Is Interconnected Pore Volume Equal to Pore Size?
Novel Technique to Evaluate Internal Pore Volume of Tissue Engineering Scaffolds

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Introduction: In response to increasing demand for tissue engineering applications, macroporous scaffolds composed of biodegradable polymers have been extensively used as 3D substrates to provide physical and mechanical support for cell proliferation and ingrowth. [1] Because current methods provide different definitions of "pore size" or "total porosity", there is a lack of a standardized measuring methodologies to evaluate the volume of the interconnected open space within the scaffolds for comparison purposes. Current methods typically employ *in vitro* cell culturing study to evaluate the efficiency of a porous structure, which involves a lot of time, money and resources. We are proposing a novel technical concept to describe the relationship between cytocompatibility and the open internal space of a tissue engineering scaffold. This technique will be able to provide a standard against which to design and evaluate porous scaffolds without the need for a specific cell culture study. It can also be applied to predict and evaluate changes in the internal volume or space during scaffold degradation. The long term goals are 1) to design a methodology to systematically compare the open internal space of scaffolds fabricated from various methods, and 2) to address the relationship between the open internal space of a scaffold and its cytocompatibility with respect to a specific cell line based on statistical analysis.

Materials and Methods: To achieve these goals we have already fabricated different scaffold prototypes with round and 4DG degradable poly(lactic acid) (PLA) yarns (Figure 1a, 1b). Tubular structures were employed to study the longitudinal oriented pores. A bi-component scaffold system (Figure 1c) was fabricated to provide a complex morphology of 100% interconnected open pores. In order to characterize the porous structure of this bi-component scaffold system, specimens were embedded in resin, sectioned in different directions (Wp, Wf) in the outer and spacer layers (Ot, Sp), polished and photographed under an optical microscope. The mean pore size was measured on these 2D images. A cell culture assay was also performed for 15 days using human dermal fibroblast (HDF) using an initial seeding density of 5,000 cells/ml on the same 3D spacer fabric scaffold (Figure 1c).

Results: The preliminary results of 2D pore size measurements for each direction in each layer are shown in Table 1. The MTT cell viability data after 15 days of culture are illustrated in Figure 2.

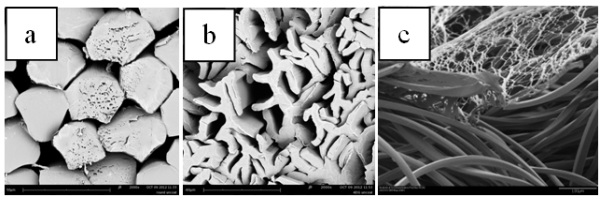

Figure 1. a) PLA tubular scaffold with round yarns. b) PLA tubular scaffold with 4DG yarns. c) bi-component scaffold system with PLCL electrospun top layer and a PET spacer fabric.

Sectioning Direction	WpOt	WfOt	WpSp	WfSp
Mean (mm)	0.036	0.045	0.242	0.468
St. Dev (mm)	0.017	0.013	0.100	0.153

Table 1. Preliminary 2D pore size measurement

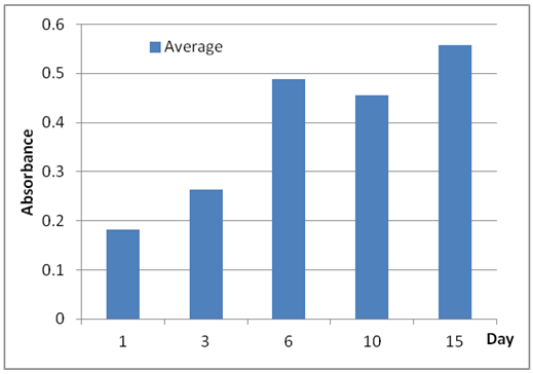

Figure 2. MTT viability assay results at 5 different timing during 15 days cell culturing.

Conclusions and Future Work: The 2D results in Table 1 will be used by Skyscan™ CT-Analyzer software (Bruker Micro CT, Belgium) to develop a three dimensional (3D) model of the internal pore volume during the scaffold resorption process. This novel approach will be used to evaluate the internal pore volume of a range of different types of scaffolds fabricated from various resorbable polymers.

References:

1. Scott J. Hollister, Porous scaffold design for tissue engineering, *Nature Materials* 4, 518 - 524 (2005)