Electrospun 3D multi-scale fibrous scaffold for enhanced human dermal fibroblasts infiltration

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Abstract: Electrospun polymeric nanofibrous scaffold possesses significant potential in the field of tissue engineering due to its extracellular matrix mimicking topographical features that modulate a variety of key cellular activities. However, traditional two-dimensional (2D) electrospun scaffolds are generally close-packed fiber mats which prohibit cell infiltration and proliferation. Consequently, the applications of electrospun scaffolds in regenerative medicine are limited. In this study, we detail the use of a needle collector to fabricate three-dimensional (3D) electrospun poly-ε-caprolactone (PCL) scaffolds with multi-scale fiber dimensions. The resultant pore size is 4 times larger than conventional 2D electrospun scaffolds with interweaving micro (3.3 ± 0.6 µm) and nano (240 ± 50 nm) fibers. The scaffold was surface modified by grafting with gelatin molecules. It was found that surface modification significantly improved human dermal fibroblasts (HDFs) cell infiltration throughout the 3D multi-scale scaffold. Even after an extended culture period of up to 28 days, cell proliferation was well supported in the surface-modified 3D multi-scale scaffold as confirmed by Ki67 staining. Extracellular matrix proteins secreted by the HDFs was evident on the 3D multi-scale PCL scaffold showing promising potential to facilitate tissue regeneration, in particular dermal tissue engineering.

Keywords: tissue engineering, 3D electrospinning scaffold, human dermal fibroblasts, three-dimensional scaffold, cell infiltration

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1. Introduction

Tissue engineering aims to improve the health and quality of human lives by restoring, maintaining, or enhancing the function of tissue and organ [1]. Among all, skin tissue engineering is one of the most developed areas where engineered skin substitutes are commercially available (e.g., Integra™, Dermagraft™, Apligraft™). However, they do not fully recreate the function and aesthetics of the skin. As a result, it leads to unsatisfactory treatment, especially for full thickness [2] and chronic non-healing wound [3].

Continuous effort is focused on developing biomimicry scaffolds to trigger cell response and function as those tissues of which they aim to restore. One of the approaches is to replicate extracellular matrix (ECM) environment using nanofibrous scaffold. Nanofibers have been demonstrated to promote vascularization [4] and mimic the native ECM [5,6] to potentially develop functional tissue. Recently, nanofibers which are known to have great influence over cellular behavior have been incorporated as promising scaffold feature.
for skin tissue regeneration and wound healing\cite{7}. Scaffold fabrication techniques, e.g., electrospinning, self-assembling peptides and phase separation, have been outlined as the three promising methods to create scaffold of fiber sizes close to the ECM fibrils in nanoscale\cite{8}. Among them, electrospinning offers superior versatility capable of fabricating nanofibrous scaffold of high porosity at controllable structure, low cost, and high repeatability from a wide range of polymers. Furthermore, the use of electrospinning allows tailoring of scaffold’s properties according to targeted tissue. The large surface to volume ratio of the nanofiber scaffold also promotes cell adhesion and cell migration.

Conventional electrospinning collects nanofibers on a plate collector where nanofibers are formed and collected as a 2D mat. This results in densely packed nanofibers with reduced pore size and porosity and it is challenging to build a scaffold with thickness beyond 100 µm using this conventional method\cite{9}. The limited cell infiltration due to the densely packed structure and small pore size has restricted the application of electrospun scaffold\cite{10}. Numerous approaches have been reported to increase the pore size of the traditional electrospun scaffold\cite{11,12}, including mechanical expansion\cite{13}, inclusion of porogen\cite{14}, increment of the fiber diameter\cite{15}, incorporation of sacrificial fibers\cite{16,17}, cryogenic electrospinning\cite{18}, and addition of microscale (3~10 µm)\cite{19,20} or macroscale (~300 µm)\cite{21,22} fibers into the nanoscale fiber (~600 nm) scaffold. However, the fabricated scaffold’s thickness is still limited and cellular infiltration was either not studied or limited to the surface of the scaffold.

