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γ-Irradiated Chitosan Based Electrospun Nanofibrous Scaffolds for Biomedical Applications

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Abstract

In this study, electrospun fiberous scaffolds are prepared from indigenously extracted and γ-irradiated chitosan (CS) and blended it with poly (vinyl alcohol) (PVA). The structure and morphology of the electrospun fiberous scaffolds are studied by Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM), respectively. FTIR analysis verified the deacetylation of chitin and the developed interactions between CS and PVA. SEM micrographs showed that bead on string fibers are obtained with high gamma rays dose due to the decrease in the molecular weight but no effect was observed on the size of the nanofibers. The cell cytotoxicity of the nanofibrous scaffolds was examined by indirect method using human fibroblast cells and human cancerous bone cells were employed for direct method. The obtained results explained that CS/PVA electrospun fiberous scaffolds were compatible to the cells and can be employed for different biomedical applications.

Keywords: Scaffolds, Nanofibrous, Chitosan, Biomedical Applications, PVA

1. Introduction

In modern era, electrospun scaffolds materials are significant for biomedical applications [1-3]. Tissue engineering has carried out to develop biological alternates for regeneration of imperfect organs tissues [4, 5]. In fact, it is a multidisciplinary field which fabricates artificial organs and tissues such as: blood vessels, bones, cartilage and skin [6]. Scaffolds with characteristic attributes of biocompatibility, biodegradability and suitable mechanical properties can be employed for tissue engineering and biomedical fields [7].

Chitosan (CS) is a naturally occurring polysaccharide having cationic nature and can be obtained from the chitin which is extracted from crab shells [8-10]. The extracted CS having high molecular weight with low solubility and irradiated with gamma rays can improve its solubility and purity [11]. It is not easy to electrospun the CS for fibrous structure due to its increased surface tension resulting from the polycationic nature. This requires a high voltage to produce electrospun fibrous structure [12]. So, CS is blended or mixed with synthetic polymers for better electrospinning such as: poly(lactic acid) (PLA), poly(vinyl alcohol) (PVA), poly (ethylene oxide) (PEO), etc. [13-17]. PVA is a non-toxic and biodegradable synthetic polymer and forms electrospun scaffolds with CS which have significant use in biomedical applications [8, 9, 18, 19].

Electrospinning is helpful for fabricating electrospun nanofibrous material used for many biomedical applications. This is an excellent process for nanofibers production at fairly low

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cost. Different polymers were electrospun for the production of fibers of varying sizes [11, 20, 21].

The present work includes the extraction of chitin from crab shells, deacetylation of chitin to produce CS, irradiation of CS by gamma rays at different doses and then the electrospinning of this CS with PVA to produce electrospun fiberous scaffolds [22]. The novelty of work was the use of self-extracted γ-irradiated CS. FTIR spectroscopy was used to confirm the deacetylation of chitin into CS and other incorporated components. Then, the morphology of electrospun CS/PVA nanofibrous scaffolds is investigated using scanning electron microscopy (SEM). The cytotoxicity of the selected scaffolds is carried by indirect method using human fibroblasts cells (F84, F192 and F121) and human cancerous bone cells (HCBC) (MG63) for direct method.

2. Materials and Methods

2.1. Materials

γ- irradiation of chitosan (degree of deacetylation = 75%), indigenously synthesized from chitin of crab shells was reported elsewhere [22]. PVA (Mw: 85,000-90,000 g/mol), industrial methylated spirit, tetraethoxysilane (TEOS), acetic acid (99.7%), methylthiazolydiphenyl-tetrazolium bromide (MTT) and were bought from Sigma Aldrich, USA.

2.2. Preparation of blended solution

Gamma irradiated CS was solubilized in 40 mL of 0.5 M acetic acid. PVA was solubilized in distilled water at 80 ºC and both solutions were blended together at ambient temperature with persistent stirring for 2 h. The mole ratio of CS and PVA was set as 1:4. Total amount of chitosan and PVA in polymer solution was 7 and 9 wt %. The codes of the fabricated fiberous scaffolds are provided in Table 1.

Table 1. Codes and composition of the electrospun nanofibrous scaffolds.

Scaffold	NFC 7	NFC 9	NF (25) 7	NF (25) 9	NF (50) 7	NF (50) 9	NF (75) 7	NF (75) 9	NF (100) 7	NF (100) 9
CS/PVA weight (%)	7	9	7	9	7	9	7	9	7	9

2.3. Electrospinning of blended solutions

The equipment with which nanofibers were prepared contained two electrodes, one was fixed to the aluminum plate and other was attached to the needle tip. Aluminum plate was placed at 5 cm away from the needle tip. A high electric charge potential was provided at the tip of syringe needle (2.0 kV/cm rate) in this procedure. The flow rate of the polymer solution was set at 0.2 mL/min [23].

