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Formation of nanostructured poly(lactic-*co*-glycolic acid)/chitin matrix and its cellular response to normal human keratinocytes and fibroblasts

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Abstract

Electrospinning of poly(lactic-*co*-glycolic acid) (PLGA) and chitin in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and formic acid, respectively, was investigated to fabricate a biodegradable nanostructured composite matrix for tissue engineering. The average fiber diameter (310 nm) of PLGA nanofibers, obtained by electrospinning a 15 wt% PLGA solution in polar HFIP, was much smaller than the diameter (760 nm) of nanofibers electrospun at the same concentration in nonpolar chloroform. On the contrary, chitin was electrospun into nanosized particles at a lower concentration with negligible chain entanglement, because it can not produce continuous fibers by the electrospinning process even at high concentrations. In order to fabricate the biomimetic scaffolds for human keratinocytes and fibroblasts, we prepared the composite matrix (PLGA/chitin = 80/20, w/w) of chitin nanoparticles embedded within a PLGA nanofiber matrix by a newly-designed simultaneous electrospinning process using two polymer solutions. Chitin nanoparticles were distributed uniformly in the PLGA fibrous structure, and appeared to adhere strongly to PLGA nanofibers by simultaneous electrospinning. To assay the cytocompatibility and cell behavior on the PLGA and PLGA/chitin matrices, cell attachment and spreading of both normal human keratinocytes and fibroblasts seeded on the matrices were studied. Our results indicate that the PLGA/chitin composite matrix may be a better candidate than the PLGA matrix in terms of cell adhesion and spreading for normal human keratinocytes, and that the PLGA and PLGA/chitin matrices are good matrices for normal human fibroblasts.

Keywords: Chitin; Poly(lactic-*co*-glycolic acid) (PLGA); Nanostructure; Electrospinning; Matrix

1. Introduction

Considerable efforts have attempted to develop scaffolds over the last decade to develop tissue engineering, using biodegradable and biocompatible synthetic or natural polymers. Principally, the scaffold's design should mimic the structure and biological function of native extracellular matrix (ECM) proteins, which provide mechanical support and regulate cell activities. A nonwoven-type matrix composed of nanofibers is architecturally similar to the collagen structure of the ECM, in which collagen multifibrils of a nanofiber scale (50–500 nm) are composed of a three-dimensional network structure together with proteoglycans (Nishido, Yasumoto, Otori, & Desaki, 1988). The matrix texture, as well as the nature of the biomaterial, was also reported to control cell adhesion, proliferation, shape, and function (Chen, Mrksich, Huang, Whitesides, & Ingber, 1997; Patel et al., 1998; van Kooten, Whitesides, & Ingber, 1998).

Recently, many researchers are trying to employ the electrospinning technique to prepare microporous biodegradable or biocompatible scaffolds (Kenawy et al., 2003; Li, Laurencin, Caterson, Tuan, & Ko, 2002; Luu, Kim, Hsiao, Chu, & Hadjiargyrou, 2003; Matthews, Wnek, Simpson, & Bowlin, 2002; Yoshimoto, Shin, Terai, & Vacanti, 2003). Li et al. (2002) prepared poly(D,L-lactide-*co*-glycolide) ultrafine fibers via electrospinning that showed a morphological similarity to the ECM of natural tissue with a diameter range from 500 to 800 nm. Yoshimoto et al. (2003) reported that the electrospun nonwoven poly(ε-caprolactone) could be a useful scaffold for

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bone tissue engineering. Luu et al. (2003) fabricated synthetic polymers (PLGA and PLA–PEO copolymer)/DNA composite scaffolds for therapeutic application in gene delivery for tissue engineering. Matthews et al. (2002) studied how electrospinning can be adapted to produce tissue engineering scaffolds composed of collagen nanofibers (a matrix composed of 100 nm fiber). They found that the structural properties of electrospun collagen varied with the tissue of origin, the isotope, and the concentration of the collagen solution, which was used to spin the fibers.

Among the biodegradable polymers, poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers have extended their applications to surgical sutures, implant materials, drug carriers, and scaffolds for tissue engineering. Chitin has structural characteristics similar to glycosaminoglycans (GAGs) such as condroitin sulfate and hyaluronic acid in the ECM. It also has good biocompatibility and biodegradability, as well as various biofunctionalities including antithrombogenic, hemostatic immunity enhancing, and wound healing properties (Yusof, Wee, Lim, & Khor, 2003).

In this study, the nanostructured matrices of poly(lactic-*co*-glycolic acid) (PLGA) and PLGA/chitin (80/20, w/w), which is composed of PLGA nanofibers and chitin nanoparticles, were produced via electrospinning to develop biodegradable and biomimetic scaffolds. In addition, we examined the effect of PLGA and PLGA/chitin matrices on the cell adhesion and spreading of normal human keratinocytes and fibroblasts. To further evaluate the effect of ECM proteins, the cellular responses were investigated on PLGA and PLGA/chitin matrices, which used fibronectin, or laminin as type I collagen, and were adsorbed onto the matrices as substrates.

