MPa based on 10 vol% alumina slurry [83]. At low ceramic loading, the green body collapsed even in the presence of a binder; at high concentrations, the body lost the interconnectivity and open porous structure, although this was improved by using powder with small particle sizes ($2.4\text{ }\mu\text{m}$) [79]. The strength of the scaffold varied from 10 MPa to 60 MPa depending on the cooling rate

(<2 °Cmin⁻¹ versus >5 °Cmin⁻¹) [79].

This technique is limited by the fundamentals of the physics of ice, in particular the development of the unavoidable sequence leading to a steady lamellar ice front that leads to the formation of two very different architectures to be obtained in the final structure (see Figure 2.6a). This poor interconnectivity as showing in Figure 2.6b, lead to little interest in a biomedical point of view.

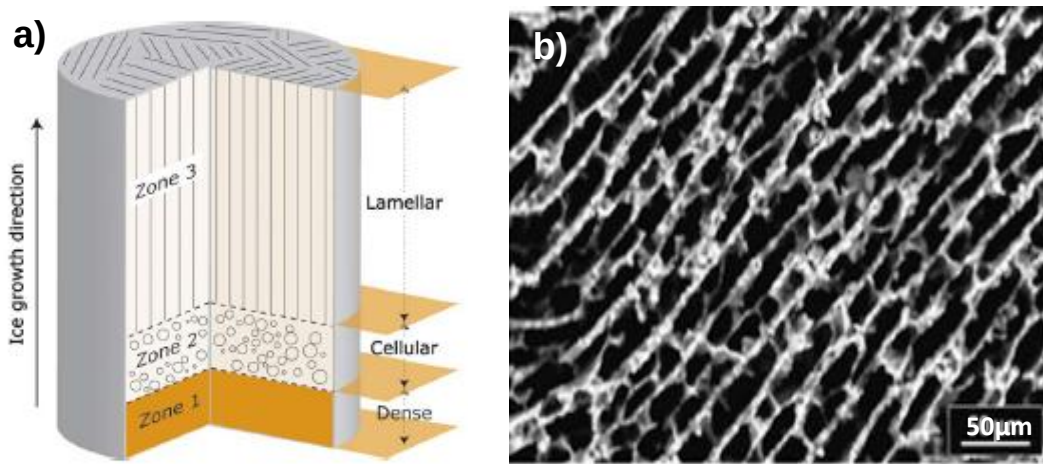


Figure 2.6 a) general microstructure of freeze dried samples, the dimensions of different zones are depended on the processing conditions, b) SEM image of porous HAP samples with 64% porosity parallel to the ice front [79]

2.6.2 Polymeric sponge impregnated with ceramic slurry

This method involves the use of polymeric foam (preferably polyurethane, PU) as a template, in order to produce scaffolds with highly ordered pore network, high porosity, high interconnectivity and controlled pore size [84-87]. The sponge is to be immersed thoroughly into a ceramic slurry followed by

squeezing the foam to remove excess slurry and drying. The removal of the polymeric sponge was achieved by firing the sample at a high temperature (sintering temperature of the particles), which would burn off the organic part during processing.

The advantage of this technique is the guaranteed high porosity and an accurate open pore structure dictated by the polymer sponge template. Figure 2.7a and 2.7b clearly shows the porous structure of the sponge is maintained through the whole process. Chen *et al.*, using Bioglass[®] [10] and Fu *et al.*, using 13-93 melt-derived bioactive glass [88] have both applied this technique to create porous scaffolds using melt-derived bioactive glasses. However because Chen *et al.* used the Bioglass[®] composition, the glass crystallized into a glass ceramic. They reported the compressive strength of the resulting glass-ceramic scaffolds to be very low (0.5 MPa at 86% porosity and 0.1 MPa at 95% porosity). This was due to inefficient sintering, because the glass have crystallized before the flow between particle was complete, leaving micropores and making hollow struts inevitable (Figure 2.8b) [10]. However, the glass was not fully crystallized, and a mixture of glass and crystalline regions may cause unpredictable properties due to preferential dissolution of the amorphous regions.

Fu *et al.* achieved dense struts with an impressive strength at 11 ± 1 MPa (porosity = $85 \pm 2\%$) because 13-93 was a tailored composition aimed to expand the sintering window and was indeed showed much improved sintering. However its bioactivity was not ideal as it has a high NC value, for more detailed discussion please see Chapter 3.

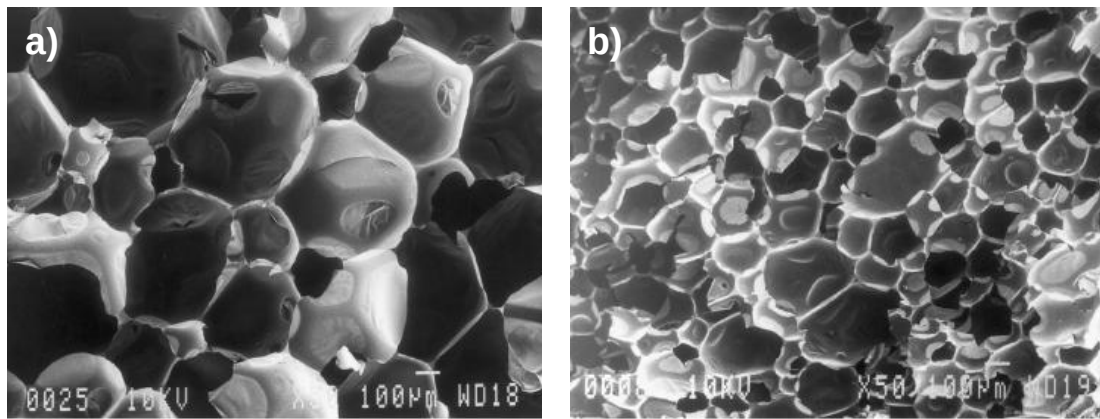


Figure 2.7 Comparison of microstructure of (a) the unfilled PU foam and (b) the PU-20 vol% Al_2O_3 foam [86]

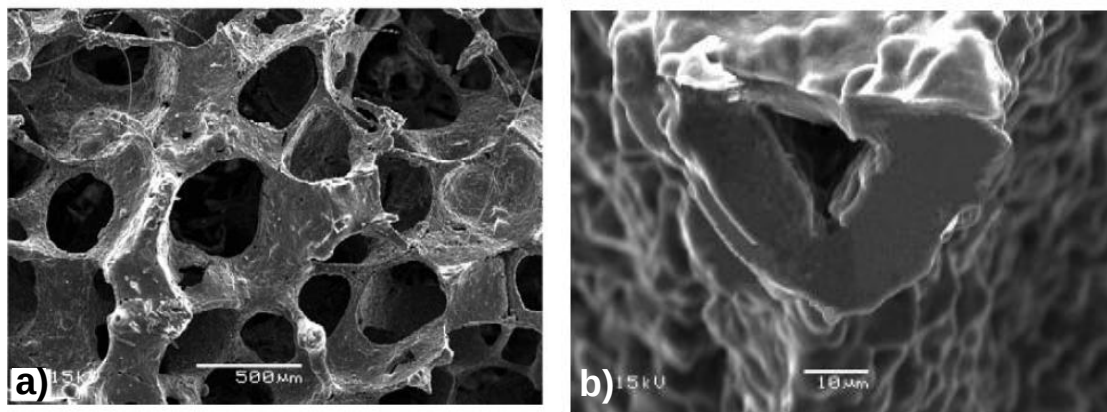


Figure 2.8 a) sintered ceramic foam and b) hollow-centered strut [10]

In addition to the problem of hollow struts, this process is difficult to scale up for mass production. After the slurry is introduced to the polymer foam, excess slurry must be removed and there is no controlled method for doing this. The sponges are usually squeezed. If not all the excess is removed, the scaffolds suffer from the excess slurry blocking the interconnected pores.

2.6.3 Slip casting foaming process

Traditionally, slip casting is referred to as liquid clay, where a mixture of clay and water is poured into a plaster mold of desired shape and after setting a layer of clay was formed on the inside cavity of the mould. It is a mass production technique and is especially good at producing ceramics with complex shapes. Because of its industrial application and easy fabrication steps, it has been modified to produce porous foams via slip-casting where the slip was to be foamed before pouring into the mold and was immediately set and dried, followed by the removal of green body and high temperature firing.

Cyster *et al.* applied a modified slip casting technique to produce a porous hydroxyapatite (HA) scaffold [89], where the HA powder suspension was dispersed and agitated by vigorous stirring, followed by the addition of the setting agent and the foaming agent, in this case methylcellulose and Tergitol TMN10 respectively, into the slurry. The foam was set and dried at 65 °C overnight. After the removal of the green body it was fired at 3 °Cmin⁻¹ up to 250 °C, and was held for 30 min to enable complete burnout of the organic components. The ceramics were then heated at the same ramp rate up to the sintering temperature of 1350 °C and held for 3h followed by cooling to room temperature. Figure 2.9 shows SEM images of such scaffold. A very good interconnectivity was established at the macroscopic level (Figure 2.9a) and Figure 2.9b showed excellent sintering was achieved.

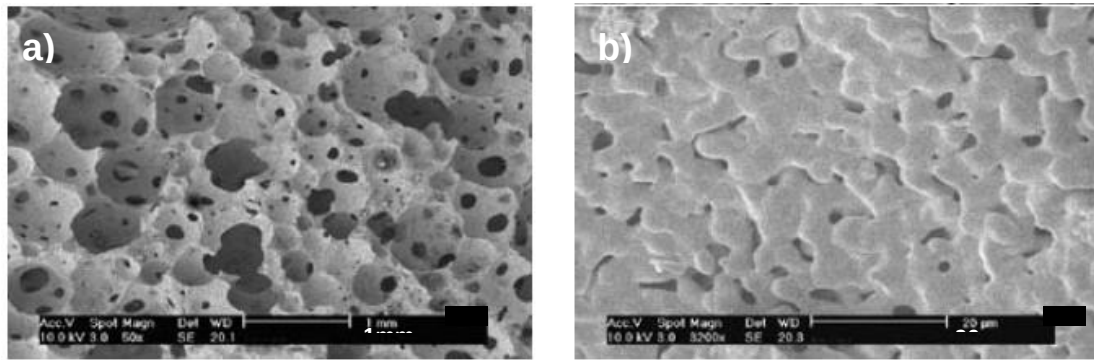


Figure 2.9 SEM micrographs showing the a) the pore morphology and b) the grain structure of HA scaffolds prepared dispersant concentration of 80 mgg^{-1} [89]

Their method produced scaffolds with a steady 85% porosity but the compressive strengths were relatively low (between 1.07-1.76 MPa). The best result was obtained at 80 mgg^{-1} dispersant concentration (85% porosity and 1.76 MPa). The pore size was between 391-495 μm and the interconnect aperture size was between 74 and 133 μm . The major disadvantage of this process is the unavoidable partial collapsing of the foam, due to excess dispersant within the slip. It is hard to avoid as the porosity is largely dependent on the dispersant concentrations.

2.6.4 Gel-casting foaming technique

Gel-casting combines the traditional slip casting with polymer chemistry. It was developed in early 90 s by Omatete *et al.* and Janney *et al.* as a new technique for fabricating dense ceramic foams to replace the conventional way of producing light ceramic tile used as construction material, which has many functions such as insulation, lightness, machinability and sound/thermal insulation [90-91].

The initial gel-casting process was developed by dissolving multifunctional acrylate monomers in solvents, water was the most popular choice as it was preferred by most ceramics. As a result, water soluble monomers were desired, which eventually led to the development of the acrylamide gel system [90, 92]. The main monomer has only a single double bond so if it was to polymerize alone would result in formation of a linear polymer; whereas the second monomer (the crosslinker) has at least 2 double bonds and when the in situ polymerization occurs, it forms a crosslinked polymer based gel.

Originally the monomer was acrylamide and the crosslinker was *N,N'* – methylene bisacrylamide, but the acrylamide was found to be neurotoxic, which is a great liability when handling it during the process, and was later replaced by the methacrylamide [92-93], a much safer candidate. The crosslinker was not as toxic, although it could be replaced by poly(ethylene glycol) dimethacrylate,

which has a minimum toxicity and a larger molecular weight [90]. The organic part was to be burnt out in order to obtain the final porous ceramic foam hence the toxicity was not important in this case. As it is a free radical polymerization, an initiator is needed, which in this case is ammonium persulfate (APS). The initiator creates free radicals by division of the molecule into two fragments along a single bond. A Catalyst is added to speed up the reaction, *N,N,N',N'*-tetramethylethylenediamine (TEMED) was used. The key step in gel-casting is the in situ gelation of a slurry by polymerization of organic additives.

Verma *et al.* produced dense HA by gel-casting [94] while Sepulveda *et al.* was the first person to introduce foaming into the gel-cast process to produce porous ceramic foams made alumina [95-96] and HA[97-98], their results suggested that gelling time was closely related to the initiation system [95]. To produce a porous scaffold, organic monomers are added to a ceramic slurry and the mixture is vigorously agitated with the aid of a surfactant, followed by pouring into a mold prior immediately prior to in-situ polymerization. The viscosity increase due to the progression of the polymerization reaction is critical to allow stabilization of the bubbles. As a result, a composite of an organic gelled foam containing inorganic powder is formed. The gel is then dried to remove the solvent (water), followed by sintering to remove the polymer and densification purposes (Figure 2.10).

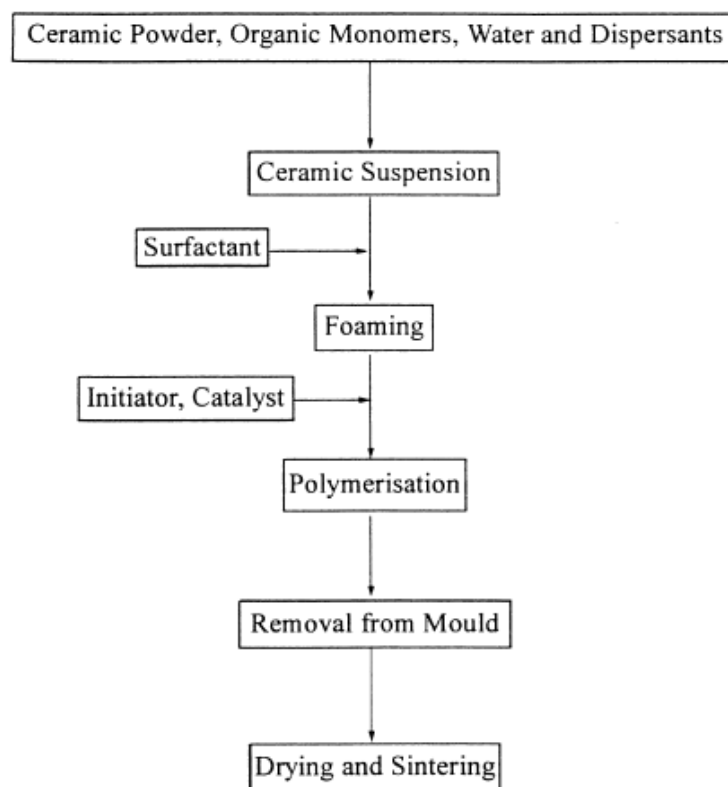


Figure 2.10 Detailed flowchart of the gel-casting foaming process [95]

In Sepulveda's work, a surfactant (Tergitol TMN10) (Fluka Chemie AG) was used to lower the surface tension of a liquid, to maintain the foam structure for a short period until it gelled. It played an important role on influencing the foam body value, which was closely related to porosity of the foam [97].

A good control of induction time (the time between the addition of reagents and the beginning of the polymerization) allowed enough time for bubble enlargement and lamella thinning [95-96], and variation in the induction time length greatly changed the pore morphology, which was clearly demonstrated in Figure 2.11; where the pore sizes in Figure 2.11a, which gelled in 90 seconds,

was much smaller than those in 2.11b (gelled in 200 s) however the interconnect size seemed not to be affected as much. A long induction time would lead to foam collapse and structure weakening, therefore it was the most vital parameter in the whole process [99] along with the foam body value, both played key roles in determining the final cell size distribution and the strut thickness.

In addition, Sepulveda *et al.* also studied how the persulfate-amine system influenced the parameters in the gel-cast foaming system and concluded that the induction time and foam body volume was mainly controlled by the initiator and catalyst volume [100], which also greatly influenced other minor factors such as the temperature, pH and oxygen content. Faster polymerization reactions with shorter induction times could be produced at higher and more alkaline pHs, higher temperatures, and lower atmosphere oxygen contents. The type of surfactant also influenced the microstructure of the foam as shown in work done by Fuji *et al.* [101]. The anionic surfactants performed better than the non-ionic ones, and ammonium lauryl sulphate was the best in foaming ability and produced alumina foams with highest porosity and pore size distribution. Compared to the unfoamed systems, foaming caused a significant change in polymerization kinetics, reaction rates were reduced and the systems were more sensitive to small temperature variations [96]. There might be cracking at the drying stage, which could be reduced by addition of a plasticizer [102-103].

The HA foams were characterized and the pore size varied between 20 – 1000 μm , the porosity was up to 90% and the mechanical strength at 80% porosity was 4.4 – 4.7 MPa. The pore size and porosity was directly linked to the specimen density.

The slip-cast foaming process in Section 2.5.2 is very similar to this process however the slip-casting generates weak green-bodies and the processing time is lengthy. Furthermore, the gel-cast foams in Figure 2.11 were much more porous at microscopic level than those in Figure 2.9, and there was no problem with excess dispersant in the system, in a way gel-cast foaming was an upgraded version of the slip-casting foaming process. With the aid of sinterable melt-derived bioactive glasses, it is possible to produce scaffolds by this method, which is an excellent opportunity for large scale production of high bioactive and osteoproduative material with great potential for clinical application.

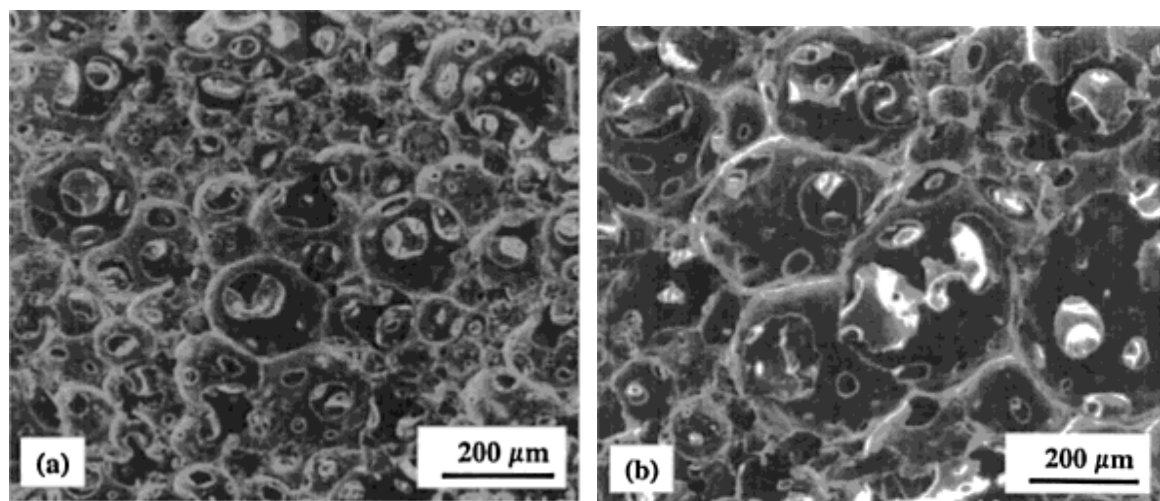


Figure 2.11 SEM images of gel-cast foamed HA scaffolds at scaffolds gelled with induction times of a) 90s and b) 200s [96]

2.6.5 Sol-gel foaming

Sol-gel BGs can be made into scaffolds simply by introducing air and surfactant into the suspension until it gels; this was termed as the sol-gel foaming process. It is very important to distinguish gel-cast foaming from the sol-gel foaming process for their similarity. They both start with a solution that was hardened into a gel at the completion of the process. In gel-casting, a high solid loading (at least 50 vol% solid) is required in order to minimize the shrinkage caused by drying and to enhance the density of the foam; whereas in sol-gel foaming, the solid was usually produced as part of the process in the form of an inorganic gel with low solid loading [104]. Figure 2.12 showed the detail of the process [67]. The foams produced had a highly uniform pore network as shown in Figure 2.13.

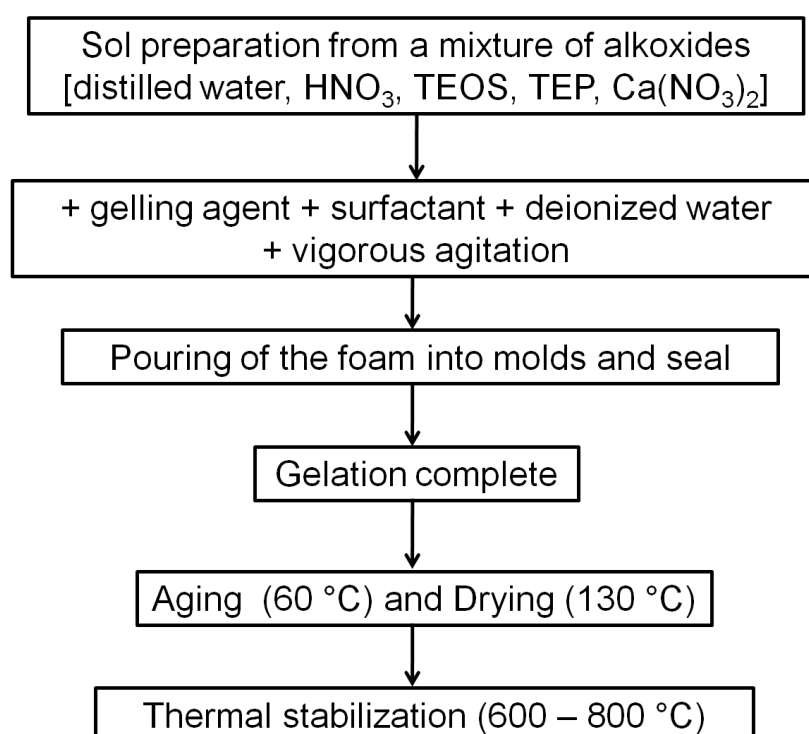


Figure 2.12 Flow-chart of sol-gel foaming process [105]

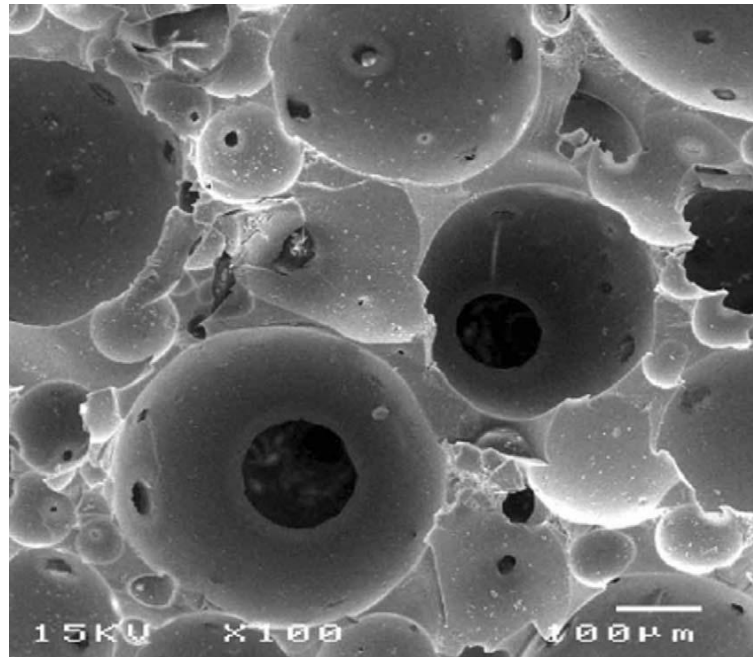


Figure 2.13 SEM micrograph of a typical pore network produced via sol-gel foaming process [106]

One major advantage of sol-gel foamed BG scaffolds is that they have extremely high surface area as a result of the mesopores, which will significantly increase their bioactivity. A downside to this is that the scaffold may disintegrate long before the tissue is regenerated in some applications. The pore distribution was homogenous and the porosity was greater than 90%. However these foams suffered low mechanical strength, when sintered at 600 °C its compressive strength was only 0.36 MPa, but with increasing sintering temperature the sol-gel BG scaffolds were able to achieve a compressive strength up to 2.26 MPa (when sintered at 800 °C for 2 hrs) [107]. The increase in sintering temperature reduced the interconnect size from 122 µm at 600 °C to 98 µm at 800 °C as well as risking potential partial crystallization ($T_{c \text{ onset}}$ of 70S30C sol-gel glass is 845 °C). The whole process was rather long: approximately 8 min to gel, 72 hr of aging, 94 hr of drying and an additional 14 hours for stabilization prior to sintering.

Handling of highly dangerous acid HF is involved as the HF is the catalyst in the system; it can be replaced by a less dangerous but stronger HNO_3 acid, which is still at its early stages of research.

In both the gel-cast foaming and the sol-gel foaming process, the changes in the foam structure prior to solidification are important because their influence in the final cell size distribution, wall thickness and microstructure of the solid foams [108].

2.6.6 Rapid prototyping (RP)

This is a relatively new technology, also known as solid freeform fabrication (SFF), developed to produce complex shapes directly from a computer generated model. Figure 2.14 shows the remarkable resemblance of the product (2.14b) to the computer model (2.14a). There are many different types of RP techniques each with their own benefits and limitations that can be classified into two categories: the direct and indirect fabrication methods. The most distinctive advantage of this technique is the ability to construct an object with customized macro- and micro-structures. The disadvantages are: first, the feasibility of the scaffold is dependent on the size of the smallest feature a RP machine can build and hence overall resolution size of the machine; secondly, to each RP technique there is a very limited choice of materials that are compatible to this specific technique, usually bio-inert materials [109-110]. Biodegradable polymers and polymer composites are the favorites in research based RP systems for their excellent processing abilities; however porous ceramic scaffolds have been produced.

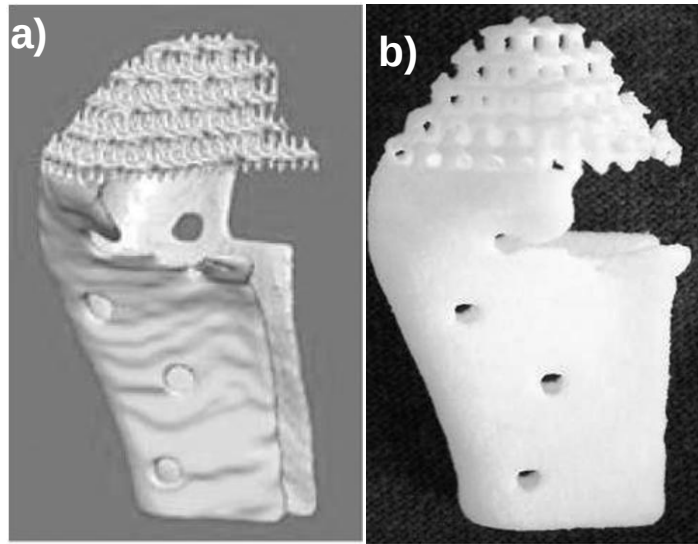


Figure 2.14 a) computer design of a polycaprolactone (PCL) scaffold, b) the scaffold produced by selective laser sintering rapid prototyping [111]

Seitz *et al.* and Leukers *et al.* have applied a 3D printing technique to produce hydroxyapatite scaffolds and evaluate their biocompatibility [112-113]; the pore size was between 450 – 570 μm with micro pores of 10 – 30 μm but the interconnectivity was extremely low; the dynamic cell culture data showed formation of continuous cell layer in both inner and outer surface of the scaffold with large number of cells in-growth to the HA granules in 7 day's time. Yang *et al.* has recently developed highly porous HA-TCP meshes by employing extrusion freeforming method and achieved a wide range of pore sizes from 50 – 500 μm [114]. The use of HA or TCP has limited the bioactivity and the dissolution rate in both cases. Rapid prototyping is considered as the next generation of tissue engineering for its immense potential in automation and direct organ/cell printing, researchers are keen to eliminate its limitations (Iwami *et al.* had recently produced a scaffold that was made with a mixture of cell and hydrogel [115]), however the cost remains high compared to the conventional fabrication techniques.

2.6.7 Summary

There are many techniques to fabricate porous ceramic foams and are summarized in Table 2.5. Gel-cast foaming is the most viable way to produce melt-derived bioactive glass scaffolds.

