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Lancing drug-reservoirs into subcutaneous fat to combat obesity and associated metabolic diseases

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Abstract

Obesity is a serious epidemic health problem that can cause many other diseases including type 2 diabetes and cardiovascular diseases. Current approaches to combat obesity suffer from low effectiveness and adverse side effects. Here, we developed a new self-administrable and minimally-invasive transdermal drug delivery strategy for home-based long-term treatment of obesity and other diseases. Specifically, ultrathin, core-shelled, and lance-shaped polymeric drug-reservoirs (micro-lances – MLs) were readily fabricated by a thermal pressing molding method and totally implanted into subcutaneous fat by lancing through the skin. Using a diet-induced obese mouse model, we show that development of obesity and associated metabolic disorders is effectively inhibited by applying therapeutic core-shelled MLs once every 2 weeks. The outstanding therapeutic effects are attributable to highly

localized and biphasic drug release, as well as combination therapy based on browning transformation of white fat and enhanced insulin sensitivity.

1. Introduction

Obesity is increasingly prevalent worldwide and it is often the root cause to many diseases such as diabetes, cardiovascular diseases, and cancers.^[1] Lifestyle intervention (diet and physical activity) alone is usually insufficient to combat obesity because of problematic long-term patient compliance, body adaptation, and the fact that obesity is the consequence of definite biological reasons.^[1-2] Although surgical intervention is effective, there are concerns about risks, side-effects, high cost, and long-term efficacy.^[3] On the other hand, only a few weight management medications are currently available. But they all act indirectly with limited efficacy, either by reducing fat absorption in gastrointestinal tract or suppressing appetite in brain. The adverse side-effects and high cost limit their use.^[4] Clearly, it is imperative to seek for new therapeutic strategy.

White adipose tissue (WAT) stores energy in the form of triglycerides. In obesity, WAT is excessively accumulated and releases various deleterious factors (e.g. free fatty acids- FFA, inflammatory cytokines, reactive oxygen species) which lead to various metabolic problems, such as insulin resistance.^[5] In contrast, brown adipose tissue (BAT) specializes in burning energy using glucose and FFA as the major fuels.^[6] Studies have demonstrated BAT as a negative regulator of adiposity and insulin resistance.^[6-7] Although BAT is scarce in adults and even less in obese people, brown-like (or beige) adipocytes have been identified in WAT, particularly in subcutaneous fat depot.^[8] And it has been found that white adipocytes can transform into brown-like adipocytes (termed as browning) upon induction by some agents, (e.g. β 3-adrenergic receptor agonist).^[7-8] Therefore, it has been proposed that stimulating browning of subcutaneous WAT (sWAT) shall be more effective strategy to combat obesity and associated metabolic diseases.^[9] Nevertheless, the clinical use of browning or anti-

obesity agents is prevented by the issues associated with the conventional drug delivery methods (e.g., oral intake, intravenous injection). Using these systemic delivery methods, the bioavailability at WAT is low due to poor GI absorption, hepatic first pass effect, renal clearance, enzymatic degradation and lack of ability to specifically target on WAT.^[10] Moreover, these methods can only give a burst release of drugs, leading to short therapeutic duration and hence the need of frequent administration. Consequently, the required high dosage and accumulation in non-targeted tissues unavoidably cause systemic side-effects. Not surprisingly, nanoparticle delivery of anti-obesity agents through the systemic route has significant off-target accumulation.^[11]

Recently, two research groups including us have demonstrated microneedle skin patches for localized and controlled transdermal delivery of browning agents to sWAT.^[12] This method offers outstanding anti-obesity efficacy with low effective dosage (thus presumably low side-effects) and minimal invasiveness (thus suitable for home-based healthcare). Despite the obvious superiority over the conventional methods, the microneedle approach still has several intrinsic limitations.^[13] Firstly, microneedle patches are typically fabricated using micro-molding method which is time-consuming and not easy to be scaled up due to involvement of centrifugation and lengthy drying process. Secondly, the length of microneedles is usually limited to be <1 mm in order to ensure painless penetration and possibility to demold microneedles without breaking.^[14] Such length is too short to reach sWAT. Therefore, drug molecules discharged from the microneedles embedded in dermis rely on diffusion into sWAT. Thirdly, loading capacity of microneedles is small. This problem is worsened by two issues: i) some molecules enter systemic circulation via dermal capillary networks before reaching sWAT; and ii) microneedles cannot fully insert into the skin (typically only ~75% of total volume being actually utilized) due to elastic deformation of skin.^[14b, 15]

Here, we developed a new transdermal delivery technique and demonstrated its use to directly deliver browning and insulin sensitizing agents into sWAT. Ultrathin, core-shelled, and lance-shaped polymeric drug reservoirs (micro-lances – MLs) were readily fabricated by thermal pressing molding and subsequent dip-coating. They can be rapidly lanced through the skin and totally implanted into sWAT for localized and biphasic drug release. The excellent therapeutic effectiveness to prevent development of obesity and associated metabolic diseases was demonstrated using an obese mouse model induced by high-fat diet. We envision that this self-administrable and minimally-invasive ML approach may also be employed for long-term home-based treatment of other chronic diseases.

2. Results

2.1. Fabrication of Core-shelled micro-lances

Polymeric micro-lances (MLs) were fabricated by a simple thermal pressing method using poly (lactic-co-glycolic acid) (PLGA), because the thermoplastic property of PLGA makes it moldable at the mobile rubbery state above the glass transition temperature ($T_g = 55\text{ }^{\circ}\text{C}$) and then becomes strong and rigid below T_g . PLGA, which has good biocompatibility and high mechanical strength, is widely used in US-FDA approved drug products and implants.^[16] The biodegradation rate of PLGA can be tailored by varying the monomer ratio (PLA/PGA). We and other studies have found that PLGA with 50:50 PLA/PGA exhibits the fastest hydrolytic degradation at the physiological condition.^[16-17] To further speed up the degradation, salt micro-particles (10 - 50 μm , Figure S1A) as porogens are added to PLGA polymer matrix. As the melting temperature of most small drug molecules is much higher than T_g of PLGA, they can be incorporated into MLs by simply mixing with PLGA molecules.

