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Biocompatibility of $CaO-Na_2O-SiO_2/TiO_2$ Glass Ceramic Scaffolds for Orthopaedic Applications.

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***Abstract* — This work aims to determine the effect of substituting TiO_2 for SiO_2 in a $0.62SiO_2$-Na_2O-0.24CaO based glass-ceramic scaffold. High temperature X-ray Diffraction (HT-XRD) was used to determine the sintering temperature (700°C). Both optical microscopy and x-ray micotomography was used to determine the average pore size (540-680μm) of each scaffold. Cytocompatibility of each scaffold was conducted using murine mesenchymal stem cells.**

Keywords-component; Glass-ceramic scaffold, bioactive glass, porosity, Stem cell.

I. Introduction

Bioactive glasses and glass–ceramic materials are proving to be suitable candidates as bone substitutes and scaffolds. 3D porous scaffolds are preferential to using traditional particulates or granules in treating large bony defects as they provide an interconnected network which permits hosts cell migration, nutrient delivery, bone ingrowth and eventually vascularization[1]. Some specific attributes required for an ideal scaffold include the ability to deliver cells to the wound site, excellent osteoconductivity, biodegradability, appropriate mechanical strength, high porosity (90 %) and a specific pore size (400–500 μm). Bioactive glasses meet a number of these criteria (excellent osteoconductivity and bioactivity, ability to deliver cells and controllable biodegradability) which makes bioactive glasses an attractive group of materials as scaffolds for tissue engineering[2]. This study investigates the substitution of TiO_2 for SiO_2 in the glass phase of the starting materials and its effect on the structural, mechanical and biological properties.

II. Materials And Methods

Three glass compositions were formulated for this study. The SiO_2 content of the glass was substituted by TiO_2 throughout the series, *Sc1* and *Sc2* (Table I). A Ti-free glass was used as a control (*ScC*) for comparison. Glasses were prepared by weighing out appropriate amounts of analytical grade reagents (Fisher Scientific, Pittsburg PA, USA) and ball milling (1 h). The powdered mixes were oven dried (100°C, 1 h) and fired (1,500°C, 1 h) in platinum crucibles and shock quenched in water. The resulting frits were dried, ground and sieved to retrieve glass powders with a particle size less than 25 μm.

Scaffolds were produced with each glass formulation denoted *ScC*, *Sc1* and *Sc2*, where *Sc2* contains the highest concentration of TiO_2. Polyvinyl alcohol (PVA, 0.008g) was initially dissolved in 9.3 ml of de-ionised water for 1 h and heated to 55°C. 12 g of glass powder was added to each flask and stirred for 1 h. 10mm x 8 mm cylindrical polyurethane foams were cut and immersed in the glass slurry, stirred with a spatula to ensure all pores were filled within the slurry. After approximately 10 min the glass embedded foam was allowed dry on a foam bed for 24 h. The scaffolds were then heat treated at ~700°C to remove the foam and sinter the suspended glass particles.

TABLE I. GLASS COMPOSITION (MOL%)

	SiO_2	TiO_2	Na_2O	CaO
ScC	0.62	0.00	0.14	0.24
Sc1	0.57	0.05	0.14	0.24
Sc2	0.52	0.10	0.14	0.24

High Temperature XRD - Siemens D5000 XRD unit with a Vantec1 linear position-sensitive detector. Cu Kα radiation was used, and measurements were collected over an angular range of 10–70° 2θ with scan rate of 2.25°/min. Patterns were measured at RT and from 400 to 800°C in steps of 20°C.

Pore Size - Olympus IX20-UCB Optical Fluorescent Microscope at 49X. Mean pore size was calculated by measuring the diameter of (1) starting polyurethane scaffolds, (2) *ScC*, (3) *Sc1* and (4) *Sc2*. 10 pores were measured from three different scaffolds (total n = *30*)/scaffold.

Murine Mesenchymal Stem Cells – Harvested from transgenic DsRed mice which constitutively express a red fluorescent protein. Scaffolds were sterilized by autoclaving at 120°C for 20 min and stored overnight in complete culture medium. Each scaffold was examined by epi-fluorescent microscopy. MSC proliferation was determined by WST-1 assay (Roche Diagnostics,

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Indianapolis, USA) according to the manufacturer's instructions[3].

