Restorative biodegradable two-layered hybrid microneedles for melanoma photothermal/chemo co-therapy and wound healing

Yue Shan†, Bowen Tan†, Min Zhang, Xi Xie and Jinfeng Liao*

Abstract

Tumor killing and wound healing are two complementary and influential processes during the treatment of melanoma. Herein, a two-layered microneedle platform was developed with bifunctional effect of chemo-photothermal synergistic melanoma therapy and skin regeneration. The bifunctional platform composed of embeddable curcumin nanodrugs/new Indocyanine Green/hyaluronic acid (Cur NDs/IR820/HA) microneedles and sodium alginate/gelatin/hyaluronic acid (SA/Ge/HA) supporting backing layer was prepared through a two-step casting process. With uniform incorporation of curcumin nanodrugs and IR820, the microneedles exhibited excellent photothermal performance under external near-infrared (NIR) light stimulation and tumor co-therapy ability. Once the embeddable microneedles were inserted into skin, they would rapidly dissolve and activate drug release successfully for tumor treatment. Moreover, the SA/Ge/HA supporting backing layer was left behind to cover the wound and promote the proliferation of endothelial and fibroblasts cells for enhanced skin regeneration. The two-layered microneedles platform can simultaneously eliminate the tumor and accelerate wounding healing, which may be potentially employed as a competitive strategy for the treatment of melanoma.

Keywords: Two-layered microneedle, Melanoma, Photothermal therapy, Skin regeneration

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Background
As the largest organ of human body, skin tissue is a crucial defense that protects the human body from outside environmental disturbance [1, 2]. Nowadays, skin tumors are diagnosed more than one million cases annually, with an increasing incidence. Melanoma, a highly malignant tumor of melanocyte origin, is one of the most aggressive skin tumors and is characterized by local invasiveness, early metastasis, repeated recurrence, and high mortality [3, 4]. Clinically, surgical resection has been the most widely applied treatment for melanoma but suffered from the high risk of recurrence [5]. Other alternative therapeutic strategies have been investigated to eradicate the melanoma cells, including the chemo/radiotherapy and photodynamic therapy (PDT) [6]. Unfortunately, melanoma is resistant to the traditional chemo/radiotherapy which could bring about endless pain to patients [7]. Moreover, a major limitation of PDT is its poor light penetration depth through pigmented lesions [8]. In addition, for complete clearance of residual tumor tissues and preventing cancer relapse, the surgical treatment for melanoma often causes a large cutaneous defect [9]. The loss of skin might cause infection and impede the wound healing process [10]. Therefore, promoting skin repair is of vital value for the treatment of melanoma. In recent decades, plenty of clinical strategies have been developed for the treatment of skin defects, such as autologous skin graft, allogeneic skin graft, and tissue engineering scaffolds, etc. Among them, wound dressing has drawn significant attentions and developed into one of the main treatment strategies for skin repair because it can mimic the native microstructure functionally, structurally, and mechanically [11]. However, there are few new biomaterials to meet the dual requirements of melanoma treatment and wound healing. Thus, it is quite urgently to develop a strategy with the integrated therapeutic effects of melanoma cure and simultaneously for accelerating skin repair.

