Electrospun polyvinyl alcohol/carbon dioxide modified polyethyleneimine composite nanofibers scaffold

Han-Bing Wu[1], David H Bremner[2], Hua-Li Nie [1], Jing Quan[1*],Li-Min Zhu[1*]

[1]*Donghua University, College of Chemistry, Chemical Engineering and Biotechnology, Shanghai 201620*

[2]*School of Science, Engineering and Technology,Kydd Building, Abertay University,Dundee DD1 1HG, Scotland, UK*

Abstract

A novel biocompatible PVA/PEI-CO_2 (polyvinyl alcohol/carbon dioxide modifiedpolyethyleneimine) composite nanofiber was fabricated by a green and facile protocol, which reduces the cytotoxicity of PEI through the surface modificationof the PEI with CO_2. The ^{13}C NMR spectrum, elemental analysis and TGA show that CO_2 has been incorporated in the PEI surface resulting in a relatively stable structure. The resulting PVA/PEI-CO_2 composite nanofibers have been characterized by ATR-FTIR, contact angle and Scanning Electron Microscopy (SEM). The results show that the average diameters of the nanofibers range from 265 ± 53 nm to 423 ± 80 nm. The cytotoxicity of PVA/PEI-CO_2 composite nanofibers was assessed by cytotoxicity evaluation using the growth and cell proliferation of normal mice Schwann cells. SEM and the MTT assay demonstrated that the promotion of cell growth and proliferation on the PVA/PEI-CO_2 composite scaffold. It suggests that PEI-CO_2 can have tremendous potential applications in biological material research.

Key words: PEI derivatives, CO_2, electrospinning, composite nanofibers, cell scaffold

Introduction

Polyethyleneimine (PEI) is a typical water-soluble polyamine and has a large number of amino nitrogen atoms in the molecular chain leading to a strong affinity to cells [1] and consequently has been widely used as a tissue engineering scaffolding material[2]; a stabilizer for nanoparticle synthesis[3, 4]; a layer component for polyelectrolyte multilayer construction[5]; and a gene delivery carrier[6, 7]. However, the high number of amino groups in PEI severely limits its further applications due to their high cytotoxicity and poor biocompatibility[8]. Thus modification of PEI in order to decrease cytotoxicity becomes important.

Many research reports have focused on modified PEI to reduce the cytotoxicity of PEI9-12. For example, Gabrielson 13 used acetic anhydride to modify PEI 25 K, by converting the primary and secondary amines on the PEI into amides. Also PEI, with different molecular weights, can be grafted and surfaced-modified which reduces the number of amino groups in the molecule, effectively decreasing cytotoxicity and improving biocompatibility 14-16. The cytotoxicity was shown to be related to size and the number of grafts, but had no direct relationship with the length of the chain of the PEG segment 14. Unfortunately, most chemical syntheses are generally not facile and green enough to allow PEI derivatization without protection/deprotection schemes and require complex multistep procedures to remove impurities, which has further limited practical applications.

Through the modification methods, I want to get a kind of green environmental protection modification methods to improve the biocompatibility of the PEI. According to reports, using PEI to capture CO2 from exhaust gases formed from fossil fuels has been developed 17-19, because that the PEI in aqueous solution shows high adsorption of CO2 since aqueous solutions of amines absorb CO2 to generate amides efficiently at ambient temperatures via an exothermic reaction 20-23. According to the definitions of green chemistry, CO2 being part of the atmosphere and having a wide variety of sources and a low price can be classified as a green chemical24-26. Therefore, carbon dioxide can be used to modify PEI to reduce its amino content, in order to achieve the purpose of reducing its cytotoxicity. This reaction is very facile and is green organic chemistry because, not only does this method reduce the number of amino groups on the surface of the PEI, but it also dispenses with complex chemical reaction processes, various kinds of catalyst, expensive modified monomers and a tedious and the time-consuming process of impurity removal. Electrospinning is a simple method for fabricating fibrous materials from a rich variety of substrates[27-29]. Nanofiber mats have very high surface areas, pore sizes ranging from several to tens of micrometers and adjustable porosities up to more than 90%. Thus, various kinds of nanofiber materials have been used for support materials[30-32] and PEI, in particular, has been systematically researched and widely used as an effective electrospun nanofiber in the field of biomaterials. The electrostatic spinning preparation of PVA/PEI nanofibers, used for environmental remediation[33-35] indicates that PEI-modified electrospun nanofibers should be a promising candidate for use in tissue engineering and medical applications.

