

# Electrospun linear polyethyleneimine scaffolds for cell growth

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## Abstract

Unique biocompatible scaffolds were produced by electrospinning cross-linked linear polyethyleneimine (PEI) with succinic anhydride and 1,4-butanediol diglycidyl ether. Nonwoven mats of PEI fibers in the range of 1600–687 nm were evaluated as interaction scaffolds for normal human fibroblast (NHF) cells. The electrospun scaffolds were characterized by Fourier transform infrared spectroscopy and ultraviolet–visible spectroscopy. The growth of the NHF cells was followed by scanning electron microscopy as well as optical and fluorescence microscopies. Cell viability was evaluated by staining with propidium iodide for dead cells and fluorescein diacetate for live cells. Immunofluorescence with fixed cells on the scaffolds was examined by staining the endoplasmic reticulum with rabbit anti-GRP 78/Alexa 488 goat anti-rabbit and staining the nuclei with 4'-6'-diamidino-2-phenylindole. Fluorescence studies confirmed that NHF cells attached and spread throughout the cross-linked linear polyethyleneimine scaffold. The attachment and spreading of cells suggests that electrospun linear polyethyleneimine scaffolds support growth of normal human fibroblasts cells. Thus, these biomaterial scaffolds may be useful in tissue engineering.

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## 1. Introduction

Millions of therapeutic procedures are performed each year to replace tissue lost to trauma or disease. These approaches can be divided into three categories: (1) synthetic prostheses, (2) drug delivery and (3) organ transplantation [1]. The search for alternative therapies has been the underlying motivation of tissue engineering [1]. The goal is an alternative approach to the repair and regeneration of damaged human tissue which avoids the need for a permanent implant [2,3]. One area in which tissue regeneration has been successful is in skin replacement. Every year, thousands of people suffer from extensive and severe burns. The common practice for small burn wounds has been to cover the area with a skin graft, usually the patient's own skin which minimizes complications such as tissue rejection.

However, if the burned area is large, finding donor skin to cover the wounds is difficult. Therefore, tissue engineering offers new hope. One way to address these problems is to create natural skin or synthetic skin substitutes through tissue engineering [4].

The human body consists of organs and tissues organized in three-dimensional (3-D) structures. The first goal of this study is to design and fabricate scaffold in such a way that it can create and maintain a space for tissue development. It is well known that the extracellular environment influences many aspects of cell behavior, morphology, functionality and cell–cell interactions [5]. The extracellular matrix (ECM) has physical structural features ranging from nanometer scale to micrometer scale. The ECM is also cell specific and provides a protective outer layer for cells. In general, scaffolds are porous, biodegradable and or biocompatible structures which are designed to mimic the extracellular matrix, acting as a physical support structure to give the cells the necessary shape. These scaffolds can then be transplanted in vivo [6]. These 3-D scaffolds

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will provide an actual physical structure for directing the growth of new replacement cells. With time, the scaffold should disintegrate leaving the body with its natural tissues. The 3-D scaffold should closely mimic the native extracellular matrix than the conventional method of growing tissues on a 2-D Petri dish surface.

Electrospinning has been used extensively as a leading technology for generation of 3-D scaffolds [7,8]. Various natural biopolymers, such as collagen, chitosan and elastin–collagen based materials, have been used [9–13]. Various synthetic biodegradable polymers and natural proteins have also been used as scaffolds for cell support [14–16]. Electrospinning is a simple and scalable method of producing fibers with diameters ranging from several microns to less than 100 nm [17–19]. The small pore size between electrospun fibers is also an attractive feature for biomimetic materials [20]. Electrospinning involves applying a high potential to a polymer solution. Once a polymer gel overcomes its surface tension, a thin jet of solution, referred to as a Taylor cone, will erupt from the surface [17,21]. This jet follows a chaotic pathway and finally deposits onto a grounded target [22]. There are several categories of variables that influence the electrospun fiber diameter including polymer solution variables, process variables and environmental variables. The effects of processing variables (applied voltage, needle tip to target distance, feed rate of the solution to the capillary tip) and solution properties such as solvent, viscosity, concentration and surface tension on controlling the fiber morphology and diameter have been extensively investigated for a variety of polymeric systems [23,24]. Environmental conditions also play a critical role in fiber morphology. Studies have shown that fibers electrospun from solutions at elevated temperatures were more uniform in diameter than those prepared at room temperature [25]. Due to the high surface-to-volume ratio of electrospun polymer fibers there are a broad range of applications, including scaffolds in tissue engineering, chemical/biological resistant protective clothing, filtration, enzyme immobilization, controlled drug release, membranes and sensors [5,19,26,27]. Polycationic polymers that contain a high density of protonated primary, secondary, tertiary and/or quaternary amines are of interest. Several researchers have reported PEI to be cytotoxic in many cells [28,29]. Free PEI has been reported to destabilize outer membranes of Gram-negative bacteria [30–32]. However, PEI in its protonated form has been perhaps the cationic polymer most widely used as a gene delivery agent due to its high charge density from the protonated amines [33,34]. Scaffolds with an excessive amount of positive charges are highly toxic *in vivo* while lower amounts of cationic charge are favorable [35–37]. The second goal of this study was to enhance scaffold biocompatibility; therefore, linear PEI was electrospun with succinic anhydride and 1,4-butanediol diglycidyl ether. Electrospun linear polyethyleneimine scaffolds promoted cell growth by mimicking the biological function of the native extracellular matrix. Normal human fibroblasts cells

were found to attach and thrive even after 5 days of cell culturing.