Table 2.5 Summary of porous ceramic foams fabrication techniques

Technique	Interconnect size/Pore size	Porosity	Advantage	Disadvantage
Freeze casting	Depend on slurry concentration >50 μm /<200 μm	Depend on slurry concentration <85% in general	Simple process, potential for mass production	Uni-directional channels, separate architecture in final structure, low interconnectivity
Slip-cast foaming	74–133 μm /391–495 μm	85%	Easy fabrication steps, industrial applicable	Unavoidable partial collapsing
Polymer foam replication	Depend on foam morphology >100 μm /<500 μm	>85%	Highly ordered pore network, high porosity, high interconnectivity, controlled pore network, potential mass production ability	Hollow centered struts, partial blockage of pores
Gel-cast foaming	>100 μm /20-1000 μm	Up to 90%	High interconnectivity, high porosity, simple process, potential mass productivity	Possible formation of small sulfate crystals which is discussed in Chapter 4 – 5.
Sol-gel cast foaming	>100 μm /10-2000 μm With mesopores 2 to 50 nm	Up to 97%	High interconnectivity, high porosity, high surface area, faster dissolution rate, potential mass productivity	In some cases dissolution rate is faster than tissue regeneration rate
Rapid prototyping	Depend on design and technique chosen	Depend on design and technique chosen	Construct object with customized macro- and micro-structures, ability for automation, direct organ/cell printing	Limited choice of material to specific technique, resolution dependent, expensive

3 Glass production and characterization

3.1 Introduction

Bioglass[®] cannot be processed due to the narrow sintering window and is therefore unable to be sintered and made into scaffolds without crystallization. To overcome this problem, more oxides were added into the composition mainly aimed to expand the sintering window but maintain the bioactivity at the same time, as well as delicate new features to the glass. The first ever melt-derived BG scaffold was produced by Fu *et al.* by applying polymer foam replication technique and 13-93 BG [88], therefore the glass was included and compared with the others. Elgayar and Hill *et al.* have evaluated the effect of adding alkali and alkali earth oxides into the glass composition on the structure of the glass and the degradation rate [37, 51]. It is from their work that glass compositions were chosen to be applied in this project, which are the ICIE 16 and ICIE 16M, a modified version of ICIE 16. These glasses have a network connectivity close to 2, which means they have very similar chemical structure to Bioglass[®], but they have a much wider processing window and are therefore applicable.

This chapter will go through the glass production process as well as analyzing the sintering behaviour of the three glasses by applying Differential Scanning Calorimetry (DSC) in Section 3.2 and the result was plotted in Figure 3.1. The network connectivity of each glass was also calculated and results summarized in Table 3.2.

3.2 Materials and methods

3.2.1 Glass composition

Table 3.1 shows the compositions of four glasses, ICIE16, ICIE 16M, the original Bioglass[®] composition and the 13-93 glass (only used as a reference because the first melt-derived BG scaffold was made from 13-93 [116] and was not used in this project). ICIE16M has the largest number of oxides and 45S5 with the simplest composition.

Table 3.1 Glass Compositions

Compositions (mol%)	Network Roles	45S5	13-93	ICIE 16	ICIE 16M
SiO ₂	Former	46.1	54.6	49.46	49.46
CaO	Modifier	26.9	22.1	36.27	27.27
Na ₂ O	Modifier	24.4	6	6.60	6.60
K ₂ O	Modifier	---	7.9	6.60	6.60
ZnO	Intermediate	---	---	---	3
MgO	Intermediate	---	7.7	---	3
SrO	Modifier	---	---	---	3
P ₂ O ₅	Former	2.6	1.7	1.07	1.07

The network connectivity (NC) was calculated using Equation 3.1 (showing example of Bioglass[®]), which is a modified version of Equation 2.1. It takes into account that phosphorus in the glass network forms Q⁰ orthophosphates (PO₄³⁻), which has 3 NBOs to each P atom and requires network modifiers to charge balance them. The presence of orthophosphates effectively polymerizes the silica

network and increase NC [73].

$$NC = 2 + \frac{2 \times \text{mol\% (SiO}_2) - 2 \times \text{mol\% (CaO + Na}_2\text{O)} + 2 \times \text{mol\% (P}_2\text{O}_5 \times 3)}{\text{mol\% SiO}_2} \quad (3.1)$$

In the presence of network intermediates, there will be two values depending on whether to assume the intermediates to behave as network modifiers or formers. In the case of 13-93, the equation would be:

$$NC = 2 + \frac{2 \times \text{mol\% (SiO}_2 + \text{MgO} + 3 \times \text{P}_2\text{O}_5) - 2 \times \text{mol\% (CaO + Na}_2\text{O} + \text{K}_2\text{O)}}{\text{mol\% (SiO}_2 + \text{MgO)}} \quad (3.2)$$

Assuming the Mg^{2+} as network formers; if assumed as modifiers, it would be:

$$NC = 2 + \frac{2 \times \text{mol\% (SiO}_2 + 3 \times \text{P}_2\text{O}_5) - 2 \times \text{mol\% (CaO + Na}_2\text{O} + \text{K}_2\text{O} + \text{MgO)}}{\text{mol\% SiO}_2} \quad (3.3)$$

The results are summarized in Table 3.2.

Table 3.2 The NC values of different glasses

Glass	NC value
Bioglass [®] 45S5	2.11
13-93 (assume Mg^{2+} as modifier)	2.59
13-93 (assume Mg^{2+} as former)	3.00
ICIE 16	2.13
ICIE 16M (assume Mg^{2+} & Zn^{2+} as modifiers)	2.13
ICIE 16M (assume Mg^{2+} & Zn^{2+} as formers)	2.55

ICIE 16 and ICIE 16M have similar NC values (2.13) as the Bioglass[®] (2.12), assuming the Mg²⁺ and Zn²⁺ ions are behaving as network modifiers in ICIE 16M. In fact this was the reason behind the design of the ICIE 16 and ICIE 16M compositions, to create glasses that have the same NC (and therefore similar bioactivity) to Bioglass[®], but a larger sintering window. 13-93 has the largest NC values, therefore a slower degradation rate and HCA formation rate is expected for 13-93 compared to ICIE 16 and ICIE 16M.

3.2.2 Glass production

All the glasses were produced by weighing and mixing the required amount of the analytical grade reagents together in a plastic container, which was well shaken. The actual weight of reagents was calculated by first multiplying mol% and molecular weight of each reagent, which were then summed up and divided by the weight of glass aimed to produce in this batch. Take Bioglass[®] as an example; for a 200g pre-fired batch of reagents (46.1% SiO₂, 24.4% Na₂O, 26.9% CaO and 2.6% P₂O₅, all in mol%), using Na₂CO₃ and CaCO₃ as sources of Na₂O and CaO, then the first step would be to calculate the mass of each oxide/carbonate :

$$\text{SiO}_2: 0.461 \times (28.09 + 16.00 \times 2) = 27.70 \text{ g}$$

$$\text{Na}_2\text{CO}_3: 0.244 \times (22.99 \times 2 + 12.01 + 16.00 \times 3) = 25.86 \text{ g}$$

$$\text{CaCO}_3: 0.269 \times (40.08 + 12.01 + 16.00 \times 3) = 26.92 \text{ g}$$

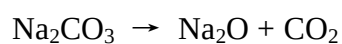
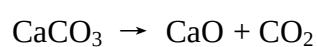
$$\text{P}_2\text{O}_5: 0.026 \times (30.97 \times 2 + 16.00 \times 5) = 3.69 \text{ g}$$

$$\Sigma = 84.17 \text{ g}$$

The actual weight of each oxide/carbonate in a 200g batch of reagents would be:

SiO ₂ : 200 × 27.70 ÷ 84.17	= 65.82 g
Na ₂ CO ₃ : 200 × 25.86 ÷ 84.17	= 61.44 g
CaCO ₃ : 200 × 26.92 ÷ 84.17	= 63.96 g
P ₂ O ₅ : 200 × 3.69 ÷ 84.17	= 8.77 g
	Σ = 199.99 g

As the glass was molten, the following reactions would take place:



which would reduce the final weight of the glass and should be taken into account.

The mol% of CO₂ in the batch would be:

mass of CO ₂ in CaCO ₃ : 26.92 × $\frac{12.01+16.00 \times 2}{40.08+12.01+16.00 \times 3}$	= 11.84 g
mass of CO ₂ in Na ₂ CO ₃ : 25.86 × $\frac{12.01+16.00 \times 2}{22.99 \times 2+12.01+16.00 \times 3}$	= 10.74 g
	Σ = 22.58 g

Therefore the actual weight of CO₂ in 200g batch of reagents would be:

$$200 \times 22.58 \div 84.17 = 54.65 \text{ g}$$

Hence, following melting and allowing for loss of CO₂ 200g batch of reagents would give roughly 146g of Bioglass®.

The mixture was molten inside a platinum crucible (5 wt% gold and 5 wt% rhodium), which was placed in a furnace to heat up to a specific temperature and holding time depending on the composition: 1370 °C and held for 1 hour for Bioglass®; 1420 °C and held for 1.5 hour for ICIE 16; 1400 °C and held for 1.5 hour for ICIE 16M. The melts were quenched in water and the coarse frit form of glass was collected and dried overnight at room temperature. The glass frit was ground and then sieved for 2 sets of 7 minutes with 5 minutes of interval in-between in order to reduce the particle sizes to <38 µm. The grinding was carried on the Glen Creston Ltd. Gy-Ro Mill grinding machine and sieving on the Endecotts EFL 2000/1 sieving machine.

The influence of different compositions (ICIE 16, ICIE 16M and Bioglass®) on sintering behaviour is studied using Differential Scanning Calorimetry (DSC). The samples were ramped at 10 °Cmin⁻¹ to a final temperature of 950 °C. The modal particle sizes (D₅₀ values) in this study were determined using particle size analysis and were around 8 µm for each glass. The influence of particle sizes and different heating rates on the glasses ICIE 16 and ICIE 16M are also studied by DSC. Three particle sizes were analyzed, the frit, >38 µm and <38 µm, the samples were each ramped at 20 °Cmin⁻¹ to 1000 °C. To evaluate the influence of the heating rates, the samples (<38 µm particles) were first heated to 550 °C at 20 °Cmin⁻¹, then to 1000 °C at various heating rates (2 °Cmin⁻¹, 5 °Cmin⁻¹, 7.5 °Cmin⁻¹, 10 °Cmin⁻¹, 20 °Cmin⁻¹ and 25 °Cmin⁻¹). The activation energies for T_{g onset}, T_{c onset} and T_p were determined from the different heating rate data by the Marotta method in section 3.3.4.

Differential Scanning Calorimetry (DSC)

The DSC is a method commonly used to determine the glass transition temperature T_g and the crystallization temperature T_c , hence the sintering window of the material, by measuring the variation of the heat flow between the sample and the reference material, the reference was alumina in this case. During phase transitions, the sample absorbs or releases more heat than the reference material, depending on whether the process is endothermic or exothermic, and a graph is plotted of the difference in the heat flow. To provide accurate results, the weight of the sample and the reference must be the same, for powders it was always 50 mg, for frit a single granule that weigh 50mg was tested. A Stanton Redcroft DSC 1500 with samples and reference in matched platinum crucibles was used.

Particle size analysis

The size of particles was determined by CILAS 1064 Particle Size Laser Analyzer, which gives the modal (most frequent value) and the mean (average value) size of the powder. Laser Particle Size Analysis measures the size of particles (powders, suspensions and emulsions) using the diffraction and diffusion of a laser beam of the light around the particle in its medium. Samples were dispersed in water (concentration = 90, measured by machine); 1 drop of Teepol together with 60s of ultrasound was applied to help further dispersion. Each size ($<38\ \mu\text{m}$ and $>38\ \mu\text{m}$) was repeated for 5 times and an average value was taken.

3.3 Results and discussion

3.3.1 Influence of composition

Figure 3.1 shows the DSC traces of the 45S5 Bioglass[®], ICIE 16 and ICIE 16M (particle size <38 μm). Compared to Bioglass[®], $T_{g \text{ onset}}$ increased from just over 500 $^{\circ}\text{C}$ to 575 $^{\circ}\text{C}$ for ICIE 16 and 550 $^{\circ}\text{C}$ for ICIE 16M; the onset temperature for crystallization ($T_{c \text{ onset}}$) increased from 575 $^{\circ}\text{C}$ for Bioglass[®] to around 750 $^{\circ}\text{C}$ whereas the T_p (peak crystallization temperature) increased from 600 $^{\circ}\text{C}$ to over 775 $^{\circ}\text{C}$ for both ICIE 16 and ICIE 16M. The sintering window is defined as $T_{c \text{ onset}} - T_{g \text{ onset}}$. For ICIE 16 it was 175 $^{\circ}\text{C}$ and 200 $^{\circ}\text{C}$ for ICIE 16M, compared to less than 75 $^{\circ}\text{C}$ for Bioglass[®]. The DSC data indicates that the ICIE 16 and ICIE 16M glasses could be sintered without crystallization at temperatures below 750 $^{\circ}\text{C}$.

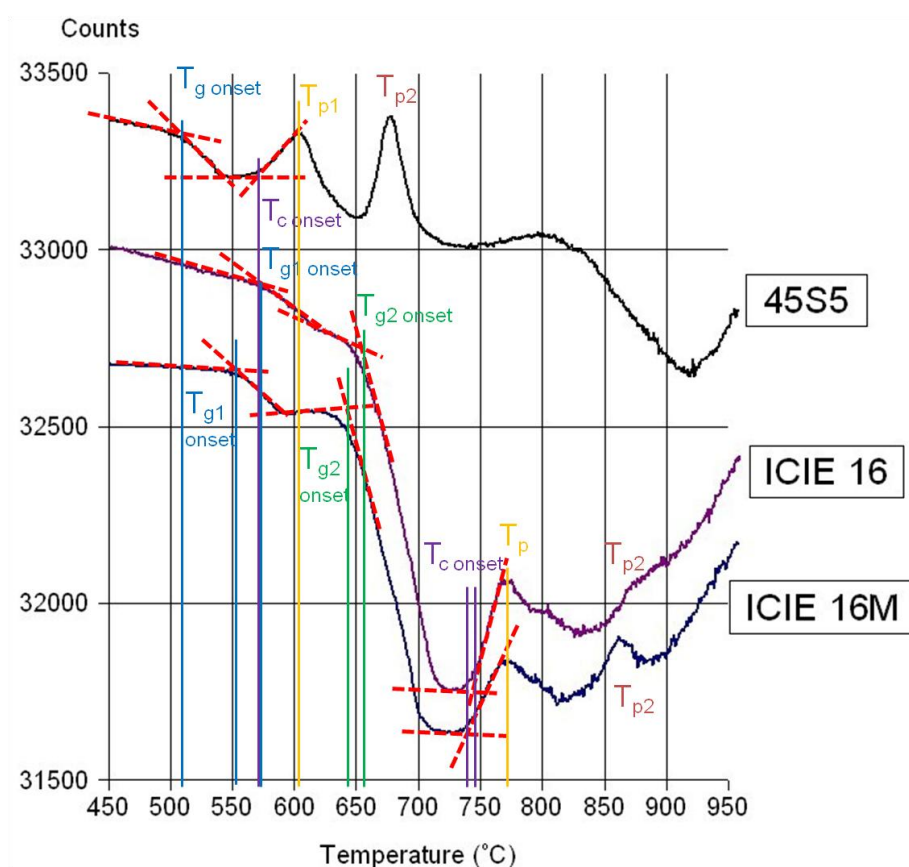


Figure 3.1 DSC traces of the 45S5 Bioglass[®], ICIE 16 and ICIE 16M¹

¹ Data provided by Donovan Nightingale

There was something interesting going on in Figure 3.1, Bioglass[®] only showed one T_g whereas ICIEI 16 and ICIE 16M showed two, but all three were showing two T_c s. In section 2.4 the role of each component in BG was discussed and P was considered to be present in the glass structure as orthophosphate form, which cannot be linked to the silica network (see Figure 2.4 for illustration). This means that all three glasses in Figure 3.1 were two phase systems and it was expected to see two T_g s and two T_c s, however Bioglass[®] would have crystallized shortly after the first T_g so the second T_g was overwhelmed by the magnitude the first T_c , which was the reason that Bioglass[®] only showed one T_g but two T_c s. There was only 1.07 ml% P in the compositions of ICIE 16 and ICIE 16M which means the orthophosphate glass phase would be reasonably small, thus the second T_g would be rather weak and difficult to detect, however it was not the case in Figure 3.1. Because T_g represents the change in heat capacity, as the glass undergoes sintering the fine particles will fuse together and form a monolith, which will increase the heat capacity by a great amount, therefore the decline in the base line slope before $T_{c \text{ onset}}$ was indicating that sintering was taking place rather than a second T_g .

3.3.2 Influence of particle sizes²

When powders undergo sintering, crystallization if any, will occur in forms of surface crystallization, where the particle size is the critical factor, different particles crystallize to different degrees. The DSC traces of ICIE 16 (Figure 3.2a) and ICIE 16M (Figure 3.2b) in different particle sizes shows exactly how the particle sizes effected the sintering parameters such as $T_{g \text{ onset}}$ and $T_{c \text{ onset}}$, and the data is summarized in Table 3.3. Overall, the $T_{c \text{ onset}}$ increased as the particle size

² Data provide by Hannah Choi

increased for both glasses, which was expected; because as the particle size becomes smaller, surface energy grows higher and the materials is more prone to surface crystallization, which means crystallization will occur at a lower temperature. The $T_{g\text{ onset}}$ on the other hand did not show any significant change, the reason being that the size of the particle would not affect the heat capacity. There was a notable decline around 520 °C (500 °C for ICIE 16M) on the <38 µm trace, the temperature was too small for T_g therefore it was suspected to be an artifact. The magnitude of T_c and T_g generally increased with larger particle size as a result of reduced sintering. The sintering widow of ICIE 16M was generally greater than ICIE 16 at all sizes, indicating better processing abilities.

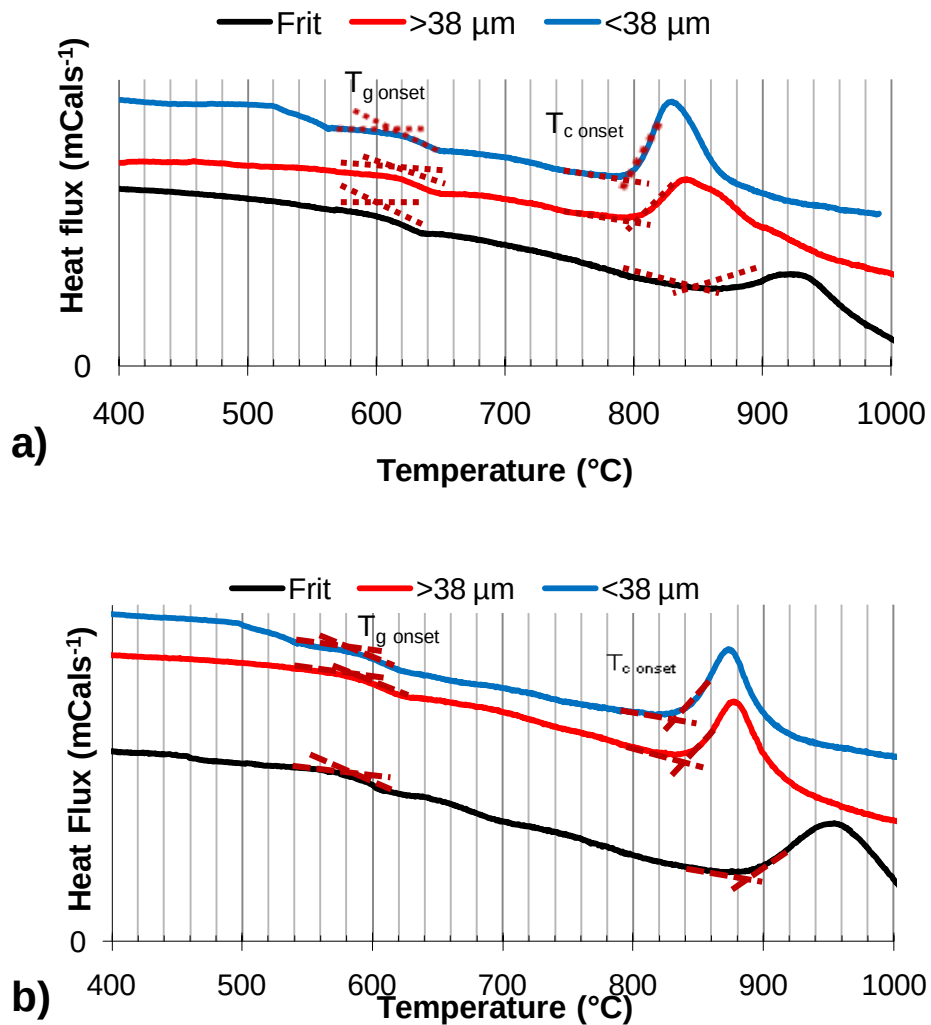
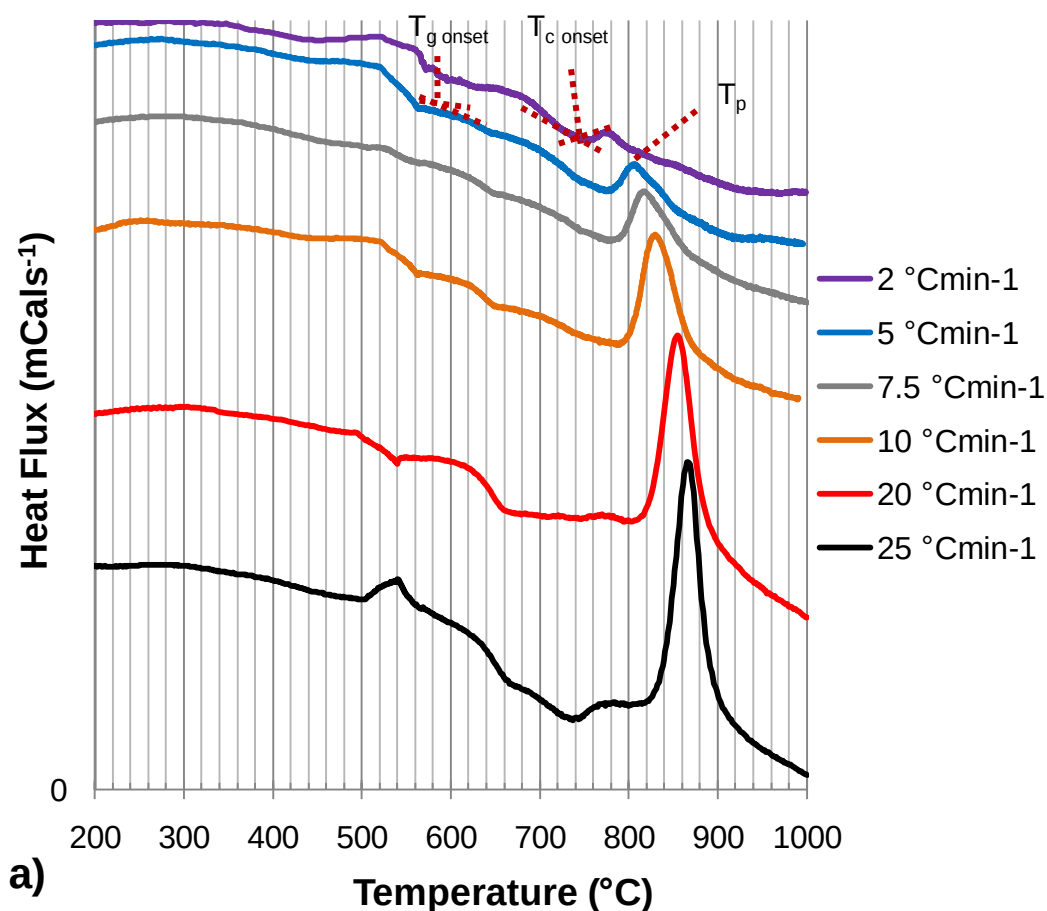


Figure 3.2 DSC traces of a) ICIE 16 and b) ICIE 16M in different particle sizes

3.3.3 Influence of heating rates²

$T_{g\text{ onset}}$, $T_{c\text{ onset}}$ and T_p of different heating rates of ICIE 16 and ICIE 16M were determined according to the way shown in Figure 3.3a and Figure 3.3b but on a full sized diagram. Note that there was a change in the baseline slope around 550 °C, this was caused by the changing of heating rate and should not be counted towards the result. In general, $T_{g\text{ onset}}$, $T_{c\text{ onset}}$ and T_p all decreased with decreasing heating rate, at higher heating rates (25 °Cmin⁻¹ and 20 °Cmin⁻¹) there were two T_g s and the magnitude of T_c was greater than slower heating rates, this was because higher heating rates allowed less time for sintering to take place. As a result it was impossible to measure $T_{g2\text{ onset}}$ from this data.



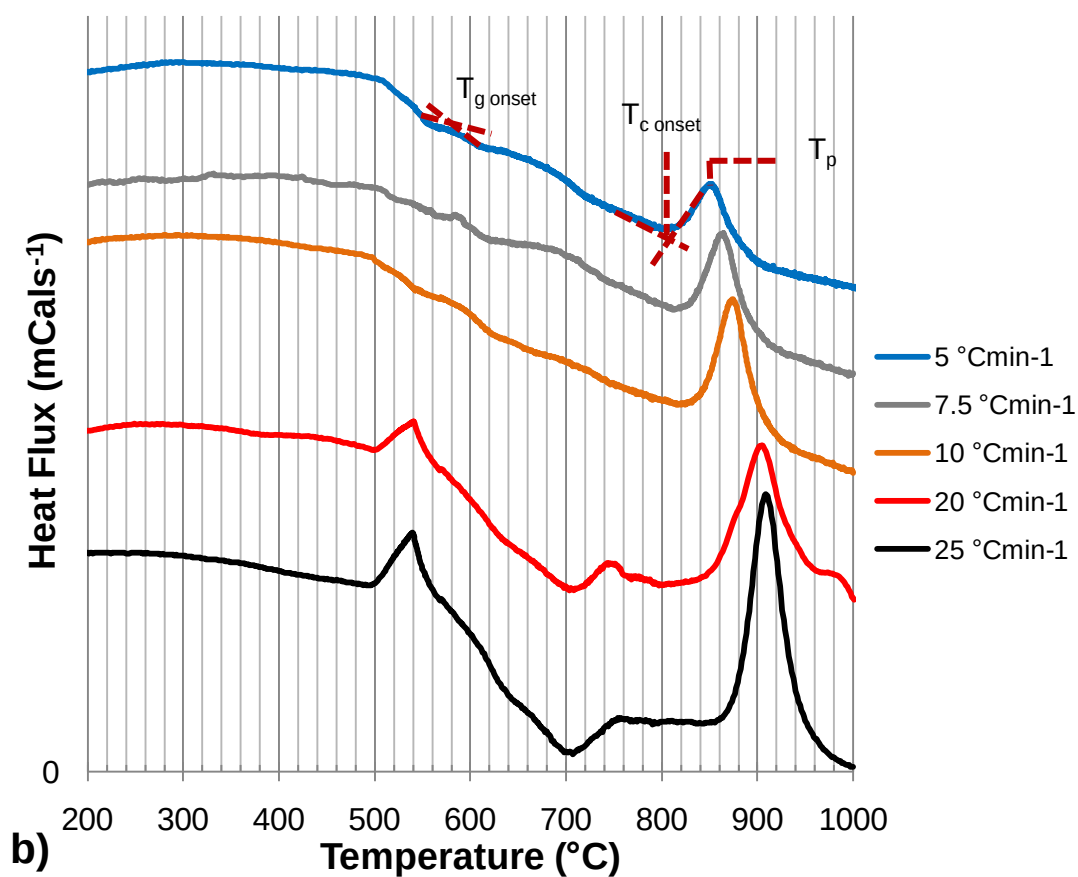


Figure 3.3 DSC traces of different sintering rates on a) ICIE 16 and b) ICIE 16M (<38 μm)

Table 3.3 $T_{\text{g onset}}$, $T_{\text{c onset}}$ and T_{p} values of ICIE 16 and 16M against different heating rates

Heating rate ($^{\circ}\text{Cmin}^{-1}$)	ICIE 16			ICIE 16M		
	$T_{\text{g onset}}$ (K)	$T_{\text{c onset}}$ (K)	T_{p} (K)	$T_{\text{g onset}}$ (K)	$T_{\text{c onset}}$ (K)	T_{p} (K)
2	850	1016	1033	---	---	---
5	861	1039	1068	839	1078	1116
7.5	863	1048	1083	849	1084	1129
10	863	1059	1096	853	1094	1140
20	870	1082	1123	860	1105	1171
25	873	1086	1137	868	1128	1178

3.3.4 Determining the activation energies (Ea) and sintering window

Crystallization is a combination of nucleation and crystal growth processes; it is temperature dependant and its Ea can be determined by heating the sample at different rates. The Marotta method [117], which is based on the relationship between heating rate and the crystallization temperature, it takes into account surface crystallization only and is affected by both nucleation and growth processes. The equation employed is given as below:

$$\ln \frac{1}{T} = - \frac{E_a}{RT_p} \quad (3.1)$$

where T is the heating rate, Ea is the activation energy, R is the gas constant (8.314 JK⁻¹mol⁻¹) and Tp is the peak crystallization temperature. A graph was plotted with ln heating rate against 1/T (data from Table 3.3), from which Ea could be determined by multiplying the negative value of gas constant with the gradient of the trend line from Figure 3.4a for ICIE 16 and Figure 3.4b for ICIE 16M, and the results were summarized in Table 3.4. In order to define the sintering window, the extrapolated temperature of 1/T_{g onset} and 1/T_{c onset} at 0 Kmin⁻¹ ln heat rate was the minimum and the maximum sintering temperature respectively, the values were recorded in Table 3.5 and the difference between them would be the sintering window.

