Restorative Biodegradable Two-layered Hybrid Microneedles for Melanoma Photothermal/Chemo Co-therapy and Wound Healing

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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 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 back layer was prepared through a two-step casting process. With uniform incorporation of Cur NDs and IR820, the microneedles exhibited nice photothermal performance under external near-infrared (NIR) light stimulation and excellent tumor co-therapy ability. Once the embeddable microneedles insert into skin, they rapidly dissolve and activate drug release successfully for tumor treatment. Moreover, the SA/Ge/HA supporting back layer 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.

Background

Skin tissue is a crucial defense that protects the human body from outside environmental disturbance.¹ Nowadays, skin tumors is 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.^{2,3} Clinically, surgical resection has been the most widely applied treatment for melanoma but suffered from the high risk of recurrence.⁴ Other alternative therapeutic strategies have been investigated to eradicate the melanoma cells, including the chemo/radiotherapy and photodynamic therapy (PDT). Unfortunately, melanoma is resistant to the traditional radio/chemotherapy which could bring about endless pain to patients.⁵ Moreover, a major limitation of PDT is its poor light penetration depth through pigmented lesions.⁶ In addition, for complete clearance of residual tumor tissues and preventing cancer relapse, the surgical treatment for melanoma often causes a large cutaneous defect.⁷ The loss of skin might cause infection and impede the healing process.⁸ Therefore, promoting skin repair is of vital value for the treatment of melanoma. However, there are few new biomaterials and strategies 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.

For address this issue, microneedle as a transdermal drug delivery system is a good choose for melanoma therapy and skin regeneration, which we designed a two-layered microneedle platform with a dissolvable microneedle part and a biodegradable backing cover. Microneedle is famous for its use in biomacromolecules delivery for diabetes therapy,⁹⁻¹¹ due to its high safety, painless invasion, and simple administration.¹²⁻¹⁴ In particular, dissolving MNs can reliably pierce into skin, rapidly dissolve, and then release the encapsulated drug into skin within minutes.^{15,16} Due to its excellent mechanical property,

water-solubility, biocompatibility, and biodegradability of hyaluronic acid (HA), we choose HA to prepare dissolvable MNs. In the MN part, IR820 and Curcumin nanoparticles were loaded for photothermal and chemo-co-therapy for melanoma treatment. 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.^{17, 18} PTT is based on photothermal agents (PTAs), which can convert near-infrared (NIR) light energy into heat to kill the tumor cells.¹⁹ New Indocyanine Green (IR820), a derivative of Indocyanine green (ICG, approved by the Food and Drug Administration (FDA)),²⁰ is capable of higher photo-thermal conversion rate and better stability under exposure of NIR light than ICG.^{21, 22} However, IR820 cannot be retained locally and 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.²³ Curcumin (Cur) possesses diverse pharmacological effects, including antitumor,²⁴ anti-inflammatory,²⁵ anti-angiogenic²⁶ properties and osteogenic induction.²⁷ However, the low bioavailability and poor aqueous solubility have restricted its clinical use.²⁸ To overcome these disadvantages, a facile and green method has recently been developed to synthesize carrier-free curcumin nanodrugs (Cur NDs), which exhibited good stability in physiological environments and better therapeutic efficacy for cancer than free Cur.^{29, 30}

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.³¹ Although some previous biomaterials such as injectable hydrogels,³² electrospun membrane,³³ and nanofibers³⁴ 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.³⁵ Simultaneously, the supporting back layer of MNs can be designed with tissue induced activity to promote the proliferation of skin cells, thus accelerating the skin repair. Therefore, 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 rapidly spread into the surrounding skin, local therapy by MNs along with PTT is a more suitable method to melanoma than the systemic therapy system. The local therapy strategy can enhance delivery efficiency to the target tumor site, avoid excessive circulation and lessen side effects to normal tissues.^{36, 37} 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 back layer for skin regeneration. As displayed in Figure 1(A), 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 back 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

