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Transdermal delivery of anti-obesity compounds to subcutaneous adipose tissue with polymeric microneedle patches

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Abstract: Excess white adipose tissue (WAT) or obesity is the leading cause of many diseases. Combating obesity is however challenged by the fact that the laboratory-proven anti-obesity compounds lose effectiveness or/and cause severe side-effects while being delivered via conventional routes. We report a new strategy using disposable transdermal patches equipped with detachable polymeric microneedle (MN) arrays for painless and bloodless drug delivery to subcutaneous WAT. In contrast to the current methods to reduce energy intake, we use MN-patches to deliver anti-obesity compounds to increase energy expenditure by transforming calorie-storing white fat into calorie-burning brown fat. Specifically, we demonstrate the prominent WAT browning and reduction effects by $\beta 3$ -adrenoceptor agonist and thyroid hormone T3 transdermally delivered from rapidly-dissolving MNs on mice. Furthermore, using a diet-induced obese mouse model, we show that $\beta 3$ -adrenoceptor agonist released by slowly-dissolving MNs can effectively promote WAT browning, and suppress gaining of body fat and weight, without the need of daily administration. Such MN approach can achieve much lower effective dose as compared to systemic administration and enables long-term home-based treatment.

1. Introduction

Sedentary lifestyle and excess consumption of calorie-rich food have fueled the increasing epidemic of obesity. Excess body fat (white adipose tissue - WAT) is the notorious risk factor for many health problems, such as diabetes, hypertension, coronary heart diseases, stroke and some cancers (e.g., colorectal cancer)^[1, 2]. According to World Health Organization, at least 2.8 million people die worldwide each year as a result of being obese, and an estimated 35.8 million (2.3%) of global disability-adjusted life years are caused by obesity^[3]. There is thus a growing demand for obesity management and treatment. However, only a few drugs are FDA-approved as the adjunct therapy for weight loss along with diet and exercise^[4]. The current anti-obesity drugs act either by suppressing appetite (e.g., Lorcaserin) or reducing fat absorption from the gastrointestinal (GI) tract (Orlistat)^[4]. But their effectiveness is limited. Tremendous effort has been paid seeking for anti-obesity drugs. Some compounds (e.g., β 3-adrenoceptor agonist) demonstrate good effectiveness^[5-8]. However, their practical uses are prevented by poor absorption, dilution and enzymatic degradation if being administrated by the conventional delivery routes (oral, nasal, pulmonary, intramuscular or intravenous routes)^[9]. In addition, these conventional delivery methods often produce systemic side-effects because of large-dose required and accumulation in non-targeted body parts. FDA-approved drugs, which all rely on oral intake, are also not immune to side-effects^[4]. For examples, Lorcaserin may cause headache and depression^[10]; Orlistat is infamous for its GI side-effects^[11]. Some anti-obesity drugs marketed before have been withdrawn because of the serious side-effects^[4, 12]. For instances, Fen-Phen (combination of two anorectics) has a potential to cause pulmonary hypertension and heart-valve problems; Sibutramine (an anorectic drug) could trigger severe cardiovascular problems. Therefore, it is imperative to seek safe and effective therapeutic strategies against obesity.

Obese WAT is the major source of many deleterious factors (e.g., free fatty acids, reactive oxygen species)^[13-15]. In contrast to the notorious WAT, brown adipose tissue (BAT) has unique capacity to increase body energy expenditure through the metabolically-active heat-producing brown adipocytes^[16]. Although BAT is rare in adult human, brown adipocyte-like cells (beige cells) have been identified in WAT, especially in subcutaneous WAT which represents ~85% of total body fat^[17, 18]. Studies have demonstrated that WAT browning (conversion of white adipocytes to beige cells) enhances body energy consumption, implying a novel strategy to combat obesity^[17, 19]. Adipose is a dynamic tissue. Drugs directly targeting on adipose tissue to promote its ameliorating self-modeling (e.g., browning, less macrophage infiltration, improved vascularization, etc.) are highly desired. However, drug delivery to adipose tissue using the conventional routes is difficult because of the lack of adipocyte-specific surface markers^[20, 21]. Although subcutaneous injection directly to adipose tissue using conventional hypodermic needle is possible, it is not practical for long-term home-based treatment due to poor patient compliance because of pain^[22] and possible infection at the injection site^[23]. Since obesity is not immediately life-threatening, it is especially important to make the treatment tolerable, safe and convenient.

To address the urgent and unmet need, this article reports a new strategy using disposable transdermal patches equipped with detachable polymeric microneedle (MN) array of varying degradation profile, for localized delivery of anti-obesity compounds directly targeting on subcutaneous WAT (Figure 1). As a proof-of-concept, we demonstrate the prominent anti-obesity effects of $\beta 3$ adrenoceptor agonist and thyroid hormone T3 transdermally delivered from rapidly-dissolving hyaluronic acid based MNs on mice. Comparing to the conventional intraperitoneal (IP) injection, lower dose and localized treatment are realized. Furthermore, using a diet-induced obese mouse model, we show that $\beta 3$ -adrenergic agonist released by slowly-dissolving poly(lactic-co-glycolic acid) based MNs can effectively suppress weight

gaining. These compounds act directly on subcutaneous WAT and nudge it towards energy expenditure status (brown-like adipose tissue). This painless and bloodless delivery strategy promises high drug integrity, low therapeutic dose, low side-effects, and amenability for home-based self-administration. We envision that such microneedle approach could be paradigm-shifting for home-based long-term management and prevention of obesity and associated diseases.

