Supplementary Information

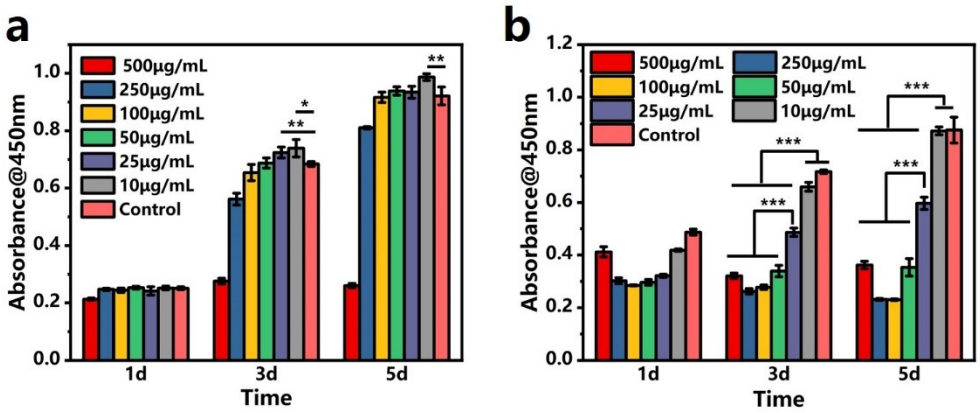

Fig. S1 The proliferation behaviors of HUVECs cultured with (a) MS extracts or (b) MS-containing dispersions with gradient concentrations. *p < 0.05, **p < 0.01, ***p < 0.001, n = 4.

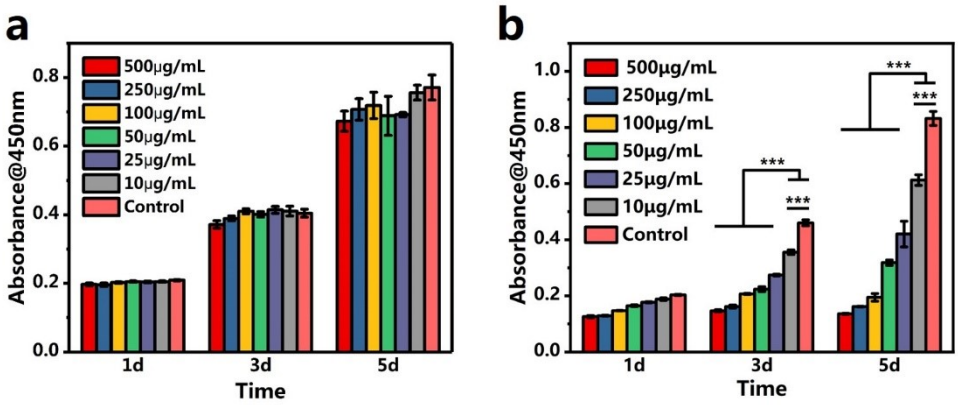

Fig. S2 The proliferation behaviors of HHDPCs cultured with (a) MS extracts or (b) MS-containing dispersions with gradient concentrations. ***$p < 0.001$, $n = 4$.

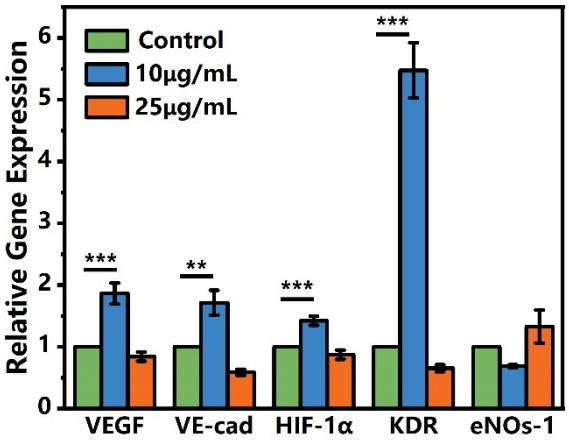

Fig. S3 The expression of angiogenesis-related genes in HUVECs cultured with different concentrations (0, 10, 25 μg/mL) of MS dispersions for 5 days. **P < 0.01, ***P < 0.001, n = 3.

