ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

Prevascularized cell sheets enhance therapeutic effect of extracellular matrix scaffolds in a one-stage skin grafting rat model

Supplementary File

(A) Supplemental methods: preparation of dECM powder

1. Preparation of split-thickness skin: Animal skin is processed into tomographic skin sheets with a thickness of 0.1–2.0 mm using a dermatome. The split-thickness skin sheets are then transformed into sheets of varying shapes using a hole-punching device.
2. Viral inactivation process: The split-thickness skin is inactivated with a viral inactivation solution comprising peroxyacetic acid at a mass concentration of 0.1–2.0% and sodium chloride at a mass concentration of 1.0–5.0% for a period of 30–60 min. The mass ratio of the skin slices to the viral inactivation solution is 1: 4-7.
3. Degreasing process: This process utilizes a mixed degreasing agent consisting of sodium carbonate at a mass concentration of 1.0–4.0%, caustic soda at a mass concentration of 1.0–4.0%, and a surfactant at a mass concentration of 0.1–1.0%. The surfactant used in this process can be one of the following: peregal, sodium dodecylaminopropionate, fatty alcohol polyoxyethylene ether, or traction ketone. The mass ratio of the skin to the mixed degreasing agent ranges from 1:4 to 1:7.
4. Decellularization process: The decellularization solution contains trypsin at a mass concentration of 0.1–0.5%, an activator with a mass concentration of 0.1–0.3%, and a degreasing agent at a mass concentration of 0.2–0.5%. This solution is utilized for decellularization over a duration of 30–60 min. The activator, comprising ammonium sulfate, is maintained at a mass concentration of 0.1–0.3%, while the degreasing agent, Peregal, is used at a concentration of 0.2–0.5%. Additionally, sodium dodecyl sulfate is incorporated at a concentration of 0.2–0.5%. The mass ratio of skin flakes to the decellularization solution ranges from 1:4 to 1:7.
5. Preparation of dECM powder: The uncrosslinked heterogeneous decellularized dermal matrix is lyophilized, ultramicro-porphyrized, and shifted to obtain fine dECM powder with a diameter of 30–750 microns.

(B) Supplementary figures

a.