2. Results

2.1 Fabrication and characterization of HA-based MN-patches

Hyaluronic acid (HA) is a glycosaminoglycan distributed abundantly in skin and other connective tissues. HA-based MNs have been employed recently for administration of anti-diabetic drugs (insulin and exenatide)^[24, 25]. Here, HA-based MN-patches were used for quick and localized delivery of anti-obesity compounds into subcutaneous WAT.

A simple two-step micromolding method was used to fabricate MN-patches (Figure 2a). Note that the therapeutic molecules are only loaded in the MNs but not in the much-larger supporting substrate; whereby avoiding the waste of these costly compounds. The patch (~8x8 mm) consists of a 10x10 array of MNs which are sharp-pointed pyramidal shape with the base width of ~250µm and height of ~550µm (Figure 2b - d). The design is based on the rationales that pyramidally-pointed MNs with the aspect ratio of 2 : 1 (height to base diameter) and the interspacing between MN tips of 700 µm are optimal for skin penetration^[26-28]. A typical patch with Cy5-loaded HA-MNs is displayed in Figure 2c. Fluorescent Cy5 molecules are uniformly distributed in the MNs and completely absent in the supporting substrate.

Although HA-MN is hard enough to penetrate skin, its compressive strength could be a issue. To relieve such concern, we show that both drug-free and drug-loaded HA-MNs did not break

until compression reached $\sim 350\text{ }\mu\text{m}$ (Figure 2e). The attained mechanical force ($\sim 0.05\text{ N}$ per needle) is sufficient for skin penetration^[26, 29].

2.2 *In vitro* and *in vivo* dissolution of drug-loaded HA-MN patches

In vitro release kinetics was examined by monitoring the release profile of Cy5 encapsulated in HA-MNs. As shown in Figure 3a, Cy5 molecules can be quickly released in both PBS solution (time constant τ of $\sim 3.5\text{ sec}$) and mouse serum ($\tau \sim 5.6\text{ sec}$) because of fast dissolving of HA-MNs. When a MN-patch was applied onto porcine skin simply by thumb pressing ($\sim 1.9\text{ N}$), the loaded CL316,243 ($\beta 3$ - adrenoceptor agonist) can also be quickly released in skin ($\tau \sim 8.6\text{ sec}$) (Figure 3b). With comparable thickness of epidermis and dermis, and abundant subcutaneous fat in hypodermal layer, porcine skin closely resembles human skin and widely used as the skin model^[30]. As demonstrated in Figure 3c - e, CL316,243-laden HA-MNs were well-pierced into the skin, leaving arrayed marks and completely dissolving in porcine skin after $\sim 2\text{ min}$ insertion. Evidently, therapeutic drugs can be swiftly and fully delivered into skin using such microneedle approach. Histological examination shows $\sim 300\text{ }\mu\text{m}$ deep cavities into the dermal layer at the insertion sites (Figure 3f), which is about half of the MN height, owing to the skin elastic property and MN deformation during insertion^[24, 31].

In vivo distribution of released molecules from MN-patch was also examined. As shown in Figure 3g, the fluorescence intensity of Cy5 was strong in the treated skin region. And most of Cy5 molecules only accumulated just beneath the MN-insertion sites *via* local diffusion while some reached liver *via* systemic circulation (Figure 3h). Fluorescence was absent in other areas (e.g., heart, head and neck), except some autofluorescence in the stomach and intestine caused by the food inside^[32]. Cy5 reached inguinal WAT (IgWAT) underneath the insertion sites within 2 hr, and continued to accumulate (Figure 3i and j). It is noteworthy that only after 18 hr some Cy5 molecules reached liver and a small amount reached the other side of IgWAT *via* systemic circulation. In other words, the local accumulation in IgWAT just

underneath the MN penetration sites dominated. This is in contrast to the IP-injected Cy5 molecules, most of which were quickly accumulated in liver and kidney. Since molecules absorbed into the peritoneal capillary network reach the portal circulation first, these Cy5 molecules ended up passing through the liver (thus experiencing first-pass metabolism) before entering systemic circulation. Taken together, the local diffusion and accumulation of transdermally-delivered molecules allow targeted delivery into subcutaneous WAT.

The pharmacokinetics was assessed by analysing the serum level of the released molecules (Figure 3k). Thyroid T3 was used as the representative encapsulated-cargo as its serum level can be readily determined by ELISA. The conventional IP injection quickly led to a burst increase of serum level due to rapid absorption, followed by fast decay. In comparison, with an initial delay (~1 h) after MN insertion, both increase and subsequent decrease of serum level of T3 were kinetically much slower (longer time to reach the maximum concentration - $T_{\max}$ and longer elimination half-life - $T_{1/2}$) (Figure 3k, Supplemental Figure 1). This is attributable to slower absorption, because transdermally discharged T3 has to diffuse through the extracellular matrix of dermis and hypodermis before entering the blood capillaries^[33]. Because of the long lasting effect, transdermal delivery would allow avoidance of bolus administration required by systemic injection.

2.3 *In vivo* studies of the drug-loaded fast-dissolving MN-patches for obesity treatment

MN-patches have been recently investigated as an efficient transdermal delivery approach for various therapeutic applications, such as anti-diabetic treatment, vaccination, etc^[34]. Here, we demonstrate the advantages of microneedle approach for obesity treatment. CL316,243 (β 3-adrenoceptor agonist) and thyroid hormone T3 were used as the model anti-obesity agents in this study.

Studies have shown that β -adrenergic and thyroid hormones are effective for weight loss mainly through their stimulation on adipose browning, thermogenic and metabolic activities^[35-37]. But their off-target effects (e.g., cardiovascular problems) associated with systemic administration deter their practical usage^[36, 37]. We conceive that these adverse effects can be largely bypassed by direct and local drug release to subcutaneous WAT using MN-patches. The hypothesis is tested on mice by applying HA-based MN-patches at inguinal area where IgWAT, the major subcutaneous fat depot in rodents, loosely attaches just below the skin (Figure 4a). The skin penetration was evidenced by the micropores created on the skin by HA-MNs which rapidly dissolved within ~2 mins (Figure 4a). No obvious skin erythema, swelling and infection were observed.

