

Fabrication and Preclinical Evaluation of Hyaluronic Acid/Aminoclay Nanocomposite Microneedles for Noninvasive Delivery of Semaglutide in Anti-Obesity Therapy

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Introduction: Injectable formulations are common for protein-based therapeutics. However, non-injectable formulations are crucial for improving patient compliance. This study aims to develop hyaluronic acid/aminoclay-based dissolving microneedles as a noninvasive delivery system for semaglutide in the treatment of obesity.

Methods: The core nanocomplex (Sema-AC) was formed by combining semaglutide (Sema) with aminoclay (AC) via electrostatic interaction. This nanocomplex was then mixed with hyaluronic acid (HA) of varying molecular weights and poured into a reverse polydimethylsiloxane (PDMS) mold to create dissolving microneedles (MNs). Various in vitro and in vivo studies were performed to evaluate the physicochemical properties and therapeutic efficacy of the MNs.

Results: Among the developed MNs, Sema-AC/HA10 demonstrated a mechanical strength of 0.37 ± 0.020 N/needle and retained stable physicochemical and morphological properties at 25°C during storage. Encapsulated Sema retained its conformational stability within the MNs. Sema-AC/HA10 dissolved rapidly upon skin insertion, enabling efficient transdermal drug absorption. The MNs containing Sema-AC nanocomplex significantly enhanced the systemic drug exposure compared to the MN loaded with the free drug. Consequently, Sema-AC/HA10 demonstrated in vivo efficacy comparable to that of subcutaneous Sema injection in type 2 diabetic rats, significantly reducing blood glucose, HbA1c, total cholesterol, triglycerides, food intake, water consumption, and body weight.

Conclusion: These findings suggest that Sema-AC/HA10 is an effective transdermal delivery system for semaglutide.

Keywords: organic clay, nanocomplex, obesity, transdermal delivery, dissolving microneedles

Introduction

Type 2 diabetes accounts for approximately 90% of all diabetes cases worldwide and is increasing at an alarming rate, posing a significant public health concern.^{1,2} The rising prevalence of type 2 diabetes is closely associated with the global increase in obesity rates.¹ Obesity-induced insulin resistance is a major risk factor for the development and progression of type 2 diabetes. Excess adipose tissue disrupts normal metabolic processes and impairs glucose regulation.² Consequently, numerous therapeutic strategies have been explored to manage type 2 diabetes and obesity. Among these, glucagon-like peptide-1 (GLP-1) receptor agonists have attracted considerable attention for their combined benefits in improving glycemic control and promoting weight loss.^{3,4} GLP-1 exerts its beneficial effects through multiple physiological mechanisms. It stimulates glucose-dependent insulin secretion from pancreatic β -cells, suppresses inappropriate glucagon release from α -cells, delays gastric emptying, and promotes satiety by influencing appetite-regulating pathways in the central nervous system.³ These multifaceted actions make GLP-1 receptor agonists a valuable therapeutic option for managing hyperglycemia and obesity. However, the clinical utility of endogenous GLP-1 is limited by its short plasma half-life, as it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) following intravenous administration.⁵ To address this metabolic instability, GLP-1 analogues with improved pharmacokinetic properties

have been developed, including liraglutide, semaglutide, albiglutide, and dulaglutide.⁶ These analogues are resistant to degradation by DPP-4, thereby extending their duration of action and improving clinical efficacy. Among available GLP-1 analogues, semaglutide (Sema) is significant for its extended half-life of approximately 165 h, enabling convenient once-weekly dosing and improving patient compliance.⁷ Clinical trials have demonstrated that Sema achieves greater reductions in glycated hemoglobin (HbA1c) levels and induces more substantial weight loss than other GLP-1 receptor agonists.⁷ Consequently, Sema is widely used to manage type 2 diabetes and obesity, offering a comprehensive treatment approach for these interconnected metabolic conditions. In clinical practice, Sema is administered either as a subcutaneous (SC) injection or as an oral tablet.⁸ Although the oral dosage form provides an alternative to injections, its systemic bioavailability is extremely low due to enzymatic degradation and poor intestinal absorption, requiring co-formulation with absorption enhancers such as sodium N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC) under fasting conditions.^{9,10} In general, oral delivery of large biomolecules, including GLP-1 receptor agonists, is limited by metabolic instability and variable absorption.^{11,12} Transdermal formulations have been investigated as alternatives;^{13,14} however, they often encounter insufficient skin permeation and instability during production and storage.^{15,16} Likewise, nasal and pulmonary delivery allow rapid systemic entry but their effectiveness is restricted by enzymatic degradation at the mucosal surface, local irritation, and short residence times.^{17–23} Collectively, these limitations emphasize the need for more reliable non-invasive delivery systems for GLP-1 receptor agonists that can achieve adequate bioavailability, stability, and patient adherence compared with injections.

Among non-injectable formulation strategies, microneedles (MNs) have emerged as a promising technique for the efficient transdermal delivery of poorly permeable macromolecules.²⁴ After insertion into the skin, MNs mechanically penetrate the stratum corneum and deliver drugs directly into the viable epidermis and dermis, where passive diffusion across the stratum corneum would otherwise be limited.²⁵ Compared with conventional transdermal patches, MNs thereby facilitate more efficient delivery of macromolecules such as Sema and promote faster entry into the systemic circulation, leading to a more rapid onset of action. In addition, MNs bypass gastrointestinal degradation and hepatic first-pass metabolism, both of which restrict the oral delivery of peptides and proteins.²⁶ Consequently, this approach can achieve therapeutic plasma concentrations with smaller doses than oral formulations, thereby reducing overall treatment costs.²⁷ Moreover, compared with conventional injections, MNs can improve patient adherence by minimizing pain and discomfort and enabling convenient self-administration. Their high acceptability has been confirmed in clinical studies;^{28–30} for example, the previous study observed that 93% of human subjects favored a dissolving MN patch over injections because of reduced pain and self-medication.²⁸ Likewise, MN-based influenza vaccination was associated with higher satisfaction and willingness to adopt compared with hypodermic needles.^{29,30} Collectively, these results underscore the potential of MNs to enhance compliance and therapeutic outcomes in chronic diseases such as diabetes and obesity, where long-term treatment is essential. Therefore, MNs represent a compelling alternative to both subcutaneous injections and oral semaglutide formulations for the management of these conditions.

