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Characterization of modified cellulose (MC)/ poly (vinyl alcohol) electrospun nanofibers for bone tissue engineering

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Abstract

In bone tissue engineering a variety of polymers were used to develop a suitable artificial bioactive scaffold for bone tissue regeneration. In this present study, we were using modified cellulose. Randomly oriented nanofibrous scaffolds of MC and poly (vinyl alcohol) (PVA) were synthesized by electrospinning technique. The blend solutions of MC/PVA with different weight ratio of MC to PVA were prepared using water as solvent to fabricate nanofibers. The morphology, diameter of electrospun nanofibers was studied using SEM. The crystalline and thermal properties of nanofibers were investigated by DSC and chemical characterization by FTIR analysis. These results showed that MC/PVA nanofibrous scaffold provides a beneficial frame for bone tissue engineering.

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Keywords: Modified cellulose, poly (vinyl alcohol), electrospinning, nanofibers scaffolds, bone tissue engineering

1. Introduction

Bone tissue engineering provides an alternative approach to repairing diseased or damaged tissue, enabling full recovery of the original state and function [1]. Bone is a complex tissue that serves multiple functions, including supporting the body, protecting organs, and storing nutrients. It has a highly anisotropic in nature, which results in a range of mechanical properties in every direction. When considering engineering bone tissue, particularly for musculo-skeletal tissues, matching the anisotropy and properties of the tissue scaffold is a key [2, 3].One of the most challenging goals in bone tissue engineering is the design of scaffolds as extracellular matrix (ECM) able to guide the process of tissue regeneration. ECMs should be biocompatible and non-toxic, and have a desired degradation rate, with high porosity, and good mechanical properties and should not cause foreign body reactions. The engineered artificial material support and guide cells to proliferate organize and produce their own extracellular matrix (ECM) to regenerate healthy tissue. Various type of materials like natural, synthetic, semi-synthetic and composite be there as scaffolds for bone tissue regeneration [4]. In addition, aligning the scaffolds can assist in the guided growth of the cells as well as increased cell proliferation [5] and higher natural extracellular matrix (ECM) production [6], compared to random fibers. Recently, electrospinning has emerged as a promising technique for fabricating scaffolds with nanofibrous features which can mimic the ECM. The inherent non-woven nature of the electrospun nanofibers results in interconnected pores sufficient for cell attachment and nutrient transfer [7,8].

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The natural bone matrix is the combination of organic/inorganic material and it consists of naturally occurring proteins specially collagen and a biological mineral which is apatite. Collagen makes a framework of fibers for cell attachment, growth and the inorganic mineral particles are embedded in between protein fibers and helps in the proliferation of cells and mineralization. In tissue engineering a wide range of polymeric scaffolds which are generally composed of natural occurring polymer (polysaccharides and proteins) and synthetic polymer such as poly (α-hydroxy esters). Although several biomaterials have been used as a bone scaffold system only a few biopolymers closely mimic the ECM by having polysaccharide chains in them. The commonly used polymers are in fabricating scaffolds such as poly (lactic acid) [9], poly (glycolic acid) [10, 11], poly (lactic-co-glycolic acid) [12], poly (caprolactone) [13], and natural polymer such as collagen [14], gelatin [15], silk [16] and chitosan [17,18]. Our intention was to use a biocompatible polymer with chemical structure similar to GAGs with polysaccharides chains and that is soluble in water.

So we have chosen modified cellulose for our studies. It is a non-ionic polymer with β (1→4) glycosidic linkage held together with H-bonds. It is a biocompatible water soluble polysaccharide material with protective colloidal action. It is not expensive and widely used in various pharmaceutical compositions, wound dressing and wound healing applications [19]. It was difficult to electrospun MC alone, so it was blended with PVA and electrospun. PVA has better fibre forming property through electrospinning and it is a poly (hydroxyl) water soluble polymer. It is biocompatible and biodegradable and widely used in biomedical applications which includes cervical dilators, drug delivery reservoirs, resorbable surgical sponges, orthopaedic stabilization splints, blood contacting material etc, [20]. There are several reports on PVA nanofibrous mats are prepared by electrospinning aqueous PVA solution [21].

In the present work, modified cellulose/ PVA nanofibrous scaffolds were fabricated by electrospinning technique using water as the solvent. PVA increased the spin ability of cellulose and increased the mechanical strength of the nanofibers. The nanostructure of the obtained nanofibers was analysed by scanning electron microscopy (SEM).

