Optimization of Process Parameters of PLGA/CS Nano-bio-composite as Tissue Engineering Scaffold

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1- Introduction

In recent years, autogenetic and allogenetic tissues have been used as substitutes in the treatment of damaged tissues. However, secondary surgery would bring donor site morbidity and lead to infections or immune responses [1]. Cells should be able to adhere and proliferate in a suitable scaffold in order for the damaged tissues to regenerate. Viable substitutes can be implanted into the functioning tissue surrounding an injured area [4]. For this purpose, biodegradable and biocompatible synthetic or natural polymers have been used to develop scaffolds especially designed to mimic the structure and biological function of the native extracellular matrix (ECM) proteins. Such scaffolds provide the mechanical support required and enhance the cell attachment and proliferation rate [2-5]. Among the current methods used for fabricating tissue engineering scaffolds, electrospinning has many advantages. The polymeric scaffolds fabricated by electrospinning are promising materials or substrates with high specific surface area, high porosity, and interconnect pores similar to the natural extracellular matrix (ECM) which can regulate cell activities. The current study describes a processing method, reported here for the first time, for the fabrication of PLGA/CS biocomposites. This method describes the deposition of chitosan nanoparticles on electrospun PLGA nanofibers by electro-spraying the chitosan solution. Randomly-oriented and aligned PLGA and PLGA/CS biocomposite nanofibrous matrices (with the different PLGA/CS ratios of 90/10, 80/20 and 70/30 (w/w) %) were successfully prepared by this methods. The scaffolds produced were subsequently characterized in terms of their morphology by SEM.

2- Experimental

The PLGA/CS nano-biocomposites were electrospun onto a rotating collector from a PLGA solution (24% w/v in TFE). The deposition of chitosan nanoparticles on the electrospun PLGA fibers was initially optimized by electro-spraying the chitosan solution into nano-sized particles at a lower concentration with negligible chain entanglement. This is because continuous fibers cannot be produced by electrospinning at high concentrations. Chitosan nanoparticles were distributed uniformly on the PLGA nonofibrous structure by simultaneous electrospinning, and the nanoparticles appeared to adhere strongly to the PLGA nanofibers. In this method, chitosan was dissolved in TFA at a concentration of 2% w/v before being sprayed onto the target by electrostatic charge and at a feeding rate of 0.33 ml/hr and an applied voltage of 13-14 kV. The distance between the spraying nozzle and the mat was 10 cm. In the double-nozzle method, the PLGA and chitosan solutions were simultaneously electrospun from two different syringes and mixed on the rotating drum to prepare the nanofibrous biocomposite membrane. A 90/10 weight ratio of PLGA/CS was obtained by simultaneous electrospinning of the two solutions. To obtain the 80/20 and 70/30 weight ratios of PLGA/CS, the duration of electro-spraying the chitosan solution was increased without electrospinning the PLGA solution in multi-steps. Randomly-oriented PLGA/CS nano-biocomposites and aligned nanofibers were formed using a rotating drum at 50 and 4000 rpm, respectively. The fabricated scaffolds were dried overnight under vacuum at room temperature. Electrospun nanofibrous membranes were sputtered with gold, and their morphology was observed using a scanning electron microscope (SEM, Seron Technology AIS 2500, India). The diameters of the resulting nanofibers were determined using the Image J software from the SEM micrographs.

3- Results

Using the Image J software for the SEM micrographs, the average particle size and fiber diameters of the CS and CS/PLGA were determined. Chitosan nanoparticles obtained under this condition were highly uniform. However, the average diameter of chitosan particles was 91 ± 8 nm in the optimum condition.