MNs can be fabricated from various materials, including silicon, stainless steel, titanium, and biocompatible polymers.^{31,32} In addition, MNs are customizable in size, shape, and functionality to meet specific therapeutic needs.^{31,32} There are several types of MNs, including solid, coated, hollow, dissolving, and hydrogel-forming MNs.³³ Among these, dissolving MNs offer distinct advantages: they eliminate sharp medical waste after administration, allow for high drug-loading capacity, and are relatively simple to manufacture.³⁴ The incorporation of biocompatible and biodegradable polymers in dissolving MNs enhances drug stability while enabling controlled and sustained drug release.³⁵ Common polymers used in dissolving MNs include poly (lactic-co-glycolic acid) (PLGA), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polycaprolactone (PCL), and hyaluronic acid (HA).^{34–38} Each material demonstrates distinct strengths and limitations. For example, PLGA enables controlled drug release but may yield acidic degradation products during hydrolysis.³⁶ PVP dissolves rapidly, although its hygroscopicity can compromise stability.³⁴ PVA provides good mechanical robustness but dissolves more slowly than PVP.³⁷ PCL ensures long-term stability but degrades at an extremely slow rate, restricting its application for rapid drug release.³⁸ On the other hand, HA is highly biocompatible, naturally abundant in skin, and dissolves rapidly, making it one of the most clinically relevant polymers for dissolving microneedle.^{37,39–41} The biocompatibility of HA minimizes the risk of skin irritation or immune responses. In addition, its inherent moisturizing and wound-healing properties promote skin recovery after MN application.³⁹ The viscoelastic characteristics of HA further enhance its utility in MN fabrication by improving mechanical strength without compromising dissolution efficiency.⁴² The molecular weight of HA is a key factor influencing the mechanical strength of MNs, which directly affects their ability to penetrate the skin and deliver drugs into the systemic

circulation.⁴³ Therefore, this study employed HAs with different molecular weights in fabricating the dissolving MNs and evaluated how these variations affect their physicochemical and pharmacokinetic properties.

Besides matrix polymers, the formation of drug-polymer nanocomplex can significantly influence the systemic drug exposure achieved through MNs. Previous studies have demonstrated that clay-based nanocomplexes enhance the cellular uptake of macromolecules by facilitating endocytosis and inducing reversible tight junction opening.^{44–46} In addition, when incorporated into polymeric MNs, clay improves needle stiffness, thereby increasing their mechanical strength.⁴⁷ Notably, dissolving MNs incorporating organic clays exhibit reinforced mechanical properties, ensuring reliable skin penetration while enabling sustained and controlled drug release.^{47,48} Among various clays, aminoclay (3-aminopropyl-functionalized magnesium phyllosilicate; AC) is a cationic organic clay that disperses readily in aqueous media and forms nanocomplexes with negatively charged proteins.^{44–46} Such interactions improve the structural stability and cellular internalization of peptide drugs. Accordingly, encapsulation of AC-based nanocomplexes within MNs can enhance the bioavailability of protein drugs.⁴⁹ Based on this rationale, the present study fabricated a nanocomplex composed of Sema and AC, which was subsequently incorporated into HA-based MNs to maximize the transdermal absorption of Sema. For comparison, MNs containing free Sema were also fabricated. The *in vitro* and *in vivo* characteristics of the developed MNs were thoroughly evaluated to identify the optimal formulation that maximizes the transdermal delivery of Sema.

Materials and Methods

Sema and Cy5-labeled bovine serum albumin (Cy5-BSA) were obtained from Hangzhou Peptide Biotechnology Co., Ltd. (Hangzhou, China) and Protein Mods (Waunakee, WI, USA), respectively. Streptozotocin (STZ), paraformaldehyde, and sucrose were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HA with molecular weights of 10 kDa (HA10) and 33 kDa (HA33) were sourced from Lifecore Biomedical, Inc. (Chaska, MN, USA) and Bloomage Biotech Co., Ltd. (Jinan, China), respectively. A polydimethylsiloxane (PDMS) mold was acquired from Micropoint Technologies Pte Ltd. (Pioneer Junction, Singapore). Pig cadaver skin and a Hematoxylin and Eosin (H&E) staining kit were purchased from Apures Co., Ltd. (Pyeongtaek, Korea) and TissuePro Technology (Gainesville, Florida, USA), respectively. Aminoclay (3-aminopropyl-functionalized magnesium phyllosilicate) was synthesized as previously described.⁵⁰ Additional chemicals were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan), and high-performance liquid chromatography (HPLC)-grade solvents, including acetonitrile and methanol, were purchased from Avantor Co. (Radnor, PA, USA).

Fabrication of Dissolving Microneedles

The nanocomplex of Sema and AC (Sema-AC) was prepared using a previously established method.⁵¹ An aqueous AC solution (10 mg/mL) was gradually added to a Sema solution (10 mg/mL in distilled water) at a clay-to-drug ratio of 2:1 (v/v), with continuous stirring at 300 rpm for 1 hour. The resulting precipitate was collected by centrifugation at $22,250 \times g$ for 15 min and subsequently washed three times with distilled water. The obtained precipitate was then dried under vacuum at room temperature, yielding a white powder form of the Sema-AC nanocomplex.

To fabricate MNs, the Sema-AC nanocomplex (10 mg/mL) was dispersed in distilled water and mixed with an aqueous HA solution (150 mg/mL) at a 4:1 volume ratio under continuous stirring at 100 rpm. The mixture was then introduced into PDMS molds using two different fabrication techniques: (1) centrifugation, in which the molds were spun at $3265 \times g$ at 4°C for 15 min, or (2) vacuum processing, where the molds were exposed to a vacuum pressure (0.8 bar) for 20 min. Following this initial loading step, the MNs were air-dried at room temperature for 12 hours. To create a backing layer, an additional HA solution (150 mg/mL) was applied to the molds, followed by another 12-h drying period at room temperature. For comparison, MNs containing free Sema (without AC complexation) were prepared using the same procedure.

Physicochemical and Morphological Characterization of Drug-Loaded Microneedles

The particle size, polydispersity index (PDI), and zeta potential of the Sema-AC nanocomplex were measured using dynamic light scattering (DLS) with a Zetasizer (Nano-ZS90, Malvern Instruments, Malvern, UK). The encapsulation efficiency (EE) of the Sema-AC nanocomplex was determined using the following formula:

$$EE (\%) = [(initial\ drug\ amount\ added - drug\ amount\ in\ supernatant) / initial\ drug\ amount\ added] \times 100.$$

The structural integrity of Sema within the MNs was assessed using circular dichroism (CD) spectroscopy (Chirascan™-Plus Spectrometer, Applied Photophysics, Surrey, UK). Spectral measurements of Sema released from the MNs in PBS buffer (pH 7.4) were recorded within a wavelength range of 200–260 nm at 25°C. The light path length and bandwidth were set to 0.5 mm and 1 nm, respectively. To evaluate the size distribution of Sema-AC within the MNs, the MNs were first dissolved in distilled water. The resulting suspension was then centrifuged at $2000 \times g$ for 20 min using Amicon Ultra-4 centrifugal filters (Millipore, Billerica, MA) to isolate the nanoparticles.⁵² The collected nanoparticles were resuspended in distilled water, and their size distribution was measured using a Zetasizer (Nano-ZS90, Malvern Instruments, Malvern, UK).

The morphology of the fabricated dissolving MNs was examined using scanning electron microscopy (SEM) (CLARA LMH, TESCAN Brno, Czech Republic). The mechanical strength of the MNs was measured using a texture analyzer (TA-XT express, Stable Micro Systems, UK). The MNs were fixed on the flat aluminum baseplate of the analyzer with their needle tips facing upward. During the analysis, the top probe advanced toward the MNs at a controlled speed of 0.05 mm/s, and the trigger force applied to initiate measurement was set to 0.049 N.

Storage Stability of the Microneedles

The fabricated MNs were stored in airtight containers with or without silica gel at 25°C and $60 \pm 5\%$ relative humidity (RH) for 7 days. Key properties including mechanical strength, weight, drug integrity, and drug release were monitored throughout the storage period and compared to those observed on day 0. Additionally, the size distribution of the Sema-AC within the MNs was assessed on Days 0 and 7 to evaluate any potential aggregation or degradation over time.