2. Materials and Methods

2.1. Preparation of blends solution

Modified cellulose (high molecular weight) purchased from Merck, and 95% hydrolyzed poly (vinyl alcohol) (average molecular weight 95,000) was purchased from Acros Organics. All the chemicals were of the highest purity and used without further purification. Prior to experiment all the glassware was thoroughly washed with detergent and copiously rinsed with deionized water. MC solution (4wt %) was prepared by dissolving MC powder in deionized water at RT with continuous stirring for 12 h, to get homogenous solution. PVA solutions (10 wt %) were prepared using PVA powder and deionized water with continuous stirring at 80° C for 2 h. Blend solutions of MC and PVA were prepared by mixing them at various ratios, 50: 50 to 60: 40 (MC : PVA) with continuously stirring for 12 hrs.

2.2. Electrospinning

The MC/PVA polymer solution to be electrospun was taken in a plastic syringe (5 ml) with a hypodermic needle with a flat-filed tip, with ID of 0.8 mm. It was ensured that there were no air bubbles in the capillary containing the polymer solution. The flow rate of the polymer solution was maintained constant at 1 ml/h using a syringe pump. The tip-to-collector distance was set at 10 cm. The needle was connected to the positive electrode of the high voltage power supply using copper wires and the negative electrode was connected to the Al foil covered rotating drum which served as the collector. The polymer solutions were electrospun at 25 kV. The electrospun nanofibers were collected on the aluminum foil.

2.3. Scanning electron microscopy (SEM)

The morphology of the ultrafine MC/PVA nanofibers was observed on a ZEISS-EVO 50 scanning electron microscope (SEM). The electrospun nanofibers were collected and placed on glass cover slip. The glass cover slip were mounted on copper stubs with a double-sided conducting carbon tape and carefully coated with a thin layer of platinum (2 nm) using sputter coater and examined.

2.4. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) measurements have been performed using TA Q 1000 DSC using modulated mode. The heat evolved during isothermal crystallization was recorded as a function of temperature -50^{o}C to 200^{o}C under the nitrogen flow environment at flow rate 50 ml/s at a heating rate of 5^{o}C/min. The sample1mg were prepared in aluminium crucible.
The degree of relative crystallinity, X_C was estimated from the endothermic area by using following equation (1):

$$X_C = \Delta H_f / \Delta H^o_f \quad (1)$$

Where ΔH_f is the measure enthalpy of fusion from DSC thermograms and ΔH^o_f is the enthalpy of fusion for 100% crystalline PVA (ΔH_m = 138.6 J/g from literature [22].

2.5. Fourier Transform infrared spectroscopy (FTIR)

FTIR spectra of the electrospun nanofibers were obtained using FTIR Spectrometer S2000, Perkin-Elmer with absorbance range of 400-4000cm^{-1} with a resolution of 2 cm^{-1}.

3. Results and Discussion

3.1. Morphology of electrospun nanofibers

MC (4 wt. %) was blended with different ratio of PVA (10 wt. %) and electrospun in order to obtain nanofibers with bead-free morphology. Though the viscosity of cellulose was higher than PVA it was impossible to electrospun MC alone without adding PVA. Lower concentration of MC (below 4 wt. %) led to electro spraying resulting in droplets rather than fibers. It is well known that lower concentrated solutions lead to electro spraying without any fibre formation [23]. Higher concentration of MC (above 4 wt. %) was thick and had difficulty in coming out of the needle during electrospinning. MC nanofibers with bead free morphology were successfully obtained. The time required for blending the two polymers plays very important role. The polymers have to be stirred for at least 12 h to make it homogeneous and to get good fibers. The morphology and structure of 50:50 ratio of modified cellulose: PVA has shown in Fig.1 and for 60:40 MC: PVA nanofibers shown in Fig 2.

