Effect of Architecture on the Long Term *In Vivo* Degradation of Designed PLLA Porous Scaffolds

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Statement of Purpose: Porous scaffold architecture plays a critical role not only for bone tissue regeneration but also in scaffold degradation. Degradation studies of many porous scaffolds have been previously reported, but the wide range of pore diameters, poorly or non-connected pores have made it difficult to interpret the relationship between architecture and degradation. The goal of this study was to determine the influence of scaffold architecture, specifically pore size, strut size, porosity and surface area, on scaffold degradation *in vivo* to improve scaffold design for a desired degradation. Indirect solid free form fabrication (SFF) methods[1] was used to control these parameters. We designed and fabricated poly (L-lactic acid) (PLLA) porous scaffolds and bulk cylinders, then subcutaneously implanted them into mice for up to 21 weeks. The relationship between the scaffolds' degradation and their architectures were determined by changes in scaffold mass, compressive modulus and polymer crystallinity.

Methods:

Sample Design and Fabrication: PLLA porous scaffolds having large (DL), medium (DM) and small (DS) pore sizes were designed using image-based techniques and fabricated by indirect SFF technique[1] (Table 1). PLLA bulk cylinders (DC) were also fabricated. All samples had the same outer diameter (5mm) and height (3mm).

Implantation: The samples were sterilized in 70% ethanol overnight and then subcutaneously implanted into immuno-compromised mice for 0, 6, 12 and 21 weeks.

Micro-Computed Tomography: All fabricated samples were scanned using a MS-130 high resolution Micro-CT Scanner (GE Medical Systems, CAN) before and after implantation to measure pore sizes, strut sizes, porosities and surface areas.

Mass, Mechanical Properties and Crystallinity Analysis: After removing surrounding tissues using type 1 collagenase solution, the samples were lyophilized using the FreeZone 6 (Labconco Corp.) and weighed using an analytical balance. Compression moduli were determined using a MTS Alliance RT30 Electromechanical test frame (MTS Systems Corp., MN) with a cross head speed of 1mm/min. To determine crystallinity, the enthalpy of the polymers was measured using a differential scanning calorimeter (Perkin-Elmer DSC-7). One-way ANOVA ($p < 0.05$) was performed using SPSS (SPSS Inc.) (N = 3-6).

Results: The fabricated samples had defined pore and strut sizes, porosities, and surface areas dependent upon their design groups (Table 1 and Fig.1 (a)).

Table 1: Pore size, strut size, porosity, and surface area of the fabricated samples from Micro-CT measurements.

	DL	DM	DS	DC
Pore size (μm)	800 ± 44	555 ± 51	285 ± 26	
Strut size (μm)	1094 ± 304	712 ± 171	417 ± 17	
Porosity (%)	47.5 ± 1.4	44.9 ± 1.9	29.8 ± 2.2	1.1 ± 0.6
Surface Area (mm2)	143.9 ± 14.8	175.3 ± 12.5	189.4 ± 5.4	99.1 ± 3.1

External and internal architectures were maintained up to 21 weeks, as confirmed visually and from Micro-CT, respectively (Fig.1). However, all samples lost mass. DC lost significantly more mass than all groups at 12 and 21 weeks, and additionally with DS at 6 weeks. In spite of its smaller pore size and porosity, DS showed significantly less weight loss than DM and DL at 21 weeks.

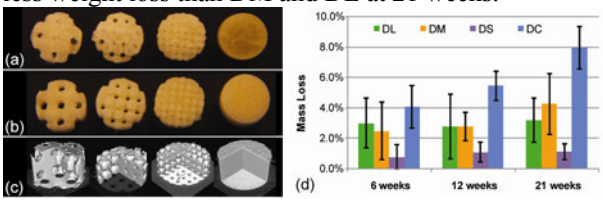

Fig 1: Pictures of the samples (DL, DM, DS, DC from Left to Right) at 0 week (a), 21week (b), rendering and cross-section images from CT data at 21 weeks (c), and mass loss of the samples (d)

Among the samples' design parameters, larger surface areas showed a linear correlation with less mass loss (Fig.2). Stronger relations were seen at 12 (R^2=0.681) and 21 (R^2=0.671) weeks than at 6 weeks (R^2=0.397).

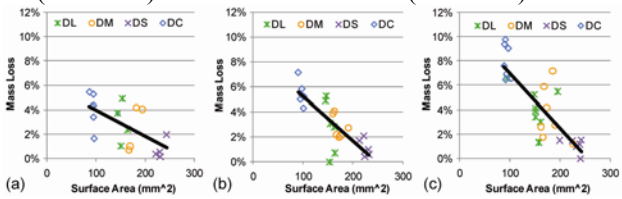

Fig 2: Correlation between samples' initial surface areas and their mass losses at 6 (a), 12 (b) and 21 (c) weeks

Enthalpy increased in all porous scaffolds (Fig 3 (a)) up to 21 weeks due to degradation of their amorphous regions. Meanwhile, enthalpy of DC increased up to 12 weeks and decreased thereafter; indicating that DC degraded faster than the porous scaffolds with its crystal regions degrading after its amorphous regions.

Percentage decrease of compressive moduli was not significantly different between all groups (Fig 3 (b)) over time. The moduli of the porous scaffolds were maintained between 50 and 90 MPa at 21 weeks.

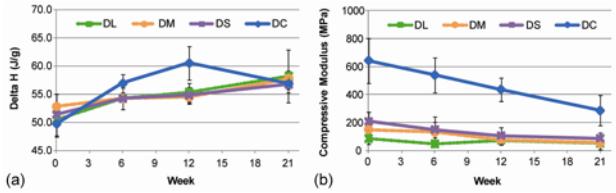

Fig 3: Enthalpy (a) and compressive moduli (b) changes along to the implantation periods

Conclusions: The present study showed that the ratio of *in vivo* long term degradation can be controlled by the designed scaffolds' initial architectures, and suggests that porous scaffolds with smaller strut sizes and larger surface area have slower degradation. Slower degradation in the smaller strut size and/or large surface area scaffolds may be attributed to reduced autocatalysis from the ability to remove acidic degradation by-products. The mechanical properties of the porous scaffolds were maintained in the lower range of human trabecular bone at 21 weeks.

Acknowledgments: NIH R01 AR 053379.

References:1.Taboas JM et al., Biomaterials, 2003;24:181-94