Carbohydrate Polymers 136 (2016) 1061–1064

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Short communication

Peptide-conjugated hyaluronic acid surface for the culture of human induced pluripotent stem cells under defined conditions

Xiaohong Zhang[a,b,1], Ping Zhou[b,1], Yinghui Zhao[b], Mengke Wang[a], Shicheng Wei[a,b,*]

[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 100871, China

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 7 September 2015
Accepted 23 September 2015
Available online 22 October 2015

Keywords:
Hyaluronic acid
Peptide
Human induced pluripotent stem cell
Defined

Chemical compounds studied in this article:
Sodium hyaluronate (PubChem CID: 3084049)
Methacryloyl anhydride (PubChem CID: 12974)
N-hydroxysuccinimide (PubChem CID: 80170)
1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (PubChem CID: 15908)

ABSTRACT

Hyaluronic acid (HA) has been cross-linked to form hydrogel for potential applications in the self-renewal and differentiation of human pluripotent stem cells (hPSCs) for years. However, HA hydrogel with improved residence time and mechanical integrity that allows the survival of hPSCs under defined conditions is still much needed for clinical applications. In this study, HA was modified with methacrylate functional groups (MeHA) and cross-linked by photo-crosslinking method. After subsequent conjugation with adhesive peptide, these MeHA surfaces demonstrated performance in facilitating human induced pluripotent stem cells (hiPSCs) proliferation, and good pluripotency maintenance of hiPSCs under defined conditions. Moreover, MeHA films on glass-slides exhibited long residence time and mechanical stability throughout hiPSC culture. Our photo-crosslinkable MeHA possesses great value in accelerating the application of HA hydrogel in hiPSCs proliferation and differentiation with the conjugation of adhesive peptides.

1. Introduction

Human induced pluripotent stem cells (hiPSCs) are widely used in regenerative medicine since they have the remarkable capacity to self-renew and differentiate into all major lineages of somatic cells with no ethical issues. Generally, hiPSCs are maintained on Matrigel that contains a broad variety of unknown proteins, which exhibit problems related to immunogenicity, animal pathogens and variability of experimental results (Hughes, Postovit, & Lajoie, 2010). Advancement in developing defined conditions for hiPSCs proliferation is required for its clinic application. Many natural and recombinant proteins, including their fragments, have been explored for the self-renewal of hiPSCs. Knowing these biological materials are too expensive for large scale cultivating of hiPSCs, effort was made in exploring economical and easily fabricated synthetic surfaces (Brafman et al., 2010).

Hyaluronic acid (HA), a major glycosaminoglycan of ECM with excellent biocompatibility, has been applied for the culture and differentiation of human pluripotent stem cells (hPSCs) for following reasons: (1) the structure and biomechanical performance of HA hydrogel are similar to native ECM (Collins & Birkinshaw, 2013a; Oommen, Wang, Hilborn, & Varghese, 2014); (2) HA plays an important role in early embryogenesis and feeder layer cultures of hPSCs (Ghatak, Misra, & Toole, 2002); (3) HA is a promising basic material for 3D culture of hPSCs (Gerecht et al., 2007; Liu et al., 2012). However, clinical application of HA hydrogel in hPSCs culture has been hampered by its short residence time and lack of mechanical integrity in an aqueous environment. It also needs an additional incubation with conditioned medium for the adhesion and proliferation of hPSCs (Collins & Birkinshaw, 2013b).

In this study, HA with methacrylate functional groups (MeHA) was prepared and covalently cross-linked using radical initiating method (Smeds, Pfister-Serres, Hatchell, & Grinstaff, 1999). Then, a synthetic peptide from vitronectin (VN peptide),

* Corresponding author at: School and Hospital of Stomatology, Peking University, No. 22 Zhong-Guan-Cun South Road, Hai-Dian District, Beijing 100081, China. Tel.: +86 10 82195780; fax: +86 10 82195780.
E-mail addresses: zhangxiaohong@pku.edu.cn (X. Zhang), zhp07@pku.edu.cn (P. Zhou), zhaoyinghui1987@gmail.com (Y. Zhao), 874912103@qq.com (M. Wang), sc-wei@pku.edu.cn (S. Wei).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.carbpol.2015.09.081
0144-8617/

effective element for the survival of hiPSCs, was immobilized onto MeHA surface. This modified surface was evaluated for its role in supporting the proliferation and pluripotency maintenance of hiPSCs under defined conditions. We believe our chemically defined substrate will not only help to accelerate the translational medicine of hiPSCs, but also provide a platform to investigate the underlying molecular mechanisms that regulate stem cell proliferation and differentiation.