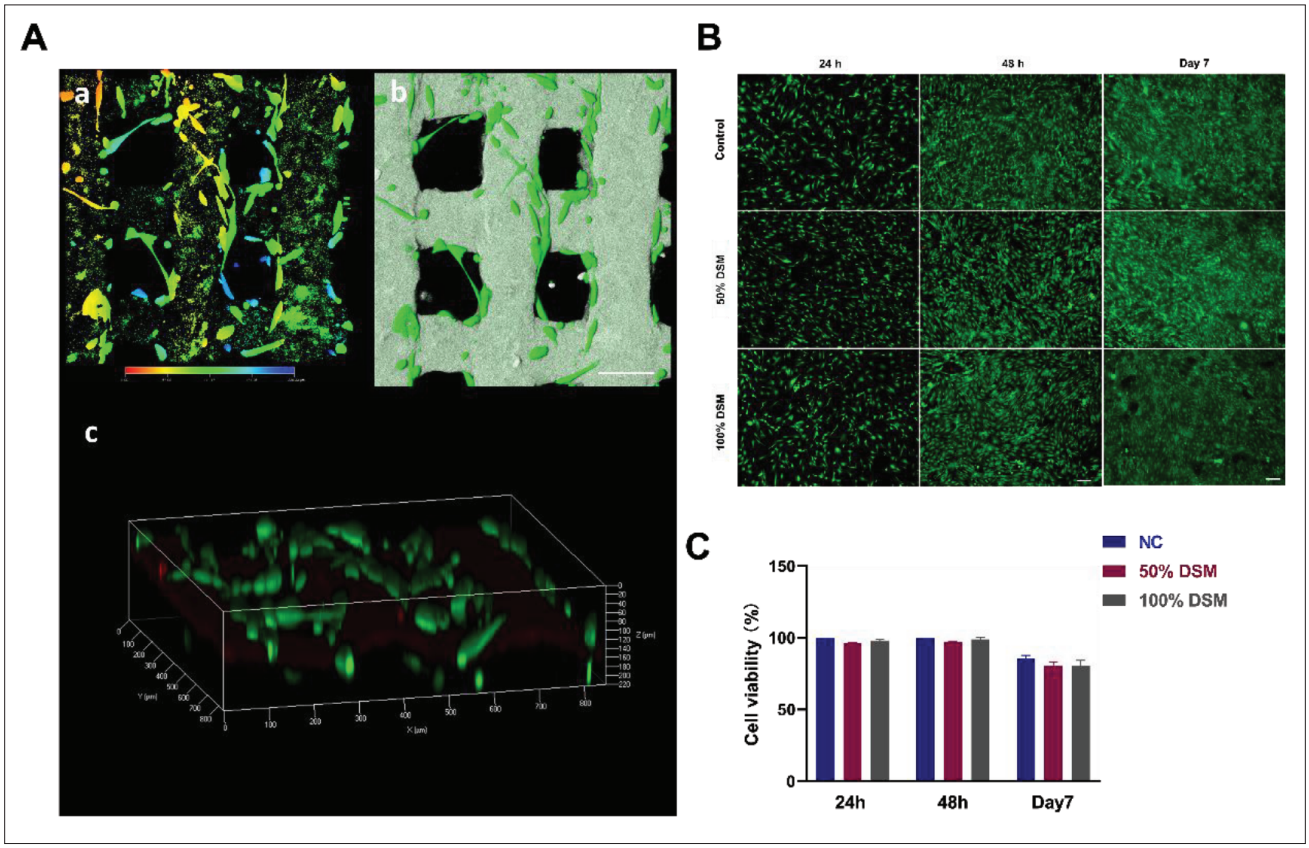

Figure S1. (A) The different 3D images after a 3-day co-culture of hBMSCs and DS. (a) Fluorescent signal Intensity image; (b) maximal intensity projection image; (c) three-dimensional reconstruction image. Scale bar = 100 μm. (B) Representative live/dead cell staining images of hBMSCs cultured with DS-conditioned medium. Scale bar = 100 μm. (C) CCK assay of hBMSC viability. All data are expressed as the mean ± SD. Abbreviations: DS, dermal substitute; DSM, DS-conditioned medium; hBMSCs, human bone-marrow mesenchymal stem cells.