A thicker scaffold, with versatility to be optimized to the dimension of wound size, may be helpful for treatment of deep skin injury where greater structural support is required to enhance wound healing. To overcome this inherent limitation associated with traditional electrospinning technique, several variants of electrospinning have been devised\cite{23,24}. In recent studies, collector design has been changed from traditional flat surface to protruded shape to increase pore size in electrospun scaffold\cite{23,25}. For example, fabrication of cotton ball-like 3D scaffold called FLUF (Focused, Low density, and Uncompressed nanoFibrous) mesh used an array of point collectors embedded in a spherical dish\cite{23}. The pore size of the FLUF mesh was increased from typical <1 µm to between 2 µm to 5 µm as viewed under scanning electron microscopy (SEM). The cell infiltration was demonstrated at ~300 µm below scaffold surface using rat insulinoma cell line INS-1. This research demonstrated the feasibility to produce porous nanofibrous 3D scaffold using electrospinning with customized collector. Even though the response of human cells was not investigated in the FLUF mesh, this study has proven the concept of changing collection method in a way of changing the electrical field to collect electrospinning fibers in 3D. However, such a collector must be tailor-made to individual electrospinning setup due to the difference in the dimension and environment which may interfere with the electrical field. Practically this system is difficult to be implemented to different kinds of conventional setup as there are too many parameters which may affect the fiber formation. These parameters include the diameter, thickness and material of the spherical dish, position, number, length and diameter of the needles.

In this work, we aim to fabricate 3D poly-ε-caprolactone (PCL) scaffold with multi-scale fibers via an improved electrospinning process based on the conventional setup. The method is easy to set up and can be adapted by any conventional electrospinning setup. The scaffold fabricated was then surface modified to improve the hyrophilicity of the PCL material for better cell adhesion and penetration. Human dermal fibroblasts (HDFs) were used to check the effectiveness of the 3D multi-scale scaffold for cell infiltration and ECM protein deposition. This strategy provides a cost-effective and feasible solution for overcoming the current challenges based on conventional electrospinning to produce 3D instead of 2D scaffold and has great potential across a wide range of tissue engineering applications\cite{26}.

2. Materials and Methods

2.1 Materials

Poly (ε-caprolactone) (PCL) (Mn 80,000) granules, Type A gelatin derived from porcine skin, 25% glutaraldehyde, and ethylenediamine (Fluka) were purchased from Sigma Aldrich. Organic solvent dichloromethane (DCM) was purchased from TEDIA, USA. N,N-Dimethylformamide (DMF) was purchased from Merck, USA. HDFs were purchased from Life Technologies, USA. Phosphate buffer saline (PBS), low glucose Dulbecco’s Modified Eagle Medium (DMEM), high glucose DMEM, gold fetal bovine serum (FBS), L-glutamine and 1% penicillin-streptomycin were purchased from PAA Laboratories, Pasching, Austria.
Minisart High Flow 0.2 µm syringe filter unit was purchased from Sartorius Stedim Biotech S.A., Aubagne, France. Jung tissue freezing medium was purchased from Leica Instruments, Germany.

2.2 Fabrication of Electrospun Scaffold

Electrospun scaffolds were fabricated using electrosprinning chamber, Nanon-01A (Mecc Co. Ltd, Japan). Briefly, PCL was dissolved in DCM and DMF at 3.5:6.5 (v/v) ratio to obtain 13% (w/v) solution. The polymer solution was spun through 21G metal nozzle at accelerating voltage 18kV and flow rate 0.5 mL/h. Working distance was set at 15 cm for 2D scaffold collected on collector plate, and 7.5 cm for 3D scaffold collected on a 7.5 cm stainless steel medical hypodermic 18G needle insulated from ground. Electrospinning was carried out for 4 hours for collection of 2D scaffold and 30 minutes for collection of 3D scaffold. Collected electrospun scaffolds were dried in vacuum oven at 37°C for 1 week to remove any residual solvent.

2.3 Surface Modification

Gelatin was grafted onto the electrospun fiber surface through aminolysis.[27] Briefly, scaffolds were washed in 70% ethanol and deionized (DI) water, followed by immersion in 40%(v/v) ethylenediamine at room temperature for 14 hours. Scaffolds were then rinsed 3 times with DI water for 10 minutes to remove free ethylenediamine. After that, scaffolds were immersed in 2.5% (by weight) glutaraldehyde (GA) for 4 hours at room temperature, followed by washing 3 times with DI water for 10 minutes. GA grafted scaffolds were then incubated in 3 mg/mL filtered gelatin at 37°C for 24 hours. Gelatin immobilized scaffolds were then rinsed 3 times with DI water for 10 minutes to remove free gelatin. Lastly, prior to cell culture, scaffolds were sterilized under ultraviolet light for 10 minutes.