## 2. Materials and methods

### 2.1. Materials

Succinic anhydride (97%) and 1,4-butanediol diglycidyl ether (95%) were purchased from Sigma–Aldrich (St. Louis, MO) and used to prepare linear PEI scaffold. Normal human fibroblasts (NHF) were obtained from the American Type Tissue Culture Collection (Manassas, VA). Trypsin–EDTA solution and Dulbecco's modified Eagle's medium (DMEM) were purchased from Irvine Scientific Inc. (Santa Ana, CA), fetal bovine calf serum (FBS) was purchased from HyClone (Logan, UT) and bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO). Fluorescein diacetate (FDA) (98%), propidium iodide (PI) (95%) and 4'-6'-diamidino-2-phenylindole (DAPI) (98%) were purchased from Sigma. Paraformaldehyde (95%, Sigma–Aldrich), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (99%, Fisher) and sodium azide (99.5%, Sigma) were also purchased. The penicillin and streptomycin used in cell culture studies were from Sigma (St. Louis, MO). Rabbit anti-Grp78 was purchased from StressGen Biotechnologies Corp. (San Diego, CA), Alexa 488 goat anti-rabbit was purchased from Molecular Probes (Carlsbad, CA) and Fluoromount G was purchased from Fisher.

### 2.2. Scaffold fabrication

Linear PEI was synthesized from poly(oxazoline) via a modification of a Tanaka et al. procedure [38]. In this procedure, 15 g of poly(oxazoline) (MW of 500,000) was dissolved in 125 ml of deionized water. Then 190 ml of 12 M HCl was slowly added to the solution, which was stirred for 5 days at 100 °C. After 24 h of stirring a white precipitate formed. After 5 days the slurry was cooled and 50 ml of a 5 M NaOH solution was added with stirring to give a pH of 14. The white solid was collected by vacuum filtration and washed with deionized water until the pH of the filtrate was 7. The white solids were recrystallized from 500 ml of hot ethanol. The precipitate was filtered and vacuum dried at 75 °C for 12 h. A semi-transparent white solid was obtained (yield ~90%, based on a calculated MW of 215,000). Linear PEI (400 mg) was dissolved in 2 ml of methanol. Then 80 mg of succinic anhydride (SA) was added to the polymer solution and the bath sonicated for 30 min. The clear solution was stirred at room temperature in a 10 ml vial to evaporate methanol until the viscosity was 1620 cP. Then 0.25 ml of 1,4-butanediol diglycidyl ether was added to the viscous gel to give a molar ratio of 1 PEI:0.09 SA:0.18 1,4-butanediol diglycidyl ether. This was stirred for 10 min at room temperature to further cross-link the polymer. The general scheme for cross-linking PEI is shown in Fig. 1. Approximately 2 ml

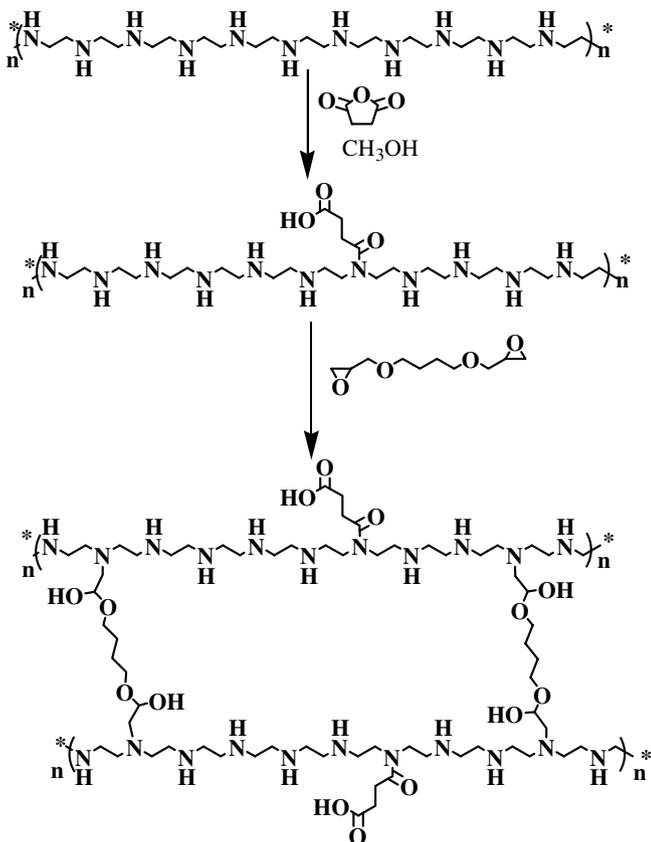


Fig. 1. Cross-linked linear PEI scaffold.

of this solution was added to a 3 ml plastic syringe barrel which was mounted in a KD Scientific syringe pump. The syringe pump was to control the feed rate of the polymer solution at  $0.01 \text{ ml min}^{-1}$ . The rotating mandrel (width and outside diameter were 14 and 15 cm, respectively) was spun at 0.7 Hz. The syringe with a flat tip stainless steel needle, 0.644 mm in width and 3.8 cm in length, was attached to an alligator clip and connected to a power supply (Sorenson H. V. Supply model #1020-30). A high DC voltage of 18 kV was applied to the viscous gel. The other contact was attached to a rotating target. This electric field induces a drop of the polymer solution to form at the needle tip. When the particles in the clear gel overcome the surface tension they form a fine jet known as a Taylor cone. The linear PEI fibers were collected for 10–15 min onto 25 mm glass cover slips (Fisher Scientific) attached to an aluminum foil target which was 10 cm away from the needle. Electrospun fibers were dried under vacuum at  $45^\circ\text{C}$  overnight to remove solvent.