2. Experimental

2.1. Materials

PLGA (50/50) copolymer (MW = 25,000) was kindly supplied by Purac Co. The chitin powder (100–500 μm) from crab shellsthat was used in this work was supplied by Kumho Chemical Co. (Korea). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), phosphoric acid (85 wt%), and formic acid (96%) were purchased from Aldrich Co. and used as received.

2.2. Preparation of regenerated chitin

Regenerated chitin was prepared according to Vincendon's method (Vincendon, 1997). Chitin solution was prepared by stirring the chitin powder in 85% phosphoric acid at room temperature for 30 min and then filtered on a glass funnel. Regenerated chitin from phosphoric acid solutions was obtained by precipitation in a cold 0.1 M NaOH solution. The precipitate was washed with water until neutral, then with ethanol and dried under vacuum.

2.3. Electrospinning

A schematic diagram of the electrospinning apparatus for producing ultrafine PLGA fibers and PLGA/chitin composite fibers is shown in Fig. 1. The electrospinning setup utilized in this study consisted of one or two syringe and needle (ID = 0.84 mm), a ground electrode (d = 21.5 cm, stainless steel sheet on a drum whose rotation speed can be varied), and a high voltage supply (Chungpa EMT, CPS-40K03). The needle was connected to the high voltage supply, which was able to generate positive DC voltages up

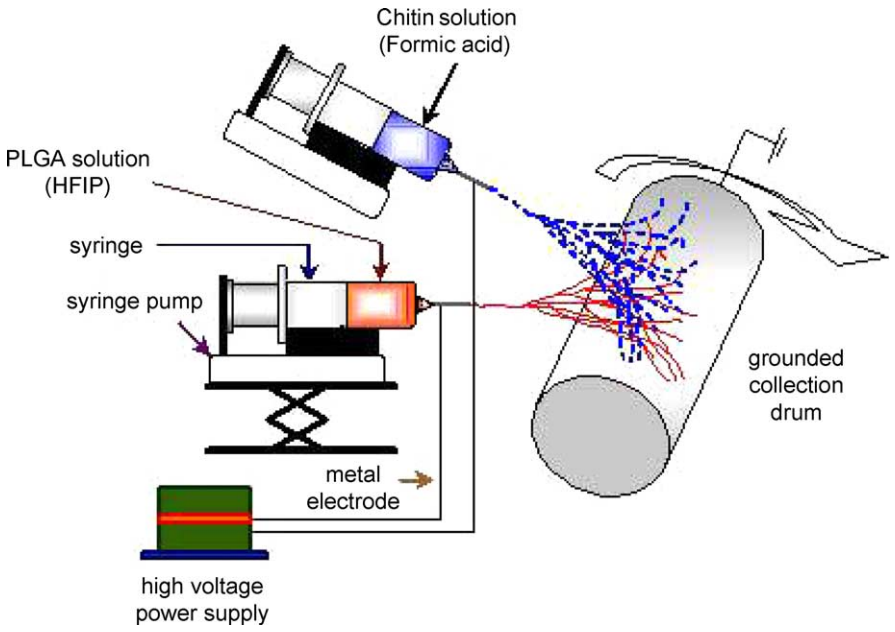

Fig. 1. Schematic diagram of the electrospinning apparatus for the PLGA/chitin composite matrix.

to 40 kV. For the electrospinning of PLGA/chitin composite fibers, PLGA and regenerated chitin were dissolved in HFIP and formic acid, respectively, and delivered by a two syringe pump (KD Scientific, Model 100) with the mass flow rate of 1–2 and 4–6 ml/h, respectively. The distance between the needle tip and the ground electrode was in the range of 5–10 cm and the positive voltage applied to the polymer solutions was in the range of 15–20 kV. All experiments were carried out at room temperature.

2.4. Measurements

The morphology of electrospun PLGA/chitin composite fibers was observed on a scanning electron microscope (SEM) (Hidachi S-2350) after gold coating. The average diameter and diameter distribution were obtained by analyzing SEM images with a custom code image analysis program (Scope Eye II).

