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Three-Dimensional Tissue Scaffolds from Interbonded Poly(ε-Caprolactone) Fibrous Matrices with Controlled Porosity

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In this article, we report on the preparation and cell culture performance of a novel fibrous matrix that has an interbonded fiber architecture, excellent pore interconnectivity, and controlled pore size and porosity. The fibrous matrices were prepared by combining melt-bonding of short synthetic fibers with a template leaching technique. The microcomputed tomography and scanning electron microscopy imaging verified that the fibers in the matrix were highly bonded, forming unique isotropic pore architectures. The average pore size and porosity of the fibrous matrices were controlled by the fiber/template ratio. The matrices having the average pore size of 120, 207, 813, and 994 μm, with the respective porosity of 73%, 88%, 96%, and 97%, were investigated. The applicability of the matrix as a three-dimensional (3D) tissue scaffold for cell culture was demonstrated with two cell lines, rat skin fibroblast and Chinese hamster ovary, and the influences of the matrix porosity and surface area on the cell culture performance were examined. Both cell lines grew successfully in the matrices, but they showed different preferences in pore size and porosity. Compared with two-dimensional tissue culture plates, the cell number on 3D fibrous matrices was increased by 97.27% for the Chinese hamster ovary cells and 49.46% for the fibroblasts after 21 days of culture. The fibroblasts in the matrices not only grew along the fiber surface but also bridged among the fibers, which was much different from those on two-dimensional scaffolds. Such an interbonded fibrous matrix may be useful for developing new fiber-based 3D tissue scaffolds for various cell culture applications.

Introduction

A considerable effort has been made over recent decades to develop three-dimensional (3D) scaffolding materials for tissue engineering applications. Reported techniques to produce 3D tissue scaffolds include phase separation,1 self-assembly,2 template and solvent leaching,3 supercritical foaming,4 3D printing,5 fused deposition modeling,6 and fiber and textile technology.7−10 However, the resulting 3D scaffolds either meet problems with controlling the pore size or interconnectivity or have issues in cytotoxic contamination or pore stability,11,12 which prevent or restrict their uses in clinic. It remains a challenge to produce a 3D scaffold mimicking native organ tissue to provide structure and physical stimuli to cells.

In cell biology, both pore size and the interconnectivity are critical for a scaffold to be integrated with the surrounding tissues.13,14 In vivo applications require the pores in a scaffold to not only encourage cell growth but also facilitate the vascularization and the exchange of nutrients/waste with outside environment. In general, cell growth requires small pores, the size of which is compatible to or slightly larger than cells,15,16 whereas nutrient/waste exchange needs larger pores. For example, pores larger than 300 μm are recommended for new bone formation17,18 and larger than 500 μm for the rapid formation of fibrovascular tissue.19 The pore size and morphology also affect the initial cell attachment, adhesion, and cell penetration inside the scaffolds,13,19 besides new tissue regeneration.20,21 Cells of different types may prefer different porous environments to grow.22 Fibrous matrices are excellent porous materials showing great potential in advanced biomedical and engineering applications. Fibrous matrices normally have large surface areas with a high porosity. They are especially advantageous in pore interconnectivity because all pores in fibrous matrix are naturally connected in such a way that they can even be regarded as a continuous phase. A wide availability of fibrous materials and fiber-processing techniques has enabled the development of various fibrous matrices for tissue scaffolding applications. So far, the cell culture performance of several types of fibrous matrices has been examined, including braided, woven, knitted, and nonwoven fibrous matrices.23,10,24

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Nonwoven matrices are most promising implant materials because of their simple and inexpensive manufacturing process and special porous structure. They have been widely used in medical areas such as wound dressing, artificial skin, bone tissue, soft tissue, blood compatible interfaces, and heart valves. The issues related to nonwoven scaffolds are the difficulties in adjusting pore size and porosity and the unstable pore structure as a result of interfiber slippage during use.10,25 Nonwoven scaffolds with stabilized porous structure and adjustable pore size and porosity have not been demonstrated in the research literatures.