To evaluate the drug release profiles, the MNs were immersed in 5 mL of phosphate-buffered saline (PBS, pH 7.4) and incubated in a shaking water bath at 37°C, with continuous agitation at 100 rpm. At specified time interval, samples were collected and centrifuged at $22,250 \times g$ for 15 min at 4°C. The amount of released drug was quantified using HPLC, as described previously.⁵¹

In-vitro Skin Penetration Properties of the Microneedles

The surface of the pig cadaver skin was cleaned with ethanol to remove moisture prior to the insertion of the MNs, followed by a 5-min drying period. The MNs were inserted into pig cadaver skin and maintained in place for 1 min. Following the removal of the MNs, the skin was fixed in a 4% paraformaldehyde solution and stored at 4°C for 12 h. Following fixation, the sample was sequentially dehydrated in 20% and 30% sucrose solutions at 4°C. It was subsequently embedded in an optimal cutting temperature (OCT) compound and sectioned into 15 μm -thick cryosections using a cryomicrotome (Leica, Nussloch, Germany). The tissue sections were stained with H&E and examined using an Eclipse Ti-U inverted microscope (Nikon, Tokyo, Japan). The dissolution profiles of the MNs were also assessed in pig cadaver skin. Following the insertion procedure described earlier, the MNs were removed at predetermined intervals (0, 3, 5, and 15 min) for morphological analysis using scanning electron microscopy (SEM, CLARA LMH, TESCAN Brno, Czech Republic).

The in-vivo Studies Using Animal Models

Assessment of Skin Recovery After Microneedle Insertion in Rats

Skin resealing following MN insertion was assessed in male Sprague–Dawley rats (180–200g) obtained from Orient Bio Inc. (Seongnam, Korea). The study protocol was approved by the Review Committee of Dongguk University (IACUC-2023-040-1). The MNs were applied to the dorsal area of the rats for 1 min. After the removal of the MNs, the morphology of the resulting penetration sites was examined at predetermined intervals (0, 0.5, 1, and 2 h) using a digital camera.

Evaluation of Transdermal Drug Permeation in Rats

To visualize transdermal drug diffusion through dissolving MNs, Cy5-BSA was used as a fluorescent probe in place of Sema and incorporated into the MNs. Male Sprague–Dawley rats (180–200 g) were obtained from Orient Bio Inc. (Seongnam, Korea), and the study protocol was approved by the Institutional Animal Care and Use Committee (IACUC)

of Dongguk University (IACUC-2023-040-1). The rats were divided into two groups (three rats per group). One group was administered Cy5-BSA-AC loaded MNs, while the other was administered a solution of free Cy5-BSA, both applied to the dorsal skin at a dose equivalent to 0.08 mg/kg of Cy5-BSA. At the predetermined intervals (0.5, 1, and 2 h), the rats were sacrificed, and their dorsal skin was immediately excised. The excised skin samples were fixed in a 4% paraformaldehyde solution for 8 h, then dehydrated in a 30% sucrose solution at 4°C for 24 h. After dehydration, the samples were embedded in an OCT compound and cryosectioned at 15 μ m thickness using a cryomicrotome (Leica, Nussloch, Germany). The samples were examined using confocal microscopy (Nikon C1, Nikon, Tokyo, Japan).

Pharmacokinetic Studies in Rats

The pharmacokinetic properties of the MNs were evaluated in rats, following an experimental protocol approved by the Review Committee of Dongguk University (IACUC-2023-040-1). Male Sprague–Dawley rats (180–200 g) were obtained from Orient Bio Inc. (Seongnam, Korea) and assigned to three groups ($n = 6$ per group). Each group received one of the following MNs: Sema/HA33, Sema-AC/HA33, or Sema-AC/HA10 at a dose equivalent to 400 μ g/kg of Sema. Blood samples were collected from the jugular vein at specified time points. The samples were centrifuged at $16,600 \times g$ for 10 min at 4°C. The resulting plasma samples were stored at -80°C until further analysis. Plasma drug concentrations were quantified using LC-MS/MS analysis, as described previously.⁵³

Evaluation of the Therapeutic Efficacy in Type 2 Diabetic Rats

The in vivo effectiveness of the developed MNs for obesity treatment was evaluated in a rat model of type 2 diabetes. The experimental protocol was approved by the Review Committee of Dongguk University (IACUC-2023-040-1). The type 2 diabetic rat model was established following a previously described method.⁵⁴ Rats were fed a high-fat diet for 3 weeks, followed by a 6–8 h fasting period. After fasting, a streptozotocin (STZ) was administered intraperitoneally at a dose of 40 mg/kg in 50 mM citrate buffer (pH 4.5). Ten days after the STZ injection, blood glucose levels were measured using a blood glucose meter (ACCU-CHEK[®] guide). Rats weighing 450–500 g with blood glucose levels ≥ 240 mg/dL were selected for the in vivo efficacy study of the MNs.

STZ-induced type 2 diabetic rats were categorized into three groups ($n = 6$ per group), with each group receiving one of the following treatments: a subcutaneous (SC) injection of saline, an SC injection of Sema (0.4 mg/kg), or insertion of Sema-AC-loaded MNs (delivering an equivalent dose of 0.4 mg/kg Sema). Each treatment was administered once daily for 30 days. Throughout the study, various physiological parameters, including blood glucose, HbA1c, total cholesterol (TC), triglyceride (TG), food intake, water consumption, and body weight, were monitored regularly. The rats were provided with 200 ± 1 g of food and 500 ± 1 mL of normal tap water each day. After 24 h, the remaining food and water were measured. Blood samples were collected from the jugular vein, and blood glucose levels were quantified using the ACCU-CHEK[®] Guide. HbA1c levels were assessed with the A1C EZ 2.0[™] glycated hemoglobin meter (BioHermes, Wuxi, China). TC and TG levels were measured using a Barozon lipid meter (Handok, Seoul, Republic of Korea).

Pharmacokinetic and Statistical Analyses

Pharmacokinetic parameters were assessed using noncompartmental analysis. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined through visual inspection of the experimental data. The elimination rate constant (K_{el}) was determined through regression analysis using the slope of the line of best fit. The half-life ($T_{1/2}$) was subsequently calculated using the equation of $0.693/K_{\text{el}}$.

All data are presented as mean values with standard deviation (mean $\pm$ SD). One-way ANOVA, followed by Dunnett's test, was employed to assess statistical differences among treatment groups, with a p -value < 0.05 indicating statistical significance.

Results and Discussion

Fabrication of HA/AC-Based Dissolving Microneedles

Sema carries a negative charge at neutral pH owing to its isoelectric point of 5.4.⁵⁵ Consequently, the nanocomplex (Sema-AC) formed spontaneously through electrostatic interaction between negatively charged Sema and positively

charged AC. Sema-AC demonstrated an entrapment efficiency of $\geq 90\%$ and had an average particle size of 217 ± 4.78 nm. The FT-IR and CD analysis confirmed the formation of nanocomplex and the conformational stability of encapsulated Sema (Figure S1). Given that Sema-AC significantly increases the drug transport across the cell membrane,⁵¹ incorporating Sema-AC into MNs is expected to improve the transdermal delivery of Sema. Therefore, the MNs were prepared with either Sema-AC or free Sema to evaluate the advantage of Sema-AC. In addition, the preparation methods and the molecular weights of HA were varied to examine their effect on the mechanical strength of the MNs.