All chemical treatment and washing processes involving the use of solution were carried out within vacuum chamber to ensure complete perfusion of the solution. Prior to subsequent characterization, all scaffolds were freeze-dried to ensure the volume and shape of the scaffolds remained intact, and no moisture was trapped within the scaffolds.

2.4 Contact Angle Measurement

Static water contact angle measurements on 2D electrospun scaffold membranes were carried out using FTA 200 (First Ten Angstroms, USA) via the sessile drop method with 0.5 µL of DI dispensed from the syringe of the system.

2.5 Mechanical Testing

The mechanical properties of 2D electrospun scaffolds were investigated using Instron microtester 5848. The samples were die cut into dumbbell shapes according to ASTM D638. The sample was tested under a crosshead speed of 10 mm/min at room temperature. 5 surface modified scaffolds and 5 surface non-modified scaffolds were tested to investigate the effect of surface modification on the mechanical properties of the scaffolds.

2.6 SEM Characterization and Fiber Diameter Measurements

The electrospun PCL scaffolds were sputter coated with gold for 30 seconds at 18mA. The size and morphology of scaffolds were observed using SEM (JEOL JSM-5310). Fiber diameters were measured using ImageJ[28] from triplicates. At least 50 measurements were taken at random locations in SEM micrograph.

2.7 Porosity and Pore Size Measurements

Micrometritics Autopore IV 9500 Mercury Porosimeter was used to investigate pore size and porosimetry of non-modified 3D and 2D electrospun scaffold. The range of pore diameters, \(d_p\), could be calculated using the Washburn equation (Equation (1)).

\[
d_p = \frac{4\gamma \cos \theta}{P}
\]  

where \(\gamma\) is the surface tension of mercury, \(\theta\) is the contact angle between the mercury and the scaffold and \(P\) is the pressure. As reported in literature[29], a contact angle of 140° between PCL and mercury in air was used. Four replicates were measured for each scaffold type.

2.8 Cell Seeding

HDFs were cultured in fibroblasts cell basal medium (high glucose DMEM supplemented with 10% Gold FBS, 1% penicillin streptomycin, 1% Amphotericin B). To prepare scaffolds for cell culture, 3D scaffolds were cut into 0.6×0.6×0.6 cm³ cube while 2D scaffolds were cut into 1.6×1.7×0.08 cm³ sheet. The cells were seeded at same density (500 cells/mm²) on 2D and 3D scaffolds. Cell culture media were changed every two days.
2.9 Cell Infiltration Characterization

Cryosectioning technique was employed to obtain the information of cell infiltration. HDFs were cultured on electrospun scaffold for 24 hours. After that, the cells were washed with phosphate buffer saline, and then fixed with 4% paraformaldehyde for 20 minutes, followed by 3 times rinsing in PBS for 10 minutes. The fixed samples were embedded in tissue embedding medium (Jung tissue freezing medium), leaving in a fridge overnight at 4°C to allow full penetration. The samples were then frozen in liquid nitrogen and cut into 5 µm thick sections in the center part via a cryostat (CM3050S, Leica Microsystems, Bannockburn, IL). All the samples were placed onto glass slides coated with 1% gelatin. The nucleus of cells were stained with 4',6-diamidino-2-phenylindole (DAPI) that emitted blue fluorescence when viewed under a fluorescent microscope (Eclipse 80i microscope, Nikon). Triplicates were viewed and captured for each scaffold type.

2.10 Detection of ECM Proteins Deposited by HDFs

Surface modified 3D multi-scale scaffold were seeded with HDFs for 21 and 28 days. The scaffolds were then immersed in Jung tissue freezing medium and frozen in liquid nitrogen before kept in a –80°C freezer. Staining of proliferation marker, Ki67 and ECM proteins, including Collagen I, Collagen III, Fibronec- tin and Elastin, were carried out according to standard protocols. Positive control (mouse multi-tissue) and negative control (samples stained in the absence of primary antibody) were stained for comparison during immunohistochemistry study.