### 2.3. Scaffold characterization

The morphology, density and thickness of fibers were evaluated using scanning electron microscopy (SEM; LEO 1530 VP). Samples for SEM were coated with a thin layer of Pd by an ion sputtering device prior to SEM observation. Fourier transform infrared (FTIR) spectra were

obtained from KBr using a Nicolet Magna 750 spectrophotometer. Diameters and pore size thickness of the scaffold were also determined from SEM images.

### 2.4. Cross-linking determination

The linear PEI scaffold was cross-linked with two molar equivalents of 1,4-butanediol diglycidyl ether. The degree of cross-linking was determined using Traut's and Ellman's reagents [39,40].

### 2.5. Cell culture

To assess cell behavior, adhesion and spreading response, the NHF cells were examined on the 3-D PEI scaffold. These results were compared with a control group of NHF cells grown on blank glass coverslips. All cells were grown in DMEM supplemented with 10 mM of HEPES and 5% FBS in an incubator with 10%  $\text{CO}_2$  maintained at  $37^\circ\text{C}$  and pH 7.4. Cells were removed from the culture dishes to seed the scaffolds using a trypsin–EDTA solution. The scaffold was placed into a 35 mm polystyrene culture dish, washed three times with phosphate-buffered saline (PBS) and soaked overnight in DMEM. The culture plate with the scaffold was filled with 2 ml of the supplemented DMEM media containing 10% FBS and  $100 \text{ mg ml}^{-1}$  each of streptomycin and penicillin, and placed into a cell culture incubator. After soaking,  $2 \times 10^5$  cells were suspended directly onto the scaffold. The plate was placed in a  $37^\circ\text{C}$  incubator. To ensure a constant pH during observation, outside of the incubator HEPES buffer was used instead of continuous  $\text{CO}_2$  flow. This also allowed the cells to be observed for extended times on the light microscope stage outside the incubator. The NHF cells were allowed to grow on the fibers and were observed daily for 5 days.

### 2.6. Determination of cell attachment to PEI scaffold

Cell viability was determined by live/dead cell assay using fluorescein diacetate (live) and propidium iodide (dead). The NHF cells were also viewed under differential interference contrast imaging (DIC). Electrospun linear PEI scaffold with fluorescence stained cells were viewed under a Nikon fluorescence microscope. Fixed cells were observed using a LEO 1530 VP field emission scanning electron microscope.

### 2.7. Immunofluorescence with fixed cells on fibers

Fibers with cells on a 25 mm coverslip were washed twice with PBS. The cells were fixed with 4% paraformaldehyde for 60 min at room temperature. Then the fixed cells were washed twice with 0.02% PBS/sodium azide and permeabilized with 0.2% saponin for 10 min. Non-specific sites were blocked by incubation in 0.02% PBS/1% BSA/0.02% sodium azide for 10 min at room temperature. The primary

antibody (anti-Grp78, also known as BiP at a 1:100 dilution) was added to the coverslip to completely cover the surface and allowed to incubate at room temperature for 45 min. The coverslip was rinsed three times with 0.02% PBS/sodium azide, and then blocked again with PBS/BSA/sodium azide for 10 min. The secondary antibody (Alexa 488 goat anti-rabbit at a 1:100 dilution) was added to the coverslip and incubated at room temperature for 45 min. The coverslip was rinsed three times with PBS/sodium azide, and then incubated in 2 ml of a DAPI solution of  $0.5 \text{ ng ml}^{-1}$  for 15 min. The coverslip was rinsed with PBS/sodium azide, then with deionized water and mounted onto a glass slide with Fluoromount G.

### 3. Results and discussion

#### 3.1. Preparation of linear PEI scaffolds

Three-dimensional scaffolds were used to investigate cell–scaffold interactions and cell behavior. There are many factors to be considered in developing a scaffold such as porosity, surface properties and chemical composition. In this study a novel 3-D PEI scaffold was developed to support growth of NHF cells. To promote interaction with the negatively charged cell membrane, linear PEI was used because of its cationic charge density [35]. Until now, linear PEI has been used extensively for drug delivery and vectors in non-viral gene delivery [31]. Our initial studies were based on both branched and linear PEI. However, attempts to electrospin branched PEI were not successful. In contrast, linear PEI was electrospun to give a high surface area to volume scaffold. However, the linear PEI fibers themselves readily dissolved in PBS, rendering them unsuitable as a 3-D support. To retain the scaffold structure and to prevent dissolution a cross-linking agent, 1,4-diglycidyl ether, was added. The pH of the cell cultures with these scaffolds immediately became basic, even after changing the cell culture medium. This caused the cells to eventually die. From cell studies it became apparent that cross-linking alone was insufficient for maintaining cell viability and that the chemical composition needed to be modified to make the scaffold compatible for cell growth. Polyethyleneimines are weak bases with  $pK_{a,s}$  of their conjugate acids typically around 7. In order to tune the cationic charge density of the scaffold, the linear PEI was treated with succinic anhydride. This converts some of the amines to amides, thus decreasing the overall basicity of the scaffold.

Electrospun linear PEI with 20 wt.% of succinic anhydride revealed a porous structure consisting of a 3-D network of fibers. The morphological variation of the fibers may be due to viscosity of the polymer gel and the applied potential. The effect of the applied potential on the morphology and size of the electrospun fiber mesh was investigated. All other electrospinning parameters, including the distance between the needle tip and the collector, solution flow rates, and syringe and needle diameters were held constant. Linear PEI was electrospun under applied potentials

of 16, 18 and 20 kV over a collection distance of 10 cm. Electrospinning of linear PEI scaffolds was carried out at a viscosity of 1620 cP measured by a Brookfield Viscometer. Smooth fibers without the presence of beads were obtained. The average diameters of the obtained electrospun fibers were  $1143 \pm 255 \text{ nm}$  (16 kV),  $687 \pm 71 \text{ nm}$  (18 kV) and  $462 \pm 158 \text{ nm}$  (20 kV), as determined from by SEM from top-down views.

