EVALUATION OF GELLAN BASED ELECTROSPUN NANOFIBERS FOR WOUND HEALING

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Abstract

In recent years, specialized high technology materials such as polymeric nanofibers with biomimetic properties have emerged as a viable alternate to traditionally used simple natural coverings. In this investigation, wound healing ability of electrospun gellan-based nanofibers has been evaluated for the first time. The gellan-based ultrafine nanofibers were fabricated by using a blend mixture of gellan and polyvinyl alcohol (PVA). As determined by field emission scanning electron microscopy (FESEM), the fiber morphology and average diameter of the electrospun nanofiber was found to be influenced by solution viscosity, surface tension, specific gravity, flow rate, tip-to-collector distance and applied voltage. The equal ratio (1:1) of gellan-to-PVA was documented as an optimum polymeric ratio to fabricate uniform bead free nanofibers with an average diameter of 40±15.8nm. Differential scanning calorimetry (DSC) and Fourier transformed infrared (FTIR) analysis confirmed the stability as well as the crosslinking within the polymers. The crystalline nature of gellan-PVA nanofibers was assessed using X-ray diffraction (XRD) analysis. Furthermore, Human dermal fibroblast (HDF) cells were cultivated on fabricated blend nanofibers in order to investigate the effect of gellan-based nanofibers on the differentiation and proliferation of fibroblast cells. This study revealed that gellan-based nanofibers could be employed as a novel wound healing material.

Keywords: Nanofiber, Electrospinning, Gellan, Wound healing.

1. INTRODUCTION

Gellan is a FDA-approved biocompatible polysaccharide which is composed of a linear chain of repeating tetrasaccharide units of D-glucose, D-glucuronic acid and L-rhamnose residues in 2:1:1 ratio [1-3].The significant structural features such as presence of free carboxylic group and bio-adhesiveness properties of gellan makes it a favorable candidate for drug delivery, tissue engineering and wound healing applications [4]. A wide range of gellan based formulations (eg. microsized fibers, nanoparticles, implanting hydrogels and thin film) has been reported earlier by various researchers throughout the world [5-9]. However, despite advantageous features of gellan, only limited studies regarding gellan based nano formulations have been scrutinized. The nano-sized formulations possess unique properties such as high surface area-to- volume ratio, which distinguish them from their macro-sized counterparts [10, 11]. Therefore, in this investigation, the gellan based electrospun nanofibers were fabricated using electrospinning technique which is recognized as a versatile, inexpensive, progressive and most innovative technique to produce fibers of diameter ranging from nano to sub-micrometer with distinct properties such as very high aspect ratio, porosity and special structural properties [12-14]. Electrospun nonwoven fibrous materials of natural polymers such as alginate, chitosan, hyaluronic acid, gelatin, collagen etc. have been synthesized and characterized with respect to their application as biocompatible materials [15-17]. However, due to the limited solubility, highly coiled chain conformations, repulsive forces and hydrodynamic responses between the polyanions present in the solution, the reproducibility and efficacy of these natural polysaccharides as an electrospun nanofibrous material always remains a challenge thus restrict their practical applications [18]. Like most of the natural polysaccharides, gellan also shows the complex sol-gel behavior and a non-typical solvation in water resulting in micro-aggregating gels which make the gellan aqueous solution very difficult to be electrospun. The above hurdle is overcome by preparing a blend solution of gellan with other water soluble, non-toxic, biocompatible and synthetic polymer i.e. PVA, which reduced the viscosity as well as the repulsive forces of

resultant solution. This study provided a detailed insight about the physiochemical properties of gellan which can be exploited for its usage in wound healing.

