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NANOFIBER DESIGN FOR HUMAN STEM CELL CULTURE

Hanwen Sun[1,2], Yancong Zhang[1,2], Linbo Dou[3], Xinfeng Song[1,2], Xiangling Gu[1,2] and Chunhua Fu[1,2]

[1]Dezhou University, China
[2]Shandong provincal engineering laboratory of new pharmaceutical excipients, sustained and controlled release technology, China
[3]Dezhou people's hospital, China

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Abstract. This review describes the recent developments in natural and synthetic nanofibers to support human stem cell (hSC) propagation and differentiation. Nanofibers are promising substrates for regenerative medicine because of their morphologies that structurally mimic the in vivo microenvironment, that is, the extracellular matrix (ECM). Cellular behavior, including attachment, proliferation, and differentiation, can be modulated by modifying nanofiber properties. Nanofibers that have been successfully used for the culture of including human pluripotent stem cells are classified as oligopeptide-immobilized nanofibers prepared from synthetic polymers,or natural polymers, and ECM-modified nanofibers. The combination of human ECM proteins or cell adhesion molecules and nanofibers with well-designed properties provides an alternative to traditional 2D media for the culture and maintenance of the pluripotency or the differentiation of hSCs. Nanofibers with macroporous networks could enhance cell infiltration. Three-dimensional nanofiber scaffolds offer an opportunity for functional tissue regeneration. This review explores the effects of nanofibers on human stem cell pluripotency and their fates.

1. INTRODUCTION

Human stem cells (hSCs), including human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and humaninduced pluripotent stem cells, and adult stem cells, such as mesenchymal stem cells (MSCs) [1-3], have promising potential for drug discovery, therapy, and regenerative medicine. The development of biomaterials for hSC culture is anticipated to affect tissue engineering and the use of hSCs significantly. The most common method used for hSC culture involves Matrigel [4,5].

Matrigel is composed of substrates isolated from the sarcomas of Engelbreth–Holm–Swarm mice; these substrates include laminin, collagen IV, heparan sulfate proteoglycans, enactin, and growth factors [6,7]. However, xenogenic components hinder the clinical application of hSCs. The key factors that influence hSC growth include growth media and environmental factors. Surface-modified nanofibers can provide a suitable living environment for hSCs. Micro- to nanoscale nanofibers have a similar fibrous environment as the extracellular matrix (ECM). Synthetic or natural biopolymer nanofibers have been applied to support the long-term culture of hPSCs because of their high surface-to-volume ratios. These nanostructures can potentially and accurately mimic the natural ECM and provide an optimal space on the subcellular scale for stem-cell attachment and propagation [1,8,9].

2. NANOFIBER POLYMERS THAT SUPPORT hSC CULTURE

A wide variety of polymers that have excellent biocompatibilities can be fabricated into nanofibers.

Corresponding author: Hanwen Sun, e-mail: hanwen916@163.com

Table 1. Polymers used for nanofibers to support stem cell.

Polymer	Cell culture substrate	Stem cell	Ref. (year)
PEG-PCL	O	PMSCs	[15](2012)
PLGA	PDGF-BB	ASCs	[16](2013)
Collagen	O	NSCs	[21](2014)
PCL/chitosan	O	BCSC	[27](2014)
PCL/gelatin	O	MSCs	[28](2014)
PCL	ECM proteins	MSCs	[39](2011)
PLLA	cRGD DGEA	NSCs	[41](2010) [43](2009) [44](2014)

PMSCs, plasma derived mesenchymal cells;
ASCs, adipose-derived mesenchymal cells;
DGEA, collagen I derived Asp-Gly-Glu-Ala-peptide

Polymers used to form nanofibers can generally be divided into two classes, namely, synthetic and natural polymers (Table 1).

2.1. Synthetic polymers

Synthetic polymers, such as poly-L-lactic acid (PLLA) [10], poly(lactic-co-glycolic acid) (PLGA) [11], poly(caprolactone) (PCL) [12], and polyhydroxyalkanoate (PHA) [13], have good mechanical capacity, biodegradability, and stability in vivo and are easy to process and modify (Fig. 1).