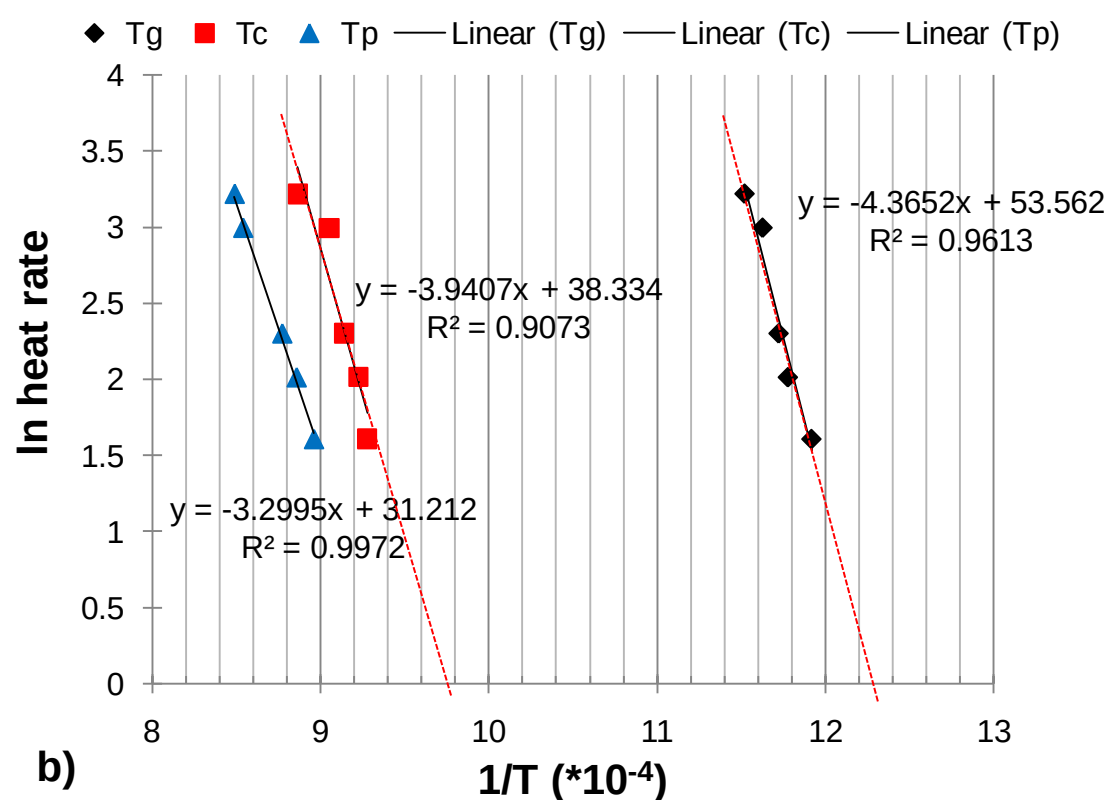
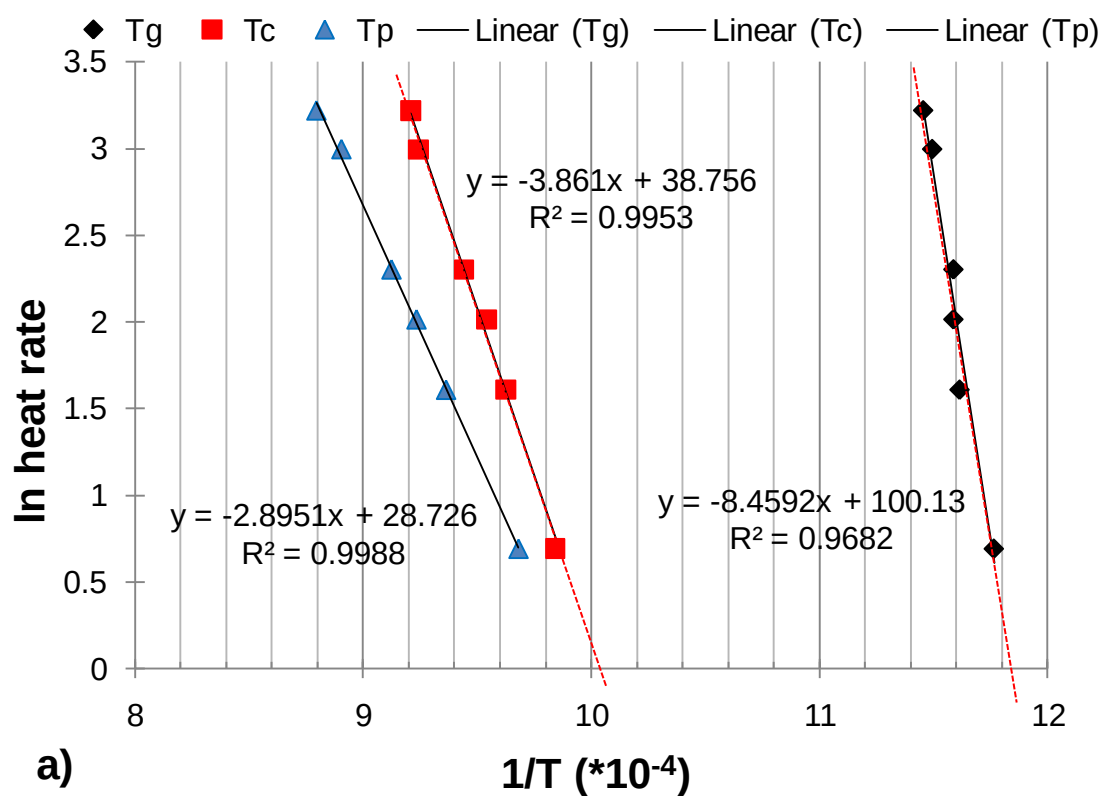


Figure 3.4 Plots of $\ln$ heat rate against $1/T_{g \text{ onset}}$, $1/T_{c \text{ onset}}$ and $1/T_p$ of a) ICIE 16 and b) ICIE 16M, and the extrapolation of $1/T_g$ onset and $1/T_c$ onset at $0Kmin^{-1}$ $\ln$ heating rate

Table 3.4 Summary of E_a for $T_{g \text{ onset}}$, $T_{c \text{ onset}}$ and T_p derived from Figure 3.4 using the Marotta method

	E_a (kJmol ⁻¹) for		
	$T_{g \text{ onset}}$	$T_{c \text{ onset}}$	T_p
ICIE 16	703	321	241
ICIE 16M	363	328	274

The E_a of ICIE 16M for T_p and $T_{c \text{ onset}}$ is greater than that of ICIE 16, which again confirms ICIE 16M is better than ICIE 16 in terms of suppressing crystallization. The lower E_a $T_{g \text{ onset}}$ value of ICIE 16M means its viscous flow and sintering occurs more readily compared to ICIE 16.

Table 3.5 Summary of $T_{g \text{ onset}}$, $T_{c \text{ onset}}$ and T_p of ICIE 16 and ICIE 16M derived from the extrapolated data

	$T_{g \text{ onset}}$ (°C)	$T_{c \text{ onset}}$ (°C)	T_p (°C)
ICIE 16	572	723	735
ICIE 16M	542	756	785

As expected, ICIE 16M had a larger working range (209 °C) than ICIE 16 (151 °C), which means the ICIE 16M is better at suppressing crystallization than ICIE 16.

3.4 Summary

The network connectivity value was the same for ICIE 16 and Bioglass[®] (both at 2.1). It was also the same for ICIE 16M if assuming the Mg^{2+} and Zn^{2+} ions were behaving as network modifiers. If they were to behave as network formers, the NC value of ICIE 16M would be 2.7, leading to a slower degradation and HCA formation rate. On DSC graphs there were two subtle T_g s on the traces of ICIE 16 and ICIE 16M but not Bioglass[®], this was because Bioglass[®] had crystallized shortly after the first T_g when the other two glasses had undergone sintering; meanwhile all three glasses showed two T_c s, which was an indication of two-phased system (an amorphous silica phase and an orthophosphate phase). Increase in particle size and heating rate would suppress sintering and enhance the DSC trace. The sintering windows of ICIE 16 and ICIE 16M were 175 °C and 200 °C respectively, which were both much wider than Bioglass[®] (75 °C). For both ICIE glasses, particle size did not affect $T_{c\ onset}$ but the $T_{c\ onset}$, which increased as particle size grew larger. The $T_{c\ onset} - T_{g\ onset}$ value was greater for ICIE 16M (209 °C) than ICIE 16 (151 °C), which represented a wider sintering window. The activation energy of $T_{c\ onset}$ and T_p was higher for ICIE 16M (328 and 274 kJmol⁻¹) than ICIE 16 (321 and 241 kJmol⁻¹), which means 16M was better at suppressing crystallization; the E_a of $T_{g\ onset}$ however was lower for ICIE 16M (363 kJmol⁻¹) than of ICIE 16 (703 kJmol⁻¹), indicating 16M was better at viscous flow and would sinter more readily than ICIE 16. ICIE 16 could be sintered without crystallization at any temperatures below 723 °C, which for ICIE 16M it was 756 °C. All results indicated that ICIE 16M was better at sintering than ICIE 16.

4 Identifying and optimizing of the standard protocol of the gel-cast foaming process

4.1 Introduction

Gel-cast foaming process was introduced by Sepulveda *et al.* to produce porous HA foams but was never applied to melt-derived bioactive glasses. The aim of this chapter is therefore to develop a protocol in producing such scaffolds. Pilot studies were carried out by Cichy *et al.* [118] on ICIE 16M (<53 μm) glasses where two gelling systems were analyzed: the methyl methacrylate (MMA) as monomer with benzoyl peroxide as initiator, N,N-dimethyl-p-toluidine as catalyst and ethylene glycol dimethacrylate as cross-linker; the other with methacrylamide as monomer, ammonium persulfate (APS) as initiator, N,N,N',N'-tetramethylethylene diamine (TEMED) as catalyst and N,N'-methylenebisacrylamide as cross-linker. Moreover, the influence of various types of surfactants (Tergitol TMN10, Teepol and Triton X100) on the gelling system was investigated. According to the result, the MMA gelling system failed to foam or gel and was therefore discarded. Out of the three surfactants, Triton X100 achieved the best foaming ability and was therefore chosen to be incorporated into the methacrylamide gelling system instead of the Tergitol TMN10, which was originally used by Sepulveda *et al.*. The scaffolds obtained was 72% porous with small (<100 μm) pores and did not sinter well.

Necessary changes were made to the protocol that Cichy *et al.* used, it was found that in order to create an interconnected pore network suitable for bone regeneration, maintaining a reasonable volume of foam during agitation (e.g. 130 ml from an original suspension of 40 ml) until polymerization occurs (e.g. 90 seconds) was the key. This is because too high a foam volume would result in very large pores and low mechanical strength whereas too little would give insufficient pore and interconnect size. Therefore to investigate the effect of process variables on the foam structure, the effect of the process variables on foam body volume was investigated initially, before relating it to pore size. The gelation time is critical because a rapid gelling time was not sufficient for the foam to develop but with too much time in agitation the foam collapsed and lost its stability. How to determine the gelling time and the foam body volume is explained in section 4.2. As shown in Table 4.1, there are 8 variables to consider. From pilot tests, it was concluded that the type of the glass did not affect the gelling time and the foam body. The monomer to cross-linker ratio was set to be 2:1. Water, catalyst and initiator content, on the other hand, played a significant role.

The aim of this study was to devise a processing protocol for the production of scaffolds suitable for bone regeneration and to optimize the structure for maximum mechanical strength while maintaining an open porous network.

Table 4.1 Gel-cast foaming process variables

Order	Components	Role
1	Glass powder	Material
2	Ultra-purified water	Medium
3	Methacrylamide	Monomer
4	N,N'-methylene bisacrylamide	Crosslinker
5	APS solution	Initiator
6	Dispex	Dispersant
7	Triton X100	Surfactant
8	TEMED	Catalyst

4.2 Experimental

As shown in Figure 4.1, the first step of the process was to mix the glass powder with deionised (18Ω) water, the monomer (acrylamide) and the cross linker (N, N'-methylene bisacrylamide) to produce a slurry. The dispersant (Dispex) was added to help further dispersion of glass powder in the solution. The mixture was then vigorously agitated (Kenwood hand mixer) to produce a foam with the aid of the surfactant (Triton X100). Polymerization was activated by the addition of the initiator (ammonium persulfate solution with a concentration of 0.52 gml^{-1}) and the catalyst (tetramethylethylene diamine), which reacted with each other releasing a free radical particle. This free radical then reacted with both the acrylamide and the N, N'-methylene bisacrylamide, forming a polymer network. Gelling time was recorded from the moment the catalyst was introduced to the suspension until the moment it had turned into a gel, which was when poured the suspension would not flow, typically in about one and half minute. After 50 seconds the whisks were

taken out, the agitation was paused and the plastic beaker was set down to take measure of the foam body at the side of the beaker, however once the readings were taken agitation must be resumed as soon as possible to avoid foam collapsing. Immediately (~2 or 3 seconds) prior to the gelation, the foam was cast into moulds, in this case 60ml screw-cap polypropylene containers, caps must be screwed on to avoid cracking on the surface of the gel that was induced by rapid cooling. The moulds were set in room temperature for around half an hour allowing the pore structure to be stabilized by the completion of gelation; they were then dried and sintered to burn out the organic green body, leaving the porous glass network.

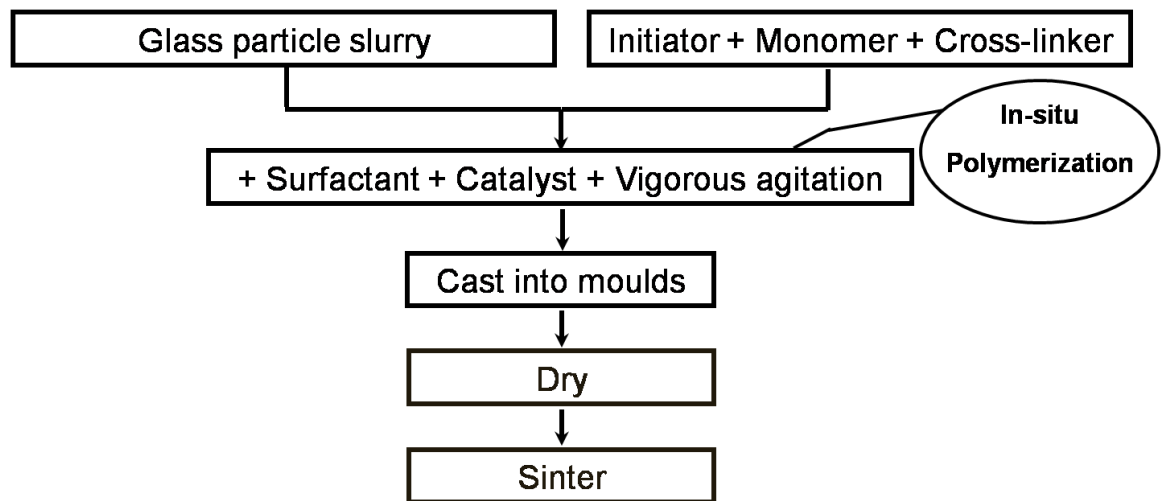


Figure 4.1 Flow-chart of the gel-cast foaming process

After many failed attempts, a base protocol was devised from the one that worked the best for Cichy *et al.* [118], which did not work when it was repeated; the detail is shown in Table 4.2. It can be noted that certain ratio of components in the slurry was changed for the protocol to become operable.

Table 4.2 The Cichy E19 protocol and the base protocol derived from it

	E19	Base Protocol
Glass powder (g)	7	14
Water (ml)	5	20
Methacrylamide (g)	1.5	6
N,N'-methylene bisacrylamide (g)	0.5	3
APS solution (ml)	1	4
Dispex	2 drops	2 drops
Triton X100 (ml)	0.05	0.1
TEMED (,ml)	2	6

Apart from the monomer (6 g), the crosslinker (3 g), the dispersant (2 drops) and the surfactant (0.1 ml), which were all kept constant throughout, the influence of all other components on both the gelling time and the foam body was investigated systematically. These components were verified unsystematically and the values chosen were the minimum amount of each component needed to be present in the system to function. Using the base protocol the effect of varying water content (10 ml, 12 ml, 14 ml, 16ml, 18 ml, 20 ml, 22 ml, 26 ml, 28 ml and 30 ml) and catalyst content (3 ml, 4 ml, 5 ml and 6 ml) were investigated. The glass powder loading in the slurry (10 g, 14 g, 20 g and 30 g) in 20 ml water with 3 ml catalyst was then investigated. APS is highly hygroscopic (absorb or adsorb water from the environment), it dissolves in water very easily, but by doing so it loses its reactivity drastically. Therefore the effect of initiator freshness was also evaluated.

As a result of this study, the base protocol was modified to: 20 g glass, 18 ml water, 6 g monomer, 3 g crosslinker, 4 ml catalyst, 4 ml initiator, 2 drops of dispersant and 0.1 ml surfactant. The effect of initiator content (1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml and 7 ml) on gelling time and foam body volume was then verified. Each data point was repeated at least 3 times to test the reproducibility of the experiments.

4.3 Results and discussion

4.3.1 Effect of Water

The amount of water in the system had a large impact on the gelling time and foam body. Figure 4.2 shows that as the water content increased from 10 to 30 ml, the average gelling time also increased from 50 to 114 seconds (127% increase), and the foam body increased from 50 to 192 ml (283% increase) respectively. Too little water significantly shortens the gelling time and it does not give a sufficient foam body volume, on the other hand too much water would weaken the structure after gelation, which would collapse after drying. This means that somewhere in the middle (18 to 20 ml) was the sensible choice. Error bars were largest at 18 ml, which was because it has been used for most of the times, and the freshness of APS solution was not taken into account until later.

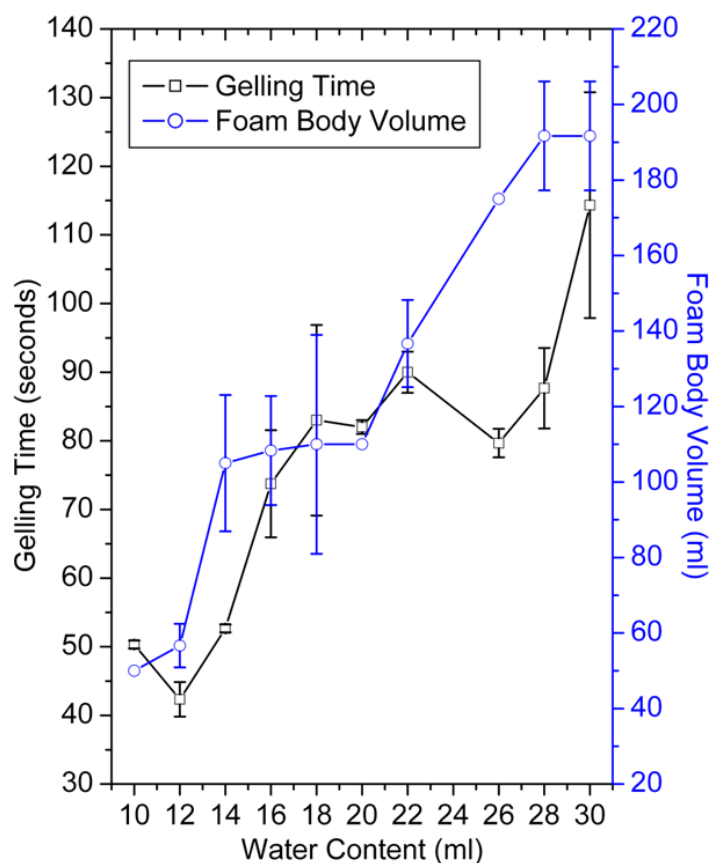


Figure 4.2 Influence of water content on the gelling time and the foam body

4.3.2 Effect of Catalyst

The effect of catalyst concentration was different to water (Figure 4.3). As catalyst content increased from 3 to 4 ml, there was a sharp decline in the gelling time from 126 to 96 s. The gelation time was approximately constant (83 s) as the catalyst content increased from 5 to 6 ml. The foam volume reduced gradually from 170 to 167 ml as the catalyst content increased from 3 to 4 ml and decreased sharply (from 158 to 110 ml) as the catalyst volume increased from 5 to 6 ml, even though the gelling time was approximately the same at these two data points. The ideal foam body was around 130 ml and an ideal gelling time for producing a stable foam body was around 90 s, so 18-22 ml of water was suitable for the process. 18 ml of water and 4 ml of catalyst were chosen to be the standard quantities.

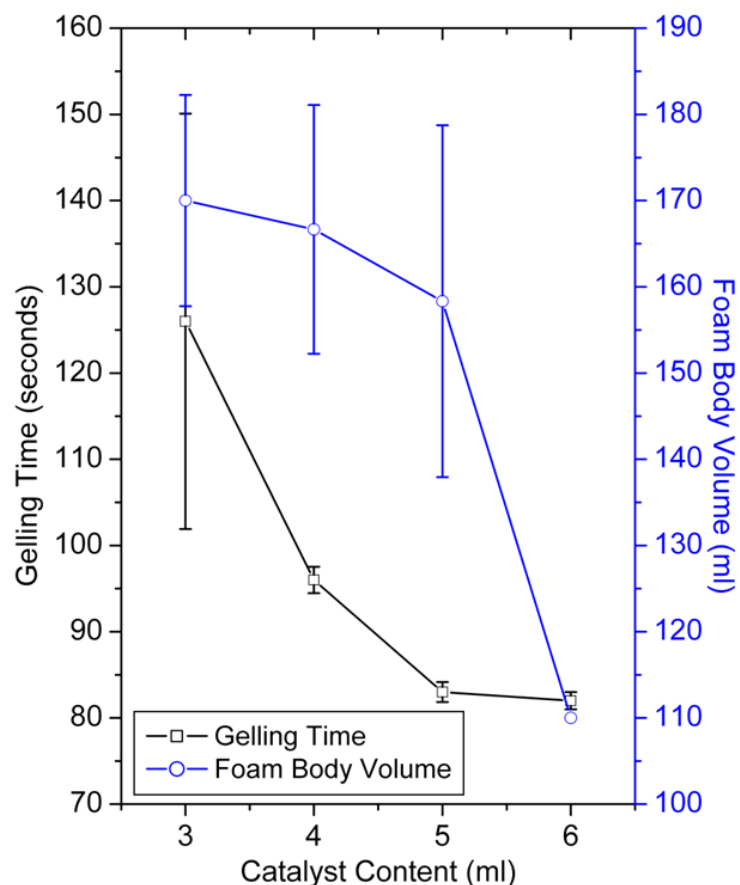


Figure 4.3 Influence of catalyst content on the gelling time and foam body

4.3.3 Effect of Initiator

As the initiator content increased from 1 to 7 ml, the gelling time decreased (182 to 51 s) and the foam body volume increased from 130 to 170 ml (Figure 4.4). The relationship was not linear over the range of concentrations used: as initiator content increased from 1 to 3 ml there was a sharp decline in gelling time from 182 to 84 s whilst the foam volume changed little, reducing from 130 to 124 ml. Then, as initiator content increased from 3 to 7 ml the gelation time decreased more slowly from 84 to 51 s and the relationship was approximately linear. The foam volume increased approximately linearly from 124 to 170 ml.

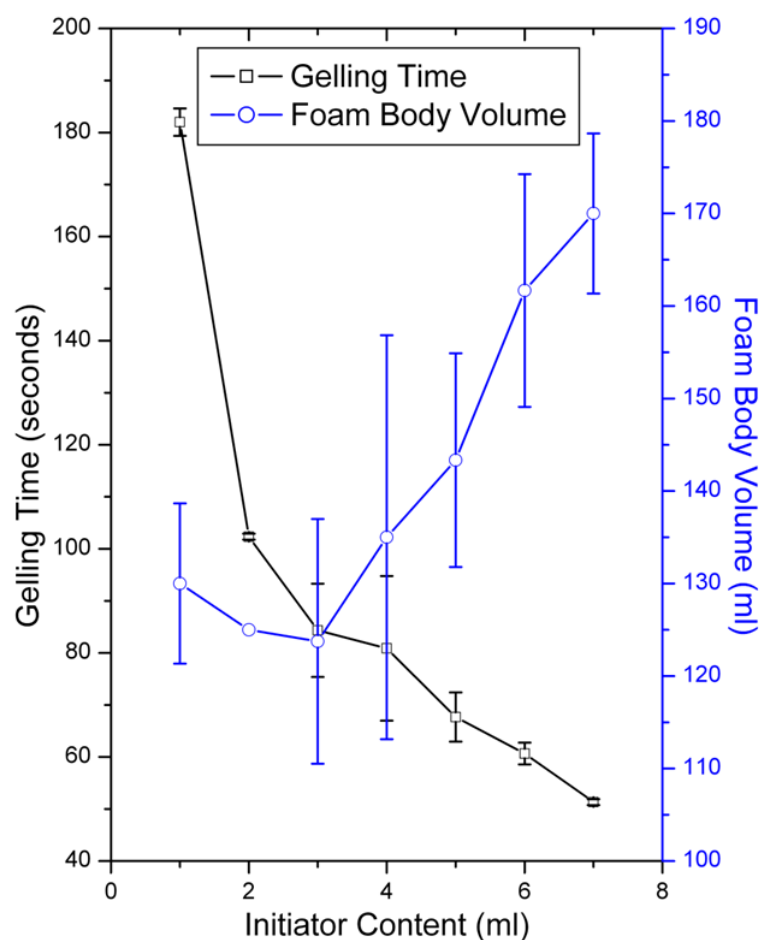


Figure 4.4 Influence of initiator content on the gelling time and foam body

In addition to the above components, the vitality of the initiator (APS) solution influenced the gelling time and the foam body volume of the suspension significantly. After mixing the APS powder with distilled water, it had to be left for at least 3 h before it could be used, otherwise the suspension would not foam at all, even though the gelling time was not affected. The solution became less reactive as time passed; therefore it was remade after 1 week. If the solution was left more than 1 week the gelling time was significantly prolonged.

A side effect of using APS as the initiator was that crystallites were present in the glass after sintering, these were not due to crystallization of the glass, but were identified by EDX (Figure 4.5) and XRD to be potassium sodium sulfate ($K_3Na(SO_4)_2$) (01-074-0398), Figure 4.6 shows a matched pattern of the XRD trace. Due to the glass being in contact with water during the process, sodium and potassium ions were dissolved from the glass in an ion exchange mechanism. The ions reacted with the sulfate, which was a by-product of the initiator (APS), causing nucleation of crystals on the surface of the glass particles. As the scaffold was sintered, the organic part was burnt out, leaving glass particles stacked in a porous glass structure, which then fused together as the temperature increased further. The crystals were therefore incorporated into the glass. Although the gelling time was short (90 s), the glass remained in contact with water during drying. Small amounts of $K_3Na(SO_4)_2$ may not affect biological properties and its dissolution behaviour was studied in Chapter 6.3.3.2, but methods to eliminate its formation were devised regardless.

