Heparin-modified acellular dermal matrix/chitosan-doped polydopamine nanoparticles composite sponge scaffold loaded with CGF: promoting full-thickness defect wound healing

Lei Yang (yuanyang@smu.edu.cn)  
Southern Medical University Nanfang Hospital

Lianglong Chen  
Southern Medical University Nanfang Hospital

Chaoyang Huang  
Southern Medical University Nanfang Hospital

Yu Zhong  
Southern Medical University Nanfang Hospital

Yujia Chen  
Southern Medical University Nanfang Hospital

Huihui Zhang  
Southern Medical University Nanfang Hospital

Zijun Zheng  
Southern Medical University Nanfang Hospital

Ziwei Jiang  
Southern Medical University Nanfang Hospital

Xuerong Wei  
Southern Medical University Nanfang Hospital

Yujie Peng  
Southern Medical University Nanfang Hospital

Lei Huang  
Southern Medical University Nanfang Hospital

Libin Niu  
Southern Medical University Nanfang Hospital

Yanbin Gao  
Southern Medical University Nanfang Hospital

Jun Ma  
Southern Medical University Nanfang Hospital
Abstract

Background

Although acellular dermal matrix (ADM) and concentrated growth factor (CGF) are both used in regenerative medicine, CGF has been hampered by problems such as burst release, and ADM is only used as an outer dressing for wound healing. In this study, we aim to prepare composite scaffolds (CADMS-G-2-Hep) with good NIR photothermal response, antibacterial activity, and drug delivery ability by introducing polydopamine nanoparticles (PDA NPS) with excellent NIR photothermal conversion ability into ADM and chitosan (CS) interpenetrating network structure and heparinization modification.

Method

Firstly, a physical-chemical double cross-linked composite network was constructed by using chemical cross-sections and the mutual entanglement of ADM and CS chains under freezing conditions. Secondly, the heparinization modification made the composite scaffold with good biocompatibility and cell/tissue affinity, significantly promoting cell adhesion and proliferation and achieving adequate fixation and slow release of CGF. Finally, PDA-NPS have excellent NIR photothermal conversion ability and can significantly promote the survival of autologous skin grafts in rats. One-way ANOVA with Bonferroni posthoc analysis was used to determine whether a statistically, significant difference exists between groups.

Result

In vivo experiments, this composite biomaterial promoted angiogenesis, cell proliferation, and adhesion, improved skin survival of full-thickness skin defect wounds, reduced graft shrinkage and scar formation and promoted wound healing and tissue remodeling.

Conclusion

CADMS-G-2-Hep has the potential as a novel scaffold material capable of delivering CGF, which can promote full-layer skin defect healing through a one-step strategy.

1. Introduction

Skin is the body's largest organ, with immune, thermoregulatory and metabolic functions[1]. Early skin grafting or skin flap transfer is essential for full-thickness skin defects[2]. However, the treatment has complex operation, severe donor site injury, high failure rate, and may lead to scar contracture of donor or flap site[3]. At present, artificial dermis substitute (artificial dermis) is a double-layer scaffold material synthesized by collagen, polysaccharide, extracellular matrix and other substances, and medical silica gel layer[4], its principle is that in situ cell scaffold materials can stimulate proliferation, migration, adhesion,
or immune regulating behavior, through the use of the activity of material itself rather than cell activity to promote wound healing, For example, Lando® in China, Integra® in the United States and Pelnac® in Japan[5, 6]. The essence of this product is the Collagen sponge scaffold (CSS), with a three-dimensional porous structure, which can reduce the formation of contracture and purpura marks, restore appearance and function, and reduce donor site damage[7]. However, the clinical treatment of skin and soft tissue defects with artificial dermis is often facing with the following problems[8, 9]: (1) Permanent tissue transplantation is a crucial method to repair skin and soft tissue defects, but tissue-engineered skin products are limited by ischemia and fail to vascularize and thus fail to survive effectively entirely; (2) Although the artificial dermal scaffold acts as a template for skin regeneration, it has poor mechanical properties, serious damage to the microstructure after implantation, and severe shrinkage of the scaffold after the second-stage skin grafting, requiring repeated skin grafting; (3) The artificial dermal scaffold has a single component and cannot provide enough extracellular matrix for reepithelialization, and there is a risk of infection in the later stage of transplantation, leading to the failure of skin transplantation.

Decellularized dermal matrix (ADM) is a kind of leather made of allograft skin substitutes that can remove the cell composition and retain only the cell composition and biocompatibility. And the composition is similar to human skin and immunological aspects. Therefore ADM has been used to repair the wound over the years[10], However, after ADM is implanted into the defective wound, it is difficult for nutrition to pass through ADM, and it cannot effectively maintain the early energy supply of grafted skin grafts and epidermal cells, resulting in an insufficient number of new blood vessels, and it takes too long for new blood vessels to reach the dermis. All these factors can lead to the insufficient blood supply of autologous skin grafts and epidermal cells, and eventually slow cell proliferation and peeling of the skin grafts [11, 12]. Therefore, improving its permeability and porosity is the key to improving graft survival.

Concentrated growth factor (CGF) is a third-generation growth factor blood product with a wide range of clinical applications and a mature production process. Its most significant characteristic is that it contains a higher concentration, which is 5 ~ 6 times the whole blood platelet concentration. Moreover, platelet can activate with the centrifugal process, releasing much decisive growth factor, which can stimulate cell proliferation differentiation and promote tissue repair [13]; In addition, CGF is easy to make, with a lower cost and minor damage to the body. Each growth factor plays its role and coordinates with each other, promoting wound repair, and providing a suitable wound bed microenvironment for granulation tissue growth and epithelial crawling[14]. Due to the sudden release of CGF, although the secreted GFs promote angiogenesis and the proliferation and migration of skin-associated cells, this release process will inevitably lead to the waste of a large amount of GFs [15]. Therefore, how to release GFs slowly and reduce waste is a hot issue that needs to be solved in the process of use.

In recent years, as a new type of NIR photothermal material, polydopamine nanoparticles (PDA-NPS) inspired by mussels have attracted extensive attention from researchers due to their high photothermal conversion efficiency, hydrophilicity, and good dispersity [16]. Most importantly, PDA is the main component of melanin and has excellent biocompatibility and high cell/tissue affinity, which can
significantly promote cell adhesion and proliferation [17]. In addition, PDA-NPS can easily bind to mercaptan or amine groups, which is conducive to better distribution and entanglements in polymer networks. Therefore, PDA-NPS can be introduced into the network structure of natural polymer materials as NIR photothermal materials to form skin substitutes with good NIR photothermal properties for wound healing [18].

This study introduced PDA-NPS into an acellular dermal matrix - chitosan interpenetrating network. We modified it with heparin to prepare NIR photothermal responsive drug-delivery composite scaffolds for skin tissue repair. The antibacterial activity, NIR photothermal conversion ability, and the effects on CGF fixation and release behavior of the composite scaffolds were evaluated. Finally, the effects of NIR photothermal therapy, CGF promoting graft survival, and synergistic effects on skin tissue regeneration were investigated in the full-thickness defect wound of rats combined with a phase I autologous skin transplantation.