2.1.1. PCL

Zhang et al. [14] developed poly(ethylene glycol)-PCL (PEG-PCL) amphiphilic block copolymer nanofiber scaffolds (NFS) as supporting materials for in vitro human placenta-derived mesenchymal stem cells (PMSCs). They demonstrated that PMSCs proliferate robustly and can be effectively differentiated into osteogenic-like cells on the NFS.

2.1.2. PLGA

Manning et al. [15] developed an electrospun nanofiber PLGA scaffold layered by platelet-derived growth factor BB along with adipose-derived mesenchymal stem cells. In vitro studies verified that the cells remained viable and that sustained growth factor release was achieved. In vivo studies on a large animal tendon model verified that the approach is clinically relevant, and that the cells remain viable in the tendon repair environment. However, most of these polymers have hydrophobic surfaces and lack binding sites for cell adhesion.

2.1.3. PLLA

Biodegradable PLLA nanofiber can be prepared by carbon dioxide (CO_2) laser supersonic drawing [16]

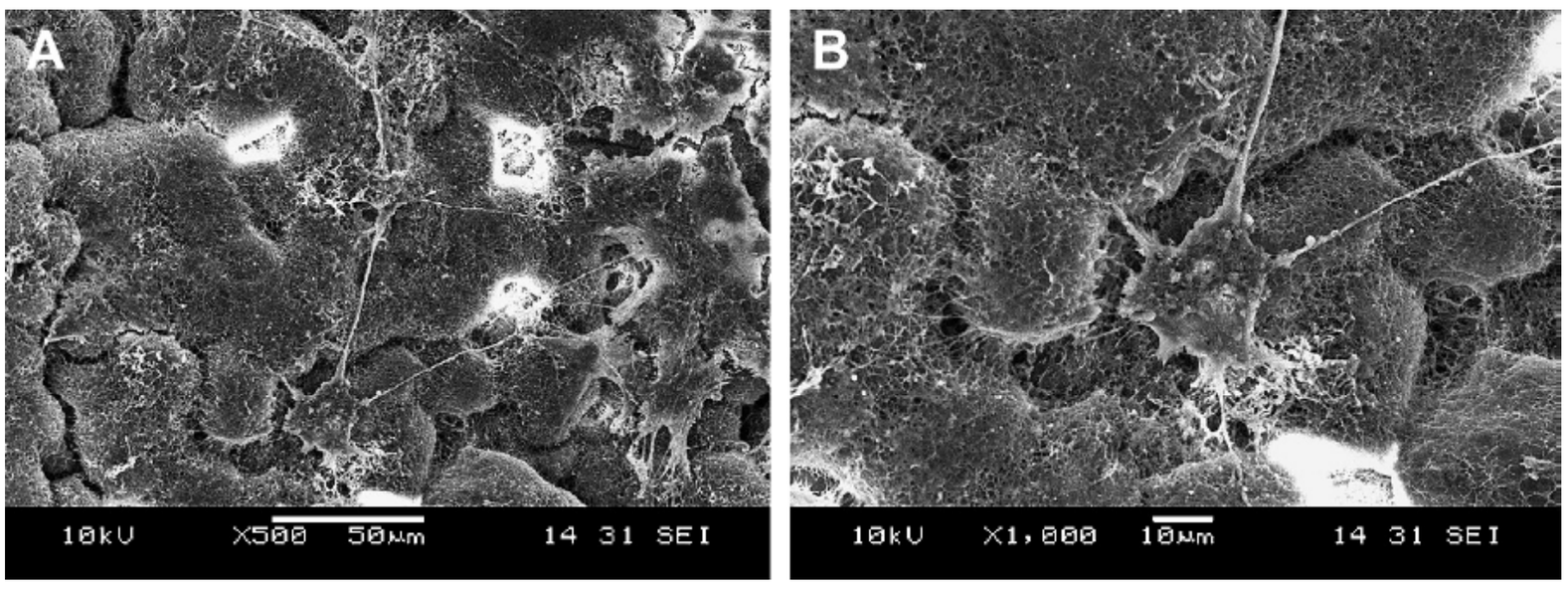

Fig. 1. NSCs grown on the PHBHHx nanofiber scaffold cultured for 7 days: magnification for A: ×500; and for B: ×1000. Reprinted with permission from X.Y. Xu, X.T. Li, S.W. Peng, J.F. Xiao, C. Liu, G. Fang, K.C. Chen and G.Q. Chen // *Biomaterials* **31** (2010) 3967, © 2010 Elsevier.

