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Cross-Linking Effect On Electrospun Hydroxyethyl Cellulose/Poly(Vinyl Alcohol) Nanofibrous Scaffolds

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Abstract

The electrospinning of hydroxyethyl cellulose/poly(vinyl alcohol) (HEC/PVA) was carried out with glutaraldehyde as a cross linker to fabricate water insoluble nanofibers. The concentration of HEC (5wt%) and PVA (15wt%) was prepared and blended in different weight ratios of HEC to PVA (50:50, 40:60 and 70:30) were electrospun to get nanofibers. The microstructure of the obtained nanofibers were analysed by scanning electron microscopy (SEM) and X-ray diffraction (XRD) before and after crosslinking. SEM images showed that there was no swelling or remarkable changes in the surface morphology after treated with glutaraldehyde. XRD analysis illustrated the effect of crosslinking on the crystallinity of the nanofibers. The results showed that these crosslinked HEC/PVA fibers were suitable for variety of applications such as tissue engineering scaffolds, drug delivery and medical prostheses.

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Keywords: Crosslinking; Electrospinning; Hydroxyethyl cellulose; Nanofibers; Poly(vinyl alcohol).

1. Introduction

Tissue engineering is an interdisciplinary field that involved principles of engineering and life sciences to understand the structure-function relationships and the development of biological substitutes that restore, maintain or improve tissue function [1, 2]. A suitable material scaffold is very important in all the strategies to engineer tissues. Scaffolds are designed to serve as a temporary, artificial extracellular matrix (ECM) in order to support cell attachment and guide three-dimensional (3D) tissue formation [3,4]. Therefore, an ideal scaffold should mimic the characteristics of natural ECM, which is a structural and support material that surrounds the tissue cells [5-8] .The most critical factor to be considered here is the interaction of cells with the scaffolds, which is the template to direct the growth of the tissue. The cells should receive all the signals from the scaffolds for cell and tissue differentiation, cell proliferation, cell adhesion, cell migration as well as tissue regeneration and repair. ECM consists of fibrous collagen in nanometer length and proteoglycans. Proteoglycans are made of proteins and polysaccharide chains known as glycosaminoglycans (GAG). The interaction between the cells and the ECM is very important in biological systems [9]. The scaffolds should provide mechanical support to resist physiological forces and prevent pores from collapsing, suitable surface chemistry for cell attachment and cell proliferation and have morphology with large surface–area to volume ratio for significant cell-surface interactions [10].

Many natural [11-13] and synthetic polymers [7, 14-16] have been used as scaffolds for studies in biomaterials tissue engineering due to their biocompatibility and biodegradability. Electrospinning is a technique that produces fibers in nanometer length and interconnected pores that closely resemble the topography features of ECM. It is a versatile technique to produce nano and micro fibers from polymer solutions or melts in the range of 30-2000 nm, through the action of high

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electric field [17-19]. When the electric field overcomes the surface tension the polymer solution in the capillary is ejected as jets. The jets solidify as it travels towards the collector and is collected as nonwoven fabric [20]. The morphology and properties of the nanofibers can be varied by changing the process parameters, such as solution viscosity and conductivity, applied voltage, average molecular weight of the polymer and the distance between the needle and the collector plate [21]. The main attractions of the electrospun nanofibers are their unique properties such as high surface area-to-volume ratio, high porosity and their diameter, which is in the nanometer range. Recently there is great interest in the electrospinning of biomaterials as scaffolds. Although there is a wide range of biomaterials being electrospun and used as scaffolds only a few biopolymers closely mimic the ECM by having polysaccharide chains in them. The most commonly electrospun biopolymer with chemical structure similar to GAGs in ECM is chitosan, which is a biocompatible and wound healing material [13]. Chitosan is electrospun by dissolving in trifluoroacetic acid/dichloromethane solvent and concentrated acid as homogeneous fibers or by blending with poly (ethylene oxide) (PEO), poly (vinyl alcohol) (PVA) or silk fibroin. It is also known that the cell attachment and spreading is demonstrated to be greater in hydrophilic surfaces compared to the hydrophobic surfaces [22]. Moreover hydrogels with large amount of water interposed in their three dimensional polymeric network are highly needed in the biomedical fields [23] and employed as scaffold materials. They are composed of hydrophilic polymer chains and their structural integrity depends on the crosslinks by various chemical bonds and physical interactions. The most significant feature to be considered in hydrogels is that they have mechanical and structural properties similar to all most all the tissues and ECM. It is also well known that polysaccharides containing materials are very promising in wound-dressing and wound-healing applications [24]. Our intension 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 hydroxyethyl cellulose (HEC) for our studies.

