Effect of permeability and pore size on mechanical performance of hydroxyapatite scaffolds post in vitro culture

Teja Guda, Mark Appleford, Jun Sik Son, Sunho Oh, Joo Ong .
The University of Texas at San Antonio.

Statement of Purpose There is a tremendous demand for tissue engineered bone graft substitutes because of the problems associated with the availability and use of autografts and allografts for functional bone replacements for compromised bone tissue. The desired qualities in the artificial grafting material would be to provide suitable mechanical properties and preferably demonstrate osteoconductive and/or osteoinductive qualities. In this regard, hydroxyapatite based scaffolds show tremendous promise as they encourage new bone formation[1]. It needs to be determined however whether they have the initial mechanical properties to match physiological needs and the transport properties to sustain new bone growth.

Methods: Highly porous interconnected polymer sponges were twice coated with hydroxyapatite (HA) slurry and sintered to 1230 ^{o}C to ash the polymer template and prepare a 100% crystalline surface as confirmed by XRD. To mimic natural trabecular bone, templates were chosen such that the final scaffolds had four different pore sizes: 200, 250, 340 and 450 um. Scaffolds were then tested to failure under monotonic compression hydrated at 37^{o}C or after 14 days of static in vitro culture with osteoblast precursor cells. Osteogenic media was changed initially every 3 days till day 6 and then at 2 day intervals. Media was collected at day 7 and 14 to measure biomarkers. Scaffold porosity was measured using pycnometry and permeability was calculated from a custom flow apparatus and applying Darcy's Law. A sample size of n=6 was used for each group. The data was analyzed using a two tailed student's t-test to check for statistical significance and before and after cell culture. ANOVA was used to check for significance between groups and Tukey's test was used for post hoc comparison. All significance is reported at p=0.5 level.

Results: Representative micrographs of the four architecture types tested are shown in Fig 1. The porosity of the 450 μm, 340 μm, 250 μm and 200 μm pore size scaffolds was 84%, 76%, 64% and 63% and their permeability was measured at 9.36e^{-10} m^{-2}, 4.99e^{-10} m^{-2}, 2.36e^{-10} m^{-2} and 2.85e^{-10} m^{-2} respectively. For a comparison, the porosity of human trabecular bone ranges from 70-80% and the permeability ranges from 1e^{-8} m^{-2} to 1e^{-11} m^{-2} [2]. The mechanical properties before and after cell culture are listed in tables 1 and 2 as mean (SD).

Table 1. Before Cell Culture

Pore Size	Strength (MPa)	Elastic Modulus (MPa)	Toughness (kPa)
450 μm	0.3 (0.09)	63.5 (12.8)	4.66 (4.0)
340 μm	0.56 (0.1)	78.1 (16.8)	8.4 (2.93)
250 μm	1.15 (0.13)	184.3 (57)	30.3 (12.8)
200 μm	1.37 (0.3)	149 (56.7)	51.8 (14.4)

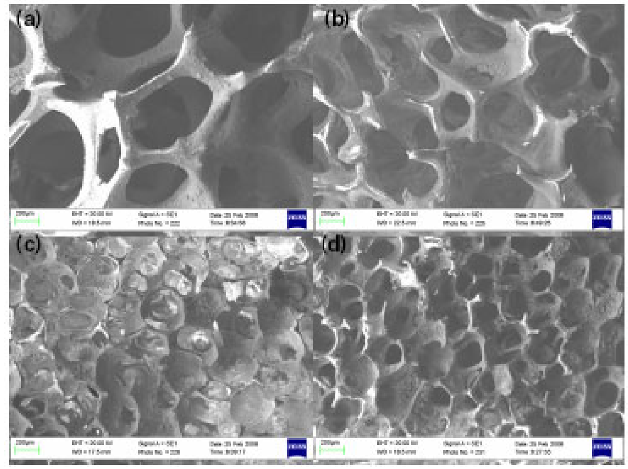

Figure 1. SEM images of the (a) 450 (b) 340 (c) 250 and (d) 200 μm pore size hydroxyapatite scaffolds

Table 2. After Cell Culture

Pore Size	Strength (MPa)	Elastic Modulus (MPa)	Toughness (kPa)
450 μm	0.18 (0.07)	39.7 (8.1)	3.30 (1.79)
340 μm	0.32 (0.1)	80.9 (14.8)	8.24 (4.66)
250 μm	1.35 (0.38)	247.6 (118)	66.11 (39.4)
200 μm	2.08 (1.18)	197 (96.2)	112.5 (52.2)

Between groups, no significant differences were observed between the 250 and 200 μm scaffolds, but these were significantly different from the 450 and 340 μm scaffolds for all properties measured except elastic modulus.

Conclusions: Porosity and permeability significantly impact the mechanical properties of the constructs as shown by the differences between the different pore sizes investigated. All architectures compare well with human trabecular bone tissue. Large scatter was observed in the elastic modulus measurements rendering them unsuitable for observing changes post culture. A trend of reducing strength and no difference in toughness (strain energy density) was observed post culture for the 450 and 340 μm pore sizes. For the 200 and 250 μm pore sizes the increases in strength seen after 14 days of cell culture are not significant but a two-fold increase in toughness is observed. Toughness might thus serve as a better predictor for mechanical functionality of scaffolds used for bone tissue engineering. From this study it is seen that the 200 and 250 μm pore size hydroxyapatite scaffolds would serve as the best platforms for bone grafts from the perspective of providing mechanical support.

References:

[1] Unger et al, Biomaterials, 2007, 28, 3965-76.
[2] Cowin SC, Bone Mechanics Handbook, CRC, 2001.