Biochemical and Biophysical Research Communications 423 (2012) 50–54

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cellular infiltration on nanofibrous scaffolds using a modified electrospinning technique

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ARTICLE INFO

Article history:
Received 8 May 2012
Available online 19 May 2012

Keywords:
Electrospinning
Three-dimensional
Infiltration
Tissue engineering

ABSTRACT

Electrospinning is currently used to fabricate nanofibrous scaffolds for tissue engineering applications. The major problem of these scaffolds is their intrinsically two-dimensional nature which inhibits cellular migration and in-growth. In this study, we have introduced a modified setup of electrospinning to produce three-dimensional nanofibrous scaffolds which allows improved infiltration of cells. An array of focused halogen light bulbs was used to localize the heat in the path of electrospun jet near the collector. The fabricated mats were then seeded with cells in order to evaluate migration and infiltration. After 14 days of culture, a homogenous distribution of cells was observed throughout the scaffolds and showed the three-dimensional architecture of nanofibrous mats. By this novel and simple setup, the prepared electrospun mats will allow the seeded cells to obtain a three-dimensional arrangement which is ideal for tissue engineering applications.

1. Introduction

In the past two decades, electrospinning has attracted much attention for application in the field of tissue engineering and regenerative medicine [1–4]. The main reason is that the architecture of electrospun nanofibers greatly mimics the structure of the natural extracellular matrix (ECM) in the body [5]. Nanofibrous scaffolds prepared via electrospinning benefit from a very high surface-to-volume ratio which is excellent for cell attachment, proliferation and differentiation. In addition, physical and chemical characteristics of these scaffolds can be modified efficiently [6,7]. The cell-seeded electrospun nanofibrous scaffolds suffer from a major drawback; they act as two-dimensional (2D) surfaces which the cells can only attach to the top layer of nanofibers and do not sense a three-dimensional (3D) microenvironment [8]. According to the 3D structure of natural tissues which is composed of specific cells and ECM nanofibrils [9], this problem is critical and should be addressed to fabricate a real biomimetic scaffold for efficient nutrient and waste transport, vascularization and tissue in-growth [10]. In general, there are two proposed approaches to create a 3D combination of cells and nanofibers; the first is to incorporate cells into the spaces between nanofibers during the process of electrospinning [11,12] and the second is to fabricate an intrinsically 3D assembly of nanofibers via fine tuning or process modification of electrospinning [13–17]. In the latter, seeded-cells are forced to migrate inside the scaffolds in a perfusion bioreactor system or naturally persuaded to infiltrate due to intrinsic characteristics of nanofibers. Many attempts have been conducted to use these approaches. Ekaputra et al. showed that cells could penetrate into micro-sized electrospun fiber scaffolds filled with bioactive ECM molecules [18]. Pham et al. fabricated multilayer scaffolds consisted of nano and microfibers which were electrospun layer by layer [13]. They showed that increasing the thickness of nanofiber layer resulted in reduced infiltration. Li et al. demonstrated that blending natural polymers such as elastin and gelatin with PLGA enhanced the migration of MSC inside the scaffold compared with PLGA nanofibers [19]. Zhu et al. used a cylinder with a frame consisted of metal struts as the collector of nanofibers and produced porous electrospun mats with large pores, which allowed cell ingrowth [16]. Leong et al. also introduced cryogenic electrospinning in which the mandrel cooled up to −30 °C to form a pattern of ice crystal on which the nanofibers deposited [20]. After sublimation to remove ice crystals, an electrospun mat with large pores was produced and enhanced cell infiltration was observed in comparison to conventional electrospinning. These large pores were also achieved by Nam et al. that combined salt leaching with electrospinning [17]. In these studies, two main reasons were noted for the infiltration hindrance in electrospun nanofibers: pore size (<5 μm) smaller than cell dimensions and highly densely packed fibers. For penetration into interior spaces, these fibers should be pushed

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0006-291X/$ - see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2012.05.069

aside by seeded-cells. In our previous studies, we used a biochemical approach via application of a bioactive molecule like collagen to enhance cellular in-growth [21]. In this study, we addressed the issue of fiber packing density to fabricate 3D electrospun nanofibrous scaffolds for application in tissue engineering. We hypothesized that loosely packed nanofibers would enable cells to migrate smartly through micro-sized interconnected pores. Loosely packed nanofibers were achieved through a simple modification of the electrospinning process using an array of focused light bulbs.