As illustrated in **Figure 1**, a dry homogeneous mixture of PLGA molecules, drug compounds, and NaCl micro-particles was firstly placed on top of a negative mold made of polydimethylsiloxane (PDMS), and subsequently heated at $90\text{ }^{\circ}\text{C}$ for 10 min to make it

rubbery and pressed into the mold with a compression force of ~20 kg for 2 h. After cooling at room temperature for 30 min, PLGA-MLs were demolded. Such thermal pressing method is simple, fast and inexpensive, and amenable for automated massive production. Moreover, to realize biphasic release of one kind of drug molecules or release two different kinds of drug molecules at distinct rates, a fast-dissolving and biocompatible layer of carboxymethyl cellulose (CMC) was coated onto a PLGA-ML simply by dip-coating to produce a core-shelled ML. In fact, CMC is the most commonly used additive in foods and beverages. As revealed by optical microscopy, the fabricated MLs are cylindrical with a diameter of ~0.23 mm, length of ~4 mm (**Figure 2A and B**) and a sharp-pointed tip ~10 μm (Figure S1B). Such dimensions are optimized based on the balanced consideration of drug loading capacity, penetration ability, and invasiveness. These MLs have similar dimensions as stainless-steel needles or lancets (32 gauge) which are widely used at home for insulin injection or blood sampling and considered virtually painless.^[18]

2.2. MLs offer bi-phasic drug delivery and strong mechanical strength

As shown in Figure 2A, confocal fluorescence imaging confirms the ability of the MLs to pack different fluorescence molecules as the model drug molecules (red cy5 in inner PLGA core, green fluorescein in outer CMC shell). To examine in vitro release kinetics, MLs were inserted into agarose hydrogel as the skin mimics, and continuously monitored. Immediately after insertion, fast-dissolving outer-shell of CMC quickly released its cargo within 5 minutes (Figure 2B and S1C), while the salt-mixed PLGA inner-core (50% salt) gradually degraded, and discharged its cargo over several weeks (Figure 2B). As revealed by scanning electron microscopy (SEM) (Figure S1D - G), PLGA MLs became porous after dissolution of NaCl micro-particles, and gradually degraded over time. Using high performance liquid chromatography (HPLC), in vitro drug release kinetics was further tested by monitoring the release profile of a browning agent (CL316,243 – a β 3-adrenergic receptor agonist)

encapsulated in inner-core or outer-shell or both in PBS solution. As shown in Figure 2C, release from the shell is fast while release from the inner core has the time constant of ~12 days based on exponential curve fitting. In comparison, drug release from PLGA ML without the salt porogens exhibits a much slower release time constant of ~27 days. More specifically, ~15% was released from PLGA-only ML while ~50% was released from PLGA ML containing 50% salt in 2 weeks. At week 6 - 7, salt-mixed MLs were totally disintegrated into highly-porous microspheres (Figure S2A) which then will be degraded into its two monomers (lactic and glycolic acids) and metabolized into carbon dioxide and water via the tricarboxylic acid cycle.^[16] Taken together, core-shelled MLs provide bi-phasic release kinetics, i.e., the initial burst release followed by the sustained release over several weeks.

As ML needs to be strong enough to penetrate through skin and implant entirely into underlying sWAT, ML's mechanical strength was assessed by compression test. As shown in Figure 2D, PLGA MLs with or without 50% salt micro-particles can sustain a compressive force of ~0.9 N, without significant deformation. It means that these MLs are strong enough for skin penetration because the force required for a sharp needle (~30 μ m tip) to penetrate human skin is <0.1 N.^[19] Addition of 50% salt micro-particles only slightly compromises the mechanical strength. But salt-blended ML is more brittle because it breaks beyond the critical compression load (~1.5N) instead of elastic bending as pure PLGA MLs (Figure S3A and B). Higher salt percentage accelerates ML degradation and drug release (Figure S2B and C), however with the price of reduced mechanical strength (Figure S3C and D). For example, MLs with 75% salt are too brittle for skin penetration. Additional CMC coating does not reinforce the mechanical strength (Figure S3E), indicating that the mechanic property is dictated by the PLGA inner core. In addition, drug loading (10%) does not significantly compromise the mechanical properties of MLs (Figure S3F).

2.3. Lancing MLs into sWAT

Mechanical failure of a needle during skin penetration is often caused by lateral bending.^[20] It is reported that the presence of lateral support can largely increase the critical buckling load.^[20] For example, the lateral support of mosquito's labium (a protective sheath surrounding the fascicle) increases the critical load of fascicle (a microneedle made of chitin) by a factor of 5.^[21] Moreover, a straight fascicle can penetrate more easily. Inspired by such a strategy from nature, ML can be laterally supported by a rigid tube. On the other hand, in order to reduce the penetration duration and hence discomfort, the speed of a piercing ML should be fast. To fulfill the above two requirements, we designed a high-speed lancing-device or applicator, comprising of a spring and a shaft connected with a stainless-steel rod. The rod is fitted into a stainless-steel tube holder, into which a ML can be snugly loaded (Figure S4A). Once the compressive force of the spring is released, the rod instantly strikes the ML, lancing ML swiftly into skin. The lancing speed is ~ 1 m/s, and duration is only ~ 50 ms (Figure S4B). These values are comparable to those achieved by the lancet lancing devices used by diabetes patients at home for blood sampling.^[22] The lancing speed, thus the skin penetration depth, can be adjusted by varying the spring compression.

Using the applicator, a core-shelled ML can be easily lanced into skin-mimicking agarose gel (4%), without buckling or fracture (Figure S4C). The distance between the penetration point on the surface and the blunted end of ML is 2 - 3 mm (greater than the thickness of human skin of ~ 2 mm at abdominal area), suggesting that MLs can be embedded deep into sWAT. Notably, the embedded ML is tilted $\sim 22^\circ$, because of the resistive force from the tissue acted on the bevelled tip of the ML (Figure S4C - F). This is consistent with the previous finding that the insertion trajectory of bevel-tipped needles is curved.^[23] Porcine skin closely resembles human skin and is the widely used skin model. Similar to human sWAT, porcine sWAT is continuously associated with dermal layer. As demonstrated in **Figure 3A**, ML can be easily lanced into sWAT (tip reaches 5 - 6 mm deep from the skin

surface). Subsequent histological study shows the narrow insertion path, and the implanted ML (Figure 3B). The insertion force for ML upon its penetration into the porcine skin is ~0.1 N (Figure S3G) as indicated by a small dip in the force-displacement curve followed by a steeper slope.^[19]