There was no significant difference in body weight (Figure 4b) or food intake of the drug-treated and control groups. But the total weight of IgWATs (both right and left sides) was significantly decreased in CL316,243-treated mice (Figure 4e and f). Although CL316,243-loaded MN-patch was only applied at one side (either left or right), IgWATs at both sides were reduced in size and weight comparing to the control ones (Figure 4g). In addition, CL316,243-treated mice exhibited significantly reduced epididymal WAT (EpiWAT, visceral fat). These suggest that CL316,243 transdermally released at one inguinal region eventually arrived these adipose tissues *via* circulation.

In contrast to CL316,243, only the IgWAT beneath the application site of T3-loaded MN-patch (but not the IgWAT on the other side) was significantly smaller and lighter than the control group (Figure 4e - g). On the other hand, EpiWAT was not obviously different. The serum levels of T3 and thyroid stimulating hormone (TSH) remained normal (Figure 4c and d), suggesting that transdermally delivered T3 confined the actions on the nearby subcutaneous IgWAT because T3 molecules reaching circulation were either bound to the T3-binding proteins (e.g., globulin)^[38] or degraded in the liver. In comparison, IP injection of the

same dose had no effects on IgWAT weight (Figure 5a and b). T3 molecules locally accumulate in subcutaneous WAT after transdermal delivery because they can be readily uptaken by adipocytes through membrane transporters to activate its nuclear receptors^[39]. While CL316,243 molecules only bind to membrane-bound β 3-adrenoceptors selectively expressed on adipocytes without being internalized^[40], and hence much of them enters systemic circulation. But for both CL316,243 and T3, preferential targeting on adipocytes is realized.

Both β 3-adrenoceptor agonist and T3 hormone are known to promote WAT browning^[17]. Consistently, expressions of UCP1 (thermogenic and brown-adipocyte specific protein), COX1 (mitochondrial biogenesis marker) and PGC1 α (transcriptional factor essential for thermogenic and brown-specific genes expression) were markedly increased in both IgWATs of CL316,243 treated mice and only the IgWAT underneath the MN-insertion site of T3-treated mice (Figure 4h).

2.4 Comparing the anti-obesity effects of transdermally- and systemically- delivered CL316,243

Due to hepatic metabolism and renal clearance, systemic administration of drugs usually produce low basal blood level soon after its initial peak, and hence, is not feasible for sustained drug effects. In contrast, as a result of slow entrance into circulation and avoidance of the first-pass effect, transdermal delivery is able to achieve prolonged effect, thus allowing a lower effective dose^[41].

In contrast to the microneedle approach, the same dose of CL316,243 (0.1 mg/kg/day) applied by IP injection exerted inappreciable effect on adipose tissue weight (Figure 5a and b). Only when IP injection dose was boosted by 5 times, similar adipose reduction was attained as MN delivery (Figure 5c - e). Both high-dose IP injection and low-dose MN delivery similarly

caused increase of body surface temperature (Figure 5f and g), which is attributable to transformation of adipose tissue into energy expenditure status (browning)^[17]. Indeed, both treatments caused significant increase of browning markers (UCP1, COX1 and PGC1a) and increased appearance of multilocular brown-like adipose cells in IgWAT to a similar extent (Figure 5h and i, Supplemental Figure 2). These observations testify that lower effective dose can be achieved *via* MN-based transdermal delivery as compared to systemic injection, therefore promising lower systemic side-effects of β 3-adrenergic agonists (such as increased heart rate and blood pressure^[35, 36]).

2.5 Characterization of slowly-dissolving PLGA-MN patches

PLGA is a biocompatible and biodegradable polymer that has been extensively adopted in the delivery vehicles for drugs and biomacromolecules^[42]. The mechanic properties and drug release kinetics of PLGA-MNs can be readily tailored by choosing or mixing different PLGA molecules with different structure, chain length and co-polymer ratio.

We fabricated different PLGA-MNs on HA substrate (Figure 6a). Microneedles were made with long-chain PLGA (756S), short-chain PLGA (502), small-branched (star) PLGA, or mixture of two species of the above. Immediately after insertion, the slowly-dissolving PLGA-MNs would detach from HA-substrate and remain in the skin because HA molecules at the MN-substrate junction dissolve quickly once interstitial fluid reaches there through capillary effect (Figure 6a). To illustrate the morphology and drug release characteristics, Cy5 molecules were loaded into PLGA-MNs but not HA-substrate. As shown in Figure 6b, MNs made with long-chain PLGA exhibited sharp-pointed pyramidal shape whereas short-chain or star PLGA-based MNs showed poor morphology. But dissolution of the long-chain PLGA, hence the release of the Cy5, was very slow. Specifically, it was well-preserved its pyramidal shape even after immersion in PBS solution for 7 days (Figure 6b); and only ~8.6% or ~37.3% of Cy5 was released after 4 days or 2 weeks (Figure 6c). In comparison, after 7

days, MN with short-chain PLGA shrunk into a small ball and the MN with star-PLGA largely disintegrated (Figure 6b). Both of them discharged >70% of Cy5 molecules after 2 weeks (Figure 6c).

