Fabrication of Novel Polylactic Acid/Amorphous Magnesium Phosphate Bionanocomposite Fibers for Tissue Engineering Applications via Electrospinning

Maryam Nabiyouni, Huan Zhou, Sarit B Bhaduri.
University of Toledo, Toledo, OH 43606

Statement of Purpose: This paper reports, for the first time, the successful in-vitro evaluation of polylactic acid (PLA)/ amorphous magnesium phosphate (AMP) nanocomposite fibers. As opposed to the great interest in amorphous calcium phosphates in bone regeneration, AMPs have received minimal attention. Recently, there has been a great deal of interest in using magnesium phosphates in orthopedics. It is well known that inorganic ions like calcium, and magnesium, the 3rd and the 4th most abundant ions in humans respectively, are involved in bone growth, mineralization, and regeneration. So, AMPs have great potential as biocompatible bone substitutes. In this study, fibrous nanocomposites consisting of AMP nanospheres and PLA were fabricated by electrospinning for two significant reasons. First, to the best of our knowledge, it is the first attempt to fabricate the biocompatible, biodegradable AMP/biopolymer composite by adding AMP to PLA fibers. Second, our results clearly illustrates the negative effect of 12-hydroxysteric acid (HSA), used as a dispersant of nanosphers, on osteoblast proliferation in contrast to the previous report by Kim et al [1].

Methods: The AMP nanospheres were synthesized using a microwave technique. The reaction solutions were prepared by mixing $MgCl_2.6H_2O$, KH_2PO_4 and $NaHCO_3$ in 1L DI water with a 1:1 Mg/P molar ratio. The solution containing beakers were placed into a household microwave (Emerson, 800 W, 2450 MHZ) heating at the maximum power for 5 min. The precipitates were moved to a cold bath, centrifuged and dried in 37 ˚C oven overnight. The as-synthesized AMP nanospheres were added to the mixture of PLA-HSA and were electrospun. A horizontal electrospinning setup was used to fabricate fibrous composite scaffold with the pumping rate of 1 ml/h, working distance of 20 cm, the needle size of 22, and the voltage of 20 kV. Fibers were collected and dried in a fume hood to remove residual solvents. XRD, SEM, EDX, TEM, and DSC methods were used for physical characterisations. Finally, MC3T3 cells were used for in-vitro studies and monitoring of osteocalcin (OCN), osteopontin (OPN), alkaline phosphatase (ALP), collagen type-I (Col I), and beta actin (β actin) gene expressions.

Results: The XRD and SEM images of AMP nanospheres and PLA fibers are shown in Figure 1. PLA pellets examined by XRD showed some crystallinity with a strong peak observed at 16.5˚. After electrospining, PLA lost its crystallinity. The as-synthesized AMP particles are highly amorphous. The SEM images illustrated the biocompatibility of the resulting PLA-AMP fibers showing high cell growth (Figure 2). The cell growth was much higher in PLA-MgP (Figure 2.b) fibers than the PLA (Figure 2.a) and PLA-MgP-HSA (Figure 2.c) fibers. Lastly, fold change in expression level of different genes grown on PLA-MgP and PLA-MgP-HSA fibers are shown (Figure 3). The expression levels were normalized to the beta actin expression of preosteoblasts grown on the PLA fibers.

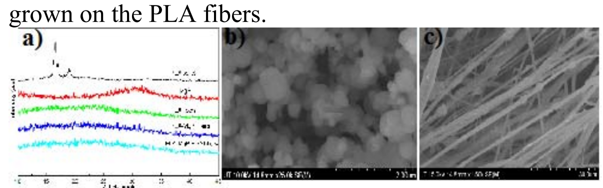

Figure 1. a) XRD patterns of AMP nanospheres and PLA fibers, b) AMP nanospheres, c) PLA fibers

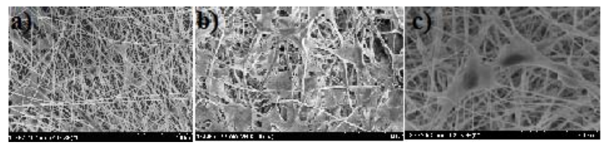

Figure2. SEM images of (a) PLA, (b) PLA-MgP, (c) PLA-MgP-HSA fibers

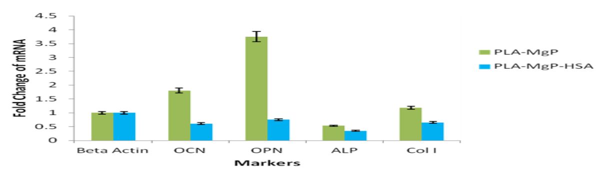

Figure 3. PCR results of OCN, OPN, ALP, and Col- I gene expression level of MC3T3 cells cultured on PLA-MgP and PLA-MgP-HSA fibers normalized to the β actin housekeeping gene

Conclusions: We have successfully fabricated fibrous composites of PLA and AMP nanospheres via electrospinning. This study provides significant data regarding the physical and biological properties of PLA-AMP composites and their in vitro interaction with preosteoblast cells. The presence of AMP nanospheres in PLA matrix is beneficial to bone formation by increasing the gene expression of OCN, Col-I and OPN of preosteoblast cells. Additionally, it is observed that HSA, a surfactant used to improve ceramic dispersion in polymer matrix, shows adverse effects to preosteoblast cells when present in conjunction with AMP (MgP). It is believed AMP can be a potential candidate in bone tissue engineering applications. Future studies will be focused on the effect of AMP on the bone tissue regeneration and its related mechanism.

References:

[1] S. R. Kim, J. H. Lee, Y. T. Kim, D. H. Riu, S. J. Jung, Y. J. Lee, S. C. Chung, and Y. H. Kim, "Synthesis of Si, Mg substituted hydroxyapatites and their sintering behaviors," *Biomaterials*, vol. 24, no. 8, pp. 1389–1398, 2003.

Abstract #853