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Electrospun sodium alginate/poly(ethylene oxide) core–shell nanofibers scaffolds potential for tissue engineering applications

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ABSTRACT

Core–shell structure nanofibers of sodium alginate/poly(ethylene oxide) were prepared via electrospinning their dispersions in water solution. The core–shell structure morphology of the obtained nanofibers was viewed under scanning electron microscope (SEM) and transmission electron microscope (TEM), and X-ray photoelectron spectroscopy (XPS) analysis was used to further quantify the chemical composition of the core–shell composite SA/PEO nanofibers surface in detail. Furthermore, one-step cross-linking method through being immersed in $CaCl_2$ solution was investigated to improve the anti-water property of the electrospun nanofibers mats in order to facilitate their practical applications as tissue engineering scaffolds, and the changes of the structural of nanofibers before and after cross-linking was characterized by Fourier transform infrared (FT-IR). Indirect cytotoxicity assessment indicated that SA/PEO nanofibers membrane was nontoxic to the fibroblasts cells, and cell culture suggested that SA/PEO nanofibers tended to promote fibroblasts cells attachment and proliferation. It was assumed that the nanofibers membrane of electrospun SA/PEO could be used for tissue engineering scaffolds.

1. Introduction

Electrospinning is a recently explored simple, least expensive, rapid prototyping, and effective fabrication technique to produce nano to microscale fibers with high surface-to volume ratio, and high porosity, depending on the solution properties, equipment and processing variables. They could have a great potential in the fields such as filter (Desai et al., 2009; Sambaer, Zatloukal, & Kimmer, 2011), sensor and protective clothing, composite-fiber-reinforced materials (Magniez, Lavigne, & Fox, 2010), and especially the biomedical applications such as tissue engineering scaffolds (Boland et al., 2006), drug delivery carriers (Ignatova, Manolova, & Rashkov, 2007), and wound dressing. Recently, there have been much interest in extending this technique to produce uniform fibers with novel compositions and uniform morphologies, such as fibers with pore (Kovacina et al., 2011; Yang et al., 2011), core–shell (Liao & Leong, 2011; Sun, Zussman, Yarin, Wendorff, & Greiner, 2003), hollow structures (Ou et al., 2011) and manufacturing uniaxially aligned electrospun fibers which can provide the performance and unique benefits. Up to now, several methods have been developed to prepare core–shell structure of polymeric nanofibers via electrospinning which could be used as tissue engineering application, and drug release by encapsulating drugs into the core of nanofibers, and the controlled release of active agents could be achieved, which could owing to the shell fluid is able to be processed with electrospinning, while the core fluid is not electrospinnable (Saraf, Baggett, Raphael, Kasper, & Mikos, 2010; Zhang et al., 2006).

For example, in the approach of template, polymeric fibers (template) were produced by ordinary electrospinning, and then the fibers were coated with the shell component by chemical vapor deposition (CVD) (Graeser, Pippel, Greiner, & Wendorff, 2007), core–shell structure nanofibers via coaxial electrospinning methods (Jiang et al., 2005; Zhao et al., 2007), and surface initiated atom transfer radical polymerization (ATRP) (Fu et al., 2008). Recently, the core–shell structure fibers using a single nozzle instead of double coaxial capillaries to get from an emulsified polymer solution were reported (Gentsch et al., 2011; Qi, Hu, Xu, & Wang, 2006; Xu et al., 2005, 2006). But there few report and research are reported about the core–shell structure fibers using single nozzle which could be caused by the reason of phase separation (Zhang et al., 2009), or electric field (Yu, Dai, & Lan, 2011), which was considered as a simple method that eliminates the needs of a special apparatus and careful selection of operation parameters.