2. Materials And Methods

2.1 Preparation of acellular dermal matrix - chitosan composite sponge scaffold

Acellular dermal matrix was prepared by hyperosmotic salt-alkali method with the full-layer skin of a white Tibetan miniature pig as raw material. The acellular dermal matrix was prepared with 0.5%, 1%, and 2% acetic acid (Sigma-Aldrich, USA) solutions. The PADMS and acetic acid solutions were added to the beaker at a mass ratio of 1:10 and stirred at 1500rpm for 48 hours. Acellular dermal matrix gel was formed after filtration (groups were labeled 0.5%, 1%, 2%) and stored at 4°C for later use. Acellular dermal matrix gel (group marked as 10%PADM) was mixed with 3% chitosan solution (group marked as CADMS) at different volume ratios and crosslinked with glutaraldehyde solution to make the content of crosslinking agent in the solution 0.02 V/V. % (group marked as CADMS-G). Acellular dermal matrix and chitosan composite gel were prepared (groups were labeled as 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 2:1, 3:1, 4:1, 5:1). The gel was freeze-dried to prepare the composite sponge scaffold, soaked in 70% ethanol solution for 1 hour to remove residual acetic acid. After full cleaning, the composite sponge scaffold was freeze-dried again for subsequent physical and chemical properties characterization.

2.2 Preparation of heparin sodium modified composite sponge scaffold

EDC and NHS (Sigma-Aldrich, USA) were added to 0.1mol /L MES buffer (Sigma-Aldrich, USA) and the final concentrations were 2g/L and 0.5g/L, respectively, with pH = 5.0. Heparin was added to the prepared reaction solution, and the reaction was 20 min. The lyophilized acellular dermal matrix chitosan composite sponge was added to the prepared reaction solution and reacted at room temperature for 24 h, followed by washing with 0.1mol /L Na₂HPO₄ buffer for 1 h, and finally washed with deionized water three times for 30 min each. Freeze-drying for stand-by use (marked group CADMS-G-Hep).
2.3 Preparation of composite sponge scaffolds doped with polydopamine nanospheres

The specific experimental method is as follows: Firstly, ammonia water (0.8 mL, 28%~30%), anhydrous ethanol (40 mL) (Sigma-Aldrich, USA), and deionized water (90 mL) are stirred and mixed evenly. Dopamine HCL (0.5 g, Sigma-Aldrich, USA) was dissolved in distilled water (10 mL), and the dopamine HCL solution was added drop by drop to the above mixture. The polydopamine nanoparticles (PDA-NPS) were obtained by centrifugation (10000 RPM) for 15 min after stirring for 30 h. The PDA-NPS were washed with distilled water three times and freeze-dried for later use. Composite sponge scaffolds with concentrations of 1g/L, 2g/L, and 4g/L were prepared by adding PDA-NPS into 10 mL acellular dermal matrix-chitosan composite gel (groups were labeled as CADMS-G-1-Hep, CADMS-G-2-Hep, and CADMS-G-4-Hep, respectively).

2.4 Preparation of concentrated growth factor delivery sustained-release scaffold

A healthy male white Tibetan miniature pig weighing about 40kg was anesthetized by applying isoflurane to oxygen inhaled by the mask. The whole blood was collected from the internal jugular vein and quickly distributed to the anticoagulant collecting vessels containing sodium meerkat. The blood was gently rotated, mixed, and centrifuged in a CGF centrifuge(CGF-BH200, China) for about 14min to obtain the supernatant CGF solution. Moreover, the composite sponge scaffold after irradiation sterilization of Co was immersed in CGF solution for 1 h to adsorb growth factors fully, and the sponge drug delivery scaffold (group labeled as CADMS-G-2-Hep-CGF) was obtained.

2.5 Physical and chemical properties characterization of materials

Fourier infrared spectroscopy (FTIR)

the sample and potassium bromide are mixed and ground into a uniform powder of 0.1% ~ 0.5%. Then, the tablet is pressed and determined by the infrared spectrometer. Infrared spectroscopy (Nicolet IS50FI-IR) detection range of 500 ~ 4000 cm\(^{-1}\), resolution of 4 cm\(^{-1}\).

Scanning electron microscope

round pieces of dry samples with diameters of 6mm in different proportions were glued to the test bench by a conductive adhesive. Gold spraying was carried out under vacuum by a sputtering machine under 1 kV and 5 mA conditions. Scanning electron microscopy (SEM; TM3030, HITACHI) observed the material's microstructure under the condition of 20kV acceleration voltage.

Water absorption
Water absorption: take an appropriate amount of scaffold material, weigh the mass as $M_1$, immerse the sample in purified water and soak it at room temperature for 24 hours. After it absorbs enough water, take out and absorb the excess water on the material’s surface, weigh the wet mass as $M_2$, and calculate the water absorption according to the following formula. Water absorption = $(M_2-M_1) / M_1 \times 100\%$

**Porosity**

The centrifuge tube with a volume of 10 mL was filled with ethanol and weighed (denoted as $W_1$). The sample was weighed (denoted as $W_S$) and immersed in the ethanol-filled centrifuge tube. The sample was placed into the ultrasonic instrument for 30 min and then filled with ethanol and weighed (denoted as $W_2$). After the sample filled with ethanol was taken out, the remaining ethanol and the centrifuge tube were weighed ($W_3$). The experiment was repeated three times, and the mean value was taken. Calculate the porosity = $(W_2-W_3-W_S) / (W_1-W_3) \times 100\%$.

**Moisture retention**

accurately weigh the sample mass $W_{dry}$ and immerse it in distilled water to make it fully absorb water and swell. Put it into the centrifuge tube, centrifuge at 500r / min for 3min, and take out the weighing W. Repeat the measurement three times in each group. Calculate the moisture retention of the material: $WR = (W-W_{dry}) / W_{dry}$

**Mechanical strength test**

electronic universal testing machine (UTM100, Wanchen) was selected to test the static mechanical properties of the samples in a dry state through the tensile test. First, different proportions of materials ($n = 3$) with a thickness of about 1mm were cut into rectangular samples with specifications of 4×1cm. The loading rate was 10mm/min, and the elastic modulus was calculated according to the slope of the stress-strain curve.

**Determination of residual dsDNA**

Deoxyribonucleic acid (DNA) was extracted from PADMS using a DNA kit (Tiangen Biotech(Beijing) CO., LTD DP304). The residual DNA content was then measured with an ultramicro spectrophotometer (Thermo, NanoDrop2000) and normalized to the dry weight of each sample.

**In vitro degradation experiment**

The samples were placed in a 15mL centrifuge tube, and 4mL of 50mg/mL type I collagenase (Solarbio, China) solution was added and taken out at five-time points ($n = 3$) at 2h, 4h, 12h, 16h, 21h, and 24h. After freeze-drying, the samples were weighed. The degradation rate was calculated according to the quality difference before and after degradation.

**In vivo degradation experiment**
Male SD rats aged 12 weeks were anesthetized with 0.03% pentobarbital sodium, and the sample (diameter 12mm, thickness about 2mm) was implanted subcutaneously, and the wound surface was sutured discontinuously. Photographs were taken at 2, 6, 8, and 16 weeks, and H&E staining was performed.

**Toluidine blue staining**

The toluidine blue staining solution was diluted to 10%, and the materials were put into the toluidine blue staining solution dyeing VAT, soaked for 20 ~ 30 min. After the staining, the materials were removed, and the tissues were thoroughly washed with deionized water. The absorbance of OD\textsubscript{600} was measured by direct photographing or enzyme-plate meter.

**Composite bracket NIR photothermal properties**

Will have the same size (diameter: 10 mm, height: 10 mm); samples were placed in 2 mL distilled water and irradiated with 808 nm NIR laser (0.8 W· cm\textsuperscript{-2}) for 30 min. The water temperature was measured and recorded with a thermometer every 5 min. An infrared thermal imager (XINTEST, China) was used to photograph the temperature distribution around the composite scaffold at different time points.