2. EXPERIMENTAL

Electrospinning process was carried out to fabricate gellan-PVA nanofibers as reported earlier [3]. Briefly, nanofibers' fabrication from the gellan-PVA blend solution were done at fixed electrospinning parameters (Voltage=21kV; Feeding rate=0.1ml/h; Tip-to-collector distance=18cm; PVA concentration=10 wt % and 1.5 wt% concentration of gellan). The nanofibrous samples were then subjected to morphological analysis using FESEM. IR absorptions of test samples were obtained at a resolution of 4 cm^{-1} in the scanning range of 4000-500 cm^{-1}. For thermogravimetric analysis the precisely weighed samples were heated from 25°C to 500°C at a scanning rate of 10°C/ min under a nitrogen gas atmosphere. The physical state electrospun nanofibers assessed using XRD analysis. For cytocompatibility evaluation, the sterilized nanofibrous formulations were seeded with HDF cells (1×10^5 cells ml^{-1}) along with DMEM medium. The cell morphologies for each sample were first observed through scanning electron microscopy.

3. RESULTS AND DISCUSSION

3.1 Physiochemical characterization of electrospun gellan-PVA nanofibers

The scanning electron micrograph of gellan-PVA blend nanofibers fabricated at equal ration of gellan and PVA is shown in Figure 1. The nanofibers were successfully electrospun at 1.5wt% concentration of gellan blended with 10 wt% concentration of PVA. The corresponding histogram (Figure 1) depicted the average diameters of about 40 ± 15.8 nm for gellan-PVA nanofibers

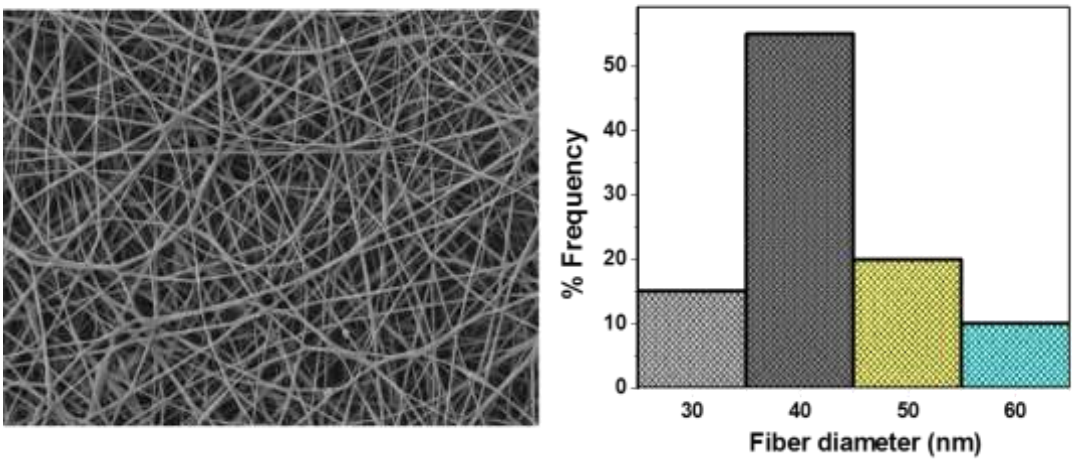

Fig. 1 FESEM micrographs and corresponding diameter distribution histograms of gellan–PVA electrospun nanofibers. Scale bar = 1µm

The FTIR spectrum (Figure 2A) of gellan-PVA nanofibers confirmed the presence of -OH interactions between gellan and PVA which were found to be in agreement with the findings of Sudhamani et al. (2003) who indicated similar interactions in gellan-PVA blend films [19].

Differential scanning calorimetry (DSC) revealed the thermal stability of gellan-PVA nanofibers (Figure 2B). The thermograms of gellan-PVA nanofibers exhibited a single decomposition peak at 306 °C with the shifting towards higher temperature as compared to the native gellan. This shifting characterizes the increased thermal stability of blend nanofibers which could be due to the formation of interpenetrating polymer network structure.

The XRD spectrum of electrospun gellan-PVA nanofibers were found to have intense peaks at around 2θ = 60° and 74°. The obtained sharp peaks indicated the crystalline nature of fabricated nanofibers as compared to native gellan and PVA. It is significant to state that the XRD data confirmed the realignment of gellan chains as well as the presence of interactions between gellan and PVA molecular chains.