2. Materials and methods

2.1. Preparation of methacrylated HA

Methacryloyl anhydride was slowly added into 1 wt% HA solution at a ratio of 1:5. After dialysised in distilled water for 72 h, the solution was vacuum dried at −80 °C. PBS buffer (pH = 7.4) that containing 2 wt% methacrylated HA and 0.05 wt% photoinitiator IHT-PI 659 was spin coated onto glass-slides at 3000 r/min for 30 s. These glass-slides were immediately cross-linked using a full features UV oven at 232 nm with an intensity of 100 nw/cm^2 for 10 min.

2.2. Immobilization of VN peptide

After incubated in 0.1 M MES solution containing 2 mM EDC and 5 mM NHS for 40 min at room temperature, MeHA-coated glass-slides were reacted with 1 mM VN peptide (Ac-KGGPQVTRGDVFTMP) in PBS buffer at 4 °C for overnight (denoted as NV-MeHA).

2.3. Characterization

MeHA film coated glass-slides were analyzed by FE-SEM at 10 kV after sputter-coated with gold for 1 min. FTIR was used to identify the functional groups of HA and MeHA in the form of pellets (KBr pellet), and the spectra were recorded from 4000 cm^{-1} to 400 cm^{-1}. The chemical composition of these samples was further measured using ^{1}H NMR. After rinsed with distilled water for three times, glass-slides modified by MeHA or VN-MeHA were allowed to dry and analyzed by water contact angle measurement and XPS at 15 kV.

2.4. hiPSCs culture and survival assay

VN-MeHA coated glass-slides were sterilized by 75% ethanol for 1 h. UMC-C1 hiPSCs were seeded as colonies onto VN-MeHA and Matrigel surface in mTeSR™1 medium. The morphologies of hiPSCs on these surfaces were observed using phase contrast microscope and FE-SEM. For SEM images, cells were fixed in 2.5% glutaraldehyde for 1 h, dehydrated with graded ethanol solutions and sputter-coated with gold for 1 min. The cell numbers of hiPSCs after cultured for 1, 3, 5 days were assessed using the CCK-8 reagent at 450 nm. The quantitative RT-PCR analysis was performed using an ABI 7500 RT-PCR machine. All experiments were performed in triplicate, and values were normalized on the basic of GAPDH value. The primers used in this study were listed in Table S1.

3. Results and discussion

3.1. Preparation of MeHA

Cross-linked MeHA films with porous architecture were formed on the glass-slides (Supplementary Fig. 1). The typical peaks of —CONH— at 1610 cm^{-1} in HA shifted to 1640 cm^{-1} in MeHA as detected by FTIR (Fig. 1A), because the amido bond was overlapped by the double bond after introducing methyacrylate groups onto HA. Moreover, peaks appeared at 1260 and 1230 cm^{-1} were putatively assigned to the symmetric and asymmetric stretching vibration of C—O—C in MeHA. ^{1}H-NMR spectrum of MeHA shows the acrylate peaks at 5.6 and 6.1 ppm and the methyl peak at 1.9 ppm (Fig. 1B). The methyacrylation percentage of HA was approximately 11%, which is consistent with the previously published data (Weng, Gouldstone, Wu, & Chen, 2008). These characterizations proved that photocrosslinkable MeHA was successfully prepared. MeHA films exhibit great potential in hiPSC culture since cross-linking was accomplished under mild condition with no applications of toxic organic solvents, and carboxyl groups of HA are retained for further immobilization of adhesive peptide (Collins & Birkinshaw, 2007; Schanté, Zuber, Herlin, & Vandamme, 2011).

3.2. Characterization of VN-MeHA surface

The contact angle (10.54 ± 1.92°) of VN-MeHA surface was evidently lower than that of MeHA surface (37.34 ± 2.27°) (Fig. 2A). Compared with MeHA surface, an apparently enhanced nitrogen signal at 400 eV was found on VN-MeHA surface (Fig. 2B). Moreover, the high-solution C 1s spectrum shows the area of C—O peak in MeHA reduced from 50.92% (Fig. 2C) to 36.42% (Fig. 2D) after conjugated with VN peptide, because VN peptide contains less C—O groups than MeHA. These contact angle measurement and XPS results proved that VN peptide was successfully grafted to MeHA surface.