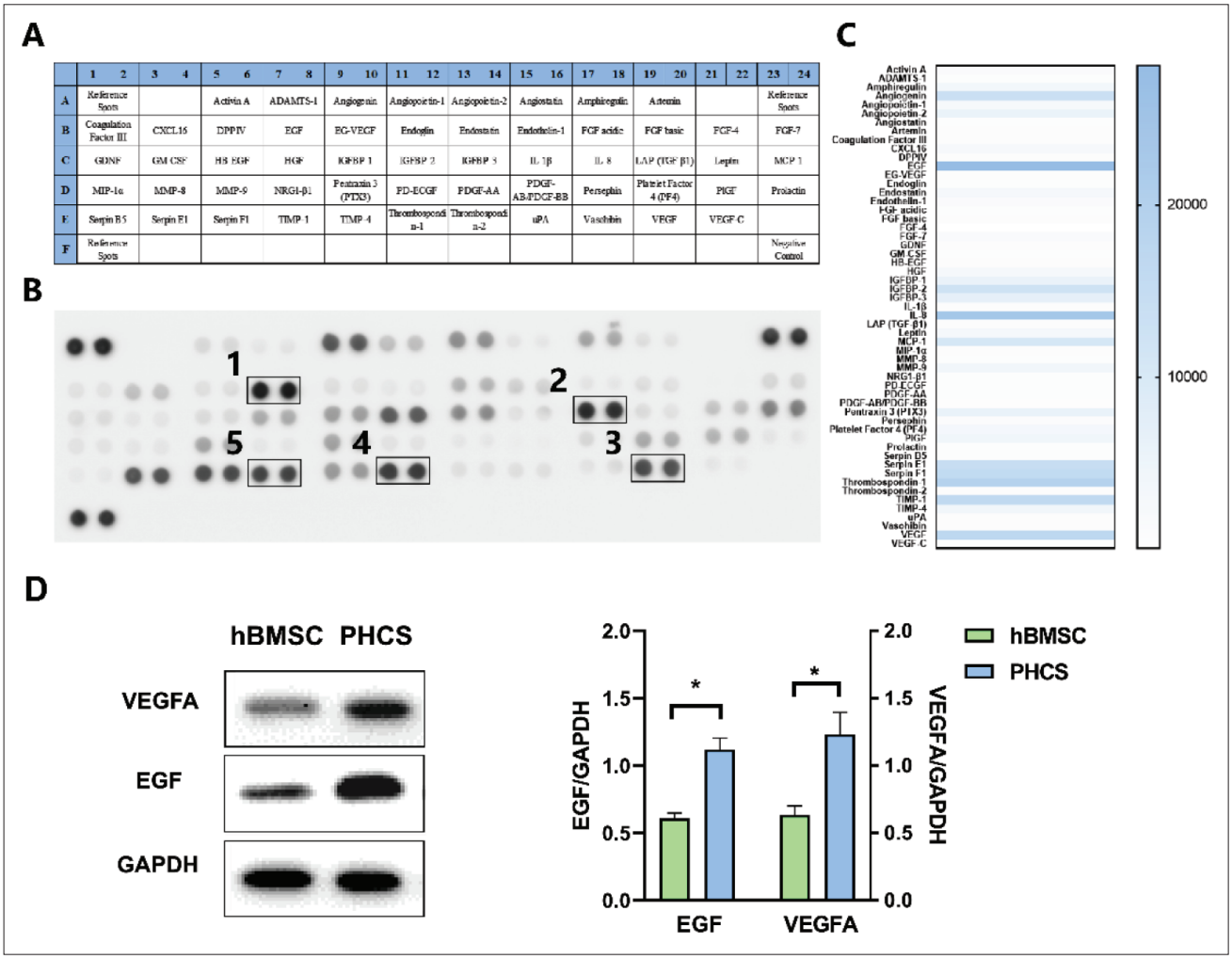

Figure S2. (A) Antibody matrix of the proteome profiler array probe. (B) Representative images of the PHCS supernatant proteome profiler array (1: EGF, 2: IL-8, 3: VEGF, 4: thrombospondin-1, 5: TIMP1). (C) Heatmap of the expression level of the results. (D) EGF and VEGFA protein expression in supernatants. The data are expressed as the mean ± SD (*$p < 0.05$). Abbreviations: EGF, epidermal growth factor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-8, Interleukin-8; hBMSC: human bone-marrow mesenchymal stem cell; PHCS, prevascularized human mesenchymal stem cell sheets; TIMP1, tissue inhibitor of metalloproteinase 1; VEGF, vascular endothelial growth factor.

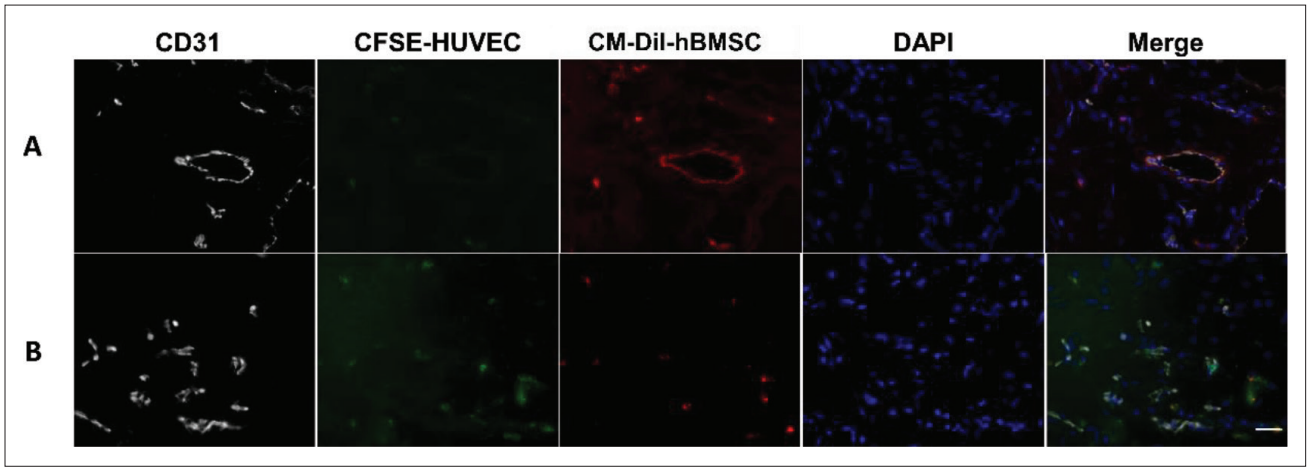

Figure S3. The tracing results of living cells on day 28 postoperatively. (A) BMSCs are involved in microvessel formation as pericytes. (B) PHCS implantation improves local vascularization. Staining colors are as follows: CD31 (white), HUVECs (green), hBMSCs (red) and DAPI (blue). Scale bar = 50 μm. Abbreviations: hBMSC: human bone-marrow mesenchymal stem cell; CFSE, carboxyfluorescein succinimidyl ester; DAPI, 4,6-diamidino-2-phenylindole; hBMSCs, human bone-marrow mesenchymal stem cells; HUVECs, human umbilical vein endothelial cells; PHCS, prevascularized human mesenchymal stem cell sheets.