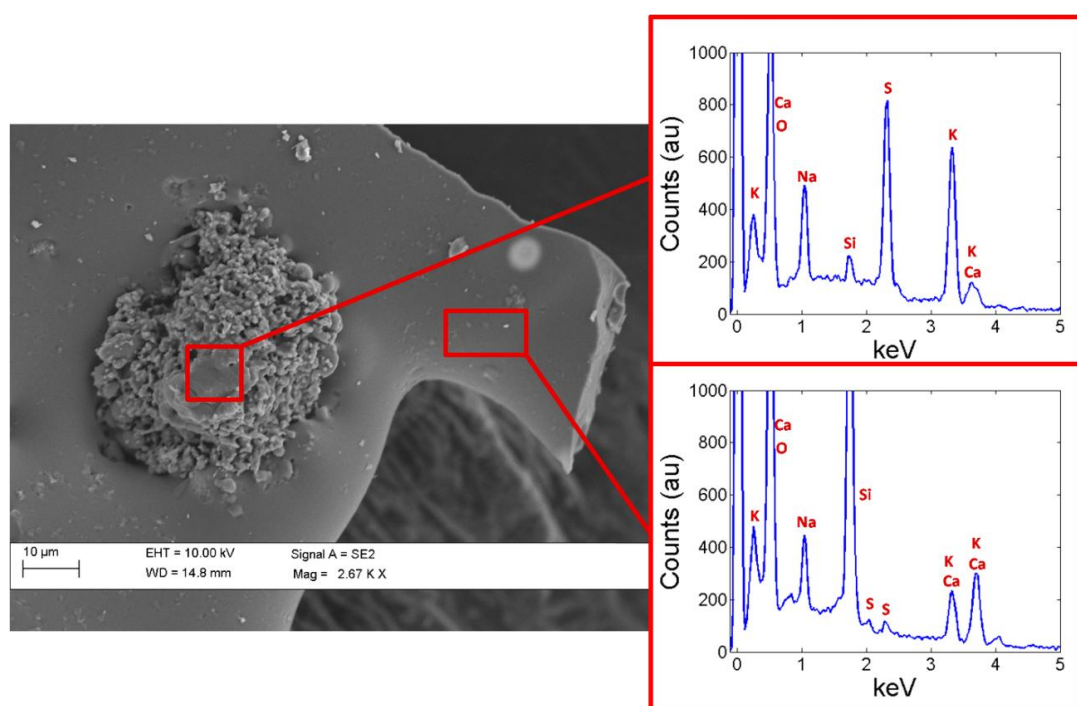


Figure 4.5 EDX analysis of a crystalline and the glass strut

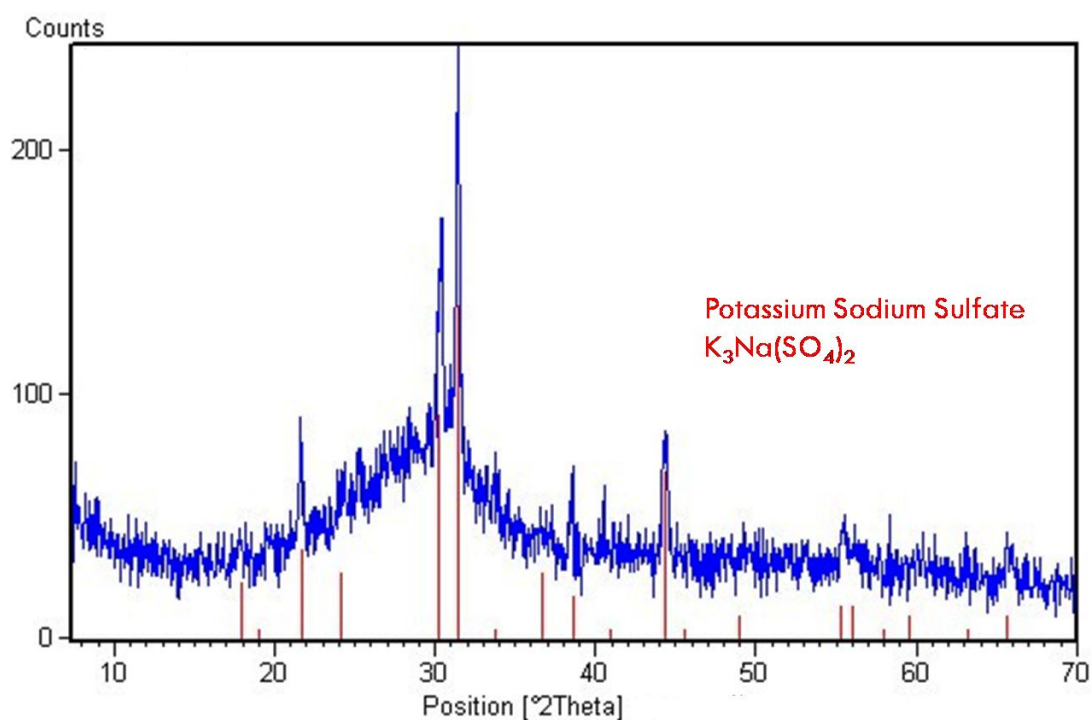
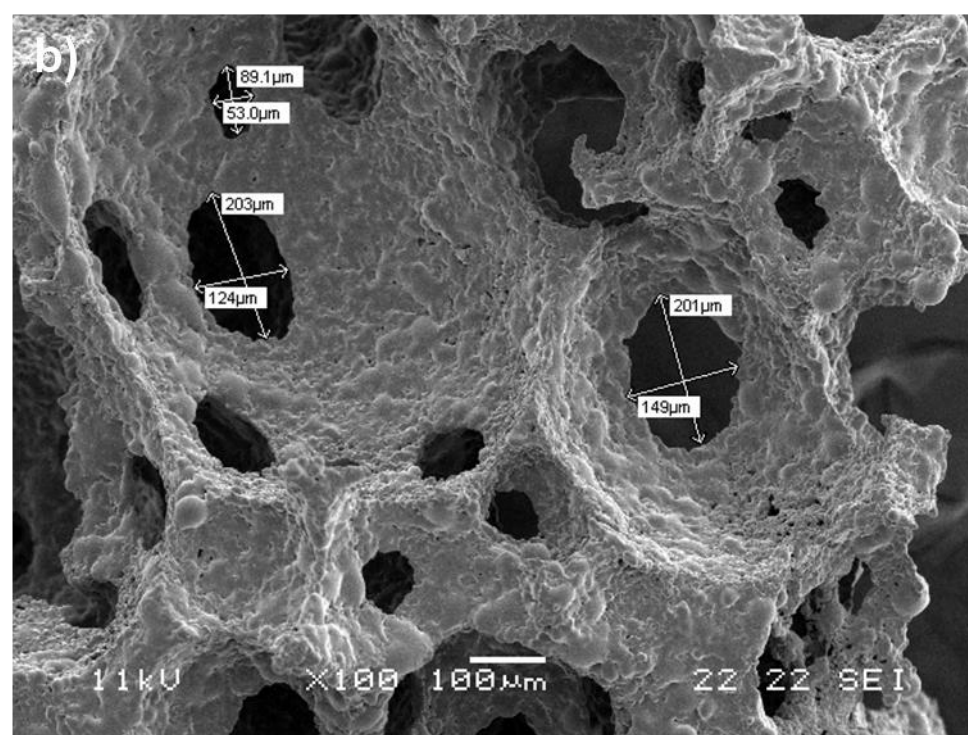
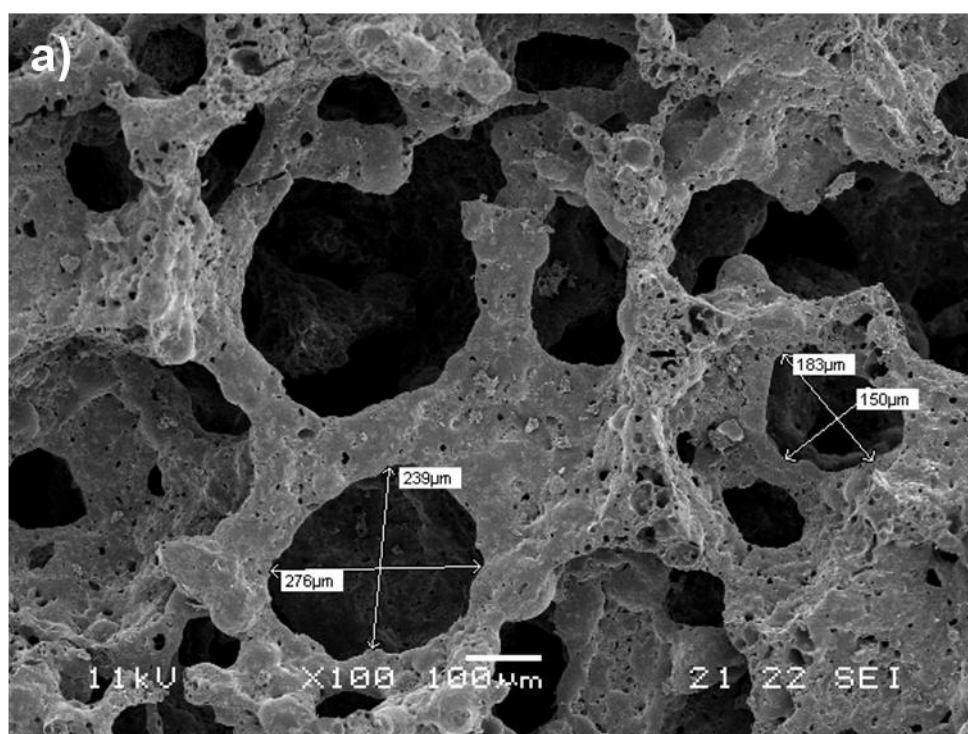


Figure 4.6 Matched pattern on the XRD trace of the scaffold after sintering

The options were to reduce the time that the glass was in contact with water (Chapter 5) or to reduce the amount of initiator used in the process. An optimised process may involve taking both of these measures. Figure 4.7a to 4.7d show that as the initiator volume increased from 1 to 4 ml, the crystallization of the $K_3Na(SO_4)_2$ was suppressed as there was less sulfate available. No crystallites were observed on scaffolds made with 1 ml APS, but with this little amount it was barely capable of gelling and the structure was rather broken as showing in Figure 4.7a. Therefore 2 ml was the optimum amount of initiator to be applied in the gel-casting process.



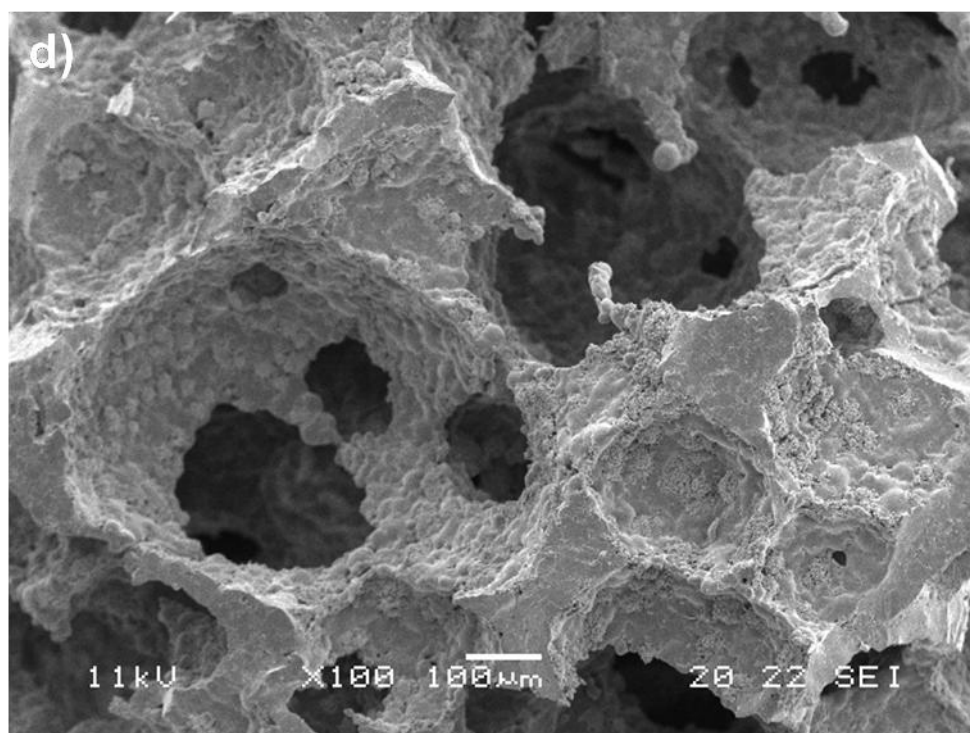
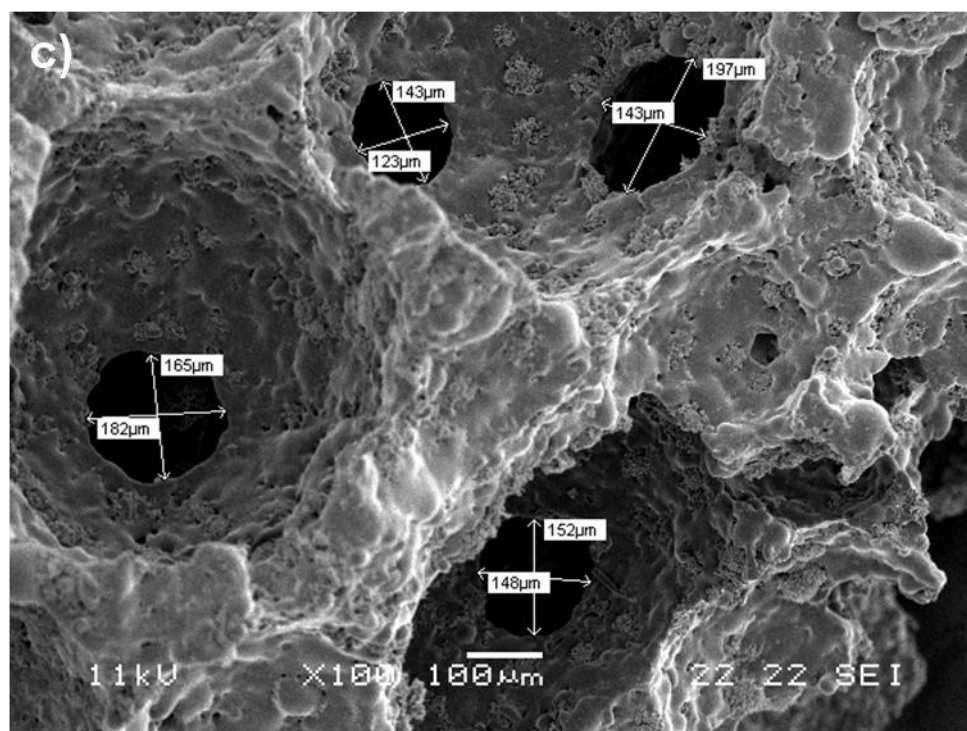


Figure 4.7 SEM images of using a) 1 ml, b) 2 ml, c) 3 ml and d) 4 ml of initiator in the gel-cast foaming process

4.4 Summary

An optimal protocol for the gel-cast foaming of ICIE 16 was therefore set up. The standard formula of the process was: 20 g glass powder, 18 ml water, 6 g methacrylamide (monomer), 3 g N,N'-methylene bisacrylamide (cross-linker), 2 ml ammonium persulfate solution (initiator), 0.1 ml Triton X (surfactant), 4 ml tetramethylethylenediamine (TEMED) (catalyst). The vitality of the APS solution is very important, it must be left to stand for at least 3 hours prior to application to prevent non-foamed suspension, and it must be freshly prepared every time as its reactivity is inversely proportional to the time. In addition, potassium sodium sulfate crystals were found on the surface of the foam after sintering, which was caused by the complex reaction between water, glass and sulphur in APS. Such crystallization could be controlled by reducing initiator content in the slurry.

5 Optimizing the drying and sintering program

5.1 Introduction

After gelation, the foam is a composite of bioactive glass particles in a polymer matrix (Figure 5.1). They must be dried and a thermal process had to be devised to remove the polymer without causing collapse of the structure and to allow the particles to sinter. Once the polymer is removed, the glass particles are effectively balancing on each other, a very careful thermal processing regime is therefore required to induce sintering without causing excess flow of the glass and collapse of the structure or crystallization. The thermal process also had to be optimized to inhibit possible potassium sodium sulfate formation.

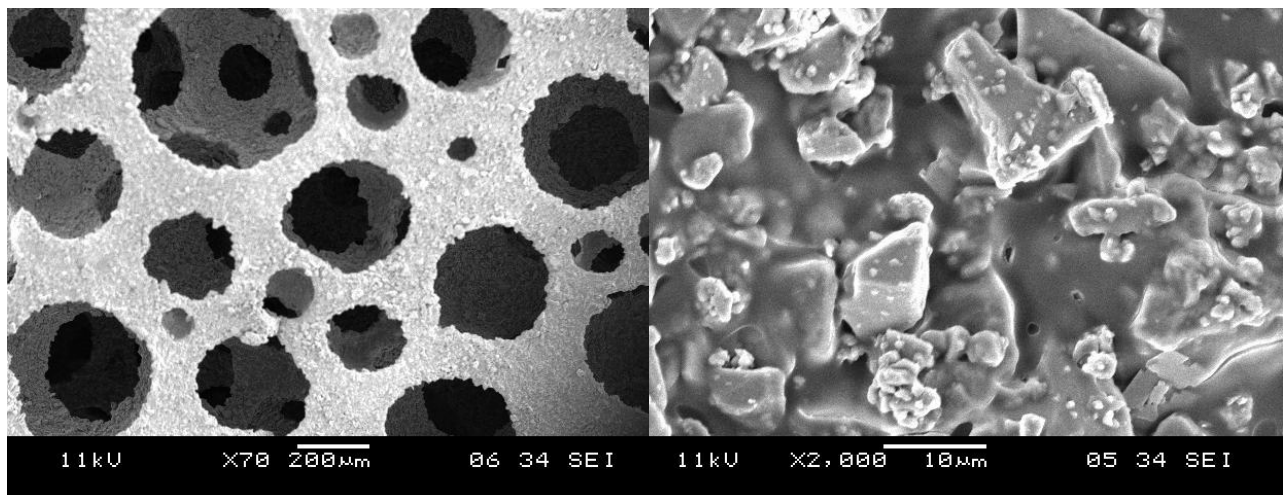


Figure 5.1 SEM image a) the whole structure and b) the surface of the dried foam (unsintered)

Provided with a wide sintering window as discussed in Chapter 3, it was difficult to predict at which conditions the scaffold best sinters. Sintering is affected by many factors, such as the particle size, the packing between particles, the ramp rate, the holding time, the sintering environment and temperature. It is thought the smaller the particle size and the higher the specific surface area, the closer the particles are packed together, the better the sintering. The ramp rate is important because high ramp rate results in rapid burn out of the polymer and collapse of the whole structure. The holding time and temperature are critical factors, if the holding time is too long or too high the temperature, the material flows too much and destroys the structure of the scaffold; but if too short or too low, the material underflows, i.e. the particles will not fuse together, which will also destroy the structure. Hence it is very important to find the suitable condition to sinter the scaffold, varying only one factor at a time.

The drying and the sintering temperatures were verified as it played an important role in the crystallization of the glass and in potassium sodium sulfate crystallization, which will be discussed in Chapter 5.3. The aim of this chapter was to find out the optimum drying and sintering temperature for gel-cast ICIE 16 and ICIE 16M glasses by employing DSC, SEM and XRD techniques.

5.2 Experimental

5.2.1 Determining the suitable sintering programme

As shown in Figure 5.2, a two stage sintering program was followed where the samples were first fired and held at a lower temperature in order to burn off the polymer in the network; which was then fired and held at a higher temperature close to T_c in order to permit the fusion of glass particles, and the sintering process was complete after the samples were furnace cooled to room temperature.

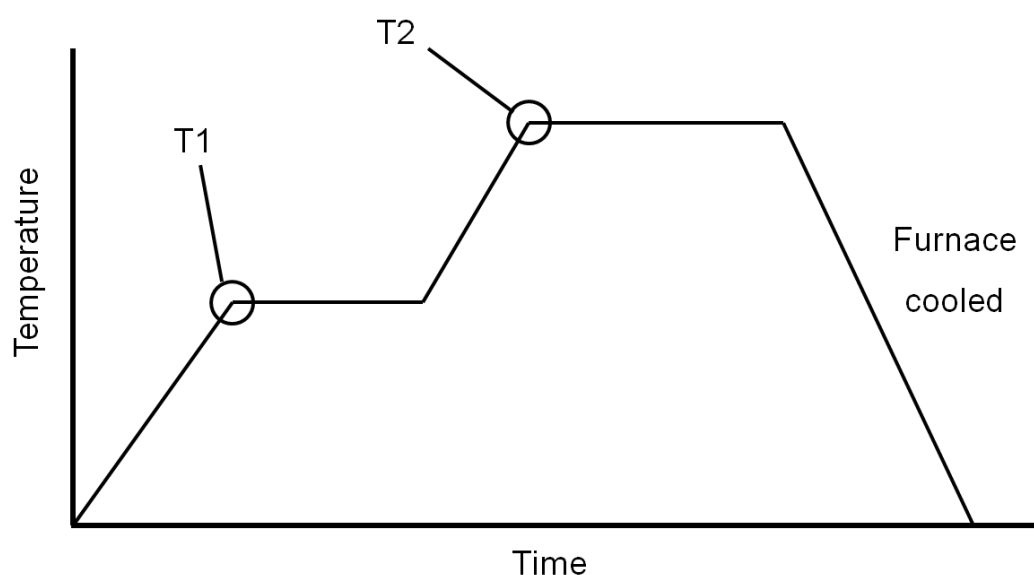


Figure 5.2 Schematic sintering program

Differential Scanning Calorimetry (DSC)

To investigate how the gel-cast structure behaved during sintering, a DSC-TGA test was carried out on a gelled foam (dried at 100 °C and unsintered) at 2 °Cmin⁻¹ and the result was shown as Figure 5.3 that showed not only the variation of heat flux but the weight loss as the temperature increased. From the figure the 1st holding temperature was chosen which is at where the first exotherm was at peak (approximately 350 °C) and thus a sintering program was derived.

Fine ICIE 16 glass powder ($<38\ \mu\text{m}$) and ground gel-cast foam (dried at $125\ ^\circ\text{C}$ and unsintered) made with ICIE 16 glass were sintered using this program on the DSC, with an extension at the end of the program to ramp to $1000\ ^\circ\text{C}$ at $20\ ^\circ\text{Cmin}^{-1}$, to investigate whether the gel-casting process affected the glass properties including the glass transition and crystallization temperature.

5.2.2 Influence of particle size

The effect of particle size was observed by applying the optimum protocol to two particle size ranges, which were collected after sieving the ground frit with a $38\ \mu\text{m}$ sieve: $>38\ \mu\text{m}$ and $<38\ \mu\text{m}$. The tests were carried out using CILAS 1064 Particle Size Laser Analyzer. Each data point was repeated 5 times in order to obtain a modal particle size value. The results were summarized in Table 5.1. Images of scaffolds made with different particle sizes were taken by employing SEM (Figure 5.4).

5.2.3 Verifying drying and sintering temperature

The effects of drying and sintering temperature were then assessed. The gelled foams were dried at different temperatures: $100\ ^\circ\text{C}$, $125\ ^\circ\text{C}$ and $150\ ^\circ\text{C}$, followed by a two stage sintering programme where the temperature was ramped to $350\ ^\circ\text{C}$ at $2\ ^\circ\text{Cmin}^{-1}$ and held for 1 h; then ramped again to either $680\ ^\circ\text{C}$, $700\ ^\circ\text{C}$, $710\ ^\circ\text{C}$ or $730\ ^\circ\text{C}$ at $2\ ^\circ\text{Cmin}^{-1}$ and held for 1 h. The results were analyzed by using SEM and XRD.

Scanning Electron Microscopy (SEM)

SEM is a powerful imaging method to view the sample surface and particle arrangements at great detail. The disadvantage of SEM is being destructive as well as the ability to produce 2D images only. JEOL JSM-5610 and JEOL-6500F LEO FEG SEM were used to obtain high resolution imaging and carry out Energy-dispersive X-ray Spectroscopy (EDS or EDX) analysis, which was aimed to find out the composition of the area of interest. EDX is a fundamental analytical technique to determine the composition of a specimen in elemental level. For JEOL JSM-5610 SEM, samples were cut into 2 × 2 mm pieces using small surgical scalpel blades and were coated with gold in EMITECH K550 Gold Coater, operating time 3 min at 30 mA. For the JEOL-6500F LEO FEG SEM, samples were cut into the same size but were coated with chromium, operating time 1 min at 65 mA. To analyze sample by JEOL JSM-5610 SEM, the working distance was kept as 20 mm and the operating voltage vary between 11 to 20 kV, magnification was kept at ×100 except for Figure 5.5, where it was enlarged to ×500 to allow closer inspection of the structure.

X-ray Diffraction Spectroscopy (XRD)

The scaffolds were ground into fine powders and were analyzed by XRD (Philips PW 1729 X-ray generator and PW1710 control system with Cu k- α source, operating at 40 kV and 40 mA, data was collected between 2 θ angles at 5 to 70 ° at an increment of 0.04 °). Diffraction patterns were obtained to evaluate the degree of crystallinity. The diffraction patterns were matched using the PANalytical X'Pert HighScore Plus (version 2.2b) software program.

5.3 Results and discussion

5.3.1 Determining the suitable sintering programme

In Figure 5.3, the DSC trace shows that there is a large exotherm at 375 °C followed by a larger one at 560 °C. The TGA trace shows constant weight loss until it reached a constant at 580 °C. Originally it was thought that the two exotherms represented the burning off of the polymer network and the oxidizing of remaining carbon inside the scaffold structure, and a preferential sintering program was drawn according to the data, starting with ramping to 350 °C at 2 °Cmin⁻¹ and held there for 1 h, followed by ramping again at 2 °Cmin⁻¹ to 730 °C and held for another hour. The first holding temperature was lowered to 350 °C from 375 °C given the long holding time. Sintering at higher temperature for a short period of time works the same as to sintering at lower temperature for a longer time. The sintering temperature was chosen based on the DSC data obtained from the as-melt-quenched glasses Figure 3.1, where both glasses were thought to be capable of undergoing sintering at any temperatures below 750 °C. However in practice there was large scale of crystallization when the samples were sintered at 730 °C, which is likely due to the presence of water and the slow ramp rate reducing the onset crystallization temperature.

Despite the disadvantage, slow ramp rate was needed as it would provide a relatively steady environment for polymer burn off and particle fusion. Thus, the sintering temperature had to be reduced to minimize crystallization of the glass. The aim of this chapter was to optimise the sintering temperature (too low would not sinter the particles efficiently).

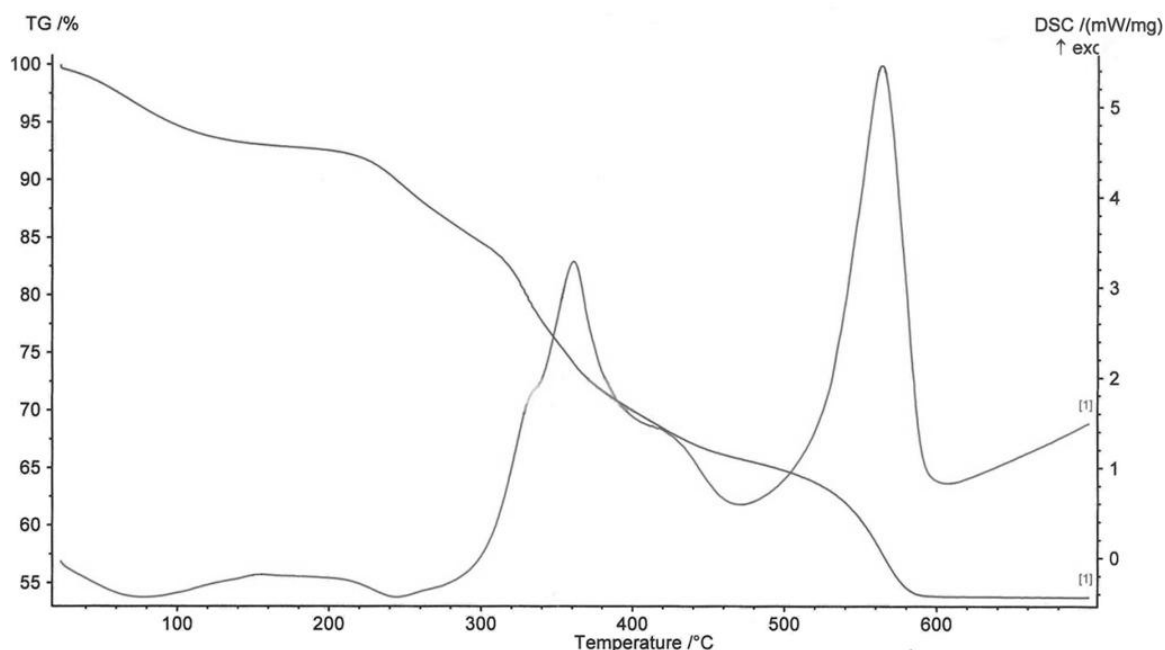


Figure 5.3 DSC-TGA trace of ICIE 16 gel-cast scaffold up to 700 °C (unsintered)

In Figure 5.4, despite the small fluctuation in the baseline that was present due to the slow heating rate, there was a distinctive double exothermic peak (marked point A and B) at 292 °C and 324 °C. There was another large peak (C) at 429 °C. None of these were present in the trace for the as-melt-quenched glass powder. The exotherms represented the burning out of the polymer network (292 °C and 324 °C) and the crystallization of the potassium sodium sulfate (429 °C). Above 429 °C, both traces kept the baseline readings until 700 °C was reached where there was a small dip (D), which was due to the sudden change in the ramp rate (from 2 °Cmin⁻¹ to 20 °Cmi⁻¹). The T_g was designated by a small endotherm in the fine glass powder trace around 650 °C whereas this endotherm occurred at 675 °C for the gel-cast glass powder. The T_c was marked by a third exothermic peak, which occurred around 820 °C (E) for the gel-cast glass powder and at 780 °C (F) for the fine glass powder.

