Mechanical Properties of Cell Seeded Porous Hydroxyapatite Scaffolds

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Statement of Purpose: Natural ceramic materials are of particular interest for bone substitutes as they are well recognized by host tissues and can provide moderate levels of mechanical integrity during fracture repair and new bone formation. Porous hydroxyapatite scaffolds permit new bone and blood vessel in-growth enhancing mechanical fixation. However, there is limited information on the time required before living tissues can affect the mechanical characteristics of replacement scaffolds. As the scaffold degrades it is of interest to determine when living tissue may influence and recover the mechanical properties of a biodegradable bone replacement.

Methods: Interconnected polymer sponges were twice coated with HA slurry and sintered at 1230°C to ash the polymer template and prepare a 100% crystalline surface as confirmed by XRD. A nano-HA SOL-Gel solution was precipitated to the surface followed by 2hr heat treatment at 650°C. The scaffolds have an average porosity value of 90% measured by Micro-CT and 400µm average pore size by SEM, Figure 1(a) and by optical microscopy with cells (b).

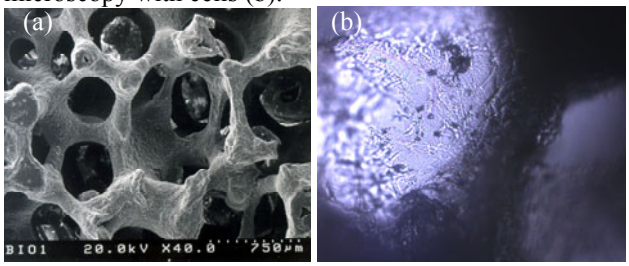

Figure 1. SEM image of HA scaffold with interconnected pore structure (a) and cellular encapsulation of an individual strut after 14 days cell culture (b).

Cell Culture: ATCC CRL 1486 human embryonic palatal mesenchyme cells, an osteoblast precursor cell line were cultured in DMEM with 7% fetal bovine serum, 2mM L-glutamine and 1% antibiotic-mycotic solution. Cells were seeded at two densities; 900,000 cells (1X), and 8.5 million cells per scaffold (10X). Cell suspension was applied in 600µL dropwise to three lengths of the scaffold (15mm length x 7mm diameter). Media was changed every two days until day 7, followed by daily changes to day 14 as the cells metabolic demand increased.

Mechanical Evaluation: Following cell culture or media control, scaffolds were washed twice with phosphate buffered saline (PBS). Sample ends were fixed into aluminum end caps to reduce edge artifacts during testing. Samples were loaded into a chamber with circulating PBS at 37°C and equilibrated for 5 minutes before compressed to failure at 1mm/min. Control scaffold fatigue was performed at 1Hz with 0.25MPa normalized stress and failure defined by 10% drop in E_{sec}/E_o modulus. Material characteristics are illustrated in Figure 2.

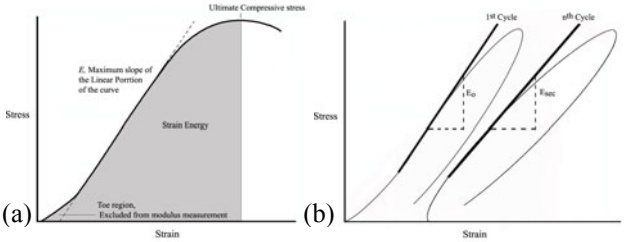

Figure 2. Stress strain curve for compression test (a) and fatigue curves showing E_o and E_{sec} measurements (b).

Discussion: A primary concern when using brittle ceramic phosphates for bone repair is the weak strain energy of the material and the potential for brittle fracture. In Figure 3, the HA scaffold exhibits a ceramic characteristic of material failure with an immediate reduction in fatigue compressive modulus.

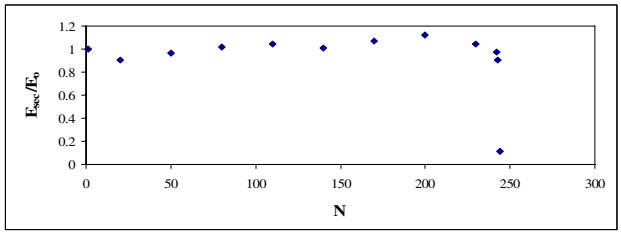

Figure 3. Fatigue behavior of secant to original compressive modulus of HA scaffolds at 0.25MPa stress.

While cell seeding has few effects on compressive modulus or strength as the scaffold properties are already approaching that of trabecular bone, it may influence the strain energy. From day 7 to 14, a moderate decrease is seen for the control scaffold while the application of cells increases these values, Table 1.

Table 1. Mechanical properties of control and cell seeded scaffolds (n=4 per group).

Day 7	Compressive Modulus (MPa)	Compressive Strength (MPa)	Compressive Strain (mm/mm)	Strain Energy (kPa)
Control Scaffold	195.5	0.766	7.19E-03	2.510
1X Seeded Scaffold	252.8	0.985	5.56E-03	2.574
10X Seeded Scaffold	107.3	0.469	5.11E-03	1.321
Day 14				
Control Scaffold	179.6	0.616	3.83E-03	1.200
1X seeded Scaffold	169.9	1.031	6.66E-03	4.312
10X Seeded Scaffold	160.2	0.771	4.57E-03	1.846

Conclusion: Despite the early reduction in mechanical properties with applied cells, cell matrix production may offset the scaffold degradation improving toughness over time. Seeding density can influence these properties as excessive cell number may cause low local pH, increasing degradation and further reducing mechanical properties.

References: 1) T. Moore. J. Biomech Engr. 2004,126:321-329. 2) T. Keaveny. J. Biomech 1994,27,9:1127-1136. 3) S. Oh. Key Eng. Mater Vols. 2001,192:91-94.