2. Materials and methods

2.1. Nanofiber fabrication

The conventional electrospinning was performed to prepare 2D nanofibrous scaffolds as described previously [4]. A 12% (w/v) solution of PLLA (M_w = 157,000, Sigma–Aldrich) in dichloromethane/dimethylformamide (Merck, Germany) was placed in a 10 mL syringe which was connected to a 21-gauge needle through an extension tube. A steel grounded collector was used to collect the electrospun nanofibers. The solution was fed through the tube into the needle by a syringe pump with a rate of 1 mL/h. Application of 20 kV-voltage between the needle and collector, forced the solution droplet to leave the needle and deposit on the cylinder in the form of ultrafine fibers. The conventional setup was modified to fabricate 3D nanofibrous scaffolds (Fig. 1). In this novel setup, localized heat was induced by halogen light bulbs focused on the last one-third of the distance between the nozzle and the collector. All other parameters were kept constant at the same values as applied in conventional electrospinning.

2.2. Characterization of nanofibers

The nanofibrous scaffolds were characterized using scanning electron microscopy (SEM). The specimens were coated with gold via a sputter coater and were imaged under a scanning electron microscope (Philips XL30, Netherlands).

2.3. Cell seeding

The human cord-blood derived unrestricted somatic stem cells (USSC) were used for cell culture studies. Collection, isolation, and expansion of USSC were performed as described previously [3]. Briefly, cord blood was obtained from the umbilical cord vein after informed consent of the mother, and the mononuclear cell fraction was separated by density centrifugation over a Ficoll-Hypaque gradient (Pharmacia-Amersham, d = 1.077 g/ml). Growth and expansion of the cells were performed in low glucose Dulbecco's modified Eagle medium (DMEM, GIBCO-BRL, Grand Island, NY, USA) supplemented with 30% fetal bovine serum (FBS, Gibco), dexamethasone (100 nM, Sigma–Aldrich), penicillin (100 U/ml, Gibco), streptomycin (0.1 mg/ml, Gibco), and L-glutamine (2 mM, Gibco). After almost 2 weeks, USSC colonies were appeared, then the cells were detached with 0.25% Trypsin–EDTA (Gibco) and replated. USSC were cultured and extensively propagated for several days in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. For further cell culture, the medium was supplemented with 10% FBS. Prior to cell seeding, the mats were cut into 15 mm-diameter circular scaffolds and were sterilized in 70% ethanol. For SEM analysis, cell-loaded scaffolds were rinsed with PBS and fixed in 2.5% glutaraldehyde for one hour. For dehydration, the scaffolds were placed stepwise in alcohol gradient concentrations and then dried.

2.4. Histological staining

To study the distribution of stem cells throughout the scaffolds, cross-sections were prepared from cell-loaded scaffolds and were stained with hematoxylin and eosin (H&E). The slides were then analyzed under a light microscope.

3. Results and discussion

The ECM-mimicking approach to create tissue-engineered scaffolds has been increasingly attracted the attention of the researchers in this field. Nanofibrous structures can efficiently play this mimicking role if they form 3D fibrillar network for appropriate cell attachment, proliferation and differentiation [5]. Infiltration and 3D distribution of cells throughout a scaffold may be the most important criteria which should be fulfilled by the physical and chemical characteristics of the scaffold. Electrospinning as a versatile, simple and cost-effective method has been used to fabricate the tissue-engineered scaffolds with nanofibrous structure [22]. Compared with other types of scaffolds, electrospun nanofibers have promoted the cell behavior like adhesion, proliferation [23] and differentiation into specific lineages such as osteoblasts [3,4], chondrocytes [24], neural cells [25,26]. However, migration of cultured cells from the surface toward the inner nanofibrous layers of an electrospun membrane has been rarely shown. In some reports, increased infiltration of cultured cells has been demonstrated via different modifications of the process parameters or setup components [13–20]. Most of these studies have focused on the modification of the collector which have influenced the size of surface pores and enhanced the ingrowth of cells through the spaces between