To address this issue, we designed a two-layered microneedle platform with a dissolvable microneedle part and a biodegradable backing cover. As a kind of transdermal drug delivery system, microneedle (MN) is famous for its use in biomacromolecules delivery [12–14], due to its high safety, painless invasion, and simple administration [15–17]. In particular, dissolving MNS can reliably pierce into skin, rapidly dissolve, and then release the encapsulated drug into skin within minutes [18, 19]. Because of its excellent mechanical property, water-solubility, biocompatibility, and biodegradability, hyaluronic acid (HA) was chosen to prepare dissolvable MNs. In the MN part, IR820 and curcumin nanoparticles were loaded for chemo-photothermal therapy of...
melanoma. In recent years, the integration of photothermal therapy (PTT) and chemotherapy has emerged as an effective and popular treatment technique for a variety of cancers, which could improve the efficiency of cancer therapeutics and decrease the side effects [20, 21]. PTT, which is based on photothermal agents (PTAs), can convert near-infrared (NIR) light energy into heat to kill the tumor cells [22]. New Indocyanine Green (IR820), a derivative of Indocyanine green [ICG, approved by the Food and Drug Administration (FDA)] [23], is blessed with higher photo-thermal conversion rate and better stability under exposure of NIR light than ICG [24, 25]. However, IR820 cannot be retained locally and can easily spread all over the body because of water-solubility. With the help of microneedles for localized delivery, IR820 can be centralized to maximize the therapeutic effects and reduce the usage dose [26]. As a natural product from traditional Chinese herb Curcuma longa, curcumin (Cur) possesses low systemic toxicity and diverse pharmacological effects, including antitumor [27], anti-inflammatory [28], anti-angiogenic [29] properties and osteogenic induction [30]. However, the low bioavailability and poor aqueous solubility restricted its clinical use [31]. To overcome these disadvantages, a facile and green method has recently been developed to synthesize carrier-free curcumin nanodrugs (Cur NDs) without using any toxic solvents. The Cur NDs exhibited good stability in physiological environments and better therapeutic efficacy for cancer than free Cur [32, 33].

Surgical resection is the most common modality for treating melanoma in clinic. The large skin defect from surgery is difficult to heal and could cause chronic wounds. Unfortunately, few studies addressed the issues of tumor-induced skin repair [34]. Although some previous biomaterials such as injectable hydrogels [35], electrospun membrane [36], and nanofibers [37] have been reported to promote skin-tumor-induced skin repair, the fabrication processes were complicated and restricted their use. However, MNs are easily prepared, conveniently used, and can access the target site in a painless and non-invasive way [38]. Simultaneously, the dissolvable microneedle part and biodegradable backing cover composed the dual function system with efficient anti-tumor and enhancing tissue regeneration ability.

Because melanoma is a superficial skin cancer, which could rapidly spread into the surrounding skin, local therapy by MNs along with PTT is a more suitable method to melanoma than the systemic therapy. The local therapy strategy can enhance delivery efficiency to the target tumor site, avoid excessive circulation and side effects to normal tissues [39, 40]. In this study, we
developed a bifunctional two-layered microneedles (MNs) system that consists of dissolving microneedle for chemo-photothermal therapy of melanoma, and a biodegradable supporting backing layer for skin regeneration. As displayed in Fig. 1A, the chemotherapeutic Cur NDs and photothermal agent of IR820 were dispersed into the HA solution to endow the microneedle with anticancer efficiency. Furthermore, the supporting backing layer was prepared by adding the sodium alginate/gelatin/hyaluronic acid (SA/Ge/HA) solution onto the microneedle. Subsequently, calcium ions were used to cross-link with sodium alginate and generate the network structure [41]. When the two-layered Cur NDs/IR820/HA microneedle was inserted into skin and irradiated by the NIR light, it would dissolve and release Cur NDs and IR820 to achieve the chemo-photothermal effect, and the remaining SA/Ge/HA backing layer could stimulate regeneration of skin tissue (Fig. 1B). The synthesis, physicochemical structure and properties, antitumor treatment and skin repair efficacy of two-layered MNs were investigated with in vitro and in vivo experiments.

**Results and discussion**

**Preparation and characterization of microneedles**

Herein, a two-layered MN system was developed via a two-step casting process. As displayed in Fig. 2A, the MNs were differently colored because of the addition of different composition. After the incorporation of Cur NDs and IR820, the color of Cur NDs/HA MNs and IR820/HA MNs microneedles turned white to orange and dark green, respectively. While the Cur NDs/IR820/HA MNs displayed the color of black green for the mixture of Cur NDs and IR820. Furthermore, Fig. 2B, C displayed the bright-field microscopic and SEM images of MNs patch, respectively. Each patch consisted of 400 (20 × 20) microneedles on the base cover with size of 1.5 cm × 1.5 cm. The pyramid-shaped microneedles were line up in order with tip-to-tip distance of 700 μm and height of 540 μm.