2.4. Safe, pain-less and minimally-invasive implantation of MLs as embedded drug reservoirs

Consistent with the well-known biocompatibility of PLGA and CMC polymers, the fabricated MLs are highly biocompatible to human cells, as evidenced by the well-preserved morphology and viability of human white adipocytes (Figure S5A and B). For in vivo experiments, MLs were administrated by the applicator at the inguinal area to implant MLs into the sWAT (inguinal WAT --IgWAT). IgWAT, which lies just 1 - 2 mm below the skin, is the largest sWAT in rodents. As shown in Figure 3C, a tiny mark (~0.17 mm²) was observed immediately after insertion, and completely disappeared by day 2, without leaving any scar or other abnormalities (e.g. skin discoloration, swelling, bruising, hemorrhage or infection). The mark left by ML is similar to those (~0.15 mm²) created by stainless-steel needles of 32-gauge size.^[22] During 1 week of observation period, there were no significant differences in body weight and food intake between the control and tested groups (Figure 3D and E). In addition, no sign of pain was observed from the mice administrated with ML, as assessed by the grimace scoring (Figure 3F).^[24] The pain-free penetration is attributable to the sharp tip, small diameter, good mechanical strength, and fast lancing speed of MLs. No abnormal behavior (e.g. scratching due to itching) was noted in any of the tested mice. Furthermore, as shown in Figure 3G, a ML penetrated into IgWAT and did not cause any noticeable tissue reactions (e.g. inflammatory reaction) at the implanting site. Previous studies demonstrated that even a large-sized PLGA implant (>1 cm) was highly inert in the body with no evidence of inflammatory infiltration.^[25] Similar to the degradation in the

buffer solution (Figure S2A), MLs were mostly degraded in vivo after 6 weeks (Figure S6). All these observations indicate that ML penetration and implantation is minimally-invasive and painless, without causing harmful effects on the targeted tissue and body.

In vivo release of dye molecules from MLs was also examined. As shown in Figure 3H, fluorescence signal of Cy5 encapsulated in the ML was strong at the penetration site. When Cy5 was loaded only in the shell, the fluorescence faded away within a day; when it was loaded in the core, the fluorescence remains strong even at day 7. Cy5 molecules released from either the shell or core only accumulated in the ML-embedded IgWAT, but not in the IgWAT on the other side or other organs (Figure 3I). In contrast, Cy5 molecules administrated systemically, i.e., intraperitoneal (IP) injection predominately accumulated in liver and kidney, but not in IgWAT. At day 7, only the IgWAT embedded with a ML having a Cy5-loaded core still showed strong fluorescence. These experiments demonstrate that core-shelled ML can act as a drug reservoir inside sWAT to give highly localized biphasic drug release.

2.5. Effective anti-obesity treatment enabled by ML approach

CL316,243, a β 3-adrenergic receptor agonist, was used as the browning agent in our mouse experiments because of its proven browning effect on rodent sWAT.^[12a, 26] It is worthy to mention that a US-FDA approved human β 3-adrenergic receptor agonist (mirabegron) is clinically used for overactive bladder syndrome. Therefore, repurposing it for anti-obesity will largely lower the translational hurdle because its toxicity is well characterized. We firstly tested the bioactivity of mirabegron molecules (1 μ g) released from MLs using human white adipocytes, and found that browning effects can be induced by mirabegron-laden MLs, similar to the same amount of free mirabegron molecules added in the culture medium, as evidenced by the increased expression of UCP1 protein which is a key biomarker of brown adipocytes responsible for energy consumption (Figure S5C and D). This experiment

demonstrates that ML encapsulation does not affect the drug bioactivity, and mirabegron can serve as the browning agent for human.

Anti-obesity effects of CL316,243 loaded MLs were investigated on diet-induced obese mouse model, which was induced by feeding the mice (6-week old male) with high-fat diet (60% kcal from fat). Obese mice (~30 g) were treated once every 1 or 2 weeks with CL316,243 delivered through IP injection or ML application (but with the same dosage of 1 mg/kg per week). Notably, mice with different treatments had similar food intake as the untreated ones (Figure S7A and B). After 6 weeks, mice continuously fed with high-fat diet gained ~23% of body weight (**Figure 4A - C**) and ~70% of the gain was due to the increase of white fat mass including IgWAT and epididymal WAT – EpiWAT (Figure 4D - E). Consistent with the previous studies,^[12a, 27] IP injection of CL316,243 failed to significantly suppress weight gain. Specifically, ~14% and 18% of weight gain were still observed in the groups received IP injection once a week or once two weeks (Figure 4C). Fluctuation of body weight changes was observed in the latter group presumably because doubled dosage caused weight loss in the initial a few days, and weight gain then restored quickly afterwards. The weight re-gaining is likely because drug molecules cannot retain in the fat tissue for a long time.

In comparison, treatment by core-shelled MLs with CL316,243 loaded in both core and shell virtually halted weight gain induced by high-fat diet. Specifically, only ~4% and ~6 % of weight gain was observed in once-a-week and once-2-week groups, respectively (Figure 4A - C). Consistently, white fat masses (IgWAT and EpiWAT) were reduced by ~50% and ~40% in these groups (Figure 4D and E). Such outstanding efficacy is attributable to the localized and controlled drug delivery enabled by MLs, which not only provide the initial bolus dose from its fast-releasing shell to quickly reach the therapeutic level, but also maintain therapeutic effect for a long period via its slow-releasing core. The intimate link

between obesity and type 2 diabetes is well recognized because excessive fat accumulation causes insulin resistance mainly due to decrease of insulin-stimulated glucose transport and metabolism in adiposes and skeletal muscles. Consistently, we found that glucose clearance rate in blood is much slower in obese mice compared to normal mice. As shown in Figure 4F, insulin resistance in the two ML treated groups was alleviated. Furthermore, the mice in the ML treated groups, but not untreated or IP treated groups, showed increased body surface temperature (Figure 4G and H). Both enhanced insulin sensitivity and elevated body temperature can be ascribed to the transformation of sWAT into thermogenic brown-like adipose tissue.

Supportively, histological analyses revealed abundant multilocular brown-like adipocytes in IgWATs of ML treated mice, but not in IP injection and control groups (**Figure 5A**). Brown-like adipocytes contain multiple lipid droplets and abundant mitochondria, and express thermogenic proteins including UCP1.^[26, 28] In addition, white adipocytes in IgWATs of ML treated mice became much smaller than that in untreated mice due to size reduction of the lipid droplets inside. Conceivably, this is because lipid storage in the white adipocytes was largely consumed by the nearby brown-like cells. As demonstrated in Figure 5B and C, the transcription factors that direct browning (PGC1 α and PRDM16) as well as brown-specific UCP1 were upregulated in the IgWAT of ML treated mice, further confirming CL316,243 induced adipose browning.