HEC 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 non expensive and widely used in various pharmaceutical compositions, wound dressing and wound healing applications [1]. It was difficult to electrospin HEC alone, so it was blended with PVA and electrospun. PVA is a polyhydroxy 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, [25]. There are several reports on PVA nanofibrous mats are prepared by electrospinning aqueous PVA solution [12].

In the present work, HEC/ PVA nanofibrous membranes were fabricated by electrospinning technique using water as the solvent. The nanofibrous membranes were crosslinked using glutaraldehyde. PVA increased the spinnability of HEC and increased the mechanical strength of the nanofibers. The microstructures of the obtained nanofibers were analyzed by scanning electron microscopy (SEM). Human fetal osteoblasts (hFoB) morphology on these nanofibers was studied. state the units for each quantity that you use in an equation.

2. Experimental

2.1 Materials

Hydroxyethyl cellulose was purchased from Merck-Schumardt, Germany. Poly(vinyl alcohol) (95% hydrolized with molecular weight 95,000) was purchased from ACROS, New Jersey, USA. Analytical reagent grade glutaraldehyde aqueous solution was purchased from Merck-Schumardt, Germany, Phosphoric acid was purchased from Merck.KGaA-Damstadt, Germany. Acetone were purchased from R&M Marketing, Essex, UK. Phospate buffer saline (PBS) was purchased from Gibco Life Technologies, USA. All the chemicals were of highest purity and used without further purification. Water used was distilled and Millipore.

2.2 Preparation of electrospinning solution.

The HEC solution with concentration 5 wt% was prepared by dissolving 5 g of HEC powder in 100 ml Millipore water for 2 h at room temperature until a clear solution was obtained with a slight increase in viscosity. PVA solution of 15 wt% was prepared by dissolving 15 g of PVA granules in 100 ml Millipore water with moderate stirring at 80 °C for 2 h. Both solutions was stirred continuously for 12 h at room temperature to ensure a complete dissolution and eventually obtained homogeneous solution. HEC was then blended in PVA solution with 3 different weight ratios of HEC:PVA such as 50:50 , 40:60 and 30:70 and stirred overnight to get homogeneous mixture for electrospinning.

2.3 Electrospinning of nanofibrous scaffolds.

Electrospinning was carried out at room temperature for all the concentrations of HEC/PVA (Model) . The polymer solution was filled in a 5 ml syringe fitted with a blunt steel needle of 0.8 mm inner diameter and flow rate of 1 ml/h. The needle tip of the syringe was connected with the applied voltage of 25 kV. The electrospun nanofibers were collected using a rotating drum collector wrapped with aluminium foil with 12 cm of distance from tip-to-collector at a rotation speed of 1000 rpm. The humidity of 50 % was preserved (humidier model) in the room. The collected electrospun nanofibers were stored in dessicator for futher use.

2.4 Cross-linking studies.

The nanofibers scaffolds were peeled and cut into 4 cm² pieces and allowed to keep in a Petri dish with diameter of (90 mm) . Glutaraldehyde in acetone followed by phosphoric acid were putted in the Petri dish contained of nanofibers for 24 h. Water resistance of the scaffolds was evaluated by immersing it in distilled water. The intergrity of the nanofibers were checked by immersing in PBS for 7 days.

2.5 Characterization of nanofibrous scaffolds.

2.5.1 Scanning Electron Microscopy (SEM) study.

The surface morphology of HEC/PVA electrospun nanofibers scaffold before and after cross-linking was observed by a Scanning Electron Microscopy (SEM) (ZEISS EVO 50) at an accelerating voltage of 15 kV. The electrospun nanofibers were sputter coated with a thin layer of platinum in double 30 s consecutive cycles at 45 mA to reduce charging and produce conductive surfaces (BALTEC SCD 005 Sputter Coater – BALTEC).

