Templated Synthesis of Biomimetic Materials for Bone Tissue Repair and Regeneration

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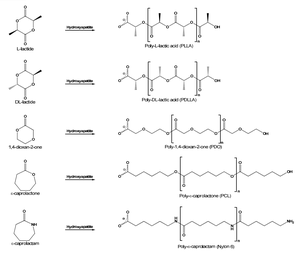
Abstract

Polymerization of L-lactide within the pores of anorganic mammalian bone is described. The resulting composites exhibit macroscopic morphologies and mechanical properties similar to that of the original bone. We observe an average compressive strength of 194 MPa and an elastic modulus of 8.8 GPa for composites comprised of poly-L-lactide and anorganic bone derived from bovine femurs. In addition, the surface initiated chemical vapor deposition polymerization of L-lactide onto a porous hydroxyapatite substrate is described. The compressive strength of the resulting composites increase as the percent of the original void volume filled with poly-L-lactide increases. Finally, a method for preparing synthetic demineralized bone matrix is described.

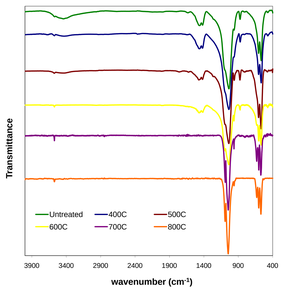
Introduction

The medical response to traumatized bone is important issue on the modern battlefield. Soldiers in ongoing armed conflicts are experiencing high rates of orthopedic trauma, such as segmental bone defects caused by improvised explosive devices (IEDs). Significant challenges confront materials scientists and physicians interested in viable sources of materials for tissue repair and replacement. Tissues for same-species allografts are frequently in short supply. For some applications, xenogeneic tissues are a viable alternative; however, immunological barriers prevent routine interspecies transplantation of tissue. Although the use of tissues from species genetically similar to humans lessens the severity of rejection, tissues from these species pose a threat because of the higher likelihood of pathogen transmission. Research into bioceramic composites is playing an increasingly important role in the repair, reconstruction, and replacement of hard tissues. Although synthetic materials lessen the threat of certain forms of rejection, challenges to the development of biomaterials that exhibit desirable chemical, physical, and mechanical properties remain daunting. The morphologies of the desired materials are often quite complex, and even if the desired structures can be constructed, the biological and biomechanical viability is not certain a priori.

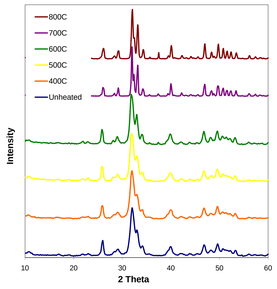


Common polymers used in biomaterials and their respective monomers.

Hydroxyapatite Preparation and Consequences

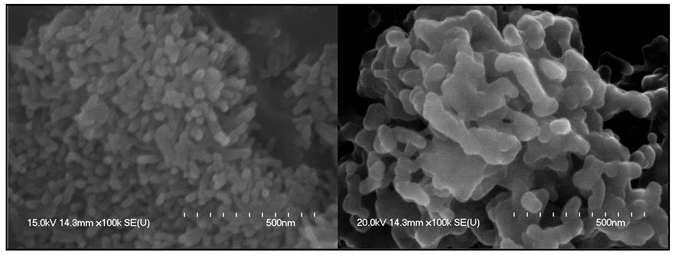


FTIR spectra of bovine derived HA sintered for 16 hours at various temperatures

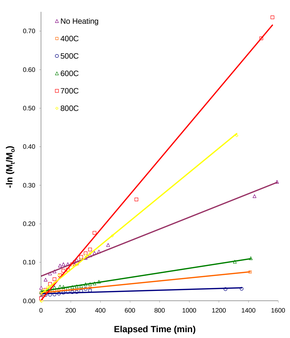


X-Ray diffraction patterns of bovine derived HA sintered for 16 hours at various temperatures

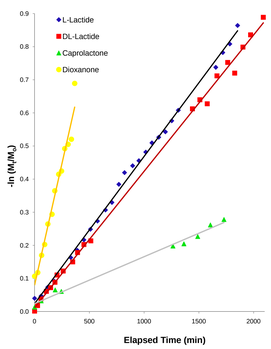
Biologically derived hydroxyapatite (HA) contains between 5 – 8% carbonate. Sintering at various temperatures changes the chemical composition and morphology. X-Ray powder diffraction and FTIR show that at 600°C and below the carbonate phase remains. As the temperature is increased the carbonate decomposes to hydroxide and oxide phases. Along with the chemical changes, the HA transforms into larger more crystalline particles, causing a decrease in particle surface area and decrease in sample dimensions. Biologically derived HA gives the highest rate of reaction when sintered at 700°C. This temperature offers the highest concentration of surface exposed hydroxide and oxide to initiate the ring opening polymerization reaction. Lower temperatures that have higher surface areas still contain large amounts of carbonate which is not nucleophilic.