There are some natural biopolymers, such as, chitosan (CS) (Jessica & Caroline, 2007; Rakkapao, Soongnern, Masubuchi, & Watanabe, 2011), hyaluronic acid (HA) (Liu, Ma, & Nie, 2011),

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and, sodium alginate (SA) (Boninoa et al., 2011), were electrospun into nanofibers via electrospinning, which can be achieved in blend form polymer by blending with synthetic polymer PEO (Bhattarai, Edmondson, Veiseh, Matsen, & Zhang, 2005), PVA (Jeun, Jeon, Nho, & Kang, 2009), and PVP (Fang, Liu, Nie, & Ma, 2011), which improves their electrospun processability while maintaining their biocompatibility. The linear anionic polysaccharide sodium alginate consists of (1–4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) units in various composition and sequence and exists widely in many species of brown seaweeds. Due to its nontoxicity, compatibility, and biodegradability, SA has been studied extensively in tissue engineering, including the regeneration of skin, cartilage, bone, and cardiac tissue (Dausse et al., 2003; Kanakis, Malkaj, Petroheilos, & Dalas, 2001; Thorvaldsson, Stenhamre, Gatenholm, & Walkenström, 2008). Water-soluble polymer formed gel in the presence of certain bivalent cation such as Ca^{2+} through ion exchange (Kim, Yoon, & Ko, 2000), which is different from the other cross-linking method via photopolymerization or chemical cross-linking, resulted into harmful substances or residues (Kim, Kim, Nair, & Moore, 2005).

Poly(ethylene oxide) (PEO) is a unique class of water-soluble biodegradable biopolymer. Because of its excellent biocompatibility, biodegradability, and very low toxicity, the potential use of PEO in biomedical applications has attracted a great attention from both the industrial and scientific points of view. Fiber-forming biopolymers such as PEO (Son, Youk, Lee, & Park, 2004), and the mix solution of PEO and SA (Christopher et al., 2011; Nie et al., 2009), SA (Fang et al., 2011) have been successfully electrospun, however, to the best of our knowledge, core–shell structure electrospinning involving SA has not yet been reported.

This study investigated the core–shell structure nanofibers generated via electrospinning from mixed solution of PEO and SA. The morphologies of the fabricated nanofibers were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Furthermore, cross-linking method with Ca^{2+} was investigated to improve the anti-water property of the electrospun membranes in order to facilitate the practical tissue engineering applications. The structure and interaction of the nanofibers were subjected to detailed analysis by Fourier transform infrared (FT-IR). The potential use of the as-spun nanofibers as a scaffolding material for tissue engineering was evaluated in vitro against fibroblasts cells.

2. Experimental

2.1. Materials

Sodium alginate and poly(ethylene oxide) (100 kDa) were purchased from Xilong Chemical Engineering (China) and Alfa Aesar, respectively, and used without further purification.

2.2. Preparation of the electrospinning solutions

Aqueous solutions of SA/PEO with different concentration and ratio were prepared, respectively, by dissolving SA and PEO in deionized water, respectively. The mixtures were stirred at room temperature with magnetic stirring for 6 h to obtain homogeneous solutions.

2.3. Electrospinning

The schematic set up and photographic image of the equipment used in this research are shown in Fig. 1. Each electrospun solution was placed into a 10 mL glass syringe capped with a steel capillary 0.69 mm diameter. The syringe was placed in a syringe pump which permitted adjustment and control of solution flow rates. The positive lead from a high voltage supply was connected via an alligator clip to the external surface of the needle. A rectangular aluminum foil was used as a static collector and connected to the ground. The tip-to-collector distance could adjust from 10 to 30 cm. The syringe pump delivered polymer solution at a controlled flow rate of 1 mL/h, while the voltage was maintained at 20 kV, the temperature was fixed at 25 °C, and humidity was 25%.

