3-DIMENSIONAL POLY(LACTIDE-*CO*-GLYCOLIDE)/HYDROXYAPATITE CONSTRUCT FOR BONE TISSUE ENGINEERING: OPTIMIZATION STUDIES

Michelle D. Kofron (1,2), Omar Fisher (1,2), Cato T. Laurencin (1,2,3)

(1) Center for Advanced Biomaterials and Tissue Engineering
Drexel University
Philadelphia, PA

(2) School of Biomedical Engineering, Science, and Health Systems
Drexel University
Philadelphia, PA

(3) Department of Orthopaedic Surgery
Drexel School of Medicine
Philadelphia, PA

INTRODUCTION

Poly(lactide-*co*-glycolide) co-polymers (PLAGA) have been widely investigated and FDA approved for select biomaterials applications [1]. Most recently, these polymers have been used to fabricate three-dimensional (3D) scaffolds for bone repair [2-4]. If the polymer is combined with hydroxyapatite to form a composite material, the implant will possess an increased potential for integration with the surrounding bone upon implantation at an osseous defect site. Our laboratory has previously combined PLAGA microspheres with particulate hydroxyapatite (HA) using the gel microsphere method [2] and solvent aggregation method [2] to form 3D, biodegradable composite scaffolds as constructs for bone tissue engineering. In this study, an alternative method, that first forms composite PLAGA/HA microspheres, was used. PLAGA/HA microspheres were fabricated and thermally fused to form a 3D-PLAGA/HA sintered microsphere matrix. We hypothesized that by varying the time and temperature of sintering composite PLAGA/HA microspheres, an osteoconductive scaffold with pore sizes and mechanical properties within the range of human trabecular bone could be formed.

MATERIALS AND METHODS

Composite microspheres were prepared using a modified solvent evaporation technique [2]. Briefly, [85:15] PLAGA (Mw = 125,000, Alkermes, Cincinnati, OH) and HA particles (53-150 μm, Howmedica, Rutherford, NJ) were added in a 1:1 ratio to methylene chloride. Once the polymer was completely dissolved, the mixture was emulsified by pouring into a 1% poly(vinyl alcohol) solution stirring at 450 rpm for 24 hours. Microspheres were isolated, washed with deionized water, dried, and sieved. Microsphere morphology was characterized using scanning electron microscopy (SEM, Amray-D4, operated at 20 keV).

DSC measurements were made (model DSC 2010, TA Instruments) to ensure the microspheres would be sintered at a temperature above the polymer's glass transition temperature. Composite microspheres, 600-710 μm in diameter, were placed into a 3D cylindrical mold, 5 x 10 mm, and sintered at 80, 85, 90, 95, 100, 105, 110, and 120°C for 2 hours. The resulting matrices were characterized using SEM, mechanically tested in compression (model 5544, Instron), and porosimetry tested (model Autopore III 9400 Mercury Porosimeter, Micromeritics). A single scaffold (105°C) was selected and then re-tested after sintering for 1, 2, and 3 hours to determine if varying the sintering time would enhance the scaffold properties.

RESULTS AND DISCUSSION

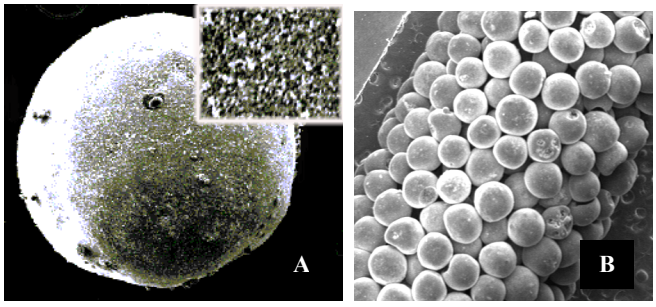


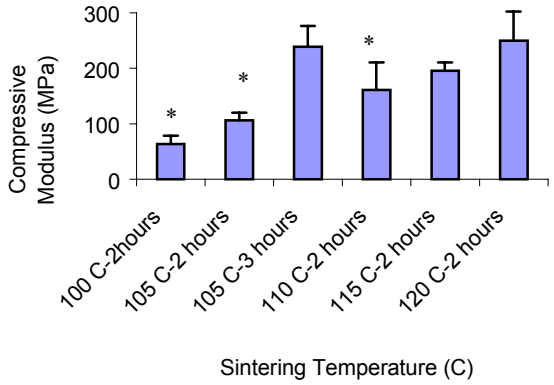
Figure A: SEM micrograph of an individual PLAGA/HA composite microsphere, 600-710 μm diameter, with 87 wt% HA particle encapsulation efficiency. Magnification 97.5X, inset 640X.

Figure B: SEM micrograph of the PLAGA/HA microsphere matrix sintered at 105°C for 2 hours. Magnification 10X.

Matrix composition, mechanical properties, and pore size are three facets important in the design of a construct to support the neogenesis of bone [3]. The use of the biodegradable polymer PLAGA means material degradation may be matched to new bone formation, and the newly formed tissue will undergo gradual loading with physiological load levels as the scaffold is resorbed. The addition of HA improves the biocompatibility of the scaffold by enhancing the adsorption of serum proteins at the defect site. Furthermore, HA tends

to buffer the acidic by-products, detrimental to cell viability, created by polymer degradation [4]. In this study, initial composite microsphere fabrication resulted in a 53 wt% HA particle loading (not shown). The subsequent modified method of composite microsphere fabrication increased the HA particle loading efficiency to 87 wt% (Figure A). The later microspheres were sintered at the various temperatures and times to form composite sintered microsphere matrices (Figure B).

