CHARACTERISATION OF POROUS BIOMATERIALS AND ROLE OF POROSITY IN THEIR PERFORMANCE

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Introduction. Tissue scaffolds provide a temporary matrix for culturing cells to form functional tissue. Providing a quantitative assessment of scaffolds manufactured from soft porous synthetic polymers or hydrated biopolymers is critical for both optimisation and quality control. This work describes experimental approaches to assessing the structure of soft polymeric and hydrogel tissue scaffolds using different techniques.

Materials and Methods. Three soft porous polymeric scaffolds were compared. A porous polycaprolactone (PCL) scaffold was prepared using the solvent casting/porogen leaching technique. A macro-porous hydrogel (MH) was synthesised by cross-linking polymerisation of hydroxyethyl methacrylate (HEMA) using a cryogelation technique (1). A commercially available hydrogel based on collagen cross-linked with glycosaminoglycan (CG), Integra Life Science Corp., USA, was investigated in its original hydrated form (98.5% w/w of water).

A range of microscopic methods were employed to investigate materials including confocal microscopy and environmental SEM. Additional techniques such as mercury porosimetry and gas flow porometry were used to study the structure of the PCL matrix. Micro-computer tomography (CT) in combination with reconstruction, 3D rendering and 3D-analysis software was applied to quantify the MH porosity and analyse pore interconnectivity by quantifying the number and size of connected pore-space domains using HR Scanner and 3D image analysis software (1). The approach based on the nuclear magnetic resonance (¹H NMR) method combined with freezing-out of bulk water was applied to study the nanoporous structure of the hydrogel. Molecular probes including aprotinin (6.7 kDa), bovine serum albumin (BSA-67 kDa) and fibrinogen (Fg-340 kDa) were used with a diffusion cell to assess diffusion driven molecular transport.

Results and discussion. The structural information derived from the imaging and invasive methods depend on the sensitivity of the technique, instrument resolution and limitations of the underlying theory. Mercury porosimetry and gas flow porometry allow measurement of the mean pore size and pore size distribution. However relating these data to those produced from structural images could be
misinterpreted because gas flow porometry is sensitive to the narrowest point along a pore which may not be even observed in an SEM or confocal microscopy image. The sensitivity of images to the thresholding methodology used to differentiate between pores and walls is a significant cause of variance in comparing data from different samples and imaging techniques. Highly hydrated materials are also subject to artefacts e.g. ice damage and distortion produced during sample preparation for electron microscopy.

There are no available techniques for measuring the nanostructure of the hydrated gels. Analysis of the thermodynamic properties of bulk and interfacial water using a layer-by-layer freezing-out technique (1H NMR) provided valuable information about the nanopores of CG hydrogels in the range up to 30nm. However all of the above mentioned tools do not produce structural information about the transparency of the matrix to molecular diffusion. μCT analysis provided information about the interconnectivity of the porous structure. Analysis of HEMA showed the total porosity of the matrix (94-96%), the pore size range (2.46-66.4mm) and allowed calculation of the total pore area ($39 \pm 5 \mu m^2/mm^2$). The scaffold permeability for nutrients was investigated by studying diffusion of molecular probes through the matrix. A range of biomolecules that differ in shape, size and charge distribution migrate through the matrix with the apparent diffusion coefficient which can be used to monitor scaffold permeability. Diffusion through the scaffold is a non-destructive measurement that has potential as a quality control method.

**Conclusions.** Assessment of the tissue scaffold performance is a challenging problem and could be only solved by using different types of invasive and non-invasive techniques. Measurement of the scaffold porosity and analysis of its microstructure should be combined with the complementary approaches to characterise the scaffold permeability to the molecular probes and interaction of the scaffold matrix with biomolecules as cell attractants.

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