To ensure good morphology for penetration and a not-too-slow release kinetics, long-chain PLGA were mixed with short-chain or star PLGA (Figure 6b and c). We found 1:1 mixture of long-chain and short-chain PLGAs most desirable because such MNs (PLGA 756S+502) were nicely sharp-pointed and able to release ~31.8% of Cy5 molecules at day4 which is comparable to MNs with pure short-chain or star PLGA. The mechanical strength of MNs with the pure long-chain PLGA is the greatest among all (Figure 6d). But the mechanical strength of PLGA (756S+502)-based MNs is beyond the threshold for skin penetration because it can exert 10mN on a MN tip without breaking. It has been shown that <5 mN or <10 mN is enough for penetration into the porcine skin^[27, 29] or human skin^[31], respectively. The PLGA (756S+502) MN-patches were therefore used for all the following experiments. As revealed by SEM, these microneedles had the base width of ~250 μm and height of ~570 μm (Figure 6e). After a brief exposure to PBS solution (~1 min), PLGA-MNs were rapidly detached from HA substrate (Figure 6e). They were able to insert into porcine skin by thumb press, leaving arrayed marks on skin surface with a penetration depth of ~300 μm (Figure 6e). Hence, with its slowly-dissolvable property and appropriate mechanical strength, drug-loaded PLGA-MNs could be used as an implanted cutaneous drug-depot which provides a sustained release of drugs.

2.6 CL316,243-loaded slowly-dissolving MN-patches to prevent diet-induced obesity

We have demonstrated the anti-obesity effect of transdermally-delivered CL316,243 on mice by daily application of drug-loaded fast-dissolving MN-patches (Figure 4 and 5). But daily application is not patient friendly for home-based long-term healthcare and pulsed delivery from the fast-dissolving MNs is not capable of ensuring sustained drug effects. Hence we

further investigated the ability of CL316,243-loaded slowly-dissolving MN-patches (applied twice per week) to prevent diet-induced obesity development.

Similar to the previous experiments using HA-MN patches, PLGA-based MN-patches were applied at inguinal area of diet-induced obese (DIO) mice. Microneedle penetration left arrayed marks on the skin (Figure 7a), yet caused no obvious abnormal skin conditions. It was observed that noticeable fluorescence signal was retained at the insertion site for 5 days following the application of Cy5-loaded PLGA-based MN-patch (Figure 7b). Such retention time is comparable to the epidermal turnover time of mice (8-10 days)^[43]. Evidently, the implanted PLGA-MNs act as long-live drug-depots for sustained release.

As shown in Figure 7c, treatment with CL316,243-laden MN-patch largely inhibited body weight gain by ~15% as compared with the control mice treated with drug-free MN-patches. In comparison, IP injection of the same dose (~0.3 mg/kg, twice weekly) did not significantly prevent weight gain. The three groups of mice had similar food intake (Figure 7d).

Consistently, MN-released CL316,243, but not IP injected CL316,243, significantly reduced both IgWAT and EpiWAT (Figure 7e and f). Furthermore, MN-delivered CL316,243 also led to elevation of body surface temperature (Figure 7g), and increased appearance of multilocular brown-like adipose cells as well as expression of UCP1 in IgWAT (Figure 8a and b). These observation unambiguously confirms the adipose browning effects of transdermally-delivered CL316,243. Clearly, the sustained transdermal release is superior to systemic administration. Just like human, obese mice exhibit metabolic syndrome including high levels of total cholesterol, free fatty acid, insulin, glucose, and triglyceride, which are high risk factors for various metabolic diseases such as diabetes. As shown in Figure 8c - g, our microneedle treatment was able to relieve metabolic syndrome by reducing the serum levels of the first three compounds.

3. Conclusions

Combating obesity is challenged by the fact that the laboratory-proven anti-obesity compounds lose effectiveness or/and cause severe side-effects while being delivered *via* conventional routes, as well as the fact that this chronic yet devastating disease demands long-term home-based treatments with good patient compliance. To tackle these challenges, we demonstrate herein a promising microneedle strategy. We envision that a microneedle patch equipped with thousands of microneedles can be readily applied on belly fat (largest depot of WAT in humans) daily or regularly by the patient at home without pain and need of skills. After thumb-pressing for 2 minutes or so, drug-loaded microneedles will detach from the supporting-patch and be embedded in the skin serving as the transdermal drug reservoirs. As patches do not need to remain attached, skin discomfort and irritation are avoided. The drug release kinetics can be readily tailored by engineering the polymeric microneedles. In principle, the array format of the microneedles permits transdermal release of multiple drugs with varying optimal kinetics to achieve synergistic effects. As obesity is often the root of all evils, such microneedle approach may also be employed for obesity-associated metabolic diseases. Instead of taking pills, making painful intravenous injection, or paying frequent clinic visits, patients can conveniently and effectively manage obesity or other metabolic diseases at home using microneedle patches.

We conceive that anti-obesity compounds directly targeting on WAT to promote its ameliorative re-modeling, particularly, those that can turn calorie-storing white fat into calorie-burning brown fat, are desirable. As we demonstrated, microneedle-enabled transdermal delivery grants much lower effective dose (hence much less side-effects) as compared to systemic administration, hence making many anti-obesity compounds now clinically feasible. As the proof-of-demonstrations, we tested β 3-adrenoceptor agonist and thyroid hormone. Other laboratory-tested compounds (e.g., FGF21, BMP7, apelin, PPAR γ -

agonist, AT2R-agonist)^[7, 8, 17] that can promote browning or reduce inflammation in obese fat could also be explored. In addition, compounds (e.g., microRNAs) that are not able to survive through systemic administration become possible.