2.5.2 X-Ray Diffraction (XRD) study

Crystallinity of HEC/PVA nanofibrous scaffolds before and after cross-linking was evaluated by XRD measurements by using X-ray diffractometer (Rigaku MiniFlex II) with Cu target and Kα radiation generated at 30 kV and 15 mA at a scanning rate of 1 °min^{-1} enabled differences in the crystal structure of the nanofibers scaffolds to be studied.

2.6 Cell culture studies.

2.6.1 Cell expansion and seeding.

hFoB cells were cultured in DMEM/F-12 medium (1:1) containing 10 % FBS and 1 % cocktail antibiotic in 75 cm² cell culture flasks. The osteoblasts cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 3 days. The cross-linked HEC/PVA (50:50) electrospun scaffolds were soaked in 100 % ethanol for 24 h, and then sterilized under UV light for 3 h. These scaffolds were again sterilized with 70 % ethanol for 30 min then washed with PBS for 15 min three times and subsequently immersed in cell culture medium overnight. hFoB cells grown in 75 cm² cell culture flasks were detached on confluency by adding 1 ml of 0.25 % trypsin containing 0.1 % EDTA. Detached cells were centrifuged and counted by Trypan blue using haemocytometer, seeded on electrospun HEC/PVA nanofibers scaffold at a density of (1 x 10^4) cells/cm² and incubated to facilitate cell growth.

2.6.2 Cell morphology studies.

After 7 and 14 days of cell culture, hFoBs grown on scaffolds were rinsed twice with PBS and fixed in 3% glutaraldehyde for 3 h. Thereafter, the scaffolds were dehydrated with increasing concentrations of alcohol and finally dried into hexamethyldisilazan (HMDS) overnight. The HMDS were air dried by keeping the samples in a fume hood. Lastly, the scaffolds were sputter coated with platinum and observed using SEM (ZEISS EVO 50) at an accelerating voltage of 10 kV.

3. Results and Discussions

3.1 Electrospinning

Fig. 1 shows the surface morphology of electrospun nanofibers of HEC blended in PVA for various weight ratios. Generally, electrospinning produces non-woven matrices with randomly arranged, ultrafine fibers with nanometer diameters. As shown in Fig. 1, HEC/PVA nanofibers had smooth surface morphology. The diameter of fibers were in the range of 150-500 nm. The fibers in Fig. 1(a) and 1(b) were straight meanwhile some fibers of Fig. 1(c) were curled at certain area. Fig. 1(c) also showed thinner fiber compared to both Fig. 1(a) and (b). During electrospinning process, the collection of nanofibers was easier to fabricate with the increases of PVA ratios. When electospun, it was hard to get nanofibers for 100 % of HEC, therefore PVA was chose to increase the efficiency of fiber formation.

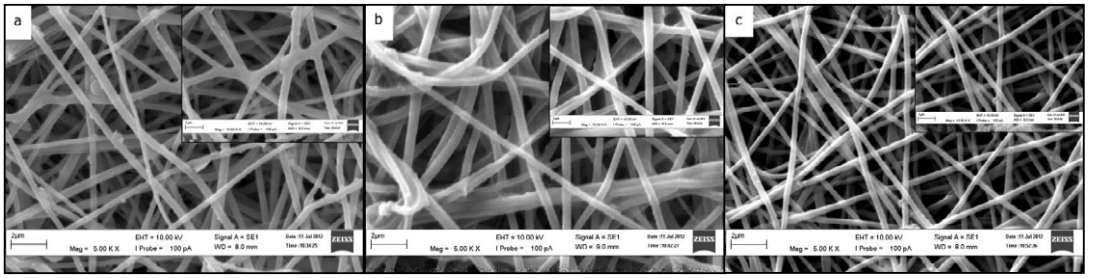

Fig 1. SEM images of electrospun nanofibers of 5 wt % HEC and 15 wt% PVA in different HEC:PVA weight ratios before cross-linking: (a) 50:50 (b) 40:60 (c) 30:70 at 5k magnification. The inlet shows image at 10k magnification.