While serving as a scaffold for tissue regeneration, an ideal construct for bone regeneration will possess similar mechanical properties to the natural tissue that it is replacing [3]. 3D-PLAGA/HA microsphere matrices sintered above the polymer's glass transition temperature (Tg = 50-51ºC) at 80, 85, 90, and 95°C for 2 hours and at 105°C for 1 hour possessed mechanical properties in compression unsuitable for use as a bone replacement scaffold. The scaffolds sintered at 100, 105, 110, 115, and 120°C were mechanically tested in compression (Graph 1). The results indicated the compressive modului of the scaffolds was within the lower range of human trabecular bone (10-2000 MPa) [2] and significantly increased with an increase in sintering temperature or time ($p<0.03$).



Graph 1: 3D-PLAGA/HA scaffolds were tested under compression at the ramp speed of 1mm/min. * denotes statistical significance when compared to the matrix sintered at 120°C using Student's t-Test. $p<0.03$, n=6

In addition to serving as an architectural support, an ideal bone replacement construct will possess a porous structure that allows for tissue and vascular ingrowth. As PLAGA/HA matrices sintered below 100°C for 2 hours or at 105°C for 1 hour showed inadequate mechanical properties, the porosity of these scaffolds was not examined. Hulbert et al. [5] examined connective tissue, bone, and blood vessel ingrowth into porous ceramic scaffolds of various pore sizes (10 to 200 μm) implanted in canine femurs. Their results demonstrated constructs with a pore size range 150-200 μm possessed the greatest bony tissue and vascular ingrowth and interior calcification and was used here as the criteria for sufficient pore size of the 3D-PLAGA/HA scaffolds. Mercury intrusion porosimetry results, on the composite matrices sintered at 100, 105, 110, 115, and 120°C, indicated the mean pore diameter ranged from 153-184 μm with no significant differences between matrices sintered at the various temperatures (Table I).

Sintering Temp / Time	Mean Pore Diameter (μm)
100 ºC / 2 hours	183.88 + 21.68
105 ºC / 2 hours	161.47 + 3.73
105 ºC / 3 hours	146.54 + 6.38
110 ºC / 2 hours	166.81 + 3.58
115 ºC / 2 hours	152.82 + 8.00
120 ºC / 2 hours	153.18 + 28.50

Table I: Mercury porosimetry results demonstrated the mean pore diameter of the PLAGA/HA sintered microsphere matrices was within the optimal size range for bony tissue and vascular ingrowth established by Hulbert, et al. n=6.

Although all of the PLAGA/HA microsphere matrices demonstrated suitable compressive moduli and mean pore diameters, scanning electron micrographs of the scaffolds sintered at 110, 115, and 120ºC for 2 hours and 105ºC for 3 hours revealed areas on the surface of the scaffold where the microspheres were completely sintered together as well as areas where the pores were maintained. Because uniform sintering did not occur, these scaffolds were deemed less suitable for tissue ingrowth. As the composite matrix sintered at 100ºC possessed the lowest compressive modulus, the 3D-PLAGA/HA microsphere matrix sintered at 105ºC for 2 hours was selected as the optimal scaffold fabricated in this study.

CONCLUSIONS

In this study, a composite matrix was fabricated by sintering PLAGA/HA microspheres at various temperatures and times. The matrices were characterized using SEM, mechanically tested in compression, and porosimetry tested to compare the physical properties of the scaffolds to the properties of human trabecular bone sited in the literature. Scaffolds sintered below 100ºC possessed insufficient mechanical properties for use as a construct for bone tissue engineering. Although all scaffolds demonstrated a mean pore diameter within the optimal size range (150-200 μm) for bony tissue and vascular ingrowth [5], SEM revealed scaffolds sintered at 110 or 120ºC for 2 hours or at 105ºC for 3 hours did not possess uniform sintering. Sintering the PLAGA/HA composite microspheres at 105°C for 2 hours produced a matrix with a compressive mechanical strength within the range of human trabecular bone and pore size suitable for bone tissue ingrowth.

REFERENCES

1. Athanasiou, K. A., Agrawal, C. M., Barber, F.A., Burkhart, S. S., 1998, "Orthopaedic Applications for PLA-PGA Biodegradable Polymers," Arthroscopy, 14, pp. 726-737.
2. Borden, M. D., Attawia, M.A., Khan, Y., and Laurencin, C. T., 2002, "Tissue engineered microsphere based matrices for bone repair," Biomaterials, 23, pp. 551-559.
3. Hutmacher, D. W., 2000, "Scaffolds in tissue engineering bone and cartilage," Biomaterials, 21, 2529-2543.
4. Burg, K. J. L., Porter, S., and Kellam, J. F., 2000, "Biomaterial developments for bone tissue engineering," 21, 2347-2359.
5. Hulbert, S. F., Young, F. A., Mathews, R. S., Klawitter, J. J., Talbert, C. T., and Stelling, F. H., 1970, "Potential of Ceramic Materials as Permanently Implantable Skeletal Prostheses," Journal of Biomedical Materials Research, 4, pp. 433-456.