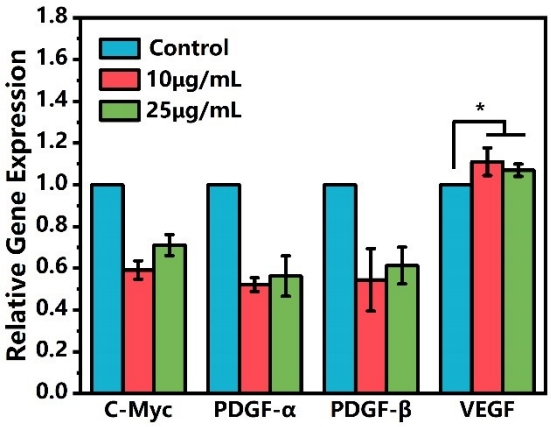

Fig. S4 The expression of genes related to hair follicle differentiation in HHDPCs cultured with different concentrations (0, 10, 25 μg/mL) of MS dispersions for 5 days. *$P < 0.05$, $n = 3$.

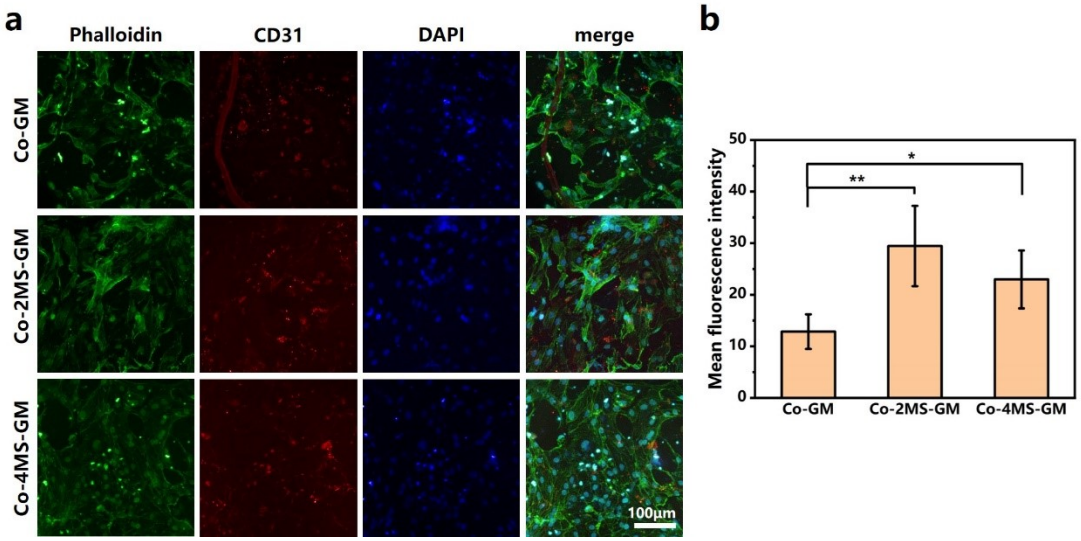

Fig. S5 (a) The immunofluorescence staining of CD31 protein in HUVECs printed in the 3D micropatterns after culturing for 14 days. Scale bar: 100 μm. (b) Statistical analysis of the fluorescence intensity of CD31 expression. *$p < 0.05$, **$p < 0.01$, $n = 4$.