Comparing to normal mice, untreated obese mice expectedly had hyperglycaemia, hypercholesterolemia, hypertriglyceridemia, and hyperinsulinemia.^[29] These problems can cause various diseases including type 2 diabetes, atherosclerosis and other cardiovascular diseases.^[1] Intriguingly, ML treated mice showed a significant reduction in the serum levels of cholesterol, triglycerides, and insulin compared to the untreated group (Figure 5D - G).

Taken together, ML treatment can suppress the development of obesity and associated metabolic diseases by inducing browning of sWAT through localized and controlled delivery of browning agent. In addition, we found that fat browning and reduction effects from biphasic delivery of CL316,243 (in both shell and core) are much better than those from fast releasing of CL316,243 (same amount but in shell only). Specifically, ~14% weight gain was still observed in the group received with the latter treatment (Figure S8A). Consistently, larger fat mass (Figure S8B) and lesser brown-like adipocytes (Figure S8C) were observed in this group than in the group treated with biphasic delivery of CL316,243. This is because the drug molecules released from ML shell can only dwell within fat tissue for ~1 day (Figure 3H and I). On the other hand, the same amount of CL316,243 encapsulated in the core for slow release only was also inferior to the biphasic drug administration (~13% weight gain, larger fat mass, and lesser brown-like cells) (Figure S8A - C), conceivably because without the initial burst release, the therapeutic level for browning is not reached. As fat transformation is a reversible process, a sufficient therapeutic level for browning is essential to rectify the whitening effect induced by high-fat diet.^[30] Consistently, we found that metabolic and insulin sensitizing effects from biphasic delivery of CL316,243 are much better than those from fast only or slow only releasing of CL316,243 (same amount in shell only or core only) (Figure S8D-H). These experiments testify the advantages of biphasic release.

2.6. Combination therapy for obesity and associated metabolic disorders

Combination therapy that involves co-delivery of several drugs for synergistic or multiple therapeutic effects are highly desired. To simultaneously tackle obesity and the resulting insulin resistance, core and shell of MLs are differentially loaded with adipose browning agent (CL316,243) and insulin-sensitizing drug (rosiglitazone which is an agonist of the peroxisome proliferator-activated receptor- γ , PPAR γ). Rosiglitazone not only is a US-FDA approved anti-diabetic drug but also is recognized as a browning agent by some studies. As

shown in **Figure 6A**, mice treated once every 2 weeks for 6 weeks, with MLs loaded with 40 μ g CL316,243 in inner-core and 20 μ g rosiglitazone in outer-shell (equivalent to the combined dosage of 1 mg/kg per week) significantly reduced weight gain induced by high-fat diet (only ~8% weight gain vs. ~24% in control group). Subcutaneous injection of both CL316,243 and rosiglitazone with the same dosage and frequency failed to exert obvious weight control effect (~20% weight gain). Interestingly, the ML treatment with CL316,243 in the shell and rosiglitazone in the core failed to control the weight gain (~19% gain, Figure 6A). The much better treatment offered by the burst release of rosiglitazone followed by sustained release of CL316,243 than the otherwise may be explained as follows. Hydrophilic CL316,243 acts on the cell surface via binding with β 3-adrenergic receptor and it is vulnerable to fast tissue clearance. In contrast, rosiglitazone which has a polar head and a hydrophobic tail can readily enter the cell and act intracellularly via binding with the nuclear PPAR γ receptor. In addition, rosiglitazone promotes angiogenesis that supports the browning process.^[31]

Consistently, as compared with other treatments (subcutaneous injection of rosiglitazone and CL316,243, or MLs with rosiglitazone in the core and CL316,243 in the shell), MLs with CL316,243 in the core and rosiglitazone in the shell performed the best in reducing adipose masses (IgWAT and EpiWAT) (Figure 6B and C), increasing WAT browning as evidenced by increased multilocular brown-like adipocytes in IgWAT (Figure 6D), and enhancing energy expenditure as evidenced by increased body surface temperature (Figure S7C), and decreasing serum levels of cholesterol and triglycerides (Figure 6E and F). Although the browning effects from such combination therapy was comparable to the treatment by ML with CL316,243 in both core and shell, it enabled faster glucose clearance rate (Figure 6I and J) and more reduction of serum insulin level (~4 ng/ml vs. ~6 ng/ml) (Figure 5G and 6H). Furthermore, the adipose insulin resistance index (IR = insulin x FFA) was lowest in the mice

received the combination therapy (Figure S7D and E). Evidently, the combination therapy not only exerts significant anti-obesity effects via inducing browning but also enhances the insulin sensitivity whereby reducing the risks to develop obesity associated metabolic diseases.

3. Conclusion

Skin, which is the largest organ, contains a vast network of capillaries and lymphatic vessels and has various tissues lying underneath including subcutaneous fat (sWAT), muscles, tendons, ligaments, and fibrous joint capsules. As the outer covering of the body, it offers a conveniently accessible interface for drug administration. Transdermal drug delivery can bypass gastrointestinal tract absorption and hepatic first pass effect, and be locally applied at the site just above the targeted or suitable tissues.^[13b, 32] Here, we invented a new method, that is, lancing polymeric micro-lances (MLs) through the skin for total implantation inside the underlying tissue to give localized and controlled drug release.

This work demonstrates the use of such paradigm-shifting approach for combating obesity and associated metabolic diseases via inducing adipose browning and increasing insulin sensitivity. Using an obese mouse model, the outstanding therapeutic effects are evidenced by halted weight gain under high-fat diet, reduced fat masses, appearance of multilocular brown-like adipocytes, upregulated expression of brown-specific proteins including thermogenic UCP1, elevated body surface temperature, increased glucose clearance rate, as well as decreased serum levels of cholesterol, triglycerides and insulin. This ML approach significantly outperforms the conventional systemic administration methods (IP or subcutaneous injection) because of high drug bioavailability owing to localized and direct delivery, as well as biphasic drug release kinetics and combination therapy enabled by core-shelled MLs. It is also superior to our previously reported microneedle-based transdermal delivery approach in terms of much less frequent treatment (here only once every 2 weeks)

and less weight gain using the same weekly dosage of CL316,243. This is attributable to the total implantation of ML inside sWAT and more desirable drug release profiles.