The DSC results suggested that the gel-casting process actually helped postponing the crystallization of the glass and therefore it should not crystallize when sintered at 700 °C. However, the glasses crystallised when the gel-cast foams were sintered at 750 °C (Section 5.3.3). This is again thought to be due to the presence of water at the early stage of the process, which assisted the nucleation of crystals on the surface of glass [119-122].

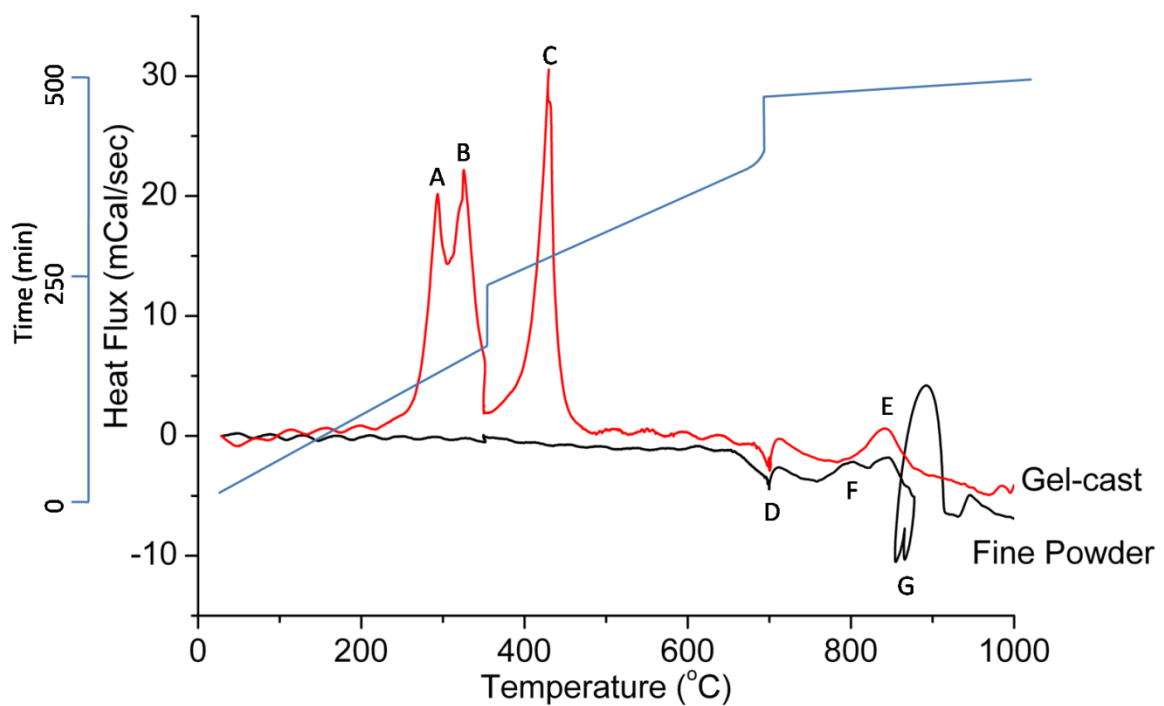


Figure 5.4 DSC of ICIE 16 glass powder (<38 µm) and ground gel-cast foam (unsintered)

5.3.2 Influence of particle size on the scaffold structure

The size of the glass particles played a critical role on sintering. From Table 5.1, the mean values were deduced and the most important value is the D_{50} of each type of the powder, which is the modal size distribution value.

Table 5.1 Particle size analysis data for ICIE 16 and ICIE 16M glass powders

	D₁₀ mean	standard	D₅₀ mean	standard	D₉₀ mean	standard
	(μm)	deviation	(μm)	deviation	(μm)	deviation
ICIE 16 <38 μm	1.23	0.03	7.70	0.68	32.84	5.38
ICIE 16 >38 μm	41.52	1.71	74.19	7.60	124.84	10.5
ICIE 16M <38 μm	1.19	0.19	6.14	0.56	22.65	2.83
ICIE 16M >38 μm	40.48	2.88	73.58	1.81	129.29	2.35

When >38 μm particles were used, sintering was not completed at 700 °C (optimum sintering temperature, see Section 5.3.4 for more details) (Figure 5.5a). The foam struts were not fully dense and consisted of large particles embedded in a sea of smaller particles, which were loosely attached to one another, and the scaffold structure was fragile. The pore network collapsed during sintering.

When <38 μm particles were used, the structure was intact and the scaffold was well sintered (Figure 5.5b). Particle packing and stacking during sintering was better for the <38 μm particles. The smaller particles have a higher specific surface area and a greater driving force for sintering and must be used in order to allow efficient sintering at 700 °C. However, smaller particles have a larger surface area for crystal nucleation that are also likely to promote crystallization at lower temperatures, which means the T_c is reduced and the sintering window shortened, but only to a small extent.

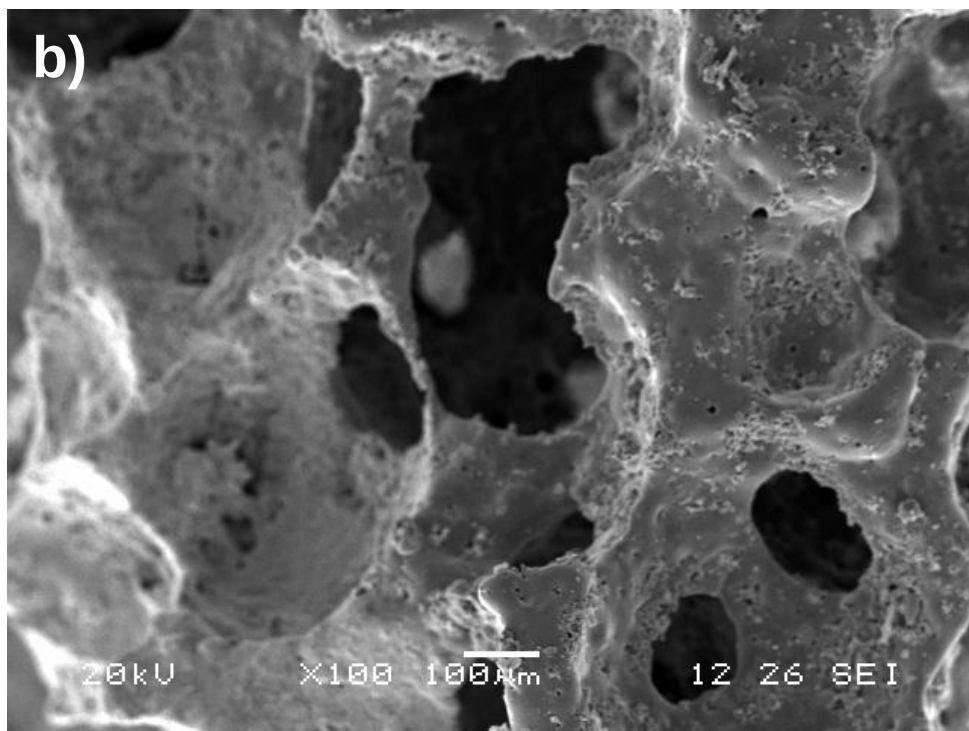
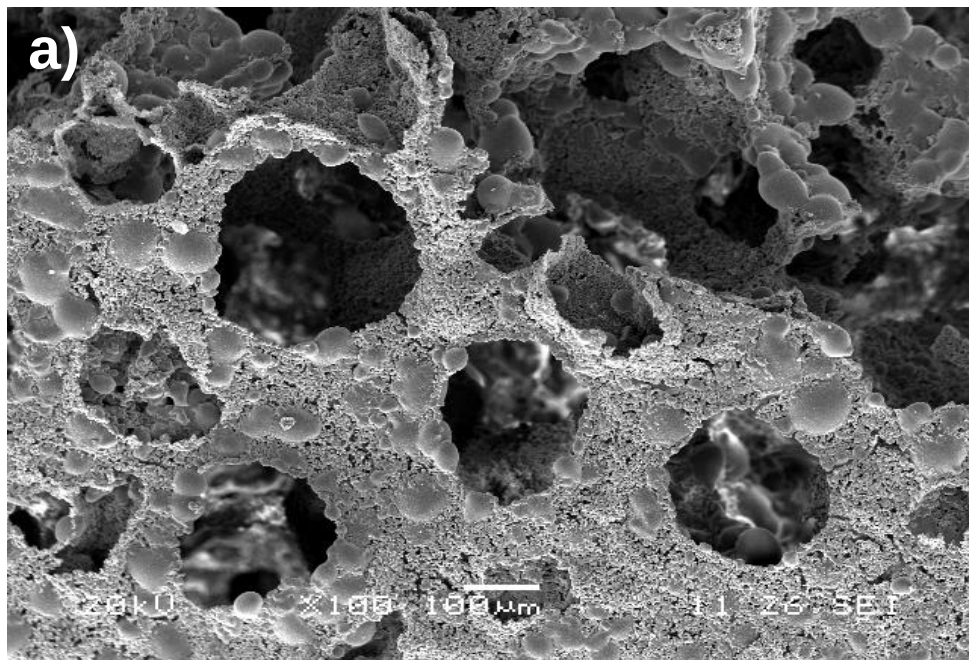
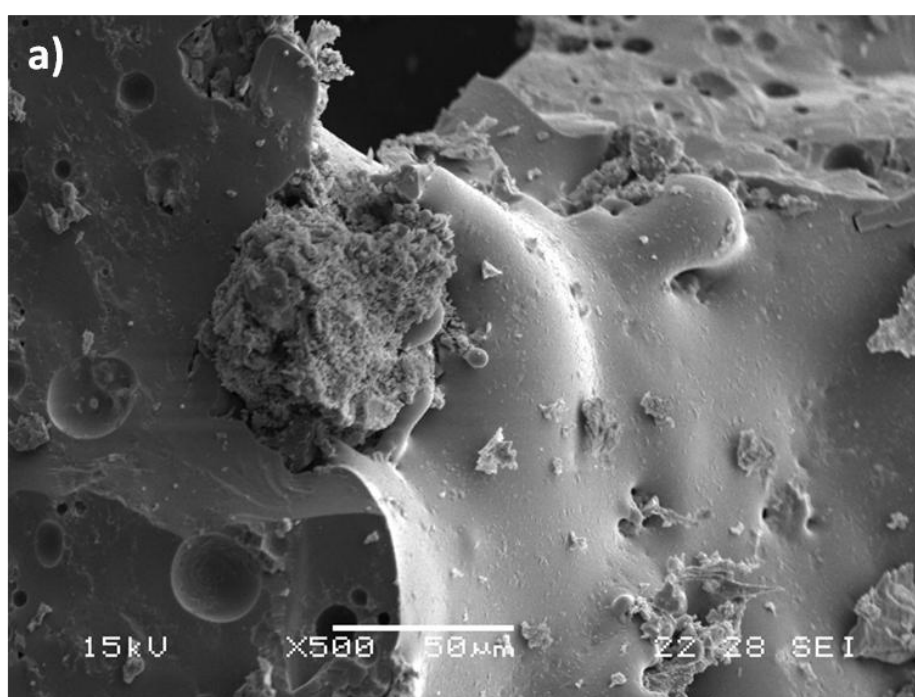


Figure 5.5 SEM images of scaffolds, sintered at 700 °C, made with a) >38 μm and b) < 38 μm glass particles

5.3.3 Influence of the drying temperature

Attempts were made to eliminate the $\text{K}_3\text{Na}(\text{SO}_4)_2$ formation by reducing the amount of water present and the amount of time the water was in contact with the glass, by increasing the drying temperature. Water could not be removed from the initial stages of the gel-cast foaming as the surfactant can only stabilise bubbles in the presence of water and surfactants rarely works well with other mediums such as ethanol. Figure 5.6 shows SEM micrographs of the surface for ICIE 16 scaffolds sintered at 700 °C but dried at 100 °C, 125 °C and 150 °C. The size of the $\text{K}_3\text{Na}(\text{SO}_4)_2$ crystals was significantly reduced from ~70 µm when dried at 100 °C (Figure 5.6a) to ~20 µm by drying at 150 °C (Figure 5.6c). In theory drying at higher temperature should reduce the amount of crystallization; however in practice it promoted glass crystallization, possibly due to accelerated ion diffusion that led to fast nucleation, which developed over the long sintering time. In addition, a high drying temperature risks early breakdown of the polymer network, thus the maximum drying temperature was taken to be 125 °C. More detailed discussions are in the next section.



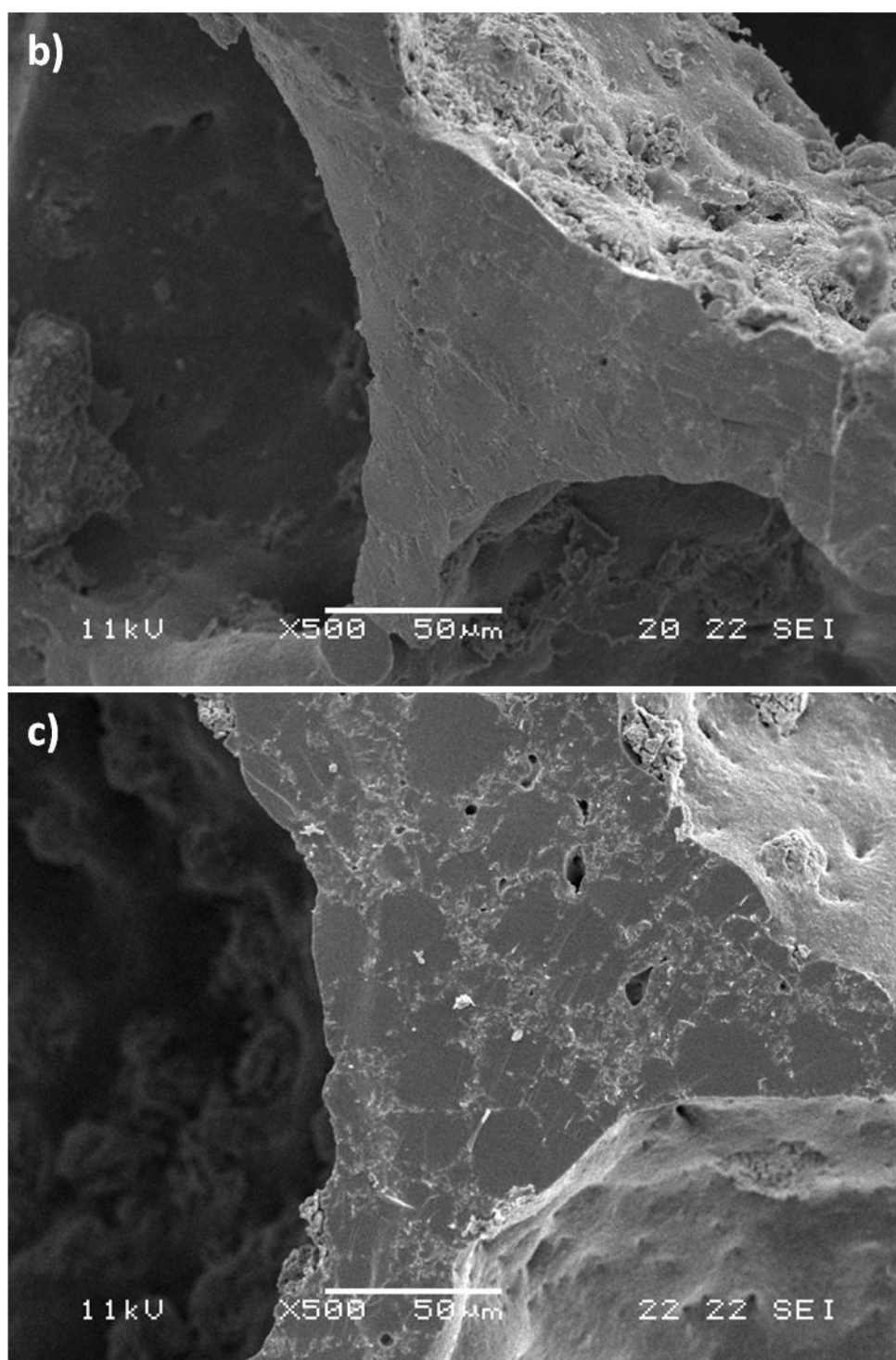
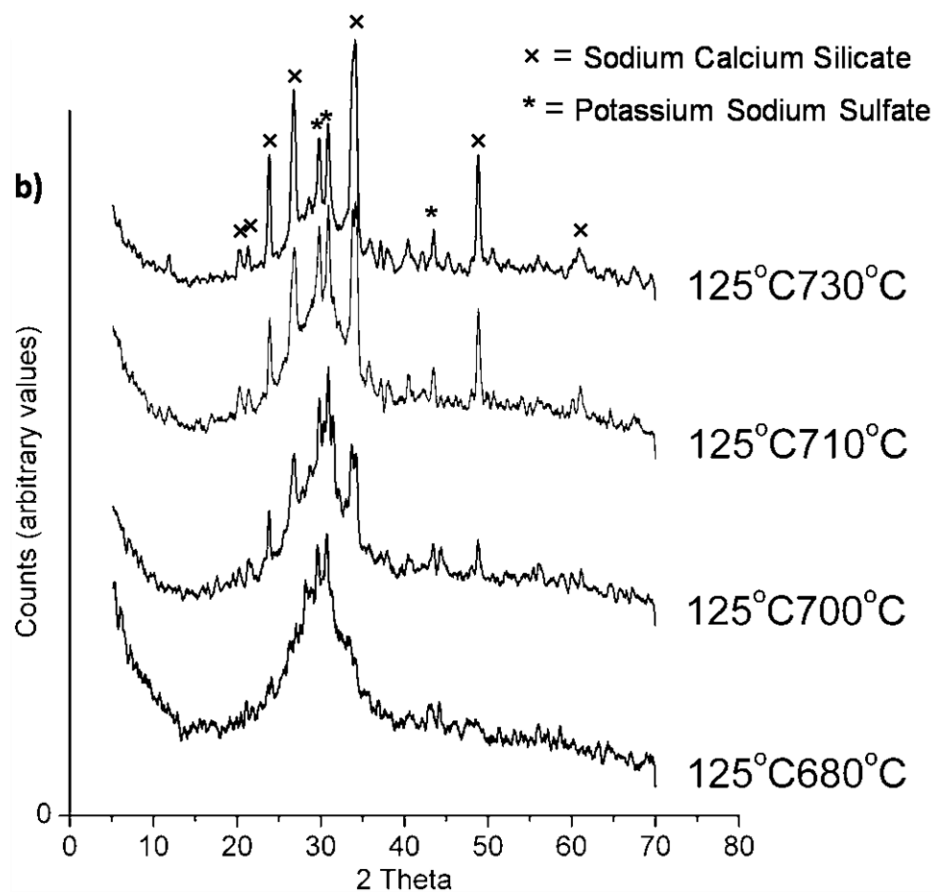
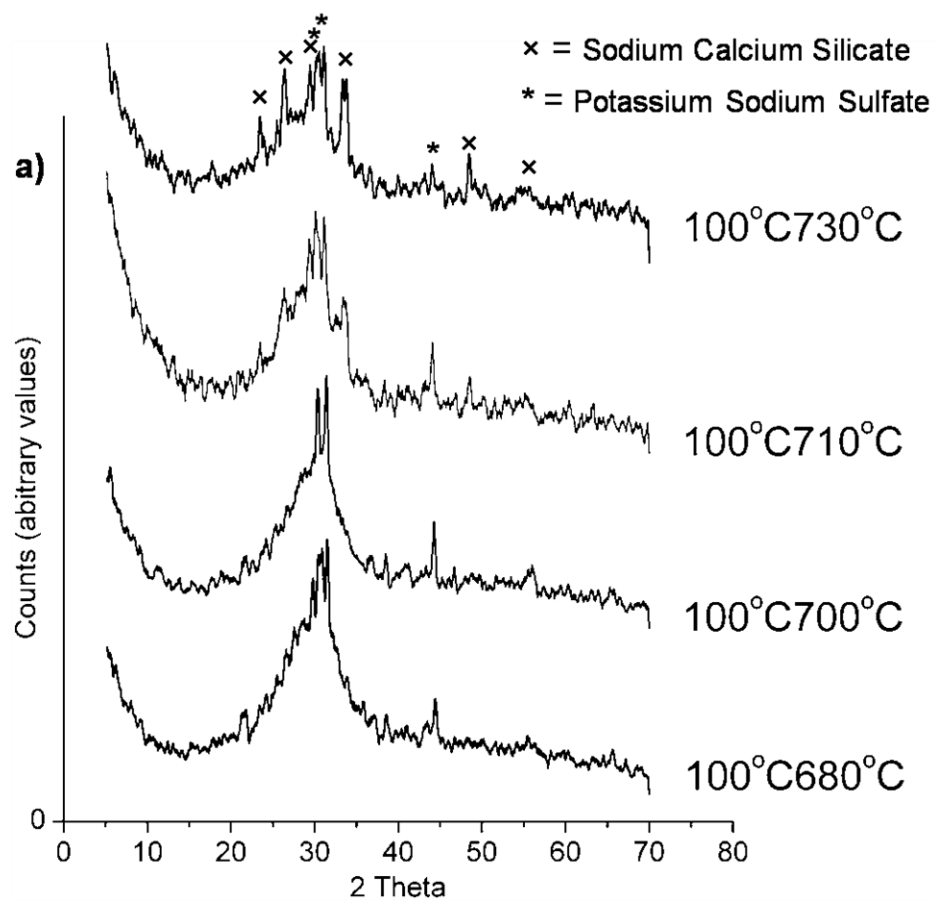


Figure 5.6 SEM images of scaffolds sintered at 700 °C having been dried at a) 100 °C, b) 125 °C and c) 150 °C

5.3.4 Influence of the sintering temperature

Figure 5.7 shows XRD traces for the ICIE 16 scaffolds dried at 100 °C, 125 °C and 150 °C for each different sintering temperature, while Figure 5.8 shows the corresponding SEM images. According to Figure 5.8, drying at 125 °C suppressed the $K_3Na(SO_4)_2$ formation to a minimum, however this contradicted with the XRD data (Figure 5.7). The XRD data suggested drying at 100 °C was the optimum (Figure 5.7a), the degree of crystallinity increased as the drying temperature increased to 125 °C (Figure 5.7b), and was similar to that of 150 °C (Figure 5.7c). This means the crystallisation temperature of the glass was reduced and drying at higher temperature promoted the onset of glass crystallisation by increasing the ion diffusion rate. As the sintering temperature increased above 700 °C, scaffolds dried at all temperatures began to crystallise, the amount of crystallization was small as the amorphous halos were still present. The crystal phases were a mixture of potassium sodium sulfate ($K_3Na(SO_4)_2$), and sodium calcium silicate ($Na_2CaSi_3O_8$) (01-077-2189). This means that the gel-casting process and the presence of water, combined with the slower heating rate had reduced the $T_{c\ onset}$ of the glass to a lower temperature (<700 °C) compared to the $T_{c\ onset}$ that was derived from the DSC trace in Figure 3.1 (725 °C).

A low sintering temperature could suppress crystallisation of the glass and growth of the $K_3Na(SO_4)_2$ crystals, but sintering was not efficient at temperatures below 680 °C and therefore the desired sintering temperature was between 680 °C and 700 °C for both glass compositions.



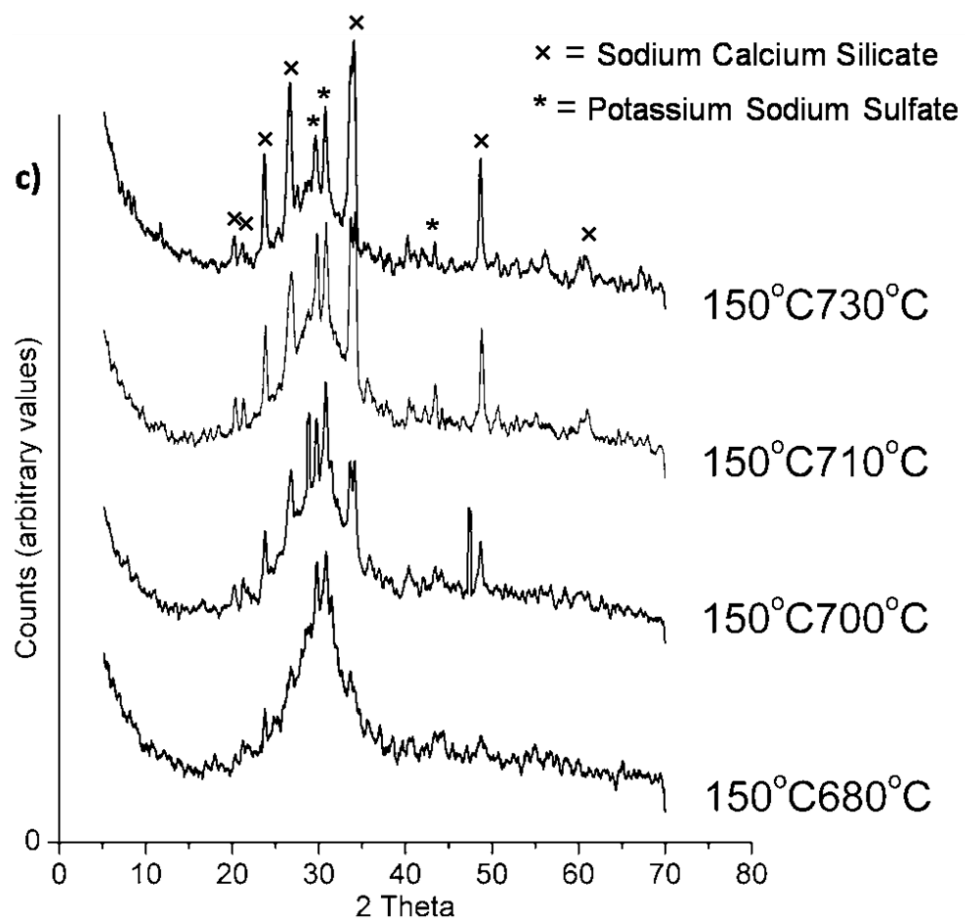


Figure 5.7 XRD traces of ICIE 16 scaffolds dried at a) 100 °C, b) 125 °C and c) 150 °C and sintered at different temperatures

