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Research Article

In Vitro Biocompatibility of Electrospun Chitosan/Collagen Scaffold

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Chitosan/collagen composite nanofibrous scaffold has been greatly concerned in recent years for its favorable physicochemical properties which mimic the native extracellular matrix (ECM) both morphologically and chemically. In a previous study, we had successfully fabricated nanofibrous chitosan/collagen composite by electrospinning. In the present study, we further investigate the biocompatibility of such chitosan/collagen composite nanofiber to be used as scaffolds in vascular tissue engineering. The porcine iliac artery endothelial cells (PIECs) were employed for morphogenesis, attachment, proliferation, and phenotypic studies. Four characteristic EC markers, including two types of cell adhesion molecules, one proliferation molecule (PCNA), and one function molecule (p53), were studied by semiquantitative RT-PCR. Results showed that the chitosan/collagen composite nanofibrous scaffold could enhance the attachment, spreading, and proliferation of PIECs and preserve the EC phenotype. Our work provides profound proofs for the applicable potency of scaffolds made from chitosan/collagen composite nanofiber to be used in vascular tissue engineering.

1. Introduction

Vascular disease is the main cause of death in Western societies [1]. Coronary artery and peripheral vascular diseases are the leading causes of mortality, necessitating surgical interventions including small-diameter bypass grafting with autologous veins or arteries [2, 3]. However, adequate autologous vessels for bypass conduits are lacking in many patients. Compliance mismatch between veins and arteries contributes to myointimal hyperplasia, particularly at anastomotic sites, whilst thrombosis is a common problem. Thus, the need for compatible tissue-engineered small-diameter (<6 mm diameter) vascular grafts is great. Tissue-engineered vascular grafts require a compliant polymer scaffold to which endothelial cells (ECs) can adhere, form an antithrombogenic luminal surface [4], exhibit vasoactive properties, and improve patency [5, 6], and within which smooth muscle cells (SMCs) can migrate, deposit function vascular ECM, and become contractile. Compliance mismatch between vessel and graft scaffold must be minimized to limit intimal hyperplasia.

A recurring problem with artificial materials used for small-diameter bypass grafts is that they often fail to support strong ECs attachment and monolayer formation and are thus highly thrombogenic [4]. To address ECs attachment and function, scaffold materials have been coated with cell-adhesive proteins such as fibronectin, vitronectin, and laminin. However, such modifications can also provide good substrates for platelet adhesion, thrombus formation [7].

Chitosan (CS) is a naturally derived polysaccharide. It has gained much attention as a biomaterial in diverse tissue-engineering applications due to its low cost, large-scale availability, antimicrobial activity, biodegradability and biocompatibility [8]. Chitosan scaffolds with various geometries, pore sizes, and pore orientations can be obtained using controlled-rate freezing and lyophilization [9]. Chitosan films are brittle with a strain at break of 40–50% in the wet state [10].

Furthermore, chitosan degradation depends on the degree of deacetylation, local pH, and homogeneity of the source. Although the deacetylation and the homogeneity of source can be controlled during polymer processing to regulate biomechanical properties, the range is marginal.

For improving the mechanical and biological properties of chitosan over a broad range, blending with other polymers is widely investigated, since the native ECM is the complex of polysaccharide and polyprotein with nanofibrous porous structure. Collagen (Col) as the polyprotein has been widely used for tissue-engineering scaffolding, owing to having a wealth of merits such as biological origin, nonimmunogenicity, excellent biocompatibility, and biodegradability [11, 12]. Chitosan is a polysaccharide, which can form a polyelectrolyte complex with collagen. Thus, it is possible to stabilize collagen with chitosan, since chitosan can function as a bridge owing to the large number of aminogroups in its molecular chain. Chitosan collagen composite scaffolds have favorable physicochemical properties needed for many biomedical applications. In the current investigation collagen and chitosan are used to develop scaffolds in the form of interpenetrating polymeric network using glutaraldehyde as a crosslinking agent. It has also been reported that the hybrids of chitosan collagen manufactured by crosslinking, wet/dry spinning, and freeze-drying have biological and mechanical benefits to be used as the tissue-engineering scaffold [13, 14].

Recently, many researchers are trying to employ the electrospinning technique to fabricate microporous biodegradable or biocompatible polymer scaffolds which are generally composed of nanosized fibers [15]. Also, there are plenty of papers on the electrospinning of biocompatible polymer blends. The electrospinning of chitosan/collagen complex has been achieved to mimic the native ECM as we previously described [16].