The human clinical studies of both β 3-adrenergic receptor agonists (CL316,243 and mirabegron) using conventional systemic delivery were disappointing. Specifically, chronic and high-dose oral intake of CL316,243 (1500 mg/day, 8 weeks) only enhanced insulin action and fatty acid oxidation, but without any effects on body weight, fat mass and metabolic activity.^[33] Mirabegron is more potent than CL316,243. Although, oral intake of mirabegron (50 mg/day, 10 weeks) increased the expression of several brown-specific proteins, no changes on body weight and fat mass were observed.^[34] A study has shown that only ~30% of orally intaken mirabegron reaches circulation with a plasma half-life of only ~40 h.^[35] A very small percentage of the circulating mirabegron actually goes to adipose tissue while the majority is absorbed by other tissues. High-dose oral intake of mirabegron (e.g. 200 mg/day) causes adverse cardiovascular problems, such as increasing heart rate and blood pressure.^[36] Our ML approach may make mirabegron as well as other browning agents clinically feasible by reducing the effective dosage because of high drug bioavailability and optimal release kinetics. Abdominal and gluteofemoral areas are suitable sites for ML application because they are the largest sWAT depots in human and the least pain sensitive compared to other body parts.^[37]

Lancing ML into these fat depots should be more patient friendly than lancing the stainless-steel lancets into the most pain-sensitive fingertips for blood sampling. For the latter, a study reported that >90% patients considered painless.^[18] In addition to frequent blood sampling, diabetes patients also need to do insulin injection using an insulin needle (e.g. 33G x 4 mm) several times a day. Owing to its sustainable releasing property, ML treatment can be much less frequent (e.g. once every 2 weeks). Using a simple applicator, ML can be easily self-administrated. ML wholly implanted in sWAT can then serve as micro-

drug-reservoir. In comparison to microneedle-based transdermal drug delivery, total implantation of ML in sWAT ensures a higher loading capacity, 100% bioavailability in the subcutaneous tissues, precise drug dosage, and minimized skin reactions. In addition, implantation depth can be controlled by simply adjusting the applicator's spring force or injection angle into the skin. Powder or liquid jet injection has been demonstrated for transdermal delivery.^[38] But for these methods, ~10% of sprayed drugs are loss at the skin surface and the majority of the remaining are dispersed in epidermis and dermis, which are easily adsorbed into circulation before reaching subcutaneous tissues (e.g., sWAT). In addition, as the velocity of jet stream requires at least 100 m/s in order to penetrate the skin, such method not only needs a sophisticated high-pressure jet injector, but also often causes pain and bruising due to the high impact.^[39] Having a unique lance-shaped structure with a sharp tip ~10 μm , our ML can be easily and swiftly lanced into sWAT using a simple applicator with the lancing speed of ~1 m/s, without causing skin injury and pain, and losing drug on skin surface or into circulation. And the applicator can be further devised to simultaneously lance an array of MLs. MLs may also be designed using different polymers. For example, shell can be made of crosslinked hyaluronic acids (Figure S9) which gives a prolonged release (~3 days)^[40] as compared to CMC. In addition, other drug combinations and loading of multiple drugs in shell or core or both can be explored.

ML approach promises for other diseases as well, for examples, implanting anti-diabetic agents (e.g. sulfonylureas- glimepiride) for diabetes, synthetic hormonal agents (e.g. levonorgestrel) for contraception and hormone replacement therapy, anti-psychotic agents (e.g. risperidone) for schizophrenia and other mental disorders, anticancer drugs to tumor tissues (e.g., tamoxifen for breast cancer). It may also be useful for intraocular drug delivery by bypassing anatomical barriers (e.g. cornea, sclera). For example, MLs can be implanted into sclera for sustained delivery of corticosteroids for uveitis or β -adrenergic receptor

blockers for glaucoma. In summary, such safe, painless and convenient transdermal delivery method may provide unprecedented long-term home-based solution for obesity and many other diseases.

4. Experimental Section

Materials

Poly (D, L-lactide-co-glycolide) (PLGA, 50:50 ratio, Mw 54,000-69,000), carboxymethylcellulose (CMC), poloxamer 188 (Pluronic F68), sodium chloride (NaCl), polydimethylsiloxane (PDMS, SYLGARD 184) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich. CL316,243 (a selective β 3-adrenergic receptor agonist), rosiglitazone (a selective PPAR γ agonist) and insulin (human recombinant) were obtained from Tocris Bioscience. Cyanine5 (Cy5) and fluorescein were acquired from Lumiprobe. DMEM/F12, fetal bovine serum (FBS), bovine serum albumin (BSA), phosphate buffered saline (PBS), penicillin-streptomycin, trypsin-EDTA and AlamarBlue cell viability reagent were obtained from ThermoFisher Scientific.

Fabrication of polymeric micro-lances

Micro-lances (MLs) were prepared via a simple thermal pressing method. Firstly, NaCl micro-particles were obtained by grinding the powder and filtering through a fine mesh with a pore size of $\sim 50\ \mu\text{m}$. The mixture of PLGA and salt micro-particles was then prepared using the solvent casting method. Briefly, PLGA was dissolved in acetone before salt micro-particles were added. After thorough mixing and complete evaporation of the solvent at room temperature, a homogeneous solid mixture was obtained. Rosiglitazone or fluorescent dyes were added directly into the salt-polymer solution. For hydrophilic molecules like CL316,243 and mirabegron, the deionized water solution of the drug was thoroughly mixed with acetone solution of PLGA. Then salt micro-particles were added, following by complete evaporation of solvent and water. The solid mixture was then placed on top of the PDMS mold, which is

the reverse replica of a home-made stainless-steel master-mold having a vertical array of 32G ultrafine needle (diameter of 0.23 mm and height of 4 mm), before heating at ~90 °C for 10 minutes and pressing with a compression force of ~20 kg for 2 h. PDMS mold, filled with drug-salt-PLGA mixture, was then heated for another 15 min without pressing to allow the PDMS mold to restore to its original shape. After cooling at room temperature for 30 min, polymeric MLs were individually taken out using forceps. Subsequently, MLs were gradually dipped into a 20 μ l DI water solution containing 0.6 mg CMC and 0.2 mg Pluronic F68 and dried in air for 15 min to form CMC-PLGA core-shelled MLs. The coating process was repeated for 3 times.

In vitro characterization

The morphology of MLs was examined using a field-emission scanning electron microscope (FESEM; JSM-6700, JEOL) and a digital microscope (Leica DVM6). MLs loaded with different fluorescence dyes were visualized with a confocal laser scanning microscope (LSM800, Carl Zeiss). Drug loading amount in ML was determined using a UV-Vis spectroscopy (Shimadzu UV-1800). The mechanical property of MLs was tested using an Instron 5543 Tensile Tester. Briefly, a vertical force was applied onto a ML using a flat-headed stainless-steel cylindrical probe at a constant speed of 0.5 mm/min and the force exerted on the MN was continuously recorded.