In this study, a novel biocompatible PVA/PEI-CO_2 composite nanofiber substrate, which can decrease the cytotoxicity of PEI through the surface modification of the PEI with CO_2, was fabricated by a green and facile protocol. The literature data38, 41 show that ultrafine PEI/PVA nanofibers can be formed using an electrospinning technology; thus, the PVA is an excellent host material for PEI-CO2, and has been widely used in different areas of the biomedical field due to its excellent chemical and physical properties, ease of processing and low cytotoxicity[34, 35]. The CO_2 modified PEI was prepared and the resulting electrospun PVA/PEI-CO_2 composite nanofiber materials utilized as a substrate for cell growth. Cell toxicity experiments prove that the nanofibers can promote cell growth and the modified PEI can effectively reduce cytotoxicity. Also, the new composite nanofibers were studied to determine the cellular biocompatibility for their future potential biomedical applications.

2 Materials and methods

2.1. Materials

PEI (branched, MW = 250,000) was purchased from Sigma-Aldrich. PVA (88% hydrolyzed, MW = 88,000) was obtained from J&K Chemical. Glutaraldehyde (25%, aqueous solution), ethyl alcohol, DMSO and paraformaldehyde were purchased from Sinopharm Chemical Reagent Co., Ltd (China). High purity carbon dioxide (> 99.999%) was purchased from Shanghai Chlorine min Gas Co. Ltd and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) from Sigma-Aldrich. Schwann cells were obtained from the Institute of Chemistry, Chemical Engineering and Biotechnology (Donghua University, Shanghai, China). Dulbecco's modified Eagle's medium (DMEM; high glucose with L-glutamine and sodium pyruvate), fetal bovine serum (FBS), 0.25% trypsin-EDTA, phosphate buffer saline (PBS), penicillin (10000 U/mL) and streptomycin (10 mg/mL) were purchased from HangzhouJinuo Biomedical Technology (Hangzhou, China). An aqueous solution of 4% paraformaldehyde (PFA) was purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. All other chemicals used were analytical grade and water was doubly distilled before use.

2.2. Modification of PEI with CO_2

The solution was prepared by dissolving branched PEI(3g) in 15mL water with heating and stirring. Carbon dioxide was bubbled into this solution at ambient temperature and stirring was continued for 5 hours until the reaction was complete. The contents were transferred to an EP tube and freeze dried to form solid PEI-CO_2 which was then ground into a fine yellow powder and stored at 4 ^{o}C in a refrigerator. The polymer was characterized using ^{13}C NMR spectrometry and spectra were recorded on a Jeol JNMECS 400(100 MHz for ^{13}C with tetramethylsilane as an internal standard). ATR-FTIR spectra were recorded using a Nicolet 5700 FTIR spectrometer (Thermo Nicolet Corporation, USA) at ambient conditions. Thermogravimetric analysis was conducted with an EXSTARTG/DTA6200 instrument (Seiko Instruments)and elemental analysis was performed on an Elmentar Vario ELIII elemental analyzer, Germany.

PEI:^{13}C NMR(100 MHz, D_2O); δ (ppm) = 56.00, 53.30, 50.87, 47.53, 45.65, 39.31, 37.50(-CH2-); PEI-CO_2 :^{13}C NMR(100 MHz, D_2O); δ (ppm) = 164.92, 163.6, 160.3 (C=O), 50.1, 44.5, 39.0, 37.8, 36.4 (-CH2-). Elemental compositions- PEI: C 55.09%; H 12.30%; N 34.48% and PEI-CO_2: C 42.92%; H 8.22%; N 21.37%.

