Micro-porous PLLA scaffolds with Nanodiamond Reinforcement for Orthopedic Surgical Fixation Devices

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Abstract

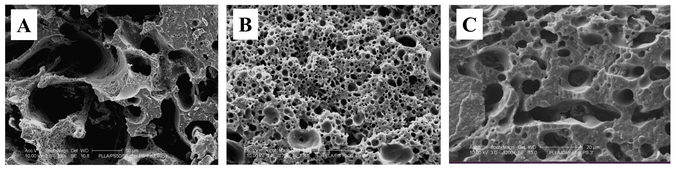
Biodegradable polymers have been used as engineered scaffolds for hard and soft tissues, such as bone and blood vessels for many years. More recently biodegradable biopolymers have also found applications in surgical fixation devices. However, currently used biopolymers are not strong enough mechanically, especially when used as surgical fixation devices and bone scaffolds. In this work, a novel composite material had been produced utilizing poly(L-lactic acid) (PLLA) and octadecylamine-functionalized nanodiamond (ND-ODA), and PLLA scaffolds with micro-porous structure were prepared. The co-continuous structure of produced scaffolds was observed under SEM. In order to evaluate the functional and cell biological applicability of the micro-porous structural PLLA scaffolds, cytotoxicity in vitro and biodegradation in vivo upon subcutaneous implantation in a rat model were tested. The micro-porous structural PLLA scaffolds were found to be non-toxic, and showed a mild foreign body reaction and complete fibrous encapsulation after implantation. Due to concise manufacturing specifications for mechanics loading, the following modeling system has been proposed to optimize the appropriate material processing for an assortment of orthopedic applications. ND-ODA/PLLA composites were prepared by mixing PLLA/chloroform solution with chloroform suspension of nanodiamond at concentrations of 0-10 by weight percent. The dispersion of ND-ODA was evaluated by transmission electron microscopy (TEM). The mechanical strength of ND-ODA/PLLA materials was tested by nanoindentation and the cytotoxicity of the composites was assessed in vitro using cultured murine osteoblasts. The results showed that the addition of ND-ODA even at high concentrations had only minimal influence on cell growth, which indicates the composites have good biocompatibility. Taken together, these results suggest that ND-ODA/PLLA with micro-porous structure might have potential applications for musculoskeletal tissue engineering and regenerative medicine.

Specific Design

Gradient porous structures: from coarse to fine pores on the screw wall

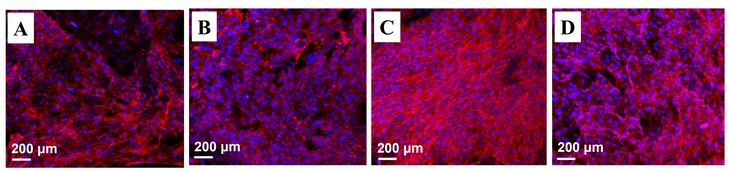
Hydrogel fillings: Embedded with healing drug, growth factor, and cells

Porous Structure Fabrication



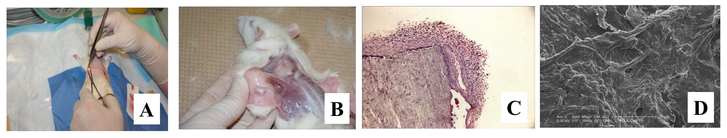
Scanning electron microscope (SEM) micrographs of co-continuous porous structure of PLLA with 50% porosity (A), 30% porosity (B), and PLLA/HA with 40% porosity (C). Original magnifications are: A: 350x, B and C: 1200 x.