In this article, we first report on a new approach for fabricating fibrous scaffolds that have stable porous structures and controlled pore sizes, and the applicability of these fibrous matrices for in vitro cell culture using two cell lines, Chinese hamster ovary (CHO) and fibroblast. The significant difference of our approach is that melt-bonding of short fibers (length 2 mm) in the presence of a powder template material is used, and the porous structure is stabilized by fiber–fiber interbonding, whereas the pore size is adjusted through fiber/template ratio. The unique porous characteristics were characterized by microcomputed tomography (μ-CT) and scanning electron microscopy. We found that both cell lines were grown nicely in the fibrous matrices, but showed different preferences in pore size and porosity.

Materials and Methods

Materials

Poly(ε-caprolactone) (PCL, purity >99%, $M_n = 80,000$; Aldrich), Dulbecco’s phosphate-buffered saline powder (Gibco), phenol-red–free media (Roswell Park Memorial Institute [RPMI]; Invitrogen), glutaraldehyde (2.1%) in phosphate-buffered saline (PBS, 10 mM, pH 7.4) (Whiteley Medical, Aidal Plus), osmium tetroxide (2% in water; SPI Suppliers), tannic acid (Aldrich), mapping to see (MTS)-kit (Promega), phenazine methosulphate (PMS; Aldrich), and sodium chloride (≥99.5%; Aldrich) were used as received. Rat skin fibroblasts and CHO cells (donated by Barwon Health Hospital, Australia) were cultured in RPMI and F-12 media, respectively (Invitrogen), both supplemented by 10% fetal bovine serum (FBS; Bovogen) and 1% penicillin–streptomycin solutions (10,000 U/mL penicillin, 10,000 μg/mL streptomycin; Aldrich).

Measurements

Cell morphology was observed using a scanning electron microscope (SEM, Leica S440) and a laser scanning confocal microscopy (Leica TCS SP5) equipped with Argon lasers. X-ray μ-CT (XCT-XRadia, Inc.) was employed to examine the 3D structure of the fibrous matrices. μ-CT–based 3D images were reconstructed using image-processing software (cone beam reconstruction, Angouleme). The porosity, pore distribution, and surface area were calculated based on the μ-CT results using 3D image analysis software (Mimics Pore Analysis, Materilise Software).

Preparation and sterilization of scaffolding materials

Short PCL filaments (diameter 51.9 ± 3.7 μm, length 2 mm) prepared by the Deakin Textile team were blended with sucrose powder (granular sugar, size 300–600 μm) in different ratios. The mixture was then placed into a metallic mold and oven heated at 62°C for 30 min to allow the fibers to bond with each other. After cooling to room temperature, the heated fiber–sugar blend was washed extensively with deionized water to leach off the sugar. Four matrices were prepared under the fiber/sugar ratio of 1:5, 1:10, 1:20, and 1:30 (wt/wt), which were marked as PCL-5, PCL-10, PCL-20, and PCL-30, respectively. The thickness of the matrices was controlled to 2 mm. PCL films (thickness 0.85 ± 0.03 mm) prepared by hot-pressing were used as two-dimensional (2D) control. To match the size of a single well in 24-well cell culture plates, both 3D fibrous matrix and 2D film were wad punched to disks of 1.4 cm in diameter. The scaffold samples were sterilized by rinsing with 70% ethanol, saturated sodium chloride aqueous solution, and Milli Q water (R 18.2 MΩ) and then placed individually in the wells of tissue culture plates, filled with 70% ethanol, and shaken for 1 h on an orbital shaker (100 rpm). The scaffold samples were finally rinsed with fresh 70% ethanol for 5 min, dried in a biohazard hood, and stored in sterile containers for further use.