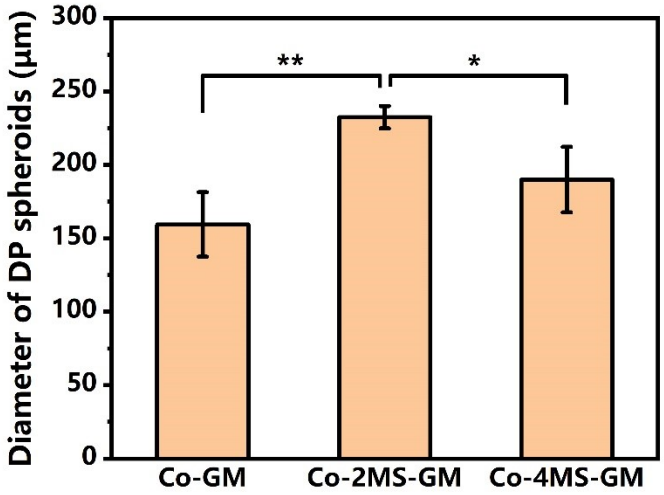

Fig. S6 Statistical analysis of the diameter of DP spheroids in the Co-GM, Co-2MS-GM, and Co-4MS-GM micropatterns. *p < 0.05, **p < 0.01, n = 3.

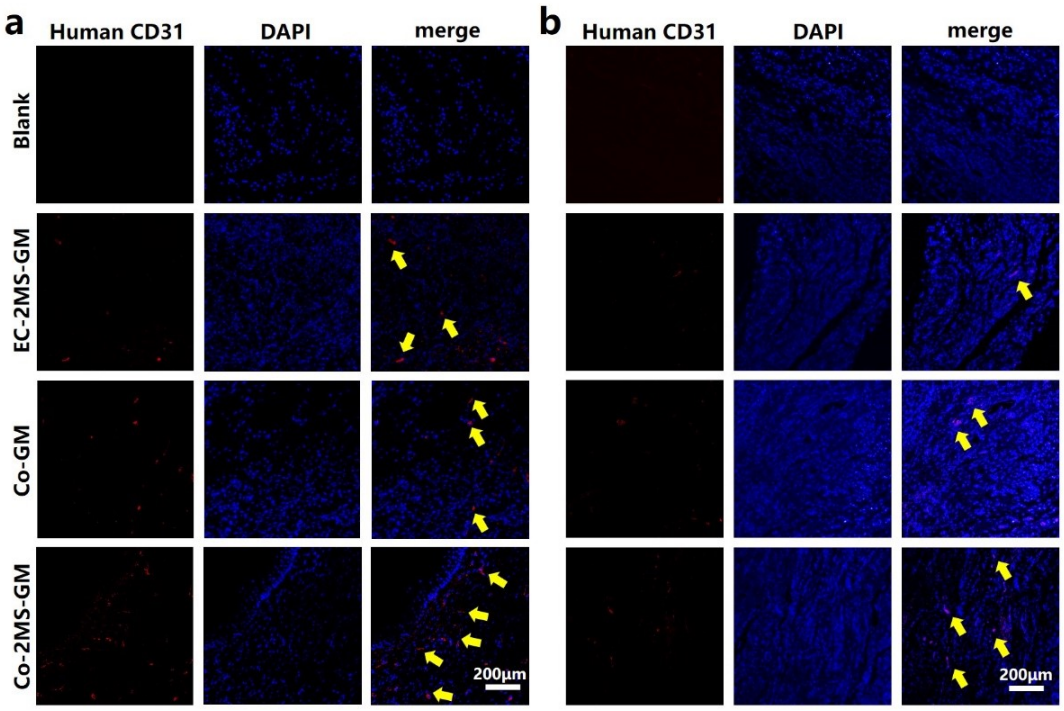

Fig. S7 The human-specific CD31 immunofluorescent staining images of the skin samples on (a) day 7 and (b) day 14. The CD31-positive blood vessels (yellow arrows) were observed in EC-2MS-GM, Co-GM, and Co-2MS-GM groups (red: CD31, blue: nucleus). Scale bar: 200 μm.

