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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

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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.

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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

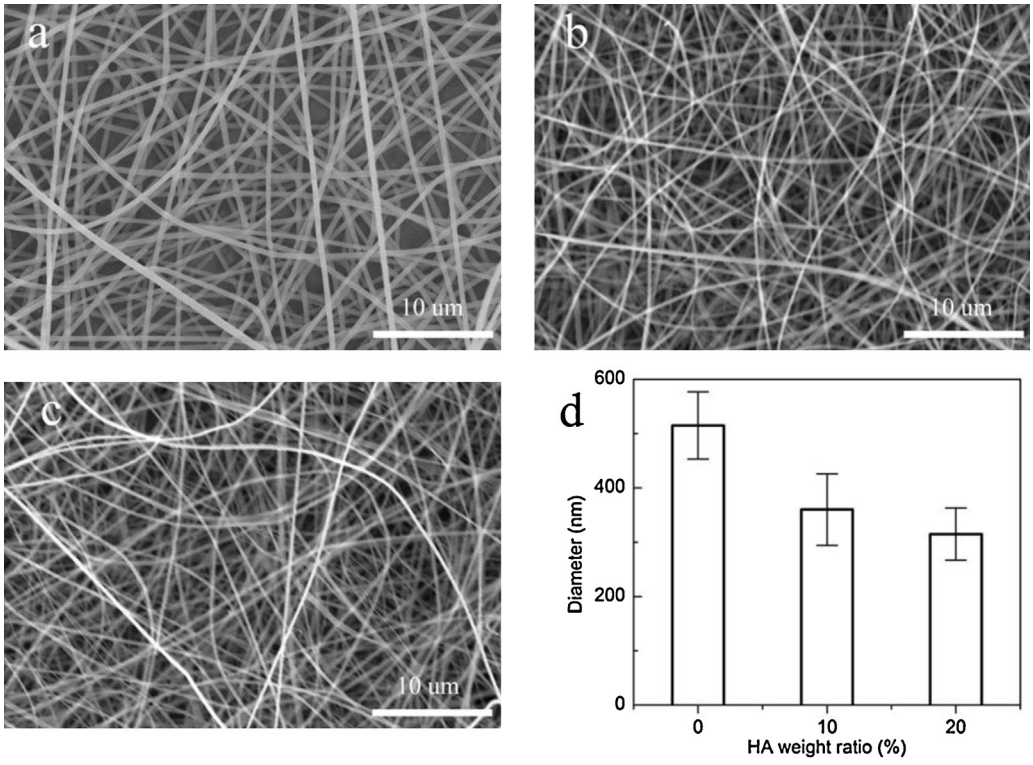

Fig. 1. SEM images of nanofibers: (a) PVA_{100}, (b) $PVA_{90}HA_{10}$, (c) PVA_{80}/HA_{20}, and (d) their diameters.

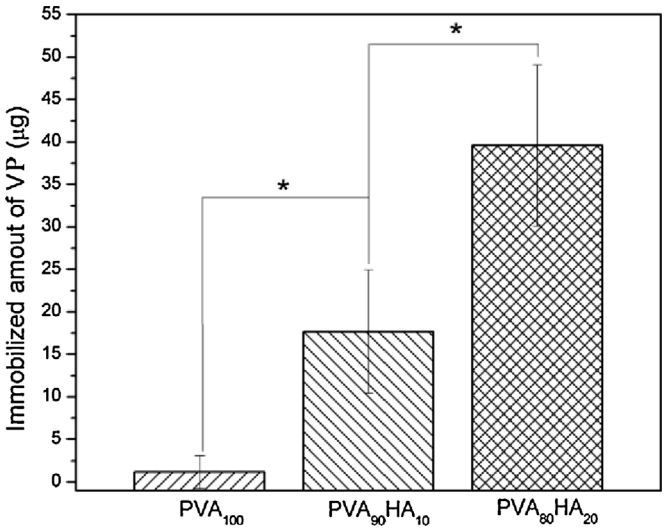

Fig. 2. The amount of VP immobilized on the fibers measured by fluorescamine assay. *Represents $p < 0.05$.

decreased with increasing HA content as shown in Fig. 1. Various weight ratios of PVA/HA were tried and at higher concentrations of HA (>20%) the solutions could not be spun anymore.