Cell seeding

A conventional static seeding method was used to seed cells onto the scaffolds.26–27 Briefly, 50 μL cell suspension containing about $1 \times 10^5$ cells was cultivated on every scaffold and incubated for 1.5 h, allowing cells to attach to fiber under standard culture conditions (i.e., at 37°C, in a humidified atmosphere containing 5% CO2 and 95% air). The remaining 950 μL of media was then added to the wells. After 24 h, the nonadhering cells were washed off with PBS for three times and the samples were transferred to new tissue culture plates. The numbers of cells remaining in the fibrous matrices, in the culture media, and on the tissue culture plate wells were measured by MTS assay.28 The seeding efficiency was calculated according to equation (1).

\[
\text{Seeding efficiency (\%)} = \frac{\text{Number of cells remaining in the matrix}}{\text{Number of cells seeded}} \times 100\% \tag{1}
\]

Cell culture and MTS assay

The cell-seeded scaffolds experienced a 21-day static culture. In the culture period, the media were first replenished after the first 7 days and then every 3 days. During the culture period, MTS assays28 were performed on the samples at four time points (3, 7, 14, and 21 days).

The cultured scaffolds were rinsed with PBS to remove nonadhering cells and were transferred to new wells containing 300 μL of phenol-red–free RPMI media (Invitrogen) and 100 μL of MTS–PMS in PBS solution (0.046 mg/mL PMS and 2 mg/mL MTS). The samples were then incubated in a 37°C incubator for 1 h, after which 100 μL of the solution was withdrawn to measure the absorption at 490 nm on a microplate reader. The numbers of cells were quantified by comparing the absorbance of a series of known numbers of viable cells.

Cytotoxicity

A typical cytotoxicity test was implemented for 3 days [DS/EN ISO10993-5] with rat skin fibroblasts. In brief,
sterilized samples were put in a 24-well tissue culture plate with 1 mL of culture media and left overnight, after which the matrices were removed and discarded. Fibroblasts were then static-seeded in the wells containing the media, using a calculated density of $1 \times 10^4$ cells/well. After 3 days of culture, the number of cells was counted by hemocytometer using a Trypan blue dye (Sigma-Aldrich). Cell viability was calculated by equation (2).

\[
\text{Cell viability} = \frac{\text{Cell number (viable)}}{\text{Total cell number (viable + dead)}} \times 100\%
\]

Statistical analysis

All experiments were conducted in triplicates and data are expressed as mean ± standard deviation. The cytotoxicity and MTS data were analyzed on SPSS (SPSS Statistics 17.0) by using ANOVA and post hoc multiple comparison tests. A p-value of less than 0.05 was considered statistically significant. The error bars in the figures are the standard deviation of the data.

Results and Discussion

Figure 1a illustrates a typical procedure for preparing the fibrous matrices. Here, short fibers of length 2 mm were used, which made it easy to mix with the powdery template because of the very low fiber–fiber entanglement. The sugar template regulates the pore size and ensures the fiber bonding just at the initial contact points. A significant advantage of using sugar as the template is its nonhazardous nature to cells. It causes no cytotoxic effects even if its removal from the matrix is incomplete during the leaching process.

The SEM images of the fibrous matrices are shown in Figure 1b. The fibers in the matrices appeared uniformly and randomly distributed without preferential orientation. They were exclusively bonded with each other in the contact parts. Such an interbonded fiber structure stabilizes the fibrous architecture and the porous network, which is unique and has not been reported elsewhere.

X-ray μ-CT is a noninvasive and nondestructive technique to obtain precise quantitative and qualitative information about the 3D microarchitecture. Here, this technique was employed to explore the 3D structure of the fibrous matrices. The μ-CT images indicated that the matrix contained two types of pores: randomly oriented small pores arising from fiber accumulation and larger pores due to the sugar template. All fibrous matrices had an excellent porous structure (Fig. 1c). All pores in the fibrous matrices were well interconnected, without any dead-end pores. Videos produced from micro-CT scanning of samples PLC-5 and PCL-10 are provided in Supplementary Data (Video S1 and Video S2). Such an excellent pore interconnectivity will facilitate the transport of nutrients and the formation of neotissues. Unlike the conventional nonwovens in which the pores are oriented along the X–Y plane (matrix surface), the pores in our fibrous matrices showed no preferential orientation, which can be proven by the 2D μ-CT images in X, Y, and Z directions shown in Supplementary Data (available online at www.liebertonline.com/ten). Such an anisotropic pore structure possibly derives from the use of short fibers in the matrix preparation, enabling the fibers to orient in different directions.