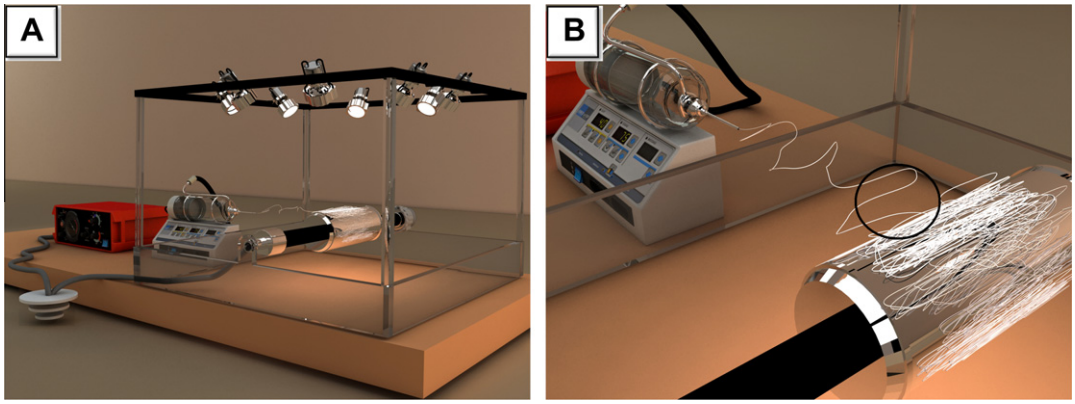

Fig. 1. Scheme for light emission array electrospinning, (A) the whole setup, (B) the location of the focused light in the path of the electrospun jet near the collector (marked by a circle).

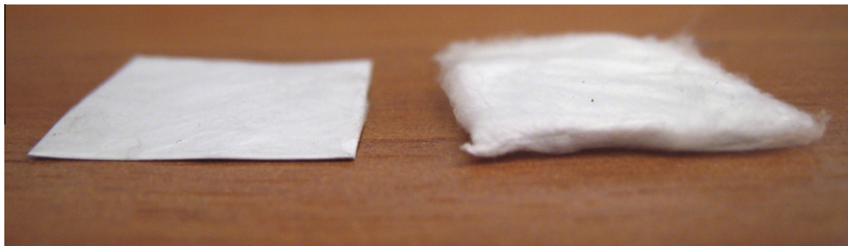

Fig. 2. The gross view of electrospun mats fabricated via conventional (left) or light emission array electrospinning (right).

nanofibers [16–20]. In the present study, we aimed to influence the packing density of nanofibers by insertion of an innovative component into the electrospinning setup. Fig. 1 shows the experimental setup that includes a series of halogen lamps in addition to the conventional setup (CE). The lamps were arrayed in a manner that their light was focused on the last one-third of the tip-to-collector distance. During the process, air circulation was performed to maintain the temperature of the workstation constant. Electrospinning was performed with or without focused halogen lamps while all other parameters were kept constant. The process that included a series of halogen lamps is referred to as light emission array electrospinning (LEAE). Macroscopic photographs from fabricated 2D and 3D mats are shown in Fig. 2. The thickness of the mat from CE was about one-tenth of the mat from LEAE. Qualitatively, it was clearly obvious that nanofibers fabricated by CE showed a much more dense structure compared to those fabricated by LEAE. Despite this, as shown in Fig. 3A (LEAE) and B (CE), there was no significant difference between the sizes of pores in top layers of the scaffolds. These are the pores through which the seeded cells must penetrate after smartly pushing the nanofibers aside. This is necessary for in-growth while the cells are larger than pores about 10 times [27]. Improved infiltration of stem cells on LEAE mats was demonstrated in static culture condition. Fig. 3C and D shows the images taken from the surface of nanofibrous membranes at 14 days after cell seeding. The infiltration of stem cells through the surface pores is obvious in LEAE scaffold (Fig. 3C). This was also confirmed by H&E stained cross-sections of cell-seeded membranes on days 1 and 14 of culture (Fig. 4). Higher amount of infiltration is clearly obvious in mats fabricated by LEAE (Fig. 4B) compared with those produced by CE (Fig. 4D). It