Cur NDs were synthesized via a reprecipitation approach. Due to strong intermolecular interaction, the Cur molecules self-aggregate and precipitate to form Cur NDs [32, 33]. As demonstrated in Fig. 2D, the Cur NDs were monodispersed and well-defined nanospheres. The result of dynamic light scattering measurement in Fig. 2E indicated that the average hydrodynamic diameter of Cur NDs was 149.4 nm and the polydispersity index (PDI) value was 0.174.

The SEM image of lyophilized SA/Ge/HA scaffold of the backing layer presented a porous and interconnected structure, which had uniform size and distributed homogenously (Fig. 2F). The average pore dimension of the backing layer scaffold measured by Smile-View was 204±76 μm. The porous structure favors the transport of nutrients and the growth of endothelial and fibroblasts cells, thus accelerating the skin repair [42, 43].

**Dissolution characteristics of microneedle arrays**

Figure 3A demonstrates the brightfield micrographs of MNs after the application into a full thickness porcine skin for 15, 30, and 45 min, respectively. For the first 15 min, approximately 3/4 of the needles dissolved. After 30 min of insertion, there were only the bottom of needles remained on the base layer and all of the needles completely dissolved within 45 min. The results indicated that the HA MNs appeared to easily dissolve upon insertion into skin, which contributed to the fast release of drugs.

**Mechanical strength test**

To clarify whether the HA MNs are mechanically strong enough to penetrate through the stratum corneum, the morphology changes of MNs tips against static force were observed. As displayed in Fig. 3B, the sharp tips of MNs presented more and more bending after putting with 10 g (~2.45 × 10⁻⁴ N/needle) to 500 g (~1.225 × 10⁻² N/needle) weights on MNs [44]. The microneedles did not break even though they were pressed by 500 g weight. The mechanical strength experiment illustrated that the mechanical strength of HA MNs was competent for transdermal drug delivery.

**In vitro skin insertion test**

To verify whether the HA MNs could successfully penetrate into the skin, the MNs were applied to nude mice skin. The brightfield photograph of mice skin displayed the uniform microchannels created due to the insertion of HA MNs (Fig. 3C). The rows of blue dots by trypan blue staining in Fig. 3D corresponded to the puncture sites of the microneedle arrays. H&E histological sections further proved that the MNs enabled complete penetration into the stratum corneum. The results indicated that the MNs possessed good skin insertion ability, which was an essential requirement for the transdermal drug delivery system to piece through the stratum corneum barrier and inserted into the skin.

**In vitro photothermal performance of MN**

As a harmless and efficient photosensitizer, IR820 could generate heat under the NIR laser irradiation and was widely used in PTT. To evaluate the in vitro heating efficacy of Cur NDs/IR820/HA MNs, the real-time
Fig. 2  

A Photograph of A1 HA MN, A2 Cur NDs/HA MN, A3 IR820/HA MN, and A4 Cur NDs/IR820/HA MN. 

B The bright-field micrographs of HA MN (B1–B3). 

C SEM images of Cur NDs/IR820/HA MN. 

D SEM image of Cur NDs. 

E Size distribution of Cur NDs. 

F SEM image of the SA/Ge/HA backing layer scaffold.
thermographic images (Fig. 4A) of different MNs under NIR light (808 nm 0.75 W/cm²) were captured by the near-infrared thermal camera and the temperature change curves (Fig. 4B) were plotted. The temperature of MNs with IR820 (Cur NDs/IR820/HA MNs and IR820/HA MNs) rapidly reached to 50 °C within 30 s. In contrast, the temperature of the MNs without IR820 (HA MNs and Cur NDs/HA MNs) remained approximately constant at room temperature under irradiation. The results demonstrated the remarkable light-to-heat transduction efficacy of the IR820-containing MNs.