In studying cell behavior on biomaterials, it is possible to recognize distinct chronological steps, which can be investigated individually in suitable *in vitro* evaluation [17]. A key parameter is cell adhesion, a process which involves molecular recognition of the proteins adsorbed on the biomaterial surface by specific receptors in the plasma membrane (cell adhesion molecules). If cell colonization of a biomaterial is a central aim, cell proliferation ranks among the key parameters which can be assayed. Nevertheless, a well-colonized biomaterial matrix is no guarantee of success, as it must be demonstrated that these cells have the desired synthetic functions (i.e., physiological phenotype). Thus, evaluation of cell function is therefore a vital part of relevant testing scheme.

The aim of this study was to evaluate the biocompatibility of electrospun chitosan/collagen complex as scaffold in vascular tissue engineering to support cell adhesion, proliferation, and phenotypic expression of endothelial cell markers by PIECs *in vitro*.

2. Materials and Methods

2.1. Materials. Chitosan ($M\eta \approx 10^6$, minimum deacetylation degree of 85%) was obtained from Jinan Haidebei Marine Bioengineering Co., Ltd (China). Collagen I (mol. wt, 0.8~1 $\times$ 10^5 Da) was purchased from Sichuan Mingrang Bio-Tech Co., Ltd (China). Two kinds of electrospinning solvents, 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and trifluoroacetic acid (TFA), were purchased from Fluorochem Ltd. (United Kingdom) and Sinopharm Chemical Reagent Co., Ltd. 0.25% trypsin-EDTA and Dulbecco's modification of Eagle's medium (DMEM) culture medium were purchased from Genom BioMed Technology lnc. Fetal bovine serum (FBS), penicillin/streptomycin, and TRIZOL were obtained from Invitrogen Corporation. Glutaraldehyde, ethyl alcohol absolute, dimethyl sulfoxide, and chloroform were also purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Scaffold Electrospinning and Stabilizing. Chitosan/collagen composite scaffolds were prepared using different ratios of chitosan and collagen as we have previously described [16]. Briefly, collagen was dissolved in HFP and chitosan in HFP/TFA mixture (v/v, 80/20) at a concentration of 8% and 10% (w/v), respectively. The electrospinning experiments were performed at room temperature. The polymer solution was placed into a 1 mL syringe with a needle having an inner diameter of 0.4 mm. A clamp connected with high voltage power supplier, which can supply positive voltage from 0 to 80 kV, was attached to the needle. A piece of aluminum foil was placed below the needle at the distance of 12 cm as grounded collector. The polymer jets were generated from the needle by high voltage field to the grounded collector and formed the nanofiber mesh on coverslip for morphological and *in vitro* cellular analysis. The applied voltage and flow rate of the solution were fixed at 20 kV and 0.8 mL/h, respectively.

The electrospun scaffolds were divided into five groups: group 1, the pure chitosan scaffold group (CS); group 2, the composite chitosan/collagen (w/w, 80/20) scaffold group (CS8Col2); group 3, the composite chitosan/collagen (w/w, 50/50) scaffold group (CS5Col5); group 4, the composite chitosan/collagen (w/w, 20/80) scaffold group (CS2Col8); group 5, the pure collagen scaffold group (Col).

To maintain nanofiber morphology in wet stage, the electrospun scaffolds were stabilized by glutaraldehyde vapor from a 25% glutaraldehyde aqueous solution in vacuum pump for 12 h at room temperature.

2.3. Cell Culture and Seeding. PIECs were purchased from the Chinese Academy of Sciences Cell Bank of Type Culture Collection (Shanghai, China) and cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained in a humidified incubator at 37°C, 5% CO_2 atmosphere.

Cell seedings were performed in 24-well tissue culture plates (Corning Incorporated, USA), and electrospun chitosan/collagen scaffolds were placed in the center of the wells. Scaffolds were neutralized in 75% ethanol and washed with PBS three times. To achieve uniform distribution of cells, the sterilized scaffolds described previously were incubated with 0.1 mL growth medium in an incubator at 37°C overnight. 200 μL cell suspension of desired seeding number was evenly introduced into each well, and the cell-seeded scaffolds were cultured under a 5% CO_2 atmosphere at 37°C allowing attachment of cells to scaffolds. Then, the medium was added, 200 μL per well for further culture. The medium had to be

sucked off before each cell assay. In addition, medium was changed daily.

2.4. Morphological Analysis. The morphologies of the stabilized electrospun chitosan/collagen scaffolds were monitored using scanning electron microscope (SEM) at an accelerating voltage of 10–15 kV.