Table S1 The primer sequences of angiogenesis-related genes

Gene name	Sequences
GAPDH	5′-ACGGATTTGGTCGTATTGGGCG-3′
	5′-CTCCTGGAAGATGGTGATGG-3′
VEGF	5′-GCGAGTCTGTGTTTTTGCAG-3′
	5′-TCTTCAAGCCATCCTGCGTG-3′
VE-cad	5′-GGCTCAGACATCCACATAACC-3′
	5′-CTTACCAGGGCGTTCAGGGAC-3′
eNOS-1	5′-TGTCCAACATGCTGCTGGAAATTG-3′
	5′-AGGAGGTCTTCTTCCTGGTGATGCC-3′
HIF-1α	5′-CCATGTGACCATGAGGAAAT-3′
	5′-CGGCTAGTTAGGGTACACTT-3′
KDR	5′-CCCAGGCTCAGCATACAAAAAGAC-3′
	5′-CCAGTACAAGTCCCTCTGTCCC-3′
bFGF	5′-CAATTCCCATGTGCTGTGAC-3′
	5′-ACCTTGACCTCTCAGCCTCA-3′

Table S2 The primer sequences of genes related to hair follicle formation

Gene name	Sequences
GAPDH	5′-TGGCAAATTCCATGCAC-3′
	5′-CCATGGTGGTGAAGACGC-3′
VEGF	5′-GCGAGTCTGTGTTTTTGCAG-3′
	5′-TCTTCAAGCCATCCTGCGTG-3′
PDGF-α	5′-GAATCATAGCTCTCTCCTCGCAC-3′
	5′-GATTCCTCCAAAGCCTCATAGCAG-3′
PDGF-β	5′-CAGTCCTGCCTGTCCTTCTACTC-3′
	5′-GGATCTGGCACAAAGATGTAGAGC-3′
C-Myc	5′-AATAGAGCTGCTTCGCCTAGA-3′
	5′-GAGGTGGTTCATACTGAGCAAG-3′

Experimental

Materials

Tetraethyl orthosilicate (TEOS), ammonium hydroxide ($NH_3 \cdot H_2O$), ammonium chloride (NH_4Cl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), ethyl alcohol (CH_3CH_2OH), and methyl cellulose (MC) were purchased from Sinopharm Chemical Reagent Co., Ltd. Gelatin and lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP) were purchased from Sigma-Aldrich (USA). Methacrylic anhydride was purchased from Shanghai Titan Scientific Co.,Ltd. Phosphate buffered solution (PBS) was purchased from Sangon Biotech (Shanghai) Co., Ltd.

Preparation of magnesium silicate hollow nanospheres

Magnesium silicate hollow nanospheres (MS) were synthesized by hard-template directed method. The silicon dioxide nanospheres were used as sacrificial templates.

First, the silicon dioxide nanospheres were synthesized as follows. 64 g ethyl alcohol and 24 g ammonium hydroxide were added in a beaker to be stirred for 10 min at 30 °C. Then, 4.2 mL of tetraethyl orthosilicate was decanted into the beaker and constantly stirred for 1 h under sealed conditions. After that, the mixture was centrifuged at 5000 rpm/min for 5 min to obtain the white precipitates. After ultrasonic washing by deionized water and ethyl alcohol, each for three times, the precipitates were dried at 60 °C to obtain the SiO_2 templates.

Subsequently, the magnesium silicate (MS) hollow nanospheres were prepared as follows. 0.1 g of SiO_2 templates were added into deionized water to be ultrasonic dispersed for 30 min. The SiO_2-dispersed liquid was denoted as liquid A. Meanwhile, 0.15 g magnesium chloride hexahydrate and 0.54 g ammonium chloride were dissolved into 30 mL of deionized water. Then, 1 mL of ammonium hydroxide was slowly dropped into the magnesium chloride-ammonium chloride mixed solution by using a syringe and stirred for 30 min. The solution after the reaction was denoted as liquid B. Then, Liquid A and 25 mL ethyl alcohol were quickly poured into liquid B and stirred for 1 h. The mixed liquid was sealed into polytetrafluoroethylene hydrothermal autoclave reactors and reacted at 140 °C for 12 h. After the hydrothermal reaction, the white precipitates were harvested and washed by deionized water and ethyl alcohol,

each for three times. Finally, the precipitates were dried at 60 °C to obtain the MS hollow nanospheres.