3. Results and Discussion

3.1 Surface Modification of 2D Electrospun PCL Scaffold

When hydrophobic PCL was electrospun into a 2D fiber mat with high surface roughness and pores, the wettability was significantly decreased further. The contact angle of this 2D electrospun PCL fiber mat measured with sessile drops method was 116°, showing the high hydrophobicity of the surface. The superhydrophobic nature of 2D electrospun PCL fiber mat limits diffusion of polar fluid such as cell culture medium and cell containing solution into electrospun PCL fiber mat, thereby limiting the functions of a scaffold in promoting cellular infiltration and mass transfer. In order to address this concern, surface modification was carried out in this study. Here, polar amino group was introduced onto the fiber surface by aminolysis. Subsequently, glutaraldehyde was introduced as bifunctional linker to link proteins to fiber surface. Therefore, this allows the modified PCL nanofiber to couple with various hydrophilic biomolecules (e.g., collagen, gelatin, peptides) that would be recognized by cell receptor. Among all, gelatin is chosen in this study because it is recognized as one of the most cost-effective peptides with great potential to promote epithelization and granulation tissue formation during wound healing [30]. After grafting with gelatin molecules, the contact angle measured on 2D electrospun PCL fiber mat was significantly reduced from 116° to 46°, showing enhanced surface wettability. This enhanced surface wettability and bioactivity provided by the gelatin molecules would be essential to promote cell infiltration and proliferation, as well as nutrient exchange within the scaffold.
Figure 1. SEM micrographs of electrospun PCL without and with surface modification reveal no physical deterioration despite the chemical treatment.

Table 1. Table showing similar mechanical properties observed between 2D electrospun PCL scaffold with or without surface modification

<table>
<thead>
<tr>
<th></th>
<th>Young’s modulus, $E$ [Mpa]</th>
<th>Yield stress, $\sigma_y$ [Mpa]</th>
<th>Ultimate tensile stress, $\sigma_{UTS}$ [Mpa]</th>
<th>Yield strain [%]</th>
<th>Elongation at break, $\varepsilon_f$ [%]</th>
</tr>
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<tbody>
<tr>
<td>Surface modified</td>
<td>15.86±4.00</td>
<td>3.14±0.48</td>
<td>5.91±0.17</td>
<td>20.34±2.98</td>
<td>321.13±118.25</td>
</tr>
<tr>
<td>Non modified</td>
<td>13.96±5.35</td>
<td>2.48±0.28</td>
<td>6.28±1.47</td>
<td>19.14±5.06</td>
<td>260.79±58.65</td>
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3.3 Fabrication of 3D Multi-scale Scaffold Using Hypodermic Needle Collector

Replacing the plate collector with a hypodermic needle collector completely transforms the physical structure of fibrous scaffold. Instead of a dense 2D mat as shown, the structure collected using the needle collector was a fluffy ball of loosely interwoven fibers as shown in Figure 2A. The fiber diameter of such 3D scaffold was measured to be a mixture of micro- (3.3 ± 0.6 µm) and nano- (240 ± 50 nm) fibers. The collected scaffold also replicates the inherent micro-nanoscale features in ECM that is essential in triggering series of cell activities [31–36]. The scaffold is therefore termed 3D multi-scale scaffold herein. In comparison, the 2D electrospun PCL collected on traditional plate collector has a relatively uniform fiber diameter with a value of 0.7 ± 0.3 µm (Figure 2C). Mercury porosimetry measurements provided insights into the pore structure of the scaffold collected on the two different scaffolds (Figure 3). Remarkably, the use of a needle collector resulted in an approximately four-fold increment in scaffold pore size (~42 µm) while maintaining the high porosity of about 92%.

The function of the hypodermic needle is to disrupt the closely packed fiber deposition pattern collected using a conventional electrospinning setup (Figure 4A). Hypodermic needle collector has a much smaller collector area compared to plate collector. It confines the electric field from spreading but form into spindle shape [37]. The fibers are therefore randomly deposited around the needle tip which has the highest electric field. When the positively charged polymer solution is deposited onto the needle collector, these positive charges are not discharged rapidly enough due to insulation from grounded conductor plate, and inherent poor conductivity of polymer fibers. As a result, the deposited fibers have residue positive charges. Therefore, repulsive static force exists between the deposited polymer fiber and the subsequent depositing polymer solution jet. This results in the formation of loosely packed electrospun fibers. Over time, the loose structure increased in volume on the needle collector. At the same time, during the collection of fiber on the needle collector, the non-conducted positive charges on needle tip, as well as the movement of spinneret, created a dynamic electrical field that drives the polymer solution to spin into fibers of different diameters.