2.3. Electrospinning

2.3.1 Fabrication of electrospun PVA/PEI-$CO_2$and PEI/PVA nanofibers

The PVA solution (12 wt%) was prepared by dissolving PVA powder (12g) in water (100mL) at 80 °C for 3 h under magnetic stirring and the solution was cooled to room temperature before use [36, 37]. The PEI solution (50 wt %) was prepared by dissolving the viscous liquid PEI (10 g) in water (20 mL) at ambient temperature. PVA and PEI aqueous solutions were mixed together with a total polymer concentration of 11 wt% according to the literature [34, 38] to prepare homogeneous solutions with PVA/PEI weight ratios of 95:5, 85:15, 75:25 and 65:35, respectively. PVA aqueous solutions and PEI-CO_2 powder were mixed together with a total polymer concentration of 11 wt% to prepare a homogeneous solution with PVA/PEI-CO_2 weight ratios of 95:5, 85:15, 75:25 and 65:35, respectively. The appropriate solutions were then constantly and controllably injected into the electrospin unit at a flow rate ranging from 0.1 to 0.2 mL/h via a syringe pump (JZB-1800, Jian Yuan MedicalTechnology Co., Ltd, China). The distance of the nozzle to collector was set at either 17 or 20 cm and the voltage was either 12.5 or 13 kV [34]. The high voltage supply (GBB40/2, Institute of Beijing High Voltage Technology, China) was connected to a stainless steel needle and the collector was earthed[39]. Under the applied electrospinning conditions, the polymer jet produced ultrafine fibers, which were ultimately deposited onto the collector, forming a nanofibrous mat.

2.3.3 Crosslinking of PVA/PEI-CO_2 and PVA/PEI nanofibers

A Petri dish containing glutaraldehyde (GA) solution (25% aqueous solution, 20 mL) was placed at the bottom of a desiccator and the electrospun PVA/PEI-CO_2 or PVA/PEI nanofibers were put onto the top ceramic plate of the desiccator. Vacuum was applied for 24 h and then the nanofibrous mats, which were treated using both the GA solution and GA vapor, were rinsed with water 3 times to remove the excess GA. All the treated mats were cut into 15 mm diameter circles, placed into 24-well plates and washed three times with PBS solution to simulate the environment found in the body.

2.3.4 Characterization

Morphologies of the electrospun PVA/PEI-CO_2 and PEI/PVA nanofibrous mats were determined using SEM (JSM-5600LV, JEOL Ltd, Japan) with an operating voltage of 10 kV or 15 kV. Prior to measurements, the nanofibrous mats were sputter coated with a 10 nm-thick gold film. The mean diameter of the nanofibers was measured using ImageJ 1.40G software(http://rsb.info.nih.gov/ij/download.html) with at least 200 different fibers in SEM images being selected for the analysis. ATR-FTIR spectra of the composite fiber membranes were recorded before and after crosslinking. The contact angle values of the electrospun nanofibers were measured with an optical contact angle goniometer (CAM 100, KSV Instruments Ltd., Helsinki, Finland). This compact video-based instrument measured contact angles between 1° and 180° with an accuracy of ±1° and also allowed photographs to be taken of the measured contact angle values on the surfaces of the nanofibers.

2.5 Investigation of cytotoxicity

Cytotoxicity was evaluated by using a MTT assay against rat Schwann Cells (RSC96). The cells were maintained in high glucose DMEM（4.5 g/L glucose） supplemented with 10% FBS and penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 ^{o}C in a 5% CO_2 humidified atmosphere. For cytotoxicity analysis, cells were seeded on the sterilized surface of PVA/PEI-CO_2 or PVA/PEI nanofiber matrixes in 24-well plates at a density of 2×10^4 cells per well. The cells were washed with phosphate-buffered saline (PBS) on days 1, 3, 5 and 7. Aliquots of 400 μL of MTT solution in DMEM (2 mg/mL) were added to each well. Plates were incubated for an additional 2 h at 37 °C. The MTT-containing medium was removed and 400μL of DMSO was added to dissolve the formazan crystals formed by living cells[40]. This solution was then transferred to 96 well plates and the optical density (OD) was measured at a wavelength of 570 nm using an enzyme-linked immune adsorbent assay plate reader (MK3, Thermo, Thermoelectric (Shanghai) Instrument Co., Ltd.). On day 5, two specimens of each scaffold were characterized using SEM. Every specimen was rinsed with PBS, fixed with an aqueous solution of 4% paraformaldehyde (400 μL) and incubated at 4 ^{o}C in a refrigerator for 2 h. The specimens with fixed cells were dehydrated using a graded ethanol series and critical point dried. All specimens were then sputter coated with gold-palladium and the cell morphology on different substrates was observed using SEM at an acceleration voltage of 2 keV.

3. Results and discussion

3.1 The modification of PEI

After carbon dioxide was bubbled into the aqueous PEI the color changed from clear to light yellow, due to adsorption of the CO_2 by PEI (Fig. 1A). The reaction is an exothermic process and the mechanism has been described previously[23]. The ^{13}C NMR spectrum of the D_2O solution showed the peaks at lower chemical shift look significantly different before and after CO_2 bubbling. According to the literature on the peaks should be the carbamoyl carbon atom[19], which also supports the formation of the carbamic acid group in PEI (Fig. 1B, I and II). This demonstrates that the capture of CO_2 by PEI in solution forms new chemical bonds. The solid yellow PEI-CO_2 compound was then produced through freeze drying.