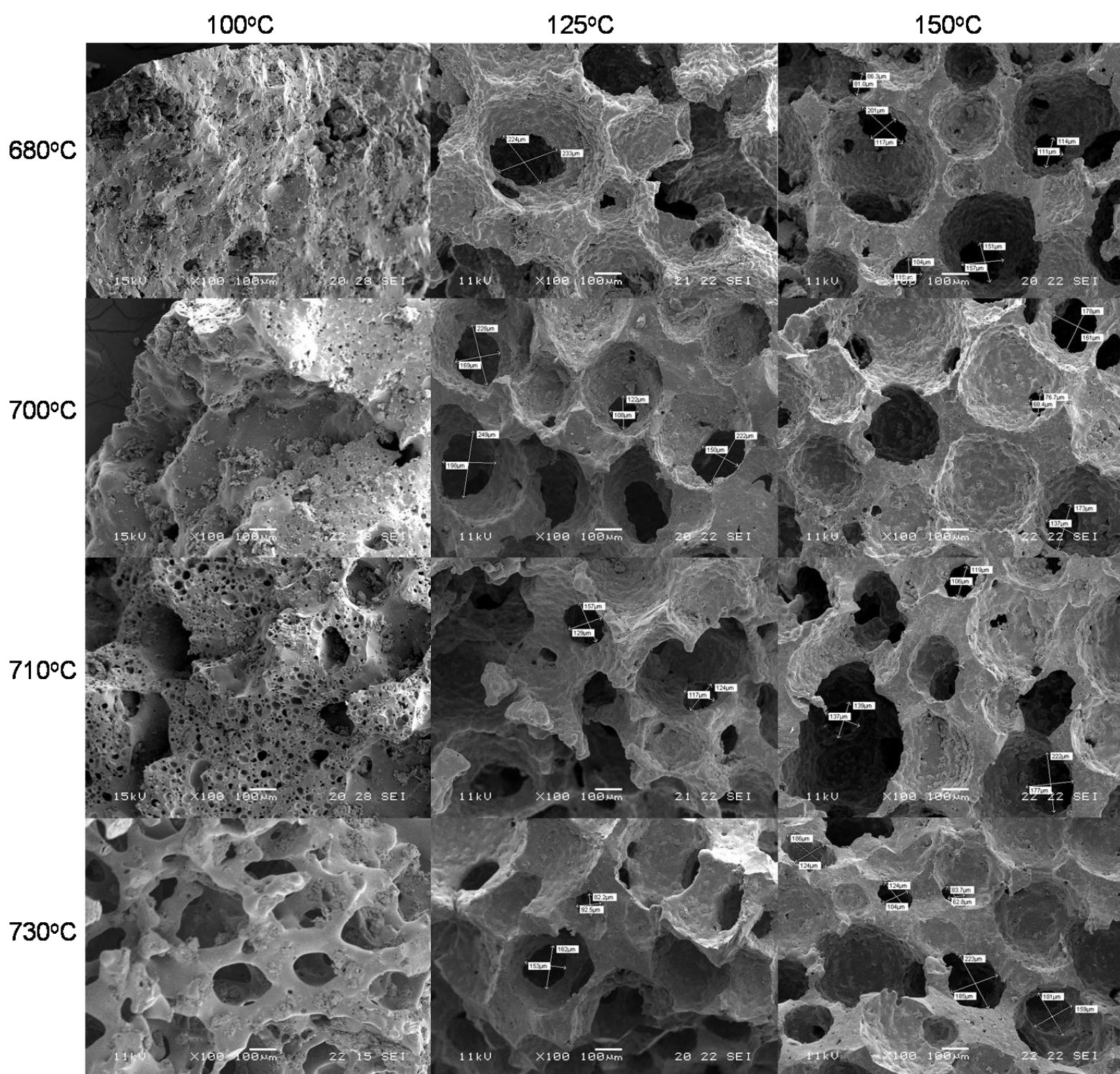
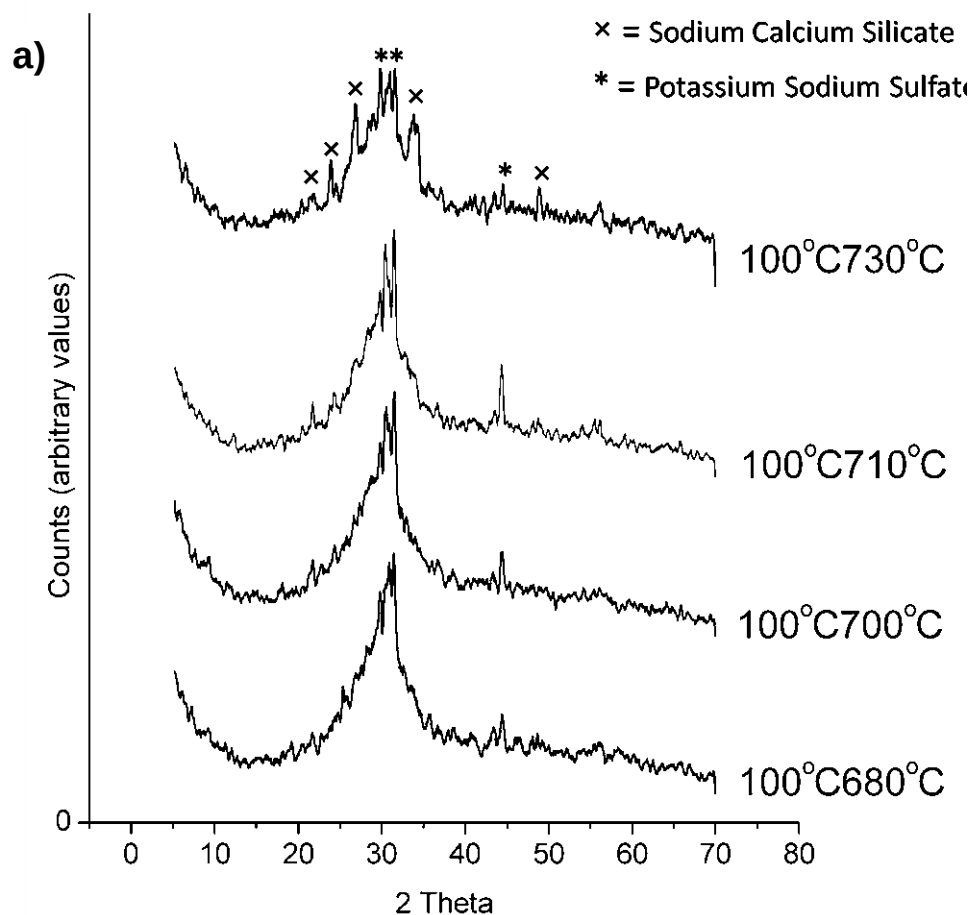


Figure 5.8 SEM images of ICIE 16 dried and sintered at different temperatures

For the ICIE 16M samples, a different result was observed. Whilst drying at 100 °C (Figure 5.9a) provides the least crystallization, an increase in drying temperature from 125 °C (Figure 5.9b) to 150 °C (Figure 5.9c) reduced crystallization. However the corresponding SEM analysis disagreed with the above statement, in Figure 5.10, the best structure was observed in the middle, where drying temperature was at 125 °C and 150 °C while the sintering temperature was 700 °C and 710 °C. In general, less crystallization was seen under all conditions in ICIE 16M compared to ICIE 16. This indicated that ICIE 16M sinters better and has a higher crystallization temperature than ICIE 16.



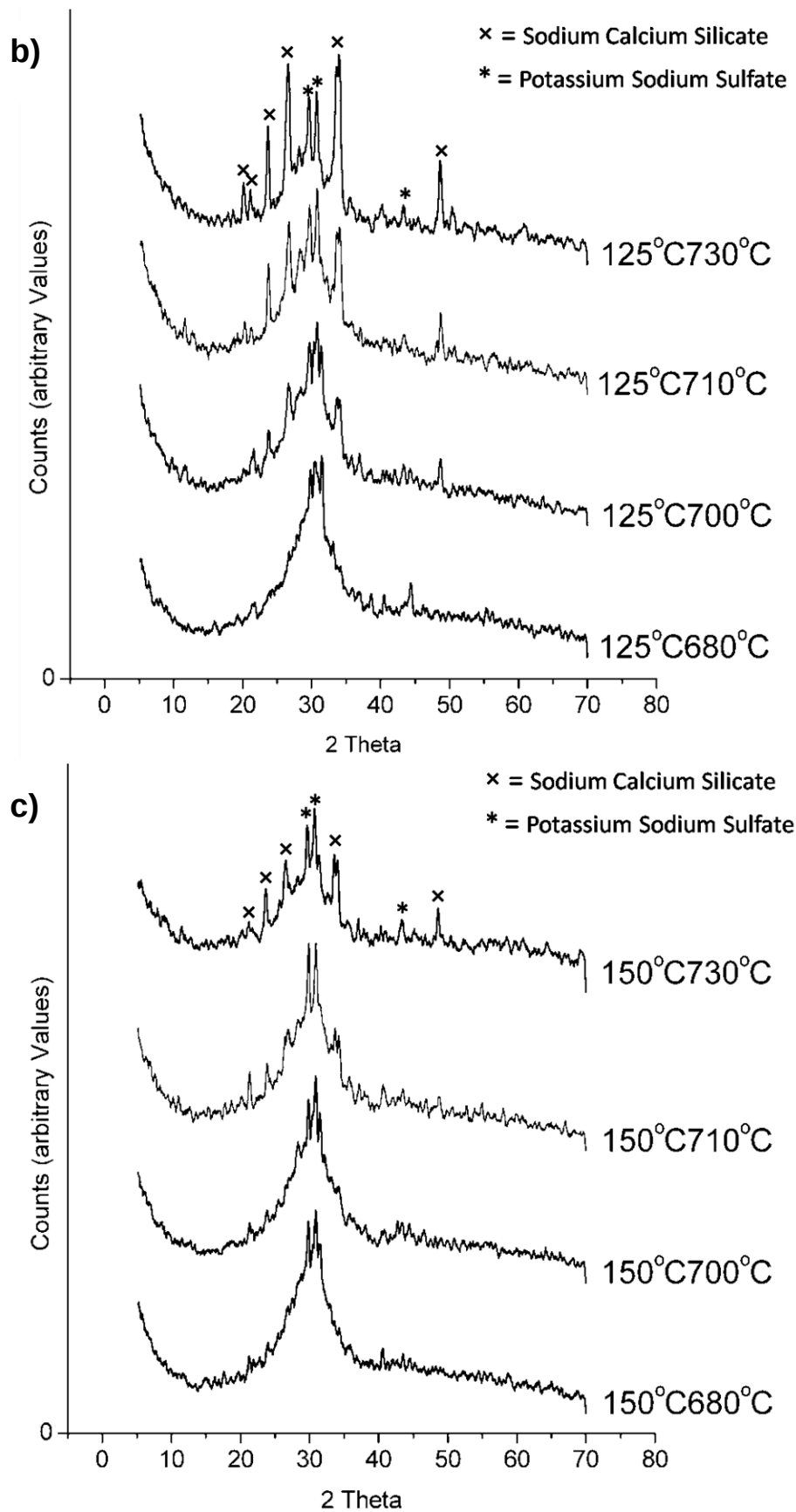


Figure 5.9 XRD traces of ICIE 16M scaffolds dried at a) 100 °C, b) 125 °C and c) 150 °C and sintered at different temperatures

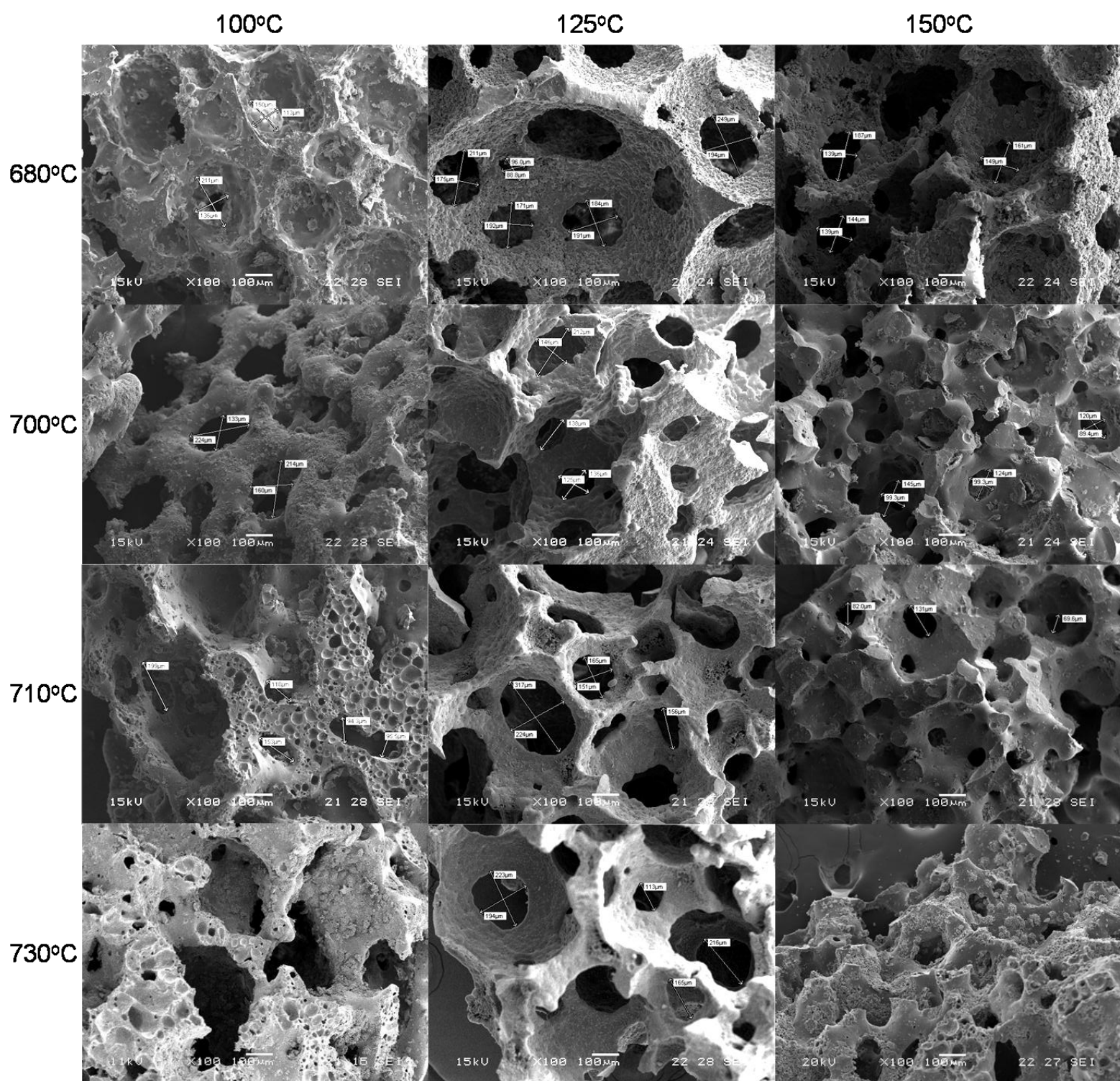


Figure 5.10 SEM images of ICIE 16M dried and sintered at different temperatures

Although in XRD data there was little improvement seen by increase the drying temperature, in ICIE 16M there were even more crystallization occurring at 125 °C than at 100 °C. In the SEM graphs, sodium potassium sulphate crystallization were clearly visible in all the scaffolds dried at 100 °C, which was improved by increasing the drying temperature 125 °C, until it re-appeared again at 150 °C. A possible reason for this disagreement between data is that at 125 °C the crystal growth was suppressed but not the nucleation, resulting in smaller crystals but greater in numbers. In dissolution the crystalline and amorphous phase dissolve at different speeds, larger crystals dissolve slowly and may cause very uneven breakdown of the network and unsteady ion release, whereas smaller crystals would not have such problems. Hence 125 °C was chosen to be the optimum drying temperature (could be increased to 150 °C for ICIE 16M). At 700 °C there was minimum crystallization of both the glass and the sodium potassium sulphate, therefore it was chosen as the optimum sintering temperature, for ICIE 16 it was lower (between 680 °C and 690 °C), as ICIE 16 had a lower T_c and smaller sintering window than ICIE 16M, as discussed in Chapter 3.

5.4 Summary

Optimum thermal processing program (as shown in Figure 5.11) was deduced to promote sintering while suppressing crystallization of sodium potassium sulfate. The optimum drying temperature was 125 °C and can be increased to 150 °C for ICIE 16M. Drying at higher temperature promoted the onset of glass crystallisation by increasing the ion diffusion rate, however crystal growth rate seemed to be suppressed, resulting the presence of large amount of small crystals. The optimum sintering temperature was 700 °C for ICIE 16M and between 680 – 690 °C for ICIE 16 as it had a lower T_c and smaller sintering window. Less crystallization was observed in ICIE 16M than ICIE 16, which confirmed with the results derived in Chapter 3, ICIE 16M is better at sintering than ICIE 16. For optimum sintering, the size of the glass powder must be around 10 μm , otherwise the structure would collapse and the particles would not sinter together due to mismatched packing. However it would slightly shorten the sintering window.

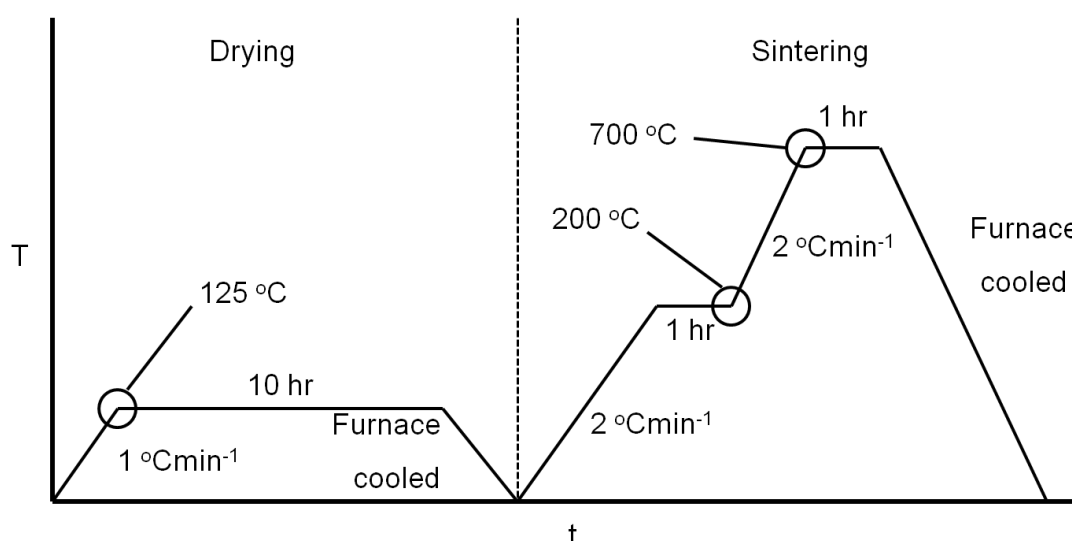


Figure 5.11 The optimized thermal process program

6 Characterization of the optimized gel-cast scaffold

6.1 Introduction

This chapter aims to characterize the optimised scaffold to investigate whether it meets the standard of an ideal scaffold for bone regeneration. First the compressive strength was examined; then the interconnect size was determined to see if it is sufficient for blood vessel penetration and tissue ingrowth, i.e. greater than 100 μm by Mercury Intrusion Porosimetry (MIP) and X-ray microtomography (μCT); finally, the rate of formation of the apatite layer on the scaffold and the ion release rate in SBF were studied by applying FTIR, XRD and ICP.

6.2 Experimental

6.2.1 Compressive Strength

Bone is a load bearing material hence it is important for the biomaterial to match the compressive strength of porous cancellous bone (2-10 MPa [107]). The compressive strength of the optimum scaffold was determined using a Zwick/Roell Z2.5 tester with a 2 kN load cell and a strain rate of 5 mm/min. Samples were cylinders, 6 mm in diameter 11 mm in height and the test was carried out on 10 samples for ICIE 16 and 5 for ICIE 16M. The results were summarized in Table 6.1.

6.2.2 Pore and Interconnect Sizes

Micro-CT

μ CT imaging can image objects in 3D and is non-destructive. Novel image processing algorithms can be applied to the images for quantitative characterizations. It is possible to calculate the key macroscopic structural features of pore and interconnect aperture size [123], as well as the porosity plus the homogeneity of the foam [124]. The 3D images can also be converted into computational fluid dynamic models to calculate the permeability of the scaffold. The resolution of μ CT scanning is mainly limited by its field of view, which is approximately 1/1000 of the sample diameter. The critical resolution required for trabecular bone and tissue scaffolds studies is 10 micron, which is one tenth of the minimum interconnect diameter required for an ideal scaffold ($>100\ \mu\text{m}$). For looking at the pore distribution and homogeneity of the foam, it was possible to scan the whole scaffold (maximum 10 mm in diameter with preferable resolution) without destroying it; however for the calculations and quantifications, higher resolution was required. A 5 mm cubic specimen was μ CT scanned using a laboratory based system (Phoenix X-ray Systems and Services GmbH) operating at 100 kV and 100 μA . Scans and image analysis were carried out by Sheng Yue, due to the complexity of the interconnected pore structure involved. The procedure for scaffold quantification was improved from previous studies, which quantified sol-gel foam scaffolds with approximately spherical pores [123-125]. Details are in Appendix A.

Mercury Intrusion Porosimetry (MIP)

It is a physical process of quantitative analysis of pore size distribution within a sample. The analysis is based on the fact that mercury is a strongly non-wetting liquid on most substrates and it has a high surface tension, which would allow it to penetrate through the whole structure with ease under pressure. The Washburn equation (Equation 6.1) is applied and the pores are assumed to be spherical [126].

$$\Delta P = \frac{\gamma_{\text{Mer}} \cos \theta}{D_{\text{pore}}} \quad (6.1)$$

where ΔP is the change in pressure, D is the pore diameter, γ_{Mer} is the surface tension of mercury (480 dyne cm^{-1} in air at 20 °C) and the contact angle θ is 140°.

The interconnect size is determined by measuring the pressure difference at the threshold of the pore. As the mercury was flushed towards an opening of a pore (an interconnect), pressure would build up as the flow path was narrowed, once it passed through the threshold, the pressure was released and would remain the same until another interconnect came up. The pressure required to intrude mercury into the pores is inversely proportional to the size of the interconnects [76]. Samples (cylinders with 6 mm in diameter and 10mm in height) were analyzed by Quantachrome PoreMaster 33. Compared to μCT , MIP has several disadvantages, firstly it is a destructive method and the samples are at constant risk of collapsing under pressure; secondly the assumptions used are accompanied with large errors, which means it can only used for comparative studies instead of quantitative analysis; thirdly it does not taken into account any closed pores, nor does it provide any shape information; lastly as it only measures the opening towards the pore (interconnect size), the actual pore size remains undetermined [126].

6.2.3 Bioactivity and dissolution studies

There are only a few materials that can directly bond to living bone without the formation of detectable apatite on their surfaces [127]. Despite this limitation, examination of apatite formation on the surface of a material in SBF solution is useful as an initial screening test for the bioactivity of the material [127-128]. The details in how to prepare 1000 ml SBF solution are summarized in Table 6.1.

Table 6.1 SBF ingredients

Step	Ingredients	Amount
1	Deionized Water	750ml
2	NaCl	7.996g
3	NaHCO ₃	0.350g
4	KCl	0.224g
5	K ₂ HPO ₄ 3H ₂ O	0.228g
6	MgCl ₂ 6H ₂ O	0.305g
7	Diluted HCl aqueous solution (1M)	30ml
8	CaCl ₂ 2H ₂ O	0.368g
9	Na ₂ SO ₄	0.071g
10	(CH ₂ OH) ₃ CNH ₂	6.057g
11	Diluted HCl aqueous solution (1M)	Heat SBF to 37 °C adjust pH to 7.26 with HCl drops
12	Deionized water	Fill the bottle to the 1 litre mark and leave to agitate for overnight

Steps must be strictly followed in order because SBF is supersaturated with apatite and any inappropriate move during the preparation would result in the precipitation of apatite in the solution. The reagents are never to be dissolved simultaneously but only after the previous one (if any) is completely dissolved.

$$\text{The volume of SBF needed} = \text{sample weight (g)} \times 50\text{ml} \div 0.075\text{g} \quad (6.2)$$

The samples were weighed and the amount of SBF required for each sample was calculated according to Equation 6.2, which was then measured using disposable plastic pipettes. Note the ratio of material versus SBF (3:2) must be kept because it was the critical value at which the active ion release rate was at maximum without the compensation in HCA layer formation rate [129]. The sample in SBF was each held in a small screw-cap polypropylene container (60ml), which was then placed inside the New Brunswick Scientific classic series C24 incubator shaker (set to 37 °C & 120 rpm agitation rate), only to be taken out (samples filtered and dried) when the required time period was met. The variation in SBF pH values (pH at the end of time point – pH before sample immersion) and % weight loss (weight before immersion – weight after drying) was recorded in Figure 6.4.

Fourier Transmission Infra-red Spectroscopy (FTIR)

FTIR is a simple but powerful tool to identify specimens quickly; it works by shining a beam that is composed of many different combinations of frequencies onto a specimen and measures how well it absorbs the beam. Each material response to a particular frequency sequence in its unique way and can hence be identified by matching the result to the database. [130]

After bioactivity tests, the dried specimens were mixed with potassium bromide (KBr) in a ratio of 1:100 and ground into powder form, which was then pressed into thin pellets. The pellets were scanned by a Bruker Vector 22 machine and a WinFirst LITE software for FTIR analysis. The reason to use KBr as a background material was because it is an inert, infra-red transparent material [130].

Inductively Coupled Plasma Spectroscopy (ICP)

ICP (ICP-OES, Thermo Scientific ICP 6000 series) was used to measure the ion-release rate in the SBF, mainly the change in ion concentration of silicon, calcium and phosphorus ions as a function of time. In ICP the ions are burnt over a stream of plasma in an aqueous solution, the ions will lose or gain electrons during the burning, corresponding electromagnetic radiation are emitted. The intensity of the radiation is measured to determine the ion concentration inside the solution accordingly. In the case of ICIE 16 and 16M, extra ions such as K, Na, Zn, Mg, Sr and S were also taken into account. Each data point was repeated at least 3 times in order to verify the error.

After the completion of bioactivity tests the solution was collected and kept in a fridge to keep extra ion activities to a minimum. They needed to be diluted 10 times (1 ml sample + 9 ml 2M HNO₃) and standards (0, 2, 5 and 20 ppm) needed to be made before testing. A standard of 0 ppm was the medium 2M HNO₃, and to make 2, 5 and 20 ppm standards, the standards of the ions that were to be tested were mixed with 3.2 ml concentrated HNO₃ and water to make up to a solution with 25 ml in total volume. The amount of each ion standards increased as the ppm value increased (50 µl for 2 ppm, 125 µl for 5 ppm and 500 µl for 20 ppm).

6.3 Results and discussion

6.3.1 Compressive Strength

The individual values are summarized in Table 6.2. Figure 6.1 (% porosity versus maximum stress) is plotted for aiding a clearer view to the table. Equation 6.3 was used to calculate the porosity:

$$\Phi = 1 - \frac{\rho_{\text{bulk}}}{\rho_{\text{true}}} \quad (6.3)$$

where Φ is the porosity of the scaffold, ρ_{bulk} is the bulk density of the scaffold (measured by weight $\div$ volume) and ρ_{true} is the theoretical density of the material from which the scaffold is made, in this case it is the density of the glass and is taken to be 2.74 gcm^{-3} for ICIE 16 and 2.6 gcm^{-3} for ICIE 16M (the average true densities obtained by Keswani *et al.* [131]).

For ICIE 16, a scaffold with 79% porosity, modal pore size of $\sim 345 \text{ }\mu\text{m}$ and modal interconnect size of $144 \text{ }\mu\text{m}$, its compressive strength was 1.9 MPa; in the case of ICIE 16M, an 80% porosity with pore size of $\sim 436 \text{ }\mu\text{m}$ and interconnect size of $113 \text{ }\mu\text{m}$, the compressive strength is 2.5 MPa. ICIE 16M is therefore stronger in compression than ICIE 16, which was unsurprising as it was proven in Chapter 3 and 5 that ICIE 16M sintered better than ICIE 16. Note that the results are largely depended on the shape of the samples, i.e. whether the top and bottom surfaces were absolutely parallel, which was very difficult to achieve for porous glasses and the reason of the large deviation in the results. Overall the compressive strength increased as the porosity decreased.