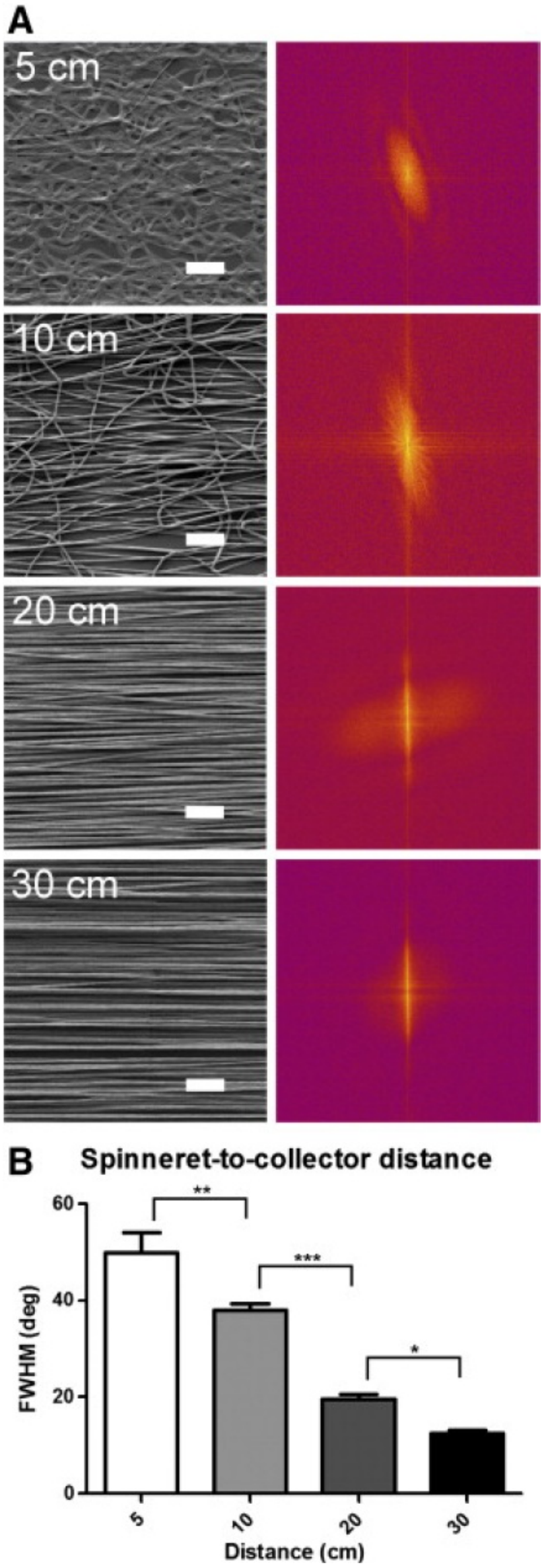

Fig. 2. SEM image and representative FFT image of PLLA nanofiber(A). There is significantly higher alignment with greater tip-to-collector distances (B). reprinted with permission from S.J. Tuck, M. K. Leach, Zhang-Qi Feng and J.M.·Corey // *Materials Science and Engineering C* **32** (2012) 1779, (c) 2012 Elsevier.

or by electrospining (Fig. 2) [17]. PLLA/keratin nonwoven fibrous composite membrane was produced and evaluated by monitoring osteoblast cellular behavior. After 7 d of culture, more cells were observed on PLLA/keratin membranes than on pure PLLA membranes [18].