2.4. Characterizations

2.4.1. Structural analysis

Structural analysis was performed to evaluate the confirmation of functional groups in the nanofibrous scaffolds using FTIR spectrophotometer (Nicolet, 6700) from Thermo Electron Corporation, USA using attenuated total reflectance mode having diamond crystal. The spectra were taken in the range of 4000–400 cm^{-1} with average of 200 scans at a resolution of 6.0 cm^{-1}. FTIR spectra of different irradiated CS with control and pure PVA with commercially available CS were recorded.

2.4.2. Morphological analysis

SEM, Model JEOL JSM 6490 (LA) was used to inspect the morphology of electrospun nanofibrous scaffolds. The gold coated specimens were examined at different magnifications**.**

2.4.3. Cytotoxicity tests

The cytotoxicity of CS/PVA electrospun fibrous scaffolds was assessed by MTT assay. The human cancerous bone cells (MG63) were used for direct method while cell lines of human fibroblast cells (F84, F192 and F121) were used for indirect method. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) feeded with fetal calf serum in tissue cultured plastic (TCP) flasks. In the culture, 5% CO_2 atmosphere was maintained at 37 °C. When cells attained 80% confluencey i.e. allows estimating that there were enough cells, they were placed in DMEM (1 mL) in well plates having nanofibers scaffolds in the transfer filters within the well plates (density of the cells was approximately 5000 cells/well). The prepared scaffolds were rinsed firstly with methylated spirit and then with phosphate buffer saline (PBS) solution before putting in transfer filters for indirect method and directly in the well plates in case of direct method. Media (300 µL) was supplemented in each well plate and kept at 37 °C and 5% CO_2 atmosphere in incubator for 1 day.

MTT assay was carried out to find out the cells compatibilities. It was a colorimetric based assay that showed purple coloured formazan on reduction showing the intensity of cell metabolism. Plates were taken out from the incubator after 1 day and eliminated all the media, transfer filters were discarded. The wells were cleaned with PBS solution and added I mL of MTT solution (0.5 mg/mL in PBS) in each well. The well plates were then put in the incubator for 1 h and then MTT solution was eliminated. 300 µL of 2-ethoxy methanol was added in each well for the elution of purple formazan. 150 µL of this solution from wells was shifted to the 96-well plate. The optical density was evaluated at wavelength of 562 nm by Spectrophotometer Biotek Instruments. The protein reference was used at 630 nm. The cells and nanofibrous scaffolds were seeded to DMEM as negative and positive control.

3. Results and Discussion

3.1. FTIR spectroscopy

Figure 1 presented the FTIR spectra of different CS/PVA electrospun nanofiberous scaffolds. All spectra demonstrated wide band at 3600-3200 cm^{-1} correspond to the hydroxyl stretching of intra- and inter-molecular H-bonding. The intensity of this band is enhanced which indicated the rise in the hydrogen bonding in nanofibers due to increased PVA and irradiated CS content. The peak at 3000-2800 cm^{-1} is ascribed to stretching frequency of alkyl groups while the peaks at 1413 and 1382 cm^{-1} are attributed to $-CH_2$ deformation and symmetrical vibrational modes, respectively. The pyranose (cyclic ester)

ring and saccharide structure of CS is confirmed by band at 893 and 1152 cm^{-1} [18, 19], respectively, in nanofibers. The absorption band of $–NH_2$ group is noticed at 1255 cm^{-1} [2]. The amide I and amide III peaks are noticed at 1648 and 1322 cm^{-1}, respectively while band at 1560 cm^{-1} attributed to amide II. The appearance of siloxane linkage in nanofibers produced by the crosslinker reaction is endorsed by the existence of bands at 1060 cm^{-1} (Si-O-C) and 1020 (Si-O-Si) [18].