The pores in the matrices were measured in the range of 100–1000 μm. Such a pore-size range is ideal for in vivo cell culture because smaller pores are effective in providing sites for cell adhesion and tissue growth, whereas larger pores facilitate efficient mass transport of nutrients and metabolic waste and enable the formation of a vascularized network throughout the scaffold.

By changing fiber/sugar ratio for the matrix preparation, the pore sizes and distributions in the fibrous matrices can be adjusted. As shown in Figure 1d, when the fiber/sugar ratio changes from 1.5 to 1.30 (wt/wt), the porosity increased from 73% to 99%. The increase in porosity also led to a decrease in surface-to-volume ratio, from 4.1 to 0.4 mm $^{-1}$, but an increase in the average pore size, from 150 to 1000 μm. These porous characteristics constitute the main advantage of this template-aided fiber-bonding technique over the conventional nonwoven and template-leaching techniques. The conventional nonwovens have a lower degree of bonding across the fabric thickness direction and very limited space to adjust the pore size and porosity, whereas the conventional template-leaching techniques typically result in low pore interconnectedness.

Both tensile and compression properties of the as-prepared fibrous matrices were measured and showed dependency on the fiber/sugar ratio (Supplementary Data Fig. S6). The tensile properties showed no directional preference. PCL-5 has the largest tensile strength (6.7 MPa) and the smallest compression strength (0.64 MPa). PCL-30 with the highest porosity had the smallest tensile strength (0.75 MPa) and the highest compression strength (0.13 MPa). Higher porosity could give larger space for compression, but reduced the tensile strength. For comparison, the tensile strength of a conventional nonwoven fabric (needle-punched) was also tested, which showed direction dependence (Supplementary Data Fig. S7). The nonwoven web along the processing direction had larger tensile strength than that along the width direction. The tensile strength along the width direction was much lower than that of our fibrous matrices. For the conventional nonwoven, its bulk density (0.27 g/cm$^3$) is relatively high. However, its tensile strength along the processing direction was much lower than that of the interbonded fibrous matrix having a similar bulk density (PCL-5, density = 0.208 g/cm$^3$). For the lab-made nonbonded nonwoven having a similar density and porosity to PCL-30, it had almost no strength (Supplementary Data). The interfiber bonding improved mechanical properties and facilitated to stabilize the porous structure.

To prove the pore stability, the fibrous matrices were immersed in water and stirred mechanically for 1 day, but no structural change was observed after the treatment. However, for the conventional nonwoven fabric and the lab-made nonbonded nonwoven having a similar porosity, lots of fibers were detached from the matrices during the test, indicating the weak ability to maintain the porous structure.

The hydraulic permeability of the fibrous matrices was tested (Supplementary Data Fig. S8). All the samples had a very high hydraulic permeability, in the range of $2 \times 10^{-8}$ to $10 \times 10^{-8}$ m$^4$ N$^{-1}$ s$^{-1}$, which was between the reported woven tissue scaffold ($\sim 1 \times 10^{-15}$ m$^2$ N$^{-1}$ s$^{-1}$) and the nonwoven tissue scaffold ($\sim 5 \times 10^{-6}$ m$^4$ N$^{-1}$ s$^{-1}$). Such a porous structure possibly derives from the use of short fibers in the matrix preparation, enabling the fibers to orient in different directions. The pores in the matrices were measured in the range of 100–1000 μm. Such a pore-size range is ideal for in vivo cell culture because smaller pores are effective in providing sites for cell adhesion and tissue growth, whereas larger pores facilitate efficient mass transport of nutrients and metabolic waste and enable the formation of a vascularized network throughout the scaffold.

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FIG. 1. (a) Typical procedure for preparing the fibrous matrices; (b) scanning electron microscopic images of different fibrous matrices; (c) µ-CT images, isometric and front views of the fibrous matrices; (d) pore size, porosity, and surface-to-volume ratio of the fibrous matrices. The scale bars in the images = 250 μm. PCL, poly(ε-caprolactone); µ-CT, microcomputed tomography. Color images available online at www.liebertonline.com/ten.
hydraulic permeability is sufficient to allow the exchange of nutrient and metabolic wastes and, hence, regulates cell migration to a deeper level of the fibrous scaffolds.