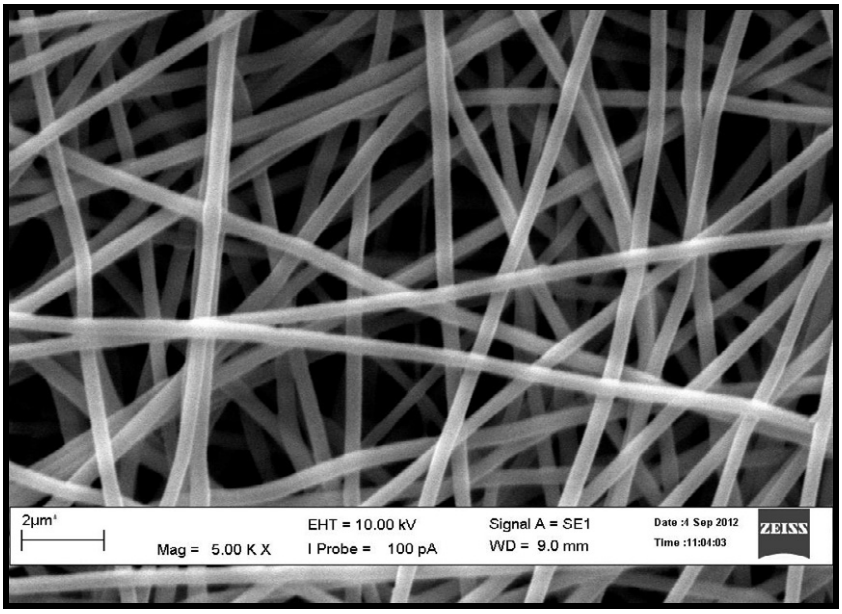

Fig. 1. Randomly oriented electrospun nanofibers of MC: PVA 50:50

The average diameter for 50:50 blend solution was 300 nm with fine structure but increasing the percentage of modified cellulose 60:40, the average diameter of fibers decreased to 250 nm and fiber structure was less fine. The variation in diameter suggests that the conductivity and visco-elastic properties are changing due to interaction between these two polymers, since all other variables were kept constant.

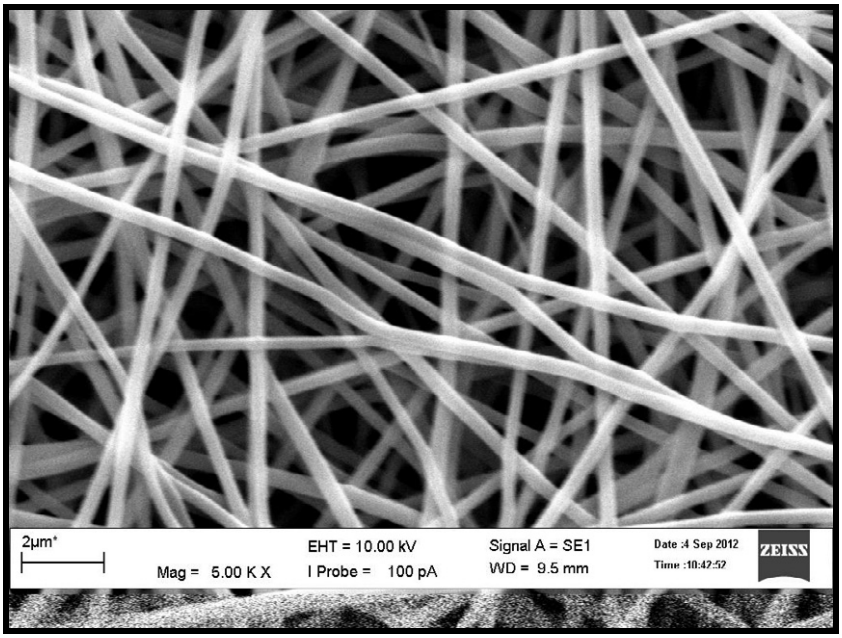

Fig. 2. Randomly oriented electrospun nanofibers of MC: PVA 60:40

3.2. Differential Scanning calorimetry (DSC) analysis

Figure 3 shows the heat flow verses temperature curves of isothermal peaks of MC: PVA nanofibers by using DSC instrument. The glass transition (T_g) temperature for 50:50 ratio is at 11.50°C and for 60:40 is at 15.6°C higher then 50:50, because as MC percentage is increased the T_g is increased. The peaks temperature in both the cases was 36.56°C and 39.26 °C respectively.

