Carbohydrate Polymers 101 (2014) 36–39

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Short communication

Peptide-decorated polyvinyl alcohol/hyaluronan nanofibers for human induced pluripotent stem cell culture

Yi Deng[a,b,1], Xiaohong Zhang[b,1], Yinghui Zhao[b], Shangshang Liang[b], Anxiu Xu[c], Xiang Gao[c], Feng Deng[c], Jing Fang[b,**], Shicheng Wei[a,b,c,*]

[a] Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Peking University, Beijing 100081, China
[b] Center for Biomedical Materials and Tissue Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100081, China
[c] Chongqing Key Laboratory for Oral Diseases and Biomedical Sciences, Chongqing Medical University, Chongqing 401147, China

ARTICLE INFO

Article history:
Received 4 June 2013
Received in revised form
10 September 2013
Accepted 13 September 2013
Available online xxx

Keywords:
Polyvinyl alcohol
Hyaluronan
Nanofibers
Human induced pluripotent stem cells
Cell culture

ABSTRACT

Realization of the full potential of human induced pluripotent stem cells (hiPSCs) in clinical applications requires development of well-defined conditions for their growth and differentiation. A novel fully defined polyvinyl alcohol/hyaluronan (PVA/HA) polysaccharide nanofiber was developed for hiPSCs culture in commercially available xeno-free, chemically defined medium. Vitronectin peptide (VP) was immobilized to PVA/HA nanofibers through NHS/EDC chemistry. The hiPSCs successfully grew and proliferated on the VP-decorated PVA/HA nanofibers, similar to those on Matrigel™. Such well-defined, xeno-free and safe nanofiber substrate that supports culture of hiPSCs will not only help to accelerate the translational perspectives of hiPSCs, but also provide a platform to investigate the cell–nanofiber interaction mechanisms that regulate stem cell proliferation and differentiation.

1. Introduction

Because hiPSCs have the potential to differentiate into all major lineages of somatic cells, they are being studied as important resources for regenerative medicine (Nelson, Martinez-Fernandez, & Terzic, 2010). Successful integration of hiPSCs into clinic applications requires well-defined xeno-free conditions for their growth and differentiation. However, currently Matrigel is the most popular substrate for hiPSCs culture based on the literature and reports. Matrigel is an extraction from Engelbreth–Holm–Swarm mouse sarcomas containing not only basement membrane components, but also numerous growth factors, inhibitors and a broad variety of unknown proteins (Saha et al., 2011). The significant quality variance of Matrigel from lot-to-lot confounds basic research to dissect the molecular mechanisms. Additionally, the presence of animal proteins cause problems related to immunogenicity, microbial and viral contamination (Klim, Li, Wrighton, Piekarczyk, & Kiessling, 2010), which limits clinical applications.

Recently, several 3D polysaccharide nanofibers have been developed for modulating growth and improving differentiation of hiPSCs (Lu, Narayanan, Lim, Gao, Leong, & Wan, 2012; Soman et al., 2012). However, Most of these studies always use Matrigel to help hiPSCs attachment. PVA nanofibers are widely used as biomaterials due to excellent hydrophilicity, high biocompatibility as well as sound mechanical properties (Khatri, Wei, Kim, & Kim, 2012). Vitronectin, one component of extracellular matrix (ECM), is reported to promote proliferation of human embryonic stem cells (hESCs) and hiPSCs (Prowse et al., 2010). In order to graft VP onto PVA nanofibers through standard NHS/EDC chemistry, a polysaccharide containing carboxyl groups was introduced to PVA nanofibers. HA is a major glycosaminoglycan of ECM, which is composed of repeating disaccharide units of β-1,3-N-acetyl glucosamine and β-1,4-glucuronic acid with a large number of carboxyl groups. With excellent biocompatibility and non-immunogenicity, HA finds a wide-range of applications in medicine (Brenner, Schiffman, Thompson, Toth, & Schauer, 2012). Meanwhile, it is also reported that HA can support adhesion and proliferation of hESCs and hiPSCs (Gerecht, Burdick, Ferreira, Townsend, Langer, & Vunjak-Novakovic, 2007). Hence, the incorporation of HA into PVA nanofibers followed by VP immobilization

* Corresponding author at: Department of Oral and Maxillofacial Surgery, Peking University, China. Tel.: +86 10 82195780; fax: +86 10 82195780.
** Corresponding author at: Academy for Advanced Interdisciplinary Studies, Peking University, China. Tel.: +86 10 62753237; fax: +86 10 62753237.
E-mail addresses: jfang@pku.edu.cn (J. Fang), sc-wei@pku.edu.cn, weishicheng99@163.com (S. Wei).
[1] These authors contributed equally to this work.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.030

could be a useful approach to enhance growth for hiPSCs without using Matrigel.

2. Materials and methods

2.1. Preparation and characterization of VP-decorated nanofibers

PVA and sodium hyaluronate powders (Aladdin) were mixed in various weight ratios (100/0, 90/10, 80/20, 70/30, 60/40). The mixtures were dissolved in deionized water with concentration of 8 wt%. The homogenous mixture solution was placed into a syringe with a metallic needle whose inner diameter is 0.5 mm. Electrospinning was performed in a rotating collector covered with Al foil with a voltage of 15 kV (Model SL 60). The obtained nanofibers were crosslinked in glutaraldehyde vapour at 80 °C for 12 h by reacting with hydroxyl groups of PVA and HA, and heated at 100 °C for another 12 h to remove residual glutaraldehyde. Nanofibers prepared from different weight ratios of PVA/HA were named as PVA_{100}, PVA_{90}/HA_{10}, PVA_{80}/HA_{20}, PVA_{70}/HA_{30} and PVA_{60}/HA_{40}.