Characterization of magnesium silicate hollow nanospheres

Morphologies of the MS nanospheres were observed by scanning electron microscope (SU9000, Hitachi, Japan). Microstructure and elemental distribution of MS nanospheres were analyzed by transmission electron microscope (TEM, Tecnai G2 F20, FEI Electron Optics, Netherlands). Chemical phase analysis was conducted by X-ray diffraction (Rigaku D/Max-2550 V, Geigerflflex, Japan).

Cell culture

Human umbilical vein endothelial cells (HUVECs) and human hair dermal papilla cells (HHDPCs) were purchased from ScienCell Research Laboratories (Sciencell, USA). HUVECs were cultured by the Endothelial Cell Medium (ECM, Sciencell, USA), which was supplemented with 5% fetal bovine serum (FBS), 1% endothelial cell growth supplement (ECGS) and 1% penicillin-streptomycin (P/S). HHDPCs were cultured by the Mesenchymal Stem Cell Medium (MSCM, Sciencell, USA), which was supplemented with 5% fetal bovine serum (FBS), 1% mesenchymal stem cell growth supplement (MSCGS) and 1% penicillin-streptomycin (P/S). The cells were all placed in an incubator with 5% CO_2 at 37 °C.

Preparation of the extracted/dispersed solution of MS hollow nanospheres

MS nanospheres were sterilized by ultraviolet light for 1 h. The sterile nanospheres were dispersed into the ECM/MSCM at a mass-to-volume ratio of 500 μg/mL. Then, the liquid was diluted to 1/2 (250 μg/mL), 1/5 (100 μg/mL), 1/10 (50 μg/mL), 1/20 (25 μg/mL), 1/50 (10 μg/mL) to obtain the dispersed solution of MS with different concentration. In addition, the MS-dispersed solutions of different concentrations were shaken in a shaking bed at 37 °C for 48 h, and then was filtrated by the 0.22 μm filters to obtain the extracted solution of MS with different concentrations.

Effects of the extracted/dispersed solution of MS hollow nanospheres on cell proliferation

HUVECs were seeded into 96-well plates with the density of 1000 cells/well. After the cells adhered on the well, the dispersed/extracted solution of MS with different

concentration was added to culture the cells. Meanwhile, HHDPCs were seeded into 96-well plates with the density of 1500 cells/well. After the cells adhered on the well, the dispersed/extracted solution of MS with different concentration was added to culture the cells. After incubation for 1, 3, and 5 days, cell proliferation was detected by the CCK-8 assay. At each time point, the culture medium was replaced by the 10% CCK-8 solution (Beyotime, China). Being kept out of light, cells were incubated for 2 h and then the absorbance of their medium was detected at 450 nm by a microplate reader (Tecan, Germany).

Effects of the extracted/dispersed solution of MS hollow nanospheres on cell differentiation

Real-time quantitative polymerase chain reaction PCR (RT-qPCR) was used to detect the expression levels of angiogenesis-related genes in HUVECs and hair follicle differentiation-related genes in HHDPCs. HUVECs were cultured in MS-dispersed solutions at concentrations of 0,10 and 25 μg/mL. HHDPCs were cultured in MS-MSCM dispersions at concentrations of 0,10 and 25 μg/mL, respectively. After incubation for 5 days, total RNA of the cells was extracted by Trizol reagent (Invitrogen, USA). Then, reverse transcription was conducted to turn the RNA into cDNA via PrimeScript 1st Strand cDNA synthesis reagent (TOYOBO, Japan). RT-PCR processes was proceeded in the StepOnePlus Real Time Systems by using SYBR Green QPCR Master Mix reagent (TaKaRa, Japan). GAPDH were set as the reference gene. The expression levels of all target genes were analyzed by $2^{-\Delta\Delta Ct}$ method. The primer sequences of the angiogenesis-related genes and hair follicle differentiation-related used in this study were summarized in **Table S1** and **S2**.