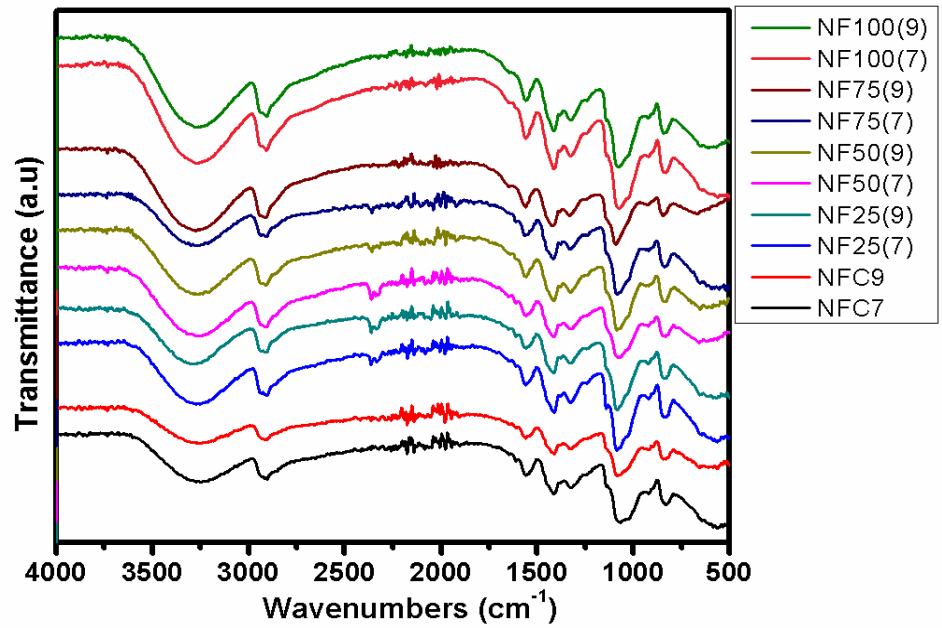

Figure 1: FTIR analysis of the prepared nanofiberous scaffolds.

3.2. Morphology

The morphological analysis of electrospun CS/PVA nanofibrous scaffolds is shown in Figure 2 which shows the two characteristic effects on the nanofibrous structure. One is the effect of increase in the gamma rays irradiation dose of extracted CS and other is the increase in PVA concentration in the polymer solution. It is remarkable to see that although with increase in the irradiation dose, the fibers are broken down but there is no effect on the size of the fibers and increasing the PVA concentration, the bead on string structure is reduced with round and uniform size fibers are formed. This may be due to the decrease in the molecular weight of the CS. The variation in size, structure and morphology of the nanofibers depends on the degradation of CS with irradiation dose and also the composition of the polymer solution. The nanofibers deteriorated at high irradiation dose is mostly due to the variation in the interaction of CS and PVA.

The parameters like flow rate, viscosity of solution, distance between collector plate and syringe and applied voltage control the size of the nanofibers. The morphology of the nanofibers is also affected by the concentration of the polymer solution [23]. It is also studied that the electrospinning of the solution became more convenient on decreasing flow rate, optimum viscosity of the polymer solution, high voltage, high molecular weight of the polymers, low flow rate and increasing the CS/PVA content from 7 - 11 wt % [23].