In vitro cell compatibility test
Cell compatibility of microneedle tip materials was tested on B16F10 and NIH3T3 cells. From the CCK-8 test results (Fig. 5A), both the two kinds of cells exhibited desirable viability after incubation with different concentrations of HA for 24 h, indicating that HA had
good cytocompatibility [45]. Besides, the in vitro biocompatibility and cell proliferation test of the SA/Ge/HA backing layer were carried out on 3T3 cells. As Fig. 5B demonstrated, the cell viability result verified that the backing layer material was non-toxic and safe. Moreover, the cytotoxicity of two-layered HA microneedle (HA microneedle tip+SA/Ge/HA backing layer) was further explored by culturing 3T3 cells with the extracts from microneedles. After incubation for 1, 2, and 3 days, the cell viability was tested through CCK-8 assay. As depicted in Fig. 5C, the results of in vitro biocompatibility test further verified the safety of the microneedle materials.

**In vitro anti-cancer cell experiments**

The in vitro anti-cancer efficacy of Cur NDs/IR820/HA MN was evaluated via live/dead staining and CCK-8 assay using B16F10 cells. In the live/dead images (Fig. 5F), the green and red fluorescence indicated the alive and dead cells, respectively. Compared with the control and HA MNs groups, the sustained release of Cur NDs in Cur NDs/HA MNs induced minor cell death, while the IR820 MNs + laser group showed a higher cell-killing efficiency. The localized hyperthermia and increased cell membrane permeability led to more tumor cell death in the Cur NDs/IR820/HA MNs group.
The quantitative cell survival rate results demonstrated that the cell viabilities were 103.88%, 86.36%, 24.73%, and 2.67% for the groups of HA MNs, Cur NDs/HA MNs, IR820/HA MNs + laser, and Cur NDs/IR820/HA MNs + laser, respectively (Fig. 5D). Besides, to evaluate the cell killing effect of MNs, we further cultured the tumor cells for 48 h after the 808 nm laser irradiation. According to the CCK-8 results (Fig. 5E), HA MNs groups showed less cell cytotoxicity, indicating that the material itself possessed good cytocompatibility. In addition, with the participation of Cur NDs in Cur NDs/HA MNs group, the cell viability of B16F10 cell decreased to 89.7%, suggesting that the antitumor efficacy of single chemotherapy was insufficient. Tumor cells in IR820/HA MNs + laser group were remarkably inhibited (23.4%), owing to the hyperthermia triggered by IR820. In contrast, the Cur NDs/IR820/HA MNs + laser group displayed the maximum antitumor efficacy (12.3%). The results exhibited the successful chemo-co-photothermal antitumor effect via curcumin and IR820.

**In vivo anti-cancer treatment**

The in vivo anti-cancer capacity was carried out on B16F10 tumor-bearing C57BL/6 mice, and the time scheme of treatment was shown in Fig. 6A. The mice were randomized into 6 groups: Control (G1), HA MNs (G2), Cur NDs/HA MNs (G3), Cur NDs/IR820/HA MNs (G4), IR820 MNs + laser (G5), and Cur NDs/IR820/HA MNs + laser (G6). The mice in different groups were inserted with the corresponding MNs. The in vivo NIR images of MNs under NIR laser irradiation (808 nm, 0.75 W/cm², 5 min) in G2, G5 and G6 were recorded, which demonstrated the changing temperature of the tumor site (Fig. 6B). As shown in Fig. 6C, G2 had no obvious temperature rise, while G5 and G6 heated up
Fig. 6  A Time scheme of animal experiment. B NIR thermal images of mice in G2, G5 and G6 with 808 nm laser irradiation (0.75 W/cm²) for 5 min. C Temperature changes of tumor site within 5 min. D Tumor volume and F body weight of mice for 14 days after treatment. E Photograph of detached tumors in each group and G their weights on day 14. G1: Control; G2: HA MNs; G3: Cur NDs/HA MNs; G4: Cur NDs/IR820/HA MNs; G5: IR820 MNs + laser; G6: Cur NDs/IR820/HA MNs + laser. *p < 0.05, **p < 0.01, ***p < 0.001
quickly in the first 30 s and kept arising slowly and steadily increased to ~ 50°C, which was sufficient to induce the ablation of tumor tissues.