The PLGA/CS electrospun fibers/electro-sprayed nanoparticles with three different ratios (90/10, 80/20, and 70/30 w/w %) were fabricated. In this method, PLGA and chitosan solutions were simultaneously electrospun from two different syringes and the electrospun PLGA nanofiber and electro-sprayed CS nanoparticles were mixed and collected on the rotating drum to prepare the nanofibrous composite membrane. As shown, the average fiber diameter of the PLGA/CS electrospun fibers prepared by electro-spraying the chitosan solution on the PLGA nanofibers is lower than the pure PLGA nanofibers but higher than the ones fabricated by the first method. This could be due to the simultaneous effect of two electrical fields. In order to prepare the aligned scaffold, a high-speed rotating drum was used as the collector at a speed of 4000 rpm. Compared with the randomly-oriented nanofibers, the aligned ones were

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smaller in diameter but no significant differences ($p<0.05$) in the diameter were observed.

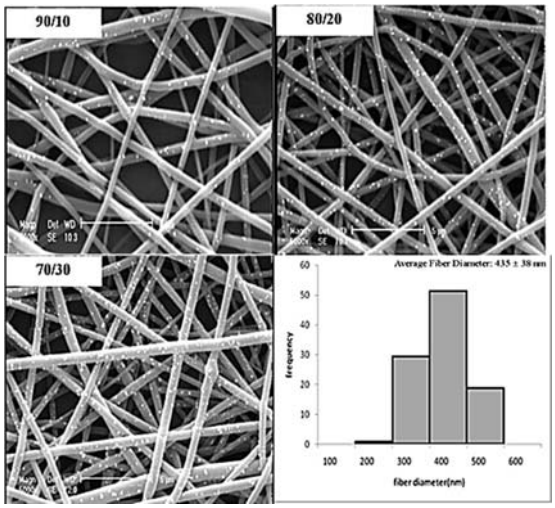

Fig. 1 SEM micrographs and the average fiber diameters of the randomly-oriented of PLGA/CS fibers

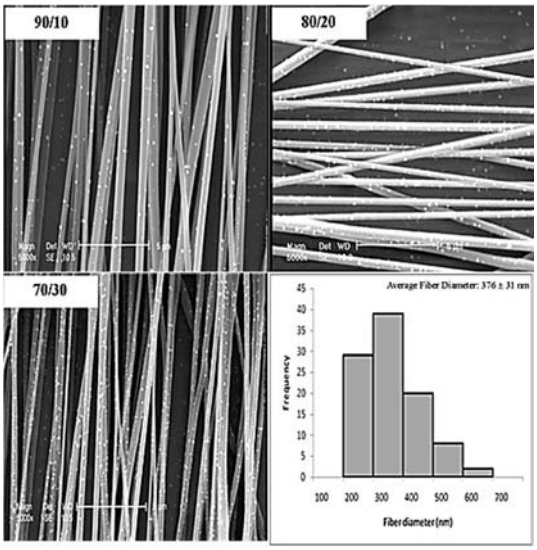

Fig. 2 SEM micrographs and the average fiber diameters of the aligned-oriented of PLGA/CS fibers

All the fabricated scaffolds were 70-80 μm in thickness as evaluated by a scanning electron microscope using a cross section prepared by the cryocut at three points and measured by the Image J software.

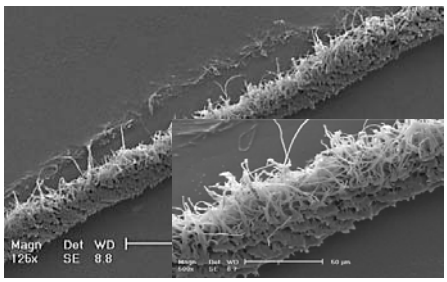

Fig. 3 TEM image of cross-section of scaffold (thickness)

4- Conclusions

In this study, both the randomly oriented and the aligned PLGA and PLGA/CS nanofibrous scaffolds were fabricated. Scaffold composed of PLGA and chitosan nano-particles provide the mechanical support required. In this study, chitosan nanoparticles and PLGA were used to fabricate the scaffolds by electrospinning method, for the first time. Nanofibrous scaffold with high percentage of chitosan nanoparticles provide a beneficial approach for tissue engineering.