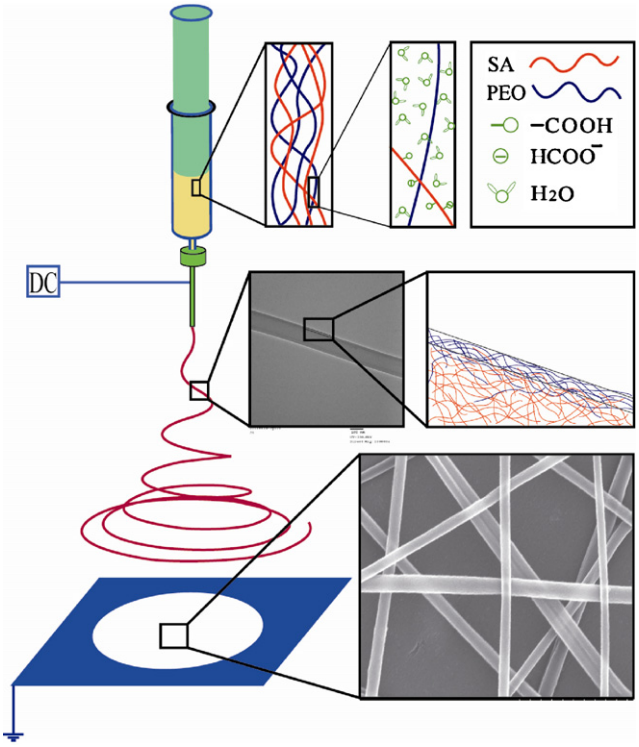

Fig. 1. Schematic of setup of the core–shell structure nanofibers electrospinning.

2.4. Sample characterization

2.4.1. Scanning electron microscope (SEM)

The morphology of the electrospun nanofibers was observed by using Hitachi S-4700 scanning electron microscope (Hitachi Company, Japan), and its accelerating voltage was 20 kV. Samples were mounted on metal stubs using a double-sided adhesive tape and vacuum-coated with a gold sputtering layer prior to examination. Diameters and distributions of the electrospun fibers were analyzed from the SEM images by using Image J analysis software (Image J, National Institutes of Health, USA).

2.4.2. FTIR analysis

Fourier transform infrared (FT-IR) spectrum was recorded on Nicolet 5700 instrument (Nicolet Instrument, Thermo Company, USA). Samples were prepared as nanofiber membranes and were scanned against air background at wave-numbers range 4000–500 cm^{-1} with resolution of 4.0 cm^{-1}.

2.4.3. Transmission electron microscopy (TEM)

The core–shell structure and cross section of the nanofiber were characterized by transmission electron microscopy (TEM, S-800 Hitachi, Tecnai G2 20 S-TWIN FEI, JEM-100CX JEOL) at 100 kV, and the samples for TEM observations were prepared by collecting the nanofibers onto carbon-coated Cu grids.

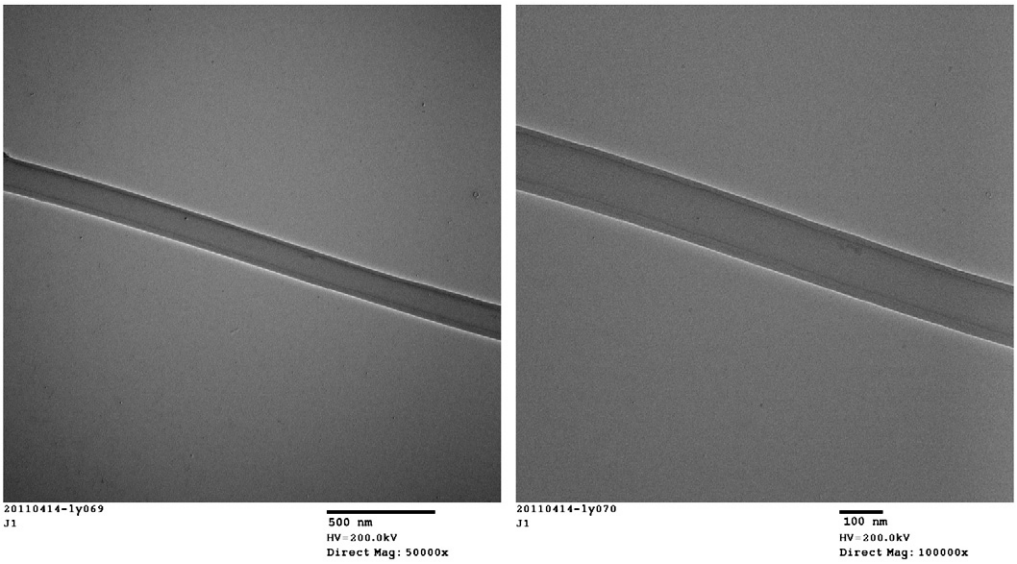

Fig. 2. TEM micrographs of core–shell structure SA/PEO nanofibers.