3.2 Cross-linking studies.

Cross-linking studies were carried out for the material to become insoluble in water. For practical purposed the nanofibrous should have good integrity in aqueous solution. The SEM images were carried out to observe any morphological changes in fibrous structure. From Fig. 2, there is no remarkable morphology changes occurred after immersed with glutaraldehyde in acetone. The nanofibers were then immersed in phosphate buffer saline solution at 37 °C for 7 days to check the intergrity of the nanofibers. The SEM images of the nanofibrous membranes after removing from the phosphate buffer solution, washed with millipore water and dried are shown in Fig. 3.

There was no swelling or change in the morphology (Fig. 3). The fibers showed good integrity in aqueous environment. This may be due to the increased resistivity of the fiber against water. The enhanced stiffness and rigidity developed by cross-linking may be responsible for bringing such kind of increased resistivity [1, 2]. The porous structure was well maintained. Pores are very important in the tissue-engineered scaffolds, since it is the structural space in which the cells reside and exchanges nutrients and metabolic wastes between the scaffold and the environment, which are the basic criteria for a successful tissue-engineered scaffold.

3.3 X-Ray diffraction studies.

The x-ray diffraction patterns of electrospun HEC/PVA scaffolds were carried out over an angle range 10-80 ° (Fig. 4). In the range of 21-26 °, the XRD patterns exhibited the broad peaks attributed to the reflection of semi-crystalline polymers. The XRD showed patterns of semi-crystalline PVA with strong peaks at 29 °. The patterns showed higher intensity of 50:50, 40:60 and 30:70 HEC/PVA after chemically cross-linking with glutaraldehyde compared with 50:50, 40:60 and 30:70 HEC/PVA before cross-linking respectively. Moreover, the decrease of PVA weight ratios contribute to less intensity of crystalline peak before and after the immersion of glutaraldehyde may be due to the amount of relative crystalline PVA solution added upon mixing with different concentration of HEC.

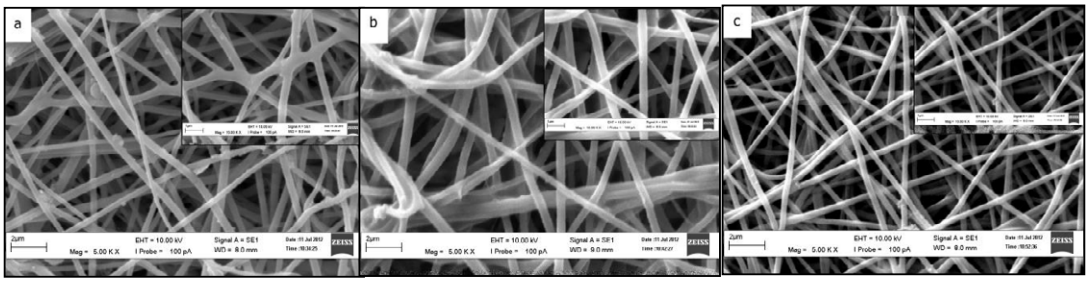

Fig 2. SEM images of the electrospun nanofibers of 5 wt% HEC and 15 wt% PVA in different HEC: PVA weight ratios after cross-linking: (a) 50:50 (b) 40:60 (c) 30:70 at 5k magnification. The inlet shows image at 10k magnification.

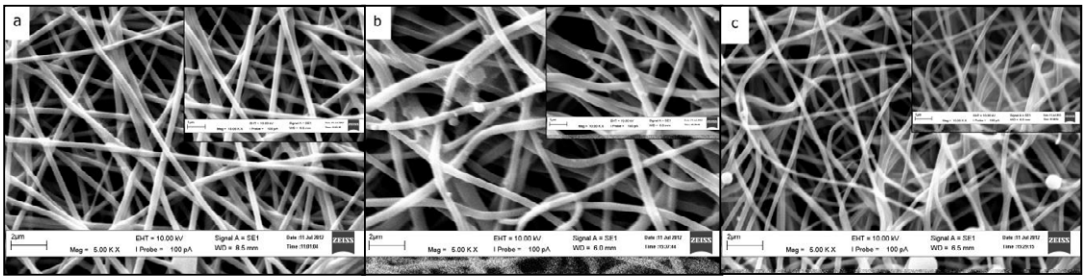

Fig 3. SEM images of the electrospun nanofibers of 5 wt% HEC and 15 wt% PVA in different HEC:PVA weight ratios after immersion in phosphate buffer saline solution for 7 days: (a) 50:50 (b) 40:60 (c) 30:70 at 5k magnification. The inlet shows image at 10k magnification.