After being secreted from thyroid gland, thyroid prohormone T4 is converted into active T3 which in turn can stimulate adipose browning and thermogenesis^[44]. Studies on both humans and animals showed that systemic administration of T3 or T4 results in a decrease of body weight and adipose tissues^[45]. However, chronic systemic administration leads to hyperthyroidism, causing widespread adverse effects on cardiovascular and nervous systems. Hence, thyroid hormones are not approved as anti-obesity agents for humans. In this study, we demonstrated that, using MN-patches, transdermal administration of low-dose T3 (0.5 µg/day for 5 days in mice) could realize localized fat reduction without producing systemic hyperthyroidism (Figure 4), because T3 molecules can quickly diffuse to and accumulate in subcutaneous WAT. Such effective dose is much lower than the previously reported using systemic administration (e.g., ~12.5 µg/day for 2 weeks)^[44, 46]. The absence of systemic effect can be attributed to the low-dose used, delayed and reduced entrance to the circulation (Figure 3i - k), and existence of buffering proteins to T3 in plasma^[38].

β3-adrenoceptor is selectively expressed in adipocytes (much lesser extent in urinary bladder), making it a favorable anti-obesity drug target^[6, 17]. A number of synthesized β3-agonists have been demonstrated as anti-obesity and anti-diabetic agents in various rodent models^[6]. But some of them lack of high specificity to β3-adrenoceptor and consequently cause sympathomimetic effects due to undesired activation of β1- and β2- receptors^[47]. For example, although energy expenditure in obese humans can be successfully promoted by a single high oral-dose (1000mg) of a β3-agonist (L-796568), systolic blood pressure is also significantly raised^[36]. Similarly, although brown and beige fat of multiple depots can be stimulated, a single high-dose (200 mg orally) of β3-agonist mirabegron (FDA-approved drug for

overactive bladder syndrome) induces an increase of heart rate and blood pressure^[35].

CL316,243 used in this study is believed to be more clinically promising for human, because, similar to mirabegron, it has high binding specificity to human β 3-adrenoceptor^[6, 48] and browning effects on human white adipocytes (Supplemental Figure 3). A clinical study in humans showed that CL316,243 (1500 mg/day orally, 8 weeks) increased insulin action and fatty acid oxidation, without altering heart rate and blood pressure^[48]. But such chronic oral intake of CL316,243 failed to reduce body weight and fat mass, because of its poor oral bioavailability, poor GI absorption, and first-pass hepatic destruction^{[48], [6]}. We show that transdermal delivery of CL316,243 to subcutaneous WAT using microneedles is superior to systemic administration, and can achieve much lower effective dose (estimated as 5 mg/day) assuming the same weight-normalized effective dose as we determined here in mice.

But we expect more prominent dose reduction in humans because human body is bigger with a vast circulation system; human has more subcutaneous WAT (~10% of body weight whereas <1% of mouse IgWAT); subcutaneous WAT attaches directly underneath the skin (unlike loose attachment in mice) and exists mainly in the belly fat which accounts for central obesity and metabolic diseases^[1, 2]. Lowering the therapeutic dose not only produces lesser side-effects but also reduces the cost. As mass production of MN-patches is possible, the amount of drug used dictates the cost. In summary, MN approach provides a cost-effective and home-based solution to combat obesity and associated diseases, which promises low effective dose (hence side-effects) whereby making many anti-obesity compounds now become practically possible.

4. Experimental Section

Fabrication of MN-patches: MN-patches were prepared *via* micromolding. Specifically, polydimethylsiloxane (PDMS)-micromolds were created by pouring PDMS (10:1 w/w ratio of prepolymer to curing agent) into a stainless-steel master structure (consisting of 10x10

arrays of MNs which are sharp-pointed phramidal in shape, with a height of $\sim 600\ \mu\text{m}$, interspacing of $\sim 700\ \mu\text{m}$, and base width of $\sim 300\ \mu\text{m}$; fabricated by Micropoint Technologies, Singapore), followed by degassing in vacuum oven and curing at 70°C for 2 hr.

To prepare hyaluronic acid (HA)-based MN-patches, PDMS-micromold was filled with 0.5 g HA solution (dissolved in 1 ml DI water, with or without 2.5 mg CL316,243 or 0.5 mg 3, 3', 5-triiodothyronine or 0.5 mg Cy5) through centrifugation (4000 rpm, 3 min) in a swinging bucket rotor (SCANSPEED 1580R, LaboGene). To prepare poly(lactic-co-glycolic acid) (PLGA)-based MN-patches, PLGA solution (200 mg of PLGA dissolved in 1 ml dimethylformamide, with or without 10 mg CL316,243) was filled into PDMS-micromold. After overnight drying at room temperature (RT), a second layer of bare-HA solution (0.5 g/ml) was filled and centrifuged again to create the supportive backing layer. After overnight drying (at RT) again, MN-patch was gently peeled off from the micromold, and stored at 4°C in an un-humidified condition.

Low molecular weight HA ($< 10\text{kDa}$) was obtained from Bloomage Freda Biopharm. CL316,243 (a potent and selective β_3 -adrenoceptor agonist) was obtained from Tocris Bioscience. Cyanine5 (Cy5) was acquired from Lumiprobe. 3, 3', 5 -triiodothyronine (a synthetic form of a natural thyroid hormone T3), PDMS, DMF and PLGA (Resomer RG 756S, 0.71-1.0 dL/g; Resomer RG 502, 0.16-0.24 dL/g; PLGA 5arm star with glucose core, Mn 15,000) were purchased from Sigma-Aldrich.

Characterization of MN-patches: MN-patches were examined using a field-emission scanning electron microscope (FESEM; JSM-6700F, JEOL). Cy5-loaded MN-patches were visualized with a confocal microscope (LSM800, Carl Zeiss) using the excitation wavelength of 633nm. The mechanical property of MN-patches was tested using an Instron 5543 Tensile Tester (Instron). A force was applied perpendicularly to MN tips placing upward on a flat aluminum plate, using a flat-headed stainless steel cylindrical probe (at a constant speed of

0.5 mm/min). The force was continuously recorded until a displacement of 350 μm was reached.