Cell growth was affected by the thickness of the 3-D scaffold. Fig. 2A shows a dense fiber mat that was used in early studies. Linear PEI was electrospun with 1,4-butanediol diglycidylether. Under cell media, the fiber diameter swelled to  $2.4 \pm 0.5 \mu\text{m}$ , thus nearly closing the pores. Also the pH of the media became too basic. One possible explanation is that organic matter can leach out of the scaffold. This surface morphology did not allow the cells to penetrate into the inner areas when the cell suspension was seeded on the scaffold. A membrane with less space between the fibers has a detrimental effect on cell migration in the cell growth on the surface of the scaffold

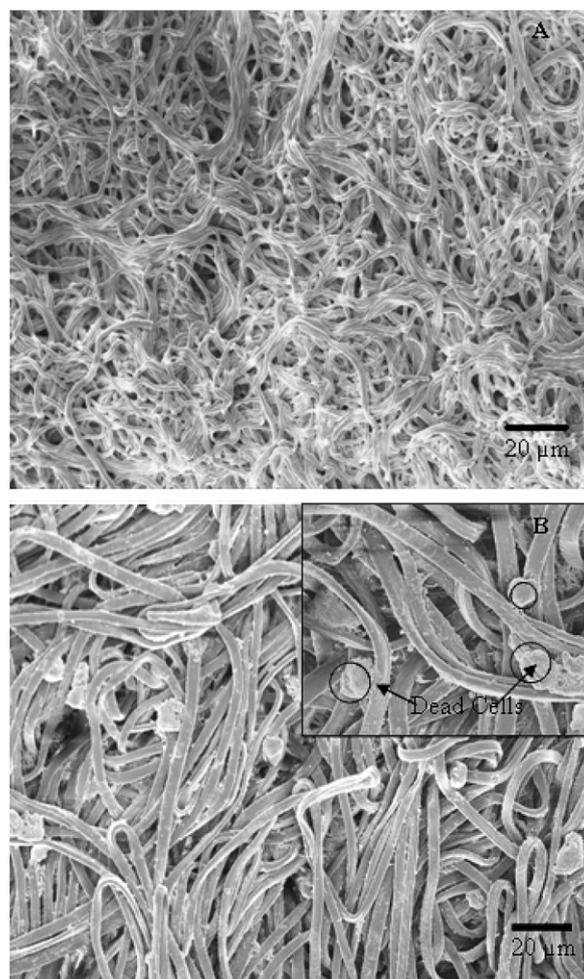


Fig. 2. SEM images of electrospun linear PEI scaffold without succinic anhydride and 1,4-butanediol diglycidyl ether: (A) thick fiber mesh before normal rat kidney cells seeding and (B) sample after 2 days of incubation (inset from a larger section of B).

[4]. The cells did not get enough nutrients and oxygen to survive under this condition. NHF cells were not able to attach and grow in the scaffold in Fig. 2A. Fig. 2B shows rounded up dead cells throughout the dense scaffold, further supporting this theory. With a small pore size, the cells were not able to deposit deep into the scaffold and effective mass transfer between the cells did not occur.

Electrospinning under higher voltages did not produce uniform fibers. At 20 kV individual fibers have a flattened appearance (Fig. 3A). This indicates a decrease in the fiber diameters with increasing applied potential, as seen in Fig. 3B. This could be a result of the increase in the transport rate of the charged jet from the nozzle by the electrostatic force acting on it. The thickness of the fiber mesh was also affected by varying the electric potential and deposition time. After 15 min of deposition time at 20 kV the electrospun scaffold thickness was 49  $\mu\text{m}$  (Fig. 3B, inset).

Electrospinning can produce PEI fibrous mats with pore diameters varying from several microns to several hundred microns. At 18 kV the average diameter of the electrospun PEI fibers was  $687 \pm 71$  nm, as shown in Fig. 4A. From a top-down SEM image, the average pore diameter was  $16.77 \pm 9.16$   $\mu\text{m}$ , compared with the average NHF cell diameter of 20.3  $\mu\text{m}$  [41]. Thus the spaces between fibers shown in Fig. 4B are ideally sized to allow NHF cells to migrate within the scaffold. The thickness of the scaffold