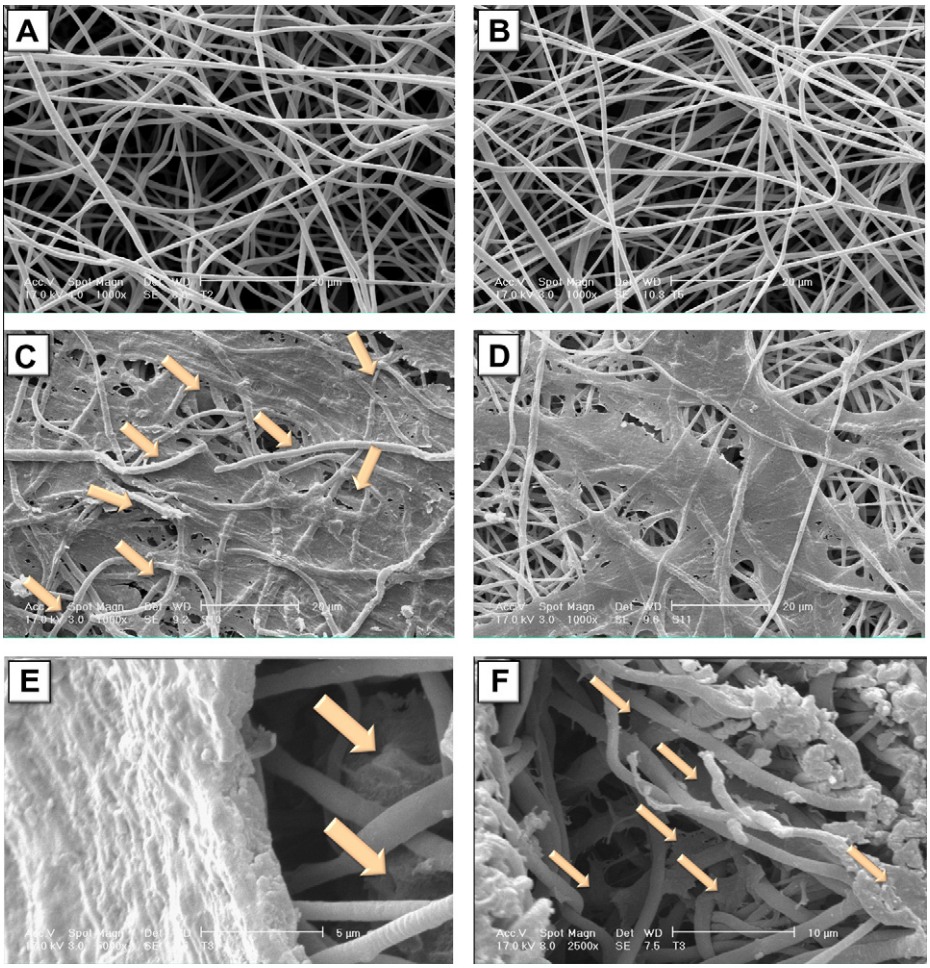

Fig. 3. SEM images of electrospun mats, (A) 3D and (B) 2D pristine nanofibrous scaffolds, (C) cell-seeded 3D and (D) 2D scaffolds after 14 days of culture, (E) the upper and (B) the lower layer of a 3D scaffold cross-section, arrows show the penetration of cells inside the electrospun 3D scaffold.