As demonstrated in Fig. 6D, F, the tumor volumes and mice body weights of G1 and G2 increased at an alarming rate. G3 and G4 showed slight tumor inhibitory effect, indicating that single Cur NDs were not sufficient for tumor therapy. In contrast, G5 and G6 both exhibited desirable tumor killing efficiency with the participation of photothermal therapy, while G6 presented better tumor inhibition effect than G5, which attributed to Cur NDs worked synergistically with IR820 for enhanced thermal-co-chemotherapy of melanoma. From day 0 to 10, the body weights of the mice in G1, G2, G3, and G4 increased rapidly compared with the other two groups (G5 and G6), which might result from the overgrowing tumors without efficient treatment. At 10 days after treatment, the body weights of G1–4 declined drastically. Besides, the emaciated figure and diminished appetite were observed, suggesting the progression to the cachectic stage. In contrast, the weights of mice in G5 and G6 were more stable than the other four groups, which proved that thermal-co-chemotherapy was efficient for tumor elimination. At 14 days, the mice were subjected to humanitarian death and the tumor tissues were removed in an integrated form. From the photograph of separated tumors (Fig. 6E), the G6 demonstrated excellent tumor growth inhibition effect. Similarly, the mean tumor weights (Fig. 6G) in G6 was the smallest compared with other groups, indicating that Cur NDs/IR820/HA MNs with laser could efficiently inhibit the tumor growth.

The histological analysis of tumor was carried out via Ki67 staining. The expression of Ki67 in tumor cells significantly reduced in G6, indicating that

![Fig. 7](A) Ki67 staining of tumor tissues and (B) H&E staining of heart, liver, spleen, lung, and kidney harvested from mice in different groups at 14 days after treatment (scale bar = 100 μm). G1: Control; G2: HA MNs; G3: Cur NDs/HA MNs; G4: Cur NDs/IR820/HA MNs; G5: IR820 MNs + laser; G6: Cur NDs/IR820/HA MNs + laser
thermal-co-chemotherapy inhibited the proliferation of tumor cell (Fig. 7A). The key organs of mice (heart, liver, spleen, lung, and kidney) were harvested for H&E staining. From the results (Fig. 7B), no significant toxicity and damages were found in these main organs. From the lung sections, obvious decrease of lung metastasis was seen in G5 and G6, indicating that photothermal therapy was effective for inhibiting tumor metastasis progression. Taken together, our results suggested that the Cur NDs/IR820/HA MNs with laser irradiation could exert chemo-photothermal therapy effect and thereby inhibit tumor proliferation without significant in vivo toxicity.

In vivo animal skin repair
In order to evaluate the skin repair efficiency of our materials, we adopted full-thickness skin defect of SD rats as animal experimental model and the rats were randomly divided into three groups (n = 8): Control group, HA MNs group, and Cur NDs/IR820/HA MNs group. At day 7, all the three groups showed a decrease of the wound area, while better wound closure was observed in HA MNs group and Cur NDs/IR820/HA MNs group compared to control group (Fig. 8A). The Cur NDs/IR820/HA MNs group presented the lowest relative wound area at day 14 (Fig. 8B). To further analyze the skin repair effect histologically, the H&E staining was performed. At day 7, inflammation response occurred in all three groups, but inflammatory cells including lymphocytes and neutrophils in control and HA MNs groups were more than that in Cur NDs/IR820/HA group (Fig. 8E). Besides, a thicker epidermal layer was observed in HA MNs group and Cur NDs/IR820/HA MNs group compared to control group (Fig. 8A). The Cur NDs/IR820/HA MNs group showed complete regeneration of skin tissue with skin appendages and increased cell density, indicating the speeding up of skin repair. In the Masson staining (Fig. 8D), the formation of collagen was analyzed to further evaluate the skin repair process. Compared with control group, a higher amount of and more tight organized collagen fibers in HA MNs group and Cur NDs/IR820/HA group suggested a higher collagen deposition level, which was beneficial for skin recovery. Moreover, the collagen level in the Cur NDs/IR820/HA MNs group was significantly higher relative to the HA MNs group. Natural polymers, including algininate, gelatin and hyaluronic acid, are widely used in the field of tissue engineering scaffolds, and in particular, for skin regeneration applications [47]. The SA/Ge/HA supporting backing layer was beneficial for new skin tissue formation [48]. Additionally, the nice repair results in Cur NDs/IR820/HA group may also attribute to anti-inflammation effects of Cur NDs released from Cur NDs/IR820/HA MNs [49].