sodium alginate and generate network structure.³⁸ When the two-layered microneedles inserted into skin and irradiated by the NIR light, the Cur NDs/IR820/HA microneedles dissolve and release Cur NDs and IR820 to achieve the chemo-photothermal effect, and the remaining SA/Ge/HA back layer could stimulate regeneration of skin tissue (Figure 1(B)). 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 Figure 2(A), 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, Figure 2(B) and (C) displayed the bright-field microscopic and SEM images of MNs patch, respectively. Each MN consisted of 400 (20 × 20) microneedles on the base patch with size of 1.5 cm × 1.5 cm. The pyramids-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. As demonstrated in Figure 2(D), the Cur NDs were monodispersed and well-defined nanospheres. The results of dynamic light scattering measurement in Figure 2(E) indicate 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 in the back layer presented a porous and interconnected structure, which had uniform size and distributed homogenously (Figure 2 (F)). The average pore dimension of the back 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.^{39, 40}

Dissolution Characteristics of Microneedle Arrays

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

Mechanical Strength Tests

To clarify whether the HA MNs are mechanically strong enough to penetrate through the stratum corneum, the morphology changes of MNs tips against static forces were observed. As displayed in Figure 3(B), the sharp tips of MNs present more and more bending after putting with 10 g ($\sim 2.45 \times 10^{-4}$ N/needle) to 500 g ($\sim 1.225 \times 10^{-2}$ N/needle) weights on MN patch⁴¹. The microneedles did not break even though they were been pressed by 500 g weight. The mechanical strength experiments illustrate that the mechanical strength of HA MNs is 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 displays the uniform microchannels created due to the insertion of HA MNs (Figure 3(C)). The rows of blue dots by trypan blue staining in Figure 3(D) 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 possess good skin insertion ability, which is an essential requirement for the transdermal drug delivery system to piece 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 thermographic images (Figure 4(A)) 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 (Figure 4(B)) were plotted. The temperature of MNs with IR820 (Cur NDs/IR820/HA MNs and IR820/HA MNs) rapidly reached to 50°C within 20 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 indicated the remarkable light-to-heat transduction efficacy of the IR820-containing MNs.

In Vitro Cell Compatibility and Anti-cancer Cell Experiment

Cell compatibility of microneedle tips and backing layer materials were tested on B16F10 and NIH3T3 cells. From the CCK-8 test results (Figure 5(A)), both the two kinds of cells exhibited desirable viability incubated with different concentrations of HA. Interestingly, high concentration of materials led to enhanced cell viability, while cell viability in lower concentration of leachates exhibited no differences between control group, indicating that HA was biocompatible and acted as nutrition for cells.⁴² Accordingly, as Figure 5(B) demonstrated, the results verified that the back layer SA/Ge/HA scaffold was also non-toxic and safe.

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 (Figure 5(C)), the green and red fluorescence indicated the alive and dead cells, respectively. Compared with control and HA MNs groups, the sustained release of Cur NDs in Cur NDs/HA MNs led to minor cell apoptosis, while the IR820 MNs + laser group triggered photothermal therapy which can generate hyperthermia and contribute to the death of majority