To examine in vitro biocompatibility of MLs, white adipocytes differentiated from the primary human pre-adipose cells (Zen-Bio Inc.) were exposed to MLs for 2 weeks, before analyzing cell morphology using an inverted microscope (IX71, Olympus, equipped with a digital camera OlympusE330) and cytotoxicity using an almarBlue cell viability assay. To evaluate in vitro bioactivity of drug molecules loaded in MLs, these adipocytes were treated with free CL316,243 or CL316,243 released from MLs (1 μ g/ml) for 7 days. Cells were then

lysed with M-PER Mammalian Protein Extraction Reagent (containing Halt protease and phosphatase inhibitor cocktail, ThermoFisher Scientific).

To evaluate the release profiles, MLs differently loaded with CL316,243 or Cy5 molecules were immersed in the phosphate buffer solution (PBS, pH 7.4), and placed in an incubator shaker (100 rpm, 37 °C). Fluorescent molecules released from MLs were determined using a fluorescence spectrometer (SpectraMax M5, Molecular Devices). CL316,243 molecules released from MLs were measured by a gradient reverse-phase high performance liquid chromatography (Agilent 1100 HPLC-DAD system) using an Agilent Poroshell 120 EC-C18 column (with a mobile phase of water and methanol, a flow rate of 0.5 mL/min and UV detection at 285 nm) and quantified based on the linear calibration curve created with different known concentrations.

Animal experiments

All the animal experiments were approved by Institutional Animal Care and Use Committee of Nanyang Technological University under protocol ARF-A18029. The mice (C57BL/6J) were housed in light and temperature-controlled facility (12-h light/12-h dark cycle, 22 °C), and allowed free access of water and standard or high-fat diet. After shaving the hairs around the inguinal region, a ML was applied using the applicator. Low-dose inhaled isoflurane was used to constrain the mice during ML application. Mice were then returned to their cages and imaged immediately to access the penetration sites. After ~5 min mice were recovered from anaesthesia and their behaviours were monitored to access their possible pain using Grimace scoring based on orbital tightening, nose bulge, check bulge, and ear position. The in vivo fluorescence from Cy5 molecules released from MLs was imaged using an in vivo imaging system (IVIS Spectrum, Perkin Elmer).

Mice (6-7-week old male) were fed on high-fat diet (60% kcal from fat, Testdiet) for 3 weeks to induce obesity and randomly divided into 5 groups for different treatments. Each

ML contains 10 µg CL316,243 in the core and 5 µg CL316,243 in the shell. One ML was applied at each left and right inguinal region once every 1 week, or 2 MLs at each region once every 2 weeks (both equivalent to 1 mg/kg/week).

Four to five mice were used in each treatment or control group, and mice were fed with high-fat diet throughout the experiments. After 5 weeks treatment, the glucose tolerance test was performed as described previously ^[41]. Briefly, the overnight-fasted mice (16 h) were injected with glucose solution (2 g per kg in PBS) via IP route, and blood glucose was monitored over time. After 6 weeks treatment, body surface temperature was monitored on the shaved skin under fully awake condition, using infrared thermal imaging camera (FLIR T420). The mice were then euthanized by a lethal dose of carbon dioxide. After measuring the body weights, fat tissues (inguinal WAT, epididymal WAT and interscapular BAT) were excised, weighted and collected for histological analyses. Some portions of IgWATs were homogenized in radioimmunoprecipitation assay buffer (containing protease inhibitor cocktail, Roche Applied Science) for immunoblot analyses.

Histological analyses

The excised tissues were immediately fixed with 4% paraformaldehyde solution for 24 h, washed with PBS, and immersed in 30% sucrose solution for 2 days to cryoprotect the tissues. After embedding in FSC22 Frozen Section Media (Leica Microsystem), tissues were frozen and sliced (10 - 20 µm thick) using a cryostat (CM1950 cryostat, Leica Microsystems). The tissue sections were finally stained with hematoxylin and eosin solutions (Sigma-Aldrich), and images were taken using a digital microscope (Leica DVM6).

Immunoblot analyses

Cell or tissue samples with equal amount of proteins (as the loading control) were separated on 12% SDS-PAGE before being transferred onto a nitrocellulose membrane. The membrane was then blocked with Superblock blocking buffer (ThermoFisher Scientific) (2 h,

at room temperature) and incubated with specific primary antibody (1:200-400 dilutions, 12 h at room temperature), before washing with Tris-buffered saline-Tween solution (TBST, 3 x 15 min) and incubation with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich) (1: 2000 – 4000; 6 h, at room temperature). 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 UCP1 (sc-6529), peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α , sc-13067), adipocyte protein-2 (aP2, sc-18661) (fatty acid binding protein 4), and actin (sc-1616) were purchased from Santa Cruz Biotechnology. Antibodies against UCP1 (PA1-24894) and PRDM16 (PA5-20872) were also obtained from ThermoFisher Scientific.

Assessment on serological parameters

Serum levels of cholesterol, triglycerides, free fatty acids, and glucose were measured by the standard assay kits from Sigma-Aldrich. Serum level of insulin was determined by a mouse insulin ELISA kit from ThermoFisher Scientific.

Statistical analyses

The data points were presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Turkey's post-hoc test. Unless otherwise stated, all experiments were performed using at least four different samples per group, and the data presented are representative of two to three independent experiments. A p value of < 0.05 was considered 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 illustration. Fabrication and application of polymeric core-shelled micro-lances.

Figure 2. Characterization of MLs. **(A)** Bright field and confocal images of salt-mixed PLGA-MLs (50% NaCl) without CMC coating, or MLs coated with CMC (core-shelled ML). The ML is loaded with red Cy5 dye (top), and the CMC shell is loaded with green FITC dye (bottom). Scale bar = 250 μ m. **(B)** Confocal images of real-time releases of Cy5 and FITC from core-shelled ML in 4% agarose hydrogel. Scale bars = 250 μ m. **(C)** In vitro release profiles (PBS, 37 °C; n = 4). From top to bottom: CL316,243 in the shell of core-shelled ML, in both core and shell of core-shelled ML, in the core of core-shelled ML, in salt-free PLGA-ML. **(D)** Mechanical compression test of MLs (n = 4). A schematic illustration of compression test is shown in inset. Each data point represents the mean value and each error bar indicates standard deviation (mean $\pm$ SD). n represents the number of experiments.