Preparation and characterization of the cell-laden composite bioinks

Firstly, GelMA hydrogel was synthesized according to the previous reports[55, 56] and set as the bioink matrix. Preparation of the HUVECs-laden bioinks: 1.2 g of GelMA and 0.05 g LAP were dissolved into 10 mL PBS at 65 °C. After GelMA was totally dissolved, 0.01 g methylcellulose was added to the GelMA solution to obtain the GelMA-MC solution. GelMA-MC was sterilized by 0.22 μm filters. Subsequently, 0 g,

0.0048 g，0.0096 g，and 0.0144 g (0, 2%, 4%, and 6% relative to the GelMA mass) of MS were ultrasonic dispersed in 2 mL of PBS and mixed with 2 mL GelMA-MC solution to obtain the hydrogel inks with different concentration of MS (GM，2MS-GM，4MS-GM，6MS-GM), respectively. Meanwhile, HUVECs, which were cultured and expanded to passage six, were digested using trypsin (Gibco, USA) and evenly mixed into the GM hydrogel inks with the concentration of 3-4 million/mL. The four kinds of HUVECs-encapsulated bioinks were denoted as EC-GM, EC-2MS-GM, EC-4MS-GM, EC-6MS-GM.

Preparation of the HHDPCs-laden bioinks: 1.2 g of GelMA and 0.05 g LAP were dissolved into 10 mL PBS at 65 °C. After the GelMA solution was filtered by 0.22 μm filters, 2 mL of it was diluted by 2 mL PBS. Then, HHDPCs, which were cultured and expanded to passage three, were digested using trypsin (Gibco, USA) and evenly mixed into the GelMA hydrogel inks with the concentration of 5 million/mL. The HHDPCs-encapsulated bioinks were denoted as DP-GelMA.

Rheological characteristics of the composite bioinks with different MS concentrations

Rheological tests of the four kinds of bioinks were conducted at 45 °C via a rotational rheometer (MCR301, Anton Paar GmbH, Austria). Viscosity profiles were obtained by adjusting the shearing rate from 0.1 s^{-1} to 10 s^{-1}.

3D bioprinting of the micropatterned multicellular scaffolds containing MS nanospheres

EC-GM，EC-2MS-GM，EC-4MS-GM，EC-6MS-GM bioinks were sealed into the stainless steel cartridge and cooled at 4 °C for 20 min, respectively. Then, the cartridges were installed on the 3D bioprinting system (BioScaffolder 3.2, GeSiM, Germany) in sequence. The temperature of the cartridges and the platform was set as 10 °C. The extrusion pressure was set in the range of 20 ~ 40 kPa. The type of the extrusion needles was 27G (inner diameter = 250 μm). Accordingly, a HUVECs-encapsulated frame structure containing different concentrations of MS was fabricated. After the HUVECs-laden frames were printed, the DP-GelMA bioinks were seeded into the holes between the struts. Then, the whole scaffolds were crosslinked by the blue

light at 450 nm for 1 min. According to the four different concentrations of MS, the multicellular scaffolds were denoted as Co-GM, Co-2MS-GM, Co-4MS-GM, and Co-6MS-GM. Each scaffold was transferred into the 12 well-plates to be cultured with 1 mL of mixed culture medium (ECM: MSCM = 1: 1).

In addition, for the printing of monocellular scaffolds, all printing parameters were consistent with the multicellular scaffolds. By canceling one of the cell-laden bioinks, scaffolds with only HUVECs (EC-MS-GM) and scaffolds with only HHDPCs (DP-MS-GM) could be printed in the same way.

Appearances of four kinds of scaffolds were recorded by digital camera. Then, the scaffolds were freeze dried for 12 h and the morphologies and elemental distribution of their cross sections were evaluated by SEM (SU8220, Hitachi, Japan) and EDS.

Cell distribution in the scaffolds

In order to characterized the micropattern of cell distribution in the scaffolds, HUVECs and HHDPCs were marked with different fluorescent (CellTracker, Invitrogen, USA), respectively. After incubating for 1, 4, and 7 days, the fluorescent-labeled scaffolds were observed by the laser confocal scanning microscope (TCS SP8, Leica, Germany). HUVECs were observed by 488 nm excitation light and HHDPCs were observed by 647 nm excitation light.