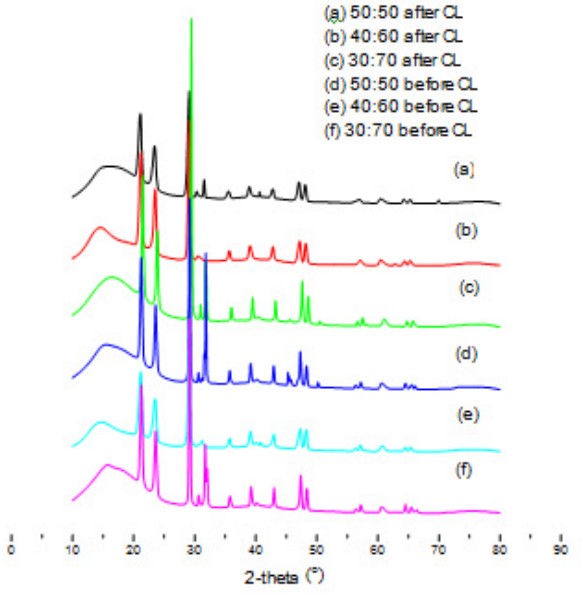

Fig 4. XRD patterns of HEC/PVA electrospun scaffolds.

3.4. Cell spreading studies.

The utilization of the nanofiber scaffold HEC/PVA (50:50) for tissue engineering was futher studies under SEM analysis. Fig. 5 shows the SEM images of cell-scaffold constructs after 7 and 14 days of culture. Seeded hFobs were adhered and spreaded well on the scaffolds. Initially, the cells begin to spread on the HEC/PVA scaffolds (Fig. 5 (a)). After 14 days, the cells spreading became more prominent and cells form in globular morphology on scaffold surfaces. These results strongly suggest that the nanofibrous scaffolds reported here is suitable for tissue engineering applications.

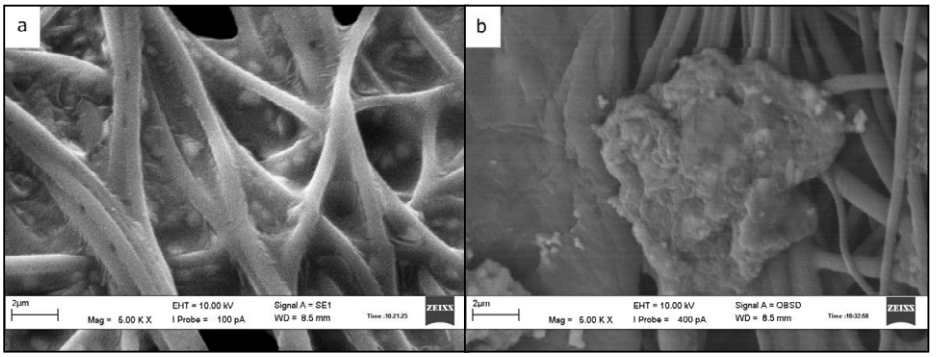

Fig 5. SEM images of hFoB cells attached on the surfaces of HEC/PVA of scaffolds after (a) 7 days and (b) 14 days.

4. Conclusion

Hydrogels, HEC is well-known as a biocompatible water soluble polymer was successfully electrospun to nanofibers through electrospinning process. PVA was selected as polymer additive to produce electrospun fiber because of its good fiber formation, biocompatibility and chemical resistance properties. The effect of weight ratios on fiber formation and the surface morphology of HEC/PVA scaffolds were studied. XRD studies represented the crystalline structure of cellulose and PVA on the HEC/PVA nanofibers scaffolds. Since both HEC and PVA are water soluble polymers, insolubility of scaffold in water was achieved by doing cross-linking with glutaraldehyde in the presence of acetone. Cell culture studies showed that hFoB cells were able to adhere and proliferate on nanofibers scaffolds. Thus, these biodegradable scaffolds may be suitable for tissue engineering applications.

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