**Histomorphological staining**

Different materials were fixed with paraformaldehyde (Leading, China), dehydrated by an automatic dehydrator, embedded in paraffin, and then paraffin sectioning. After dewaxing, hematoxylin-eosin staining, Masson staining, and toluidine blue staining were performed.

**Growth factor release experiment**

The compound sponge drug delivery stent (CADMS-G-2-Hep-CGF) was immersed in PBS buffer (Sigma-Aldrich, USA). The supernatant was rapidly absorbed at room temperature at 2, 12, 24, 36, 48, 60, and 72 h. The release curves of VEGF and PDGF-BB were quantitatively determined using an Enzyme-Linked immunosorbent assay (ELISA) kit. The sustained-release drug loading characteristics of the materials were evaluated (n = 3).

### 2.6 Cytotoxicity test

To prepare the extract, the material was immersed in a complete medium at 37°C for 24h to prepare the extract. L929 cells /HUVEC/HaCat were inoculated into 96-well plates at a density of 2×10\textsuperscript{4} cells/mL. After cell adherence, extracts were added, and a blank control group was used as the basal medium. CCK-8 reagent solution (DOJINDO, Japan) was added for 48h and incubated at 37°C for 1 h. Optical density values of each well (D) were measured at 450 nm wavelength of BioTek (China), and statistical analysis was performed (n = 3). Alternatively, the morphology of HaCat was photographed under a microscope at 36h.
The basal medium was used to dilute CGF to 5%, 10%, 15%, 20%, and 25%, and the control group was a 10% complete medium. L929 cells were inoculated into 24-well plates at a density of 3×10^4 cells/mL, and cell morphology was photographed under a microscope at 24h, 36h, 48h, and 60h, respectively.

### 2.7 Scratch test

L929 cells /HUVEC were inoculated into 6-well plates at a rate of 5 × 10^5 cells/well (n = 3) and cultured in an incubator at 37°C until confluence. Then use the pipette tip to make a scratch at a constant speed on the cell layer on the back of the plate. After three washes with PBS, the 24mm insert (micron microporous polyester film) was placed into the 6-well plate, and the material was transferred to the insert. Subsequently, a serum-free medium was added to the orifice plate until the scaffold was immersed. Alternatively, different concentrations of CGF can be added to the 6-well plate. Cell migration was recorded at 0h, 12h, 20h, 36 h, and 48h. The migration area was calculated as follows: mobility (M) (%) = (R_0 - R_n) / R_0 × 100%, where R_0 represents the initial scratch area and R_n represents the remaining unsealed scratch area.

### 2.8 Staining of living and dead cells

The staining solution was prepared from 10uL 1 mmol/L Calcein-AM reserve solution and 15uL 1.5mmol/L PI reserve solution (Solarbio, China) to 5mL PBS and frozen at 0 ° C under dark conditions for later use. Three days after HUVEC/L929 cells were inoculated into the material, the original medium was dried, and the material was immersed entirely with 100uL PI/CA staining solution. The staining solution was washed with normal saline in the cell incubator for 20min, and the inverted fluorescence microscope (Axio Observer D1, ZEISS, Germany) images of cell proliferation and adhesion at 490 nm were obtained to observe the number and state of cells. The experiment was repeated with three samples in each group.

### 2.9 Cell proliferation and adhesion experiments

HUVEC was inoculated on each group of 96-well plates at a density of 10^5 cells/mL and cultured at 37°C with a medium exchange culture every two days. On day 3 of culture, the samples were removed from the incubator, washed with PBS, and fixed with 4% paraformaldehyde at room temperature. Then the fixation solution was washed away with PBS, and the cells were sequentially permeated with 0.1% Triton X-100 and 1% bovine serum albumin. According to the manufacturer's agreement, the cytoskeleton and nucleus were subsequently stained in a dark chamber with Rhodamine-labeled Phalloidin and 4, 6-Diamidino-2-phenylindole (DAPI) solution (Abcam), respectively. Finally, laser scanning confocal microscopy (LSM 880, ZEISS, Germany) was used to obtain cell proliferation and adhesion images at 405/516 nm, recording three field images per sample.

### 2.10 In vivo wound healing in full-layer skin model

According to the ethical standards of the International Center for Animal Experiments, this study was approved by the Ethical Committee of Animal Experiments in Nanfang Hospital of Southern Medical College of China. Male SD rats (n = 24, 12 weeks of age, 250–270g) were purchased from the experimental animal center of southern medical university. Before the experiment, 0.03% pentobarbital sodium (0.2ml
/10g) was used to induce anesthesia after one week of environmental adaptation. Under aseptic surgery, full-thickness skin defect wounds of 12mm diameter were made on both sides of the back. The animals were randomly divided into CADMS-G-2-Hep-CGF, CADMS-G-2-Hep, CADMS-G, and normal saline (NS, as blank control). By removing the deep subcutaneous tissue, the full-thickness skin trims for full-thickness skin piece, then USES the cross-matching principle to optimize material into two wound beds, with a continuous suture of full-thickness skin cover, finally to disinfection of the wound, and in turn with vaseline gauze, sterile dressings pressurized packaging, external dressing change once every three days, 14 days to dismantle dressings. On days 7, 14, 21, and 28 after surgery, six rats were randomly killed by carbon dioxide inhalation (12 wounds in total, three times in each group). At the same time, rats' hearts, liver, spleen, lungs, and kidneys were collected. Then all samples were fixed with paraformaldehyde, embedded in paraffin, and stained for subsequent experimental operations.

Sterile operation conditions, on the back to build four 12 mm diameter model of full-thickness skin defect wound will clip for full-thickness skin, the skin will material implant four wound bed, with a continuous suture of full-thickness skin cover, finally to disinfection of the wound, and in turn with vaseline gauze, sterile dressings pressurized packaging, external dressing change once every three days, The dressing was removed on day 14. After the operation, the rats were randomly divided into two groups, with four rats in each group, including the NIR stimulation group (CADMS-G-2-Hep-CGF, CADMS-G-2-Hep) and the NIR stimulation group (CADMS-G-2-Hep-CGF-INR and CADMS-G-2-Hep-INR). The non-NIR stimulation group was not irradiated with a NIR lamp, while the NIR stimulation group was irradiated with a NIR lamp (0.05 ~ 0.1 W·cm⁻²) for 30 min on the back of SD rats. The wound area of the rats was recorded on days 0,3,7,14 and 21. The SD rats were sacrificed on days 14 and 28, and the tissue around the implanted sample was removed for fixation, embedding, and sectioning, and subsequent experimental operations were carried out.

### 2.11 Skin graft survival, contraction, and capillary density

The wound was photographed with a digital camera, and the unhealed area and scar area were quantified by Image J software. The graft and surrounding tissue were collected and flattened on a transparent petri dish. A white light source was set from the bottom of the dish to illuminate the sample, observe the blood vessels, and photograph and record them.

### 2.12 H&E and Masson trichromatic staining

After paraffin-embedded samples were selected, dewaxed, and rehydrated, hematoxylin and eosin (H&E) staining and Masson trichromatic staining were performed. Images were taken by an automatic section-scanner (C10730-12, HAMAMATSU) to measure the number of new vessels and collagen deposition histologically. Collagen density was measured using ImageJ software.
2.13 Immunohistochemical staining of Ki67, CD31, and α-SMA

Immunohistochemistry of Ki67, CD31, and α-smooth muscle actin (α-SMA) evaluated tissue regeneration, angiogenesis, and scar formation in wound tissue. In short, the skin tissues of each group were routinely dewaxed, repaired, washed, sealed, diluted, cleaned, incubated with antibodies, stained, and sealed, and then photographed with a fluorescence microscope or automatic sectioning scanner.