2.5. Cells and cell culture

Primary normal human oral keratinocytes (NHOK) were prepared from human gingival tissue specimens and maintained as described previously (Min, Woo, Beak, Lee, & Park, 1995). The tissue samples were obtained from three healthy individuals with ages ranging from 21–30 years while undergoing surgery. Briefly, samples were thoroughly washed with calcium- and magnesium-free Hanks' balanced salt solution (CMF-HBSS; GibcoBRL). To separate the epithelium from the underlying mucosa, the tissues were incubated in CMF-HBSS containing collagenase (type II, 1.0 mg/ml; Sigma) and dispase (grade II, 2.4 mg/ml; Boehringer–Mannheim) for 90 min at 37 °C in 95% air and 5% CO_2. Epithelial cells were isolated from the epithelial tissue by trypsin digestion. These cells were seeded onto a 60 mm Petri dish and allowed to proliferate until reaching 60–70% confluence. The primary normal human epidermal keratinocytes (NHEK) were prepared in a manner similar to the NHOK from human foreskins that were obtained from patients (ages ranging from 1 to 3) undergoing surgery. Primary NHOK and NHEK were serially subcultured, being passaged at every 70% confluence level and the second passage keratinocytes were used in the described experiments. The cells were cultured in a keratinocyte basal medium containing 0.15 mM calcium and a supplementary growth factor bullet kit (KGM; Clonetics). Primary normal human gingival fibroblasts (NHGF) were established from explant cultures of gingival connective tissue that were excised from a patient undergoing oral surgery. When the cells reached 80% confluence, they were serially subcultured, being passaged at every 80% confluence level and the fifth passage fibroblasts were used in the described experiments. The cells were cultured in Dulbecco's modified Eagle's medium (Sigma), supplemented with 10% fetal bovine serum.

2.6. Cell adhesion assay and cell spreading analysis

Cell adhesion was assayed using a modification of the method of Mould, Askari, and Humphries (2000). Briefly, PLGA and PLGA/chitin (80/20, w/w) matrices were cut out with a punch (14 mm in diameter) and put onto the 24-well culture plates (Corning). Twenty four-well culture plates containing PLGA or PLGA/chitin matrices were coated with 300 μl/well of ECM proteins, in this case type I collagen (50 μg/ml), fibronectin (1 μg/ml), or laminin (10 μg/ml) in PBS by overnight adsorption at room temperature. The wells were then washed with PBS and the unbound sites were blocked with 10 mg/ml of bovine serum albumin (BSA) in PBS. Cells were detached by trypsin digestion, 300 μl of a cell suspension containing 1×10^5 cells was placed in each well, and the cells were allowed to settle/adhere for 1 h at 37 °C in an atmosphere of 5% CO_2. Loosely adherent or unbound cells from experimental wells were removed by aspiration, the wells were washed once with PBS, and the remaining bound cells were fixed with 10% formalin in PBS for 15 min. The fixative was aspirated, the wells were washed twice with PBS, and attached cells onto the PLGA or PLGA/chitin matrices were stained with hematoxylin and eosin. The wells were gently washed three times with distilled H_2O. The PLGA and PLGA/chitin matrices were mounted, and cells attached onto the matrices were photographed. To ensure a representative count, each SF matrix was divided into quarters and two fields per quarter were photographed with an Olympus BX51 microscope at 100×. Average percentages and standard deviations were calculated from four independent experiments.

Cell spreading was analyzed with photographs that were taken in the cell adhesion assay. To ensure a representative count, each matrix was divided into quarters and two fields per each quarter were photographed with an Olympus BX51 microscope at 100×. The cells that adopted a flattened, polygonal shape, with filopodia- and lamellipodia-like extensions were regarded as spreading cells. In contrast, the cells that resisted washing and remained tethered to the plate surface were regarded as non-spreading cells. The percentage of cells displaying the spread morphology was quantified by dividing the number of spread cells by the total number of bound cells. Average percentages and standard deviations were calculated from four independent experiments.

3. Results and discussion

3.1. Electrospinning of PLGA and regenerated chitin

In the electrospinning process, the nature of the solvent, such as volatility and polarity (dielectric constant), has a significant influence on the morphology and diameter of

the electrospun fibers, similar to the influence of the conductivity of a solution. Bognitzki et al. (2001) used highly volatile dichloromethane (boiling point, 40 °C) as a solvent, and prepared PLLA fibers with a regular pore structure. On the contrary, the electrospinning of the polymer in a polar solvent generally produces ultrathin fibers with a smaller average diameter, although the effect of solvent polarity on the fiber diameters has not been systematically studied. Fig. 2(a) and (b) shows SEM micrographs of the ultrafine PLGA fibers electrospun from a 15% PLGA solution in HFIP (dielectric constant = 16.70 at 20 °C) or chloroform (dielectric constant = 4.81 at 25 °C). The distance between the needle tip and the ground electrode was 7 cm and a positive voltage was set at 17 kV. The average fiber diameter (310 nm) of nanofibers electrospun from polar HFIP was much smaller than that (760 nm) of nanofibers electrospun from nonpolar chloroform. Therefore, this difference indicates that the diameter of the electrospun PLGA nanofibers might be dependent on the dielectric constant of the solvent. As shown in Fig. 2(c), the fiber diameters of PLGA electrospun from a 15% PLGA solution in HFIP were in the range of 100–600 nm, which is a desirable size to replace the natural extracellular matrix, because the ECM is composed of randomly-oriented collagens in the diameter range of 50–500 nm. Therefore, we chose HFIP as the solvent for the PLGA nanofiber matrix.