2.2. Natural polymers

Natural polymers, such as collagen [9], gelatin [19,20], and chitosan [21], are important components of the natural ECM of human and alga. Natural polymers have inherent bioactivity with peptide sequences that promote cell adhesion.

2.2.1. Collagen

Yanling et al. [22] demonstrated that neural stem cells (NSCs) cultured on both randomly oriented and aligned collagen nanofibrous scaffolds were inhibited by an ERK inhibitor (PD98059) and showed higher frequencies of miniature excitatory postsynaptic currents than that on the collagen-coated control.

2.2.2. Gelatin

The hPSCs on electro-spun gelatin nanofibers under feeder- and serum-free culture conditions maintained pluripotency for more than 20 passages without introducing any abnormal chromosome [23].

2.2.3. Chitosan

The high viscosity of chitosan, which limits its spinability is resolved by alkali treatment. Nanofiber diameter is strongly affected by electrospinning conditions and solvent concentration [24]. The biocompatibility of the collagen–chitosan fibers is better than any single ingredient [25].

2.3. Composite polymers

However, natural polymers also have disadvantages, such as poor mechanical properties and rapid degradation rate. Blending both types of polymers is a feasible way to reduce the disadvantages of both [26,27]. Composite nanofibers, exploit the bioactivity of natural polymers and the physical properties of synthetic polymer, have been used to fabricate nanofibers.

2.3.1. Chitosan composite polymers

In the biological tests of NFS fabricated by Steffens et al. [28] using poly-D,L-lactic acid (PDLLA) associated with *Spirulina* biomass (PDLLA/Sp), stem cells were found to adhere more and had greater viability in PDLLA/Sp molds compared with the PDLLA scaffolds. Sims-Mourtada et al. [29] found that breast-cancer stem-like cells cultured on 3D

templates of electrospun PCL-chitosan nanofibers exhibited increased mammary stem cell markers and sphere-formation abilities compared with cells cultured on polystyrene culture dishes. An increase in the proliferation of stem cell populations was not observed. The increase in stemness was accompanied by increased resistance to docetaxel and doxorubicin. Leung et al. [30] used aligned chitosan-PCL nanofibers to mimic the microenvironment of native muscle ECM and showed enhancement of myogenic differentiation of hESCs.

2.3.2. Collagen composite polymers

Kun et al. [31] fabricated NFS with poly(D,L-lactide-co-glycolide) blended with collagen I and further coated with E-selectin using electrospinning, which showed that blended NFS significantly increases cell capture percentage at room temperature. Schofer et al. [32] developed a nanofiber scaffold by blending PLLA with collagen I and found that cell growth was better enhanced when blends at a ratio of PLLA-COLI 4:1 were used. hMSC growth, as well as osteoblast differentiation, was improved compared with PLLA nanofibers alone.

2.3.3. Gelatin composite polymers

Binulal et al. [33] fabricated nanofibrous scaffolds with various PCL/gelatin ratios (90:10, 80:20, 70:30, 60:40, 50:50 wt.%) using diluted acetic and ethyl acetate mixture. The results showed that the composite nanofibers containing 30 and 40 wt.% gelatin exhibited an optimum combination of hydrophilicity and degradability and also maintained the structural integrity of the scaffold. hMSCs showed favorable interaction and proliferation on the composite scaffolds. hMSC proliferation was highest in the 30 and 40 wt.% gelatin-containing composites. Ildeu et al. [34] prepared mineralized PCL/gelatin core-shell nanofibers via co-axial electrospinning and subsequent incubation in biomimetic-simulated body fluid containing ten times the calcium and phosphate ion concentrations in human blood plasma. The results suggest that these mineralized nanofibers promote osteogenic differentiation of human adipose-derived stem cells (hASCs).

3. SURFACE MODIFICATION OF NANOFIBERS

In addition to the various available materials mentioned above, nanofibers are usually biomodified for tissue regeneration. The ECM protein and some biomolecules extracted from natural ECM can be incorporated into polymer nanofibers by physical absorption or covalent surface bonding techniques. ECM molecules can modulate cell adhesion, proliferation, and differentiation. ECM interactions between cells and substrate nanofibers can be directed by functional ECM proteins.