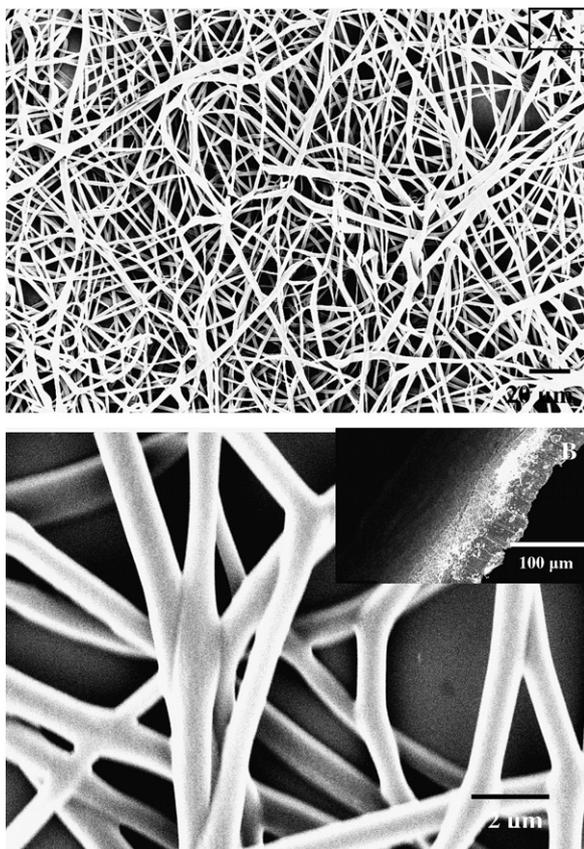


Fig. 3. SEM images of electrospun linear PEI scaffolds: (A) fiber mesh and (B) fiber diameter (inset is a cross-section of the scaffold) at 20 kV.

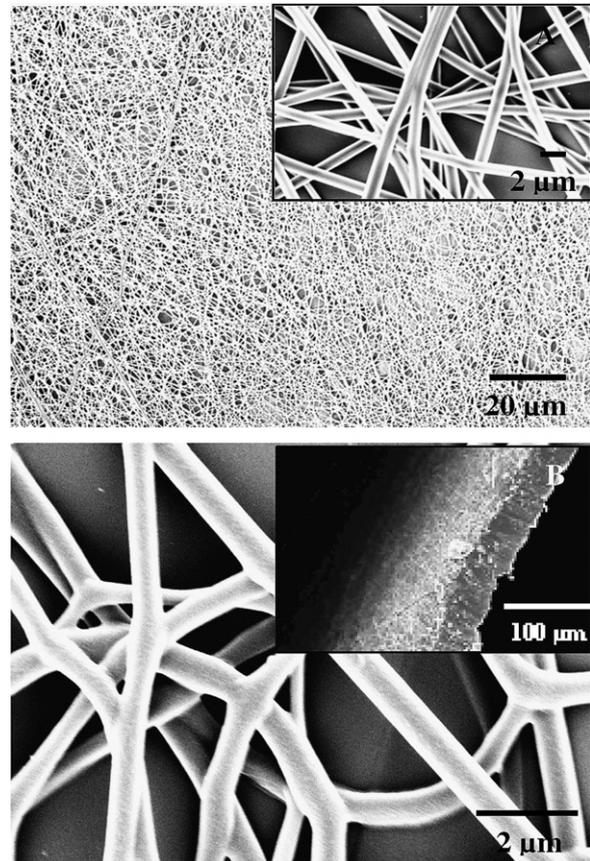


Fig. 4. SEM images of electrospun linear PEI scaffolds: (A) fiber mesh (inset fiber diameter) and (B) pore sizes (inset is the cross-section of the scaffold) at 18 kV.

electrospun at 18 kV was 30.94  $\mu\text{m}$  (Fig. 4B, inset) after 10 min of deposition.

### 3.2. Scaffold characterization

The linear PEI scaffold obtained via electrospinning contains both secondary and tertiary amines, as well as a lesser amount of terminal primary amines (Fig. 1). Based on the stoichiometry, the maximum degree of cross-linking is 26.7% (see Table 1). Additionally, only 8.1% of the PEI should be functionalized with succinic anhydride. However, to better evaluate the composition of the fiber surfaces, the scaffolds were investigated via FTIR. In Fig. 5, a strong peak at  $3430\text{ cm}^{-1}$  is indicative of N–H groups [42]. The C–H stretches were observed at 2919 and  $2847\text{ cm}^{-1}$ . The C=O peak is at  $1729\text{ cm}^{-1}$  [43]. The amide

Table 1

Per cent of primary amines, secondary amines and carboxylates in electrospun linear PEI scaffold

Chemical functionality	Theoretical (mol%)	Experimental (mol%)
PEI/SA/diglycidyl ether (amines)	26.7	25.2
PEI/SA/diglycidyl ether (carboxylates)	8.6	3.1
PEI/diglycidyl ether (amines)	18.1	17.2

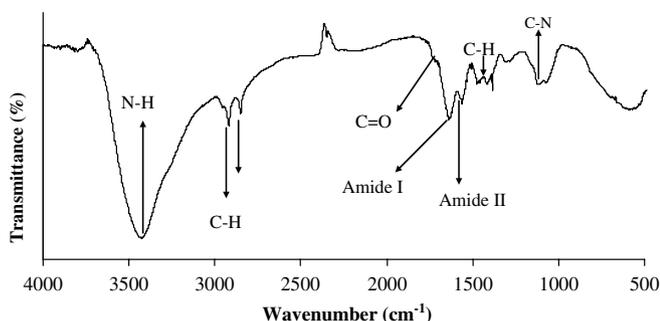


Fig. 5. FTIR-ATR of linear PEI/succinic anhydride with 1,4-butanediol diglycidyl ether scaffold.

I [42] peak is at  $1648\text{ cm}^{-1}$ , and  $1552\text{ cm}^{-1}$  corresponds with the amide II peak. These results are consistent with the structure in Fig. 1.

### 3.3. Determination of the degree of cross-linking

A colorimetric determination of reactive amino groups in the PEI scaffold was determined using Traut's and Ellman's reagents via ultraviolet–visible spectroscopy [39,40]. The experimentally determined degree of cross-linking of the amines was 25.2%, while the theoretical maximum was 26.7% (see Table 1 for calculations). The exposed carboxyl groups in the linear PEI fibers can be quantified via a similar method [40]. The amount of acces-

sible carboxylate groups in the electrospun linear PEI scaffold was determined to be 39.5% of the theoretical amount, assuming all of the succinic anhydride reacted.

