NANODIAMOND REINFORCED SMART SCAFFOLDS FOR BONE TISSUE ENGINEERING

Qingwei Zhang[1,2,3], Vadym Mochalin[4], Ioannis Neitzel[4], Isabel Knoke[4], Yury Gogotsi[2,4], Jack Zhou[2] and Peter I. Lelkes[1]
[1]School of Biomedical Engineering; [2]Department of Mechanical Engineering and Mechanics; [3]College of Medicine; [4]Department of Materials Science and Engineering, Drexel University, Philadelphia, PA 19104, USA

Introduction

Tissue engineering offers a promising approach to creating artificial constructs for regeneration of new tissue. A key challenge in bone tissue engineering is to design a proper scaffold with the mechanical strength similar to host tissue being replaced, good biocompatibility with adjacent tissue, and adjustable biodegradability so that it will be gradually replaced by the growing new tissue. Human cortical bone has Young's modulus of 5-27 GPa [1] and hardness of 0.62-0.74 GPa [2]. These unique tissue properties present a primary challenge to bone tissue engineering, *viz* to mimic the mechanical properties of natural bone with degradable, biocompatible and nontoxic materials. However, till now there are no artificial scaffolds that fully meet all of the above requirements.

Nanodiamond (ND) is an attractive nanomaterial for reinforcement of biopolymers due to its superior mechanical and chemical properties, and low biotoxicity [3]. In this study we explored the possibility of manufacturing the ND/poly(L-lactic acid) (ND/PLLA) composites with uniformly distributed ND-ODA (octadecylamine-functionalized nanodiamond) particles [4]. We emphasize the role of ND surface functionalization in achieving uniform dispersion and enhanced affinity between the components of the composite leading to improved mechanical properties. We further investigated biocompatibility of ND/PLLA composites and demonstrate that they can be used as efficient scaffolds for bone tissue engineering.

Materials and Methods

Sample preparation: PLLA (1g) (Gorinchem Inc., Netherland) was dissolved in 20 ml of chloroform (Sigma-Aldrich). Predetermined amounts of ND or ND-ODA (NanoBlox Inc., Florida, USA) with the average particle size of about 5 nm were weighed with an analytical balance, dispersed in 10 ml of chloroform and then immediately mixed with PLLA/chloroform solution. After solvent evaporation, the obtained thin film was dried under vacuum and used directly for further measurements.

Characterization: For transmission electron microscope (TEM), the samples were sectioned at about 100 nm and images obtained with a JEOL JEM 2100F TEM at 200 kV. Images at different magnifications were taken from several spots on the grid to ensure that the results shown are representative. Depth sensing indentation was performed using an MTS Nano Indenter XP with a 17 μm spherical tip. All tests were stopped at an indentation depth of 1500 nm . Each sample was tested 10 times in different locations. Cytotoxicity of ND and ND-ODA, as well as *in vitro* biocompatibility of ND-ODA/PLLA composites were tested by culturing osteoblast cell line (7F2, ATCC, CRL-12557) isolated from mouse bone marrow. Cell viability and morphology were assessed with alamarBlue™ assay (AB, Biosource, Alameda CA) and cytological staining with Hoechst 33258 (nuclei) and rhodamine phalloidin (F-actin) following published procedures [5-6].

Results and Discussion

The dispersion of ND-ODA in PLLA was observed by TEM in samples containing 1, 3 and 10% wt of ND-ODA. Low resolution TEM images (Fig. 1a-c) show that small agglomerates of ND-ODA particles are uniformly dispersed in PLLA matrix. With increasing concentration of ND-ODA, larger interconnected particle chains are formed (Fig 1c). Thus, we conclude from the TEM results in Fig. 1 that there is no strong agglomeration or phase separation of ND-ODA and PLLA in the composites up to, at least, 10% wt content of ND-ODA.

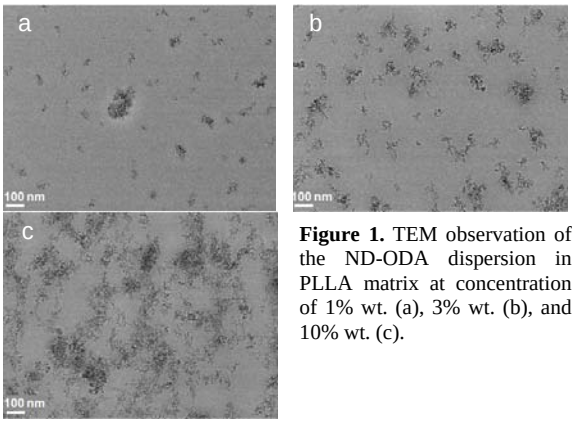

Figure 1. TEM observation of the ND-ODA dispersion in PLLA matrix at concentration of 1% wt. (a), 3% wt. (b), and 10% wt. (c).