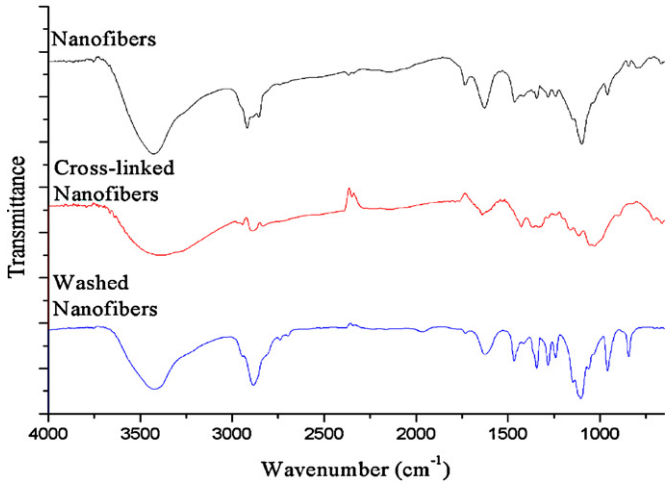

Fig. 3. FT-IR spectra of electrospun core–shell, cross-linked, and washed nanofibers.

2.4.4. X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) was carried out using a Riber LDM-32 Base System with a take off angle of 45 °C.

2.5. In vitro cytotoxicity of drug-loaded fiber mats

2.5.1. Methylthiazolydiphenyl-tetrazolium bromide (MTT) assay

The cytotoxicity of the electrospun nanofiber's membranes was evaluated based on a procedure adapted from the ISO10993-5 standard test method. Fibroblasts cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, together with 1.0% penicillin–streptomycin and 1.2% glutamine. Culture was maintained at 37 °C in a wet atmosphere containing 5% CO_2. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1 mM ethylenediamine tetraacetic acid (EDTA). The viabilities of cells were determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) assay. The level of the reduction of MTT into formazan can reflect the level of cell metabolism. For the MTT assay, the as-spun membranes were sterilized with highly compressed steam for 15 min and placed in wells of a 24-well culture plate, respectively. The samples were then incubated in 1 mL of RPMI1640 medium at 37 °C for 24 h. The extraction ratio was 6 cm^2/mL. At the end of this period, the membranes were removed and the so-called extracts were obtained and further were diluted to obtain extraction medium samples. Fibroblasts cells were seeded in wells of a 96-well plate at a density of 4×10^3 cells per well. After incubation for another 24 h, the culture medium was removed and replaced with the as-prepared extraction medium and incubated for 24 h, then 150 μL of MTT solution was added to each well. After 24 h incubation at 37 °C, 200 μL of dimethyl sulfoxide was added to dissolve the formazan crystals. The dissolved solution was swelled homogeneously for about 10 min by the shaker. The optical density of the formazan solution was detected by an ELISA reader (Multiscan MK3, Labsystem Co. Finland) at 490 nm. For reference purposes, cells were seeded to medium a fresh culture medium (negative control) under the same seeding conditions, respectively.

2.5.2. Fluorescence assay

The prepared square samples (1 cm × 1 cm) soaked in the 250 mL phosphate buffer solution (PBS pH = 7.4) for 24 h before sterilization with highly compressed steam for 15 min, then the samples were transferred to the 24-well tissue culture plate. 1 mL of fibroblasts cells suspension with 1.5×10^4 cells/mL was seeded on the samples. After 48 h of culture, collected cellular was rinsed twice with PBS to remove non-adherent cells, subsequently fixed by 75% alcohol solution, Hochest staining, and the samples were observed by the fluorescence microscope.

2.5.3. Cell culture and adhesion

Fibroblast's cells were selected for the biological assays in order to evaluate the effect of electrospun nanofiber membranes on cell culture, adhesion, and proliferation. The electrospun nanofiber membranes were fixed on the glass cover slips by using copper tapes. The sample membranes were sterilized, rinsed three times with sterile phosphate buffer solution (PBS), then transfered to individual 24-well tissue culture plates. Aliquots (1 mL) of fibroblast's cells suspension with 1.5×10^4 cells/mL were seeded on the sample membranes. After 24 h of culture, cellular constructs were harvested, rinsed twice with PBS to remove non-adherent cells. The samples were dehydrated through a series of graded ethanol solutions and dried overnight at room temperature. The dry samples were coated with gold by sputtering for further analysis cell morphology on the surface of the scaffolds by SEM.

