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Fabrication and characterization of water soluble vitamin loaded Poly (lactic-co-glycolic acid) aligned electrospun nanofibers

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Abstract

Recent trend in nano biomaterial are focusing on combination of various biological molecules with natural or synthetic polymers to improve their physiochemical and mechanical properties. In present work we have blended the water soluble vitamin (B5)with PLGA solutions and fabricated vitamin loaded aligned nanofiber mash by electrospinning process. Material was further characterized for its morphology, mechanical strength and surface wettability properties.The average diameter of PLGA and PLGA/Vt-B5 nanofiber was noted560.12±88.46nm and 305.44±58.95nm respectively. The tensile strength was noted higher in PLGA nanofibers 24.16±1.82MPa as compared to PLGA/Vt-B5 nanofibers 19.49±1.38MPa respectively.However, by the addition of vitamin contact angle measurement were decreased from 126.01° to 117.23° in PLGA/Vt-B5 nanofibers.Present studied provideda basis for further application of this nanofibrous material in tissue engineering and drug delivery applications.

Key words: Electrospinning; water soluble vitamin; PLGA; Aligned nanofibers; Biomaterial.

Introduction:

The selection of idealbiomaterial is one of the most important aspects that should have synchronised biocompatibility, biodegradability and mechanical properties. For this instancevarious biological and synthetic materials have shown their own strengths and weakness reported previously(Agarwal, Wendorff et al. 2008). An inadequate selection of material may cause incomplete recovery such as low permeability of material or chronic inflammatory response or significant deformation or degrading swelling or compatibility of mechanical properties with native tissue(Biazar 2010, Nectow, Marra et al. 2012, Carriel, Alaminos et al. 2014). In this contrast so far many natural polymers, synthetic non degradable and synthetic biodegradable materials have been tested in various tissue engineering approaches and achieved some good results but still having some short comings that should be address to meet the specific targets (Verreck, Chun et al. 2005). Likewise, hydrophobic nature of synthetic biodegradable materials limits their use as tissue engineering scaffolds so biodegradable materials should be preferred over non-degradable materials because of its chronic inflammation and compression to tissue over time characteristics(Haile, Haastert et al. 2007). The compatibility and mechanical stability of any material are two most important features for successful graft. For tissue engineering electrospun nanofibers scaffolds are preferred over phase separation and self-assembly based scaffolds due to their high surface area to

volume ratio that maximizes the chance of cell attachment and growth (Bhutto, Zhang et al. 2016). Aligned fibers have been produced by using different collectors during electrospinning process which not only enhanced the mechanical properties of scaffolds but also increase biological response (Kuihua, Chunyang et al. 2014, Zhang, Qiu et al. 2014, Huang, Kuo et al. 2015, Bhutto, Zhang et al. 2016). PLGA is the most promising polymer used as vehicle for drug delivery and tissue engineering applications(Rowlands, Lim et al. 2007, Lee, Bashur et al. 2009, Li, Wu et al. 2012, Zamani, Latifi et al. 2013).It is FDA approved polymer with diverse properties such as biocompatibility, variable mechanical properties and biodegradability with wide range of erosion time(Holy, Dang et al. 1999, Makadia and Siegel 2011).PLGA is hydrophobic in nature due to presence of methyl group of PLA(Makadia and Siegel 2011). The surface hydrophilicity of the material could be achieved by using surface modification method such as coating with ECM proteins or specific amino acid sequence on the surface (Subramanian, Krishnan et al. 2009). However, physiochemical properties could be enhanced by using some natural micronutrients such as growth factors and other biological molecules in composite manner (He, Xu et al. 2011, Sheng, Fan et al. 2013, Kuihua, Chunyang et al. 2014, Xu, Dong et al. 2014).Vitamins are one of the most important biology micronutrient required to sustain lifeand involved in a variety of reactions such as function as hormones, antioxidants, mediators for cell signalling and as regulators of cells or tissue growth and differentiation(Engelking 2015, Aqeel, Wu et al. 2016).

In present work we have blended the water soluble vitamin (B5)with PLGA solutions to produced aligned electrospun nanofiber mashes PLGA/Vt-B5 respectively, and characterized the material for its morphology by SEM, mechanical strength, and surface wettability properties.Present work provides basis for further studies of this aligned nanofibrous material in tissue engineering and drug delivery applications.

2. Experimental:

2.1 Material

The co-polymer of Poly (lactic-co-glycolic acid) (PLGA) (87:13) was purchased from Jinan Daigang Bioengineering Co. Ltd (China). 1,1,1,3,3,3,-hexafluoro-2-propanol (HFIP) was purchased from Daikin Industries Ltd (Japan). Water soluble Vitamins B5 was purchased from sigma.

2.2 Electrospinning solution

The electrospinning solution of PLGA was prepared by dissolving the 1.5 grams of co-polymer in 10ml of 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) to yield an ultimate concentration of 15% (w/v). 50mg of vitamin B5separately were added in solution with total volume of 10 mL to produce the PLGA/Vt-B5 solutions, and all prepared solutions were constantly stirred overnight.