### 3.4. Cell viability

For skin scaffolds, both surface compatibility and a 3-D porous structure to support cell attachment and growth are needed [44]. A cell viability study was used to assess potential cytotoxic cellular interactions by staining the scaffold with FDA, PI and DAPI. DAPI forms fluorescent products upon binding to DNA in the nucleus. This fluorescent probe can be used with Alexa 488 and other fluorescent probes to differentiate organelles in the cell. Fluorescein diacetate is a non-polar dye which passes through the cell membrane. Once inside the cell, intracellular esterases cleave the diacetate group to produce a fluorescent green product [44]. Therefore, the product fluorescein accumulates in those cells that have intact cell membranes. Cells that do not have an intact membrane cannot accumulate fluorescein and do not exhibit green fluorescence. PI is a non-permeant dye which can penetrate the membranes of dead or dying cells. It intercalates into the major groove of the DNA and produces highly fluorescent red adducts in non-viable cells.

In order to assess cell–scaffold interactions, the normal human fibroblasts were seeded on electrospun linear PEI for 5 days (see Fig. 6). A live/dead assay (Fig. 6) showed

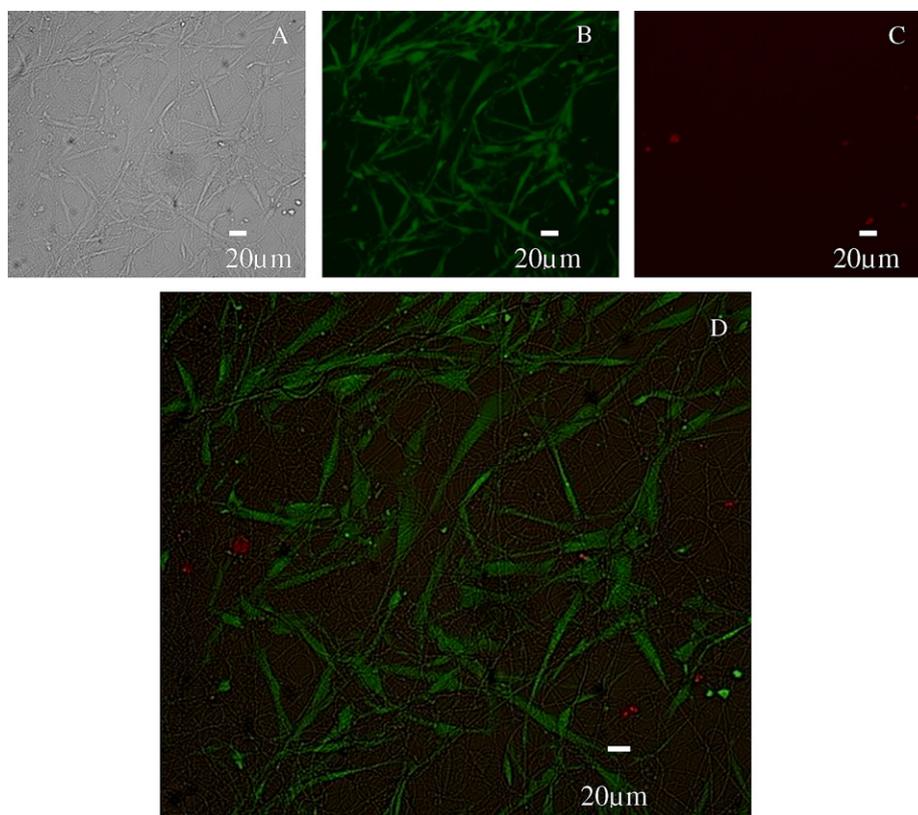


Fig. 6. Five days of culture: (A) differential interference contrast image (DIC) of NHF attached to linear PEI scaffold; (B) fluorescence microscopy image of FDA stained live normal human fibroblasts cells; (C) PI stained dead cells and (D) overlay image of B and C.

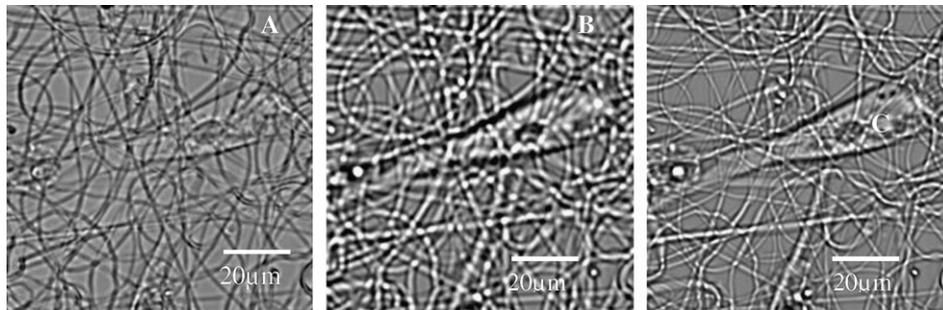


Fig. 7. Differential interference contrast images of (A) top view of normal human fibroblast and (B) top view of the 3-D scaffold where the cell is embedded inside pores focused on the fibers (5 days of culture), (C) focus on the NHF cells.

that NHF cells were able to adhere to the surface of the electrospun scaffold with excellent viability. This suggests linear PEI scaffolds were non-cytotoxic and inductive for cell growth. Fig. 6 also demonstrates the representative cell morphology on the linear PEI scaffold. Differential interference contrast (DIC) or Nomarski imaging is a modification phase microscopy. The DIC image taken by light microscopy reveals growth of NHF cells on the scaffold, as shown in Fig. 6A. The NHF cells adhered to the surface of the scaffold and maintained their lamellar shape, and were about 20.3  $\mu\text{m}$  in size (Fig. 6A) [41]. A fluorescence microscopy image of FDA stained live cells is shown in Fig. 6B. Almost all of the NHF cells were green,<sup>1</sup> indicating the cells are live. Fig. 6C shows a fluorescence microscopy image of PI stained dead cells. In the overlay image (Fig. 6D) the lack of PI stained dead cells supports the idea that normal human fibroblasts have attached to the scaffold and that the majority of the cells were viable. Even after 120 h, NHF cells can be seen growing on the PEI scaffold.

