Changes in trabecular-like hydroxyapatite scaffold permeability after *in vitro* cell culture

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Introduction: Hydroxyapatite (HA), a calcium phosphate ceramic, has shown tremendous promise as a bone graft substitute material. It provides the initial mechanical rigidity and structure, and is proven to be osteoconductive as well as angiogenic *in vivo* [1,2]. When fabricated into porous structures, HA facilitates tissue penetration and allows for perfusion and transport. *In vitro* smaller pores show a better performance due to greater surface area for cell proliferation [3]. The exact nature and effects of changes in transport properties due to colonization by cells however have not been completely understood. As such, the change in scaffold permeability with cell culture was studied to investigate the patency of scaffold fluid and waste transport for different pore sizes.

Methods: Porous HA scaffolds were fabricated by twice coating polymer sponge templates with HA slurries. The coated templates were then sintered to 1230^{o}C to ash the polymer template. Scaffolds with 4 different mean pore sizes: 450, 340, 250 and 200 μm were chosen for this study. Human fibrous osteoblast cells were cultured in osteogenic culture media for 14and 28 days in static *in vitro* culture and fixed in formalin. Scaffold permeability was calculated from a custom flow apparatus using repeated measures per specimen and applying Darcy's Law. Permeability measurements were carried out before and after 14 and 28 days of *in vitro* culture**.** A sample size of n=6 was used for each group for permeability and n=3 for histological evaluation. The data was analyzed using one way ANOVA to check for statistical significance and Tukey's test was used for post hoc comparison.

Results: Fig 1 shows the change in the permeability of scaffolds at 14 and 28 days after culture. For comparison, the permeability of human trabecular bone ranges from $1e^{-8}$ m^{-2} to $1e^{-11}$ m^{-2} [4]. Scaffold permeability was normalized to specimen porosity to magnify the effects of cell culture. Representative histological sections of the 4 architecture types were stained to show cell encapsulation of scaffold struts post *in vitro* culture (Fig 2).

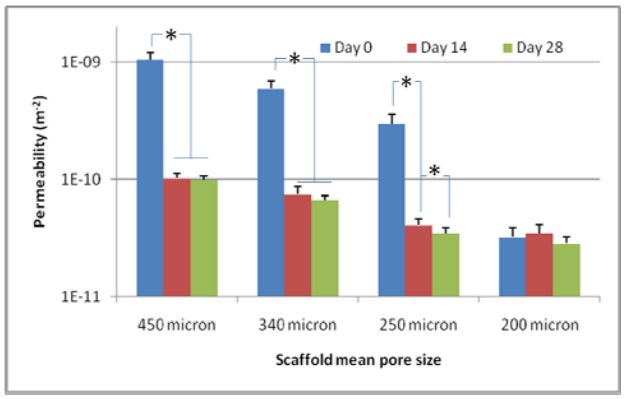

Fig 1. Permeability of the 4 different pore size architectures at 0, 14 and 28 days of *in vitro* culture.

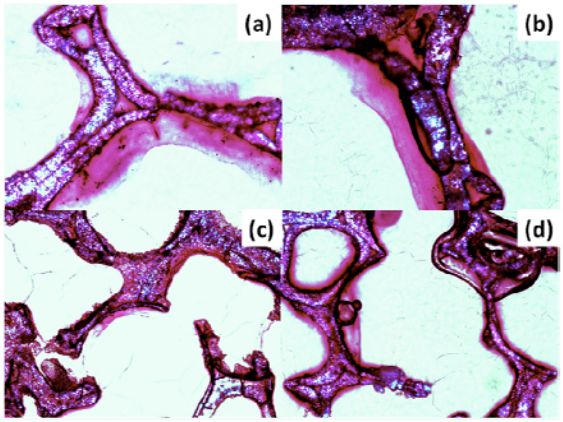

Fig 2. Representative histological sections of the (a) 450 (b) 340 (c) 250 and (d) 200 μm pore size scaffolds after 28 days of cell culture *in vitro*. (Mag: 333x).

Discussion: Within the 450, 340, 250 μm pore size groups, permeability was significantly reduced after 14 days of cell culture as compared to non cell culture controls. Although a trend of reducing permeability was observed within each pore size at day 28 compared to day 14 of cell culture, a statistically significant reduction was observed only for scaffolds within the 250 μm ($p<0.05$) pore size. No significant changes in permeability were observed within the 200 μm pore size scaffold throughout the culture period.

Prior to cell culture, significant differences in permeability between the 4 scaffold designs were observed ($p<0.01$). In comparing between the 450 μm, 340 μm and 250 μm pore size scaffolds at day 14 and day 28, significant differences in permeability were observed. No significant difference in permeability was observed when comparing the 200 μm and 250 μm after cell culture. Cell encapsulation of struts was observed from the histological sections as well as lining of the pore walls. Some small interconnections between pores were observed to be spanned by the cell extra cellular matrix production.

Conclusions:

It was concluded that HA architectures can be fabricated to not only mimic the cortical-cancellous organization, but also having comparable permeability to trabecular bone. It was also concluded that a significant reduction in permeability was observed in larger pore size scaffolds after *in vitro* cell culture and that this reduction was supported by histological evaluation.

References:

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