Figure 1A shows that all three MNs were prepared as uniform MN arrays. The MNs exhibit a pyramidal shape, characterized by a tip height of $800 \mu\text{m}$ and a base width of $200 \mu\text{m}$. The CD analysis indicated that the secondary structure of Sema remained intact throughout the manufacturing process (Figure 1B). The CD spectra of Sema released from the MNs closely matched that of the standard drug, indicating that the conformational stability of Sema was well

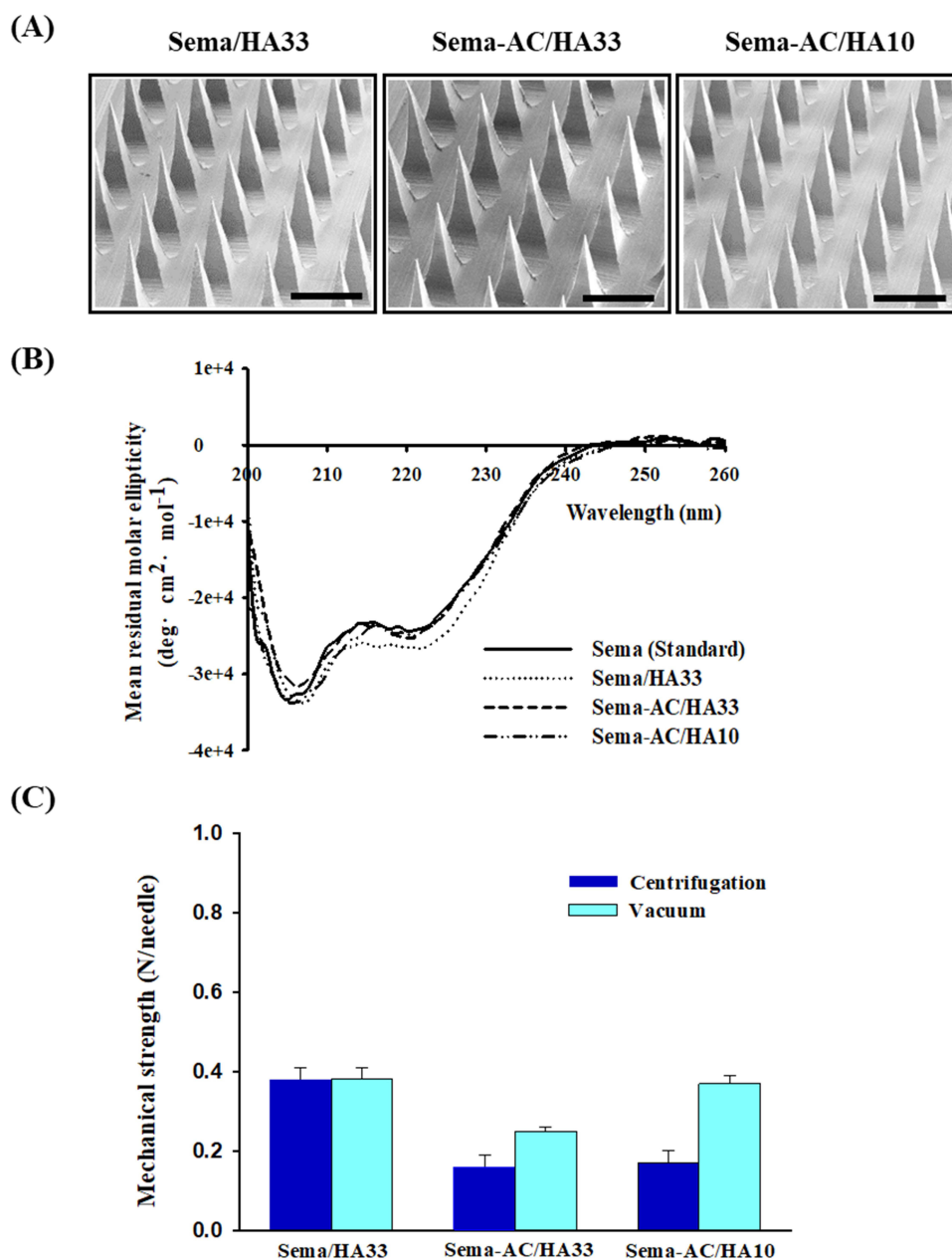


Figure 1 Physical and morphological characterization of microneedles. **(A)** SEM images of microneedles (Scale bar: $500 \mu\text{m}$), **(B)** CD spectra of semaglutide released from microneedles, and **(C)** The effect of fabrication methods on the mechanical strength of microneedles.

preserved within the MNs (Figure 1B). Effective skin penetration and drug delivery require MNs to possess a mechanical strength ≥ 0.058 N/needle.⁵⁶ Figure 1C shows that all HA-based MNs exhibited mechanical strength ranging from 0.16 to 0.38 N/needle, which is sufficient for skin penetration.⁵⁶ The mechanical strength of the MNs was influenced by three key factors: drug formulations (free drug vs nanoparticles), preparation methods (centrifugation vs vacuum), and the molecular weight of HA (10 kDa vs 33 kDa). Sema-AC-loaded MNs exhibited lower mechanical strength than MNs loaded with the free drug, with this reduction being more pronounced when centrifugation was employed during drug loading (Figure 1C). This reduction may result from nanoparticles accumulating in the lower portion of the needles during centrifugation or vacuum process, weakening the tips and decreasing the mechanical strength of the MNs. Consequently, the preparation method significantly influenced the strength of Sema-AC-loaded MNs. In contrast, MNs loaded with the free drug maintained consistent mechanical strength regardless of whether centrifugation or vacuum methods were employed (Figure 1C). The molecular weight of HA also significantly influenced the mechanical strength of the MNs. The lower molecular weight of HA achieved greater mechanical strength; the mechanical strength of Sema-AC/HA10 and Sema-AC/HA33 were 0.37 ± 0.02 N/needle and 0.25 ± 0.01 N/needle, respectively. In general, the mechanical strength of MNs tends to decrease as the molecular weight of HA increased.⁵⁷ This phenomenon is attributed to the formation of tightly packed molecular structures by low molecular weight HA during the MN solidification process, which enhances mechanical strength.⁵⁷ In contrast, the linear structure of high molecular weight HA exhibits greater conformational flexibility, resulting in increased bending and twisting during molecular packing. This inefficient molecular packing reduces mechanical strength. Chi et al⁴³ also report that MNs prepared with 10 kDa-HA demonstrated the highest mechanical strength when compared to MNs made with higher molecular weight HA (74 kDa and 290 kDa), using Rhodamine B as a model drug.

Taken together, the preparation method and molecular weight of HA significantly influenced the mechanical strength of the MNs loaded with Sema-AC nanoparticles. While the mechanical strength of all tested MNs exceeds the minimum force required for skin penetration,⁵⁶ Sema-AC/HA10 MNs, prepared using the vacuum method, demonstrated higher mechanical strength.