2.14 Statistical Analysis

All data were analyzed with three replicates and expressed as mean ± standard deviation. All quantitative data were recorded by GraphPad 8.02 software and statistically compared between groups using an independent sample T-test or one-way ANOVA and expressed as mean standard deviation. Values of P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***) were considered statistically significant, while NS was not significant.

3. Results And Discussion

3.1. Preparation and characterization of acellular dermal matrix gel

The synthesis principle of multi-functional scaffold materials is shown in Fig. 1. Polydopamine nanospheres (PDA-NPS) with excellent NIR photothermal conversion ability were introduced into the acellular dermal matrix glue (ADM) and chitosan (CS) interpermeable network structure and heparinized to prepare multi-functional composite scaffolds with good NIR photothermal response, antibacterial activity, and drug delivery ability (Fig. 1A). Heparinization modification enabled the composite scaffold to absorb CGF and achieve adequate fixation and slow release of growth factors (Fig. 1C). PDA-NPS have excellent NIR photothermal conversion ability and can significantly promote the survival of autologous skin grafts in rats (Fig. 1B).

ECM provides tissue-specific ultrastructural support and functional molecules that influence local cell behavior. Acellular dermal matrix was prepared by the high osmotic salt-caustic soda method in the team's early stage. Acellular dermal matrix was cut into pieces and then stirred and dissolved in acetic acid solutions of different concentrations to obtain acellular dermal matrix glue. As shown in Fig. 2A, acellular dermal matrix gel becomes more viscous with increasing acetic acid concentration and takes on a white, loose, and porous spongy form when freeze-dried (Fig. 2B), supporting cell growth and allowing free flow in and out of liquid components[19]. The results of Fourier transform infrared spectroscopy (Fig. 2C) showed that the attribution peaks of the characteristic amide bands dominated by collagen, including the amide I band (near 1640 cm⁻¹), the amide II band (near 1530 cm⁻¹), and the amide III band (near 1232 cm⁻¹), were almost unchanged. The results also showed that the collagen structure of
PADMS was not damaged. Figure 2D shows that sponge DNA content gradually decreased with the increase of acetic acid concentration, which were 0.15 ± 0.02 ug/mg, 0.09 ± 0 ug/mg, 0.06 ± 0 ug/mg (**P < 0.01, ***P < 0.001). The CCK-8 assay was used to evaluate the cytotoxicity of the materials. Figure 2E showed that an acellular dermal matrix prepared with 1% acetic acid had good biocompatibility, while excessively high concentrations of acetic acid (2%) caused damage to cells. Therefore, acellular dermal matrix adhesive prepared at 1% concentration was selected for subsequent experiments.

3.2 Preparation and characterization of acellular dermal matrix - chitosan composite sponge scaffolds

As shown in Fig. 3A, with the increase in chitosan proportion, the color of composite sponges gradually deepened from white to light yellow, and the distribution was uniform, indicating that chitosan was evenly dispersed[20]. Figure 3B SEM showed that the simple chitosan sponge presented layered distribution, while the other proportions of composite scaffolds presented an irregular arrangement of three-dimensional interconnected pore structure. Different proportions of PADM-chitosan composite sponges were soaked in deionized water (Fig. 3C), and Only 10%PADM, 2:1, 3:1, 4:1, and 5:1 groups were dissolved in water and had fixed morphology. The staining results of living and dead cells showed that (Fig. 3D) each group had good biocompatibility and that cells adhered along to the pore structure of the sponge. The hygroscopicity and water retention of scaffold materials are the keys to removing wound exudate, maintaining a moist environment, promoting wound healing, and preventing scabs. In contrast, the other groups were dissolved in water. The staining results of living and dead cells showed that each group had good biocompatibility and that cells adhered along to the pore structure of the sponge. The hygroscopicity and water retention of scaffold materials are the keys to removing wound exudate, maintaining a moist environment, promoting wound healing, and preventing scabs[21]. The results of Fig. 3 (E, F) showed that the group 2:1 composite sponge had good water absorption (5480.74 ± 430.14%) and moisture retention (4604.06 ± 528.87%). Therefore, adding a certain amount of chitosan to the PADM sponge can improve moisture retention and water absorption. It is well known that the pore structure and porosity of sponges are vital factors affecting wound healing because they have a significant effect on exudate and blood absorption[22]. The pore structure of the composite sponge was measured by the ethanol replacement method, as shown in Fig. 3G. The 2:1 group had excellent porosity (94.1 ± 4.52%), which indicated that adding an appropriate proportion of chitosan was beneficial to improving the physical and chemical properties of the composite sponge. In summary, group 2:1 composite sponges were selected for subsequent experiments and redefined as the CADMS group. The mechanical properties of scaffolds are very important for their operability in clinical use. Figure 3H further verifies the above conclusion from the mechanical perspective. In summary, group 2:1 composite sponges were selected for subsequent experiments and redefined as the CADMS group.

3.3 Glutaraldehyde cross-linking and heparinization modification of composite sponge scaffold
According to the literature[23, 24], 0.02 V/V. % glutaraldehyde was selected for appropriate cross-linking of the material. Figure S1A Masson's trichromatic and H&E staining showed no residual nucleus, and collagen was well preserved. In addition, Glycosaminoglycan (GAG) was preserved to some degree. After cross-linking with glutaraldehyde, the material's porosity is more significant (Figure S1B), conducive to cell incorporation, adhesion, and growth. The IR spectra of the scaffold clearly showed the characteristic peaks of the collagen/chitosan scaffold (Figure S1C) at 1645 cm$^{-1}$ (C = O stretched in the amide I), 1550 cm$^{-1}$ (N-H bent in amide II), and 1151 cm$^{-1}$ (C-O-C) there are several absorption bands of chitosan polysaccharide structure[25]. The cross-linked glutaraldehyde scaffold had an additional peak at 1652 cm$^{-1}$ (C = N bond), indicating the presence of GTA's Schiff base[26]. Representative stress-strain curves before and after glutaraldehyde cross-linking were shown in Figure S1D. The elastic modulus of scaffolds was calculated according to the stress-strain curves (Figure S1E). Glutaraldehyde cross-linking increased the tensile strain at fracture of materials in the CADMS group but did not significantly increase the elastic modulus of materials (NS, P > 0.05). CCK-8 assay confirmed (Figure S1F) that appropriate glutaraldehyde cross-linking was performed in a non-cytotoxic manner (P > 0.05).

Heparin can bind to a variety of growth factors that contain heparin-binding domains[27]. Therefore, covalently linking heparin to ECM can steadily release growth factors. The EDC/NHS reaction is the most commonly used reaction to attach heparin to collagen covalently is the EDC/NHS reaction[28]. Spectrophotometric determination of trace amounts of heparin using toluidine blue is the most widespread and mature method[29, 30]. Figure S1(G, H) shows that the toluidine's blue color gradually deepened with increased heparin concentration[31]. The absorbance reached the maximum value when the heparin concentration was 2g/L; that is, the modification of heparin reached saturation. It can be seen from the infrared spectrum (Figure S1K) that the characteristic peaks of OSO$_3^-$ and S0$_3^-$ on heparin at 1224cm$^{-1}$ and 1143cm$^{-1}$ indicate that heparin has been effectively bound to the composite scaffold of CADMS-G[30]. Figure S1J shows that the morphology and water absorption swelling of CADMS-G before and after heparin modification did not change significantly because the scaffold modified by the heparin interface was only modified at the interface and did not participate in the phase separation process[32]. CCK-8 assay confirmed (Figure S1I) that heparinized modification had no significant effect on cell proliferation (P > 0.05).