Fig. 1.

The structure of the modified PEI was further explored by determining the elemental composition. However, since only carbon, hydrogen, and nitrogen can be determined directly using elemental analysis the presence of oxygen can only be found by subtraction. Elemental analysis of PEI-CO_2 indicated that it contained about 27.56% of oxygen but due to the possible presence of water in the compound the percentage of nitrogen atoms which captured carbon dioxide was based on the amount of nitrogen and carbon present. Consequently, it was estimated that approximately 23% of the nitrogen atoms of PEI had reacted with CO_2. It was calculated using the following equation:

$$CO_2\% = \frac{[C-(N\times 24\div 14)]\times 44}{12}\times 100$$

where C is the carbon atoms in the PEI - CO_2 content, N is the nitrogen atoms in the PEI - CO_2 content.

In order to further verify the composition of the modified PEI, TGA was used to characterize the loading capacity of CO_2 immobilized into the PEI (Fig. 2). At a temperature of 400 ^{o}C and a heating rate of 10 ^{o}C/min under nitrogen, the polymer components were completely decomposed. The initial decrease of both PEI and PEI-CO_2 is probably due to the loss of water in the polymer, while the weight loss in the region of 82 ~ 187 ^{o}C is attributed to the decomposition of the PEI-CO_2 polymer and release of CO_2 which equates to about 31%. Finally, the major weight loss within the region of 270 ~ 400 ^{o}C is attributed to the decomposition of the PEI polymers. This result is similar to the conclusions from elemental analysis and proves that the modified PEI has considerable stability and is suitable for biological applications.

Fig.2.

3.2 Morphology and structure of composite nanofibers

It is well known that PVA is viscous and the addition into other polymer solutions can significantly improve the spinnability of the polymers[38, 41]. Therefore, in this study, PVA/PEI-CO_2 and PVA/ PEI fibrous membranes were selected to obtain uniform electrospun polymer nanofibers to study the cytotoxicity of the modified PEI and PVA composite nanofiber scaffold. There are many factors which can affect themorphology of the electrospun nanofibers including collection distance, voltage, flow rate, and polymer concentration. In order to obtain PVA/PEI-CO_2 and PVA/PEI nanofibers with a smooth and uniform morphology, the most crucial processing parameter, polymer concentration, was optimised. Fig. 3 shows the SEM micrographs, diameter distribution histograms and change in average diameters of the different w/w ratios of PVA/PEI-CO_2 and PVA/PEI of electrospun nanofibrous mats fabricated under fixed conditions (flow rate of 0.15 mL/h, polymer concentration of 11 wt%, applied voltage of 12.5 kV, and collection distance of 17 cm).

From Fig. 3 and table 1 it can be seen that the fiber diameter distribution of the PVA/PEI-CO_2 nanofibers is more uniform than the PEI/PVA nanofibers and, with the increase of the content of PEI-CO_2, the average diameter of the PVA/PEI-CO_2 decreases initially and then increases. This is due to the fact that PEI-CO_2 contains carboxyl and amino groups which can be ionized, forming an ionic polymer to increase the conductivity of the solution. The overall effect is equivalent to adding inorganic salts to the spinning solution. When a small amount of PEI-CO_2 or PEI is added the conductivity of the solution will be greatly improved though the solution viscosity and surface tension will remain basically unchanged. At the same time, the fiber diameter is reduced and the diameter distribution range changes and becomes narrower. However, with a further increase in polyelectrolyte (PEI-CO_2) content, the viscosity of the solution increases while the surface tension decreases, so that the viscosity of the solution is dominant resulting in the increase of fiber diameter. A similar observation can be seen in the diameter of PVA/PEI nanofibers, but is not so obvious, because the fiber diameter distribution is very uneven and fiber morphology is irregular compared to PVA/PEI-CO_2 nanofibers.

Fig.3.
Table 1.