***In vitro* and *in vivo* drug release profiles:** *In vitro* drug release profiles in solutions were tested using the Cy5-loaded MN-patches immersed in either phosphate buffered saline (PBS, pH 7.4) or mouse serum (37 °C). Cy5 release from MN-patches was sampled at different time intervals (0 – 2 min), and analyzed using a fluorescence spectrometer (SpectraMax M5, Molecular Devices) with excitation of 633 nm and emission of 660 nm, at 37 °C.

For the *ex vivo* release profile, CL316,243-loaded MN-patches were inserted into porcine skin for different time durations (0 – 4 min). The amount of CL316,243 delivered from MNs into the skin was calculated by subtracting the amount remaining in MN-tips after insertion and on the skin surface from the amount initially loaded in the MNs. CL316,243 molecules remaining in the inserted-MNs were extracted by dissolving the MNs in PBS. The residual amount left on the skin surface at the insertion site was also extracted by tape-stripping on the skin and soaking the stripped-tape in PBS. The extracted CL316,243 was then measured by a reverse-phase high performance liquid chromatography (Agilent HPLC-VWD system) (using an Agilent Poroshell 120 EC-C18 column, with a mobile phase of water: methanol 80:20 (V/V), a flow rate of 0.5 mL/min, and UV detection at 230 nm), and quantified based on the linear standard curve created with various known concentrations. To determine the *in vivo* release kinetics, serum levels of T3 from mice treated with either T3-loaded MN-patch (0.5 μg in MNs of each patch) or intraperitoneal (IP) injection of T3 (0.5 μg in 50 μl PBS) were analysed.

***In vivo* fluorescence imaging and bio-distribution of Cy5:** The mice treated with unloaded or Cy5-loaded HA-based MN-patches (0.5 μg in MNs of each patch) were imaged (1 hr after insertion) using an *in vivo* imaging system (IVIS Spectrum, Perkin Elmer). Specifically, MN-patch was applied at left or right side of the inguinal (Ig)-region, and left for ~5 min, before

removing the patch substrate and wiping the skin surface with PBS-soaked cotton balls. 6 hr after insertion, the mice were euthanized and dissected in order to image the bio-distribution of Cy5 molecules in IgWAT (just under the skin into which MN-patches were applied) and other internal organs. In some experiments, the mice treated with either Cy5-loaded HA-based MN-patch or IP injection of Cy5 (0.5 μ g in 50 μ l PBS) were euthanized 2 hr or 18 hr post-treatment, and the major organs (liver, heart, kidney, lung and IgWAT) were dissected and visualized by IVIS imaging system. Mice treated with Cy5-loaded PLGA-based MN-patches were also imaged immediately after insertion (day0) and at day5.

Anti-obesity experiments with different mouse models: All animal studies were approved by the SingHealth Research Facilities Institutional Animal Care and Use Committee (Singapore). Mice (C57BL/6J, 7 – 8 week old male) were housed in light and temperature controlled facility (12-hr light/12-hr dark cycle, 21 °C), and allowed free access of water and normal standard diet. Five to six mice were used in each treatment or control group. Mice were randomly grouped and given daily transdermal insertion for 5 days with different HA-based MN-patches: unloaded or drug-loaded MN-patches (2.5 μ g CL316,243 or 0.5 μ g T3 were loaded in 100 MNs of each patch). The mouse hairs in the inguinal region (lower left or right quadrant of dorsolateral area, adjacent to the hind limbs) were carefully shaved-off, before a MN-patch was applied at left or right side of the exposed region, and left for ~5 min to let MNs being completely dissolved. MN-patch was applied with the daily doses of 2.5 μ g for CL316,243 (~0.1 mg/kg/day) and 0.5 μ g for T3 (~20 μ g/kg/day), respectively. In the control experiments, mice were grouped and given daily intraperitoneal (IP) injection for 5 days with 2.5 μ g CL316,243 (~0.1 mg/kg/day) or 0.5 μ g T3 (~20 μ g/kg/day) (dissolved in 50 μ l PBS). In addition, to compare the effectiveness of two different methods for delivering anti-obesity agents, mice were given either daily IP injection of high-dose CL316,243 (12.5 μ g, ~0.5 mg/kg/day) or daily transdermal delivery using CL316,243-loaded HA-based MN-

patch (2.5 μg , ~ 0.1 mg/kg/day). Quantification of CL316,243 and T3 loaded in MNs were confirmed by analyzing the extracted drugs using HPLC or ELISA, respectively.

In order to test anti-obesity effect of drug-loaded MN-patch on diet-induced obese (DIO) model, high-fat diet (58Y1, 60% kcal from fat, Testdiet) was used to induce and maintain obesity in C57BL/6J mice. DIO mice (C57BL/6J, 9 – 10 week old male, ~ 30 g) were then randomly divided into 3 groups and treated twice weekly, with application of unloaded PLGA-based MN-patch (control), or CL316,243-loaded PLGA-based MN-patch (1 patch per mouse, 10 μg in MNs of each patch), or IP injection of CL316,243 (10 μg per mouse; ~ 0.3 mg/kg).