Figure 3. In vitro and in vivo studies of core-shelled MLs. **(A)** Bright field image of porcine tissue (skin epidermis, Epi; dermis, D and subcutaneous white adipose tissue, sWAT) after lancing ML. Scale bar = 2 mm. **(B)** Representative histological section of porcine tissue showing ML insertion track (arrow) and ML implanted inside sWAT. Scale bar = 250 μ m. Note that ML appears transparent because of the thin slice. **(C)** Bright field images of mouse skin, immediately (day 0) or 1 day (day 1) or 2 day (day 2) after ML application. Scale bars = 1 mm. **(D - F)** Body weight (D), food intake (E), and pain assessment (F) were examined at day 0, day 1, day 4 or day 7. Data represents mean $\pm$ SD (n = 4). **(G)** Representative histological sections of mouse tissue showing ML penetration through the skin (left) and implantation inside sWAT (right). Scale bars = 250 μ m. **(H)** In vivo imaging of the mice, at day 0, day 1 or day 7. **(I)** In vivo distribution of Cy5 delivered through intraperitoneal (IP) injection or ML application. Note that fluorescence intensity is highly concentrated at the ML

implantation site. The representative fluorescence images of dissected organs, at day 0 (2 h post-treatment) or day 7, are shown.

Figure 4. Effective anti-obesity treatment. Diet-induced obese mice were treated with MLs without drug (control), or with CL316,243 (CL, 1 mg/kg/week) via IP injection or ML application (CL316,243 loaded in both core and shell), once every 1 week (1 wk) or 2 weeks (2 wk) for 6 weeks. **(A)** Representative images of differently treated mice at week 6. **(B and C)** Relative body weight increases at week 6 (B) and over the course of treatment (C). Each data point represents mean $\pm$ SD from four to five mice in each group. **(D and E)** The statistical analyses (mean $\pm$ SD, $n = 4$) and representative images of IgWAT, EpiWAT and interscapular BAT. **(F)** Intraperitoneal glucose tolerance test at week 5 (mean $\pm$ SD, $n = 4$). **(G)** Infrared thermographic images of the representative mice at week 6. **(H)** The average body surface temperature of differently treated obese mice and non-obese mice (normal diet; dashed rectangle), measured from the indicated the areas (mean $\pm$ SD, $n = 4$). Statistical comparison among groups was performed using one-way ANOVA. * $p < 0.05$, ** $p < 0.01$ vs. control (no treatment).

Figure 5. ML approach improves browning and metabolic effects of CL316,243 in obese mice. Diet-induced obese mice were treated with ML without drug (control), or with CL316,243 (CL, ~1 mg/kg/week) via IP injection or core-shelled ML application (loaded in both core and shell), once every 1 or 2 weeks for 6 weeks. **(A)** Representative hematoxylin and eosin staining images of IgWATs isolated from differently treated mice. Asterisks * indicate white adipocytes while the circle indicates a brown-like adipocyte. Scale bar = 50 μ m. **(B and C)** Representative immunoblots of UCP1, PRDM16, PGC1 α , aP2 and actin in IgWAT, and the respective statistics (mean $\pm$ SD, $n = 4$; normalized to actin density). **(D - G)** Quantification of serum levels of cholesterol, triglyceride, free fatty acids and insulin (mean

$\pm$ SD, $n = 4$). Statistical comparison between groups was performed using one-way ANOVA.

* $p < 0.05$ vs. control.

Figure 6. Combination therapy. Diet-induced obese mice were treated ML without drug (control), or with CL316,243 and rosiglitazone (combined dosage of 1 mg/kg/week) via subcutaneous (SC) injection or ML application, once every 2 weeks for 6 weeks. MLs loaded with rosiglitazone in core (Ro-c) and CL316,243 in shell (CL-s) or MLs loaded with rosiglitazone in shell (Ro-s) and CL316,243 in core (CL-c) were used in the experiments. **(A)** Relative body weight increases of differently treated mice. Data represents mean $\pm$ SD ($n=4$ or 5). **(B and C)** Statistical analyses (mean $\pm$ SD, $n = 4$) and representative images of IgWAT, EpiWAT and interscapular BAT. **(D)** Representative hematoxylin and eosin staining images of IgWATs. Scale bar = 50 μ m. **(E - H)** Quantification of serum levels of cholesterol, triglyceride, free fatty acids and insulin (mean $\pm$ SD, $n = 4$). **(I and J)** Intraperitoneal glucose tolerance test at week5 (mean $\pm$ SD, $n = 4$). Glucose levels from non-obese mice (normal diet) and mice treated with ML (CL-c+CL-s) (CL316,243 in both core and shell) are used for comparison. Statistical comparison between groups was performed using one-way ANOVA.

* $p < 0.05$ vs. control.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

Lancing drug-reservoirs into subcutaneous fat to combat obesity and associated metabolic diseases

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Figure S1. Characterization of core-shelled MLs. (A - B) Bright field images of salt micro-particles (A), and salt-mixed PLGA-ML (50% NaCl) (B). Scale bars: 50 μm . (C) The average fluorescence intensity changes ($n = 3$) of the real-time release of FITC from ML's shell in 4% agarose hydrogel. (D - G) SEM images of salt-mixed PLGA MLs (50% NaCl) changes over time in PBS at 37 $^{\circ}\text{C}$. Scale bars = 10 μm .

Figure S2. In vitro degradation and release profiles of salt-mixed PLGA- MLs. (A) Bright field images of salt-free PLGA-ML and salt-mixed PLGA-MLs (50% salt) after 50 days of incubation in PBS at 37 °C. Scale bars = 250 μ m. (B) In vitro release profiles of Cy5 molecules from different salt-mixed PLGA-MLs (0%, 10%, 20% and 50% salt; PBS at 37 °C). Data are expressed as fluorescence intensity in arbitrary unit (a.u.) and represent the mean and standard deviation ($n = 4$). (C) Bright field images of different salt-mixed PLGA-MLs incubated in PBS at 37 °C, at day 0, day 5, day 15 and day 30.

Figure S3. Mechanical properties of polymeric MLs. (A) Representative force-displacement curves of salt-free PLGA- ML and salt-mixed PLGA-ML (50% salt). Failure load is defined as the maximum load ML can sustain without buckling or breaking (fracture). Young's modulus of salt-free PLGA- ML and salt-mixed PLGA-ML (50% salt) are ~1.4 and ~1.7 GPa, respectively. (B) Bright field images of MLs after pressing with their respective failure loads (~1.5N), showing a typical buckling of salt-free PLGA- ML and breakage of salt-mixed PLGA-ML (50% salt). (C and E) Mechanical compression tests of different salt-mixed PLGA-MLs (0%, 10%, 50% and 75% salt), without or with carboxymethyl cellulose (CMC) coating layer (D and F) The statistics of the failure loads of different salt-mixed PLGA-MLs (D) and MLs loaded with different % of CL316,243 (E). (mean $\pm$ SD; $n = 4$). (G) The representative insertion force of salt-mixed PLGA-ML (50% salt) into porcine skin. The insertion force for ML upon its penetration into the porcine skin is ~0.1 N as indicated by a small dip in the force-displacement curve followed by a steeper slope.