For cell morphologies, ECs were seeded at a density of 1×10^4/mL and cultured under a 5% CO_2 atmosphere at 37°C for 3 days. Then, ECs were fixed with 2.5% glutaraldehyde for 10 min, immersed in PBS for 10 min, washed three times, and dehydrated in successive ethanol baths (50%, 70%, 80%, 90%, and 100% in sequence for 10 min per step), followed by vacuum freeze-drying. Scaffolds were sputter-coated with gold at 40 mA prior to observing under scanning electron microscope.

2.5. Cell Adhesion Studies. The cellular activity of PIECs adhered and grown on the cultured scaffolds was estimated by a modified MTT assay [18]. In brief, ECs were seeded at a density of 1×10^5/mL, and unattached cells were warily removed by washing with PBS at different time points. In succession, 400 μL serum-free medium and 40 μL MTT solution (5 mg/mL in PBS) were added to each sample, followed by incubation at 37°C for 4 h for MTT formazan formation. The supernatant was carefully discarded, and 400 μL DMSO was added to the sample to dissolve the formazan crystals. The formazan dissolution was transferred to a 96-well plate after solubilization, and the absorbance at 490 nm was read on a microplate reader (Thermo, USA) versus untreated coverslip control. The value obtained is proportional to the number of adhered cells in each well.

2.6. Cell Proliferation Studies. Cell proliferation was quantified by MTT assay following the above-mentioned protocol. Briefly, PIECs were seeded following the procedure described for cell culture and seeding. A point distinguished from the attachment assay was the less cell seeding density. ECs at a density of 1×10^4/mL were seeded on the scaffolds in 24-well tissue culture plates and cultured for 1, 3, 5, and 7 days. Cells were incubated for 4 h with MTT reagent for formazan formation. The formazan dissolution was transferred to a 96-well plate and measured at 490 nm versus untreated coverslip control. The values obtained reflect the proliferated cell population in each well.

2.7. Semiquantitative RT-PCR Analysis. PIECs were seeded on scaffolds at the density of 1×10^5/mL in 24-well cell culture plates and incubated for 24 h at 37°C, 5% CO_2 atmosphere. Expression of a series of endothelial cell marker genes selected was examined by RT-PCR. The total RNA was prepared by isolation reagent TRIzol according to the manufacturer's instructions. One microgram of total RNA was used as template for the synthesis of cDNA with oligo-dT and AMV reverse transcriptase. Beta-2-microglobulin (B2M) primers were used in order to standardize transcription. The PCR products were electrophoresed on a 2% (w/v) agarose gel visualized by attaining with ethidium bromide, and analyzed densitometrically using Quantity One software. The relative levels of mRNA expression were quantified by comparison to the internal control (B2M). Each sample was run in triplicate.

The following primers for RT-PCR were designed using Primer3web (http://primer3.wi.mit.edu/):

ICAM-1 (forward, 5′-CGGGAGATTACAGGCACCTACCA-3′; reverse, 5′-ACCCTGAATGAGATTAGACTGAGAAGCA-3′);

VCAM-1 (forward, 5′-TGCCTATGCCCTTGCGGTG-3′; reverse, 5′-CCCAGTTCTTTCTTGGTTTATCATTTGC-3′);

PCNA (forward, 5′-GACATCATTACGCTAAGGGCAGAAGA-3′; reverse, 5′-TCTCTATGGTAACTGCTTCCTCCTCT-3′);

p53 (forward, 5′-ACCGATGTTCAAGAGAGAAGGACC-3′; reverse, 5′-GGGCTTCCGACCCAGTGTATG-3′);

B2M (forward, 5′-CCATCCGCCCCAGATTGAAA-3′; reverse, 5′-TCTCTGTGATGCCGGTTAGTGGTC-3′).