of tumor cells. The localized hyperthermia and increased cell membrane permeability assisted in inducing more tumor cells apoptosis in the Cur NDs/IR820/HA MN + laser group⁴³. The quantitative cell survival rate results demonstrate that the cell viabilities were 103.88%, 86.36%, 24.73%, and 2.67% in the groups of HA MNs, Cur NDs/HA MNs, IR820/HA MNs + laser, and Cur NDs/IR820/HA MNs + laser respectively, compared with control group (Figure 5(D)). 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 (Figure 5(E)), HA MNs groups showed less cell cytotoxicity, indicating that the material itself was cell compatible. In addition, with the participation of Cur NDs in Cur NDs/HA MNs group, the cell viability of B16F10 decreased to 89.7%, suggesting the single chemotherapy efficacy 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 suggested that the hyperthermia effects could accelerate the localized release of Cur NDs and also increase the cell membrane permeability and fluidity,⁴⁴ exhibiting the successful chemo-co-photothermal anti-cancer 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 Figure 6(A). The mice were randomized into 6 groups: Control, HA MNs, Cur NDs/HA MNs, Cur NDs/IR820/HA MNs, IR820 MNs + laser, and Cur NDs/IR820/HA MNs + laser. 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 a changing color of the tumor site (Figure 6 (B)). As shown in Figure 6 (C), G2 had no obvious temperature rise, while G5 and G6 heated up 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 Figure 6 (D) and (F), the tumor volumes and mice body weights in G1 and G2 increased at an alarming rate. G3 and G4 groups showed slight tumor inhibitory effect, indicating that single Cur NDs was not sufficient for tumor therapy. In contrast, G5 and G6 exhibited a desirable tumor killing efficiency with the participation of photothermal therapy, and G6 presented a better tumor inhibition effect, which attribute to the Cur NDs worked synergistically with IR820 for enhanced thermal-co-chemotherapy of melanoma. From day 0 to 10, the body weight of the mice in G1, G2, G3, and G4 increased rapidly compared with the other two groups, which might result from the overgrowing tumors without efficient treatment. At 10 days after treatment, the body weight of G1-4 declined drastically. Besides, an emaciated figure and diminished appetite were observed, suggesting the progression to the cachectic stage. In contrast, the weight of mice in G5 and G6 were more stable than the other four groups, which proved that thermal-co-chemotherapy is 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 digital picture of separated tumors (Figure 6 (E)), the G6 demonstrated excellent tumor growth inhibition effect. Similarly, the mean tumor weights (Figure 6 (G)) 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 the Cur NDs/IR820/HA MNs + laser group, indicating that thermal-co-chemotherapy inhibited the proliferation of tumor cell (Figure 7 (A)). The key organs of mice (heart, liver, spleen, lung and kidney) were harvested for H&E staining. From the results (Figure 7 (B)), no significant toxicity and damages were found in these main organs. From the lung sections, an 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 inhibit tumor proliferation and thereby exert chemo-photothermal therapy effect with no 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. Fortunately, better wound closure was observed in Cur NDs/IR820/HA MNs group (Figure 8 (A)). The Cur NDs/IR820/HA MNs group presented a lower relative wound area compared to other groups at day 14 (Figure 8 (B)). 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 (Figure 8 (E)). Besides, a thicker epidermal layer was observed in Cur NDs/IR820/HA group. As shown in Figure 8(C), at day 14, except the repair of epithelia tissue, the cutaneous appendages such as hair follicles also took shape. Especially, 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 (Figure 8 (D)), the collagen formation was analyzed to further evaluate the skin repair process. A higher amount of and more tight organized collagen fibers in 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 alginate, gelatin and hyaluronic acid, are widely used in the field of tissue engineering scaffolds, and in particular, for skin regeneration applications.⁴⁵ The SA/Ge/HA supporting back layer was beneficial for new skin tissues formation.⁴⁶ 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.⁴⁷

Conclusion

Considering the dilemma of clinical management for melanoma, a microneedle-based noninvasive photothermal/chemotherapy 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 and convenient, and avoids the operation of trauma, 2) Photothermal/chemotherapy efficiently controls tumor progression, 3) The curcumin nanodrugs not only

induce melanoma cells apoptosis, but also reduce inflammation and promote skin regeneration process, 4) The SA/Ge/HA back layer is biocompatible and biodegradable for promotion wound healing. This two-layered microneedle system is not only designed for skin tumor management through a noninvasive path, but also capable of treating wound defect.

Materials And Methods

Materials

Sodium hyaluronic acid ($M_n = 1.44 \times 10^3$ 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 Houston, Texas, USA.

SD rats (8 week-old, male, 200 ± 20 g) were purchased from Chengdu Dashuo Animal Experiment Co. LTD. 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.

Preparation and Characterization of Microneedles

The Cur NDs/IR820/HA MN patches were prepared through a two-step casting process. Firstly, the Cur NDs were synthesized *via* a reprecipitation approach.³⁰ In brief, Cur dissolved in ethanol solution was dropwise added into distilled water under vigorous stirring and form Cur nanoparticles. 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 polydimethylsiloxane (PDMS) microneedle mold, and 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 the backing layer. Finally, the Cur NDs/IR820/HA MN patches were peeled from the PDMS template. The Cur NDs/HA MNs and IR820/HA MNs were fabricated as mentioned above without the incorporation of IR820 or Cur NDs, respectively. The HA MNs 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.