3.1. Natural ECM modified nanofibers

hPSCs proved to be suitably cultured on several human cell lines as feeders [35,36].Thus, ECM polymers secreted by the feeder cells can be assumed to be indispensible for hPSCs adhesion. On the basis of this deduction, Matrigel is widely used for feeder-free culture of hPSCs [37]. Matrigel is composite substrates isolated from the sarcomas of Engelbreth-Holm-Swarm mice including types I and IV, which are composed of collagens, laminin, entactin, heparan sulfate proteoglycan, matrix metalloproteinases, and undefined growth factors and other compounds. Matrigel composed of xenogenic substances is limited to research purposes and cannot be used for the culture of clinical-grade hPSCs [38].

Aside from Matrigel, many ECM proteins have been developed to support hPSC self-renewal or lineage commitment. Recombinant vitronectin is a defined substrate that sustains hESC self-renewal [39]. Laminin-511 has been shown to play an important role in sustaining long-term hPSC growth [40]. These ECMs are used as coating materials or immobilized on nanofibers by covalenting.

3.1.1. Fibronectin

Kang and coworkers [41] developed fibronectin (FN)-immobilized PCL nanofibers to improve cardiac function and inhibit left ventricle (LV) remodeling in a rat model of myocardial infarction (MI). In their study, aligned nanofibers were uniformly coated with poly(glycidyl methacrylate) by initiated chemical vapor deposition followed by covalent immobilization of FN proteins. Degree of cord blood-derived mesenchymal stem cell (UCB-MSC) elongation and adhesion efficacy was improved by FN immobilization. Genes related to angiogenesis and mesenchymal differentiations were upregulated in the FN-immobilized PCL nanofibers compared with controlling PCL nanofibers in vitro.

3.1.2. BMP-2

Schofer et al. [42] fabricated an artificial PLLA-based nanofiber scaffold composed of PLLA-collagen type I or BMP-2 incorporated PLLA-collagen type I. The

incorporation of BMP-2 into PLLA-collagen type I nanofibers resulted in a decrease in diameter and pore sizes of the scaffold. MSCs showed better adherence and reduced proliferation on BMP-containing scaffolds.

3.1.3. Collagen

Hashemi et al. [43] developed collagen-grafted polyethersulfone (PES-COL) electrospun nanofibrous scaffold for mouse embryonic stem cell (mESC) culture. The mESCs cultured for seven passages on PES-COL scaffolds exhibited typical undifferentiated morphology, enhanced proliferation, stable diploid normal karyotype, and continued expression of stemness and pluripotency-associated markers Oct-4, Nanog, SSEA-1, and alkaline phosphatase (ALP) compared with PES scaffolds and gelatin-coated plate.

3.1.4. Cytokines

Malcolm et al. [44] developed a 3D electrospun PCL NFS by fiber alignment and aminolyzation modified with brain-derived neurotrophic factor (BDNF) that was found superior to the classical 2D cultureware in promoting in vitro proliferation and differentiation of cortical cells. Their findings indicate that the modified PCL nanofibrous 3D scaffolds are capable of supporting NSCs and their derivatives.

3.2. Oligopeptide-immobilized nanofibers

Although immobilized ECM proteins may be effective in providing cell binding sites and localized signals, the proteins are sensitive to physiological environment and are easily denatured during the multisteps of the immobilization processing. Thus, the application of short peptide sequences derived from the ECM proteins may offer a strategy to fabricate optimal biomaterials for tissue engineering.

3.2.1. RGD

Rudolf et al. [45] coupled the cRGD onto PLLA nanofibers using oxygen plasma combined with EDC/sulfo-NHS activation. When hMSCs were cultured onto the cRGD functionalized scaffolds, cells showed no increased proliferation or cell density but rather displayed an induction of genes associated with the osteoblast lineage. Schofer et al. [46] cultivated hMSC on a functionalized synthetic PLLA nanofiber by direct incorporation of cRGD. However, hMSC showed a better adherence on PLLA-cRGD (d). Nevertheless, this advantage was not reflected during the course of cultivation.