Table 6.2 Compression tests result summary

ICIE 16			ICIE 16M		
Bulk Density [gcm ⁻³]	Porosity [%]	$\sigma_{\max}$ [MPa]	Bulk Density [gcm ⁻³]	Porosity [%]	$\sigma_{\max}$ [MPa]
0.504	82	1.22	0.738	72	3.22
0.532	81	1.79	0.753	71	1.86
0.437	84	1.48	0.831	68	2.60
0.498	82	2.00	0.522	80	2.52
0.688	75	2.31	0.842	68	1.74
0.704	74	2.82			
0.550	80	1.67			
0.712	74	1.75			
0.578	79	1.88			
0.694	75	2.07			

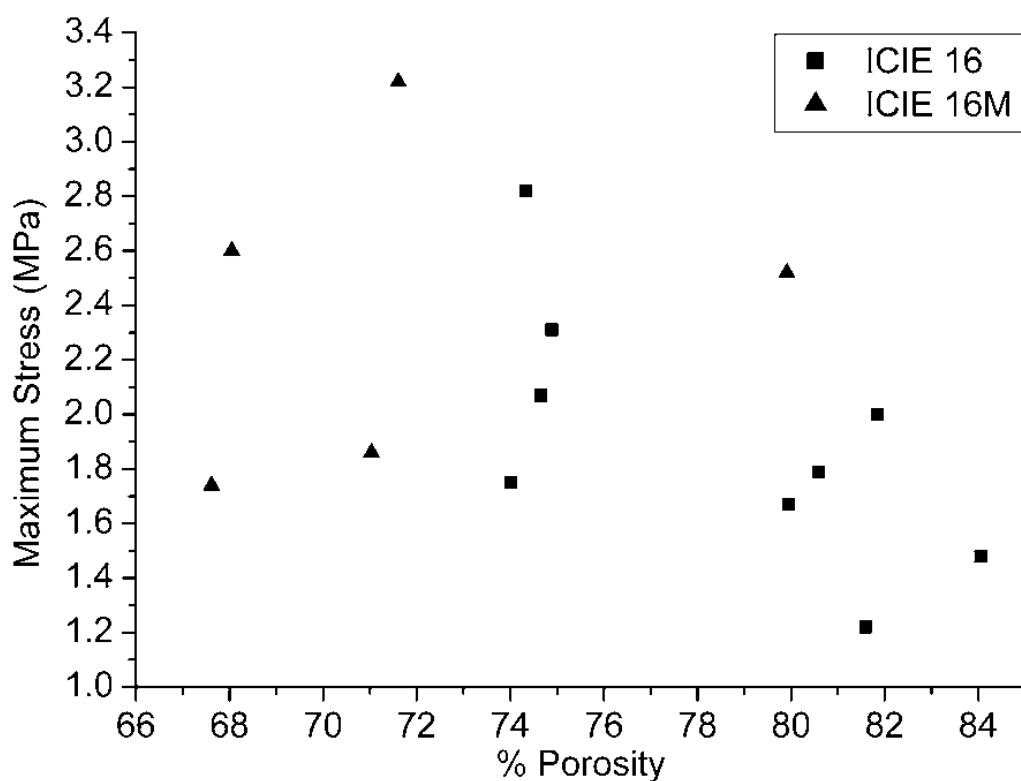
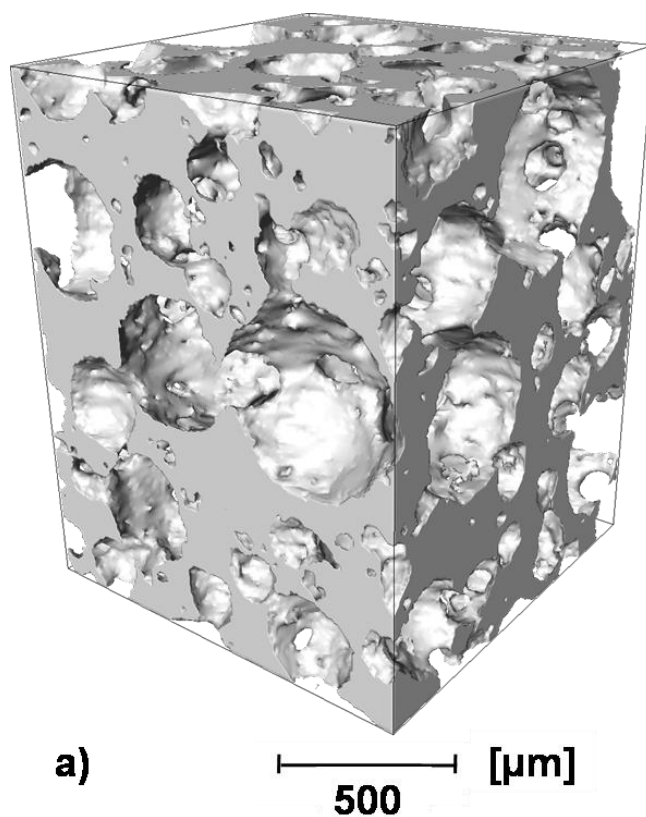


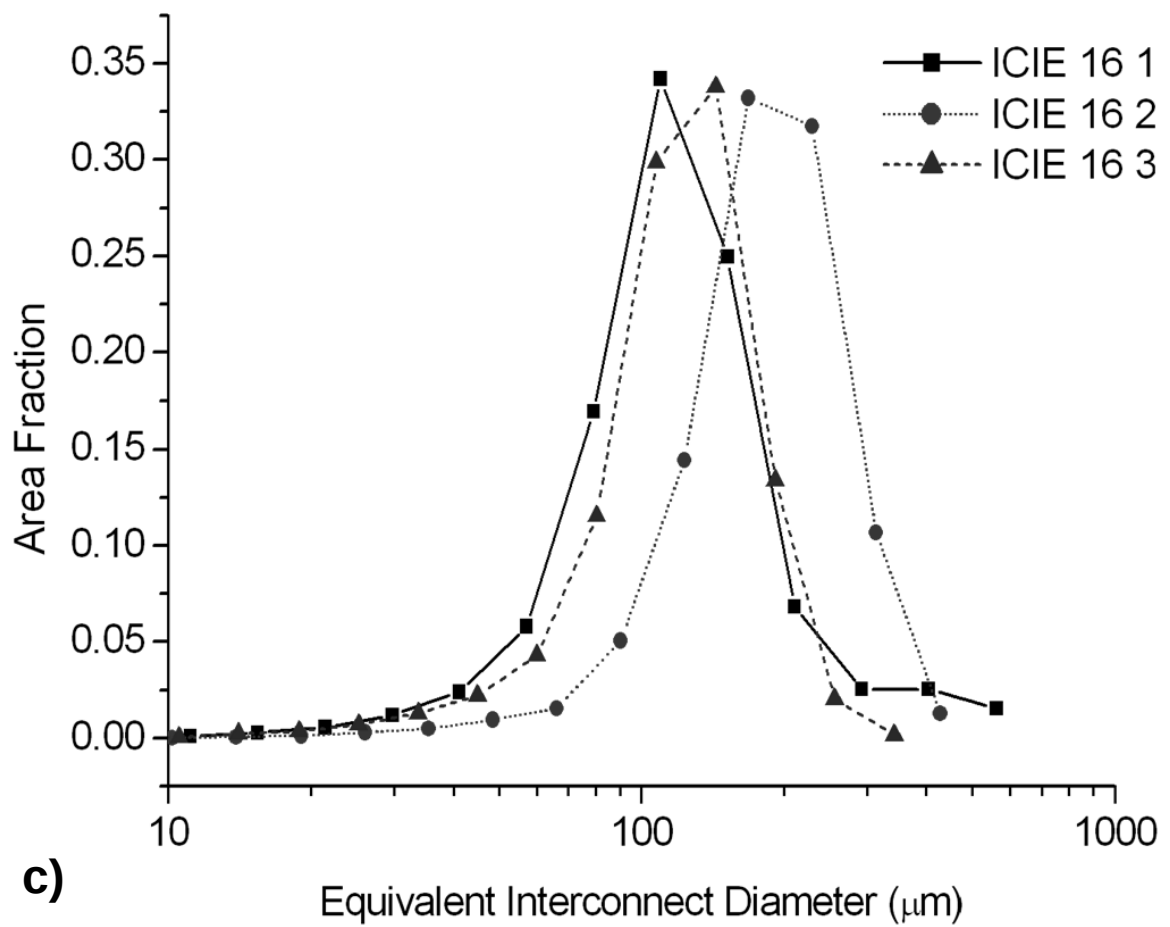
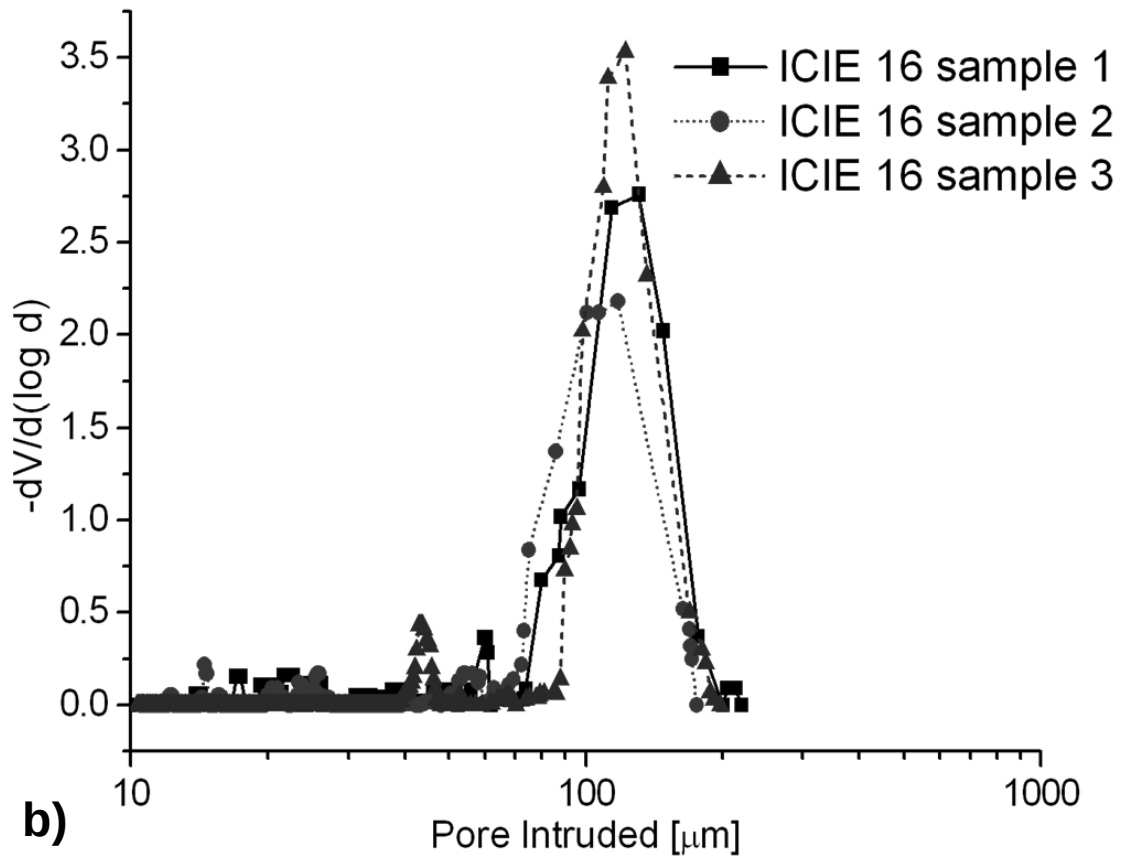
Figure 6.1 Maximum stresses against % porosity of both glasses

6.3.2 Pore and interconnect Sizes³

Figure 6.2a is a 3D image of the structure of a representative optimized scaffold and is the visual evidence of the high interconnectivity and porosity of the scaffold. Figure 6.2b shows the data obtained by MIP, from which the modal sizes of the interconnect were 128 μm , which corresponded to 80% porosity (measured geometrically). Three such scaffolds were scanned and the interconnect size distributions from μCT image analysis is shown in Figure 6.2c. ICIE16 1 is the sample imaged in Figure 6.2a, the mean modal interconnect size of the three samples was 140 μm with a standard deviation of 30.89. The image analysis of μCT images also allowed quantification of pore size (Figure 6.2d), which had a mean modal pore size of 379 μm with a standard deviation of 93.43.



³ μCT data collected and analyzed by Sheng Yue



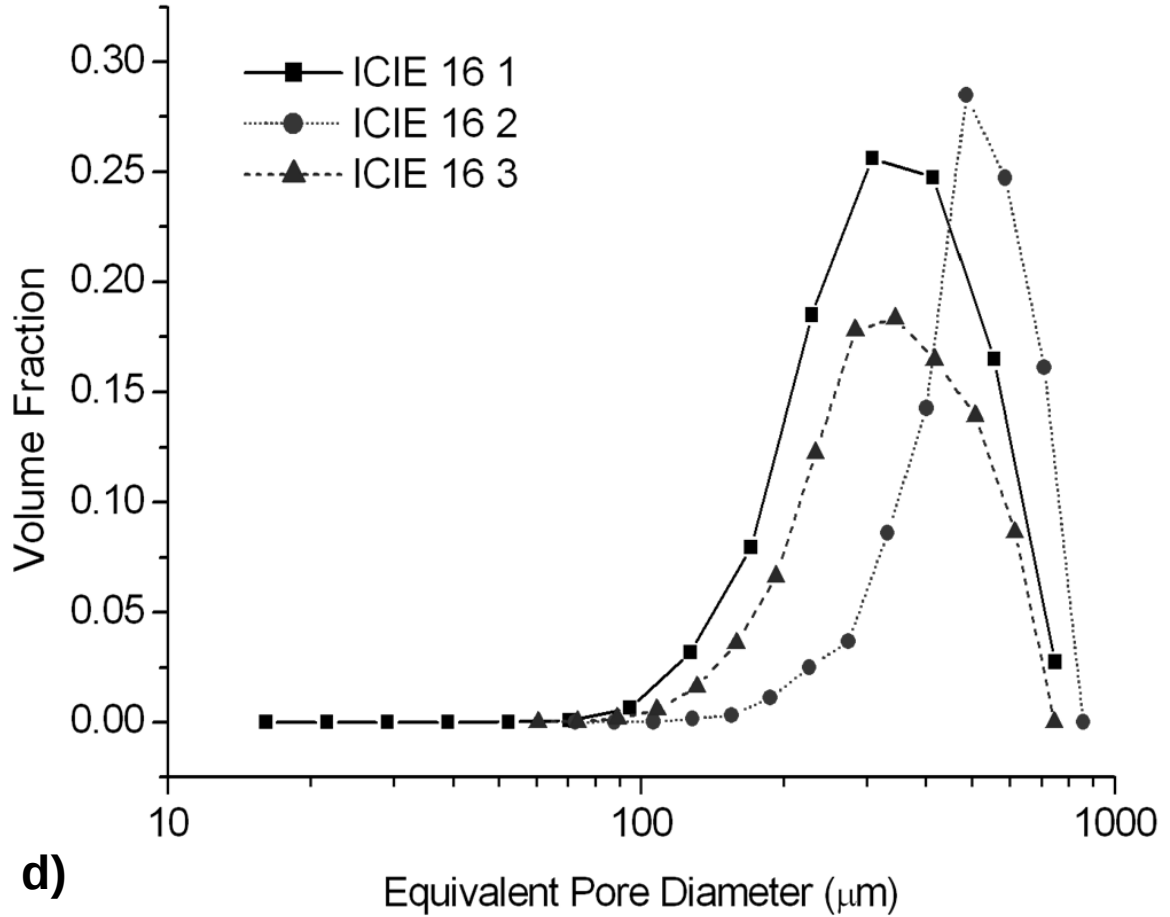
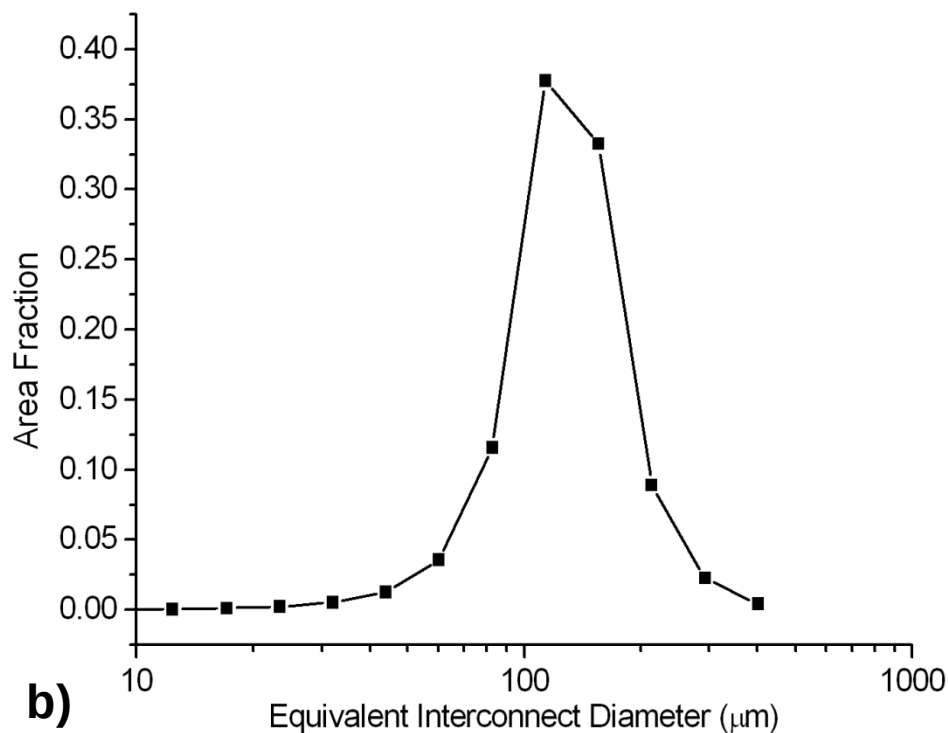
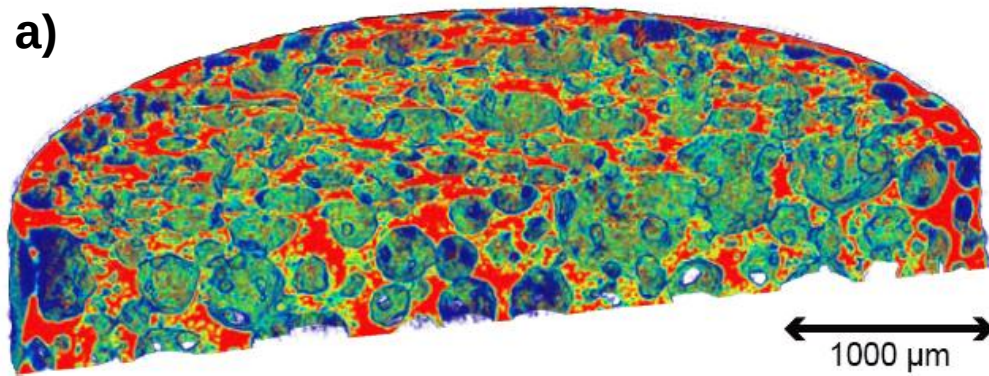


Figure 6.2 a) A 3D μ CT image of an ICIE 16 gel-cast foam scaffold; pore network quantification of ICIE 16 by b) MIP; c) the interconnect diameter and d) the pore size distributions from image analysis of μ CT images

In Figure 6.2d and 6.2d it is clear that ICIE 16-2 had larger modal interconnect size and pore size than the other two samples, this is because ICIE 16-1 and ICIE 16-3 are from the same batch whilst ICIE 16-2 is from another, although the same production protocol was followed, there is still a variation in-between batches. However this difference did not show in MIP results. MIP gave smaller values than the μ CT results, the reason is probably because the MIP is not as sensitive as μ CT in determining the changes of pore sizes.

For ICIE 16M, the μ CT analysis (Figure 6.3b and 6.3c) determined the modal interconnect size was 113 μm with a modal pore size of 436 μm . The sample had 80% porosity measured geometrically. Figure 6.3a is a 3D density distribution image of a section of the scaffold and evidently showed the edge of the scaffold was denser than the centre, indicating an inhomogeneous sintering was taking place. Overall ICIE 16M sintered better than ICIE 16, as the interconnect size and the pore size were both much smaller compared to ICIE 16, which is a result of enhanced viscous flow during sintering.



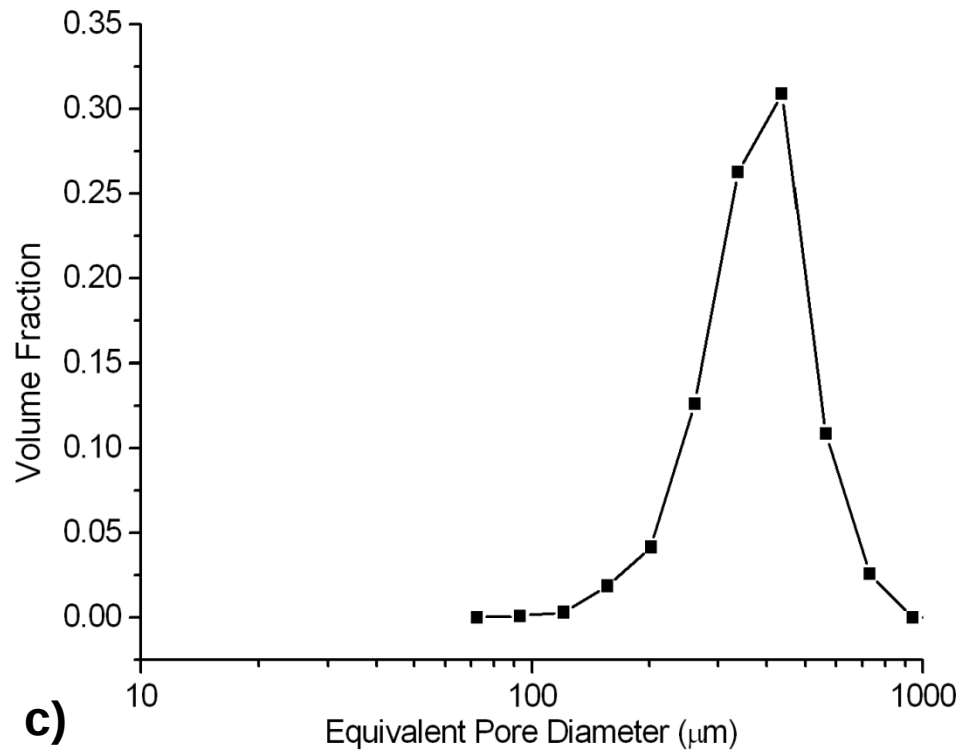


Figure 6.3 a) A 3D density distribution of a section of the ICIE 16M scaffold, with density increases as the color changes from blue (cooler color) to red (warmer color); pore network quantification of ICIE 16M by μCT imaging analysis, b) is the interconnect diameter distribution and c) the pore size distribution

6.3.3 Bioactivity and dissolution studies

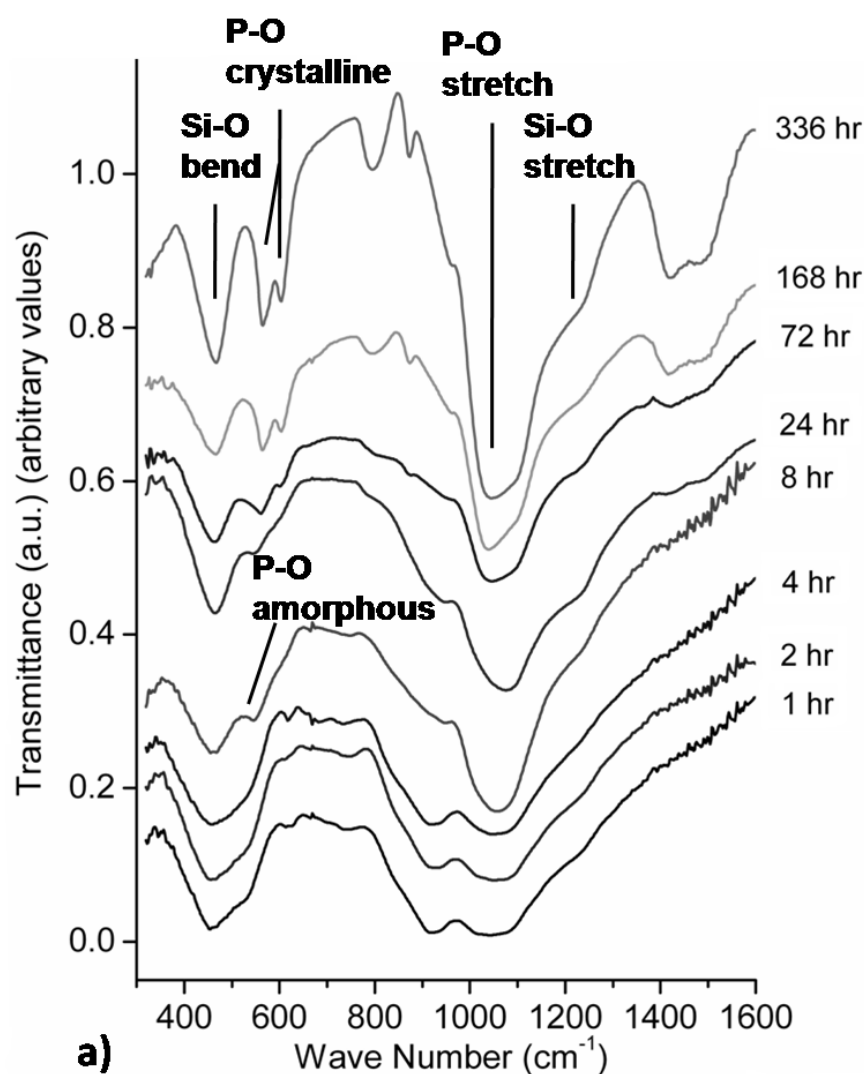
FTIR, % weight loss and change in pH

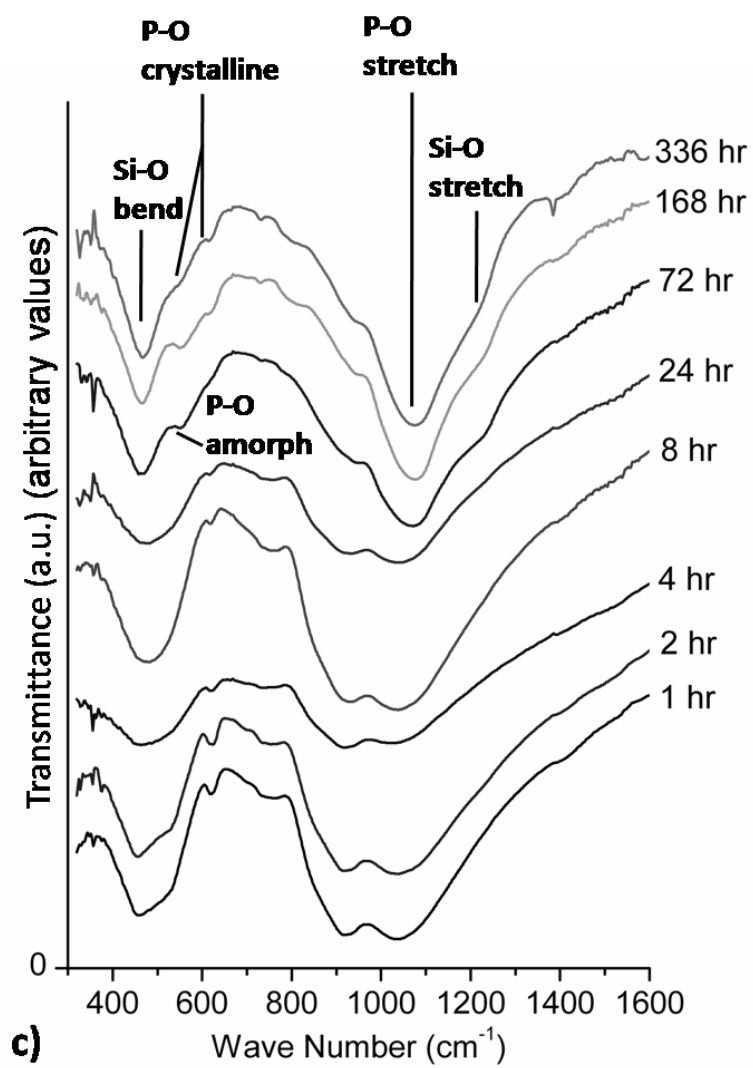
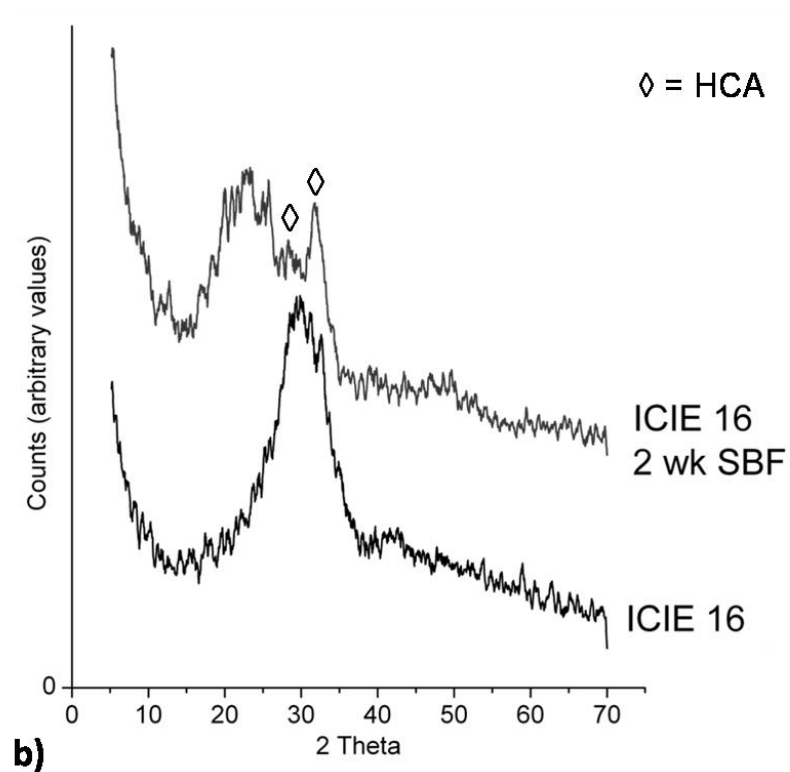
ICIE 16 scaffold with a bulk density of 0.52 gcm^{-3} and a porosity of 81% and ICIE 16M scaffold with 0.78 gcm^{-3} density and 73% porosity were immersed in SBF for the purpose of deposition and dissolution rate studies. The deposition of HCA was inspected by FTIR and the results were summarized in Figure 6.4a and 6.4c respectively.