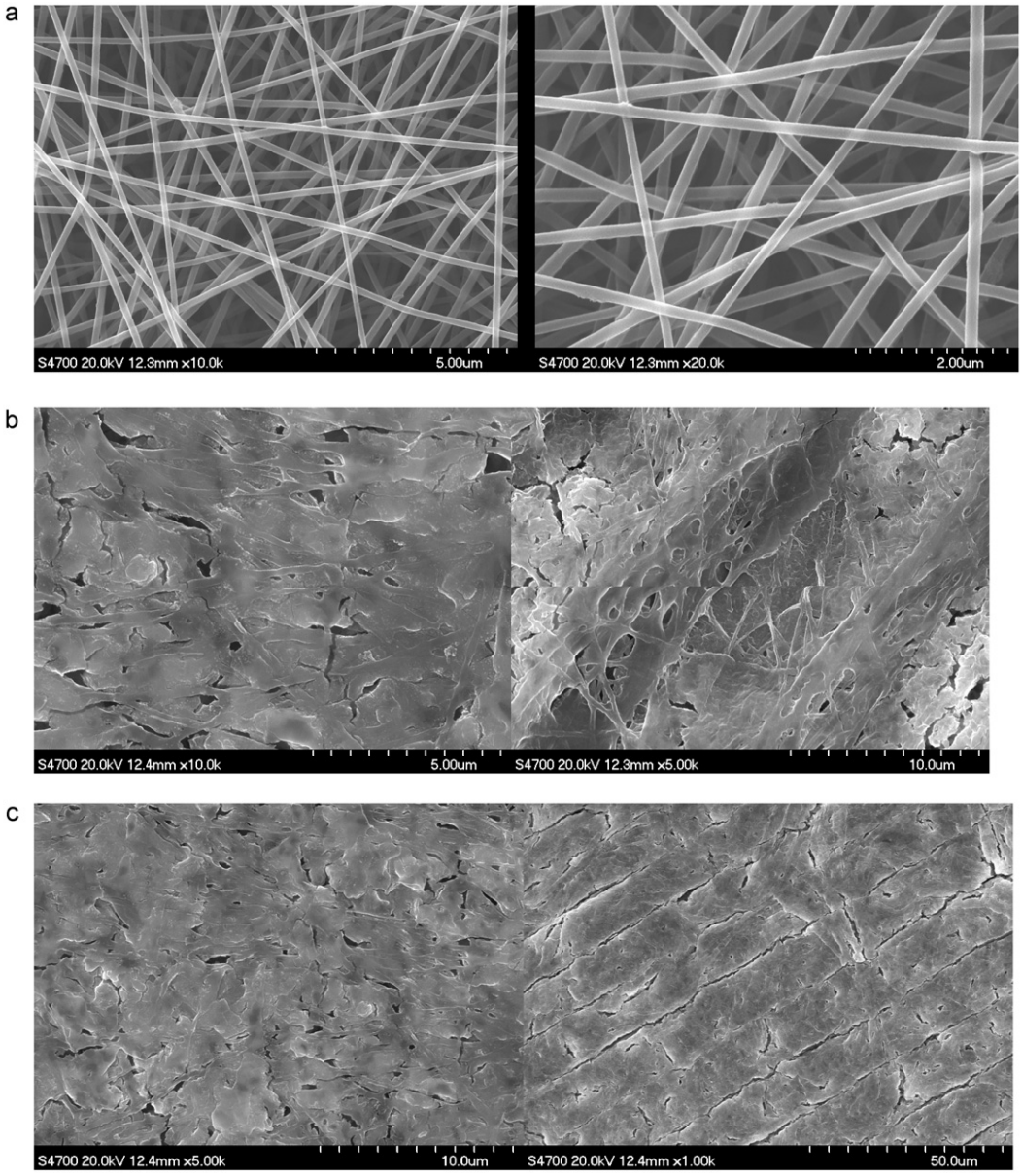

Fig. 4. SEM morphologies of uncrosslinked and crosslinked SA/PEO nanofibers for different time with $CaCl_2$ solution ((a) uncrosslinked SA/PEO nanofibers, (b) crosslinked SA/PEO nanofibers for 24 h with $CaCl_2$ solution, and (c) crosslinked SA/PEO nanofibers for 48 h with $CaCl_2$ solution).