Pharmacokinetic Evaluation of Microneedles in Rats

The pharmacokinetic profiles of Sema were evaluated in rats after drug administration via the MNs or SC injection. The relative bioavailability values of the MNs were estimated by comparing their plasma drug exposure with those achieved by SC injection (Table 1). First, the study assessed how the AC-based nanocomplex influenced the pharmacokinetics of the MNs. As summarized in Table 1, compared to Sema/HA33 loaded with the free drug, Sema-AC/HA33 containing the Sema-AC nanocomplex exhibited significantly higher systemic drug exposure, with a 1.74-fold increase in C_{max} and a 2.74-fold increase in AUC. Although it had lower mechanical strength, Sema-AC/HA33 demonstrated superior transdermal drug absorption. This improvement can be attributed to the AC-based nanocomplex, which facilitates endocytosis and induces reversible modulation of tight junctions.^{44–46} In our previous study, Sema-AC enhanced drug permeability by approximately 3.3-fold compared with free Sema in Caco-2 cells.⁵¹ Such an increase in permeability is most likely attributable to transient loosening of epithelial tight junctions, thereby promoting paracellular drug transport. In parallel, the positively charged amine

Table 1 Pharmacokinetic Parameters of Semaglutide Following the Transdermal Administration of Semaglutide via Different Formulations in Rats (Mean $\pm$ SD, n=6)

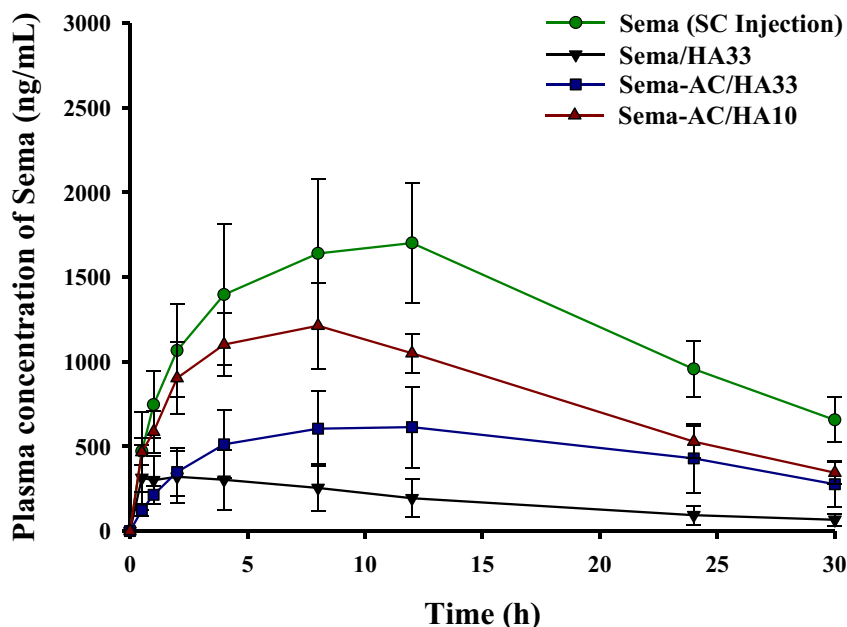
Parameters	Drug Administration			
	Sema/HA33	Sema-AC/HA33	Sema-AC/HA10	Sema (SC injection)
C_{max} (ng/mL)	383.2 $\pm$ 206.1	668.5 $\pm$ 248.8	1237 $\pm$ 238.6	1755 $\pm$ 428.9
T_{max} (h)	1.7 $\pm$ 1.2	10 $\pm$ 2.0	7.0 $\pm$ 3.2	11.3 $\pm$ 1.50
AUC (ng $\times$ h/mL)	5380 $\pm$ 2974	14,760 $\pm$ 5669	24,360 $\pm$ 2992	37,330 $\pm$ 7935
$T_{1/2}$ (h)	11.5 $\pm$ 1.50	14.1 $\pm$ 2.90	11.6 $\pm$ 2.6	13.4 $\pm$ 1.20
F (%) [*]	14.4	39.5	65.3	

Note: ^{*}Relative bioavailability of the microneedles compared to SC injection.

functionalities of Sema-AC engaged in electrostatic interactions with negatively charged membrane components, enhancing cellular association and subsequent internalization. These complementary mechanisms conferred dual enhancement of drug transport: paracellular passage via modulated tight junctions and transcellular uptake through endocytic pathways. Consequently, MNs loaded with the Sema-AC nanocomplex significantly improved the skin permeation of Sema, resulting in higher systemic drug exposure in rats (Figure 2).

The influence of the MN matrix on pharmacokinetics was also examined in rats. Sema-AC/HA10 resulted in approximately 1.65-fold higher systemic drug exposure than Sema-AC/HA33 (Table 1 and Figure 2). As previously discussed, Sema-

(A)



(B)

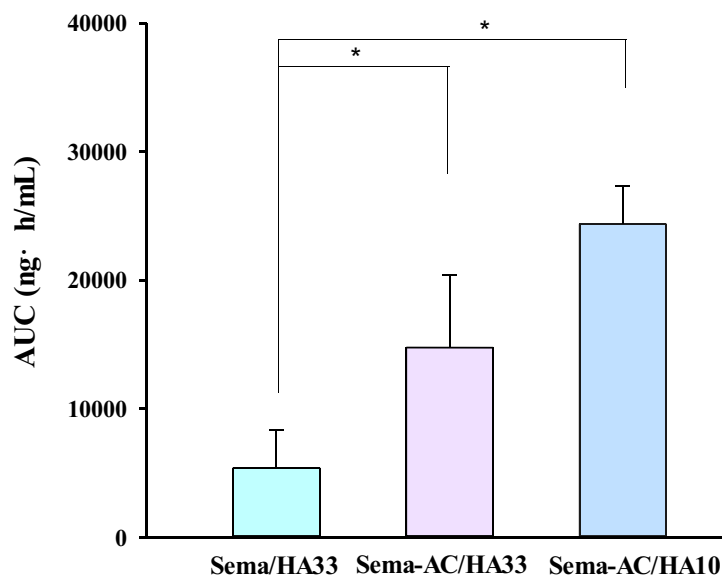


Figure 2 Pharmacokinetic profiles of semaglutide following transdermal administration via different formulations in rats (Mean $\pm$ SD, $n = 6$). The dose was equivalent to 0.4 mg/kg of semaglutide. (A) Plasma concentration-time profiles of semaglutide; (B) Comparison of AUC values achieved by different microneedles. *: $p < 0.05$.

AC/HA10 exhibited greater fracture resistance compared to Sema-AC/HA33, which likely enabled deeper skin penetration and more effective drug delivery to blood vessels in the skin layers.⁵⁷

Collectively, Sema-AC/HA10 demonstrated the highest bioavailability among the tested MNs, which might be attributed to the synergistic effects of its robust mechanical strength and the incorporation of the AC-based nanocomplex. These findings led to the selection of Sema-AC/HA10 as the optimal MN for further characterization, including assessments of skin penetration, storage stability, and in vivo efficacy.