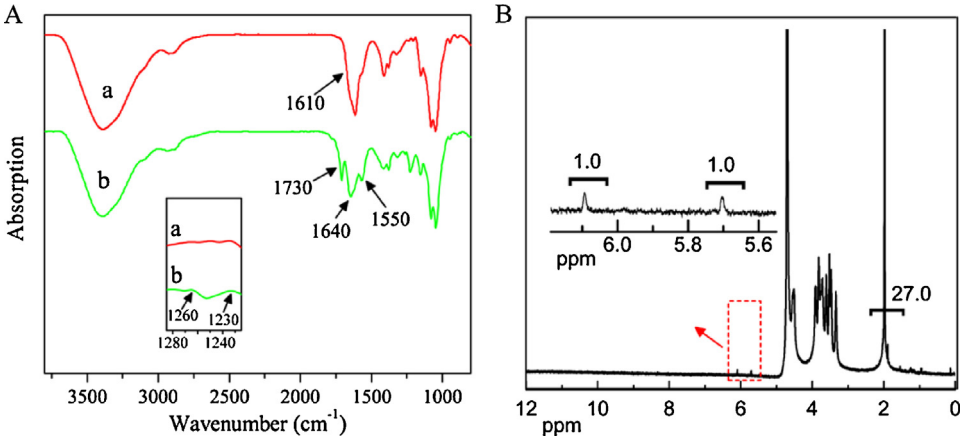

Fig. 1. (A) FTIR of HA (a) and MeHA (b). (B) ^{1}H NMR of MeHA.

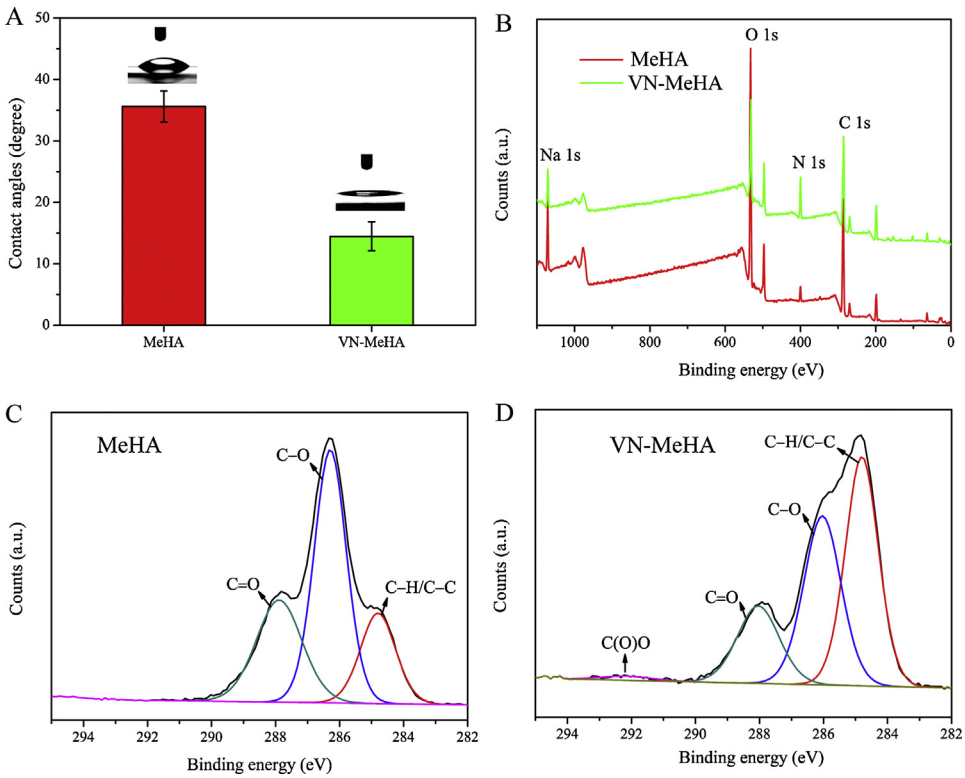

Fig. 2. Water contact angles (A) and XPS wide spectra (B) of MeHA and VN-MeHA surface. (C and D) High-resolution spectra of carbon peaks (C 1s) of these samples.