3. Results and discussion

3.1. Transmission electron microscopy (TEM)

TEM images of the obtained ultrafine SA/PEO nanofibers are shown in Fig. 2, exhibiting a core–shell structure. From Fig. 2, it could also be seen that neither the nanofibers size nor the diameters of the core and shell were uniform, and the nanofibers diameters varied with the range from 100 to 300 nm. Very fine continuous structures with clear boundaries between the core and shell components were observed. The dark regions in the TEM micrographs of the nanofibers were identified as PEO, while the light regions were analyzed as SA part.

To further quantify the chemical composition of the core–shell structure SA/PEO nanofibers surface, X-ray photoelectron spectroscopy (XPS) analysis technique was used to test the chemical characteristics on the surfaces of SA/PEO core–shell nanofibers and confirm the core and shell part of core–shell nanofibers.

Table 1
The C, O and Ca element content of nanofibers, cross-linked nanofibers, and washed nanofibers.

Sample	C (mol%)	O (mol%)	Ca (mol%)
Nanofibers	66.6	33.0	0.4
Cross-linked nanofibers	51.7	28.4	19.9
Washed nanofibers	53.4	43.2	3.4

Table 1 shows the atomic ratios of carbon, oxygen, and calcium on core–shell nanofibers and crosslinked nanofibers with Ca^{2+} from the $CaCl_2$ solution. XPS is a surface analysis method that can give the information of elemental and average chemical composition of a material on its surface in 5–10 nm depth by measuring the binding energy of electrons associated with atoms (Li et al., 2008). Moreover, the molar concentration ratio of O/C in Table 1 was also found to be in good agreement with the theoretical values of O/C in PEO. The results suggested that SA was properly wrapped by the

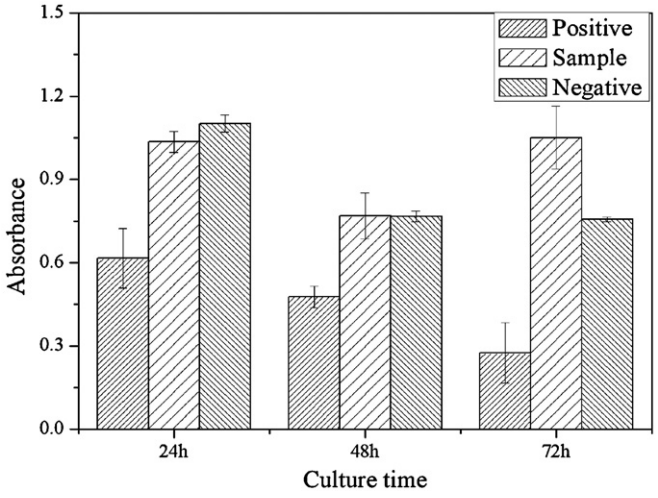

Fig. 5. Cytotoxicity test of electrospun core–shell nanofibers with positive and negative controls. ($p > 0.05$) $^{*}p > 0.05$ when compared to the negative control of indirect cytotoxicity.

shell material PEO, and the shell thickness is at least beyond the ultimate detection depth of XPS, i.e., 5–10 nm. Those results also indicated that Ca molar concentration reached up to 19.9%, at the same time, the molar concentration of O/C was 1.8 after crosslinking, lower than that before crosslinking, which was probably due to the removal of PEO from the core–shell nanofibers after crosslinking with $CaCl_2$ solution. When the cross-linked nanofibers were washed with deionized water again, the molar concentration of Ca^{2+} was lower, which assign to the deposition of Ca^{2+} washed away.