Skin Penetration Properties and Storage Stability of Sema-AC/HA10 MNs

Pig skin is considered a suitable surrogate for human skin owing to its comparable histological and physiological characteristics despite certain inherent differences.⁴⁸ Therefore, skin penetration and subsequent dissolution of the MNs were evaluated using pig cadaver skin. As shown in Figure 3A, Sema-AC/HA10 successfully penetrated the epidermis of

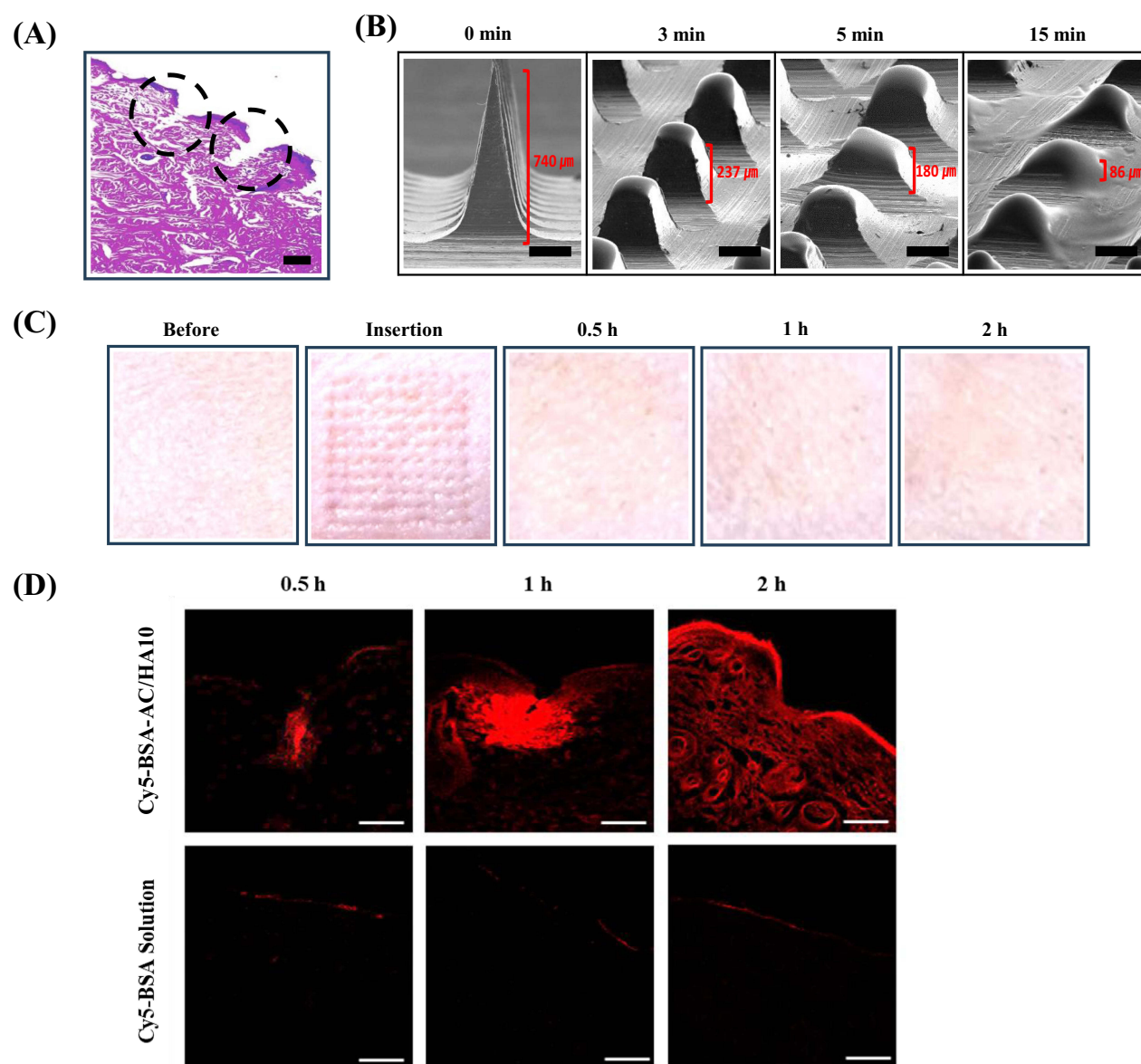


Figure 3 In-vitro and in-vivo skin penetration characteristics of microneedles. **(A)** Cross-section of H&E-stained pig cadaver skin after insertion of Sema-AC/HA10. Black circles indicate the insertion sites of microneedles. Scale bar is 200 μm . **(B)** Dissolution of Sema-AC/HA10 after application to pig cadaver skin. Red lines and numbers (740 μm , 237 μm , 180 μm , and 86 μm) indicate the length of the microneedles at each time point. Scale bar is 200 μm . **(C)** Skin recovery after Sema-AC/HA10 insertion in rats. Photographs of rat skin were obtained at different time intervals after microneedle application. **(D)** Histological cross-sections of rat skin were obtained at various time points following the application of Cy5-BSA solution or Cy5-BSA-AC/HA10 in rats. The dose was equivalent to 80 $\mu\text{g/kg}$ of Cy5-BSA. Scale bar is 200 μm .

the pig cadaver skin, reaching a depth of approximately 400 μm . The measured penetration depth was less than that of the full height of the needle tips ($\sim 800\ \mu\text{m}$). This reduced depth may be attributed to two factors: the rapid dissolution of the needle tips following skin insertion and the inherent elasticity of the skin, which resists deep penetration by the MNs.⁵⁸ As shown in **Figure 3B**, Sema-AC/HA10 dissolved rapidly upon insertion into the skin, which might facilitate immediate drug release from the MNs.

Skin recovery following MN insertion was also assessed in rats. As shown in **Figure 3C**, the micro-pores created by MN insertion sealed rapidly, with no visible openings observed as early as 2 h post-insertion. Furthermore, no signs of skin irritation or bleeding were observed. The rapid closure of micro-pores is particularly beneficial as it reduces the potential risk of infection.⁵⁹ Overall, these findings suggest that Sema-AC/HA10 can effectively deliver drugs while minimizing adverse skin reactions, ultimately enhancing patient compliance.

To evaluate drug diffusion through the skin via MNs containing the drug-AC nanocomplex, bio-imaging studies were performed using Cy5-BSA as a fluorescent probe. The MNs loaded with Cy5-BSA-AC nanocomplex (Cy5-BSA-AC/HA10) or a free Cy5-BSA solution were applied to the dorsal skin of rats. Cy5-BSA distribution across skin layers was then assessed using confocal microscopy. As shown in **Figure 3D**, free Cy5-BSA solution remained largely confined to the stratum corneum, with no significant penetration into the epidermis or dermis. This finding indicates that Cy5-BSA, a high-molecular-weight protein, cannot effectively pass through the skin barrier unaided. This aligns with that of previous studies showing that molecules $\geq 500\ \text{Da}$ have limited passive diffusion through the skin.⁶⁰ In contrast, Cy5-BSA-AC/HA10 displayed substantial drug diffusion across the skin layers (**Figure 3D**). Following MN application, strong fluorescence signals were initially concentrated around the micro-pores but gradually dispersed into the dermal area over time (**Figure 3D**). In addition, the micro-pores created by the MNs progressively recovered over time. These findings indicate that HA10 MNs loaded with the drug-AC nanocomplex offer an effective transdermal delivery system for protein drugs, overcoming the inherent barrier associated with large molecules.