Cell viability in the scaffolds

Cell viability was evaluated by conducting the live/dead assay *via* Calcein-AM/PI staining reagent (Dojindo, Japan). The live/dead staining solution was mixed by Calcein-AM/PI staining reagent, ECM and MSCM as the ratio of Calcein-AM: PI: ECM: MSCM = 2: 3: 500: 500. After 1, 7, 14, and 21 days of culture, the scaffolds were immersed into the staining solution and incubated at 37 °C for 20 min. Then, the fluorescence microscope (DMi8 S, Leica, Germany) was applied to captured the fluorescent images. The live cells were detected by 488 nm excitation light and the dead cells were detected by 552 nm excitation light.

Cell viability was calculated by respectively counting the live and dead cells. The cell survival rate (%) = the number of living cells/the total number of living cells and dead cells. Four parallel samples were analyzed from each group.

Cell morphology in the scaffolds

Morphologies of HHDPCs and HUVECs in Co-GM, Co-2MS-GM, and Co-4MS-GM scaffolds were characterized. After being cultured for 1 day and 14 days, the 3D bioprinted multicellular scaffolds were anchored by 4% paraformaldehyde for at least 30 min. After washing with PBS 3 times (5 min each time), 0.5 % Triton-X solution (5 min) and 5 % BSA solution (30 min) were applied to permeabilize and block the scaffolds, respectively. Next, the scaffolds were incubated with the solution of CD31 primary antibody (Abcam, ab28364, 1:100) at 4 °C overnight. Then the scaffolds were incubated with the second antibody (1:1000, Alexa Fluor 647) at 37 °C for 1 h after being washed with PBS. The cytoskeleton and nuclei of the cells printed in the scaffolds were stained by Alexa Fluor Plus 647 Phalloidin (Molecular Probes, USA) for 1 h and by DAPI (Sigma-Aldrich, USA) for 10 min. After that, the scaffolds were washed by PBS for 3 times and observed by the laser confocal scanning microscope (TCS SP8, Leica, Germany). The F-actin of the cells was detected by 647 nm excitation light, the CD31 protein was detected by 488 nm, and the nucleus was detected by 405 nm excitation light. At last, the semi-quantitative analyses were implemented by the ImageJ software (NIH, USA).

Relative gene expressions of HUVECs and HHDPCs in the micropatterned scaffolds

RT-PCR was used to detect the expression levels of angiogenesis-related genes in HUVECs and hair follicle differentiation-related genes in HHDPCs. After being cultured for 7 days, Co-GM, Co-2MS-GM, and Co-4MS-GM scaffolds were digested by the collagenase (0.3 mg/ml) for 2 h. After the digestion, the mixture was centrifuged at 1000 rpm/min for 5 min. 1 mL of Trizol reagent was added to the precipitated cells to extract their total RNA. Then, reverse transcription was conducted to turn the RNA into cDNA via PrimeScript 1st Strand cDNA synthesis reagent (TOYOBO, Japan). RT-PCR processes were proceeded in the StepOnePlus Real Time Systems using SYBR Green QPCR Master Mix reagent (TaKaRa, Japan). GAPDH was set as the reference gene. The expression levels of all target genes were analyzed by $2^{-\Delta\Delta Ct}$ method. The primer sequences of the angiogenesis-related genes and hair follicle differentiation-

related used in this study were summarized in Table S1 and S2.