3.2. Fourier transform infrared (FT-IR)

Fig. 3 shows FT-IR spectra of electrospun core–shell, crosslinked, and washed nanofibers. When adding PEO into the core–shell nanofibers, the asymmetrical band of carboxylate ion and the hydroxy band of sodium alginate were shifted, revealing interaction of sodium alginate and PEO through hydrogen bonding between the etheric oxygen of PEO and hydroxyl groups of sodium alginate (Lu, Zhu, Guo, Hu, & Yu, 2006). The strong and broad band at around 3440 cm^{-1} attributed to –OH stretching vibration, and the weak and broad peaks at 1320 cm^{-1}, in addition to other characteristic bands at 1080 cm^{-1} were assigned to the stretching vibration of C–O, C–O–C. Peaks that correspond to C–O–C bond stretching in PEO located at 845, 1100 cm^{-1}, and $-CH_2$ bond stretching at 1340 cm^{-1}, deceased after crosslinking with the solution of $CaCl_2$. The asymmetric and symmetric stretching of protonated carboxylic group of sodium alginate occurs at 1600 and 1430 cm^{-1}, respectively. An enlargement of the region of the spectra showed a shift of the symmetric COO– peak from 1600 cm^{-1} to higher frequencies 1615 cm^{-1} and an increase of the intensity of this peak with an increase of the amount of cross-linking Ca^{2+} ions. These variations were explained by an increase of the interaction between COO– groups of alginate and Ca^{2+} ions (Pathak, Yun, Lee, & Paeng, 2010).

3.3. Scanning electron microscope (SEM)

Fig. 4 illustrates SEM morphologies of uncrosslinked and crosslinked SA/PEO nanofibers membranes for different crosslinking time periods in $CaCl_2$ solution. It showed the smooth and round-shaped nanofibers, and SEM results exhibited the low average diameter and the narrow diameter distribution. The crosslinked nanofibers surface got rough, nanofibers diameter became uniform, and only small degree of adhesion between the nanofibers. The

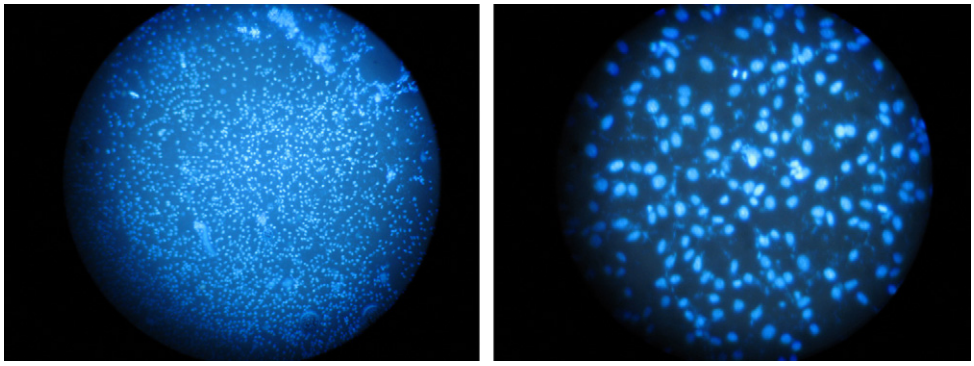

Fig. 6. Fluorescent micrographs of nuclei (blue) of cells counterstained with Hochest.

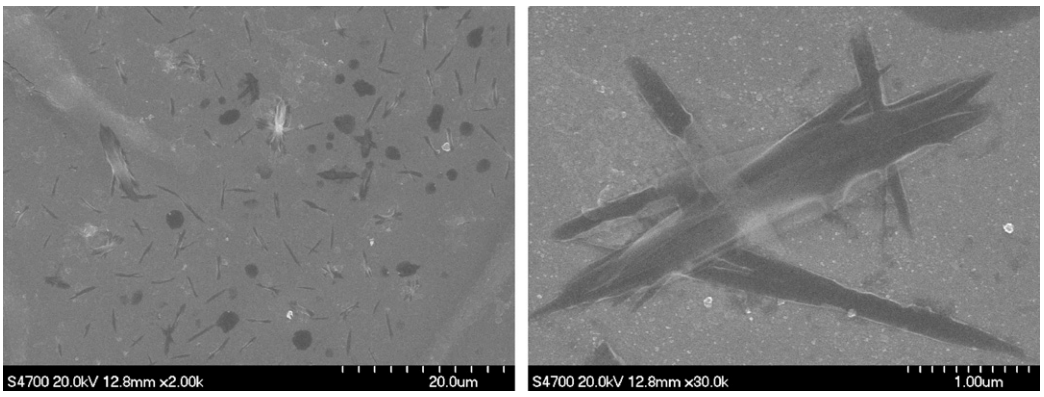

Fig. 7. SEM micrographs of fibroblasts cells cultured for 72 h on the crosslinked nanofiber membranes.