To determine the cell adhesion of NHF cells on with electrospun linear PEI scaffold, in depth DIC images were taken by fluorescence microscopy. Cells grew in an organized fashion and aligned themselves in close contact along the fibers (Fig. 7). The robust, porous PEI scaffold allowed the media to support cell growth for at least 5 days. The differential contrast image in Fig. 7 shows a cell clinging to individual fibers. Additionally, the NHF cells are growing not just on the scaffold but in between the fibers. Fig. 7B shows the top view of electrospun linear PEI scaffold and a NHF cell. Fig. 7C is focused on the normal human fibroblast cell. The internal structures of the NHF are also clearly visible in this image.

### 3.5. Cell growth daily intervals comparison

To assess the effectiveness of the PEI scaffold at promoting cell growth, NHF cells were seeded and the daily growth was monitored. The data were compared with a parallel control group. The viable cells were counted

between 24 and 140 h of incubation time. The cell viability was quantified by counting the numbers of live and dead cells from five randomly chosen areas. Fig. 8 shows the normalized cell growth curve for cells grown on the linear PEI scaffold and as well as the control cells. After 24 and 72 h, the number of viable cells on the scaffold was similar to the control. After 120 h, cells continued to grow on the scaffold. Student's *t*-test was performed on the data and the calculated *t* value of the data was 6.15 ( $t_{\text{crit}} = 2.13$ ), which allows us to reject the null hypothesis of no variance between the control group and the scaffold. Importantly, there was statistically significant enhanced growth of NHF cells on the scaffold. The enhanced growth could be attributed to succinic anhydride-decorated PEI, which improved the suitability of the surface for the cells. The cell population doubling time (PDT), i.e. the time it takes for the cell number to double, may be calculated from the formula:

$$dt = t \ln 2 / \ln(N/N_0)$$

where *dt* is the cell doubling time, *ln* is the natural log of the number, *N* is the final cell number, *N*<sub>0</sub> is the initial cell number and *t* is the time interval between *N*<sub>0</sub> and *N*. For the control study we obtain a cell doubling time of 71 h, and for the scaffolds the PDT was 74 h. The NHF cells grew at a similar rate in the PEI scaffolds compared with in the medium alone, which means that linear PEI not only

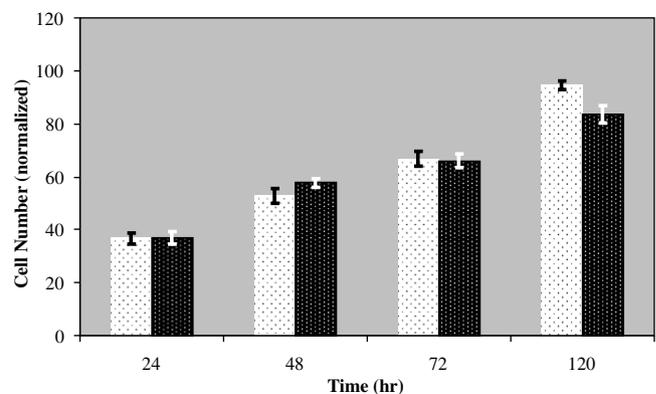


Fig. 8. Growth curve for electrospun linear PEI scaffolds (□) and control (■).

<sup>1</sup> For interpretation of color in Fig. 6, the reader is referred to the web version of this article.

allowed the cells to grow but also showed no toxicity over 120 h.

### 3.6. Fixed cell morphology

The cell morphology on the scaffold was imaged using scanning electron microscopy. As seen in Fig. 9, the fixed normal human fibroblast cells cultured on the L-PEI/succinic anhydride scaffold were attached to the fibers. The cells were spread throughout the scaffold. This is further evidenced by their pseudopodia growing along the fibers. From these images we can see these fibers closely mimic native ECM in their ability to promote cell growth.

### 3.7. Immunofluorescence with fixed cells on L-PEI/succinic anhydride scaffold

Normal human fibroblasts were fixed according to the procedure described in the experimental section. NHF cells were fixed to localize specific structures within a cell. The primary antibody (rabbit anti-Grp78; also known as BiP) was added to the cells attached to the scaffold. BiP is an endoplasmic reticulum heat shock protein. This chaperone participates in protein folding in the endoplasmic reticulum. A secondary antibody, Alexa 488 goat anti-rabbit

(fluorescent marker), was added to detect the primary antibody. Nuclei were stained with DAPI. From Fig. 10 the morphology of the normal human fibroblasts cells is clearly visible. The cells exhibit their normal phenotypic shape, indicating that the cells are spread apart. These results show that linear PEI scaffold can promote growth for this particular cell line. Additionally, the cells are adhering to the fibers and spread throughout the mesh well after 5 days of cell culture (Fig. 10A). This evidence indicates that linear PEI promotes cell–scaffold interactions. This study demonstrates that novel electrospun PEI fibers function as scaffolds and may be used for soft tissues such as a skin replacement in vivo.