The cytotoxicity was determined by growing fibroblasts in the extract of the scaffolding materials. The cell viability obtained was higher than 90% for all the samples, including PCL pellets (raw material), as-extruded PCL fibers, PCL fibrous matrices, and PCL 2D films, and there was no significant difference among the samples tested \((p > 0.05)\), indicating that all the tested PCL samples are nontoxic to cells (Supplementary Data Fig. S9).

In this study, two cell lines, CHO and fibroblast, have been used to examine the applicability of the fibrous matrices as tissue scaffold. CHO cells are the most commonly used mammalian hosts for industrial production of recombinant protein therapeutics. They were chosen mainly because they are contact inhibitive and can only grow in monolayer until confluence is reached. Fibroblasts are another popular cell line, which, unlike the CHO cells, can grow layer-on-layer to fill the remaining volume. Therefore, they are considered good cell lines to examine the cell culture ability of the 3D fibrous scaffolds. The ability to culture fibroblasts also indicates the potential application of the scaffolds for skin repair.

Seeding efficiency reveals the ability of cells to attach on a scaffold material. The seeding efficiencies of both cell lines on the fibrous matrices are listed in Table 1. The PCL-5 has the largest seeding efficiency for both fibroblast and CHO cells. The matrices having a smaller mean pore size showed larger cell population. The seeding efficiency for the fibroblasts was larger than that of the CHO cells. 2D PCL film showed lower seeding efficiency than the commercial 2D culture plate.

Figure 2 shows the confocal microscopy images of the CHO and fibroblast cells growing in the fibrous matrices. Cell distributed uniformly on the fibers, not only on the surface but also at various depths of the matrices. However, confocal microscopy has a limitation to collect clear images over 300 μm in depth. To confirm cell growth throughout the whole thickness of the matrix samples, μ-CT was used. As evident in the reconstructed μ-CT image in Figure 2c, many OsO4-stained cells (red spots) were observed on the surface of fibers (green) inside the 3D scaffolds. In contrast, a μ-CT image of the PCL fibrous matrix without containing any cells was also included (Fig. 2d), but no red spots can be seen in the matrix.

The morphologies of CHO cells growing in the PCL fibrous matrices are shown in Figure 3. The cells adhered and spread well on the surface of PCL fibers. Rounded cells, indicative of nonviable or unhealthy cells, were not observed, further demonstrating the conductive environment for cell culture. Live CHO cells mostly maintained a spindle shape, even though some cells with elliptical and polygonal morphologies were observed. The cells spread and proliferated in a random arrangement around the fibers. They wrapped around the fiber surface and grew along the fiber axis without directional preference.

The cell morphology on the fibrous scaffolds varied over time. After 3 days of seeding, CHO cells cultured on PCL-20 proliferated throughout the matrix, indicating that the matrices are suitable for the growth of CHO cells (Fig. 3a, a’). On the 7th day of culture, the cells covered more surface of the fibers and they thickened to anticipate rounding up for mitosis (Fig. 3b, b’), presumably because of the ability of CHO cells to change in response to contact with other cells in the population.34,35 With an increase in the culture time, the cell population increased dramatically (Fig. 3c, d). As a consequence of the adaptive processes, cells shape changed. Viable cells were obviously in a highly asymmetric (bipolar) form, toward spindle-like shaped, and orientated along the fiber direction due to a process called contact guidance.36–38 Cell growth on other fibrous matrices showed a similar tendency.