Dissolution Characteristics of Microneedle Arrays

For *in vitro* dissolution analysis of MNs, the Cur NDs/HA MNs 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 Tests

To evaluate the mechanical strength of HA MNs, 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. 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 and Anti-Cancer Cell Experiments

To study the cell compatibility of microneedle tip and backing layer material, 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. The cell culture medium was then replaced by HA MNs solution (dissolved in DMEM

medium, without the back 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, working solution was prepared with DMEM medium (CCK-8 kit concentration 10%), then 100 ml of working solution was added to each well. After incubation in the dark (37°C) for 1-4 hours, 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.

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/cm²). 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 of 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 (808nm, 0.75 W/cm², 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 grow to approximately 50–70 mm³, the mice were randomized into 6 groups (n = 5) as follows: 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. After anesthetization *via* isoflurane, the mice were treated with microneedles according to the grouping. The G5 and G6 groups were exposed to the NIR laser (808 nm, 0.75 W/cm², 5 min). The tumor surface temperature and thermal images were captured timely *via* the NIR thermal imaging camera. The body weight 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 (3M, St. Paul, MN, USA). At day 0, 7 and 14, the wound healing was photographed and the wound areas were measured by ImageJ 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 7 and 14 days, 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) were utilized for statistical analysis. Statistical differences of groups were analyzed by one-way ANOVA, and a P values < 0.05 was considered statistically significant.

Declarations

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Authors' contributions

JFL designed the study. YS and BWT were major contributors in conducting the experiments and writing the manuscript. YS, BWT and MZ organized and analyzed data. JFL, YS, BWT, MZ and XX reviewed and edited the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

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

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 declared no conflicts of interests.

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Figures

Figure 1

Schematic illustration of (A) The preparation of Cur NDs/IR820/HA MN. (B) Tumor photothermo-chemo therapy and tissue regeneration.

Figure 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 (B1-3). (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 SA/Ge/HA the back layer scaffold.

Figure 3

(A) Dissolution of microneedles after application onto porcine skin *in vivo* for 15 min, 30 min, and 45 min. (B) The morphology of microneedles tips with resistance to different weights (10, 50, 100, 250, and 500 g). (C) Bright micrographs of puncture sites on the back skin of nude mice after insertion of MNs. (D) (E)

Trypan blue staining of the back skin after MNs application. (E) H&E staining microneedle-treated skin section.

Figure 4

(A) Near-infrared thermal images of the MNs under irradiation by 808 nm NIR laser within 5 min (0.75 W/cm^2). (B) Heating curves of the MNs after irradiation with NIR laser.

Figure 5

(A) Cell viability of B16 and 3T3 cells cultured with different concentrations of HA. (B) Cell viability of 3T3 cells cultured with backing layer material (SA/Ge/HA) leachates. (C) LIVE/DEAD staining of B16F10 cells, at 2 h after different MNs treatment. (Scale bar = 100μm). (D) Cell survival rate of B16F10 cells after been treated with different MNs (2 h). (E) Cell viability of B16F10 cells after 808 nm laser irradiation treatment (48 h). * $p < 0.05$, *** $p < 0.001$.

Figure 6

(A) Time scheme of animal experiment. (B) NIR thermal images of mice in G2, G5 and G6 with NIR laser irradiation (808 nm, 0.75 W/cm²) for 5 min. (C) Temperature changes of tumor site within 5 min. (D) Tumor volumes and (F) body weights of mice for 14 days after treatment. (E) Digital pictures 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. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 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.

Figure 8

(A) Photographs of skin repair at 0 day, 7 days, and 14 days in control, HA MNs, and Cur NDs/IR820/HA MNs groups. (B) Relative wound area of control, HA MNs, and Cur NDs/IR820/HA MNs groups at 7 days and 14 days. (C) H&E staining images and (D) Masson staining images in control, HA MNs, and Cur NDs/IR820/HA MNs groups at 7 days and 14 days. The red arrows indicated newly formed skin appendages. (E) The statistical data of inflammatory cells at 7 days and 14 days.

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