Conclusions
Considering the dilemma of clinical management for melanoma, a microneedle-based photothermal/chemo co-therapy platform was proposed for enhanced tumor treatment and followed skin tissue repair. This hybrid system demonstrated desirable prospects including: (1) the microneedle-based system is relatively simple, convenient, and noninvasive, (2) photothermal/chemo co-therapy could efficiently control the progression of tumor, (3) the curcumin nanodrugs could not only inhibit the melanoma cells growth, but also reduce inflammation and promote skin regeneration process, (4) the SA/Ge/HA backing layer is biocompatible and could promote wound healing. This bifunctional two-layered microneedle system is a desirable candidate for photothermal/chemo skin tumor therapy and simultaneously treating wound defect.

Materials and methods
Materials
Sodium hyaluronic acid (Mn = 1.44 × 10^5 kDa) was obtained from Freda Biochem Co., Ltd (Shandong, China). Curcumin was purchased from Dalian Meilun Biological Co., Ltd. Sodium alginate, IR820, and gelatin (from porcine skin) were obtained from Sigma-Aldrich (USA). Fetal bovine serum (FBS), Dulbecco’s modified Eagle’s medium (DMEM), penicillin, and all other cell culture media and reagents were bought from Gibco™ (Grand Island, NY, USA). Live/dead cell imaging kit was acquired from Thermo Fisher Scientific Inc (Waltham, MA, USA). CCK-8 kit was purchased from from Houston, Texas, USA. The polydimethylsiloxane (PDMS) microneedle templates were acquired from Guangzhou SLLK Co. Ltd (Guangzhou, China).

SD rats (8 week-old, male, 200 ± 20 g) were purchased from Chengdu Dashuo Animal Experiment Co. Ltd (Chengdu, China). C57BL/6 mice (8 week-old, female) and nude BALB/c mice (4 week-old, female) were obtained from Beijing HFK Bioscience Co. Ltd. (Beijing, China). The animal researches were admitted and supervised by the animal committee of West China Hospital of Stomatology, Sichuan University.
Fig. 8 (See legend on previous page.)
**Preparation and characterization of microneedles**

The Cur NDs/IR820/HAMN patches were prepared through a two-step casting process. Firstly, the Cur NDs were synthesized via a reprecipitation approach [33]. In brief, Cur dissolved in ethanol solution was dropwise added into distilled water under vigorous stirring and then formed Cur NDs. The Cur NDs were concentrated by ultrafiltration and then obtained by lyophilization. Afterwards, 10 ml of HA aqueous solution (30 mg/ml) was mixed with 8 ml of Cur NDs (600 μg/ml) and 2 ml of IR820 solution (2 mg/ml). After forming a uniform solution, 1 ml of the HA mixture solution was added into the PDMS microneedle mold, and then the filled template was dried at 55 °C overnight. Subsequently, an additional 1 ml of SA/Ge/HA solution (weight ratio = 2:1:1) were casted onto the mold to form the backing layer. Afterwards, 1.0% calcium gluconate aqueous solution was sprayed to crosslink with the backing layer. Finally, the Cur NDs/IR820/HAMN patches were peeled from the PDMS template after drying process. The Cur NDs/HAMNs and IR820/HAMNs were fabricated as mentioned above without the incorporation of IR820 or Cur NDs, respectively. The HAMNs were fabricated without IR820 and Cur NDs.

