

## Collagen-Hydroxyapatite scaffolds for Hard Tissue Engineering with a predefined architecture and shape

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**INTRODUCTION:** The aim of hard tissue engineering is to produce tissue to repair damaged or diseased skeletal bones. A porous matrix (the scaffold) comprising collagen-hydroxyapatite (Col-HA) can be tailored to the shape of any defect using a 3-D printing technology and its internal architecture is controlled, through control of the porosity and internal micro-vasculature during the manufacturing process.

**METHODS:** The external and internal geometry of a mould is pre-designed (Mechanical AutoCAD 2005) and printed using solid freeform fabrication<sup>1</sup>. Hydroxyapatite powders and bovine collagen (Sigma-Aldrich) were suspended in an acidic aqueous solution at pH=5, poured into a mould and frozen. The freezing temperature (-20°C, -80°C), crosslinking by dehydrothermal treatment (DHT) and solute concentration (1-5wt% collagen) were varied in order to optimise scaffold porosity and its resistance to collagenase degradation. The mould was removed by ethanol, and the water removed by critical point drying<sup>2</sup>. A low voltage scanning electron microscopy (SEM; JSM-840F JEOL) was used to measure porosity through image analysis (Image-J); differential scanning calorimetry (METLER DSC-821e), swell ratios and resistance to collagenase were used to determine the amount of crosslinking. Finally, human osteosarcoma cells (MG63) were suspended in a culture medium and seeded ( $1 \times 10^5$  cells/scaffold) onto Col-HA scaffolds.

**RESULTS:** Figures 1 illustrates the ability to control the porosity of a Col-HA scaffold by changing the solute concentration or the freezing rate. Pore sizes varied between 10µm to 350µm. Pore size distribution curves revealed a higher percentage of large pores (mean distribution peak shifts from 20µm to 50µm), for scaffolds manufactured with low solute content and lower freezing rates. DHT has not induced any denaturation of the scaffold and has altered the thermal stability, the swell resistance and the degradation resistance to collagenase. These can be correlated to the amount of crosslinking present. The external and internal shape of the scaffolds is controlled by printing out different moulds of different size and design.

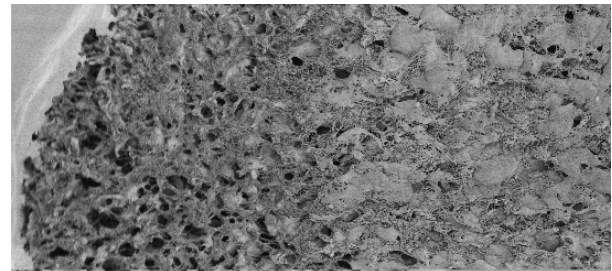


Fig. 1: Varying the pore size across a Col-HA scaffold (left: 1wt% collagen, right: 5wt% collagen)

Figure 2 illustrates the viability of osteogenic cells (MG63) on a collagen/HA scaffold surface after 14 days of culture.

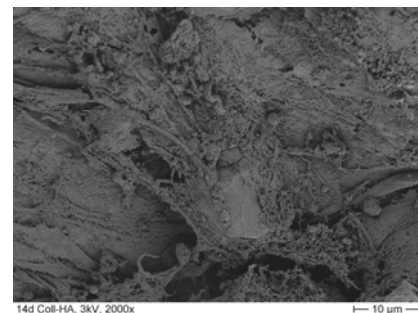


Fig.2: SEM image of cells cultured for 14 days onto a Col/HA scaffold

**DISCUSSION & CONCLUSIONS:** Choosing biocompatible materials for the moulds and scaffolds will lead to the reduction of implant rejections. The ability to control pore geometry within the scaffold has the potential to optimise mechanical and biological properties. The current issues facing tissue engineering of 3D tissues arise from the passive diffusion limitation of cells, which cause cellular necrosis within the centre of engineered scaffolds. The potential to have an internal vasculature system within a Col-HA scaffold may overcome such problems.

**REFERENCES:** <sup>1</sup> E. Sachlos and J.T. Czernuszka (2003) *Eur Cell Mater* **5**: 29-40. <sup>2</sup> E. Sachlos et al. (2003) *Biomater* **24**: 1487-97;

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