Figure S4. ML lancing application using a home-made lancing applicator. (A) Prototype image of a home-made lancing applicator, comprising a stainless-steel spring house within a chamber, and a shaft connected with a stainless-steel rod which is fitted into a tube holder. The spring loading force is ~ 1.5 kgf. (B) Time-lapse images of a salt-mixed PLGA-ML (50% salt) lancing from the applicator, recorded with 240 fps. The lancing speed is determined to be ~ 1 m/s (C) Bright field image salt-mixed PLGA-MLs (50% salt) embedded within 4% agarose hydrogel, showing a typical ML's trajectory path and insertion depth. The trajectory angle is $\sim 22^\circ$ deviated from the insertion line (white dash line), and the insertion depth is 2 - 3 mm between the gel surface and the blunted end of ML. Scale bar = 2 mm. (D) Bright field image of a salt-mixed PLGA-ML, showing its bevel angle (ϕ) of $\sim 75^\circ$. Scale bar = 250 μ m. (E and F) Illustrative diagrams of the proposed ML's trajectory mechanism: The asymmetry of the resistive forces from the agarose gel acting on ML's tip causes a moment on ML's tip, causing the whole ML to bend as it is advanced into the agarose gel. The net resultant force (F) experienced by ML is proportional to the sine of the bevel angle ($\sin\phi$).

Figure S5. In vitro studies of core-shelled MLs. (A - B) In vitro biocompatibility of CMC-coated salt-mixed PLGA-MLs (50% salt) tested on human white adipocytes. Briefly, human pre-adipose cells (Zen-Bio Inc., USA; SP-F-2) collected from subcutaneous WAT of non-diabetic human subjects (BMI 25 - 29.9) were grown till confluence in DMEM/ F-12 containing 10% FBS. The cells (defined as day 0) were then treated for 7 days with serum-free differentiation medium (DMEM/F-12 containing 0.5 μ g/ml insulin, 0.5 mM IBMX, 100 nM dexamethasone, 100 nM rosiglitazone, 33 μ M biotin, 17 μ M pantothenate, 10 μ g/ml transferrin and 1 nM triiodothyronine). The cells were then cultured for another 7 days with serum-free growth medium (DMEM/F-12 containing insulin and dexamethasone). At day 14, white adipocytes cultured in 96 wells were exposed to ML for 2 weeks. Representative bright field images of adipocytes with lipid droplets are shown in (A). Oil Red O staining confirmed the formation of lipid droplets in white adipocytes (inset). (B) The statistics of cell viability (alamarBlue assay) (%control; mean $\pm$ SD, $n = 5$). (C - D) In vitro bioactivity of mirabegron released from salt-mixed PLGA-MLs. Drug released from MLs or freshly prepared drug (~ 1 μ M, 1 μ g/2.5 ml) was used to treat white adipocytes for ~ 7 days before conducting the immunoblot experiment. The representative immunoblot images of uncoupling protein 1 (UCP1) and actin, and the statistics (mean $\pm$ SD, $n = 3$; normalized to actin density) are shown. Student's t-test: *** $p < 0.001$ vs. no treatment.

Figure S6. In vivo degradation of core-shelled MLs in mice. Bright field images, and their corresponding histological and fluorescence images of mouse IgWATs collected after 1 week (A - C) or 6 weeks (D – F) post-application of Cy5-loaded salt-mixed PLGA-MLs (50% salt). Scale bars = 250 μ m.

Figure S7. In vivo study of core-shelled MLs. (A and B) The average daily food intake of -- (A) obese mice treated without (Control) or with CL316,243 (1 mg/kg/week) via IP injection or ML application (CL316,243 loaded in both core and shell), once every 1 week (1 wk) or 2 weeks (2 wk) for 6 weeks, or (B) obese mice treated without (Control) or with CL316,243 +rosiglitazone (combined dosage of 1 mg/kg/week) via subcutaneous (SC) injection or ML application, once every 2 weeks for 6 weeks. MLs differently loaded with rosiglitazone in core/shell (Ro-c/s) and/or CL316,243 in core/shell (CL-c/s) were used in (B) (n = 4) (C) The average body surface temperatures (mean $\pm$ SD, n = 4). (D and E) The adipose insulin resistance index-- the fasting serum insulin - fatty acids product (IR = insulin x FFA) (mean $\pm$ SD, n = 4). Statistical comparison among groups was performed using one-way ANOVA. * p < 0.05, ** p < 0.01 vs. Control (no treatment).

Figure S8. In vivo study of core-shelled MLs. Diet-induced obese mice were treated without (Control) or with CL316,243 (1mg/kg per week) via ML application once every 2 weeks for 6 weeks. CL316,243 were loaded in either ML's core or shell (CL-c or CL-s) (A) Relative body weight increases over the course of treatment (means $\pm$ SD, $n = 4$). (B) The statistical analyses (mean $\pm$ SD, $n = 4$) of IgWAT, EpiWAT and interscapular BAT (relative to the respective body weight). (C) Representative hematoxylin and eosin staining images of IgWATs. Scale bar = 50 μ m. (D - G) Quantification of serum levels of cholesterol, triglyceride, free fatty acids and insulin (mean $\pm$ SD, $n = 4$). (H) Intraperitoneal glucose tolerance test at week5. Data shows mean $\pm$ SD ($n = 4$). Statistical comparison among groups was performed using one-way ANOVA. * $p < 0.05$, ** $p < 0.01$ vs. Control (no treatment).

Figure S9. ML coated with crosslinked methacrylated-hyaluronic acids (MeHA).

Confocal images of real-time releases of Cy5 (red) and FITC (green) from salt-mixed PLGA-ML and crosslinked-MeHA shell, respectively (4% agarose hydrogel). Scale bars = 250 μm . MeHA was synthesized by functionalizing HA (~300 kDa) with methacrylic anhydride. After dip-coating with MeHA solution (50 mg/ml MeHA together with 0.5 mg/ml Irgacure 2959 in deionized water) and drying, the core-shell ML was exposed to low-intensity ultraviolet light (5 min) to crosslink MeHA.