2.3 Electrospinning parameters

The 3ml of each solution were pumped through 21 gauge needle with the flow rate of 1.2ml/hour.However, the distance between the collector and needle was set 15 cm. The aligned nanofibers were collected by using the rotating drum collector at a speed of 3500rpm. A high voltage of 14KV was supplied by a high voltage power supply (BGG6-358, BMEI Co Ltd, Beijing China).

2.4 Morphological study

The electrospun nanofibers surface morphology was observed by scanning

electron microscope (SEM). Dry samples were sputter-coated with gold for 10 seconds (twice) and scanned at the accelerating voltage of 10kV for SEM imaging. For average nanofiber diameter calculationImage J software (National Institute of Health, USA) was used and from each SEM image 100 electrospun nanofibers diameters were selected and calculated the average diameter distribution.

2.5 C**haracterization of electrospun nanofibers**

The characterization of prepared scaffold was evaluated by Fourier transforms infrared spectroscopy (ATR-FTIR) as reported previously(Sun, Li et al. 2014). Infrared measurements were performed on an Avatar 380 FTIR spectrometer (Nicolet 6700, Thermo Fisher, USA) at transmission mode (32 scans) in the wavelength ranges of 500 to 4000cm^{-1}.

Surface wettability characteristics were tested by measuring the water contact angle on the surface of nanofiber matesusing a contact angle measurement instrument (OCA40, Dataphysics, Germany). 0.3ml deionized water was used for each measurement and for each sample test were conducted on 5 different positions and finally calculated the average value.

The mechanical strength of the electrospun samples (10x 30 mm^2,n=5) were tested by universal material tester (H5 K-S, Hounsfield,UK) at an ambient temperature of 20°C with 65% humid environment. The cross head speed was set at 10mm/min and the stress-strain curves were plotted for each sample. Finally tensile strength, elongation at brakewas calculated.

2.5 Statistics analysis

Statistical analysis was performed using 8.0 (Origin Lab Inc.,USA). All the values were in triplicate and expressed as means± standard deviation (SD). Statistical difference between control and different samples was determined via one way ANOVA paired test followed by Bonferroni's multiple comparison test ($p<0.05$) considered as statistically significant.

3. Results and Discussion:

3.1 Morphology of electrospun nanofibers

The electrospun nanofibers morphology and alignments are shown in Fig.1. SEM images showed that average diameter of PLGAand PLGA/Vt-B5 nanofiber was 560.12±88.46nm and 305.44±58.95nm (Fig.1A-B)respectively. It was observed that the diameter of vitamin loaded nanofibers are smaller than PLGA nanofibers, it may be due to two possible reasons, first presence of hydrophilic/charged groups of vitaminresulting the electric charges were increased during the process of electrospinning and second changing in viscosity of solutions due to addition of vitamin (50mg) and produced smaller nanofibers of PLGA/Vt-B5.Previously it is well reported that under the same electrospinning parameters if solution concentration is changed it effects on diameter of the nanofibers(Zhu, Zhang et al. 2006, Jun, Jeong et al. 2009, Wang, Zhang et al. 2011).

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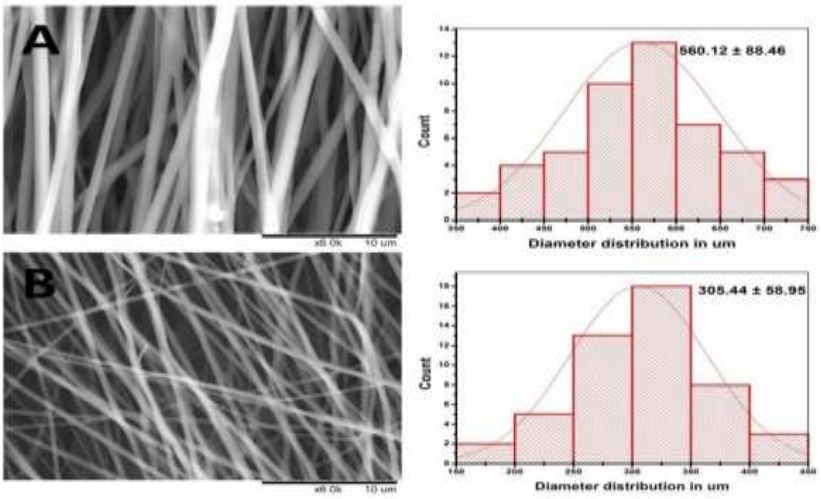

Figure 1: Sem images of A) PLGA and B) PLGA/Vt-B5 nanofibers

3.2 Characterization of electrospun nanofibers

ATR-FTIR spectrum of pure vitamin B5, PLGA and PLGA/Vt-B5 and nanofiber is shown in Fig.2. The pure vitamin B_5spectra represents the two main characteristic peaks of amide I ($\nu_{C=O}$) and amide II (δ_{NH}) at 1642 cm^{-1} and 1558 cm^{-1}respectively, whereas spectra of PLGA represent the characteristic ester group stretching peak at 1752 cm^{-1} (C=O).The appearing of all these three peaks in PLGA/Vt-B5 nanofiber spectra confirming the presence of vitamin with PLGA.