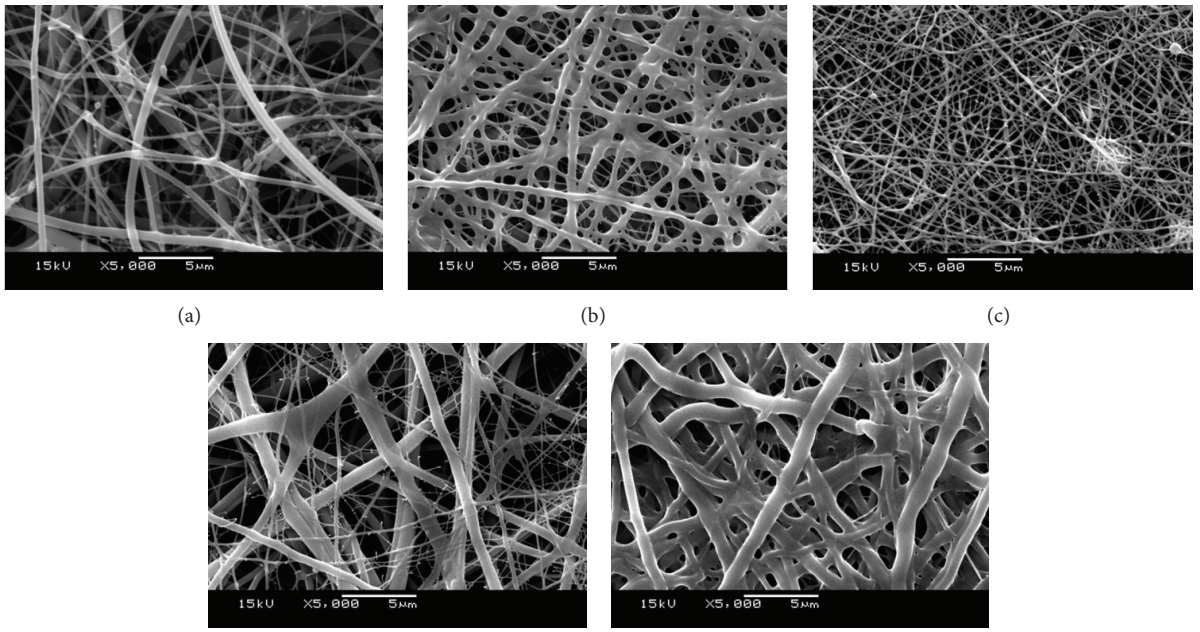
2.8. Statistical Analysis. The data are shown as mean ± S.E.M. Differences between experimental results were evaluated according to a one-way analysis of variance (ANOVA), with $P < 0.05$ considered statistically significant. All experiments were performed in triplicate.

3. Results

3.1. Stabilizing Chitosan/Collagen Scaffolds. Uncrossed-linked scaffolds were analyzed for their dimensional and structural stability in wet stage. After a few minutes, the structure collapsed. In initial experiments, the scaffolds were stabilized by cross-linking with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS); physical ultraviolet irradiation has been used. Results exhibited that the scaffolds could not keep their dimensional and structural stability in wet stage. While glutaraldehyde (GA) has been wildly used as cross-linking agent [19], the electrospun scaffolds were stabilized by cross-linking with GA vapor for 12 h at room temperature. For the purpose of purifying the residual GA to maximum extent, a further washing step was performed with PBS. The total incubation time was 2 h, during which PBS was changed every 30 min. Scanning electron microscope (SEM) analysis showed the morphology of the cross-linked chitosan/collagen scaffolds (Figure 1). The stabilized scaffolds could keep their dimensional and structural stability in wet stage. Hence, scaffolds were stabilized by cross-linking with glutaraldehyde in all subsequent experiments.

3.2. Morphology of PIECs on Nanofibrous Scaffolds. Cell morphology, phenotype and the interaction between the PIECs and the chitosan/collagen nanofibrous scaffolds were studied by SEM.

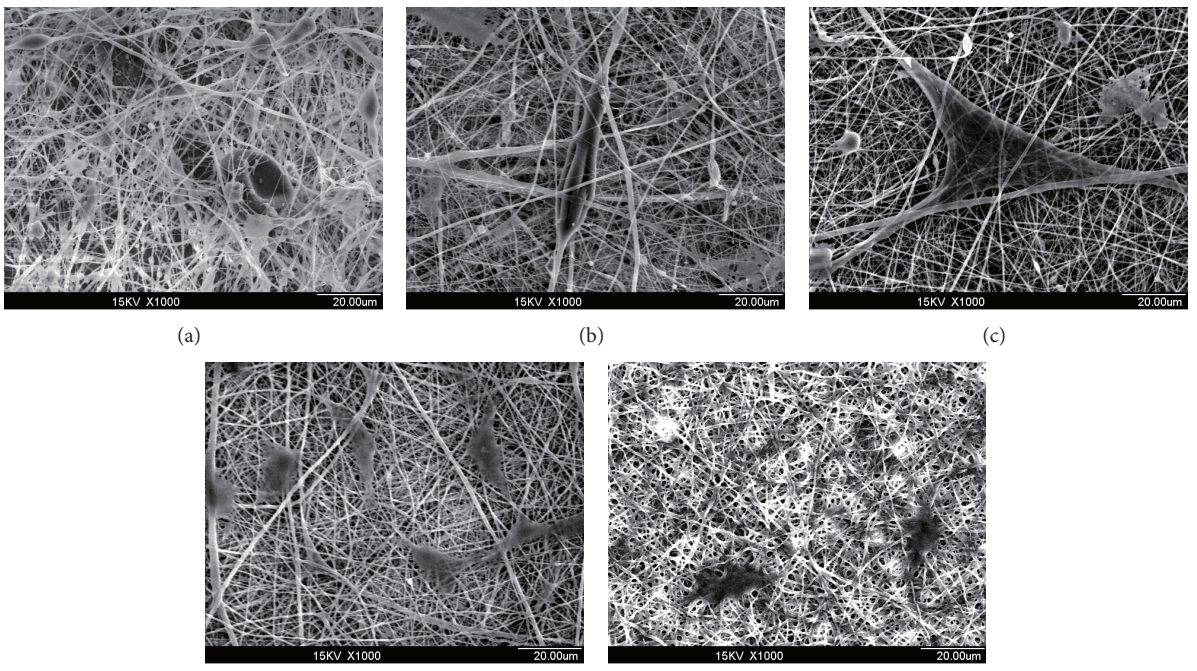
It can be seen that cells extended along the nanofibers with a spindle-shaped appearance. Cells grew to various depths in the fiber meshes. Figure 2 shows the cell-cell and