III. Results And Discussion

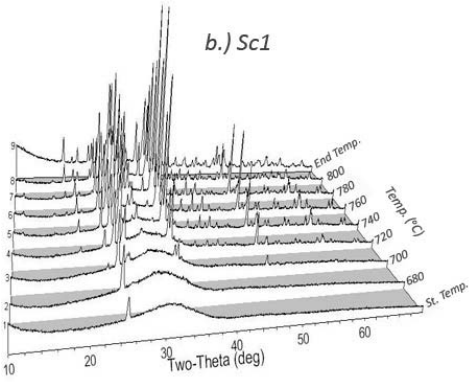

Figure 1. High temperature X-ray diffraction patterns Sc1 glass-ceramic scaffold.

Fig 1. High temperature XRD (HT-XRD) was undertaken to determine the degree of crystallization occurring at the sintering temperature during processing, and also to determine crystal phases present. HT-XRD revealed that low-level crystallinity was present at the sintering temperature; however, the characteristic amorphous trace was also retained. As was expected, with an increase in temperature (820°C), the amorphous content from each material was partially converted to exhibit crystalline phases. HT-XRD phase identification revealed $Na_2Ca_3Si_6O_{16}$, combeite (Na_6Ca_3-Si_6O_{18}) and quartz (SiO_2) phases which were present in each material after cooling. It was observed that as the concentration of TiO_2 increased, the degree of crystallinity was found to reduce to the point where *Sc2* partially retained a higher degree of its amorphous character, suggesting Ti possible role in inhibiting crystallization.

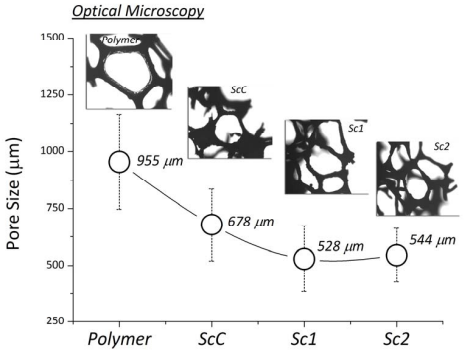

Figure 2. Porosity polymer scaffold and ScC, Sc1 and Sc2 sintered scaffolds.

Fig 2. Optical microscopy was used to determine the mean pore diameter of the starting foam, and the sintered glass/ceramic scaffolds. Measurement of the polymer foam determined a mean pore diameter of 955 mm. The Ti-free control (*ScC*) exhibited a mean pore diameter of 678 µm, while *Sc1* and *Sc2* showed a significant reduction in pore diameter when compared to the pre-processed polymer, 528 µm and 544 µm respectively. This is expected as the polymer burns out, the glass particles densify during the sintering process resulting in a reduced pore diameter. The smaller pore diameter as experienced with *Sc1* and *Sc2* suggests a higher degree of densification than the control *ScC*.

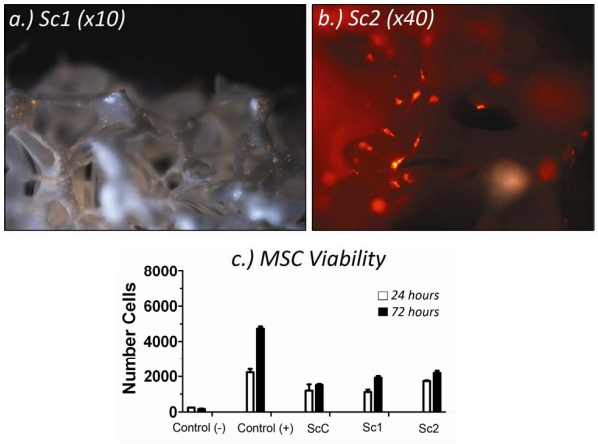

Figure 3. Stem cells attached to scaffold surface and cell viability after 24 and 72 hours..

Each scaffold was subjected to cytotoxicity testing using MSC in order to determine cell viability after 24 and 72 h exposure times. *ScC*, *Sc1* and *Sc2* were each able to support the attachment and proliferation of adult MSCs. Fig 3 shows that MSC colonized all scaffolds well and that MSC numbers increased over the 24 to 72 h culture period, which was particularly evident regarding the Ti containing scaffolds[3]. This study suggests that Ti-substituted for Si may provide a beneficial structural and therapeutic effect when fabricating glass/ceramic scaffolds for bone augmentation.

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