Establishment and treatment of full-thickness skin injury model in nude mice

All of the animal experiments were conducted to conform to the guidelines sanctified by the Institutional Animal Care and Utilization Committee of Nanjing First Hospital, Nanjing Medical University. Eight-week-old male BALB/c nude mice (SPF) were applied to conduct the experiment of repairing full-thickness skin defects. The nude mice were purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). The nude mice were randomly divided into 4 groups: Blank (without scaffolds implanted), EC-2MS-GM (MS-incorporated monocellular scaffolds loaded with HUVECs), Co-GM (micropatterned scaffolds without cells) and Co-2MS-GM (MS-incorporated micropatterned multicellular scaffolds). All scaffolds were printed with the diameter of 10 mm and height of 0.8 mm. All scaffold were incubated *in vitro* for 3 days before implantation. The nude mice were anesthetized by intraperitoneal injection of sodium pentobarbital before the operation. After sterilizing the skin, a circular full-thickness skin defect with a diameter of 10 mm was made on the back of the nude mice. After the scaffolds were implanted into the defects, they were fixed and bandaged with sutures, sterile gauze, and medical dressing (Tegaderm TM, 3M). After 0, 8, 10, 12, and 14 days of implantation, the defects were recorded by digital camera. Then, the area of defects was measured by Image J software (NIH, USA). The relative area of the defects was calculated based on the following formula:

$$\text{Relative area of the defects (\%)} = \frac{S_t}{\overline{S_0}} \times 100\%$$

S_t represented the area of defects after t days of implantation, $\overline{S_0}$ represented the average area of the defects in day 0.

After 32 days of implantation, the hair growth on the back of nude mice was recorded. On the 14th and 32nd days, the skin tissues were collected and anchored in 4% paraformaldehyde for 24 hours.

Establishment and treatment of androgenetic alopecia (AGA) model for hair growth

Eight-week-old male C57BL/6 mice were applied to establish the androgenetic

alopecia (AGA) model. The C57BL/6 mice (SPF) were purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). A homogeneous testosterone solution (5 mg/mL) was prepared for daily subcutaneous injection in the murine back. All mice were divided into 3 groups randomly: Blank (without scaffolds implanted), GM (hydrogel scaffolds without MS and cells) and Co-2MS-GM (MS-incorporated micropatterned multicellular scaffolds). On day 7 post-injection, dorsal hair was removed and a full-thickness wound with a diameter of 1 cm was created on the murine back. The operation day was recorded as day 0. The scaffolds were implanted into the skin defects and fixed with sutures, sterile gauze, and medical dressing (Tegaderm TM, 3M). Besides, the testosterone solution continued to be injected daily for three weeks. Gross photos of wound sites were taken on day0, 7, 15, 25 and 40. Skin and hair samples were obtained after treatment for 25 days and 40 days. The area of hair growth was measured and calculated by Image J software (NIH, USA). The diameter of the regrown hair was observed by SEM (Quattro S, ThermoFisher Scientific, USA). Therelative wound area calculation method was the same as the previous section.

Histological analysis

All of the skin samples were preserved in 4% paraformaldehyde solution for more than 24 h. Afterward, the samples were dehydrated and paraffin-embedded, and then cut into sections with 6 μm thickness. Next, the dewaxed sections were immersed in 0.01 M citrate buffer solution (pH = 6.0) maintained at 99 °C for 20 min for antigen retrieval. After cooling naturally, 5% bovine serum albumin (BSA) was prepared and dropped on the sections to block non-specific staining. Then, BSA solution was removed, and the tissues on the sections were covered by diluted primary antibody solution. Sections were placed in a 4 °C refrigerator for overnight incubation under a wet condition. Next, a secondary antibody solution with fluorescence was dropped on the rewarmed sections for 1-hour incubation in the dark. The neutral resin containing DAPI dye was added dropwise for mounting finally. The primary antibody used in this study were as follows: CD31 (1:100, Abcam, USA, ab28364), human CD31 (1:100, Abcam, USA, ab76533), Cytokeratin 5 (1:100, Abcam, USA, ab52635), hair cortex Cytokeratin AE13 (1:100, Abcam, USA, ab16113), Ki67 (1:200, Abcam, USA,

ab16667). The stained sections were observed by CLSM (TCS SP8, Leica, Germany). The numbers of hair follicles and blood vessels in generated skin tissue were counted in several equal-area regions randomly selected in the stained sections.

Statistical analysis

All numeric types of data in this manuscript was expressed as mean ± standard deviation in Origin software (OriginLab, USA). The statistical analysis was conducted by one-way ANOVA testing. Significantly differences were defined by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.