(a) (b) (c)

(d) (e)

Figure 1: SEM micrographs of the stabilized electrospun fibers of chitosan/collagen nanofibrous scaffolds prepared using different ratios of chitosan and collagen. (a) CS, (b) CS8Col2, (c) CS5Col5, (d) CS2Col8, and (e) Col. Original magnification is ×5,000, and the scale bar = 5 μm.



(a) (b) (c)

(d) (e)

Figure 2: Cell morphology, phenotype, and the interaction between the PIECs and the chitosan/collagen nanofibrous scaffolds prepared using different ratios of chitosan and collagen. (a) CS, (b) CS8Col2, (c) CS5Col5, (d) CS2Col8, and (e) Col. Original magnification is ×1,000, and the scale bar = 20 μm.

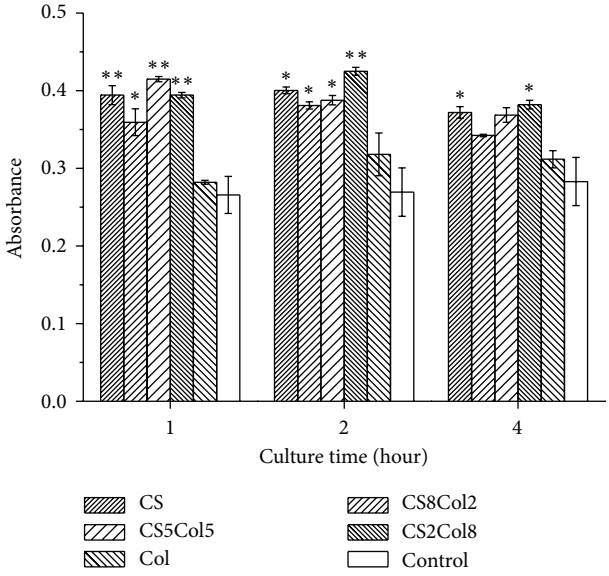


Figure 3: Adhesion profile of PIECs to untreated coverslip control and electrospun chitosan/collagen nanofibrous scaffolds prepared using different ratios of chitosan and collagen. Data were expressed as absorbance values at 490 nm ± S.E.M. of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ versus untreated coverslip control.

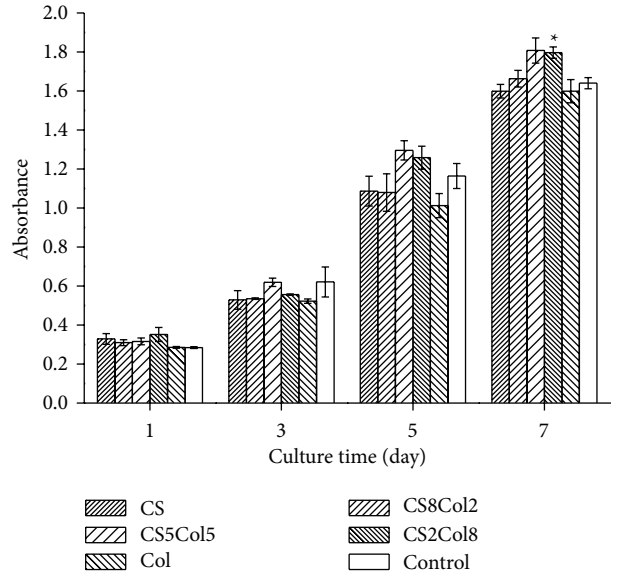


Figure 4: PIECs proliferation on untreated coverslip control and electrospun chitosan/collagen nanofibrous scaffolds prepared using different ratios of chitosan and collagen. Data were expressed as absorbance value at 490 nm ± S.E.M. of three independent experiments. $^{*}P < 0.05$ versus untreated coverslip control.