Body surface temperature (which reflects the body core temperature^[49]) of differently treated mice was monitored using infrared thermal imaging camera (FLIR T420; the thermal sensitivity, <0.045 $^{\circ}\text{C}$ and the spectral range, 7.5 - 13 μm). Sera were also collected for further analyses. The mice were then euthanized by a lethal dose of carbondioxide. After measuring the body weight, fat tissues (inguinal WAT- IgWAT, epididymal WAT and interscapular BAT) was excised and weighted. IgWAT was then homogenized in radioimmunoprecipitation assay buffer (containing protease inhibitor cocktail, Roche Applied Science) using a MagNA Lyser (Roche) for further immunoblot analyses.

Histological analyses: *In vitro* skin insertion by MNs was evaluated by analyzing the histological sections of porcine skin ~ 2 min after application with CL316,243-loaded MN-patches. The excised skin samples were immediately fixed with 4% paraformaldehyde (overnight), washed with PBS, and immersed in 30% sucrose solution to cryoprotect the tissues (24 hr). After embedding in FSC22 Frozen Section Media (Leica Microsystem), cryosections (10 μm thick) were prepared using a cryostat (CM1950, Leica Microsystems). The skin sections were finally stained with hematoxylin and eosin (H&E, Sigma-Aldrich), and images were taken using an inverted microscope (IX71, Olympus) equipped with a digital

camera (OlympusE330). Similarly, for morphological analysis of adipose tissues, both IgWAT and interscapular BAT of differently treated mice were collected, fixed and cryoprotected, before tissue sectioning (10 - 20 μ m thick), H&E staining, and imaging.

Immunoblot analyses: After a brief vortexing and centrifugation at 4 °C, tissue supernatant was collected and the protein concentration was determined by a bicinchoninic acid protein assay (Fisher). Each sample with equal amount of proteins (as the loading control) was separated on 12% SDS-PAGE before being transferred onto a nitrocellulose membrane. The membrane was then blocked (Superblock blocking buffer, 2 hr at RT) and incubated with specific primary antibody (1: 200-400 dilutions, 12 hr at RT), before washing (Tris-buffered saline-Tween solution- TBST, 3 x 15 min each) and incubation with horseradish peroxidase-conjugated secondary antibody (1: 2000 – 4000; 6 hr at RT). After washing again with TBST (3 x 15 min), the protein bands were detected in a G:BOX Chemi XT4 imaging system (Syngene) using SuperSignal WestPico Chemiluminescent Substrate (ThermoFisher Scientific). Antibodies against uncoupling protein-1 (UCP1, sc-6529), adipocyte protein-2 (aP2, sc-18661) (fatty acid binding protein 4), peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α , sc-13067), cytochrome-c oxidase subunit-I (COX1, sc-23982), and actin (sc-1616) were purchased from Santa Cruz Biotechnology. The anti-UCP1 antibody (PA1-24894) was also obtained from ThermoFisher Scientific.

Assessment on serological parameters: Serum levels of thyroid T3 and thyroid stimulating hormone (TSH) in mice treated with T3-loaded MN-patches were analyzed using a mouse triiodothyronine (T3) ELISA kit from Biovision and mouse TSH ELISA kit from Elabscience, respectively. Serum levels of free fatty acids, triglycerides and glucose from obese mice were measured by the standard assay kits from Biovision. Serum level of cholesterol and insulin from obese mice were determined by cholesterol quantitation kit from Sigma-Aldrich and mouse insulin ELISA kit from ThermoFisher Scientific, respectively.

Statistical analyses: Data as analyzed using a standard two-tailed unpaired Student's *t*-test, and the results were expressed as mean $\pm$ SEM. Unless otherwise stated, all experiments were performed using at least four different samples per group, and the data presented are representative of four independent experiments. A *p* value < 0.05 was considered to be statistically significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Figure captions

Figure 1: Schematic of delivery of adipose browning compounds to subcutaneous adipose tissue using a transdermal patch equipped with dissolvable MN array.

Figure 2: HA-MN patches. scale bars = 100 μ m. **[a]** Schematic of fabrication process of drug-loaded MN-patches. **[c]** Photograph of a HA-MN patch. **[b]** Cy5-loaded HA-MN patch. left: mergance of bright-field and confocal fluorescence image of side-view (upper) and bottom-view (lower); right: cross-sections of a microneedle. **[d]** SEM image of a HA-MN patch. **[e]** The force-displacement curves of unloaded, CL316,243-loaded or T3-loaded MN-patches (each trace is the average from 4 different samples).

Figure 3: *In vitro* and *in vivo* dissolution of HA-MNs and bio-distribution of released compounds in mice. **[a and b]** *In vitro* release profiles of [a] Cy5 from Cy5-loaded HA-MNs in phosphate buffered saline (PBS) and mice sera (at 37°C), and [b] CL316,243 from CL316,243-loaded HA-MNs in porcine skin. Data points are expressed as mean $\pm$ SEM (n = 4) and fitted by an exponential function. **[c]** Photographs of a CL316,243-loaded MN-patch applied on porcine skin (left) and the penetration marks left after patch removal (right). Scale bars = 2 mm. **[d and e]** SEM images of CL316,243-loaded HA-MNs, before and after insertion into porcine skin. Scale bars = 200 μ m. **[f]** Haematoxylin and eosin stained section of porcine skin showing the penetration of a CL316,243-loaded HA-MN. Scale bar = 200 μ m. **[g and h]** *In vivo* fluorescence imaging of mice transdermally-applied with unloaded (control) or Cy5-loaded MN-patch. [g] shows the ventral view 1 hr after insertion. [h] shows the ventral view of dissected mice 6 hr post-insertion to reveal the bio-distribution of Cy5 (blue circles: IgWATs; L: liver; H: heart; S&I: stomach and intestine). **[i and j]** *In vivo* bio-distribution of Cy5 treated with MN-patch or IP injection. The representative fluorescent images of dissected organs and the quantitative analyses of their fluorescent intensities are shown. In [i], data points are shown as mean $\pm$ SEM (n = 4). **[k]** *In vivo* pharmacokinetic profiles of thyroid T3 treated with MN-patch or IP injection (mean $\pm$ SEM; n = 4).