It is clear that for ICIE 16 the deposition of calcium phosphate occurred after 8 h of immersion in SBF, as vibrational bands for P-O bending were seen for the first time, which is likely to be amorphous calcium phosphate. Similar bands were observed at 72 h for ICIE 16M. Amorphous calcium phosphate deposition has previously been found to occur on bioactive glasses prior to HCA formation, using synchrotron X-ray diffraction studies [132-133]. The twin bands for P-O bending at 576 and 611 cm^{-1} observed after 3 days immersion for ICIE 16 indicate the calcium phosphate crystallized into HCA, whereas the crystallization was just occurring after 2 weeks of immersion for ICIE 16M. The fact that the P-O bending peaks corresponded to HCA was confirmed by XRD (Figure 6.4b and 6.4d). Apatite formation in SBF of ICIE 16 was therefore at a similar rate to sol-gel foam scaffolds that had a similar compressive strength [107], which is more rapid than the ICIE 16M and 13-93 scaffolds.

The reason is that ICIE16 has a network connectivity closer to 2 (2.13 according to Table 3.4), whereas the network connectivity of 13-93 is a lot higher, at 2.59 and 3.00 based on different assumptions [58], as the result of the higher silica content

of 13-93. The higher network connectivity means that the glass is less susceptible to ion exchange and dissolution, which are the first stages of the HCA layer formation mechanism. ICIE 16M performed similarly to 13-93, it seems that its theoretical NC value of 2.13 (or 2.55 depending on assumption) was not correct. It performed less well than the ICIE 16 scaffolds, which suggest that the network intermediates (Zn^{2+} and Mg^{2+}) were probably acting more as network formers and the NC was close to 2.53 in this case.





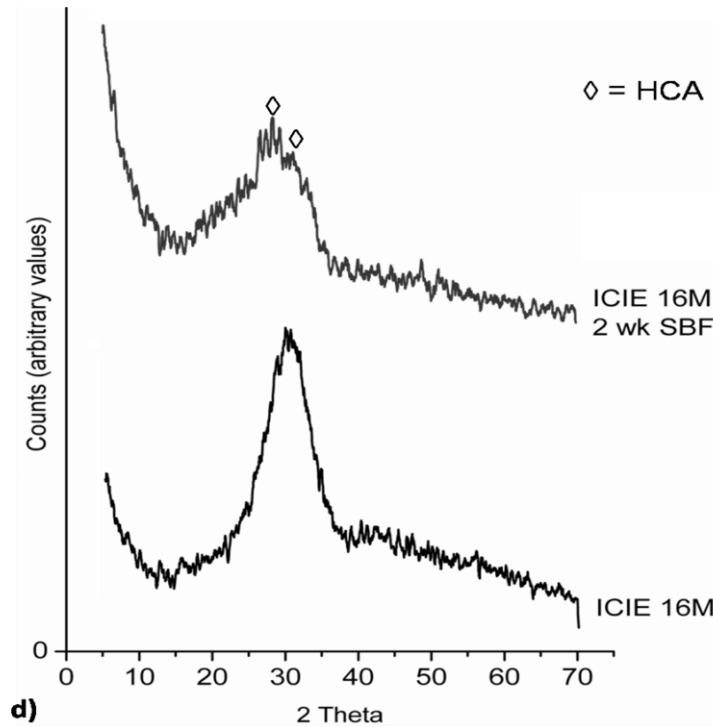


Figure 6.4 a) and c) are respectively the FTIR traces of ICIE 16 and 16M scaffolds immersed in SBF for 2 wks while b) and d) are the corresponding XRD traces of glass powder (<38 μm) and after 2 wks SBF

In Figure 6.5, it is clear that ICIE 16 lost more weight than ICIE 16M after 2 weeks immersion in SBF. ICIE 16 lost 22% of its mass within the first 8 h of immersion (10.5% to 21.5%). For ICIE 16 M, it took 24 h to lose a similar amount (from 4.8% to 23.9%). After these time points, the weight loss of ICIE 16M was small (23.9% to 27.2%), unlike ICIE 16, which the rate of weight loss was still fairly rapid (from 21.5% to 35.1%).

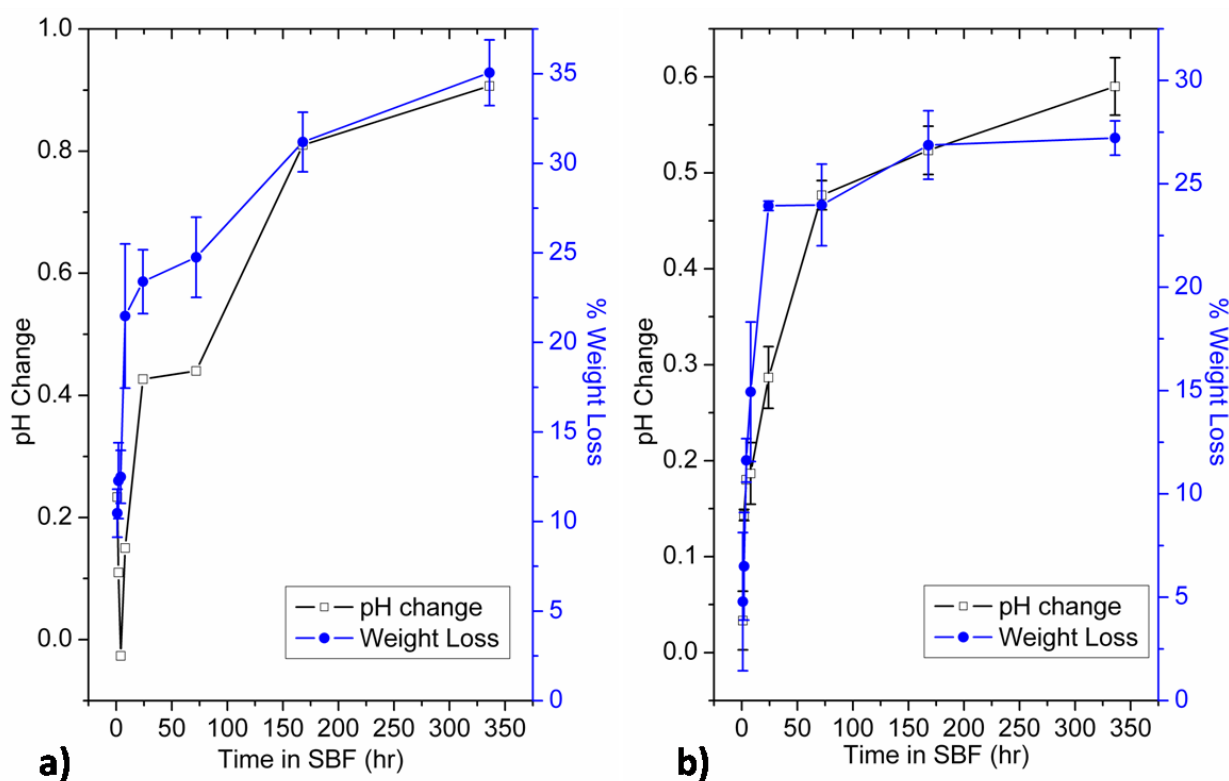


Figure 6.5 a) the pH and % weight loss of ICIE 16 throughout the time immersed in SBF and b) the same for ICIE 16M

Both scaffolds experienced an increase in pH change (pH after immersion - pH before immersion) (0.23 to 0.91 for ICIE 16 and 0.03 to 0.59 for ICIE 16M) with ICIE 16 being more active than ICIE 16M yet again. The results confirmed the FTIR data that ICIE 16 scaffolds are more bioactive than ICIE 16M scaffolds.

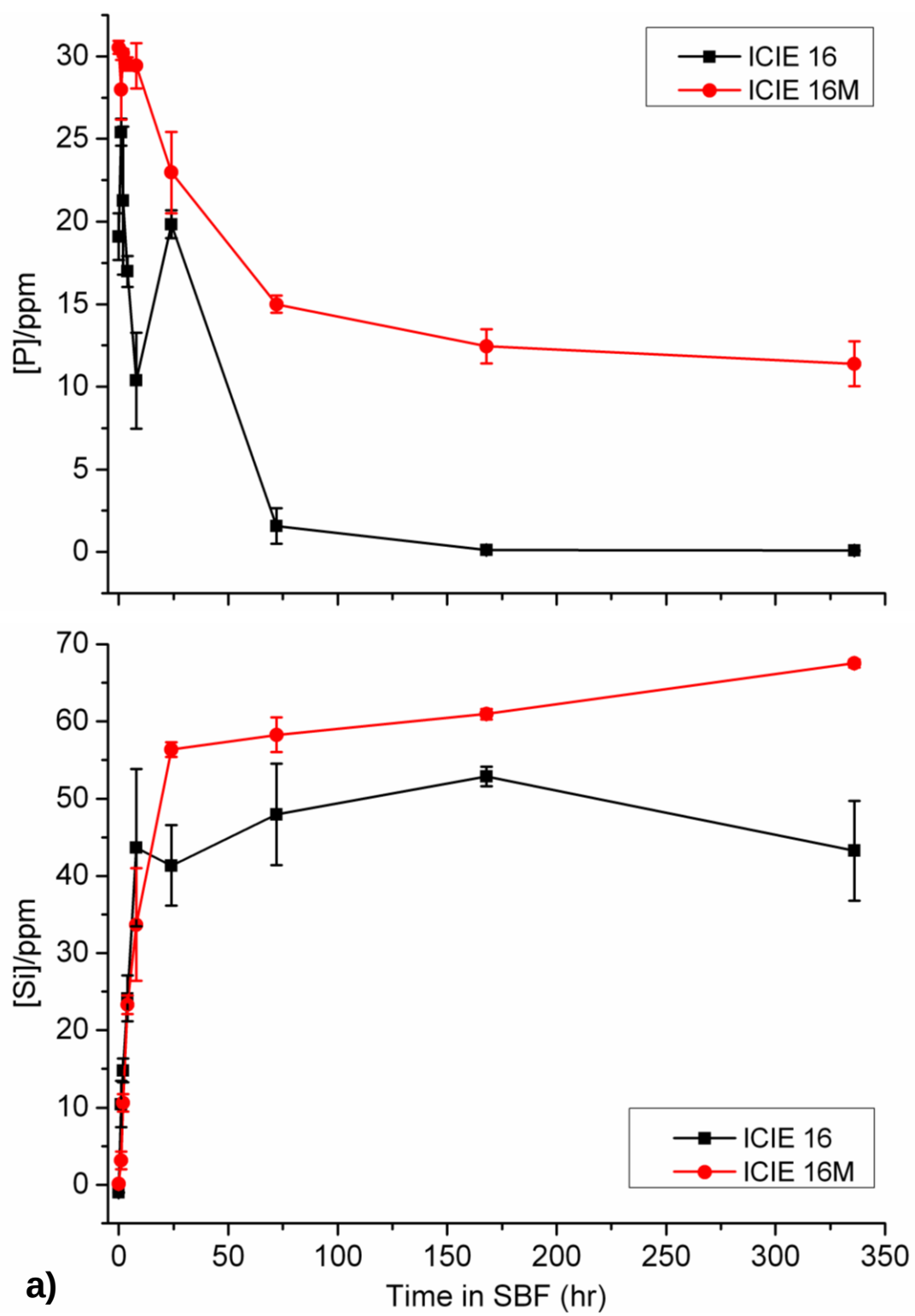
Dissolution

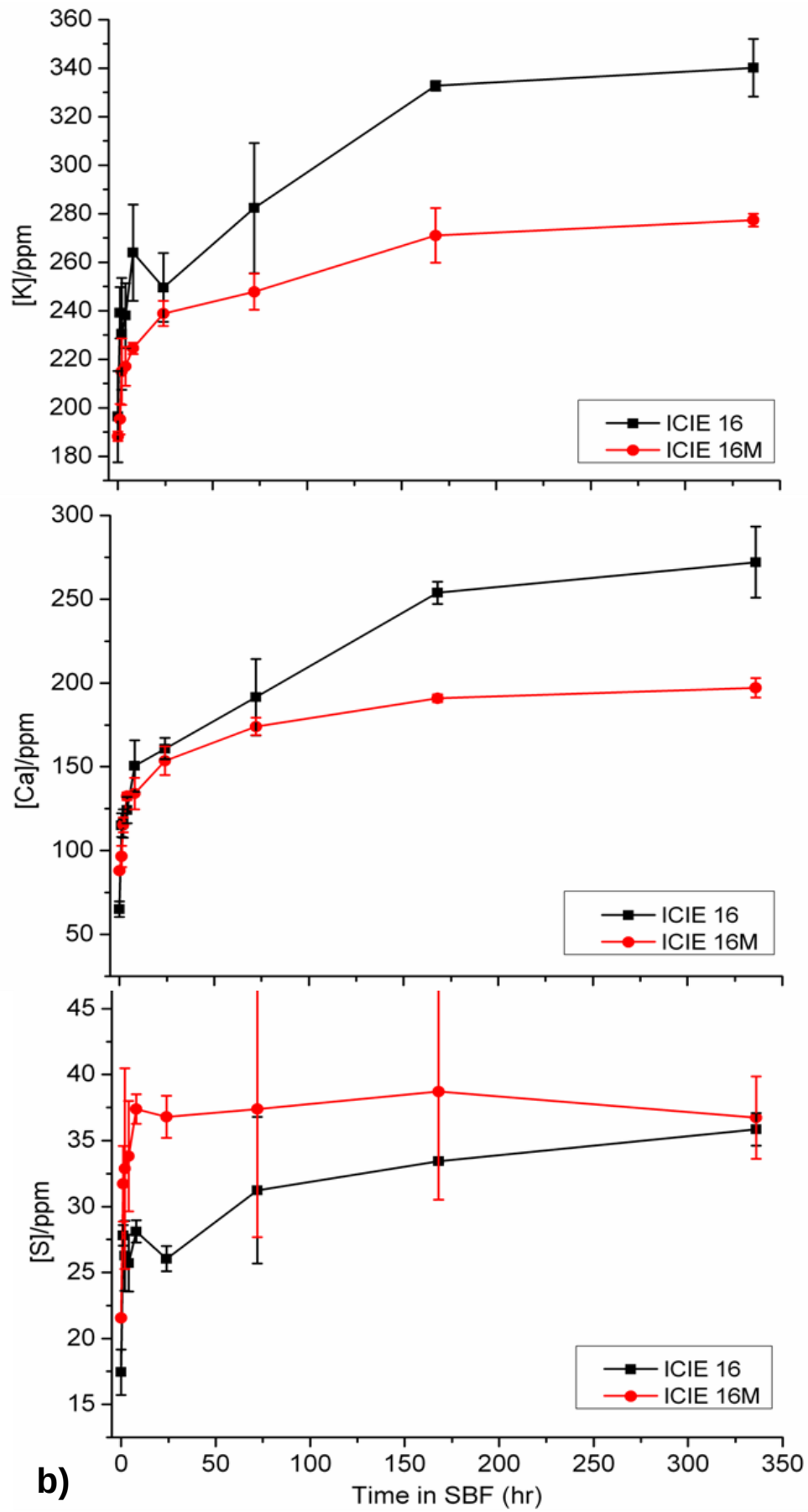
Figure 6.6 shows the dissolution (ICP) data. Ca, P, Si, K, Na and S were all released slowly into the SBF for both glasses, in addition to the extra ions such as Sr, Mg, Zn for ICIE 16M.

Theoretically the longer the soaking time of scaffold in the SBF, the higher the silicon ion concentration due to the breaking of the glass network. The concentration of calcium ions will also increase throughout time, but it will decrease again after a certain amount of time, which is when calcium starts to deposit along the glass surface. On the contrary, the phosphorus ion concentration will decrease over time, due to the formation of HCA layer on the glass surface. In practice, the phosphorus was taken up by the sample from the solution and none was left after 1 week of immersion for ICIE 16, indicating P deposition on the glass; which is not the case for ICIE 16M, after 2 weeks there was still 15 ppm of P in the solution which confirms slower apatite formation in ICIE 16M than in ICIE 16. After 1 day of immersion of the ICIE 16 scaffold in SBF, there was an increase of phosphorus in the solution but a decrease in all other elements except calcium. It is therefore possible that some amorphous calcium phosphate deposited on the glass and then redissolved before reprecipitating on the glass again. This has been observed in sol-gel derived bioactive glasses using synchrotron XRD [134-135].

Interestingly, there is a correspondent drop in pH change in Figure 6.2a at the exact time point, which could be a result of this phenomenon. Approximately 35 ppm for ICIE 16 and 37 ppm for ICIE 16M of sulfur was released into the solution after 2 weeks, which was from the potassium sodium sulfate crystallites. From Figure 6.6b it is not clear whether all the sulfur crystals were degraded or not, but the majority was released during the first 8 hours of immersion. Sulfur in its element form is non-toxic and a vital component of all living cells, at a quantity this small it is unlikely to provoke any response from the body.

Sr and Mg were released readily into the solution for ICIE 16M but Zn behaved rather strangely. Considering the mol% of the three elements were exactly the same (3 mol%) in the composition, not only was the released amount extremely low (maximum at 0.23 ppm) compared to the Sr and Mg (23 ppm and 53 ppm respectively), but after 8 h there was a sharp decrease (from 0.23 to 0.13 ppm) and after 1 day it reached a saturation at 0.07 ppm. This means that Zn was not released from the network, and after a small release of Zn ions into the solution, it was taken up again possibly by the glass or the orthophosphates and formed a stronger bond with either of them, which prohibited the further release of Zn ions. This evidently confirmed that Zn ions, unlike Mg ions which were also network intermediates, were acting as network formers in this case, and this could be the reason that ICIE 16M was very slow in forming crystalline HCA.





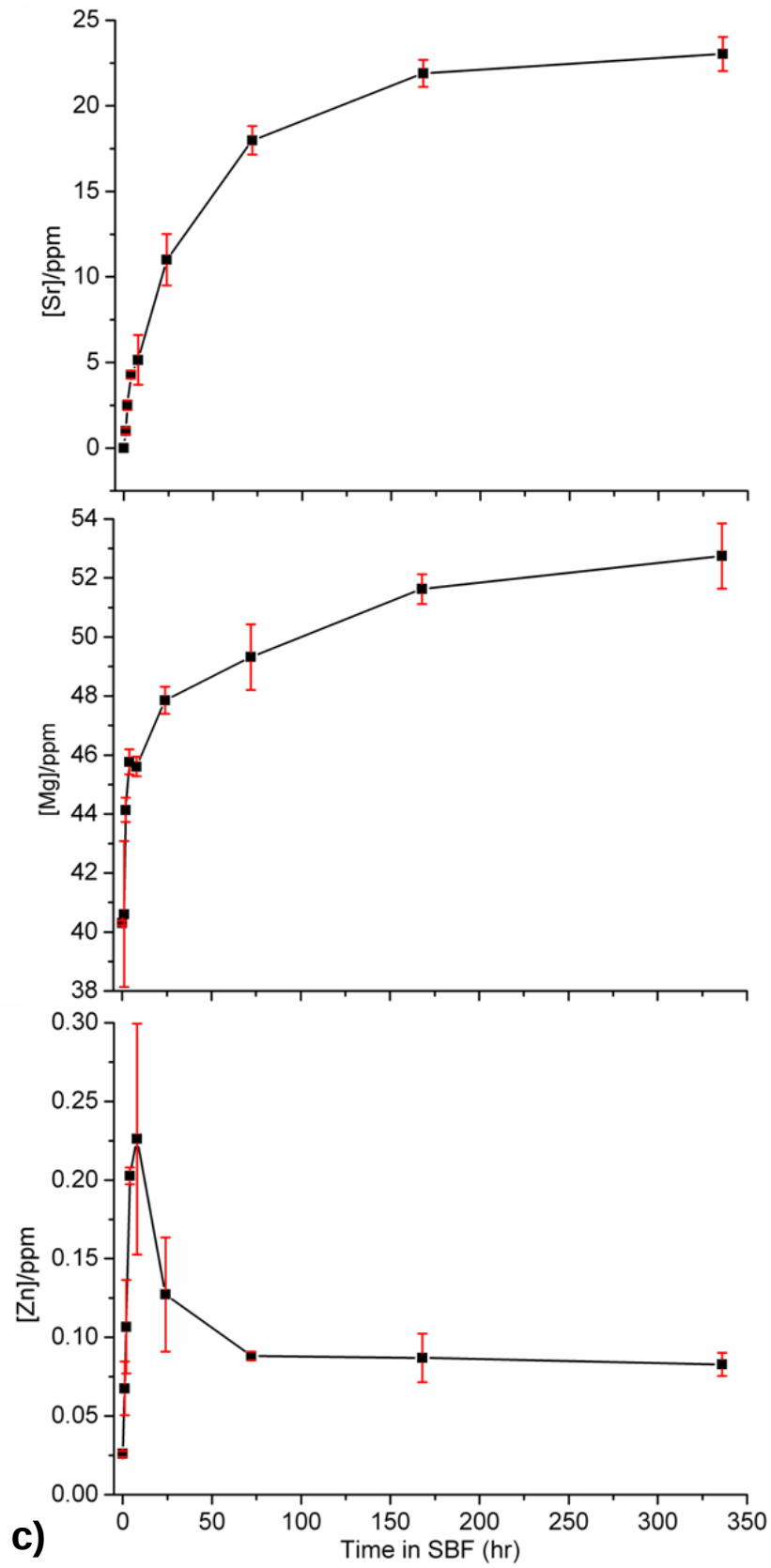


Figure 6.6 ICP traces of a) P and Si ions, b) K, Ca and S ions for ICIE 16 and ICIE 16M glass, in addition to c) Sr, Mg and Zn ions for ICIE 16M

In section 3.2 the NCs were calculated and summarized in Table 3.2, which can now be revised according to this discovery of the role of Zn in ICIE 16M, the revised version is Table 6.3:

Table 6.3 The NC values of different glasses revised

Glass	NC value
Bioglass [®] 45S5	2.11
ICIE 16	2.13
ICIE 16M (Mg ²⁺ as modifier and Zn ²⁺ as former)	2.35

6.4 Summary

The pore and interconnect sizes are different for different batch of samples produced under the same protocol and conditions. ICIE 16M scaffolds have greater compression strength than ICIE 16 scaffolds due to superior sinterbility. Both scaffolds have met the interconnect size requirement (128 μm for ICIE 16 and the 113 μm for ICIE 16M) for an ideal scaffold ($> 100 \mu\text{m}$). The pore and the interconnect sizes for ICIE 16M were both smaller than ICIE 16, as an evidence of better sinterbility of ICIE 16M. After immersion in SBF for 2 weeks, ICIE 16 (amorphous HCA formation at 8 h which crystallized at 72 h) outperformed ICIE 16M (amorphous HCA formation at 72 h and just started to crystallized at 2 weeks) in apatite formation rate in SBF, which is because the network intermediates are acting as network formers and resulting in a larger network connectivity value for ICIE 16M.

7 Conclusions and future work

7.1 Conclusions

Highly interconnected 3D porous melt-derived BG scaffolds were successfully fabricated using the gel-cast foaming technique; the standard protocol to produce optimum foam structure was derived, which consisted of 20 g glass powder, 18 ml water, 6 g acrylamide (monomer), 3 g N' N'-methylene bisacrylamide (cross-linker), 2 ml ammonium persulfate (APS) solution (initiator), 0.1 ml Triton X (surfactant) and 4 ml tetramethylethylene diamine (TEMED) (catalyst). The more water, the less catalyst and the less initiator, the longer the gelling time with a greater foam body volume; on the contrary, the more the initiator in the solution, the larger the foam body.

A suitable drying program was verified to be at 125 °C for 10 hours. A two stage sintering program for optimum sintering was also determined, where at first it was ramped at 2 °C /min to 350 °C, held for 1 hour and ramped up again at 2 °C /min to 700 °C, held for another hour, in order to burn out the organic components and sinter the glass particles.

The modal interconnect size was 128 μm for ICIE 16 scaffolds and 114 μm for ICIE 16M ones whereas the modal pore size was 379 μm and 476 μm respectively. After 8 hours immersion in SBF, amorphous HCA was formed and crystallized in 3 days for ICIE 16, and the ICIE 16M formed amorphous HCA in 3 days, which just started to crystallize after 2 weeks of immersion. The compressive strength was determined to be around 2 MPa for ICIE 16 and slightly stronger for ICIE 16M scaffolds at 2.6 MPa.

Overall, ICIE 16M was better for processing than ICIE 16 whilst ICIE 16 outperformed ICIE 16M in bioactivity studies. The ICIE 16 scaffolds would be more suitable as new artificial bone graft materials.

The gel-cast foaming process can be applied to fabricate highly porous and interconnected scaffolds made of any melt-derived BGs, with only small modifications required at specific parameters e.g. the sintering temperature, and there is immense potential for mass production.

7.2 Future Work

The role of Zn in ICIE 16M requires further investigation, the best way to do so is carry out ^{29}Si Nuclear Magnetic Resonance (NMR) spectroscopy on both ICIE 16 and ICIE 16M to see whether the addition of Zn has had an impact on glass structure. If Zn is indeed acting as a network former, there will be more NBOs in ICIE 16M than in ICIE 16, which can be identified as the peak will shift towards the negative side of the spectrum.

The crystallization of potassium sodium sulfate was unavoidable when using the methacrylamide gelling system. Melt-derived BGs other than ICIE 16 and 16M might not suffer from the same phenomenon depending on the composition, however it is likely that if a glass is reactive enough to be bioactive it is likely to suffer this problem if water is present. Although it may not be a great issue in terms of biocompatibility and can be controlled by reducing initiator content as well as modifying the drying/sintering program, it is sensible to explore other options. Many gelation systems have been developed, mainly based on water soluble epoxy resin and sodium alginate as gel formers. For epoxy resin gelling system, the gelling time was extremely long (>45 minutes in general) and the sintering temperature required for burning out the organic parts were too high (>1000 °C) [136-139]. For the sodium alginate gelling systems it is possible to control the gelling time by applying different reagents [140] but the setting time takes a few hours, despite complicate process and the prolonged preparation

time of the sodium alginate solution [141]. Therefore such systems are not suitable for the purpose of this study.

Potozeck *et al.* produced highly porous HA foams by using agarose as the gelling agent, Tergitol TMN-10 as the surfactant and agitate the slurry at 65 °C [142-143], gelation occurred when the foam was cooled to 15 °C. The pore network was controlled by the vol% of HA in the system and agarose concentration, mean pore size ranged from 130–380 µm and 37–104 µm for interconnect size. Porosity ranged from 73–92% as mechanical strength decreased from 5.9–0.8 MPa. Agarose is a substrate of red algae and have been long used as a food ingredient, therefore it is completely safe and hazard free. Gelatin based gel-cast system works in a similar way to agarose [144] where the slurry was agitated and foamed at 30 °C, then cooled to around 10 °C for gelation to take place and Fongraminox KC-B was used as the surfactant, the gelling time was about 23 min, the scaffold properties remained uninvestigated [145]. These two systems are the potential candidates for a safer, sulfate-free gel-cast foaming system that require a thorough investigation to determine the standard processing protocol.

Cell culture studies also need to be carried out to investigate the cell attachment, cytotoxicity and LIVE/DEAD viability of the scaffold.

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Appendix A – 4-step μ CT image analysis process⁴

1. A $3 \times 3 \times 3$ median filter was applied to remove the noise and then the image was binarised into two phases: the struts and the background (void spaces which were identified as the pores). A $5 \times 5 \times 5$ morphological closing operator (dilation followed by erosion) was then applied to remove the fine intra-strut porosity, which otherwise reduces the watershed accuracy (step 3 below).
2. A distance map was then generated by successive dilation of the strut phase until all the pore voxels were filled. The distance function is then the number of steps need to reach that voxel in the pore, while the final voxels to be filled, or regional maxima in the distance in the 3D distance map, were tagged as the macropore centroids [123-124].
3. A 3D watershed algorithm [146] was then applied to the distance map and centroids. A watershed algorithm uses the rise and fall the distance map values to group voxels together where any water falling on those voxels would all run down to the same local minima. Here, the distance map is inverted and the lowest values at the struts are high point ridges where the water sheds into the centroid segmenting the space between struts into different macropores. Once the macropores are defined, the interconnects are easily identified as groups of voxels touching two macropores.

⁴This information is provided by Mr. Sheng Yue, who has also carried out all the μ CT analysis.

4. After the pore space was segmented thoroughly, the sizes of each pore and interconnect could be represented by their equivalent diameters. The equivalent diameter of a pore was the diameter of a sphere that has the same volume to this pore. The equivalent diameter of an interconnect was firstly quantified by using a principal component analysis (PCA) based method [125]. This particular method found the best fit principal plane to each interconnect and projected all the voxels that belonged to that interconnect to the principal plane. The diameter of a circle with the same area of the projected voxels shade was the equivalent diameter of the interconnect.