cell-matrix interactions. It can be seen typically that the elongated cell body pulls and seizes the nanofibers (Figures 2(b), 2(c), and 2(e)), and a "bridge" is formed between the cell body (Figure 2(d)). In addition, cells on pure chitosan scaffold (Figure 2(a)) showed a bit circular morphology, similar to other reports [20].

3.3. Cell Adhesion Studies. The first hours of contact between cells and materials are critical, since it is well known that for anchorage dependent cells, adhesion to a substrate has to occur within few hours; otherwise, the cells will lose their viability [21]. In addition to anchoring cells, adhesive interactions activate various intracellular signaling pathways that direct cell viability, proliferation, and differentiation [22, 23]. Thus, to establish the ability of a biomaterial to serve as a substrate for cell culture, the adhesion efficiency of cells needs to be evaluated prior to the establishment of the long-term culture system.

In this work, we evaluated the adhesion of ECs to electrospun chitosan/collagen scaffolds, and Figure 3 presents the results compared to cell adhesion to coverslip control without surface treatment. ECs are attached to all the substrates over the 4 h period. By 1 h, the pure chitosan and chitosan/collagen (w/w; 50/50, 20/80) supported the approximate cell adhesion, and the pure collagen and untreated coverslip control supported the least. By 2 h, the number of ECs adhered to chitosan/collagen scaffold (w/w; 80/20) caught up with the number of adherent cells on the ratio of 50 to 50 scaffold and the pure chitosan. The scaffold (w/w; 20/80) had the most adherent cells, and difference was found between untreated coverslip control and the substrates except pure collagen. By the time of 4 h after seeding, the number of cells that attached to the substrate decreased slightly. When multiple comparisons were made among the substrates, a significant difference was detected between chitosan/collagen (w/w; 20/80) and pure collagen, chitosan/collagen (w/w; 80/20), and coverslip control, respectively. However, there was no significant difference when comparing chitosan/collagen (w/w; 20/80) with two other scaffolds.

The difference in adherence values probably reflects the variation in material properties, in terms of chemistry, surface charges, reactive groups, and roughness.

3.4. Cell Proliferation Studies. PIECs proliferation on the six substrates described above was studied at days 1, 3, 5, and 7 with the results shown in Figure 4. The increasing number of cells on the six substrates during the culture period demonstrated that proliferation occurred on all the substrates. When comparing chitosan/collagen (w/w; 50/50, 20/80) with other four substrates on day 7, significantly more cells were found on the chitosan/collagen (w/w; 20/80) than the other four ($P < 0.05$). There was no statistically significant difference in the cell numbers proliferated during this period between pure chitosan, chitosan/collagen (w/w; 80/20), pure collagen, and untreated coverslip control.

3.5. Molecular Analysis of Endothelial Cell Gene Expression. The regulation of endothelial cell gene expression on the molecular level was examined for cells growing on electrospun chitosan/collagen scaffolds for 24 h by semiquantitative RT-PCR. The role of selected genes was classified as adhesion molecules (ICAM-1 and VCAM-1), proliferation molecule (PCNA), and function molecules p53.