***Left:** SEM image of nonsintered bovine HA **Right:** SEM image of bovine HA sinteread at 700°C*



First order kinetics plots for ring opening polymerization of L-lactide initiated by sintered HA



First order kinetics plots for ring opening polymerization of common monomers with HA sintered at 700°C.

Temperature (°C)	Diameter (mm)	Height (mm)	Diameter Change	Height Change	Mass (g)	Total Mass Loss
	6.42	12.76	0.0%	0.0%	0.5648	0.0%
600	6.41	12.70	0.2%	0.5%	0.5262	6.8%
700	6.06	12.01	5.6%	5.9%	0.5161	8.6%
800	6.05	11.97	5.8%	6.2%	0.5129	9.2%

Sintering Temperature (°C)	Surface Area (m^2/g)
400	87.8
500	70.8
600	46.8
700	4.8
800	3.6
900	2.5

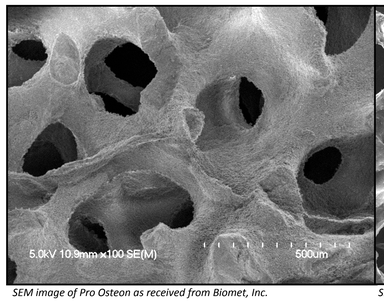
Composite Properties

***Upper Left:** Human tibia reconstituted with poly-l-lactide **Upper Right:** Poly-L-lactide / HA bone fixation screw **Lower:** Rat skull reconstituted with poly-l-lactide*

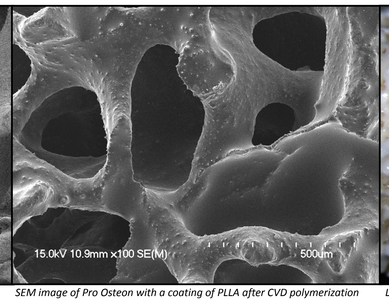
Compressive stress-strain curves for anorganic cortical bone reconstituted with PLLA reveal mechanical properties that can meet or exceed the original properties of the bone. We found the ultimate compressive strength of 10 reconstructed samples of cortical bone obtained from bovine femurs to be 194 ± 26 MPa. Compressive failure of the samples occurred via the typical brittle failure mechanism. The elastic modulus of the same set of samples was found to be 8.8 ± 0.5 GPa, and the molecular weight was 7.5 ± 1.5kD. The extent to which we can manipulate the molecular weight to control mechanical properties remains unclear, but it seems likely that both the molecular weight and the polydispersity of the resulting polymer is determined by the volume distribution of interstices within the anorganic bone. For comparative purposes, we note that Yamada reports the ultimate compressive strength of the cortical bone from wet bovine femurs to be 144 MPa and the elastic modulus to be 8.52 GPa. The compressive strength of human femurs is known to peak at 167 MPa for persons from 20 to 29 years of age, but it gradually declines to approximately 140 MPa for persons from 60 to 69 years of age. It is also noteworthy that Verheyen et al. have reported the compressive strength and elastic modulus for high molecular weight PLLA to be approximately 125 MPa and 5.3 GPa, respectively.

Chemical Vapor Deposition Polymerization

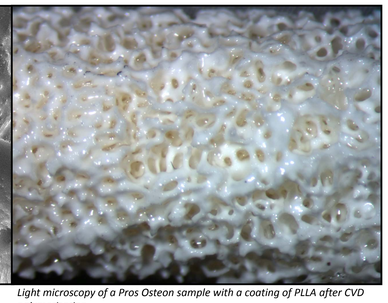
In addition to being osteoconductive and osteoinductive, an ideal bone graft substitute should contain a matrix of interconnected pores in which soft tissue can grow and bone can regenerate. These interconnected pores allow the osteogenic healing process to reach the interior of the graft without reabsorbing bone. An optimal pore size for ceramics is between 150 and 500 µm. Chemical vapor deposition (CVD) polymerization is particularly useful when coating porous substrates where one wishes to increase the mechanical strength of a graft by applying a thin polymer film, but not fill the pores. In CVD polymerization, a monomer is heated under vacuum in the presence of a nucleophilic substrate, creating an atmosphere of monomer gas. The gas phase monomer collides with the surface of the substrate and either reacts or returns to the gas phase. A thin film of polymer begins to form shortly after the reaction is initiated, and increases in thickness the longer the reaction proceeds. In our case, the monomer is a cyclic lactone (often L-lactide) and the substrate is a form of hydroxyapatite. Two examples of this type of substrate are trabecular bone and Pro Osteon, a coralline hydroxyapatite marketed by Biomet, Inc., which contains a interconnected, porous structure similar to trabecular bone.