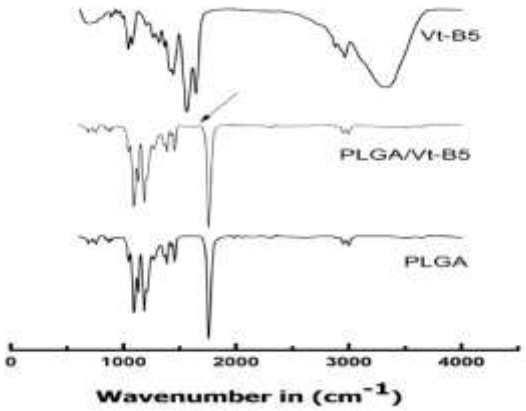

Figure 2: ATR-FTIR spectrum of pure vitamin B5, PLGA/Vt-B5 and PLGA nanofibers.

A surface wettability property of any material is very important because it directly influences on biocompatibility and biodegradability. The water contact angle measurements onPLGAand PLGA/Vt-B5nanofibers surface are illustrated in Fig.3. Results showed that PLGA nanofibers surface were hydrophobic (126.01±2.1°), however by the addition of water soluble vitamin B5the nanofibers become slightly

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towards hydrophilic and the contact angle decreasedto117.23±1.7°respectively. These results are confirming that vitamin is present on the surface of nanofiber mashes and its water soluble nature effects on PLGA nanofibers wettability properties which will be supportive in cell attachment and tissue engineering application.

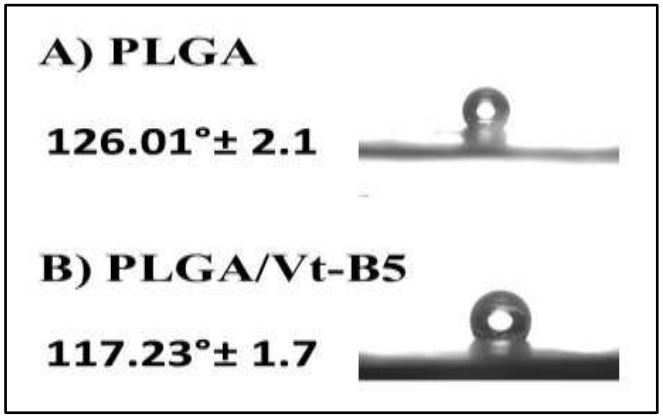

Figure 3: water contact angle measurements on PLGA and PLGA/Vt-B5 nanofibers surface

Mechanical properties of any biomaterialis very important because it providesthepreliminary biomechanical profile for the cells and tissue(Chen, Wang et al. 2010). The average thickness of PLGA and PLGA/Vt-B5 nanofibers meshes were measured 0.10 and 0.07mm, respectively. The mechanical properties of the PLGA and vitamin loaded PLGA/Vt-B5 nanofibers were characterized by stress-strain curve as shown in Fig.4.The tensile strength of PLGA nanofiber mash was observed 24.16±1.82MPa whereas by the addition of vitamin the tensile strength of PLGA/Vt-B5 nanofibers are decreaseto19.49±1.38MPa . Meanwhile the elongation at break of PLGA nanofibers were noted at 102.49 ±4.16 % which is dropped down to 50.21±5.95% in PLGA/Vt-B5 nanofibers mashes respectively.

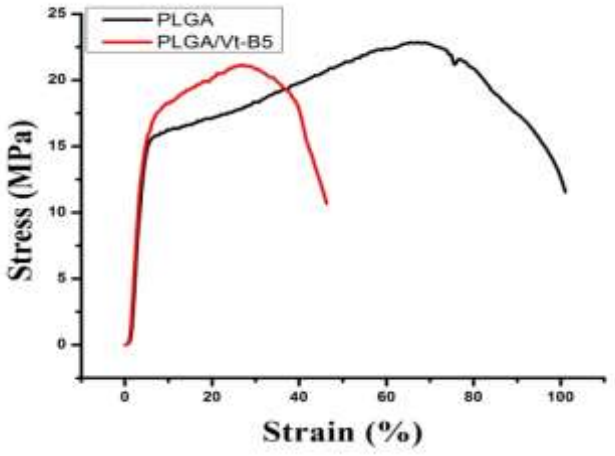

Figure 4: Stress-strain curve of PLGA and PLGA/Vt-B5 nanofibers mashes.

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4. Conclusion:

In present work we have successfully blended the water soluble vitamin (B5) with PLGA solutions and produced aligned electrospun nanofiber mashes. It is concluded that addition of vitamin B5 with PLGA solution decrease the nanofiber diameter size, elongation at break and tensile strength of the nanofibers mashes. Meanwhile, slightlyincreases in hydrophilicity were noted in PLGA/Vt-B5 nanofibers mashes as compared to PLGA alone. Present studied provideda basis for further application of this nanofibrous material in tissue engineering and drug delivery applications.

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