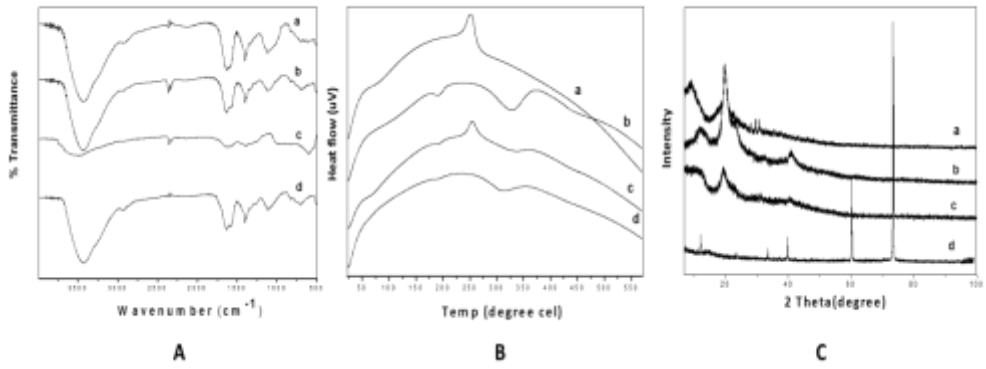

Fig. 2 (A) FTIR, (B) DSC and (C) XRD spectra of (a) raw gellan (b) raw PVA (c) gellan-PVA physical mixture and (d) gellan-PVA nanofibers

3.2 Cytocompatibility studies

The cytocompatibility of gellan-PVA nanofibers was analyzed by observing the morphology, adherence and proliferation of HDF cells on it (Figure 3). The nanofibrous scaffolds showed improved adherence, penetration, proliferation and migration of cells within the scaffolds in a similar manner to native extracellular matrix (ECM). The large surface area of nanofibers is thought to responsible for providing high cellular binding sites which in turn encouraged the enhanced adhesion of cells on electrospun nanofibers.

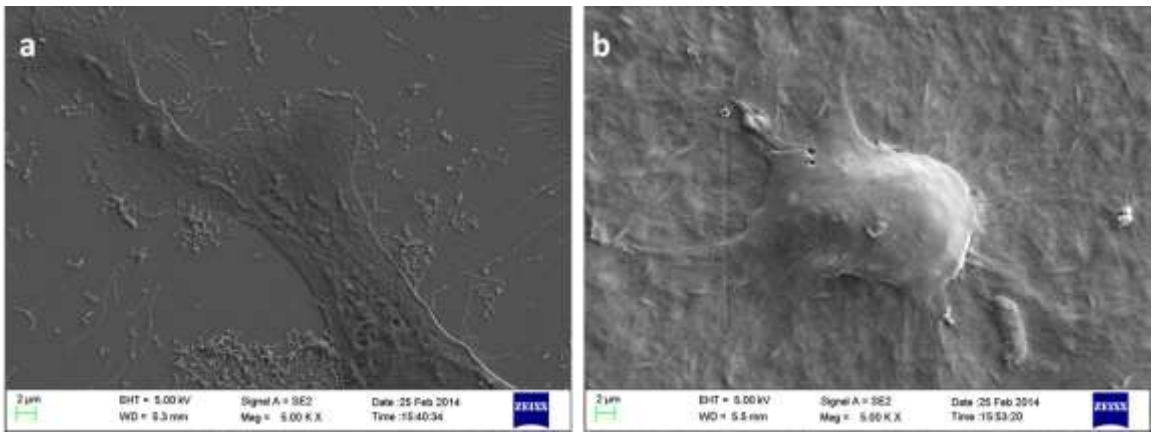

Fig. 3 FESEM micrographs of HDF cells after 24 h of cultivation on (a) control (b) gellan based nanofibers

CONCLUSION

In this investigation, the gellan-PVA blend nanofibers were successfully fabricated using a facile electrospinning approach. The developed nanofibers exhibit good uniformity, structural integrity and cytocompatibility. The nano-topographical features of electrospun gellan-PVA (such as high surface area to volume ratio) are believed to responsible for enhanced adhesion and proliferation of human dermal fibroblast cells. This property of fabricated nanofibers could be exploited for its usage for skin tissue regeneration or wound healing. In addition, the low fabrication cost of gellan-PVA nanofibrous scaffolds make them easy to process and scale-up in industrial sectors.

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