3.4 Preparation and characterization of composite sponge scaffolds doped with polydopamine nanospheres

Figure 4A is the PDA-NPS preparation process model diagram. Figure 4B shows that the solution gradually changed from colorless and transparent to a light brown solution with the passage of reaction time. After 24 h of reaction, the color of the solution was brown-black, and the color did not change, indicating that the polymerization was complete. UV spectrum shows (Fig. 4C) that the solution has a strong absorption peak at 280 nm, which is the characteristic UV absorption peak of a DA monomer. As the reaction progressed, the absorption peak of the solution at 280 nm gradually weakened, while the absorption peak at 320 nm appeared, indicating the formation of quinone bonds. The absorption peak at
370 nm indicates PDA formation, indicating intramolecular cyclization between quinone bond derivatives[33]. Scanning electron microscope results showed (Fig. 4D) that the average particle size of PDA-NPS prepared was about 0.17 ± 0.02 um, and the particle size distribution was uniform. As shown in Fig. 4E, CADMS-G gradually deepened in color with the increase of added PDA-NPS concentration. At 2g/L, CADMS-G showed homogeneous black and formed a black spongy shape after freeze-drying. Scanning electron microscopy (Fig. 4F) observed that after the addition of PDA-NPS, the composite scaffold retained a similar interpenetrating three-dimensional porous structure as a CADMS-G scaffold. PDA-NPS in the 2g/L group were evenly dispersed and embedded in the pore wall of the composite scaffold, while agglomeration occurred in the 4g/L group. CCK-8 assay confirmed (Fig. 4G) that the incorporation of PDA-NPS was performed in a non-cytotoxic manner[34]. The surface of PDA-NPS contains many hydrophilic groups, so the PDA-NPS surface should have good interaction with -OH in CADMS-G molecular chain. Fourier infrared absorption spectrum comparison showed (Fig. 4H) that more substantial -OH stretching vibration peaks appeared near 3274 cm⁻¹ and 1086 cm⁻¹ in the composite scaffold mixed with PDA-NPS, and the movement of these -OH peaks confirmed the strong hydrogen bond between PDA-NPS and CADMS-G[35].

The antibacterial activity of the compound sponge against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) was studied by the colony-forming unit (CFU) test[36]. Qualitatively shown in the picture (Fig. 4J), the number of colonies on the composite sponge with chitosan was significantly reduced compared with the control group. To better understand the effect of different samples on antibacterial activity, the bacterial killing rate was determined by counting the number of colonies. Figure 4I showed that the killing rate of composite sponges mixed with 2g /L PDA-NPS was greater than 99%, significantly higher than that of the CADMS-G group and 4g/L group. The mechanical properties test showed (Fig. 4K) that the addition of PDA-NPS increased the tensile strain at fracture but did not significantly improve the material's elastic modulus.

To study the photothermal conversion ability of the composite scaffold, the composite scaffold was placed in distilled water and then irradiated with an 808 nm NIR laser (0.8 W·cm⁻²). The temperature of distilled water was measured and recorded at a specific time interval. As shown in Figure S2 (A, B), NIR photothermal conversion of composite scaffolds depends on the content of PDA-NPS in composite scaffolds, and the higher the content of PDA-NPS in composite scaffolds the higher the temperature rise will be. After 30 min NIR laser irradiation (Figure S2C), the water temperature around the composite scaffold in 1g/L and 2g /L groups rose slowly from 22 ºC to 44 ºC. In contrast, the temperature in the 4g/L group reached 47 ºC due to local aggregation of PDA-NPS. The 2g/L group was optimized for subsequent experiments and renamed CADMS-G-2-Hep.

Studying the degradation process of collagen-based biomaterials in vivo is of great significance for improving the coordination between the degradation process and the autologous tissue formation process of the recipient, ensuring the properties of the material before tissue formation and enabling its self-degradation after tissue formation. In vitro degradation test results showed (Figure S2D) that the CADMS-G-Hep group and CADMS-G-2-hep group still had residues after 24h, while the control group had
no obvious visible residues. Their 28h degradation rates (Figure S2E) were 0.89 ± 0.02, 0.91 ± 0.02 and 0.75 ± 0.03, respectively. The in vivo degradation experiment (Figure S2F, S2G) showed that clear contours were visible in each group after two weeks. A parcel was formed between the material and the tissue, with an obvious inflammatory cell infiltration reaction around the tissue. After 16 weeks, materials in CADMS-G and CADMS-G-Hep groups were completely degraded, and only the location of materials could be determined by fuzzy judgment. However, materials in the CADMS-G-2-Hep group had an irregular shape and were completely attached to tissue fascia, which was difficult to separate. The undegraded part was thin and dense, without obvious inflammatory cell infiltration.

As shown in the microstructure (Figure S2H), fibrin fragments remained in the pores of the composite scaffold after soaking and loading CGF in the CADMS-G-2-Hep group. Results of the growth factor release experiment in vitro (Figure S2J) showed that PDGF and VEGF showed short-term explosive release of growth factor within 36h in the CGF group. The sudden release effect of the CADMS-G-Hep group was alleviated to some extent, while the CADMS-G-Hep group was the gentlest, which may be due to the rich functional groups of PDA-NPS as bioactive sites to achieve effective fixation and slow release of growth factors[37, 38].

3.5 Cell migration experiment

Wound healing involves multiple processes of cell proliferation, migration, and angiogenesis. An improved cell migration experiment was used to reflect the characteristics of wound healing and regeneration [39], as shown in Fig. 5A. A transwell chamber was placed on the adherent cells, the material was placed in the chamber, and the cell culture medium submerged the material. At 36 h after scratches, both in HUVEC and L929 cells, cells on both sides of the CADMS-G-2-Hep-CGF and CADMS-G-4-Hep-CGF groups were in contact with each other (Fig. 5D,5E) and showed significantly increased mobility (Fig. 5B,5C). The results showed that the CADMS-G-Hep-CGF group significantly promoted cell migration within 36 h (P < 0.01), and the positive effect was further enhanced by adding PDA-NPS. However, no statistical difference was observed between the CADMS-G-2-Hep-CGF group and the CADMS-G-4-Hep-CGF group (P > 0.05).

3.6 Cell adhesion and proliferation experiments

L929 cells and human umbilical vein endothelial cells (HUVEC) were inoculated for three days. As shown in Fig. 6A, cells in each group grew well, distributed along with the material's pores, and adhered to a large number of living cells, with no statistical difference between groups (Fig. 6D) (ns, P > 0.05). Co-culture of material extract and cells could reflect the cytotoxicity of the material to a certain extent[40]. Morphological results of cells in Fig. 6B showed that heparinized CADMS-G did not affect cell morphology, and the addition of PDA-NPS promoted cell proliferation to a certain extent. No difference was observed between the CADMS-G-2-Hep-CGF group and the CADMS-G-4-Hep-CGF group. Fluorescence staining results of DAPI-Photolipin (Fig. 6C) showed that HUVEC were closely attached to the pore walls of each group of materials after three days of culture, and typical polygonal cells with normal morphology could be observed. Moreover, quantitative fluorescence results showed (Fig. 6E) that HUVEC
cultured in the CADMS-G-2-Hep-CGF group and CADMS-G-4-Hep-CGF group had the most substantial proliferation ability (P < 0.001). There was no statistical difference between the CADMS-G-2-Hep-CGF group and the CADMS-G-4-Hep-CGF group (NS, P > 0.05). These results confirmed that optimized CADMS-G-2-Hep combined with CGF could promote the adhesion and proliferation of skin and soft tissue healing related cells (HUVEC and L929 cells) on the material. We are confident to speculate that CADMS-G-2-Hep-CGF may be an alternative drug-delivered scaffold with good potential as a template for skin regeneration to achieve efficient regeneration of full-thickness skin defects.