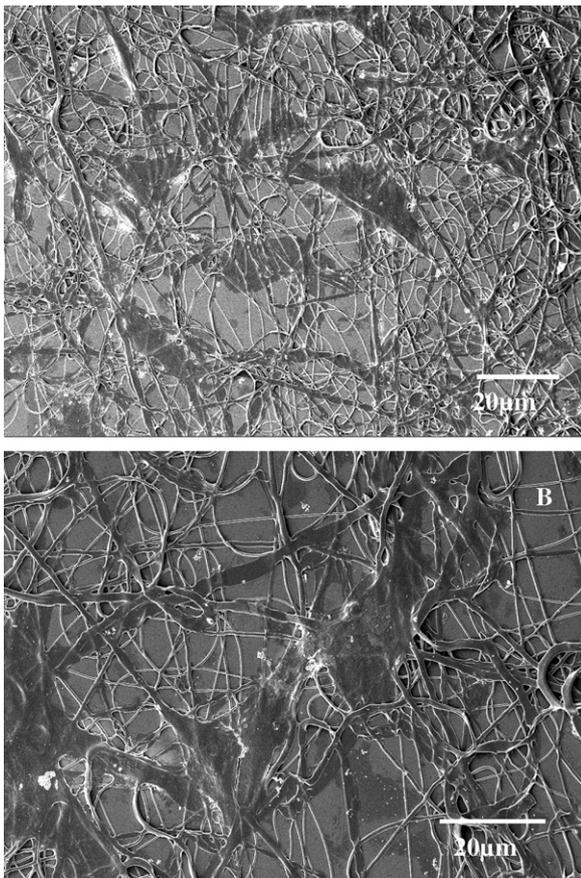


Fig. 9. SEM images of electrospun linear PEI scaffolds and fixed NHF cells after 5 days of culture (A and B are different areas).

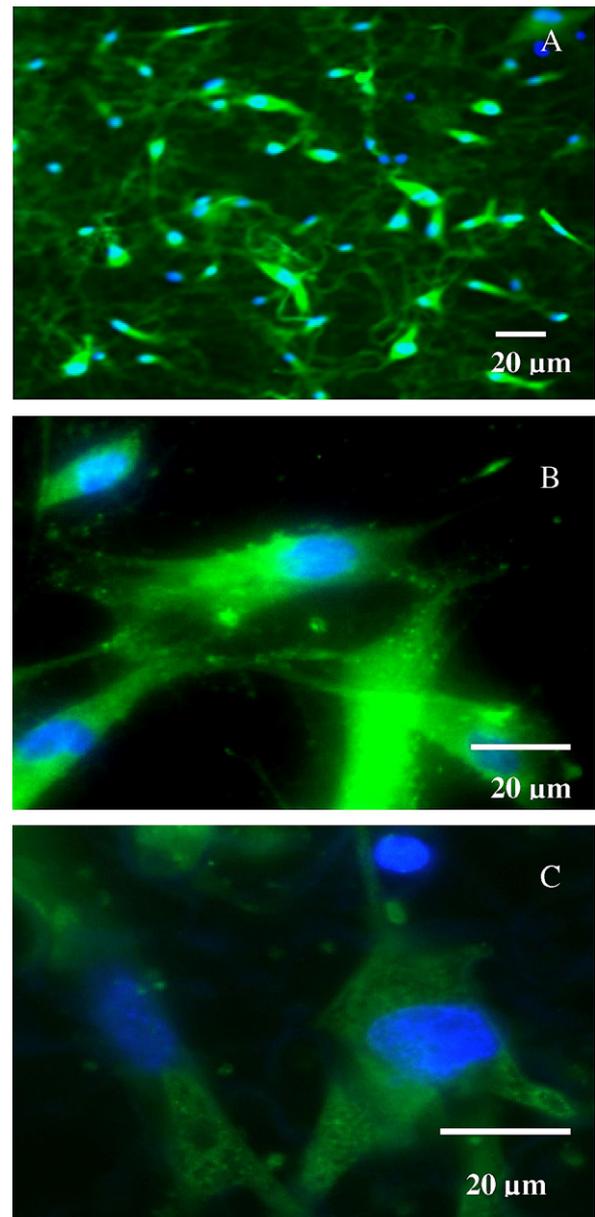


Fig. 10. Fluorescence microscopy images of fixed NHF cells on linear PEI scaffolds, with rabbit anti-GRP 78 conjugated with Alexa 488 goat anti-rabbit (green)/DAPI (blue) stained cells at (A) 10, (B) 40 and (C) 60 magnifications.

#### 4. Conclusion

The use of electrospinning for biomaterials applications was reported by Martin and Cockshott in 1977 [45]. Electrospinning polymer scaffolds have been investigated only recently. Previous studies found that cells attach to nanometer diameter fibers [46]. The electrospinning technique provides a unique and efficient approach to fabricating biomimetic scaffolds with diameters in the nanometer range. In the present study, linear PEI scaffold supported the 3-D growth of NHF cells. We have developed and standardized the scaffold, which promotes the direct formation of new tissue. Results suggest that these scaffolds may be used for animal cell culture in vivo with excellent biocompatibility. The results obtained from both fluorescence and scanning electron microscopy indicate that cells were growing on the electrospun linear PEI scaffold. This novel structure with a high surface-area-to-volume ratio favors cell attachment by providing a 3-D extracellular environment similar to that found in native tissues. In conclusion, linear PEI scaffolds mimic the native extracellular matrix and thus can serve as a skin substitute. Future studies will involve engineering suitable scaffolds with electroactive components for cardiac tissue and artificial neurons.

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