



# A Novel Hydroxyapatite Bone Tissue Engineering Scaffold Incorporating a Tri-Modal Pore Distribution



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## Introduction

The aim of scaffolds has evolved from providing an inert biocompatible construct facilitating vascularisation and cell invasion to a tissue engineered approach, whereby the construct is initially seeded with cells, cultured *in vitro*, and finally implanted. Scaffold characteristics such as interconnectivity, pore size, microporosity, macroporosity and surface roughness influence cellular responses, but they also collectively control the degree of nutrient delivery, penetration depth of cells and metabolic waste removal.

Many of the existing sponge-type scaffolds provide a single domain into which cells are seeded and, during *in vitro* culturing, proliferate and migrate to a small extent into the available pores [1]. The populating cells cannot migrate into the interior regions of the scaffold. This is believed to be due to insufficient delivery of nutrients and oxygen, and also removal of metabolic waste products [2]. In addition, the high mass transfer rates at the fluid/scaffold interface are believed to cause higher mineralization which acts as an effective diffusion barrier [3]. It is essential that a scaffold possess suitable interconnectivity and pore size to minimise diffusion limitations and pore occlusion prior to vascularisation.

## Objectives

To address these issues we have developed a hydroxyapatite (HA) ceramic scaffold with a tri-modal pore distribution in order to provide non-competing domains for (1) cell seeding and nutrient diffusion, (2) cell proliferation and mineral formation, (3) vascularisation once implanted

## Materials & Methods

### Materials

The hydroxyapatite (HA) ceramic scaffolds are fabricated from a HA powder (Plasma Biotol) and deionized water suspension, employing Darvan 811 (RT Vanderbilt) as a defloculating agent and methyl cellulose (Sigma-Aldrich) as a binding agent to improve the green body handliability and preventing collapse of the resulting porous structure during sintering.

### Methods

The ceramic suspension is cooled at a constant cooling rate to a desired final freezing temperature ( $-10^{\circ}\text{C}$ ). The frozen suspension is then sublimated under a vacuum ( $<100\text{mTorr}$ ) for 17h at a temperature of  $0^{\circ}\text{C}$  [4] which results in a micro- ( $<5\mu\text{m}$ ) and mesoporous ( $90\text{-}100\mu\text{m}$ ) structure. A unidirectional macrochannel pattern ( $500\mu\text{m}$  pore size and spacing) is introduced into the green body structure through CNC machining. Green body discs ( $\varnothing 5\text{mm}$ ) are subsequently sintered to  $1500^{\circ}\text{C}$ . To determine the average porosity of the sintered ceramics, the densities of 10 scaffolds were measured and compared to the theoretical density of HA. Saturation experiments were carried out to determine the mass water retention capacity of the mesopore phase.

Osteoblast-like (MC3T3-E1) cells are being employed to assess the biocompatibility of the scaffold and the influence of the macrochannels on the cell distribution throughout the scaffold depth. The oxygen consumption rate of MC3T3-E1 cells cultured on hydroxyapatite discs is determined using an oxygen-sensing microplate (OBS System, BD Biosciences). Effective oxygen diffusion coefficients of the mesopore phase are measured using a dissolved oxygen electrode (ISO2 probe, WPI) and a Franz diffusion cell (PermeGear). Using the data from these experiments, a finite element model will be utilised to determine the optimal pore configuration to minimise oxygen diffusion limitations within the scaffold core.

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## Results

Figure 1. demonstrates the scaffold architecture produced, showing macro channels (a-c) and finer mesopore structure (c-e) in the regions between the macrochannels, also containing a degree of microporosity (f) Figure 1e, shows a cross-section of a macrochannel, demonstrating an open interconnected structure. The total porosity of these scaffolds was calculated to be 84%, with a saturation capacity of  $150\mu\text{l}$  for scaffold discs of diameter  $10\text{mm}$  and height  $3\text{mm}$ . The shrinkage occurring due to the sintering process was calculated as 18%.

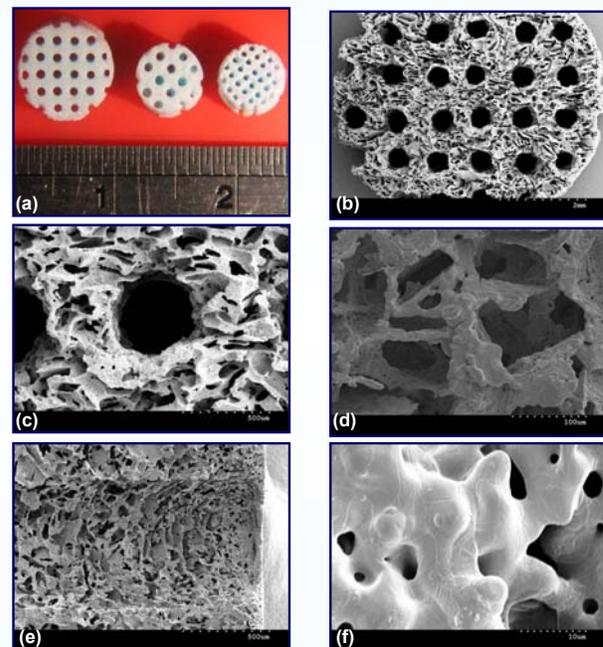


Figure 1: Porous hydroxyapatite scaffold architecture

## Discussion & Conclusion

Scaffolds with a tri-modal pore structure have been successfully fabricated. The large unidirectional, oriented macropores provide three functions. Firstly to aid in uniform cell seeding; secondly to provide a direct diffusion route for oxygen and nutrients to the scaffold interior to promote matrix formation and thirdly to permit vascularisation once implanted *in vivo*. The mesopores provide a second diffusion route as well as providing a domain for cell proliferation and migration. Finally the microporosity provides a third albeit limited diffusion route and the volume for pre-conditioning the scaffold with biochemical agents. Current work will assess the distribution and viability of MC3T3-E1 osteoblast-like cells within the mesoporous phase, and the influence of the macrochannels on the local oxygen environments.

## References

- [1] Sachlos E & Czernuszka JT, Eur Cell Mater 2003; 30:29-39. [2] Galban CJ & BR Locke, Biotechnol Bioeng 1999; 65:121-132 [3] Martin I et. al. J Orthop Res 1998; 16:181-189 [4] O'Brien FJ et. al. Biomaterials 2004 25:1077-86.