3.7 Preparation of CGF and evaluation of in vitro biocompatibility

As shown in Figure S3A, the CGF fibrin solution prepared by variable speed centrifuge is also a light yellow liquid, which will not change significantly when standing at room temperature. After freeze-drying (Figure S3B), CGF with different concentrations showed a loose and porous spongy structure with brittle quality. When CGF with different concentrations were incubated at 37°C for 24h, 10% and 15% of CGF could be seen to form a gel (Figure S3C). CCK-8 results showed (Figure S3D) that CGF concentration below 15% had no significant effect on cell proliferation, while CGF concentration above 15% inhibited cell activity. In microscopic morphology (Figure S3E), CGF with different concentrations has a particular three-dimensional fiber network structure and fragile texture. L929 cells were cultured with different concentrations of CGF (Figure S4A). CGF solution with a concentration below 20% could achieve the same effect as a 10% complete medium to maintain cell growth. However, CGF concentration above 20% could not maintain cell growth at 48h. The cell migration experiment further optimized the concentration of CGF (Figure S4B) and found that 15% CGF promoted cell migration at 36h to achieve the best effect. 10% of CGF continued to promote cell migration for 48h until contact between cells. Quantitative mobility results showed that (Figure S4C), the 48h mobility was 10%CGF > Control > 15%. In conclusion, the optimized 10%CGF has the best effect on cell proliferation and migration.

3.8 In vivo wound healing in the full-thickness skin defect model

To verify the effect of CADMS-G-2-Hep-CGF on wound healing and regeneration in vivo, a full-thickness defect wound model of rat back was constructed, and a stage autologous skin graft was performed. The surgical design is shown in Fig. 7(A, B). On the seventh postoperative day, the grafts in the CADMS-G group showed signs of erosion, ischemia, and unshed scab. In contrast, the grafts in the CADMS-G-2-Hep group showed slight necrosis, indicating that the grafts were unstable and immature, verifying that the artificial dermal stent needed a second transplant to establish a blood supply[41]. The grafted skin of the CADMS-G-2-Hep-CGF group was flat and firmly attached to the recipient bed (Fig. 7C). With the extension of time, the unhealed wounds in the CADMS-G group and CADMS-G-2-Hep group showed a decreasing trend with the progress of transplantation (Fig. 7G). Neovascularization observations of skin showed similar results. Namely, after loading CGF, the CADMS-G-2-Hep group showed more new vessels and more substantial branches, forming microvessels that could be fused with the rat vascular system (Fig. 7D).
3.9 Histological analysis

3.9.1 H&E staining and Masson trichromatic staining

Hair follicles and their attached sebaceous glands are the relevant components of reconstructed skin[42]. H&E staining enabled us to analyze structural changes in the graft epidermis. As shown in Fig. 7E, on day 14, the full-thickness skin grafts in each group mostly retained evenly distributed hair follicles. The follicles enlarge and grow hair from day 14 to day 28 as the remodeling progresses. Survival after implantation depends mainly on efficient blood circulation, which provides nutrients essential for skin repair[43]. The development of functional neovascularization early in transplantation is associated with enhanced maintenance of healthy skin grafts, preventing scarring formation and subsequent contraction[44]. Figure 7F shows that the number of microvessels (characterized by endothelialized lumen containing red blood cells) in the CADMS-G-2-Hep-CGF group was significantly higher than that in the control group on days 14 and 28 after surgery (Fig. 7H) (P < 0.001). Compared with intact skin, collagen fibers in scar tissue are densely packed and linear in the direction of tension, leading to myofibroblast proliferation and scar contraction[45]. Collagen deposition and tissue in skin grafts were analyzed by Masson trichromatic staining (blue areas represent collagen). As shown in Fig. 7F, the collagen fibers in the CADMS-G group and CADMS-G-2-Hep group were arranged compactly 14 days after surgery. In contrast, the skin of the CADMS-G-2-Hep-CGF group and the control group showed loose collagen fiber arrangement, which was conducive to reducing scar formation. The quantitative image analysis results further supported these observations (Fig. 7I). On the 14th day after surgery, collagen deposition in the CADMS-G-2-Hep-CGF group was higher than in the control group (P < 0.05). As time went by, on day 28, compared with the control group, the collagen in the CADMS-G-2-Hep-CGF group was more orderly, conducive to the migration and infiltration of fibroblasts to the wound surface thus stimulating the secretion of more and more mature collagen. In contrast, the disordered and immature collagen state in the CADMS-G group may contribute to scar hyperplasia[46].

3.9.2 Immunofluorescence staining

Ki67 is a proliferation cell-associated antigen. Its function is closely related to mitosis and is indispensable in cell proliferation[47]. Ki67 expression was significantly increased in skin grafts and hair follicles in the CADMS-G-2-Hep-CGF group (Figs. 8A and 8B). Fluorescence quantitative results showed the same trend with significant statistical differences (*P < 0.05, **P < 0.01, ***P < 0.001). These results indicate that the addition of CGF promotes the proliferation of graft skin cells, and the wound is ready to move from a repair stage characterized by reepithelialization and granulation tissue development to a functional remodeling stage[48].

3.10 Near-infrared photothermal conversion promotes the healing of wound grafts

In vivo, full-thickness skin defect repair experiments showed that the introduction of CGF promoted wound healing. Figure 9(A, B) shows the surgical design scheme of near-infrared photothermal
conversion to promote wound healing of grafted objects. The results showed that the grafts in each group had no infection and began to heal gradually after surgery. Figures 9C and F show that the CADMS-G-2-Hep-CGF-INR group had lower skin shrinkage than the CADMS-G-2-Hep group and CADMS-G-2-Hep-INR group. CADMS-G-2-Hep-CGF group had a lower skin shrinkage rate than the CADMS-G-2-Hep group. The above results showed that the addition of CGF was beneficial to skin survival, and the introduction of INR further amplified this effect. Our CADMS-G-2-Hep-CGF is also suitable for combating scar contraction in patients with significant skin defects, compared with most group engineered materials that focus on stimulating wound healing.

3.10.1 H&E staining

Locally administered stent materials can accumulate in various organs through the bloodstream, which may also cause some early and late organ toxicity[49]. Finally, each experimental animal's heart, liver, spleen, lung, and kidney were obtained on days 3 and 21 and stained with H&E for histological evaluation (Fig. 9D). The results showed no noticeable pathological changes of cell necrosis, swelling, congestion, and other lesions in all organs, only slight inflammatory cell infiltration in the liver, and no significant difference was observed in other organs, which indicated that the set dose was safe for mice. This further supports the application of CADMS-G-2-Hep-CGF in wound healing. After H&E staining, the repaired skin tissues of each group were further analyzed (Fig. 9E). For the group without NIR irradiation, the composite scaffold itself was beneficial to skin tissue regeneration, especially at the initial stage of tissue regeneration. H&E staining results showed that compared with the CADMS-G-2-Hep group, the CADMS-G-2-Hep-CGF group was more conducive to the regeneration of graft skin appendage. The new tissue infiltrated into the porous structure of the composite scaffold. For the CADMS-G-2-Hep-CGF group irradiated by NIR light, the regeneration rate of skin graft accessory was further increased, and the regeneration tissue was more mature, similar to natural hair follicle tissue. These results indicate that the composite scaffold introduced by PDA-NPS can respond to NIR stimulation and achieve NIR photothermal therapy to promote skin tissue regeneration[50, 51]. In contrast, the introduction of CGF and NIR photothermal therapy has a synergistic effect and further accelerates the wound healing rate.