The bright-field microscopic photographs of MNs were captured by a stereomicroscope (OLYMPUS, SZX16, Tokyo, Japan). The morphology of the microneedle structures, the Cur NDs and the lyophilized SA/Ge/HA backing layer were characterized by scanning electron microscopy (SEM; JSM-5900LV,JEOL, Tokyo, Japan). The hydrodynamic size of the Cur NDs was determined by a DLS instrument (Malvern Zetasizer Nano ZS). The average pore size of lyophilized SA/Ge/HA backing layer was estimated with the Smile-View software. Briefly, 100 pores were chosen randomly from the SEM images of the lyophilized SA/Ge/HA backing layer scaffold to calculate the average pore size using the “Length Measurement” tool.

**Dissolution characteristics of microneedle arrays**

For in vitro dissolution analysis of MNs, the Cur NDs/HAMNs were applied onto porcine skin and fixed with a gum tape. The MNs were taken off from the skin at indicated time intervals (15, 30, and 45 min) and imaged by brightfield stereomicroscopy to visualize the dissolution of microneedles.

**Mechanical strength test**

To evaluate the mechanical strength of HAMNs, the MNs were tested with a serial of weights. Briefly, the MN patches were positioned vertically (microneedle tips facing up), and weights of 10, 50, 100, 250, and 500 g were put on the tips of MN patches. Afterwards, the deformation of the microneedles was visualized with stereomicroscopy.

**In vitro skin insertion test**

The in vitro skin insertion ability of HA MNs was assessed by inserting the MNs into the separated back skin of nude BALB/c mice with a force of 10 N and remained for 5 min. After removal, the treated skin was observed using stereomicroscopy and trypan blue staining method. In brief, the inserted mice skin was stained with trypan blue dye for 5 min, and the residual dye was washed off with phosphate-buffered saline (PBS). The skin insertion capacity was observed by stereomicroscopy. In addition, the collected skin samples were evaluated by H&E staining.

**In vitro photothermal performance of MNs**

To evaluate the photothermal performance of MNs, the MNs were exposed to 808 nm NIR laser irradiation (MW-GX-808/5000 mW, Leishi, Changchun China) at an output power of 0.75 W/cm² for 5 min. The real-time thermal images and temperature changes of the MNs were recorded by a Fluke Ti32 Infrared thermal camera (Infrared Cameras, Fluke, Avery, WA, USA) every 30 s.

**In vitro cell compatibility test**

To study the cell compatibility of microneedle materials, two kinds of cells (B16F10 and NIH3T3 cells) were used. Firstly, the cytotoxicity of microneedle tip material (HA) was verified by B16F10 and NIH3T3 cells. B16F10 or NIH3T3 cells (5000/well) were seeded into the 96-well plates and cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in an incubator (5% CO₂, 37 °C) for 24 h, respectively. The cell culture medium was then replaced by HA MNs solution (dissolved in DMEM medium, without the backing layer) with different concentrations. After another 24 h of incubation, the CCK-8 kit was utilized to detect the cell viability according to the manufacturer’s protocol. In brief, 100 µl CCK-8 working solution (10%) was added into each well. After incubation in the dark (37 °C) for 1–4 h, the absorbance values were measured at 450 nm via Beckman DU7400 spectrophotometer (Beckman coulter, Miami, FL, USA). To investigate the cell compatibility of the backing layer material (SA/Ge/HA), the NIH3T3 cells were employed and the experimental procedure was carried out as described above. Moreover, the CCK-8 assay was also used to quantitatively analyze the NIH3T3 cell proliferation with the leaching solution from two-layered HA microneedle material (HA microneedle tip +SA/Ge/HA backing layer) for 1, 2, and 3 days after cell seeding.
In vitro anti-cancer cell experiments
The in vitro anticancer activity of MNs was evaluated using live/dead staining. B16F10 cells were seeded into a 6-well plate (2 × 10^5 cells/well), and different groups of microneedles were placed on the chambers of Transwell. Then the microneedles were irradiated with an NIR laser for 5 min (808 nm, 0.75 W/cm^2). After 2 h, the cells were stained with live-dead cell imaging kit and observed by fluorescence microscope (DM2000, Leica, Germany).