For comparison, CHO cells were also cultured on 2D culture plates in the same conditions and their morphology on the 14th day of culture is shown in Figure 3e. CHO cells appeared flattened and well spread, but not as elongated as those cultured on 3D PCL matrices. Such differences in cell morphology and cell spread should derive from the apparently different culture environment between 2D film and 3D fibrous structure.39,40

Figure 4 displays morphology of fibroblasts cultured on PCL-20. The arrows point to the cells attached to multiple fibers close to the interfiber bonding sites or where fibers came in close proximity (overlapping fibers). Dehydration and gold coating before the SEM observation are the main causes of the cracks visible in the SEM images, especially for fibroblast layers. Within 7 days of the culture, fibroblasts adhered well and showed a healthy morphology. Especially, cells were observed growing along the fiber axis and bridging between adjacent fibers (Fig. 4b). At the 14th day, the number of bridging cells increased. At day 21, layered fibroblasts were observed. These results suggest that fibroblasts attached, spread, and grew very well on the 3D fibrous scaffolds during the culture period and that they could form layers spanning the entire scaffold surface with time. By comparison, no layered cell morphology could be seen on the PCL film.

Figure 5a summarizes the mean number of CHO cells growing on the fibrous matrices over 21 days of culture. Because of the low seeding efficiency, the culture number initially on the 2D control should be higher than that on the 3D matrices. This is true for the fibroblasts. The fibroblast cells on the 2D control after 3 days of culture had a higher number than those in the 3D matrices (Fig. 5b). However, for the CHO cells, it showed a reverse trend. We found that the low CHO number on the 2D control in the early days came from the uneven distribution of the CHO cells in the culture plate. Although there was high seeding efficiency for tissue culture plate (TCP), most of the CHO cells preferred to stay on the edge area of the TCP well (Supplementary Data

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**Table 1. Seeding Efficiency of Chinese Hamster Ovary and Fibroblast Cells on Different Fibrous Matrices**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Chinese hamster ovary (%)</th>
<th>Fibroblast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Matrix</td>
<td>Well</td>
</tr>
<tr>
<td>PCL-5</td>
<td>18.3</td>
<td>32.0</td>
</tr>
<tr>
<td>PCL-10</td>
<td>13.5</td>
<td>71.1</td>
</tr>
<tr>
<td>PCL-20</td>
<td>9.6</td>
<td>46.5</td>
</tr>
<tr>
<td>PCL-30</td>
<td>3.7</td>
<td>47.9</td>
</tr>
<tr>
<td>PCL film</td>
<td>3.9</td>
<td>39.2</td>
</tr>
</tbody>
</table>

Seeding efficiency on the two-dimensional plate (TCP) is 93.6% for Chinese hamster ovary and 93.9% for fibroblasts.

PCL, poly(e-caprolactone).
Fig. S18). This led to the occurrence of cell confluence in this area just within 3 days of culture.

The higher cell numbers in the 3D matrices than on the 2D control were maintained for the whole culture period. The CHO cells in the matrices grew at a similar rate during the culture period. However, the cell number on the 2D PCL film and the culture plate was stabilized after 2 weeks of culture, indicating that the cell growth rate slowed down from day 14 of culture. Because of the contact-inhibitive nature, the CHO cell growth is surface area dependent. Larger surface area in scaffold should result in more cells to grow. This could be the main reason as to why the 2D controls accommodated fewer cells than the 3D fibrous matrices.

The sample PCL-5 had the highest surface area (265.7 cm²), the lowest porosity (73.19%), and the smallest pores (119.6 μm) among the fibrous matrices. It also showed higher seeding efficiency and larger cell number in 3 days of cell culture than the other matrices. The results that longer culture time resulted in decreased cell growth rate could be attributed to two reasons: the growing cell population reduced the pore size, which adversely led to inhibited mass transfers of nutrients and waste, and the cells were confluent in some areas of the matrix.

By comparison with PCL-5, PCL-10 provided larger pores (size above 200 μm) for the cell growth. This also facilitated the nutrient and waste exchange, allowing cell growth at a steady rate. In the first 2 weeks, the cell number in PCL-5 and PCL-10 was very similar. Longer culture time resulted in higher cell growth rate in PCL-10 than PCL-5. PCL-20 and PCL-30 contained larger pores of about 800 and 1000 μm, respectively. The mean cell number on PCL-30 was much less than that on PCL-20 after 21 days of culture, which could come from the reduced overall surface area of the fibrous matrix. These results indicated that pore size played an important role in the cell growth.