3.2.2. DGEA

Ceylan et al. [47] synthesized nanofibers functionalized with osteo-inductive collagen I-derived Asp-Gly-Glu-Ala (DGEA) peptide sequence to promote bone-like mineralization on the implant surface and found the DGEA-functionalized nanofiber to provide an advantage in the initial adhesion, spreading, and early commitment to osteogenic differentiation for hMSCs.

3.2.3. GFOGER

PCL meshes with oriented topography were created by electrospinning-aligned nanofibers coated with a triple-helical type I collagen-mimetic peptide containing glycine-phenylalanine-hydroxyproline-glycine-glutamate-arginine (GFOGER) motif. The results indicate that GFOGER significantly enhances the migration, proliferation, and osteogenic differentiation of hMSCs on nanofiber meshes. Aligned nanofiber meshes displayed increased cell migration along the direction of fiber orientation compared to the random meshes [48].

4. PHYSICAL CUES OF NANOFIBERS ON STEM CELL FATE

Biological cues, such as ECMs can guide the direction of differentiation of stem cells. Recently, researchers have carried out substantial efforts to identify the potential importance of the physical cues of biomaterials that influence stem cells. The in vivo biomechanical environment is composed of integrated ECM networks; the ECM networks and cells present in a highly aligned orientation in many tissues such as nerve, muscle and skeletal and is, thus, essential in fabricating spatially aligned nanofibers for cell culture.

A purely natural, uniform, and highly aligned nanofibrous ECM scaffold was created by removing the cellular components from the fibroblast cell sheet. The elastic modulus of the scaffold was found to be well-maintained after the decellularization process because of the preservation of elastin fibers. Reseeding hMSCs shows the excellent capacity of the scaffold in directing and supporting cell alignment and proliferation along the underlying fibers [49].