The change of composite nanofibers before and after crosslinking, which is essential for producing high-quality and stable nanofibers, was investigated through ATR-FTIR analysis (Fig. 4). The PEI (I) and PEI-CO_2 (II), the PVA/PEI (III) and PVA/PEI-CO_2 (IV) nanofibers and the crosslinking of PVA/PEI (V) and

PVA/PEI-CO_2 (VI) nanofibers were confirmed using ATR-FTIR spectra. The well-defined doublet at 3373 cm^{-1} and 3276 cm^{-1} are –NH_2 of antisymmetric and symmetric stretching vibration absorption peak in PEI and the peaks become a broad –OH band which could be overlapping with the – NH_2 peaks in PEI-CO2 (Fig. 4I and Fig. 4II), because the carbon dioxide decorates – NH_2 and –NH- into –COOH[42]. A broad peak at 3300 ~ 3350 cm-1 is the -OH of hydroxyl stretching vibration in PVA (Fig. 4 III~VI). The –CH_2 and –CH- of stretching vibration absorption peak are 2940 cm-1 and 2810 cm-1(Fig. 4I). It is generally known that due to the lone electron pair on nitrogen atom and carbonyl form p-π conjugate in the acid amides, the frequency of C=O stretching vibration reduces. The carbonyl stretching vibration absorption peak of a secondary amide is 1680 cm-1 and 1655 cm-1 (the amide I peak), the bending vibration of C-N-H is 1600 cm-1 and 1530 cm-1 (the amide II peak) and there is also a characteristic peak around 1300 cm-1(the amide III peak). Tertiary amides have no N-H bending vibration absorption around high frequency and its carbonyl stretching vibration absorption peak is 1670 cm-1 and 1630 cm-1 (the amide I peak), because there is no N-H bond in tertiary amides. There is a peak (1669 cm-1) in the PEI-CO_2, which were not evident in the PEI and the stretching vibration absorption peak of C=O is the “the amide I peak” (Fig. 4II); The peak at 1570 cm-1 is the “the amide II peak” (The bending vibration of C–N–H; Fig. 4II); there is also a peak (1300 cm-1) is characteristic absorption peak of secondary amide (the amide III peak). A peak at 1655 cm^{-1}, assigned to N-H bending of the primary amines of PEI or PEI-CO_2, still existed after crosslinking with GA vapor, suggesting that the PEI primary amine groups are available for cell sorption (Fig. 4III-VI). A weak band at 1564 cm^{-1}, representing the formation of an aldimine linkage after crosslinking is in agreement with the literature (Fig. 4V)[38,43]. The C=N- bond is formed by the reaction of amine with GA[38]. It leads to that lone electron pair of amino disappears, p-π conjugate is replaced by C=N–C=O conjugate and amide stretching vibration peak is around 1720 cm^{-1}. Therefore, the C=O stretching vibrations at 1728 cm^{-1} is more pronounced after crosslinking by GA vapor further demonstrated the successful crosslinking reaction (Fig. 4 VI,V) and the weak peak at 1021 cm^{-1} indicates an ether linkage (-O-) formation between the PVA hydroxyl groups (Fig. 4 V~VI) and the GA aldehyde groups which is also in agreement with the literature data [44].

Fig.4.

Hydrophilicity, which may be demonstrated using contact angle measurements, plays a vital role among factors influencing effectiveness of biocomposites in the growth of cells. It can be seen in Fig. 5 that all four samples showed a decrease in the average of contact angle from 59° to 30° after crosslinking of the PVA/PEI-CO_2 nanofibers when PEI-CO_2 concentration was increased, which indicates that the surfaces of the nanofiber became more hydrophilic due to decrease in PVA content.

Fig.5.

3.3 In vitro cell growth and cytotoxicity assay

The morphology of cells attaching to the surface of biomaterials reveals the cytocompatibility of the scaffold. When cells contact with biomaterials, they undergo morphological changes to adapt to the cell-material surface[35, 45]. SEM images of Schwann cells after 5 days culturing on the prepared electrospun scaffolds showed that the cells spread well and attached firmly onto the scaffold surface (Fig. 6).