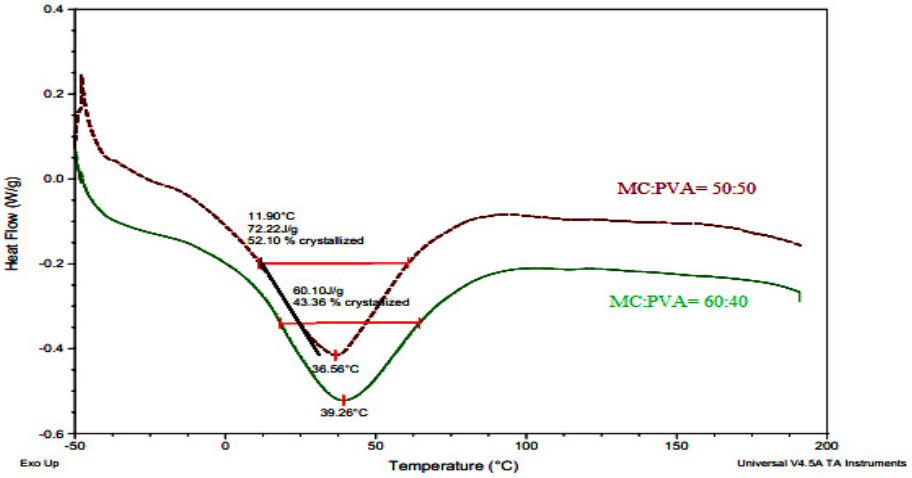

Fig. 3. The DSC spectra at 5°C/min heating rate

The enthalpy of fusion (ΔH_f) is measured the area under the DSC isothermal curves. ΔH_f for 50:50 is 72.22 J/g and for 60:40 is 60.10 J/g. As the enthalpy of fusion for 100% crystalline PVA is 138.6 J/g [22]. The degree of crystallinity of nanofibers was estimated by using equation 1. The crystalline nature of nanofibers is decreasing as the percentage of PVA is decreased for the blends solution. The degree of crystallinity for 50:50 ratio is 52.10% and 43.36% in comparison of 100% crystalline PVA. However; the nanofibers less crystalline in nature is better for bone tissue engineering it will help in improving the mechanical properties of nanofibers scaffolds.

3.3 FTIR analysis

Figure 4 shows the FTIR spectrum of nanofibers prepared using MC and PVA blends solution in different ratio. The broad peak at 3313.40 cm^{-1} of O-H stretching vibration from the intermolecular and intramolecular hydrogen bond in MC and PVA structures. The peak at 2908.90 $cm^{-1}$is attributed to C-H aliphatic stretching vibration in MC and C-H from alkyl group of PVA.

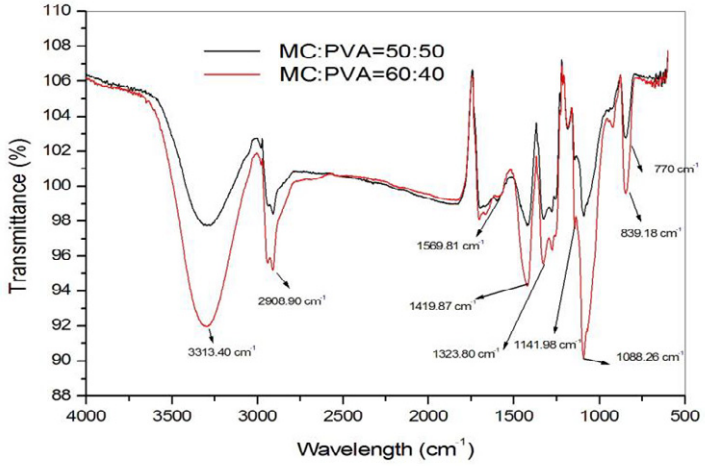

Figure 4. FTIR peaks of MC/PVA electrospun nanofibers**.**

At 1142 cm^{-1} the peak is attributed for assessment tool of PVA structure because it is a semi-crystalline synthetic polymer able to form some domains. The peak at 770 cm^{-1} is a characteristic peak of MC which shows the β (1→4) glycosidic linkage held together with H-bonds in MC structure.

4. Conclusions

Modified cellulose/PVA nanofibers were successfully prepared using electrospinning technique for bone tissue engineering application. Randomly oriented nanofibers was fabricated with an average diameter in between 117nm to 500nm. The crystallinity percentage of nanofibers are decreased as the PVA percentage decreased in comparison of pure PVA, less crystalline nanofibers make electrospun nanofibers favorable for bone tissue engineering application.

The FTIR results show the characteristic peaks of modified cellulose and PVA in electrospun nanofibers. For β (1→4) glycosidic linkage of MC structure, the peak was present at 770 cm^{-1}; this linkage is present in the natural bone structure.

In summary, electrospun nanofibers prepared form MC and PVA blends solutions can provide a very promising scaffold for bone tissue regeneration.

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