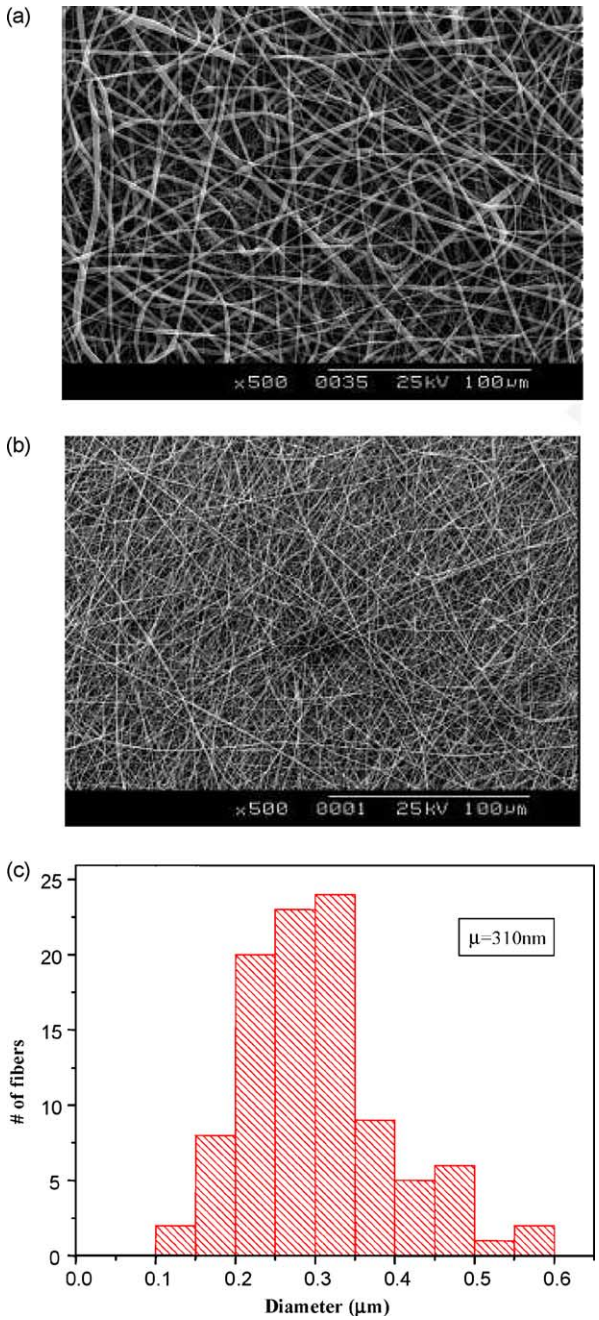

Fig. 2. SEM micrographs of the ultrafine PLGA fibers electrospun from a 15% PLGA solution. (a) solvent, HFIP; (b) solvent, chloroform; (c) fiber diameter distribution of PLGA nanofibers electrospun using HFIP.

Chitin has an acetamide group attached to the C2 position, instead of the amino group in chitosan, or instead of the hydroxyl group noted in cellulose. Chitin is insoluble in most organic solvents because the presence of amide functionality imparts chitin a rigid three-dimensional hydrogen-bonded molecular structure. However, chitin is soluble in concentrated mineral acids including 10 M hydrochloric, sulfuric and phosphoric acid solutions at room temperature. In this study, we obtained the regenerated chitin by precipitating its solution in phosphoric acid. The regenerated chitin is soluble in formic acid, and is considered a more acceptable solvent for electrospinning than phosphoric acid.

In electrospinning, the solution viscosity plays an important role in determining the range of concentrations from which continuous fibers can be obtained. Above a critical concentration, a continuous fibrous structure is obtained and its morphology is affected by the concentration of solution (Doshi & Reneker, 1995). The formation of continuous fibers is attributed to the extensive chain entanglements in the polymer solution. We electrospun the regenerated chitin solution in formic acid across a broad range of concentrations (3–17 wt%), but we failed to electrospin chitin into ultrafine fibers even at the concentrations in which the chitin chain was extensively entangled. Fig. 3 shows SEM micrograph of chitin nanoparticles electrospun at 3 and 15 wt% concentrations. Chitin particles obtained at high concentration (15 wt%) was more uniform than at low concentration (3 wt%). However, the average diameter (420 nm) of chitin particles was smaller at low concentration and its distribution was in the range of 200–1100 nm, although some particles were agglomerated. Therefore, we prepared chitin nanoparticles to be electrospun at lower concentrations in which the particle size of chitin becomes smaller.