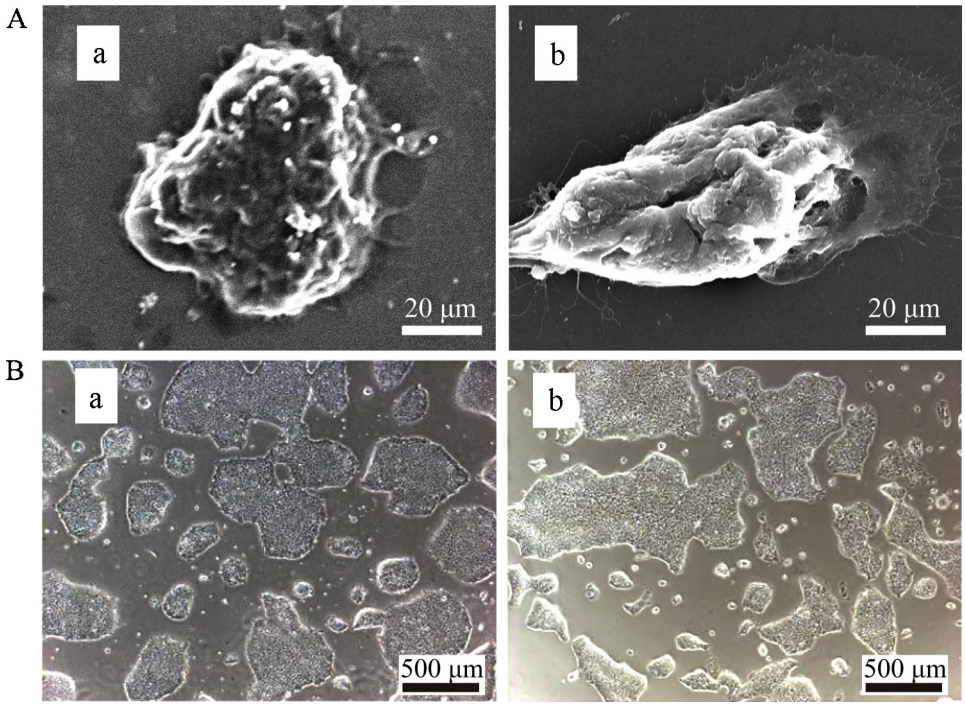

Fig. 3. The morphology of hiPSCs cultured on VN-MeHA (a) and Matrigel surface (b) for 24 h (SEM images, A) and 5 days (B), respectively.

3.3. *hiPSCs survival*

hiPSCs were robustly attached on VN-MeHA and Matrigel with remarkable spreading after seeded for 24 h (Fig. 3A), and maintained typical undifferentiated morphology at day 5 (Fig. 3B). It is worth to note that OD values of cells on VN-MeHA surface was nearly 75% of that on Matrigel surface at each time points (Fig. 4A). Moreover, these hiPSCs expressed equivalent level of pluripotency gene markers including Oct-4, Nanog and Sox-2 (Fig. 4B), proving cells maintained pluripotency after cultured on VN-MeHA and Matrigel for 5 days. Besides, VN-MeHA films were stable throughout the study, demonstrating they possess long residence time and mechanical stability in an aqueous environment.

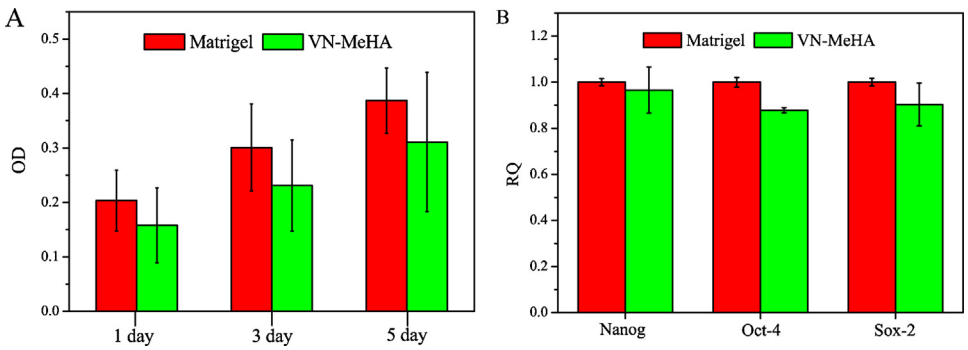

Fig. 4. (A) The OD values of hiPSCs cultured on VN-MeHA and Matrigel surface for up to 5 days. (B) The expression of pluripotent genes of these hiPSCs.

Although Matrigel, a complex mixture of ECM proteins, is benefit to the adhesion of hiPSCs in comparison with VN peptide, VN-MeHA surface has no security issues and exhibits great value in hiPSCs proliferation and tissue engineering.

4. Conclusion

In this study, a UV cross-linked methacrylated HA hydrogel was prepared on glass-slides and followed by conjugation with VN peptide. The modified HA surface could support the adhesion and proliferation of hiPSCs under defined conditions. The growth rate of hiPSCs on VN-MeHA surface was comparable to that on Matrigel surface during 5 day's culture, and the expression of pluripotency genes for these cells was similar. This modified surface is favorable for culture of hiPSC and has the potential to be applied in long-term self-renewal and 3D culture in the future.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (No. 81371697) and Peking University's 985 Grant.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.carbpol.2015.09.081.

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