After being thoroughly washed, samples were incubated in 2 mM EDC and 5 mM NHS in 0.1 M MES solution for 40 min. Then, VP solution (Ac-KGGPQVTRGDVFTMP sequence, 1 mM in sterile PBS) was incubated on the activated carboxyl-rich fibers in 4 °C refrigerator for 24 h. The nanofibers were thoroughly washed before characterization.

Chemical constituents of the nanofibers were analyzed by FTIR (Nicolet) and XPS (Kratos). The fiber morphology was observed by FE-SEM (HITACHI S-4800). Immobilized VP on the nanofibers was quantified by fluorescamine assay. The concentration of unattached peptides in the retrieved solutions was determined according to the manufacturer's instruction using a multilabel reader (Perkin Elmer Ltd.). Solutions with known VP concentration (100–1000 μM) were also reacted with fluorescamine to obtain a standard curve. Six samples in each stage were used to provide an average and standard deviation.

2.2. Culture of hiPSCs on nanofibers

All samples were sterilized using 75% ethanol. hiPSCs generated from umbilical cord mesenchymal cells (Guangzhou Institutes of Biomedicine and Health) were cultured using chemically defined mTeSR™1 media (StemCell) on VP-decorated nanofibers, Matrigel-coated nanofibers and Matrigel-coated culture plate. 600 μL diluted Matrigel™ (BD Biosciences) was pipetted to each well of 12-well plates and quickly incubated at 37 °C for 30 min.

2.3. Characterization of hiPSCs on nanofibers

The adhesion of hiPSCs to nanofibers was assessed using CCK-8 (Dojindo). The absorbance value was measured at 450 nm cultured for 1, 3, 5 days. The morphologies of cells were observed using FE-SEM. For SEM images, all samples were fixed in 2.5% glutaraldehyde for 1 h and then dehydrated with graded ethanol solutions. Then hiPSCs were also subjected to fluorescence staining. For fluorescence staining, cells were fixed using 4% paraformaldehyde for 30 min. After being washed with PBS, cells were incubated for 5 min with 10 μg/mL DAPI (Sigma–Aldrich). The stained signals were observed by a laser confocal microscopy (Carl Zeiss). At last, RT-PCR analysis was carried out using an ABI 7500 machine. Item were run in triplicate and values were normalized on the basic of GAPDH value. The primers used in this study were shown in Table S1.

3. Results and discussion

3.1. Morphology and chemical composition of nanofibers

Fig. 1 shows SEM images of PVA/HA polysaccharide nanofibers. The diameter of fibers varied with the HA contents in the fibers. A broad variety of factors, such as conductivity, viscosity and surface tension (Li, He, Zheng, & Han, 2006), can affect fiber diameter, fiber morphology, and electrospinning ability. The fiber diameter

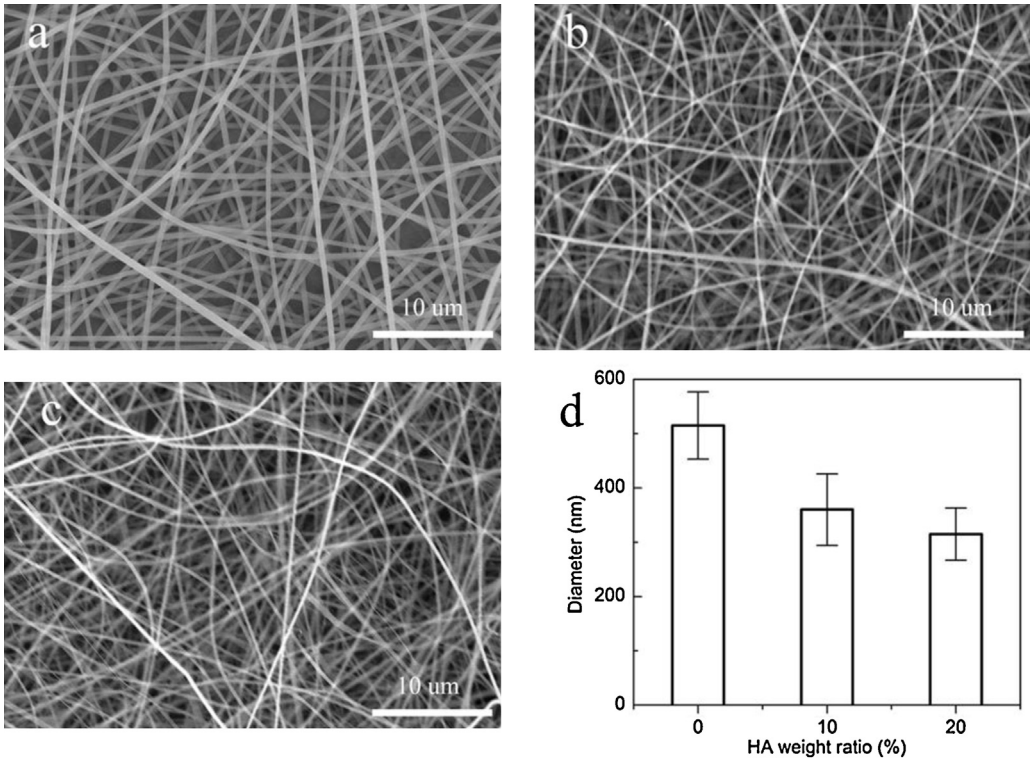

Fig. 1. SEM images of nanofibers: (a) PVA_{100}, (b) $PVA_{90}HA_{10}$, (c) PVA_{80}/HA_{20}, and (d) their diameters.