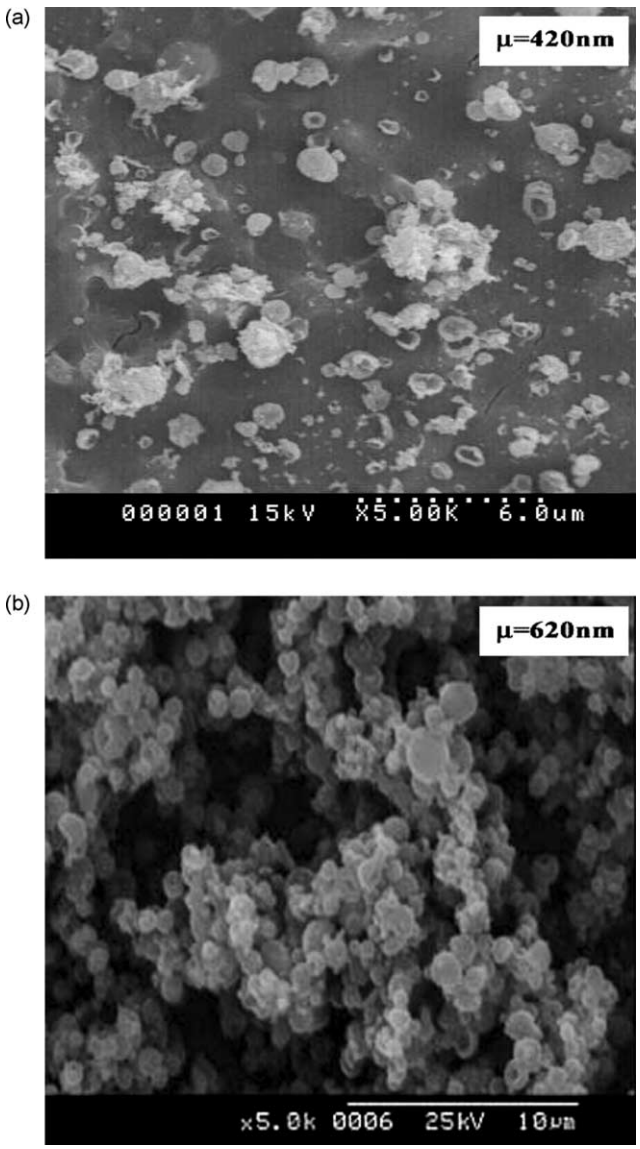

Fig. 3. SEM micrographs of chitin nanoparticles electrospun at 3 wt% (a) and 15 wt% (b) concentration in formic acid.

3.2. Composite electrospinning of PLGA/chitin

The ECM is a complex mixture of structural and functional proteins, glycoproteins, and proteoglycans arranged in a unique, tissue specific three-dimensional ultrastructure (Badylak, 2002). Structural proteins such as collagen and GAGs are especially important in mechanically supporting tissue reconstruction and in providing attachment sites for cell surface receptors. Collagen is the most abundant protein within the ECM, but its prohibitively expensive price minimizes its use as a scaffold for tissue engineering. GAGs play important roles in binding growth factors and cytokines, water retention, and the gel properties of the ECM. Interestingly, chitin has a chemical structure similar to a repeating unit of GAGs such as chondroin sulfate, heparin, heparan sulfate, and hyaluronic acid.

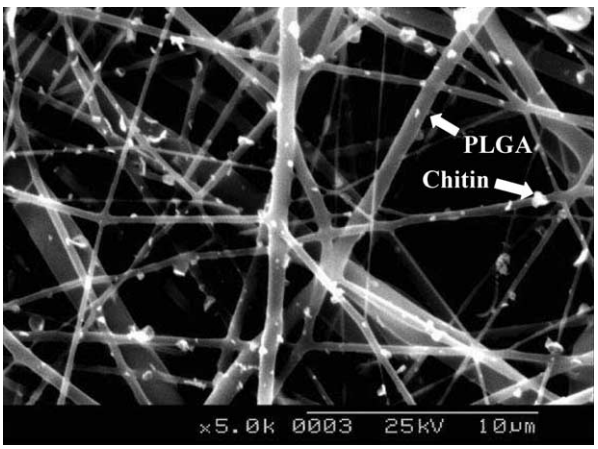

Fig. 4. SEM micrograph of the composite matrix composed of PLGA nanofibers and chitin nanoparticles (PLGA/chitin = 80/20, w/w).

Among GAGs, hyaluronic acid has been extensively investigated as a scaffold for dermal reconstruction.

We chose PLGA and chitin as substituents of collagen and GAGs in the ECM, respectively. In order to fabricate the composite structure of PLGA and chitin, we designed a novel simultaneous electrospinning using two polymer solutions as shown in Fig. 1. The spinning conditions of PLGA and chitin solutions were the same as described in Section 3.1. Fig. 4 shows a SEM micrograph of the composite matrix composed of PLGA nanofibers and chitin nanoparticles (PLGA/chitin = 80/20, w/w). As shown in Fig. 4, chitin nanoparticles were distributed uniformly in the PLGA fibrous structure, and appeared to adhere strongly to PLGA nanofibers.