In addition, CCK-8 assay was also employed to determine the cell viability after MNs treatment. The dissolved solutions of HA MNs, Cur NDs/HA MNs, IR820/HA MNs and Cur NDs/IR820/HA MNs were added into the culture wells, respectively. Subsequently, the cells were irradiated with an NIR laser light (808 nm, 0.75 W/cm^2, 5 min), and then cultured for 48 h. The cell survival rate of B16F10 was tested via the CCK-8 kit.

In vivo anti-cancer study
The subcutaneous melanoma tumor model was established by injecting 100 µl of 1 × 10^6 B16F10 cells into the right back of C57BL/6 mice (6–8 weeks old, female, ~20 g). When the tumor size grew to approximately 50–70 mm^3, the mice were randomized into 6 groups (n = 5) as follows: Control (G1); HA MNs (G2); Cur NDs/HA MNs (G3); Cur NDs/IR820/HA MNs (G4); IR820 MNs + laser (G5); Cur NDs/IR820/HA MNs + laser (G6). After anesthetization via isoflurane, the mice were treated with microneedles according to the grouping. The G5 and G6 were exposed to the NIR laser (808 nm, 0.75 W/cm^2, 5 min). The tumor surface temperature and thermal images were captured timely via the NIR thermal imaging camera. The body weight of mice and size of tumor were measured every 2 days. The tumor volume was calculated as follows: tumor volume (V) = (tumor length) × (tumor width)^2/2. At day 14 after treatment, the mice were sacrificed and the tumor tissues was harvested, weighed and photographed. After fixation with 4% paraformaldehyde, the tumor samples were dehydrated with graded ethanol series, embedded into paraffin, sectioned into serially 5 μm thick slices, and then stained with Ki67. Additionally, the key organs of mice including heart, liver, spleen, lung, and kidney were all collected for histological analysis.

In vivo animal skin repair experiment
Skin repair experiment was carried out on SD rats (8 week-old, male, 200–220 g). After anesthetization, the dorsal hair of SD rats was shaved and a 1.5 cm × 1.5 cm skin defect was constructed on the back. The rats were randomized into 3 groups (n = 8): Control group, HA MNs group, and Cur NDs/IR820/HA MNs group. The microneedles were inserted into the defect area, after which the defects of all groups were protected with Tegaderm (3 M, St. Paul, MN, USA). At day 0, 7 and 14, the wound healing was photographed and the wound areas were measured by Image J software. Subsequently, four rats were sacrificed in each group at day 7 and day 14, respectively, and the whole wound sites (including the wound and the surrounding normal skin tissues) were excised and stained with H&E and Masson staining. At day 7 and 14, the number of inflammatory cells in H&E stained micrographs was calculated through and Image J software.

Statistical analysis
All quantitative data were presented as means ± standard deviation (SD). SPSS 11.0 software (Chicago, IL, USA) was utilized for statistical analysis. Statistical differences of groups were analyzed by student’s t-test or one-way ANOVA, and the P value < 0.05 was considered to be statistically significant.

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Author contributions
JFL designed the study, analyzed data and revised the manuscript. YS and BWT performed the study, collected the data and were major contributors in writing the manuscript. YS, BWT and MZ organized and analyzed data. YS, BWT, MZ and XX reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated and analyzed during this research and included in this published article.

Declarations
Ethics approval and consent to participate
Ethics approval and consent to participate all animal experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University.

Consent for publication
All authors agree for publication.

Competing interests
The authors declare no competing interests.

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