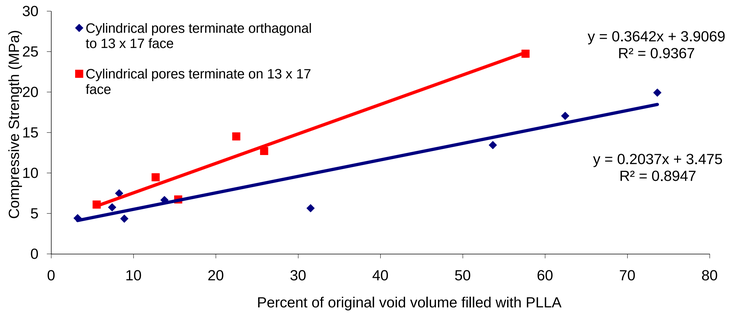
SEM image of Pro Osteon as received from Biomet, Inc.



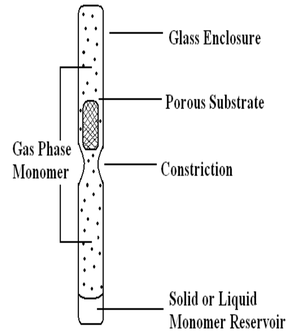
SEM image of Pro Osteon with a coating of PLLA after CVD polymerization



Light microscopy of a Pros Osteon sample with a coating of PLLA after CVD polymerization



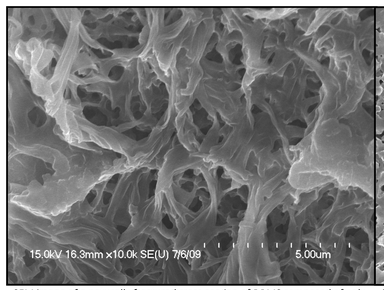
Compressive strength dependence on the percent of the original void volume filled for Pro Osteon coated via CVD polymerization



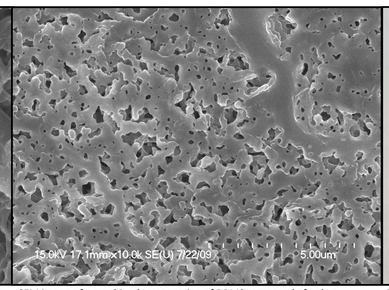
Schematic diagram of a CVD polymerization vessel

Demineralized Bone Matrix Simulant

Demineralized bone matrix (DBM) is a commonly used bone graft substitute. It is the product of acid extraction of bone. The majority of the mineralized component is dissolved, leaving collagen, noncollagenous proteins and bone growth factors. Although it has little structural strength, DBM is known to possess good osteoinductive properties. Similar to DBM, the mineralized component of demineralized bone matrix simulant (DBMS) has been extracted, but a bioresorbable polymer is substituted in place of the non-mineral components of bone. This is achieved by preparing composites of anorganic bone and a bioresorbable polymer described in previous work, followed by acid extraction.



SEM image of a naturally fractured cross section of DBMS composed of poly-ε-caprolactone (PCL)



SEM image of a machined cross section of DBMS composed of poly-ε-caprolactam (nylon-6)

DBMS composed of PCL made by acid extraction of a reconstituted bovine femur

References

1) Wiegand, T.; Karr, J.; Steinkruger, J.D.; Hiebner, K.; Simetich, B.; Beatty, M.; Redepenning, J. *Chemistry of Materials* (**2008**), 20(15), 5016-5022. 2) Fortin, J.B.; Lu, T. *Chemical Vapor Deposition Polymerization: The Growth and Properties of Parylene Thin Films*. Boston: Kluwer Academic Publishers, **2004**. 3) Sutherland, D.; Bostrom, M. Grafts and Bone Graft Substitutes. In *Bone Regeneration and Repair: Biology and Clinical Applications*; Lieberman, J.R.; Friedlaender, G.E., Ed.; Totowa, NJ: Humana Press, **2005**. 4) Owens, B. D.; Kragh, J. F.; Macaitis, J.; Svoboda, S. J. & Wenke, *J. C. J. Orthop. Trauma* (**2007**), 21, 254. 5) Ratner, B. D.; *Biomaterials Science: An Introduction to Materials in Medicine*, Boston: Elsevier, **2004**. 6) Stock, U. A.; Vacanti, *J. P. Ann. Rev. Med.* (**2001**), 52, 443. 7) Townsend, C. M.; Beauchamp, R. D.; Evers, B. M.; Mattox, K. L. Section IV. Transplantation and Immunology. In *Sabiston Textbook of Surgery, 17th ed.*, Philadelphia: Saunders, **2004**. 8) Bach, F. H. *Annu. Rev. Med.* (**1998**), 49, 301. 9) Yamada, H. Mechanical Properties of Locomotor Organs and Tissues. In *Strength of Biomaterials*; Evans, F. G., Ed.; Baltimore: William Wilkins Company, **1970**. 10) Verheyen, C. C. P. M.; de Wijn, J. R.; van Blitterswijk, C. A.; de Groot, K. J. *Biomed. Mat. Res.* (**1992**), 26, 1277.

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