

Development of Hierarchies in Scaffold Architecture

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Introduction

In-vivo tissues are extremely complex in nature and the biological and physiological methods behind their creation are ongoing topics in current research. Within the body there exists structural hierarchies that are responsible for both the mechanical and physiological properties of the tissue. [1] Compositional changes in tissue can arise as a result of variations that occur in these structural components.

Hypothesis

Biological scaffolds are homogeneously fabricated and do not take into account the diversity with which biological tissues are constructed. As tissues continually vary in structure and composition within the body there is a need for a scaffold with similar character designed to meet that of the *in-vivo* environment

Objectives

The overall goal of this project is to develop simple techniques to control scaffold architecture and use these techniques to create a functional scaffold. The objectives will be twofold:

1. To develop techniques to generate biological structures with varying morphology
2. To create structurally graded scaffolds which emulate the *in-vivo* environment.

Materials and methods

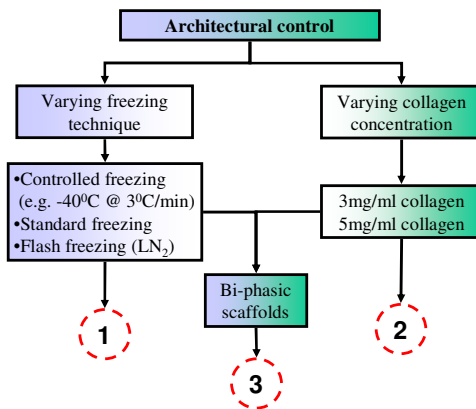


Figure 1. Methods used prior to lyophilization to create highly controlled and structurally graded scaffolds

ANALYSIS

SEM images of collagen scaffolds created with 3mg/ml and 5mg/ml solutions were analysed by stereological methods to determine pore size while porosity was calculated via a relative density method.

Serial histological sections of representative scaffolds were captured. As a feasibility study the Alcian blue stained serial sections were stacked into 3D using Image Pro Plus® (Media Cybernetics, Silver Springs, MD) to provide a visual conception of scaffold architecture

Results and discussion

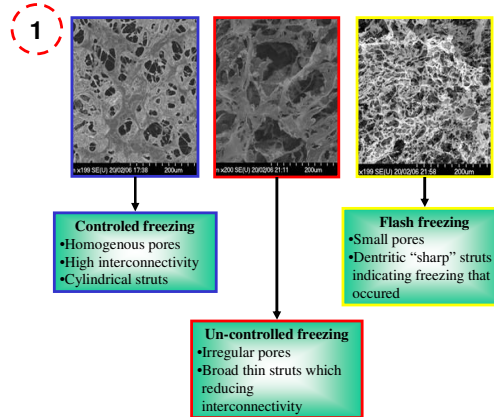


Figure 2. SEM images plus a summary of the resulting pore structures for the three different freezing cycles

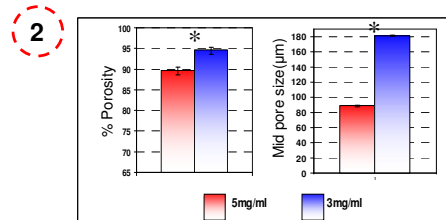


Figure 3. Porosity and mid pore sizes of 5mg/ml and 3mg/ml solutions lyophilised prior to controlled freezing. (* indicates statistical difference $p < 0.05$)

From Figure 3 it can be seen that porosity and pore size could be controlled with a high degree of accuracy by combining **controlled freezing** and varying the **collagen concentration**. With such diversity in scaffold architecture available both concentrations were combined to create single bi-phasic scaffolds whereby each concentration represents a single phase.

Bi-phasic scaffold

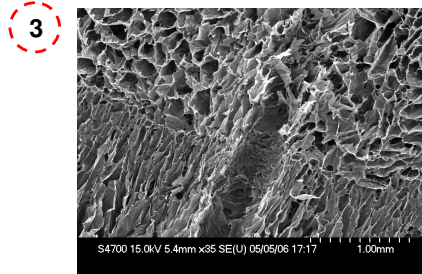


Figure 4. SEM images showing the interface between the two phases in the bi-phasic scaffold. Open pore channels have also been created during freeze drying by the incorporation of steel rods

By layering 3mg/ml and 5mg/ml solutions of collagen and lyophilizing the composite, bi-phasic scaffolds were created whereby the porous structure in each phase has been controlled.

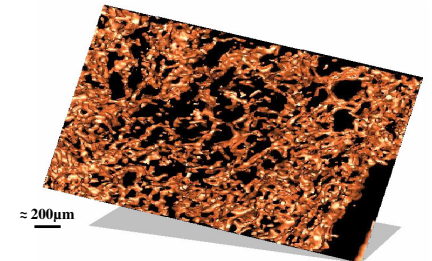


Figure 5. 3D reconstruction of serially stacked histological sections performed using 3D reconstruction software (Image Pro Plus®, Media Cybernetics, Silver Springs, MD). Alcian blue staining was used to label collagen type I under light microscope.

The collagen struts can be seen to project in all dimensions. Such 3D images can act as visual aids but with further research may also be used to determine pore size, porosity and three dimensional interconnectivity which are essential for cell infiltration.

Conclusions

Defined complex architectures were created by simple freezing techniques. These freezing techniques have been combined with varying collagen concentration to create structurally graded scaffolds designed to emulate the structural diversity that exists *in-vivo*

Such scaffolds may be of use in the regeneration of complex biological structures e.g. osteochondral junction, ligament bone interface, epidermis dermis attachment.

Future studies

Following from these promising preliminary results, future studies will include assessing the mechanical properties and characterisation of the pore morphology. Various cell types will also be seeded onto the scaffold to see the effect of different morphologies on cell phenotypes

Acknowledgements

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Reference

- [1] Liao *et al.*, J Biomed Mater Res Part B: Appl Biomater 69B: pp158–165, 2004