Since environmental conditions such as temperature and humidity can affect the mechanical strength and performance of MNs,⁶¹ the stability of Sema-AC/HA10 was evaluated during the storage at 25°C, both with and without silica gel as a desiccant. As shown in **Figure 4A** and **B**, the mechanical strength of the MNs gradually decreased over time when stored without silica gel, likely owing to moisture absorption. In contrast, when stored with silica gel, the mechanical strength of Sema-AC/HA10 remained stable, with no significant change in weight (**Figure 4A** and **B**). These findings underscore the importance of controlling moisture during storage. To further evaluate the structural stability of Sema within the MNs, CD spectral analysis was performed to assess its conformational integrity. **Figure 4C** shows that the CD spectrum of Sema released from MNs after 7-day storage closely matches the spectrum recorded at Day 0, confirming that its structural conformation remained intact in the presence of silica gel. Additionally, the dissolution properties of the MNs were unaffected during storage, indicating consistent performance over time (**Figure 4D**). The stability of the Sema-AC nanocomplex within the MNs was also examined. As shown in **Figure 4E**, the size distribution of the Sema-AC nanocomplex in the MNs remained unchanged, suggesting no significant aggregation of nanoparticles during the storage.

These findings indicate that key properties of the MNs—including mechanical strength, dissolution behavior, conformational stability of the encapsulated drug, and nanoparticle sizes—remained stable when stored in a sealed container with silica gel. In contrast, MNs stored without a desiccant experienced a gradual decline in mechanical strength due to moisture absorption. These findings highlight the importance of proper packaging strategies, including incorporating desiccants, to ensure the long-term stability of MN formulations. Future research should explore long-term stability under different environmental conditions to ensure robustness for clinical and commercial applications.

Therapeutic Efficacy of Sema-AC/HA10 MNs in Type 2 Diabetic Rats

The *in vivo* efficacy of Sema-AC/HA10 was evaluated in type 2 diabetic rats and compared with SC injection. Both MNs and SC injections were administered once daily, and their anti-diabetic and anti-obesity effects on key physiological parameters were assessed. These parameters included blood glucose, HbA1c, plasma lipids (TC and TG), food and water intake, and body weight.^{62,63}

As summarized in **Figure 5**, SC injection of Sema significantly improved glycemic control and reduced body weight, which is consistent with that of previous studies.^{63–65} The Sema-AC/HA10 produced comparable therapeutic efficacy,

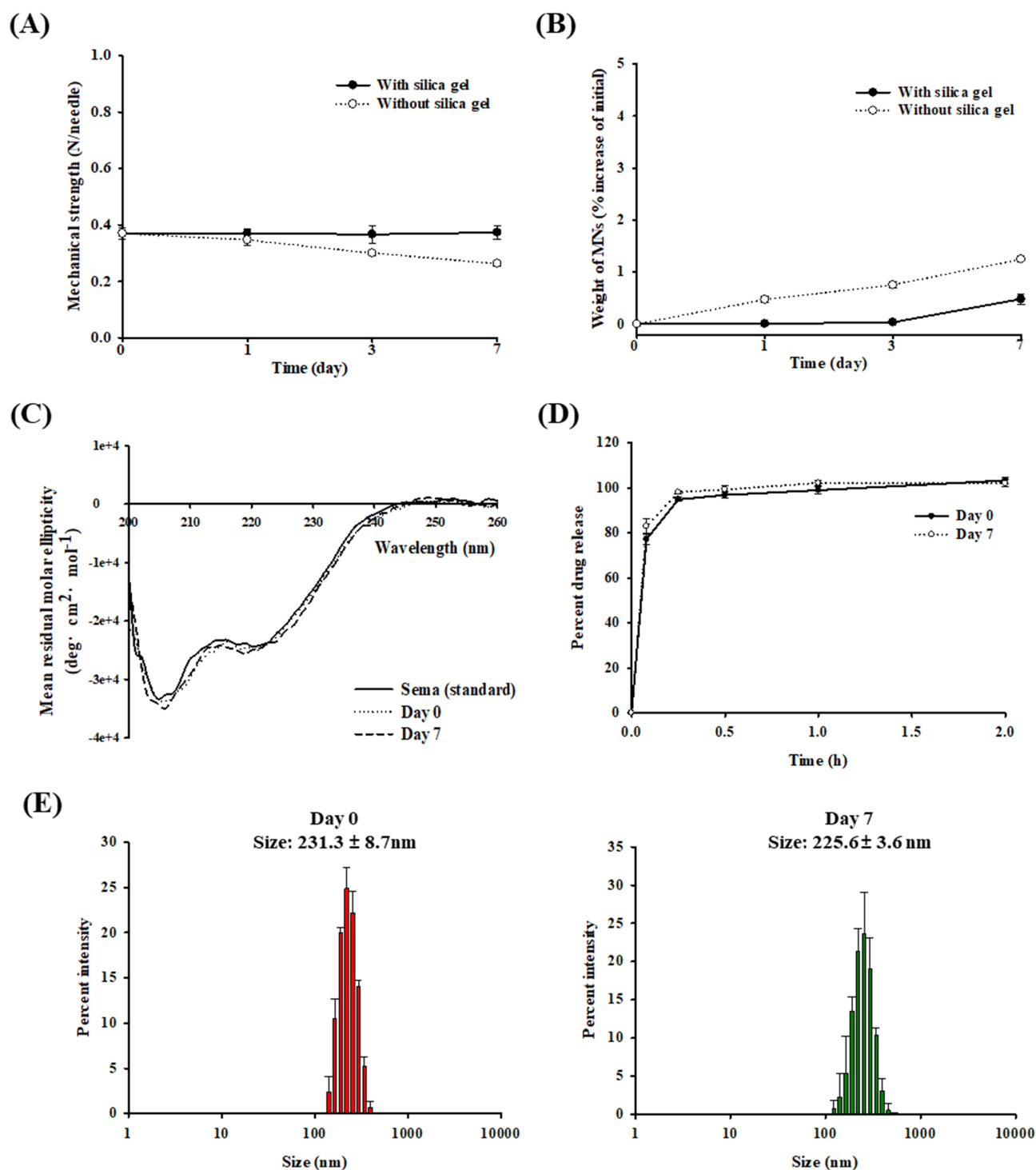


Figure 4 Stability of Sema-AC/HA10 during storage at 25 °C/ 60 ± 5% RH. **(A)** Alteration in mechanical strength (mean ± SD, n = 3), **(B)** Changes in weight (mean ± SD, n = 3), **(C)** CD spectra of semaglutide released from microneedles, **(D)** Drug release profiles in pH 7.4 PBS (mean ± SD, n = 3), and **(E)** Size distribution of Sema-AC nanocomplex released from microneedles.

effectively lowering blood glucose levels and promoting weight loss. Glycemic control was evaluated by monitoring fasting blood glucose and glycosylated hemoglobin levels over a 30-day treatment period. Following the MN application, fasting blood glucose and HbA1c levels gradually declined, reaching approximately 62% and 65% of their initial values, respectively, by day 30 (Figure 5A and B). Since glycemic control is closely related to lipid metabolism,^{66,67} plasma TC and TG levels were