3.3. Attachment and spreading of normal human keratinocytes and fibroblasts on PLGA and PLGA/chitin matrices, alone or in conjunction with ECM protein coating

It is interesting to study the cytocompatibility of the PLGA and PLGA/chitin (80/20, w/w), alone or in conjunction with ECM protein coating, in relation with a possible use as wound dressing materials and scaffolds for tissue engineering. Moreover, since the connective tissue and the epithelium play an important role in reconstructing intra- and extraoral defects, the responses of normal human epithelial cells and fibroblasts forming these tissues were analyzed in vitro. Since the absence of cytotoxicity of PLGA and chitin has already been demonstrated (Chow & Khor, 2002; Ignatius & Claes, 1996; Zange, & Kissel, 1997), and initial cell adhesion and spreading might be an important contributing factor to their potential use as wound dressings and scaffolds for tissue engineering, the initial cell attachment and spreading on these PLGA and PLGA/chitin matrices were studied. To assess cell adhesion and spreading, PLGA and PLGA/chitin matrices, alone or in conjunction with ECM protein coating, were seeded with normal human keratinocytes and fibroblasts. The adhesion of the cultured cells was evaluated using

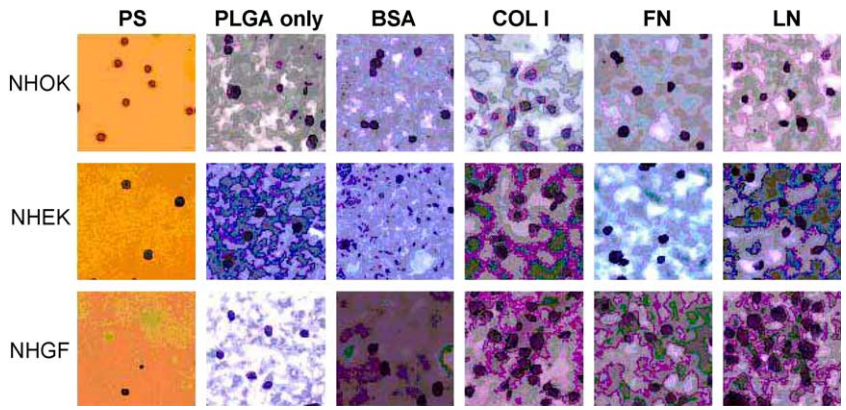

Fig. 5. Microphotographs of the cell adhesion and spreading of normal human keratinocytes (NHOK and NHEK) and fibroblasts (NHGF) to PLGA matrix, alone or in conjunction with ECM proteins: PS, polystyrene surface only; PLGA only, PLGA matrix only; BSA, bovine serum albumin-coated PLGA matrix; Col I, type I collagen-coated PLGA matrix; FN, fibronectin-coated PLGA matrix; LN, laminin-coated PLGA matrix.

the cell adhesion assay in serum-free medium, and using as type I collagen (50 μg/ml), fibronectin (1 μg/ml), or laminin (10 μg/ml), which were adsorbed onto the PLGA and PLGA/chitin matrices, as substrates. We tested the effect of 0.1–50 μg/ml of type I collagen, fibronectin, and laminin on human oral keratinocytes adhesion and spreading to the culture plate surface and found that 50 μg/ml type I collagen, 1 μg/ml fibronectin, or 10 μg/ml laminin showed approximately maximal effect in NHOK (data not shown). Rapidly proliferating normal human keratinocytes and fibroblasts adherent to PLGA and PLGA/chitin matrices were microphotographed during the adhesion assay after washing, fixing, and staining the cells with hematoxylin and eosin. Figs. 5 and 6 show representative microscopic fields for cells adhering on immobilized type I collagen, fibronectin, and laminin, which are covering PLGA and PLGA/chitin matrices, respectively. Normal human keratinocytes, such as NHOK and NHEK, were plated on the PLGA/chitin only, without added matrix molecules, and attached better than to the PLGA only. However, normal human fibroblasts also attached well in both PLGA and PLGA/chitin only under our conditions. In particular, the number of adhering cells observed on the PLGA/chitin matrix alone was approximately 2.6-fold higher in normal mucosal keratinocytes NHOK than normal epidermal keratinocytes NHEK (Figs. 6 and 8(a)). Type I collagen promoted the adhesion of proliferating normal human keratinocytes and fibroblasts tested on a PLGA matrix (Fig. 7(a)) and also promoted the adhesion of fibroblasts on the PLGA/chitin matrix, but did not in normal human keratinocytes (Fig. 8(a)). In contrast, laminin and fibronectin had no effect on the cell adhesion of normal human keratinocytes in either the PLGA or PLGA/chitin matrices. In addition, the adhesion activity profile of laminin-coating onto the PLGA and PLGA/chitin matrices for cultured human fibroblasts was very similar to that of its type I collagen counterparts, but fibronectin showed minimal adhesion activity under our conditions compared to the BSA control (Figs. 7(a) and 8(a)).