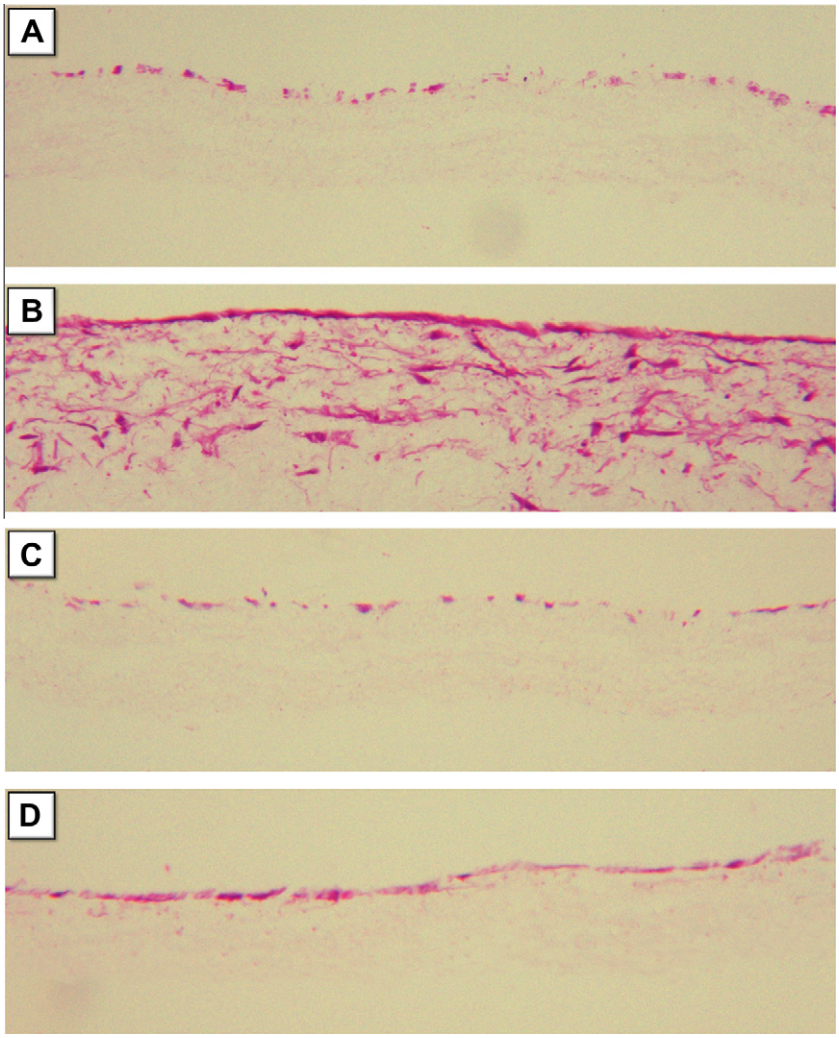

Fig. 4. The images of H&E stained cross-sections of cell-seeded nanofibrous scaffolds after 1 (A and C) and 14 (B and D) days of culture, (A and B) 3D and (C and D) 2D electrospun mats.

is worth noting that on day 1, no sign of cellular entrance forced by seeding was observed in mats. On the other hand, it is indicative that the infiltration in LEAE membranes was only the result of smart migration of stem cells during the culture period. The cell monolayer on the surface of 3D scaffolds and penetrated cells has been shown in Fig. 3E and F. This is very interesting that in contrast to previous reports, the infiltration was enhanced despite this fact that the surface pores were smaller than the size of cells. It is clearly indicative that the stem cells could smartly push the surface nanofibers aside and migrate inside the scaffold. The demonstration of this capacity for stem cells is important since their multilineage differentiation potential is critical for the construction of efficient cell-scaffold complexes in order to regenerate different damaged tissues. To the best of our knowledge, this is the first study which has proposed the fabrication of loosely packed nanofibrous mats as 3D membranes for tissue engineering applications. During the electrospinning process, the polymer solution travels as a jet from the nozzle toward the collector. Within this time, the solvent evaporates via the absorption of heat from the environment. After the formation of electrospun mat, the existence of excess solvent in mats is always a major problem. This issue can be addressed by the incubation of the mat in the vacuum [28]. When the jet reaches the collector, the remaining solvent will make the nanofibers to adhere to each other and form a densely packed electrospun mat. Therefore, the removal of excess solvent before the gathering of fibers on the collector will produce a web consisted of individual nanofibers with the least tendency to attach to or merge with each other. Here, we enhanced the evaporation rate of the solvent via the localized heat in the path of the jet near the collector. A series of halogen lamps was used to induce localized heat in the mentioned region. The spectrum of these lamps contains the wavelength of visible and infra-red (IR) radiation. The near IR emissions have thermal effects, which induce localized heat in the desired region [29].

In this study, the visible light was helpful to focus the invisible IR emissions on the partially solidified jet. Using this simple modification, we have prepared electrospun scaffolds with a high potential to provide a suitable environment for viable cells to proliferate and migrate in a 3D arrangement. This structure is highly appropriate for tissue engineering applications while enables researchers to fabricate 3D artificial tissues containing biodegradable polymers and functional cells. To the best of our knowledge, the results of our study provide new insights into the field of tissue engineering.

Acknowledgments

This work was financially supported by Stem Cell Technology Research Center (Tehran, Iran).

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