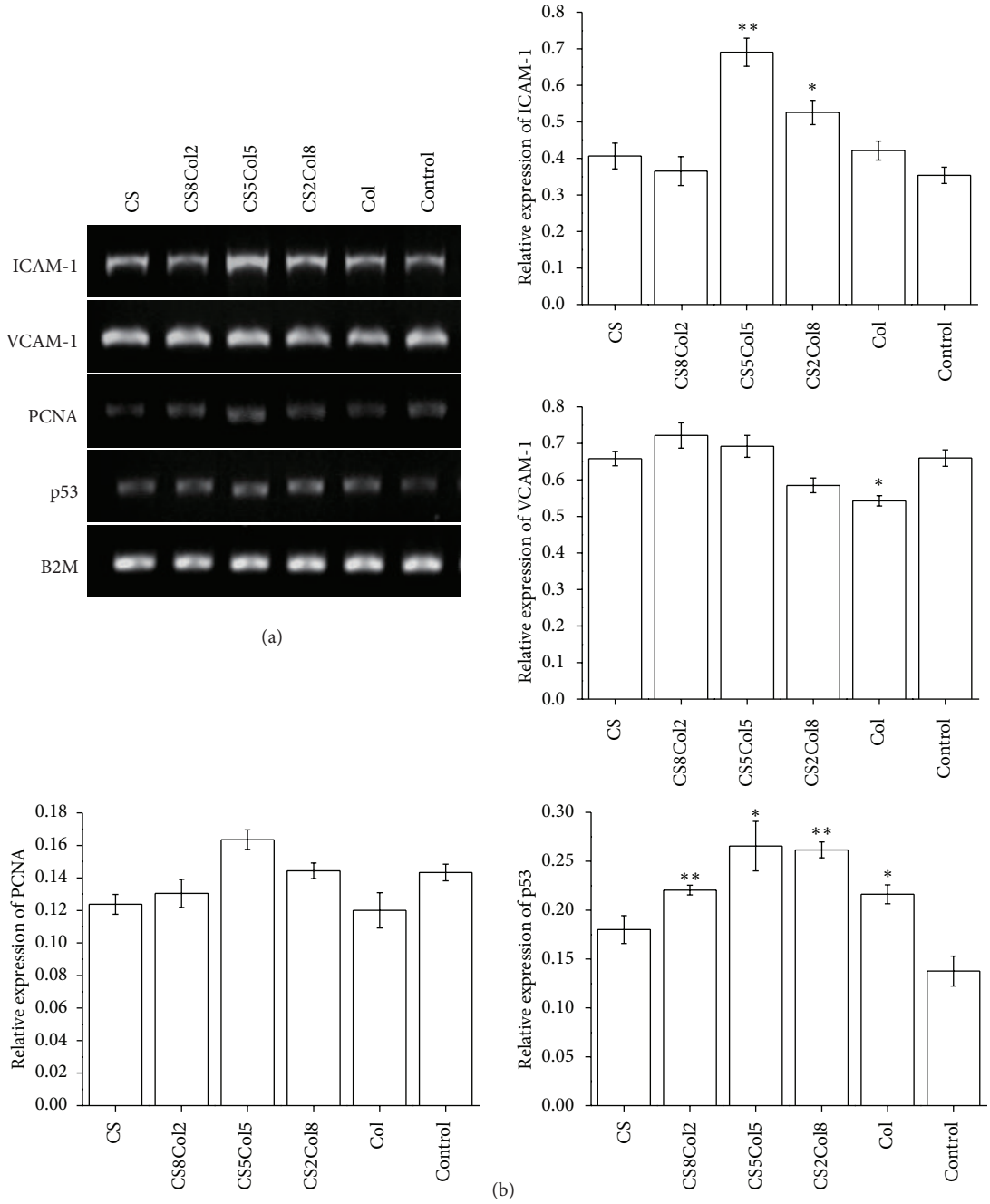


Figure 5: Semiquantitative mRNA expression of PIECs growing on untreated coverslip control and electrospun chitosan/collagen nanofibrous scaffolds prepared using different ratios of chitosan and collagen. (a) A typical agarose gel showing the products of 873 bp ICAM-1, 424 bp VCAM-1, 339 bp PCNA, 430 bp p53, and 222 bp B2M (internal control) bands. (b) The mRNA levels were quantitated semiquantitatively by Quantity One software according to three independent experiments, and B2 M mRNA was used as an internal control. The densitometric values of ICAM-1, VCAM-1, PCNA, and p53 were normalized with that of B2M, respectively, and the relative amount was presented as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ versus untreated coverslip control.

As can be seen in Figure 5, cells growing on electrospun chitosan/collagen scaffolds exhibited gene expression for adhesion molecules, both ICAM-1 and VCAM-1. In addition, chitosan/collagen (w/w; 50/50, 20/80) upregulated ICAM-1, and collagen downregulated VCAM-1 compared with coverslip control, respectively. Although PCNA was also expressed on all the substrates, no statistically significant differences were found when compared with coverslip control. The p53 tumor suppressor gene, known for encoding a nuclear phosphoprotein with cancer-inhibiting properties, was induced on scaffolds as well as on coverslip control. Furthermore, the overall tendency of the p53 mRNA expression on all

the substrates except chitosan was significantly upregulated compared with control.

