Preparation and Characterization of Poly (hydroxybutyrate co hydroxyvalerate) Nanofibers Containing Sol-gel Derived Bredigite for Bone Regeneration Applications

Monireh Kouhi[1, 2*], Mohammadhossein Fathi[1], Morteza Shamanian[1], Molamma P. Prabhakaran[2], Seeram Ramakrishna[2]

1. Biomaterials Research Group, Department of Materials Engineering, Isfahan University of Technology, Isfahan, Iran.

2. Center for Nanofibers and Nanotechnology, Faculty of Engineering, National University of Singapore, Singapore.

Statement of Purpose: Recently, electrospun nanofibrous mats served as suitable scaffolds for bone tissue engineering (BTE) thanks to large surface area, high porosity with an interconnected network, which can architecturally mimic the structure of extracellular matrix (ECM)[1,2]. Poly (3-hydroxybutyrate co hydroxyvalerate) (PHBV) is a biodegradable, biocompatible, non-toxic polyester produced by bacteria, has received great attention lately as scaffold materials in the drug delivery system and tissue regeneration applications[3]. To overcome the limitation of PHBV including the lack of bioactivity and insufficient mechanical properties for BTE scaffold, in this study, bredigite (one of the most bioactive bioceramic in the ternary system CaO-MgO-SiO_2 [4]) nanoparticles were synthesized using a sol gel method and added to PHBV nanofiber through the electrospinning process.

Methods: Bredigite nanoparticles were prepared through a sol-gel method using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS, Merck), magnesium nitrate hexahydrate($Mg(NO_3)_2.6H_2O$, Merck) and calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H2O$, Merck) as raw materials. Pure and composite nanofibers of PHBV (Tian An Enmat chemical co, China) were fabricated from 8wt% PHBV in HFIP (Sigma, Aldrich) solutions containing 5, 10 and 15% bredigite nanoparticles. X-ray diffractometer (XRD) analysis and transmission electron microscopy (TEM) were used to study the phase structure and morphology of synthesized bredigite nanoparticles. The surface morphology of the electrospun scaffolds was observed with field-emission scanning electron microscopy (FE-SEM). The nanofibrous scaffolds containing different amount of nanoparticles were tested for their mechanical strength using a table top tensile tester. In vitro bioactivity and biodegradation behavior of pure and composite nanofiber scaffolds were also evaluated by immersion the scaffolds in simulated body fluid (SBF) during predicted period of time and the surface morphology and chemistry were investigated by using SEM and Fourier transform infrared spectroscopy (FTIR).

Results and discussions: XRD pattern of prepared bredigite nanoparticles (Figure 1), was in good agreement with the standard card of bredigite (JCDP: 036-0399) with no additional peaks. TEM image and size frequency diagram of synthesized nanoparticles indicated that bredigite nanoparticles had an average particle size of 25nm. Results of SEM study showed that PHBV nanofiber containing different amount of bredigite nanoparticles had the similar fiber diameter meaning the bredigite nanoparticles did not produce significant changes in the average fiber diameter ($p<0.05$), though high amount of bredigite caused nanoparticles agglomeration. Results of mechanical test revealed that by adding and increasing the bredigite nanoparticles into the PHBV nanofibers up to 10%, The Young's modulus increased from 99MPa to 147MPa and ultimate strength increased from 4.01Mpa to 5.45MPa indicating the reinforcement of fibrous scaffold. Results also showed that strain at break decreased by adding and increasing the nanoparticles due to a quasi-brittle behavior. When bredigite content reached 15% wt., young modulus and ultimate tensile of nanofibrous scaffolds decreased due to the presence of nanoparticles agglomeration and heterogeneity of the nanofiber structure.

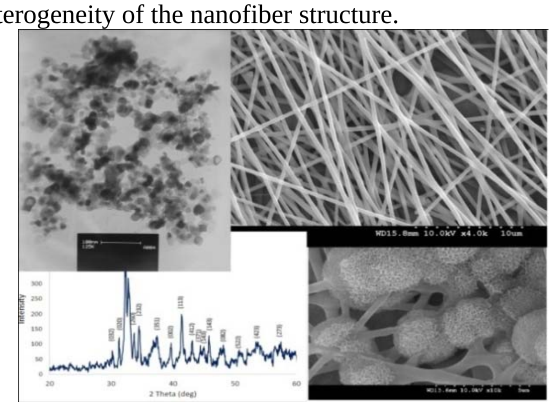

Figure1- TEM image and XRD pattern of bredigite nanoparticles and SEM images of PHBV nanofibers before and after immersion in SBF

In vitro bioactivity assay showed that PHBV nanofiber containing bredigite nanoparticles, induced formation of apatite crystal after 1 week immersion in SBF and appeared to be coated heavily over the composite fibrous scaffolds during the immersion time. In contrast, no apatite formation was evident in the pure nanofiber. Results of biodegradation behavior study revealed that PHBV/bredigite scaffold had increased degradation rate compared to pure PHBV scaffold due to presence of Si containing component of bredigite.

Conclusions: In the present work, bredigite nanoparticles and PHBV/bredigite nanofibers were successfully produced for the first time. Results showed that incorporation of bredigite into the PHBV nanofiber improved the Young's modulus and strength of composite PHBV scaffold compared to pure PHBV scaffold. Results also suggested that the incorporation of bredigite induced favorable bioactivity and biodegradation on the composite nanofibrous scaffolds.

References:

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