## Freeze-drying of Chitosan-PCL blends impregnated with microporous hydroxyapatite from algal origins

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### Introduction

Tissue engineering aims to provide a viable means of supporting bone regeneration whereby an inert biocompatible construct mimics the natural composition (collagen: mineral ⇒ polymer: ceramic) of bone. The literature describes several scaffold fabrication methods each with inherent disadvantages mainly associated with the inability to oxygenate and transfer nutrients to the interior regions of the scaffold [1]. In terms of cell culture, highly porous interconnective scaffolds are more favourable as they support angiogenesis. However, this morphology can be to the detriment of the scaffold in load-bearing situations, as the porosity compromises the mechanical properties of the construct.

The aim of this work was to fabricate a tri-phasic construct, whereby a polymeric phase was impregnated with a natural ceramic to provide a suitable morphology for bone re-growth. To improve the mechanical properties of the polymer phase, PCL was added to chitosan to form a matrix structure. Its purpose was to compensate for the deficiency of mechanical strength induced by porosity in both phases. Freeze-drying was used to induce the required macro/mesopores morphology in the polymer matrix. Furthermore, the microporosity from the natural porous ceramic provides an additional support mechanism for nano-vascularisation and has the potential to act as a drug delivery system with growth factors to further induce bone remodelling.

### Methods

Design of experiments (DOE) was used to assess the combinatorial effect of porosity on the mechanical properties of the construct. A full 2-level factorial design with 3 factors was built using Design Expert software package V5 (Stat-Ease Inc., MN, USA). The factors studied were 1) polymeric ratio, 2) polymeric concentration, and 3) percentage ceramic. The ceramic material used was derived from the calcified red algae *Corallina officinalis,* by a low-pressure synthesis technique [2]. Chitosan was purified and blended with PCL using a similar method as described by *Sarasam et al* [3]. Thereafter the polymer/ceramic blends were prepared by stirring for 2 minutes at room temperature to ensure homogeneity. The blends were injected into a mould immersed in liquid nitrogen to prevent the ceramic settling together during freezing. The moulds were then frozen at -80°C overnight, followed by freeze-dried for 5 days in a Lyophilizator. The constructs were characterised using scanning electron microscopy (SEM), micro-computer tomography (μCT) and BET nitrogen adsorption. In addition mechanical properties were determined under wet and dry conditions using compression testing.

### Results and Discussion

According to Design Expert the optimal processing conditions were achieved using a 1:5 ratio of chitosan to PCL respectively, dry blended with 30% ceramic material. Under these conditions the highest compressive modulus achievable was >1MPa. The μCT results in figure 1.1 indicate a significant decrease in porosity between the control samples (no ceramic) and the constructs, as its resolution cannot detect the algal pores of >10μm. Therefore, BET is used to complement these results by correlating variation in surface area to porosity.

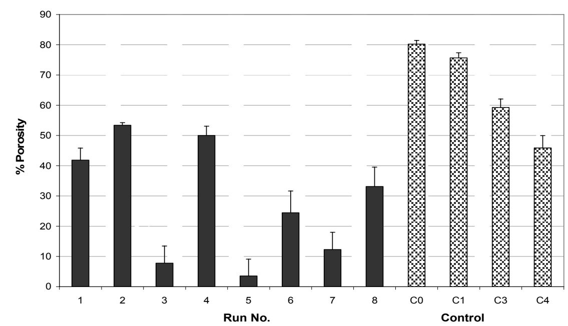


**Figure 1.1: μCT results of porosity in the constructs**

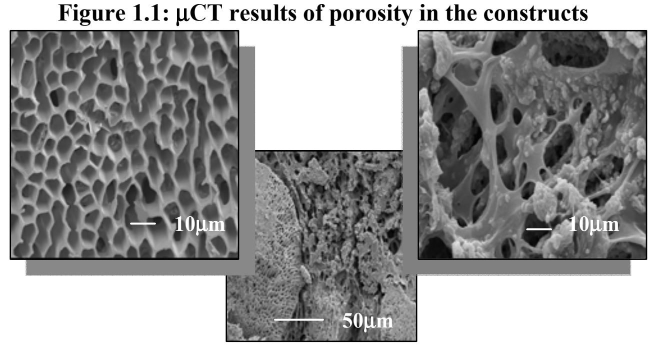


**Figure 1.2: SEM of internal morphology - a) raw algae (ceramic phase) b) and c) optimal construct**

Figure 1.2 shows the typical internal morphology achieved by freeze-drying. A highly porous interconnective structure is clearly visible.

### Conclusions

A novel process has been developed to produce interconnected tri-phasic structures ideal for bone tissue engineering applications. The scaffolds have unique porosity and properties can be tailored using DOE

**References**

1. Buckley et al., 12th BINI, Galway, 2006
2. Walsh *et al,* ESB, Sorrento, 2005
3. Sarasam *et al*, Biomaterials: 26 (2005): 5500-5508

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