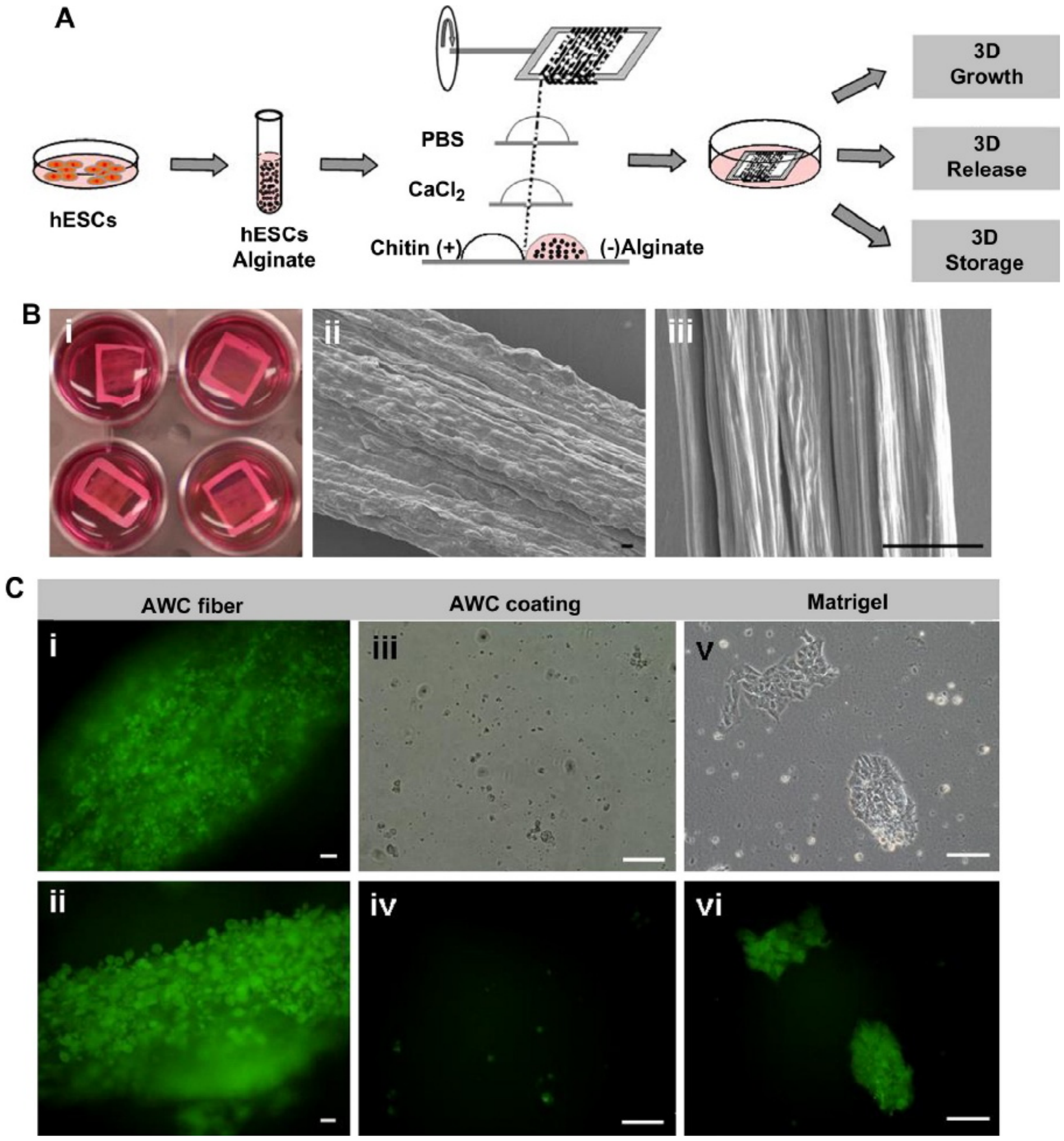

Fig. 3. (A)cell-fiber co-electrospinning process . (B)SEM images for hESC-loaded on AWC fiber (C)The morphology of BGO1V/hOG cells cultured in AWC microfibers. Reprinted with permission from B. Joddar and Y. Ito // *J. Biotechnol.* **168** (2013) 218, (c) 2013 Elsevier.

To mimic highly organized nanofibrous ECM, two kinds of electrospun nanofibrous meshes with different fiber arrangements (totally non-woven and lattice-like) were fabricated and used for in vitro culture of MSCs. Results showed that osteopontin (OPN), osteocalcin (OCN), and ALP, as well as related genes (Runx2 and Colla genes), were all expressed at higher levels on lattice-like nanofibrous meshes than on non-woven ones [50]. Random and aligned PGLA fibrous scaffolds with nanostructure were prepared and the effect of nanofiber morphology on the pluripotent P19 embryonic carcinoma cell proliferation and neural differentiation in response to retinoic acid induction was studied. The results showed that the aligned fibrous substrates provide a suitable surface for cell function and augmentation of cell differentiation because of their similarity with natural ECM [51].

5. CONCLUSION AND DISCUSSIONS

Nanofibers, which structurally mimic the architecture of the native ECM, are attractive materials for the homing and delivery of stem cells. Surface-functionalized nanofibers show advantages for stem

cell adhesion, proliferation, and differentiation, which can be directed by modifying fiber surface chemical components. Aligned nanofibers demonstrate their advantages in directing and supporting cell morphological behaviors such as cell alignment, which shows the important role of fiber alignment. NFS formed by electrospinning are porous and the mechanical properties provide enough surface space to accommodate cells. However, the presently reported nanofibrous scaffolds exhibit limitations relative to cell infiltration because the fibers are commonly electrospun before cells are seeded. Alternatively, some researchers have reported co-electrospinning stem cell incorporation into the scaffolds during nanofiber formation [38,52], the procedure of which is shown in (Fig. 3).Cell viability remains to be further improved and its feasibility needs to be further confirmed by more researchers. In addition, although a variety of studies have revealed the biochemical or physical effects on stem cells, their interactive effects are still not clear. The mutual effect of the two factors is anticipated to be determined in the future.

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