The chemical composition of nanofibers is shown in Fig. S1a. The broad peak at 3353.7 cm^{-1} is associated with —OH, and 2931.4 cm^{-1} can be assigned to asymmetric stretching vibration of $—CH_2—$ in PVA (Dilamian, Montazer, & Masoumi, 2013). It was noticed that the characteristic peak of carboxyl groups vibrations appeared and enhanced at 1614.9 cm^{-1} (Brenner et al., 2012), with the increasing of HA contents. The XPS wide scan in Fig. S1b shows the peaks of carbon and oxygen in all samples. However, after VP immobilization, the significant appearance and enhancement of nitrogen signal implied successful grafting of VP on the surface of PVA/HA nanofibers, especially for $PVA_{80}HA_{20}$ ones.

Next, we indirectly calculated the amount of VP on the surface of nanofibers. As shown in Fig. 2, the amount of VP immobilized on the fibers increased as the concentration of HA increased in PVA/HA fibers, consistent with XPS analysis. The carboxyl groups in PVA/HA nanofibers provide the reaction sites for VP immobilization. When reacted with 60 μL VP solution, approximately 40 μg VP was immobilized on 1.9 cm^2 of the $PVA_{80}HA_{20}$ in 24-well plates, which was significantly greater than the amount of VP in $PVA_{90}HA_{10}$, and almost no VP was detected in PVA_{100}.

3.2. Morphology and viability of hiPSCs

It is generally quite a challenge to promote adhesion and proliferation of hiPSCs without Matrigel. In our experiment, a good cell activity of hiPSCs was demonstrated on VP-decorated PVA/HA nanofibers, which implies relatively good adhesion of hiPSCs on VP-decorated PVA/HA nanofibers. Fig. 3 shows that hiPSCs colonies are able to grow on both Matrigel-coated and VP-decorated PVA/HA nanofiber surfaces comparably. The fluorescence staining indicated that hiPSCs cultured on VP-decorated nanofibers grew in tightly packed colonies with a lot of cells, and defined colony borders, similar to those on Matrigel-coated nanofibers. Due to 3D structure of PVA/HA nanofibers, it was difficult to focus on every single cell in a colony. Fig. 4a shows that the OD value increases as time goes on. As for Matrigel-coated nanofibers, the cell viability displayed no statistical differences regardless of the HA concentrations. However, it could be easily found that cell number was lower on surfaces that had less grafted peptide. The cell numbers greatly increased with the increase of HA contents. At 3 and 5 days, no statistical differences were displayed between VP-decorated and Matrigel-coated PVA_{80}/HA_{20} groups. By comparison, no cell adhered on "bare" PVA_{100} nanofiber surface. As mentioned above, VP is reported to support the adhesion and proliferation of hiPSCs *via* RGD–cell integrin interaction, and hiPSCs adhesion is significantly influenced by the peptide density on the surface (Prowse et al., 2010).

3.3. RT-PCR analysis

The pluripotency gene expressions of hiPSCs cultured on the surface of VP-decorated PVA/HA nanofibers were examined. The results showed that hiPSCs cultured on Matrigel-coated 2D culture plate expressed high level of three pluripotent markers (Fig. 4b). Various studies have showed that nanofibers could significantly influence the differentiation of various cell types (Ku, Lee, & Park, 2012), as well as iPSCs (Nelson et al., 2010). Meanwhile, 3D fibrous matrices could serve as temporary ECM for cell growth and differentiation. When hiPSCs were cultured on Matrigel-coated PVA/HA nanofibers compared to those on Matrigel-coated 2D culture plate, the down-regulated expression of pluripotent markers was observed on 3D nanofibers, implying that 3D nanofibers could promote the loss of pluripotency and tend to differentiate, which was consistent with previous reports (Lu et al., 2012). However, much lower pluripotent markers have been detected for VP-decorated PVA/HA nanofibers. The down-regulated expression of pluripotency of hiPSCs is in advance of cell differentiation. The PVA nanofibers are widely used in cell differentiation for bone tissue engineering (Linh & Lee, 2012). The *in vitro* study of PVA/HA nanofibers for hiPSCs culture provided primary information on the potential for bone tissue engineering. Compared with Matrigel-coated nanofibers, the VP-decorated nanofibers avoid the problems related to immunogenicity, microbial and viral contamination. As a consequence, our VP-decorated PVA/HA polysaccharide nanofibers may be suitable for human induced pluripotent stem cell culture without Matrigel coating.