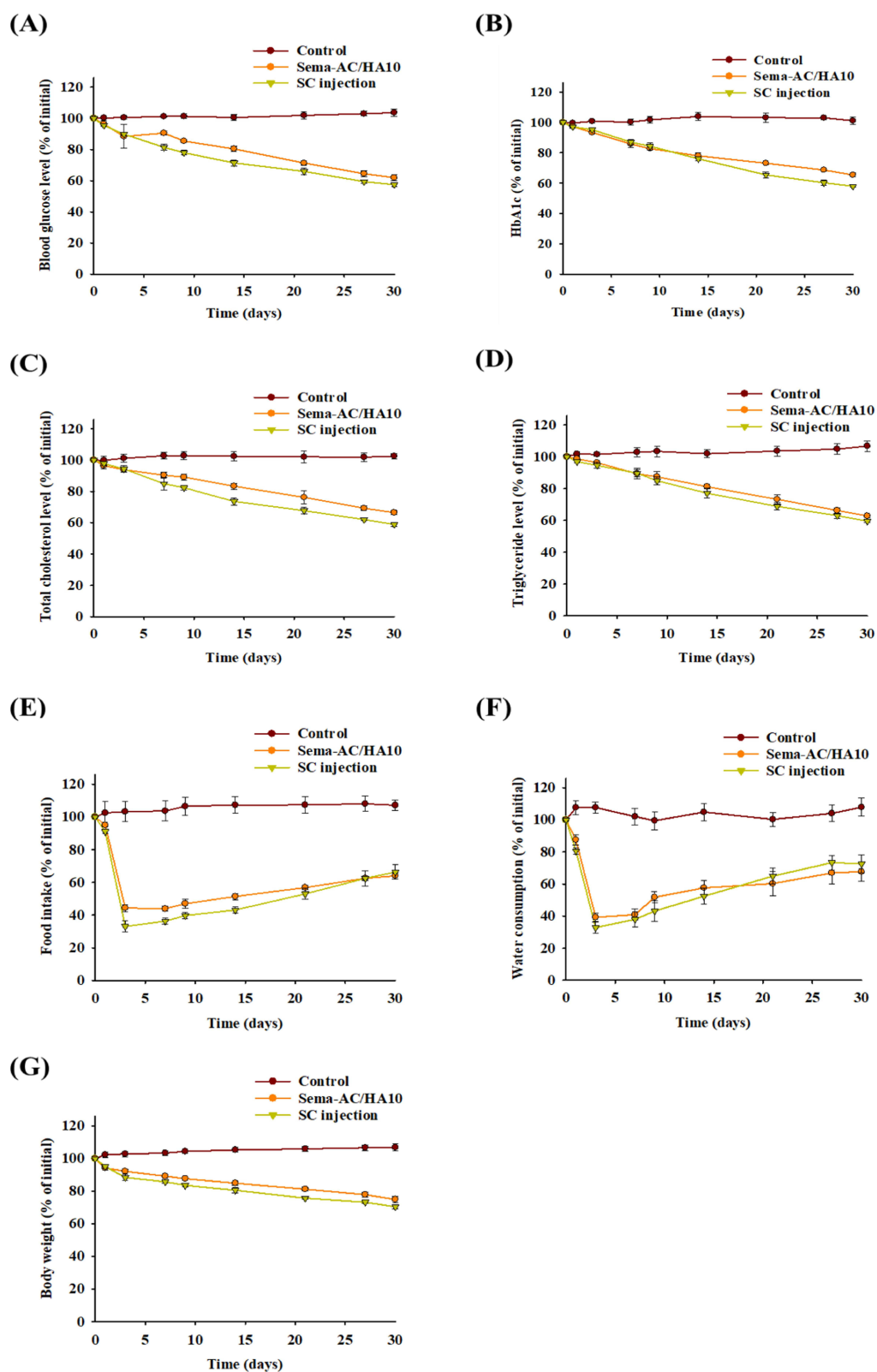


Figure 5 In-vivo efficacy of Sema-AC/HA10 in type 2 diabetic rats (Mean $\pm$ SD, n=6). Alteration in physiological parameters was monitored during the treatment period: (A) blood glucose, (B) glycosylated hemoglobin, (C) total cholesterol, (D) triglyceride, (E) food intake, (F) water consumption, and (G) body weight. Drug was administered once daily for 30 days via SC injection, or Sema-AC/HA10. The dose was equivalent to 0.4 mg/kg of semaglutide.

also measured. The MN treatment significantly reduced plasma TC and TG levels, lowering them to approximately 66% and 62% of their initial values, respectively, by day 30 (Figure 5C and D). These findings are consistent with those of previous studies indicating that Sema improves both glycemic control and lipid metabolism, potentially reducing cardiovascular risk in patients with diabetes.⁶³

Sema is recognized for its appetite-suppressing effects, which contribute to weight loss.⁶⁸ To evaluate the influence of Sema-AC/HA10 on obesity-related symptoms, food and water intake were monitored in type 2 diabetic rats. As shown in Figure 5E–G, drug administration via the MN significantly reduced food and water consumption, resulting in significant body weight loss.

Overall, Sema-AC/HA10 demonstrated therapeutic efficacy comparable to SC injection, highlighting its potential as a non-invasive alternative for managing diabetes and obesity. Although human skin is generally thicker and structurally more complex than rodent skin, the developed MNs exhibited sufficient mechanical strength to penetrate the human stratum corneum. Nevertheless, caution is warranted when extrapolating animal data to clinical translation, as variability in human skin properties such as hydration, elasticity, and regional thickness may influence insertion efficiency and drug diffusion. Moreover, therapeutic dose requirements and drug stability within the dermal environment could affect pharmacokinetics and overall efficacy. In addition to these considerations, long-term performance will be critical for clinical application, given that the present study primarily demonstrated short-term therapeutic outcomes. Repeated administration of MNs may pose challenges, including localized skin irritation, alterations in barrier integrity, or variability in efficacy over time. Therefore, future investigations should systematically assess the long-term stability of the MNs, their safety under chronic use, and the reproducibility of pharmacological effects during extended treatment to firmly establish the clinical feasibility of this microneedle platform.

Conclusion

Sema-AC/HA10 MNs were developed as an effective transdermal delivery system for Sema. Incorporating the Sema-AC nanocomplex within the MNs significantly enhanced transdermal drug absorption, resulting in higher systemic drug exposure compared to the MNs loaded with free Sema. Consequently, Sema-AC/HA10 MNs achieved therapeutic outcomes comparable to those of SC injection in type 2 diabetic rats. Once-daily application over 30 days significantly improved glycemic control, as reflected by reductions in blood glucose and HbA1c levels. Administration of Sema-AC/HA10 MNs effectively suppressed food and water intake, resulting in notable body weight loss. These findings reveal the potential of Sema-AC/HA10 MNs as a transdermal delivery system for Sema and a promising alternative to SC injections. By offering a non-invasive, patient-friendly approach, this MN system may enhance treatment adherence and improve therapeutic outcomes for individuals with diabetes and obesity.

Data Sharing Statement

The data in this study are available upon request from the corresponding author.

Ethics Approval and Consent to Participate

All animal studies were approved by the Institutional Animal Care and Use committee of Dongguk University (IACUC-2023-040-1) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (the National Research Council (US), 2011).

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Disclosure

All authors declare that they have no conflicts of interest in this work.

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