To further evaluate the adhesion of type I collagen, fibronectin, and laminin, we determined whether adherent cells were tethered to the substrate or spreading over the substrate. Similar or extremely low cell spreading was observed on either BSA- or fibronectin-coated PLGA or

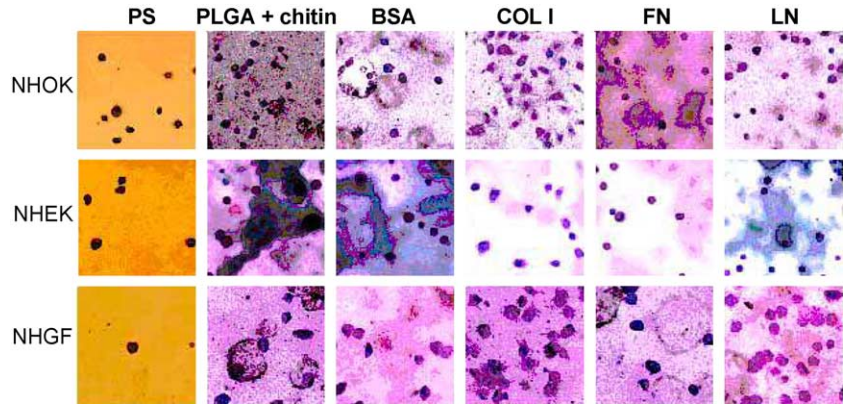

Fig. 6. Microphotographs of the cell adhesion and spreading of normal human keratinocytes (NHOK and NHEK) and fibroblasts (NHGF) to PLGA/chitin matrix, alone or in conjunction with ECM proteins: PS, polystyrene surface only; PLGA + chitin, PLGA/chitin matrix only; BSA, bovine serum albumin-coated PLGA/chitin matrix; Col I, type I collagen-coated PLGA/chitin matrix; FN, fibronectin-coated PLGA/chitin matrix; LN, laminin-coated PLGA/chitin matrix.

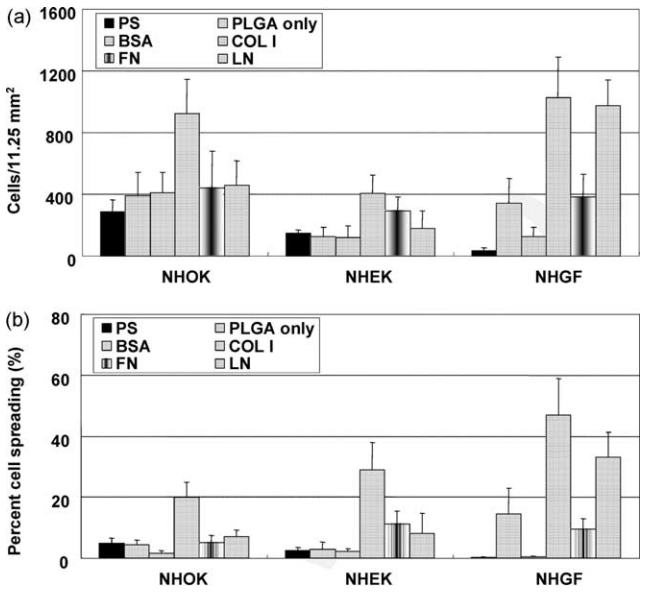

Fig. 7. The level of cell adhesion (a) and the incidence of cell spreading (b) of NHOK to normal human keratinocytes (NHOK and NHEK) and fibroblasts (NHGF) to the PLGA matrix, alone or in conjunction with ECM proteins: PS, polystyrene surface only; PLGA only, PLGA matrix only; BSA, bovine serum albumin-coated PLGA matrix; Col I, type I collagen-coated PLGA matrix; FN, fibronectin-coated PLGA matrix; LN, laminin-coated PLGA matrix.

PLGA/chitin matrices for cultured human keratinocytes and fibroblasts compared to that observed on PLGA and PLGA/chitin matrix only, except fibronectin-coated PLGA matrix for NHEK (Figs. 7(b) and 8(b)). Interestingly,