The MTS assays performed on the fibrous matrices revealed fibroblast proliferation on the 3D PCL matrices (Fig. 5b). Within the 21 days of the culture period, the number of fibroblasts continuously increased in the fibrous matrices. Comparing with the 2D tissue culture plates, the 3D matrices had less cell numbers at days 3 and 7, because of the low seeding efficiency. However, the cell population on the ma-
FIG. 3. CHO cells in fibrous scaffolds (PCL-20) after (a, a') 3 days, (b, b') 7 days, (c, c') 14 days, and (d, d', d'') 21 days of culture. (e) CHO cells after 14 days of growth on PCL film under the same culture conditions. Cells were fixed with OsO₄, and the images were taken from front or back of samples. All scale bars 50 μm.
FIG. 4. Fibroblast cells on fibrous scaffolds (PCL-20) after (a, a’) 3 days, (b, b’) 7 days, (c, c’) 14 days, and (d, d’, d”) 21 days of culture. (e) Fibroblast cells after 14 days of growth on PCL film under the same culture conditions. Cells were fixed with OsO4, and the images were taken from front or back of samples. Arrows indicate the cells bridging between the fibers. All scale bars = 50 µm. Color images available online at www.liebertonline.com/ten.
Interbonded fibrous tissue scaffolds increased continuously with time and became comparable to 2D tissue culture plates in 2 weeks and then dramatically larger by day 21. The 2D PCL film showed the lowest cell number among the sample groups after 21 days of culture. This can be explained in that the 3D fibrous matrices have a larger surface area than 2D films or well, allowing the cells to spread on the fiber surface. The 3D porous structure also facilitates the cell uptake and exchange of nutrients and disposal of waste.

Again, the porosity, pore size, and surface area of the fibrous matrices affected the fibroblasts growth. The matrix PCL-5 yielded the highest cell number. This trend was maintained for the whole 3-week culture period. This is different from what was observed for the CHO cells, in which the cell growth rate leveled off from day 14. Fibroblasts are large flat cells with a size of about 30–100 μm and a thickness of about 3–4 μm. In this case, cells growing on fiber surface had relatively smaller influence on pore volume and, hence, the media and waste exchange, compared with the CHO cells. As a result, sensibly larger cell population can be achieved on the matrix with a larger surface area, and PCL-5 disk has the largest cell population after 3 weeks of culture. It is reasonable to predict that longer culture period would lead to more fibroblasts growing in the fibrous matrices, and the inhibited mass transfer will happen once the cells take up certain pore volume.

Besides the potential use for skin repair, our more recent study has revealed that osteoblast cells can also be cultured very well on the fibrous matrices (Supplementary Data Fig. S19), which enables them to be potentially used for bone repair and regeneration.

Conclusions

We have demonstrated that melt-bonding of short synthetic fibers combined with a template-leaching technique is a promising method to produce fibrous matrices having stabilized porous network and adjustable porosity. The cell studies using CHO and fibroblast cells suggested that PCL fibrous matrices are biocompatible and conducive to cell growth. Using μ-CT and confocal microscopy, the cell migration and growth inside the fibrous matrices were clearly proven. It was also found that the pore characteristics and surface area played an important role in mediating the cell growth of CHO cells and fibroblasts inside the matrices differently. The integration of large surface area and smaller pores was suitable for the growth of fibroblasts. As the scaffold-making approach developed allows the choice of different fibers and the control of pore size and porosity, fibrous scaffolds with different pore characteristics can be produced to suit different cell lines for many different biomedical applications. As the developed fibrous matrices showed better mechanical performance and more stable pore structure than the conventional nonwoven scaffolds, they could have better performance in tissue engineering applications, which will be demonstrated in our further work.

Acknowledgments

Funding support from the Australian Research Council under its Linkage Project scheme (LP0776751), the Advanced Manufacturing Cooperative Research Centre, and Deakin University under the Central Research Grant Scheme is acknowledged.

Disclosure Statement

No competing financial interests exist.

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FIG. 5. (a, b) Mean numbers of CHO and fibroblast cells on different scaffolds.

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Received: April 14, 2010
Accepted: August 26, 2010

Online Publication Date: October 26, 2010