Unlike the design of tip collector with protruded metal struts [37] or point collector with sharp tip [38] reported earlier, hypodermic needle used in this study is a hollow tube with slanted cylindrical opening (Figure 4B). The slanted area of needle opening offered alternate landing surface for fiber deposition when the sharpest tip was occupied and reduced in electrical conductivity. Therefore, deposited fibers would fold on the needle collector when subsequent fibers landed on the
Electrospun 3D multi-scale fibrous scaffold for enhanced human dermal fibroblasts infiltration

Figure 2. Comparison between 3D multi-scale scaffold and 2D electrospun scaffold. (A) 3D multi-scale scaffold has folded into cotton ball like shape. Scanning electron image on the right shows a mixture of (i) micro-fibers of 3.3 µm diameter and (ii) nano-fibers of 0.2 µm diameter. (B) Diameters of fibers in 3D multi-scale scaffold are mostly in the range of 0.2~0.4 µm, or 2~4 µm. (C) 2D scaffold electrospun scaffold collected on aluminium foil wrapped on plate collector. Micro-fibers of 0.7 µm are densely packed in traditional 2D scaffold.
Figure 3. Mercury porosimeter revealed a four-fold increment in pore size in 3D scaffold in comparison with 2D scaffold, with little change in porosity.

slanted needle opening instead of the needle tip. Eventually, newly spun fibers would wrap around the needle tip and develop into a cotton-like scaffold. The scaffold thickness collected within 30 minutes was more than 6 mm and this is about 75 times more than that in 2D electrospun PCL scaffold. On the other hand, existence of a mixture of micro- and nano-fiber could play a part in disrupting fibers packing, resulting in an increase of the pore size of the scaffold\cite{19-22}. Larger pore size offers higher opportunities for cell infiltration and mass transfer without sacrificing the ECM mimicry nanofeatures.

3.4 Comparison of HDFs Distribution Between 2D Electrospun and 3D Multi-scale Scaffold

Both 2D electrospun and 3D multi-scale scaffold were examined to study the effect of pore size of electrospun scaffold on cellular distribution in scaffold after cell seeding. Both types of scaffolds were surface modified with the chosen surface modification method demonstrated in Section 3.1, and compared to that without surface modification. As discussed earlier, surface modification significantly enhanced hydrophilicity of the PCL scaffold without deteriorating the architectural properties. As shown in Figure 5, 3D multi-scale electrospun PCL improved in water absorption and expanded in phosphate buffer solution only after effective surface modification.

Cell culture results as shown in Figure 6 revealed the difficulty for HDFs to be seeded into traditional 2D electrospun scaffold. Traditional 2D PCL electrospun scaffold had desired porosity for tissue engineering but the dense fiber packing resulted in small pore size which restricted cell to be seeded throughout the whole scaffold (Figure 6A). Even with the aid of gelatin grafting to improve wettability and cell-scaffold interaction, no significant improvement in cellular distribution was observed. HDFs seeded on gelatin grafted 2D electrospun scaffold were found to adhere on the top surface only, despite the enhanced wettability (Figure 6B). This is a common issue that has restricted the application of electrospun scaffold\cite{9,10}. By increasing the pore size of the scaffold using needle

Figure 4. Electrospinning setup. (A) Collection of 2D electrospun scaffold on plate collector; (B) Collection of 3D electrospun scaffold on needle collector.
Figure 5. Surface modification on 3D multi-scale scaffold. PBS can penetrate into modified but not non-modified 3D multi-scale scaffold easily. Therefore, surface modified 3D multi-scale scaffold absorbs PBS and expand in size when immersed in PBS. Scale bar = 2 mm.

collector, better cellular distribution at seeding was observed even for the unmodified 3D multi-scale scaffold. The images of the cryo-sectioned scaffold demonstrated HDFs infiltration after 24 hours of cell seeding but was limited to 21% (1.2 mm) of the total depth (6 mm), presumably due to the hydrophobic surface which retarded further infiltration of medium and cells (Figure 6C). Whereas in the gelatin modified 3D multi-scale scaffold, cells were distributed through the whole thickness of the scaffold (Figure 6D). The low cell number observed here is due to the low initial seeding density which was deliberate in order to have a more definite observation of the effect of cell distribution. It is evident from this study that improvement in 3D scaffold architecture with larger pores, wettability and bioactivity enhance uniform cellular distribution throughout the thickness of the scaffold. Although some studies have reported electrospun scaffold fabricated with enlarged pore size, few have demonstrated the complete cellular distribution throughout the scaffold. Our study has highlighted that in addition to having large enough pore size, it is also important to have hydrophilic and conducive surface properties to promote cell infiltration and migration. This has bridged the electrospinning technology with its potential application by showing thorough cellular distribution and active proliferation into electrospun scaffold with a thickness of a few millimeters.