Porous Structure Fabrication

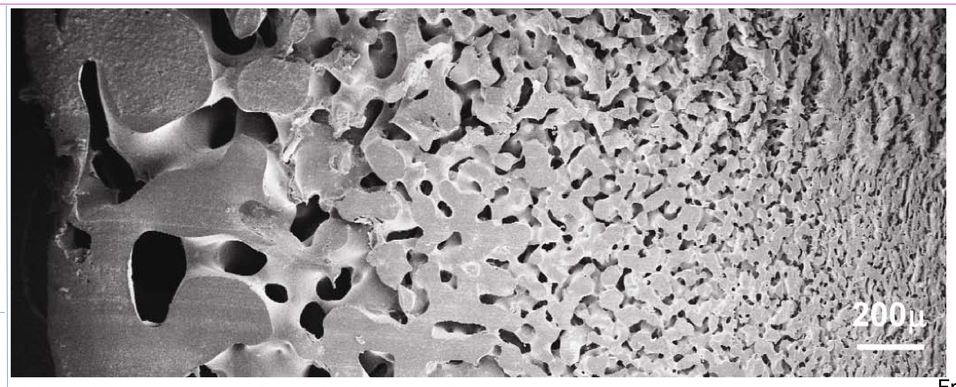


Morphology (bisbenziminde and pahlloidin staining) of 7F2 osteoblasts on solid PLLA (A), porous PLLA with 50% porosity (B), porous PLLA with 70% porosity (C), and porous PLLA/HA with 40% porosity (D) scaffolds 9 days post-seeding.

in vivo Studies

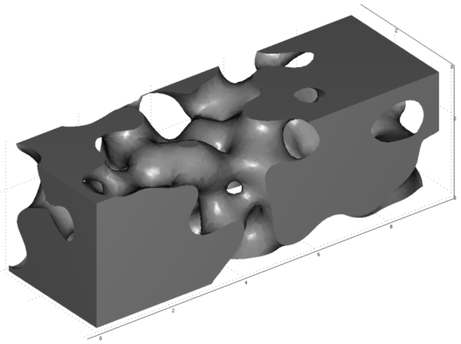


(A) Implantation of PLLA porous scaffolds (4x4x5 mm^3) in rats. The scaffolds were implanted subcutaneously from the incision at both right and left backs; (B) Scaffolds harvested 4 weeks post-implantation with the surrounding ECM attached to the scaffolds; (C) Thin section micrograph (Hematoxylin and Eosin (H&E) staining) of the porous PLLA/HA with 40% porosity retrieved from rat subcutaneous tissue 4 weeks post-implantation (200x magnification). (D) SEM micrograph of the surface of porous PLLA/HA with 40% porosity 4 weeks post-implantation.



Left: SEM image of gradient porous PLLA formed as a 50/50 mixture with polystyrene and annealed with a linear thermal gradient for 10 minutes.

Right: Model based on observed initial conditions and model equations below.



Model Equations: Coupled Level Set

$$\rho\frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u}\cdot\nabla)\mathbf{u} = \nabla\cdot[-p\mathbf{I} + \eta(\nabla\mathbf{u} + \nabla\mathbf{u}^T)] + \mathbf{F}_g + \mathbf{F}_{st} + \mathbf{F}$$

$$\frac{\partial \phi}{\partial t} + \mathbf{u}\cdot\nabla\phi = \gamma\nabla\cdot\left(\varepsilon\nabla\phi - \phi(1-\phi)\frac{\nabla\phi}{|\nabla\phi|}\right)$$

$$\nabla\cdot\mathbf{u} = 0 \qquad \kappa = -\nabla\cdot\mathbf{n}|_{\phi=0.5} \qquad \mathbf{F}_{st} = \sigma\kappa\delta\mathbf{n} \qquad \mathbf{n} = \frac{\nabla\phi}{|\nabla\phi|}\Big|_{\phi=0.5}$$