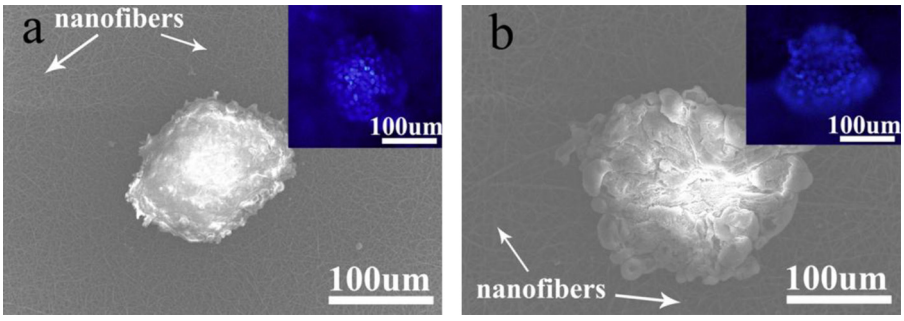

Fig. 3. SEM images of hiPSCs on (a) Matrigel-coated and (b) VP-decorated nanofibers. The insets show the immunofluorescent images.

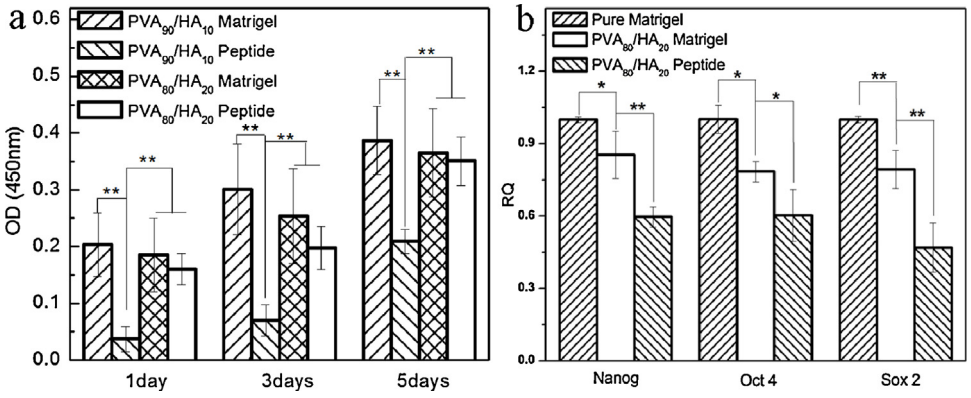

Fig. 4. (a) Viability and (b) RT-PCR results of hiPSCs on the fibers. *Represents $p < 0.05$, **represents $p < 0.01$.

4. Conclusion

In summary, a chemically defined polysaccharide nanofiber has been developed that supports hiPSCs growth and proliferation under fully defined conditions. The nanofibers were prepared by electrostatic spinning of PVA/HA followed by VP immobilization. The VP-decorated nanofibers could support hiPSCs proliferation and promote down-regulated expression of pluripotency. The culture system has advantages of eliminating risks of microbial and viral contamination picked up from animal-derived proteins. Therefore, our VP-decorated PVA/HA nanofiber is favourable for culture of hiPSCs, in addition, we provide critical information on the potential of PVA/HA polysaccharide nanofiber for applications in field of hiPSCs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.09.030.

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