4. Discussion

After implantation of a biomaterial, a neovascularization process begins with the formation and outgrowth of microvasculature from the host tissue. For this reason, the ability of a tissue-engineering scaffold to elicit an appropriate response from the host ECs is crucial for a successful vascularization of the implant. We have formerly described the electrospun chitosan/collagen scaffold as a biomaterial to mimic the native ECM and to develop functional biomaterials. Previous work has shown that this is an excellent scaffolding material. A successful implant not only requires the adhesion, growth and proliferation of the cells for a functioning tissue or organ replacement, but also needs an intact vasculature to supply these cells with oxygen and nutrients and also to remove metabolites. Therefore, in this study, the adhesion, morphology, proliferation, and endothelial marker gene expression of ECs on the electrospun chitosan/collagen scaffolds were examined. Endothelial cells are employed for testing cytocompatibility *in vitro*. Endothelial cell cultures can be derived from animals (usually mouse, pig, or calf aorta) [24], and established endothelial cell lines are also available [25]. A lot of interest has arisen in pig endothelial cells as a model of atherosclerosis, allograft rejection and accommodation, xenotransplantation, and viral hemorrhagic diseases [26], and pig endothelial cells have been validated to assess EC interaction with the biomaterial on many biomaterials [27].

In our experiments, morphological evaluation of PIECs on the electrospun chitosan/collagen scaffolds proved to be similar to the physiological evaluation, with spindle-shaped appearance. The scaffolds were biocompatible *in vitro*, since ECs adhered well to the nanofibrous surface, proliferated through the pores into nanofiber mesh, and interacted with the surrounding fibers. Cell adhesion and growth after endothelial seeding is favored by surface characteristics of the material. Chemical structure, texture, and porosity of the surface are other important factors for cell adhesion. Various cell-substrate interactions contribute to adhesion and proliferation of PIECs on electrospun chitosan/collagen scaffolds. These may include electrostatic attraction to unreacted aminogroups and integrin binding to collagen.

The study of the biochemical mechanism which governs cell adhesion is important to predict the material which permits a better endothelialization. The cell-cell and cell-biomaterial interactions are mediated by adhesion molecules, which were hypothesized to be directly involved in the adhesion mechanisms between endothelial cells and biomaterials. Adhesion molecules permit both the adhesion of a cell to another cell and the adhesion of the cell to the adhesive proteins of the substratum.

In this study, we examined the modifications induced by electrospun chitosan/collagen scaffolds on the expression of both ICAM-1 and VCAM-1. Here, the electrospun chitosan/collagen scaffolds in different weight ratios can act as stimuli. ICAM-1 is an adhesive protein of immunoglobulin superfamily, which occurs on the endothelial cell membrane, and regulates the adhesion process of the leucocytes to these cells. VCAM-1 belongs to the immunoglobulin superfamily as well. The expression of this molecule on the endothelial cell membrane increases significantly after exposure to stimuli such as endotoxin, IL-1, and TNF. In our experimental model, the expression of endothelial cell adhesion molecules after contacting with chitosan/collagen scaffolds was compared with the behavior of unexposed cultures. The modification of these adhesion molecules may represent *in vitro* the mechanism which permits the outlet of the leucocytes from the circulation stream after inflammatory stimuli.

Proliferating cell nuclear antigen, commonly known as PCNA, is a protein that acts as an auxiliary protein of DNA polymerase delta to play a fundamental role in the initiation of cell proliferation. Its level correlates directly with rates of cellular proliferation and DNA synthesis. In this research, all the cells grown on the chitosan/collagen scaffolds expressed PCNA, and the expression on chitosan/collagen (w/w; 50/50) was slightly higher when compared with others.

We also examined the potential of carcinogenic activity of the electrospun chitosan/collagen scaffolds through the expression of p53. p53, also known as protein 53, is a transcription factor that regulates the cell cycle and hence functions as a tumor suppressor. It is important in multicellular organisms as it helps to suppress cancer. The development of cancer often involves inactivation of this suppressor function via various mechanisms, such as gene deletions and point mutations. Moreover, the p53 protein is a multi-functional transcription factor involved in control cell cycle progression, DNA integrity, and cell survival in cells exposed to DNA-damaging agents. Loss of p53 activity predisposes cells to the acquisition of oncogenic mutations and may favor genetic instability. In this study, the p53 mRNA expression was observed; that is, the PIECs grown on the electrospun chitosan/collagen scaffolds maintained the activity to regulate the cell cycle and hence function as a tumor suppressor.

5. Conclusion

In conclusion, electrospun chitosan/collagen scaffold shows EC adhesion, proliferation and does not adversely affect cellular function. The chitosan/collagen (w/w; 50/50, 20/80) scaffold in particular shows the highest potential as candidate for vascular tissue engineering.

Conflict of Interests

The authors declared that they have no conflict of interests in this work.

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