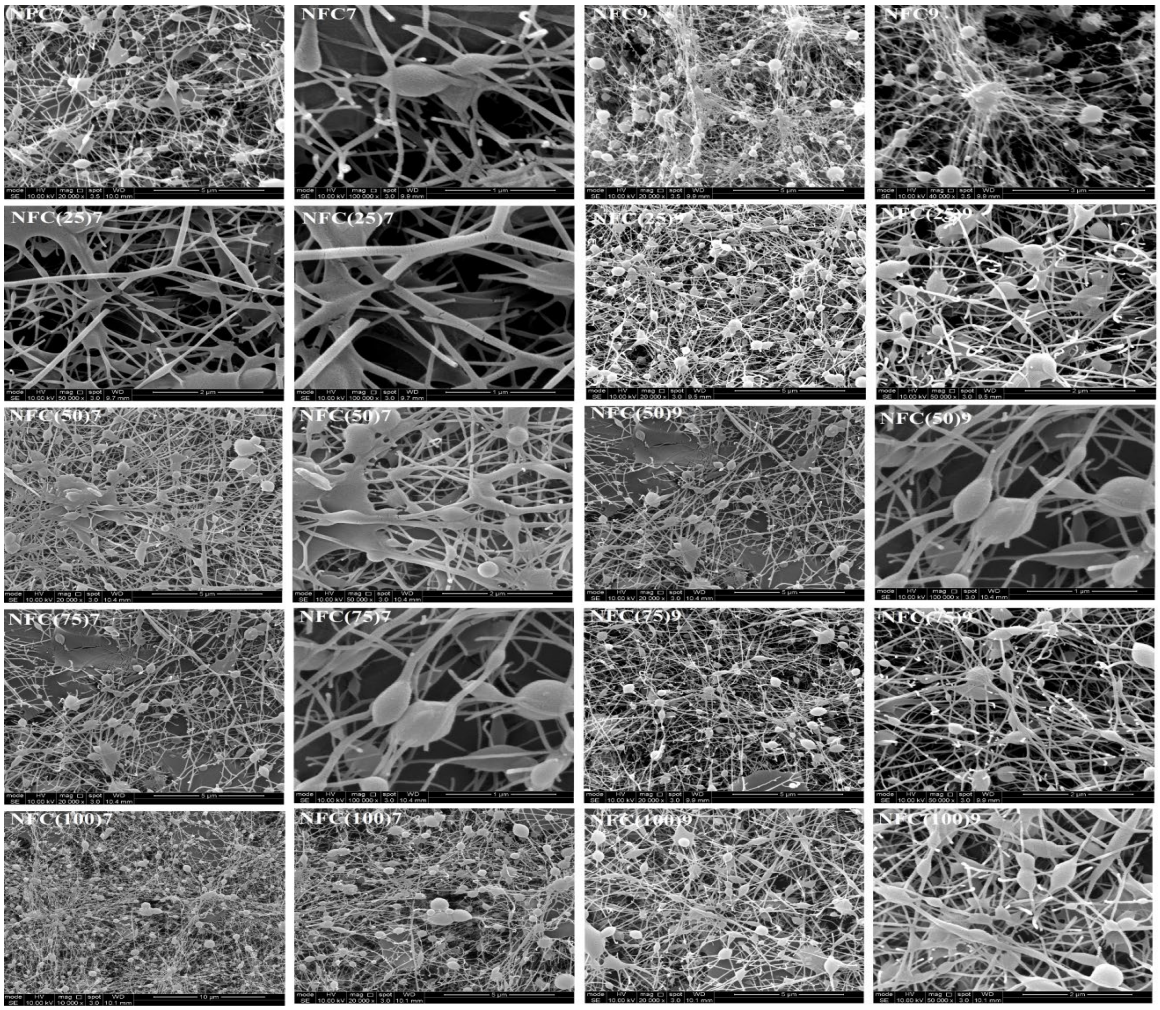

Figure 2: SEM images of fiberous scaffolds materials.

3.3. Cell toxicity/MTT assay

The cytotoxicity of electrospun scaffold materials can be determined using MTT assay. The results of electrospun scaffolds with direct and indirect method in comparison with controls (negative and positive) are shown in Figure 3. Different cell lines (F121, F192, F84 and MG63 for indirect method and MG63 for direct method) were used. It was observed that NF(75)9 shown no significant with the fibroblast as well as bone cells activity in the presence of electrospun scaffolds in comparison with positive and negative control. For F192 cell line, the cells have grown maximum and proliferated as compared to other cell lines as shown in Figure 3. For direct method, bone cells also grown in the presence of NF (75)9 and NF (25)9 electrospun scaffolds. It can be inferred that the electrospun scaffolds are non-toxic to human fibroblasts and bone cells and can be employed as biomedical applications.

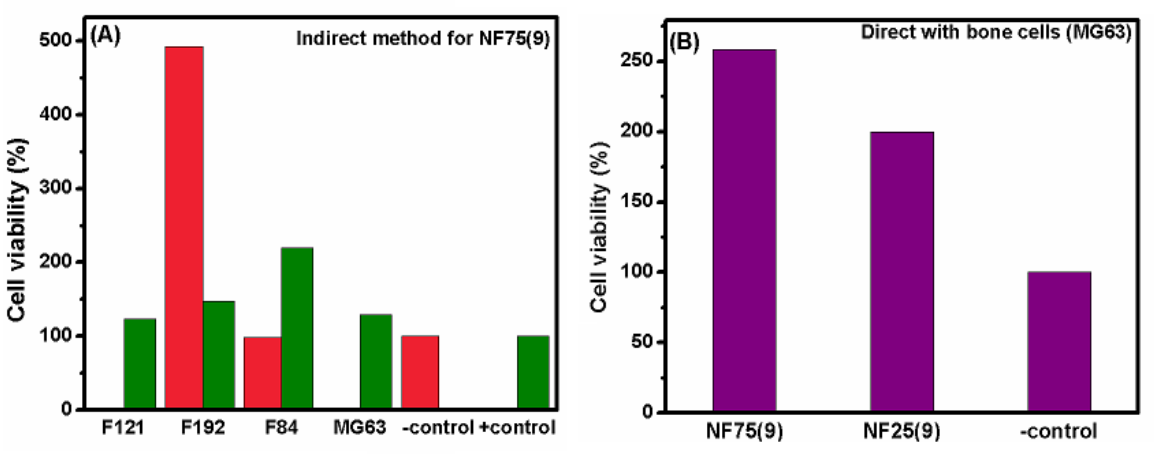

Figure 3: Cytotoxicity results of electrospun scaffolds, indirect method (A) and direct method (B).

4. Conclusions

Electrospun CS/PVA nanofibrous scaffolds were prepared by electrospinning method. FTIR analysis verified the extraction and confirmation of CS**.** SEM results exhibited that the decrease in the molecular weight of the CS by irradiation resulted in the bead on string structure of the nanofibers but the size of the fibers is not affected. Cytotoxicity analysis proved that CS/PVA nanofibrous scaffolds are compatible to the fibroblast as well as bone cells and have potential to be used in biomedical applications.

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