average crosslinked nanofibers diameter became larger when compared with that of the original uncrosslinked nanofibers (Fig. 4b, was immersed in $CaCl_2$ solution for 24 h). This phenomenon became more serious with increase of crosslinking time in Fig. 4c, which was immersed in $CaCl_2$ solution for 48 h. It was due to the PEO was removal from the composite nanofibers, and the COO– group of SA were crosslinked with Ca^{2+}. It also could be seen from Fig. 4b that the morphology of SA/PEO nanofibers membrane was still maintained the state of nanofiber shape after immersed in $CaCl_2$ solution, insolubility of the nanofibers membrane in water was achieved by crosslinking with $CaCl_2$, and the mechanical performance of the nanofiber membrane was enhanced (Christopher et al., 2011).

3.3.1. MTT assay

The level of toxicity of electrospun nanofibers membranes cultured for different periods time towards viability of fibroblasts cells was evaluated using ISO10993-5 standard test method of indirect MTT cytotoxicity assay. Fig. 5 lists the absorbance illustrating the viability of fibroblasts cells that were cultured with the extraction medium from the specimens. It could be seen that no statistically significant differences $^{*}p > 0.05$ were observed in the cell activity of fibroblasts cells culture for different periods of time in the presence of core–shell nanofibers extracts in comparison with control. The viability of fibroblasts cells cultured on nanofibers membrane decreased on 48 h compared with that of 24 h, then increased on 72 h, which meaned fibroblasts cells might occupy all available spaces on the nanofibers membranes. But the average absorbance values on 48 h were almost with that of the control condition, and higher than that of the control condition on 72 h. This result could also be verified in fluorescence microscopy experiment in Fig. 6 when the SA/PEO nanofiber membrane immersed in $CaCl_2$ solution for 48 h. Hochest-stained nanofibers membrane observed under a fluorescence microscope showed cell populations on the scaffold surface, and cells were individually distributed on the surface of nanofibers, with uniform ellipsoid and elongated structure. The results suggested that nanofibers membrane of SA/PEO was nontoxic to fibroblasts cells, was advantageous to the fibroblasts cells proliferation, and were good candidates to be used as potential for tissue engineering applications.

3.4. Cell adhesion and spreading

Fig. 7 represents the SEM micrographs of fibroblasts cells cultured for 72 h on the crosslinked nanofiber membranes. Fibroblasts cells attached on the nanofiber membranes and changed their original round shape to elongated and spindle-like shape on electrospun nanofiber membranes, and fibroblasts cells spreading were prominent and cells became in a flat morphology on the surface of electrospun scaffold. It was probably because of the short internanofiber distance and high surface density of fibers owing to the nanofibers, which could permit cells adhesion and spreading across the neighbour nanofibers (Duan et al., 2006). The results could be verified from the Curtis reports that cells recognize nanometric topologies of nanofibers or microporous structure (Curtis & Wilkinson, 1998).

4. Conclusions

The core–shell structured SA/PEO nanofibers could be prepared by electrospinning of SA/PEO dispersed solutions. The insolubility of the scaffold in water was achieved by cross linking with $CaCl_2$ solution, and the structural changes nanofibers before and after cross-linking were certificated by Fourier transform infrared. Core–shell structure and sharp interface of the nanofiber could be observed easily in the TEM images and were tested XPS. SA/PEO nanofibers membrane was nontoxic to the fibroblasts cells indirect cytotoxicity assessment, and was benefit to promote fibroblasts cells attachment and proliferation, which assumed that the nanofibers could be potentially used tissue engineering applications.

Acknowledgements

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