$$\delta = 6|\nabla\phi||\phi(1-\phi)|$$

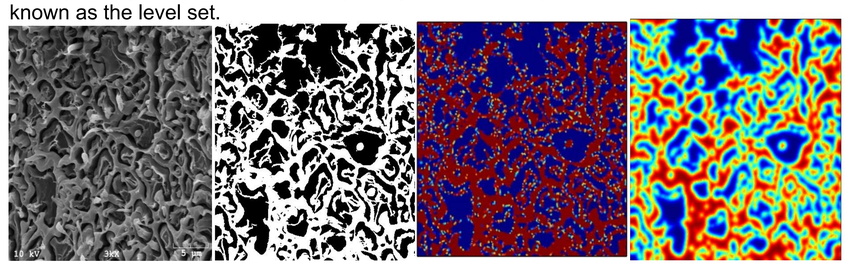
$$\mathbf{F}_g = \rho\mathbf{g}$$

Cross Viscosity Model

$$n = n_\infty + \frac{n_0 - n_\infty}{1 + (C\dot{\gamma})^m}$$

$$n_\infty(T) = a_1 e^{a_2/T}$$
$$n_0(T) = b_1 e^{b_2/T}$$
$$C(T) = c_1 e^{c_2/T}$$
$$m(T) = m_1 e^{-m_2/T}$$

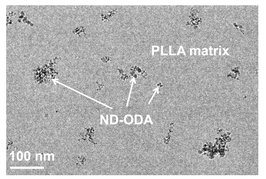
From left to right: 1) SEM images used as initial conditions in modeling by decomposing into 2) binary material selection, 3) interpolated, and 4) initialized as the material field known as the level set.



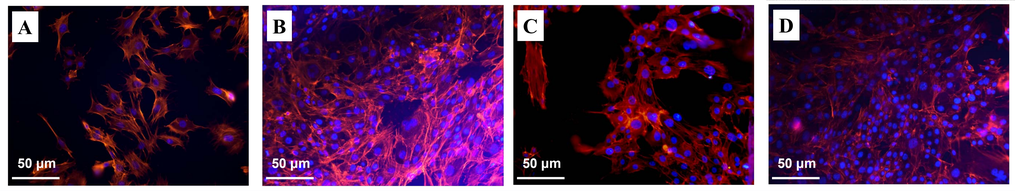
Dispersion Study and Mechanical Reinforcement of PLLA by ND-ODA

Mechanical properties of ND-ODA/PLLA determined by depth-sensing indentation

Description	Young's Modulus (GPa)	Increase (%)	Hardness (GPa)	Increase (%)
pure PLLA	2.58±0.11*	0	0.05±0.01	0
1 % wt ND-ODA/PLLA	5.34±0.24	107	0.21±0.01	320
3 % wt ND-ODA/PLLA	5.50±0.29	113	0.25±0.00	400
5 % wt ND-ODA/PLLA	5.90±0.26	129	0.26±0.00	420
7 % wt ND-ODA/PLLA	6.83±0.51	165	0.31±0.06	520
10 % wt ND-ODA/PLLA	7.89±0.09	206	0.46±0.05	820



Low resolution TEM micrograph of ND-ODA/PLLA thin cross-section shows a uniform dispersion of ND-ODA in the PLLA matrix at 1% wt concentration.



Morphology (bisbenziminde and pahlloidin staining) of 7F2 osteoblasts on PLLA (A, B) and 10% wt ND-ODA/PLLA (C, D) scaffolds 2 (A, C) and 3 (B, D) days post-seeding.

Conclusions

1. Co-continuous porous structures with different micro-pore sizes were successfully generated in PLLA which facilitate the initial cell attachment and cell proliferation.
2. *in vivo* study shows after 4 weeks of rat subcutaneous post-surgery, ECM encapsulated the scaffolds. Histological analysis indicates that cells start to grow inside the porous structure.
3. The modeling system has been proposed to optimize the porous structure manufacturing process.
4. The mechanical properties of ND-ODA/PLLA composites were improved dramatically upon the addition of ND-ODA.
5. The addition of ND-ODA had only a negligible effect on cell proliferation, which is indicative of good biocompatibility of the composites

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