3.10.2 Immunohistochemical staining

As a cytoskeletal protein, α-smooth muscle actin is a typical marker of myofibroblast contraction. The wound repair process is often accompanied by wound contraction and scar formation. α-smooth muscle actin is the material basis of scar contraction and determines the outcome of the scar[52]. Figures 10A and 10C showed that the distribution of α-smooth muscle actin in the CADMS-G-2-Hep-CGF-INR group was less than that in the other three groups, followed by the CADMS-G-2-Hep-INR group, CADMS-G-2-Hep-CGF group, and CADMS-G-2-Hep group. There was statistical significance (P < 0.05). These results indicated that the CADMS-G-2-Hep-CGF-INR group inhibited the deposition of α-smooth muscle actin and ultimately reduced scar formation. Neovascularization provides oxygen and nutrients for wound repair and affects cell tissue proliferation and remodeling. Therefore, CD31 was used to label vascular endothelial cells to analyze the degree of vascular formation during wound healing. Figures 10B and 10D
showed that the distribution of CD31 positive cells in the CADMS-G-2-Hep-INR group was more comprehensive than that in the other three groups, and the diameter of blood vessels was also more prominent, indicating that CADMS-G-2-Hep-INR group could accelerate the formation of blood vessels in the process of wound healing.

4. Discussion

The usual treatment for large-area skin defect wounds includes debridement, anti-inflammatory medications, keeping the wounds moist, and covering allogeneic and allogeneic skin[53]. Its flaw is that it can only debride necrotic tissue on the surface and suppress local inflammation; it has no advantage in promoting wound healing, such as revascularization, promoting healing factor secretion, and controlling fibroblast apoptosis and proliferation, resulting in a long or even enlarged and deeper wound treatment cycle[54]. To some extent, the development and application of skin tissue engineering can improve skin grafting treatment.Currently, after thorough wound debridement, a stent is implanted in the wound in the first stage, and skin grafts are performed in the second stage to cover the wound bed after 2 days of pre-vascularization[55]. Needless to say, requiring two surgeries is not only inconvenient and frustrating but also lengthens hospital stays and increases the risk of infection. As a result, one-step combined transplantation has promising clinical prospects. The main challenge of this strategy, however, is to promote cell adhesion, proliferation, and vascularization in the short term after surgery to achieve sustained and effective tissue regeneration with adequate vascularization to achieve optimal skin graft survival.

In this study, we investigated the NIR solar-thermal conversion coating’s ability to introduce decellularized dermal matrix (ADM) and chitosan (CS) and heparinization in interpenetrating network structure modification with good NIR onto thermal response-ability, antimicrobial properties, and multi-function scaffold drug delivery ability. The stent’s advantages are that it overcomes the shortcomings of a single function, poor vascularization ability, and no antibacterial effect in wound repair that are currently available on the market. The goal of designing an acellular dermal matrix chitosan sponge scaffold is to create a composite scaffold with both properties so that the composite sponge scaffold has high porosity and biocompatibility while also providing excellent antibacterial performance. Through the characterization of physical and chemical properties, we finally determined the optimal ratio of 2:1.

At 25°C with the collagen amino crosslinking reaction, glutaraldehyde with aldehyde group in the molecule, the activity of the two aldehyde groups is stronger, easily with collagen amino residue in amino acid and free amino in reaction, and crosslinking is very stable[56]. Crosslinked collagen is extremely stable and resistant to acid and boiling water, and its enzymatic hydrolysis stability has been greatly improved. It does, however, have obvious drawbacks, such as cytotoxicity, and its dosage is difficult to control[57]. We chose 0.02 V/v.% percent glutaraldehyde for appropriate cross-linking of the material based on the literature, and the results showed no obvious toxicity. The material’s pore size is larger after cross-linking, which promotes cell incorporation, adhesion, and growth.
Heparin can bind to a wide range of growth factors with heparin-binding domains. As a result, covalently linking heparin to ECM can continuously release growth factors[58]. The EDC/NHS reaction is the most commonly used reaction to covalently attach heparin to collagen[59]. The absorbance reached its maximum value when the heparin concentration was 2g/L, indicating that the heparin modification had reached saturation.

PDA NPS can be introduced as a NIR photothermal material into the network structure of natural polymer materials to form a skin substitute with good NIR photothermal properties for wound healing[60]. The composite scaffold retained the same interpenetrating three-dimensional porous structure as the CADMS-G scaffold after the addition of PDA-NPS. After near-infrared light irradiation, the PDA-NPS in the 2g/L group were evenly dispersed and embedded in the pore wall of the composite scaffold, and the temperature change was more gentle. Antibacterial experiments confirmed the previous findings, and the antibacterial activity of the 4g/L group decreased. We hypothesized that adding PDA-NPS to the material would improve its biocompatibility[61]. Growth factor release experiments also revealed that after heparin modification and the addition of PDA-NPS, growth factor release was slower.

Optimized CADMS-G-2-Hep combined with CGF could promote the adhesion and proliferation of skin and soft tissue healing related cells (HUVEC and L929 cells) on the material in an in vitro cell level experiment. We are confident in hypothesizing that CADMS-G-2-Hep-CGF may be an alternative drug-delivered scaffold with good potential as a skin regeneration template for achieving efficient regeneration of full-thickness skin defects[62, 63]. We used a multifunctional drug-loading scaffold combined with an autologous skin graft to test the effect of scaffold material on skin survival in a full-thickness skin defect model of rats in vivo. The CADMS-G grafts showed signs of erosion, ischemia, and unshed scab, whereas the CADMS-G-2-Hep grafts showed slight local necrosis, indicating that the grafts were unstable and immature, confirming that the artificial dermal stent required a second transplant to establish a blood supply[64]. The grafted skin in the CADMS-G-2-Hep-CGF group was flat and firmly attached to the recipient bed, with no obvious ischemic necrosis. The CADMS-G-2-Hep group had more new blood vessels and stronger branches, more regular collagen deposition, and more cell proliferation after CGF loading, according to histomorphological results. This implies that the addition of CGF promotes the proliferation and migration of graft skin cells, and the wound is now ready to transition from a repair stage characterized by reepithelialization and granulation tissue development to a functional remodeling stage[65, 66]. We repeated animal experiments to confirm the superposition effect of NIR and CGF. The results showed that CGF was beneficial to the survival of skin slices, and the addition of INR amplified this effect, and the skin slices had no obvious shrinkage, smaller scars, and more regeneration of skin appendages in the later stage.

To summarize, the positive effect of the CADMS-G-2-Hep-INR group on wound repair could be attributed to the following factors: For starters, the CADMS-G-2-Hep composite scaffold has a 3D microporous structure similar to ECM, which does not only promote cell adhesion and proliferation but also induces tissue growth[67]. Second, CADMS-G-2-Hep composite scaffolds have a high liquid absorption capacity and can absorb nutrients and components from the surrounding host tissues, promoting cell growth and
tissue regeneration[68]. Third, heparinization modifications improve the loading capacity and controlled release effect of growth factors, allowing local CGF concentrations to remain high and more effective in angiogenesis and tissue repair. Finally, CADMS-G-2-Hep exhibits excellent NIR photothermal properties, including the ability to respond to external NIR stimulation, accelerate blood flow, improve substance metabolism, and promote tissue regeneration to achieve NIR photothermal therapy[69].