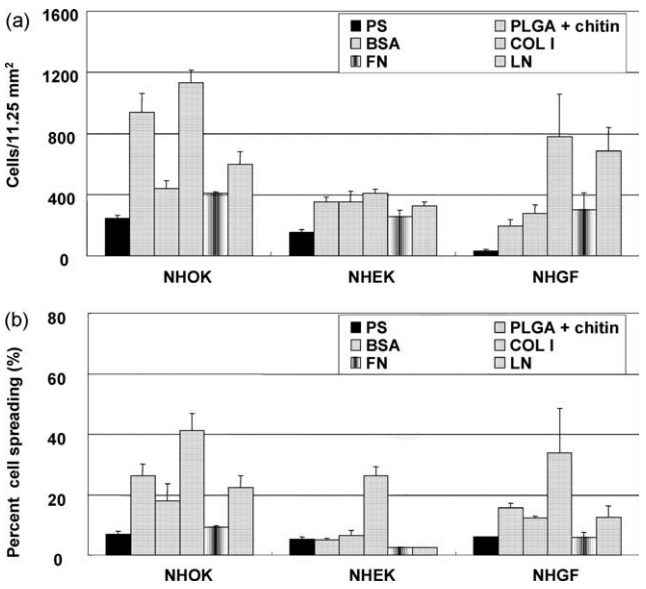

Fig. 8. The level of cell adhesion (a) and the incidence of cell spreading (b) of NHOK to normal human keratinocytes (NHOK and NHEK) and fibroblasts (NHGF) to PLGA/chitin matrix, alone or in conjunction with ECM proteins: PS, polystyrene surface only; PLGA + chitin, PLGA/chitin matrix only; BSA, bovine serum albumin-coated PLGA/chitin matrix; Col I, type I collagen-coated PLGA/chitin matrix; FN, fibronectin-coated PLGA/chitin matrix; LN, laminin-coated PLGA/chitin matrix.

NHOK attached and spread approximately 2.3- and 6.5-fold more on the PLGA/chitin matrix only than on the PLGA matrix only, respectively, but did not in NHEK (Figs. 7 and 8). This finding supports that the addition of chitin nanoparticles into the PLGA matrix promotes the cell adhesion and spreading of NHOK. On type I collagen, 20% of the proliferating NHOK and 29% of the proliferating NHEK showed a spreading morphology on the PLGA matrix, and 41% NHOK and 27% NHEK also showed a spreading morphology on the PLGA/chitin matrix, i.e. they adopted a flattened, polygonal shape, with filopodia- and lamellipodia-like extensions (Figs. 7(b) and 8(b)). The remaining non-spreading cells on integrin ligands resisted washing and remained tethered to the PLGA and PLGA/chitin surfaces. In contrast, laminin and fibronectin had no effect on cell spreading of cultured human keratinocytes on the PLGA matrix. Laminin displayed functional properties on the PLGA matrix similar to type I collagen in cultured human fibroblasts, because laminin and type I collagen demonstrated that 33 and 47% of proliferating NHGF displayed a spreading morphology (Fig. 7(b)). Coating the PLGA/chitin matrix with type I collagen promoted spreading of the cultured human fibroblasts, whereas negligible cell spreading was detected in laminin-coated PLGA/chitin compared to that observed on BSA control. We repeated this experiment four times and no gross difference was observed. These results indicate that the PLGA/chitin matrix is a better candidate than the PLGA matrix in terms of cell adhesion and spreading for normal human keratinocytes, and that the PLGA and PLGA/chitin matrices are good matrices for normal human fibroblasts. Our results also support that, in the case of the integrin ligands, type I collagen is functionally active in terms of cell responses in normal human epithelial cells and in normal human fibroblasts, and that laminin is also functionally active in normal human fibroblasts.

4. Conclusions

PLGA nanofibrous structures were obtained by electrospinning of PLGA/HFIP at a 15% concentration and the average fiber diameter was 310 nm, whereas electrospinning of PLGA/chloroform produced an average fiber diameter of 760 nm at the same concentration. Therefore, in the PLGA nanofibers electrospun from HFIP, the diameter range was found to be more desirable to mimic the natural extracellular matrix. On the contrary, chitin was electrospun into nanosized particles, not into nanofibers, even at the concentrations that resulted in extensive chain entanglements. This is an indicative that not every polymer solution can be electrospun into ultrafine fibers.

In order to fabricate the biomimetic scaffolds for human keratinocytes and fibroblast, we prepared the composite matrix composed of PLGA nanofibers and chitin nanoparticles (PLGA/chitin = 80/20, w/w) by a newly-designed

simultaneous electrospinning process using two polymer solutions. Chitin nanoparticles were distributed uniformly in the PLGA fibrous structure, and adhered strongly to PLGA nanofibers.

The cell attachment and spreading onto the PLGA and PLGA/chitin matrices, using type I collagen, as a substrate, showed relatively promising results for the normal human keratinocytes tested. In addition, the surface modifications of biomaterials with ECM proteins or peptides were also extensively tested. Keeping in mind that the final goal of scaffold design is producing an ideal structure for replacing the natural ECM proteins until host cells can repopulate and resynthesize a new natural matrix, a PLGA/chitin matrix may be a better candidate for tissue engineering scaffolds because it has a biomimetic three-dimensional structure resembling the collagen-GAGs composite structure in the ECM.

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