Table 1. Mechanical properties determined by depth-sensing indentation

Description	Young's Modulus (GPa)	Hardness (GPa)
pure PLLA	2.6±0.1*	0.05±0.01
1 % wt ND-ODA/PLLA	5.3±0.2	0.21±0.01
3 % wt ND-ODA/PLLA	5.5±0.3	0.25±0.01
5 % wt ND-ODA/PLLA	5.9±0.3	0.26±0.01
7 % wt ND-ODA/PLLA	6.8±0.5	0.31±0.06
10 % wt ND-ODA/PLLA	7.9±0.1	0.46±0.05

*- Standard deviation

Young's modulus and hardness values of the composites were calculated after effective zero point correction [7] and are presented in Table 1. The measured Young's modulus of the PLLA thin film is 2.58 GPa, which is comparable to the value for the PLLA film (2.05 GPa) reported by Martin et al. [8]. The measured average Meyers hardness was 0.05 GPa. It should be noted that the modulus and hardness of our PLLA thin film were lower than that of PLLA samples produced by injection molding (Young's modulus 4.6 GPa and hardness 0.23 GPa) [9]. These differences are probably due to different crystallinity of the

PLLA produced by solution casting (our samples) *vs.* PLLA sample subjected to elevated temperature and mechanical drawing during the injection molding process [10].

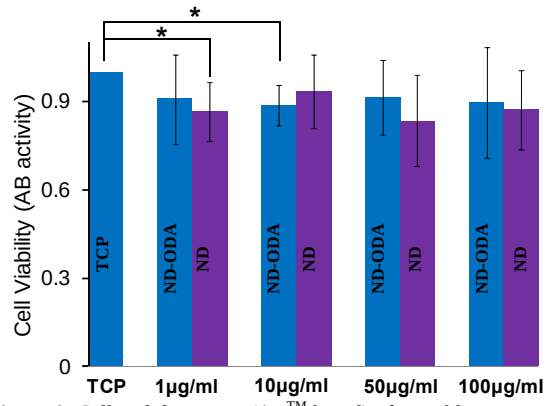

Figure 2. Cell viability assay (AB™ based) of osteoblasts grown on tissue culture plastic in the absence (TCP), or presence of, respectively, 1-100 µg/ml of ND-ODA, and ND (n=6).

Since the toxicity of ND-ODA had not been previously determined, the biocompatibility of ND and ND-ODA particles was studied in experiments with mouse bone marrow derived osteoblasts (7F2) Treatment of the osteoblasts with 1-100µg/ml of both ND and ND-ODA particles did not reduce the cell viability (Fig. 2).

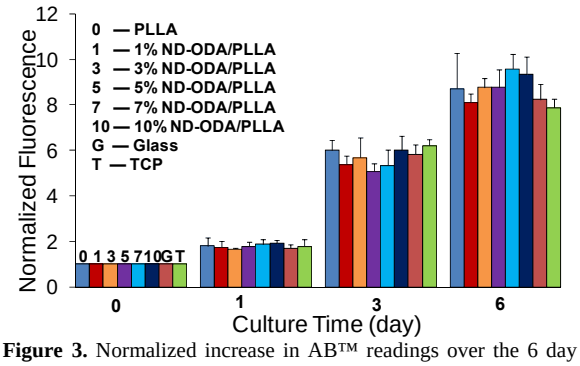

Figure 3. Normalized increase in AB™ readings over the 6 day 7F2 cell culture on pure PLLA, ND-ODA/PLLA (1-10% wt of ND-ODA), and control glass and TCP (n=6).

To illustrate the feasibility of the use of ND-ODA/PLLA as a matrix for supporting cell growth, 7F2 cells were cultured on the scaffolds for 6 days. The attachment and proliferation of 7F2 on all scaffolds were assessed by fluorescent nuclear staining with Hoechst 33258 and Alamar Blue™ assay. The results (Fig. 3) showed that the additions of ND-ODA do not influence cell growth, which is an indicative of good biocompatibility of the composites.

Conclusion

In this study we demonstrate that with ODA functionalization, ND can disperse in PLLA uniformly. Cytotoxicity tests suggest that both ND and ND-ODA are biocompatible with osteoblasts. In conjunction with the toxicity testing of nanodiamonds, 7F2 cells were grown on ND-ODA/PLLA scaffolds with different concentration of ND-ODA to examine their sustained viability over time, which provided further assurance for the utility of ND-ODA as reinforcement for PLLA in bone tissue engineering. Furthermore, the mechanical properties of ND-ODA/PLLA composites system are increased significantly with the addition of ND-ODA. Addition of 1% wt of ND-ODA can increase the hardness by the factor of 4 and 10% wt of ND-ODA lead to about an order of magnitude increase in the hardness. A 3 times higher Young's modulus and improved hardness make this material very promising for bone tissue engineering applications.

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