3.5 Gelatin Grafted 3D Multi-scale Scaffold for Dermal Tissue Engineering

HDFs were seeded in gelatin grafted 3D multi-scale scaffold for 21 and 28 days prior to characterization for its proliferation marker and ECM deposition respectively. As shown in Figure 7, HDFs were proliferating throughout 28 days of culturing as indicated by positive staining of cellular marker for proliferation, Ki67, in cell nuclei. Despite the large scaffold size and high cell density anchor on scaffold’s surface, HDFs has successfully penetrated into the scaffold and remained viable with the proliferative protein expressed. This has indicated efficient nutrient and mass transfer in and out of the millimeter-thick multi-scale scaffold. However, the cell number was observed to be on the
low side due to the low initial seeding density and therefore perhaps a longer culture time or higher seeding density would be required to resolve this issue.

ECM deposition by cells is an essential process for remodelling and repair of skin defects\textsuperscript{[39]} and therefore it is important to characterize this cellular behavior on the scaffold. Deposition of the two fibroblastic origin extracellular matrix proteins, Collagen I and Collagen III, was observed after both 21 and 28 days of culturing in gelatin grafted 3D multi-scale scaffold (Figure 7B and Figure 7C). Elastin, which determines the elasticity of the skin tissue, was also observed to increase in amount over time (Figure 7D). Fibronectin, which is involved in cell adhesion, growth, migration and differentiation, was found to increasingly deposit in bundle format within the scaffold over time, as an evidence of cell-extracellular matrix adhesion (Figure 7E). These encouraging positive stainings of ECM proteins indicated favorable interaction between 3D scaffold and HDFs, which is essential for the eventual application in tissue engineering.

Taken together, the presence of large, interconnected pores in gelatin grafted 3D electrospun scaffold promoted infiltration of HDFs throughout the millimeter-thick scaffold, and also encouraged nutrients and mass exchange which are all crucial requirements of tissue engineering scaffolds. This study has successfully demonstrated a user-friendly and cost-effective needle collector technique to produce 3D electrospinning scaffold with enlarged pores. Coupled with simple surface modification, the scaffold showed promising cellular interaction and support.

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<th>Non modified</th>
<th>Surface modified</th>
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<td>2D scaffold</td>
<td>(A,B)</td>
<td>(C,D)</td>
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<tr>
<td>3D multi-scale scaffold</td>
<td>(A)</td>
<td>(B)</td>
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<td>HDFs were seen only at the external surface of scaffold for both non-modified and surface modified 2D electrospun scaffold. (C) Cross-section of 3D multi-scale scaffold shows cells attached to only the sub-surface region of the scaffold without surface modification. (D) After surface modification, HDFs were seen to have penetrated throughout the 3D multi-scale scaffold. Solid white arrows show cells. (A,B) Scale bar = 100 µm and (C,D) Scale bar = 0.5 mm.</td>
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4. Conclusion

Application of electrospun scaffold in tissue engineering has been hindered by limited cell infiltration into the scaffold due to its dense 2D structure. This study has successfully resolved this issue via two approaches. Firstly, we improvised the collector such that the resultant scaffold would be deposited into a 3D structure with sufficiently large pores for cell infiltration. Besides enlarged pore sizes, the fibers forming 3D scaffold mimicked closely the ECM architecture with multi-scale diameters (from ~200 nm to 3 µm). Secondly, we improved the surface wettability and bioactivity of electrospun scaffold through surface modification. As a result, cell infiltration throughout the entirety of the 6 mm fibrous scaffold was observed after 24 hours of cell seeding. Proliferation and ECM deposition of HDFs in the gelatin grafted 3D scaffold were observed up to 28 days of cell culture. The conducive environment of gelatin grafted 3D multi-scale scaffold for attachment, infiltration and ECM deposition of HDFs may find wide applications in the biomedical field particularly in tissue engineering or as fillers.

Conflict of Interest and Funding

No conflict of interest has been reported by the authors.

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