For different cells, scaffolds of optimum aperture is uncertain, but dozens to hundreds of microns diameter for cell migration and internal stent ingrowth is for the most part considered necessary [46]. Proper pore size and high porosity (> 90%) and connected to the hole shape, for a large number of cell cultivation, the growth of cells and tissues, the formation of extracellular matrix, and the transport of oxygen and nutrients, metabolite excretion and the blood vessels and nerves within the growth plays a decisive role [47]. Image A1 and B1 (Fig. 6) shows cells growth is getting better and better with the increase of the PEI-CO2 content in the scaffolds. Image A2 and B2 (Fig. 6) shows cells that were grown on nanofibers produced using a high PEI content and the complete absence of cell sand the number of cells was less due to the cytotoxicity of PEI. Comparing Fig.6 B1 and B2, almost all cells get into scaffolds in B1, but B2 is not. Appear this kind of phenomenon the reason is that because the PEI-CO2 has good biocompatibility, and PEI has strong cytotoxicity. On the other hand the PEI-CO2 fiber scaffold fiber structure is neat, being helpful for cell growth.

Fig.6.

To further study the cytotoxicity of modified composite nanofibers in a cell scaffold, experiments were conducted with different mass ratios of PVA/PEI-CO_2. Fig. 7 presents the proliferation results measured using the MTT assay after culturing for 1, 3, 5, and 7 days on the different mass ratios of PVA/PEI-CO_2 nanofiber matrices. Over the incubation time of 1 ~ 7 days, the cell growth on the pure PVA scaffold and the control were very similar with the latter actually showing a decrease in growth after day 1. When the PVA/PEI-CO_2 ratio in the scaffold was increased from 95:5 to 85:15 to 75:25 there was very little difference in cell proliferation although all three matrices were better than pure PVA and the control. However when the 65:35 composite fiber was used there was a remarkable increase in the proliferation of the Schwann cells over the time period. On the seventh day, the OD value reached 0.47 which is more than double that of the other matrices containing PEI-CO_2 (OD values were 0.2, 0.17, 0.23) and 5-fold compared with the control sample and PVA alone. As discussed above, the PEI-CO_2 content in the scaffold is not cytotoxic to cells but, rather, enhances their growth.

Fig.7.

Fig. 8 shows the data for the growth of cells and the relative toxicity of the surfaces for PVA, PVA/PEI-CO_2, PVA/PEI and a control with no matrix present. There are two factors occurring: adsorption and cytotoxicity where the former is conducive to cell growth, but the latter acts in the opposite way and there is a balance between them. With low amounts of PEI in the fiber, the adsorption of the cells plays a leading role, cytotoxicity is very small, and so this is conducive to cell growth. Also if the number of free amino groups in the PVA/PEI scaffold is low, then the cells grow better. However, when the PEI content reaches 35%, the presence of the amino groups is more detrimental leading to an increase in cell toxicity. In the modified PVA/PEI-CO_2 scaffold, the number of surface amino groups is greatly decreased so cell toxicity is reduced whilst adsorption is maintained so this scaffold promotes cell growth significantly but the PVA/PEI scaffold shows the opposite effect[40, 48]. Since both nanofibers contain PVA, it is only the PEI and PEI-CO_2 content which differs, thus proving cytotoxicity of composite nanofibers can be effectively reduced by the PEI modified with CO_2 [49]. The current research shows that an increase in PEI-CO_2 content in the fiber, not only reduces the cytotoxicity but also promotes cell growth [40, 50]. This study has also demonstrated the cytotoxicity of PEI is effectively reduced by the PEI modified by carbon dioxide suggesting that PEI-CO_2 nanofibers may have enhanced applications in biological materials.

Fig.8.

4. Conclusions

The study provides a detailed description of a PEI modification methodin order to decrease the cytotoxicity of a PVA/PEI nanofiber scaffold. NMR and TGA verified that CO_2 and PEI react together to form a relatively stable modified polymer containing amide and carbamic acid groups. High voltage electrospinning using PEI modified with CO_2 and PVA as the fiber scaffold was used to prepare composite nanofibers that could provide cellular scaffold material. A comparison of PEI/PVA nanofiber, without modification, with the modified fiber showed that the latter was smoother and had a more uniform diameter. ATR-FTIR spectra demonstrated that, owing to the presence of the carbonyl bond, the composite nanofibers before and after crosslinking contain the PEI-CO_2, functionality. *In vitro* tests showed that the growth of cells on the cellular scaffold of PVA/PEI-CO_2 composite nanofibers is much more successful than those on the scaffold of PVA/PEI composite nanofibers. SEM and the MTT assay demonstrated that cells do not grow well on fibers which contain increasing amounts of PEI but the modified fiber is much more effective and allows enhanced proliferation of cells. The present study describes a simple and useful approach for the systematic design and fabrication via electrospinning of novel biomaterials which may support and enhance cellular growth *in vivo*.

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