Figure 4: *In vivo* studies of drug-loaded HA-MN patches for obesity treatment. Mice were treated for 5 days with unloaded (control) or drug-loaded (CL316,243 or T3) MN-patches applied only above one-side of inguinal region. MN: IgWAT under the MN-patch; N: IgWAT on the other side of the body. **[a]** Illustration and photographs of application of MN-patch (upon and after insertion). **[b]** Average body weights of differently treated groups (mean $\pm$ SEM, n = 5). **[c and d]** Serum levels of T3 and TSH from mice (at day5) treated with unloaded or T3-loaded MN-patches (mean $\pm$ SEM, n = 5). **[e - g]** Images and average weights (relative to body weight) of IgWAT, epididymal WAT and interscapular BAT (mean $\pm$ SEM, n = 5). **[h]** Western blot image and analyses of protein expressions in IgWAT isolated from differently treated mice. The statistics is mean $\pm$ SEM (n = 4, blot intensity normalized to actin density). Student's *t*-test: **p* < 0.05, ***p* < 0.01 vs. non-insertion site of control mice; #*p* < 0.05, ###*p* < 0.01 between indicated pairs.

Figure 5: Anti-obesity effects of CL316,243 delivered by transdermal and intraperitoneal routes. **[a and b]** Mice were given daily IP injection for 5 days with saline (control), 2.5 μ g CL316,243 or 0.5 μ g T3. Representative images of IgWAT, epididymal WAT and interscapular BAT isolated from differently treated mice are shown in [A]. Average body

weights and weight ratios of adipose tissues are shown in [B] (mean $\pm$ SEM, $n = 5$). [c - i] Mice were given daily treatment for 5 days with IP injection of saline (control) or high-dose injection of CL316,243 (12.5 μ g) or transdermal delivery of CL316,243 (2.5 μ g). Average body weights and weight ratios of adipose tissues are shown in [d] (mean $\pm$ SEM, $n = 5$). Representative images of IgWAT, epididymal WAT and BAT isolated from differently treated mice are shown in [e]. [f and g] show infrared thermographic images and the average body surface temperature (body ROIs) (mean $\pm$ SEM, $n = 5$). [h and i] shows the browning effects of CL316,243 in IgWATs isolated from differently treated mice. The representative immunoblots of UCP1, COX1, PGC1 α , aP2 and actin expressions, and their respective statistics (mean $\pm$ SEM, $n = 4$; normalized to actin density) are shown in [h]. The representative haematoxylin and eosin staining images of IgWAT from the differently treated mice (400x) are shown in (i). Student's t -test: $*p < 0.05$ vs. control.

Figure 6: PLGA-MN patches. [a] Schematic of PLGA-MN implantation, and chemical structures of linear and branched PLGA. [b] Confocal fluorescence (first column) and bright-field (second column) images of different types of Cy5-loaded PLGA-MNs (with a HA supporting substrate). Scale bar = 100 μ m. And *in vitro* dissolution of Cy5-loaded PLGA-MNs in PBS (at 37 °C), immediate (day 0, third and fourth columns) or long-term exposure in PBS (day7, fifth and sixth columns). [c] *In vitro* release profiles of Cy5 from PLGA-MNs in PBS (at 37°C; average from 4 patches). [d] The force-displacement curves of CL316,243-loaded PLGA-MN patches (average from 4 different samples). [e] SEM images of CL316,243-loaded PLGA(756S+502)-MNs, before and after insertion into the porcine skin (upper lane). Haematoxylin and eosin stained section of porcine skin penetrated by one of CL316,243-loaded PLGA-MN and bright-field photograph penetration marks left by MNs (lower lane). Scale bars = 200 μ m.

Figure 7: Anti-obesity effects of CL316,243-loaded PLGA-MN patches in diet-induced obese (DIO) mice. DIO mice were treated twice a week for 25 days: unloaded MN patch (control) or CL316,243-loaded MN-patches or IP injection of CL316,243. [a] MN penetration-marks in inguinal region. [b] *In vivo* fluorescence imaging, immediately (day0) or 5 days after application of Cy5-loaded MN-patch. [c] Relative body weight increases of differently treated obese mice (mean $\pm$ SEM, $n = 5$). [d] Average food intake per mouse per day (mean $\pm$ SEM, $n = 5$). [e and f] The representative images and average weights of IgWAT, epididymal WAT and interscapular BAT (relative to the respective body weights) isolated from differently treated obese mice (mean $\pm$ SEM, $n = 5$). [g] infrared thermographic images and the average

body surface temperature (body ROIs) (mean $\pm$ SEM, $n = 5$). Student's t -test: $**p < 0.01$ vs. control.

Figure 8: White adipose browning effects of CL316,243-loaded PLGA-MN patches in DIO mice (same experiments as in Figure 7, but analyses at cellular and molecular levels). **[a]** The representative hematoxylin and eosin staining images of IgWAT from differently treated mice (upper lane, 100x; lower lane, 400x). **[b]** The representative immunoblots of UCP1, COX1, PGC1 α , aP2 and actin in IgWAT, and their respective statistics (mean $\pm$ SEM, $n = 4$; normalized to actin density). **[c - g]** Quantification of serum levels of cholesterol, free fatty acids, triglyceride, glucose and insulin from differently treated obese mice (mean $\pm$ SEM, $n = 5$). Student's t -test: $*p < 0.05$, $**p < 0.01$ vs. control.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

FIGURE 8