This study still has some limitations. Pigs were used in this study to obtain enough blood to produce large amounts of CGF uniformly. The scaffold material was not completely degraded because the in vivo degradation experiment ended at 16 weeks, and the degradation products were not traced in vivo, which will be improved in our next study. Furthermore, because each rat had four different groups on its back, we were unable to compare the difference in GFs release in vivo and its effect on body circulation by collecting whole blood from rats. Furthermore, thin skin slices were used in the pre-experiment, but all groups experienced extensive necrosis. As a result, full-thickness skin grafts were used in informal experiments, and they demonstrated the expected survival rate and differential effect. Thin-layer skin grafts, on the other hand, were commonly used in clinical practice. Is this difference due to species differences in the skin?

5. Conclusion

We successfully prepared a novel multi-functional composite repair scaffold, CADMS-G-2-Hep, which has good cytocompatibility and tissue affinity and can synergically promote skin regeneration and wound healing in response to external NIR stimulation. The composite can effectively adhere to more CGF and achieve a durable growth factor release time. Cells in vitro and in vivo animal experiments, the compound biological material to promote angiogenesis, cell proliferation, and adhesion, improve the full-thickness skin defect wound skin, reduce the shrinkage of the implant and scar formation, promote wound healing and tissue remodeling, provides the design of the biological tissue engineering materials more choice.

Declarations

Consent for publication

Not applicable.

Authors’ contributions

LLC contributed to the conception and design of the work, interpretation of the data, and revising of the manuscript. CYH&YJC&HHZ contributed to performing the experiments, acquisition, analysis, and interpretation of data drafted and revised the manuscript. LH&ZWJ contributed to performing the experiments. ZJZ&YZ contributed to the acquisition and analysis of the data. XRW&YJP&LBN contributed to the analysis and interpretation of the data and revising of the manuscript. YBG&JM contributed to the conception and design of the work, interpretation of the data, and revising of the manuscript. LY contributed to revising the manuscript. All the authors approved the submitted version.
Funding

This work was supported by the Natural Science Foundation of Guangdong Province [No.2020A151501108], the Guangdong Province Key Field R&D Program Project [No. 2020B1111150001], the Science and Technology Innovation Project of Guangdong Province [No. 2018KJYZ005], the Natural Science Foundation of Tibet Autonomous Region [No. XZ2017ZR-ZY021].

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The procedures involving animal use for the rat full-thickness defect wound experiment were approved by the international animal care and use committee (NFYY-2022-0105).

Competing interests

The authors declare that they have no competing interests

References


30. Xie J, Wan J, Tang X, Li W, Peng B. Heparin modification improves the re-endothelialization and angiogenesis of decellularized kidney scaffolds through antithrombosis and anti-inflammation in


Figures
Figure 1

Schematic diagram of the multifunctional wound repair sponge scaffold preparation. (A) Preparation of CADMS-PDA-Hep composite sponge scaffold. (B) CADMS-PDA-Hep composite sponge scaffolds loaded with CGF exhibited synergistic effects of slow release of growth factor and photothermal conversion to accelerate wound healing under NIR stimulation. (C) Schematic diagram of the synthesis mechanism of the multifunctional composite sponge scaffold.
Figure 2

Preparation and characterization of acellular dermal matrix gel. (A) Images of acellular dermal matrix dissolved in different acetic acid concentrations (0.5%, 1%, 2%) before and after lyophilization. (B) SEM images of acellular dermal matrix sponges. (C) FTIR spectra of 0.5%, 1% and 2% groups. (D) Residual DNA content in 0.5%, 1% and 2% groups. (E) Evaluation of cytotoxicity of acellular dermal matrix sponges by CCK-8.
Figure 3

Preparation and characterization of acellular dermal matrix-chitosan composite sponge scaffolds. (A) Freez-dried morphology of 10%PADM mixed with 3% chitosan at different volume ratios. (B) SEM images of PADM-chitosan. (C) Morphology of PADM-chitosan after immersion in deionized water. (D) Living and dead cell staining evaluate the biocompatibility of different PADM-chitosan group materials.
(E) Determination of moisture retention. (F) Determination of water absorption of materials. (G) Determination of material porosity. (H) Representative tension-stress curves of materials.

Figure 4

Preparation and characterization of the composite sponge scaffold doped with polydopamine nanospheres. (A) PDA-NPS preparation process model diagram. (B) PDA-NPS synthesis process. (C)
Ultraviolet spectra of PDA-NPS. (D) SEM images of PDA-NPS. (E) The freeze-desiccation-wetting process of the sponge scaffold doped with polydopamine nanospheres. (F) SEM images of dopamine nanospheres composite sponge. (G) Relative cell viability. (H) Fourier infrared spectroscopy. (I) Antibacterial rate of Escherichia coli (a) and Staphylococcus aureus (b). (J) Photographs of antibacterial experiments in vitro by AGAR dilution method. (K) Obtain the (a) stress-strain curve and (b) elastic modulus of the material by tensile test.
**Figure 5**

The effect of multifunctional drug-delivered sponge scaffold on cell migration was evaluated. (A) Modified scratch test map; (B) Statistical quantification of HUVEC mobility (*P<0.05, **P<0.01). (B) Statistical quantification of L929 cell mobility (*P<0.05, **P<0.01). (D) Physical map of HUVEC migration and change at different time points. (E) Dynamic changes of L929 cell migration.
Figure 6

The biocompatibility of multifunctional sponge scaffolds was evaluated at the cellular level in vitro. (A) Fluorescent staining of living and dead cells; (B) morphology of keratinocytes cultured with multifunctional sponge scaffold extract for 48h. (C) Fluorescence staining of DAPI-Ghostopeptide. (D) Statistical quantification of fluorescence intensity of living and dead cells. (E) Statistical quantification of DAPI-positive fluorescence staining intensity; (*P<0.05, **P<0.01, ***P<0.001).
Figure 7

Evaluation of wound healing. (A) Schematic diagram of the application of composite sponge scaffolds in rat wounds; (B) Schematic diagram of rat autologous skin transplantation combined with multifunctional drug-delivered sponge stent transplantation; (C) Representative images of the survival of full-thickness skin grafts at different time points. The edges of the unhealed wound are circled with a red dotted line; (D) Images of neovascularization of skin grafts in each group on days 14 and 21; (E) H&E staining of skin grafts collected at different time points, hair follicles (black star), implants (black arrow). (F) Masson trichromatic staining of skin grafts, and blood vessels (red arrow). (G) Changes of unhealed wounds on postoperative days 3, 7, and 14. (H) Quantitative vascular analysis. (I) Quantitative analysis of collagen deposition by Masson trichromatic staining. (*P<0.05, ***P<0.001).
Figure 8

Immunofluorescence staining images of Ki67 in skin tissues of rats treated by different drug administration groups. (A) Full skin. (B) Hair follicles. (C) Full-thickness skin fluorescence quantification. (D) Immunofluorescence quantification of hair follicles (*P<0.05, **P<0.01, ***P<0.001).

Figure 9
Evaluation of wound healing under near-infrared photothermal therapy. (A) Application image of composite sponge stent in rat wound; (B) Schematic diagram of autologous skin transplantation in rats under near-infrared photothermal therapy; (C) Representative images of postoperative full-thickness skin graft survival at each time point. A. The edge of the graft is circled with a red dotted line; B. Vascularization image of composite sponge stent. (D) H&E staining results of rat viscera. (E) H&E staining of skin grafts collected at different time points, hair follicles (black star), implants (black arrow). (F) After the injury, shrinkage of grafted skin on days 3, 7, 14, 21, and 28.

Figure 10

Immunohistochemical analysis of different groups on day 21. (A) Immunohistochemical staining images of CD31 in each group. (B) immunohistochemical images of α-SMA in each group. (C) α-SMA quantitative analysis. (D) CD31 quantitative analysis (*P<0.05, **P<0.01, ***P<0.001).
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx