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Original Article

Clinical Evaluation of a Low-pain Long Microneedle for Subcutaneous Insulin Injection

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Abstract Microneedles (MNs) are being developed to overcome the limitations of the conventional hypodermic needle, e.g. the injection pain. In this study, we conducted an analysis of clinical pain and bleeding at the site of MN insertion and evaluated the insulin pharmacodynamic profile compared with parameters obtained with a conventional pen needle. MN insertion into the skin of 25 healthy adults or 15 patients with type 2 diabetes (T2D) revealed significantly less pain relative to a conventional hypodermic pen needle, thus reducing pain scores from 2.1 ± 1.9 to 1.3 ± 1.4 (mean ± standard deviation [SD]). Besides, no bleeding was observed when the MN was used. In the insulin pharmacodynamic assay, no significant differences were observed in the blood glucose-lowering effect between the pen needle and MN. Based on these results, the MN is expected to be a good substitute for conventional hypodermic pen needles and improve the quality of life of patients by significantly reducing the pain associated with insulin treatment.

Keywords: Microneedle, Insulin, Subcutaneous injection, Pain, Bleeding, Pharmacodynamic profile

Introduction

Diabetes mellitus is a glucose metabolic disorder caused by insufficient insulin secretion or an inability to produce insulin, resulting in an abnormally high blood glucose concentration (hyperglycaemia)[1]. Blood glucose control in diabetes is important to prevent and delay the progression of diabetic complications[2-4]. Although new classes of blood glucose-lowering agents, such as dipeptidyl peptidase (DPP)-4 inhibitors, glucagonlike peptide (GLP)-1 receptor agonists, and sodium-glucose co-transporter 2 (SGLT-2) inhibitors, were developed in recent years, insulin (which promotes the absorption of glucose from blood into the liver) is still considered the main treatment of diabetes as β-cell function declines with the progression of diabetes[5,6].

Insulin is usually administered by subcutaneous (SC) injection because oral delivery of a protein results in low bioavailability due to protein degradation in the gastrointestinal tract by proteolytic enzymes at extremely low pH as well as poor penetration of the intestinal epithelium by the protein[7,8]. Although conventional hypodermic needles or pen needles are widely used for SC insulin injections, the pain, needle phobia, and tissue trauma make patients reluctant for insulin therapy and reduce compliance, resulting in reduced quality of life of patients with diabetes[9,10]. Therefore, a minimally invasive insulin delivery system has been suggested to overcome the limitations of hypodermic-needle or pen needle usage. Although oral and inhaled insulin have been introduced as non-invasive delivery systems in diabetes care and were launched as Exubera® (Pfizer, US) and Afrezza® (MannKind, US), disappointing performance on the market because of low bioavailability and safety concerns such as acute bronchospasm and lung cancer are a challenge[11-13].

Microneedles (MNs), which have microscale dimensions, have been developed as an ideal alternative to

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conventional needles owing to a pain reduction and minimization of tissue damage[14]. Because MNs have been introduced to overcome the limitations of traditional hypodermic needles, various types of solid, dissolving, and hollow MNs have been designed and fabricated for drug injection. In a solid MN array, a topically applied drug is delivered into the skin through a micro-channel that is created by a solid MN array[15]. The life-span of such a micro-channel, however, is usually very short and thus drugs do not have sufficient time to pass through this micro-channel. This along with bioavailability variation represents the limitations of solid MNs for insulin administration. To overcome these limitations, drug-coated solid MNs and dissolving MNs have been introduced[16,17] in which a drug is delivered into skin via a solid MN and dissolving MN, respectively. Therefore, these MNs are especially useful for administration of high-molecular-weight molecules that have no time limitations on passage through skin. Nonetheless, the amount of the drug is rather limited due to the small dimensions of these MNs[18] and thus cannot always deliver the required amount to exert a pharmaceutical effect for human applications, especially regarding insulin, which is not high-potency medication.

Therefore, various hollow MNs that deliver an insulin solution have been introduced, and pharmacokinetic profiles and postprandial glycaemic responses have been compared to a conventional hypodermic needle[19,20]. In a hollow MN, a drug is delivered via a tubular bore inside the MN body after the MN pierces the skin[21], and thus there are no limitations on the quantity of insulin required to achieve a pharmaceutical effect. Although the drug is delivered through the MN bore in a manner similar to that of hypodermic needles, the length limitation of these hollow MN, which ranges from 500 to 2000 μm, makes it difficult for insulin to reach the target SC tissue layers (approximately >3 mm) resulting in insulin delivery into the dermis layer[22]. This action induces the formation of a superficial bleb on the skin surface[23,24], easily causing the drug backflow and skin damage[25]. Moreover, the rapid insulin uptake in the dermal layer may be problematic for stable insulin absorption for glucose management[26,27].

To ensure innocuous SC insulin injection, in our previous study, a long enough hollow MN was introduced to deliver insulin to the targeted deeper adipose tissue layers of the pig and thus enhanced the dosage adjustment with a stable absorption rate[25]. This hollow MN showed highly accurate and reproducible SC insulin delivery (as did a conventional hypodermic needle) and minimal tissue damage. Even with the aforementioned advantages, the main reason for patient reluctance for SC injection is the pain caused by needle insertion. Therefore, pain reduction by an MN in comparison with conventional hypodermic needles needs to be proven in clinical studies to allow the widespread use of MNs which can overcome the traditional limitations of hypodermic needles.

In this study, we carried out an analysis of clinical pain and bleeding at the site of insertion of the MN; the results showed highly accurate and reproducible SC insulin delivery with minimal tissue damage. The MN insertion into 25 healthy adults or 15 patients with type 2 diabetes (T2D) was associated with significantly less pain relative to conventional hypodermic pen needles (pain scores, 2.1 ± 1.9 with MNs and 1.3 ± 1.4 with conventional hypodermic pen needles, (mean $\pm$ standard deviation [SD])). Besides, no bleeding was observed when the MN was employed, although the pen needle resulted in three cases of bleeding among 34 patients. In addition to pain assessment and bleeding monitoring, we compared the pharmacodynamic (PD) effect of the MN with that of the conventional hypodermic pen needle, and this long MN yielded stable blood glucose management similarly to the conventional hypodermic needle. Thus, the use of this MN offering a reduction in pain and minimal tissue damage is expected to improve the quality of life of patients with diabetes, in addition to accurate and reproducible SC insulin delivery.

Results and Discussion

Fabrication of the MN Assembled with a Syringe Connector

To fabricate the MN, a heated flat glass panel was spin-coated with the SU-8 2050 polymer, and reverse drawing lithography was performed via contact by means of a 27-gauge blunt needle to form a polymer bridge between the glass panel and the blunt needle (Figure 1(a)). The polymer bridge was hardened at room temperature and then the SU-8 mould for electroplating was separated by additional drawing the hardened SU-8. Silver was deposited on the solid MN mould by means of Tollens' reagent for the electroplating seed. Nickel electroplating was carried out on the MN mould, and its upper tip was cut by a laser at a 15° bevel angle. The SU-8 polymer inside the nickel was removed by an SU-8 remover and acetone with continuous heating. The final MN was produced by parylene coating for a biocompatible surface. The size (length, outer diameter, and inner diameter) of the MN was controlled by process parameters of SU-8: temperature, drawing speed, electroplating time, and electric currents[28]. After fabrication of the MN, it was assembled with a syringe connector for mounting onto the 1 mL disposable syringe.

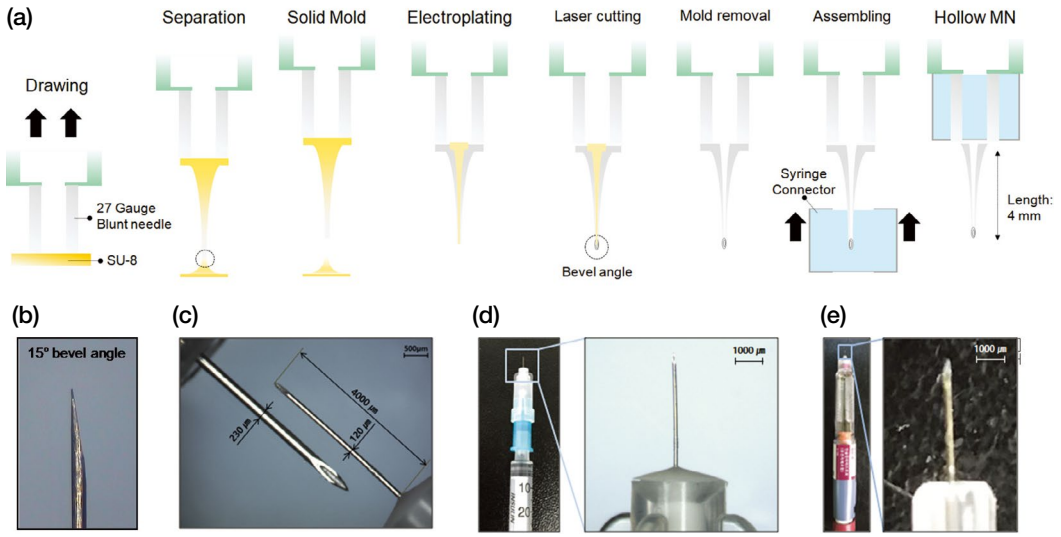

Figure 1. Schematic representation of fabrication of the MN with a syringe connector, and photographs of an assembled MN and pen needle. (a) The process of fabrication of the MN. (b) The 15° bevel angle of the MN. (c) Microscopic image of the MN with 4 mm length and 120 μm outer diameter, and a pen needle with 4 mm length and 230 μm outer diameter. (d) A photograph of the assembled MN-mounted syringe. (e) A photograph of pen needle–mounted Humalog KwikPen.

The length and outer diameter of the MN used in pain assessment and PD analysis were 4 mm and 120 μm, respectively with a 15° sharp bevel angle (Figure 1(b) and (c)). As shown in this figure, the fabricated MN had the same length as the pen needle (4 mm) with approximately half the outer diameter (120 μm) with a 32-gauge pen needle (230 μm). Besides, the MN was assembled on a 1 mL disposable syringe (Figure 1(d)) as a pen needle, which was assembled with the Humalog KwikPen (Figure 1(e)). These specifications and assembly of the MN implied that the target injection site of insulin is similar to that of the conventional pen needle, but with reduced pain induction levels owing to the same length but smaller outer diameter. The manufacturing process of the long MN used in this study is very simple, and the critical process parameters such as the temperature of the polymer and the drawing rate for the producing the solid MN mould are well controlled. Thus it is expected that the scale-up for the mass production is likely to be successful.

Mechanical Characterizations of the MN

A mechanical characterization was performed to determine whether MN was appropriate as a subcutaneous injection before conducting clinical pain evaluation and PD study. The mechanical strength of the MN assembled onto the syringe connector was analysed by driving the needles into pig skin and by applying axial load to the needles. As depicted in the typical force-displacement curve, the initial point of the sharp force drop denotes the moment of skin penetration (Figure 2(a)) and fracturing of the needle (Figure 2(b)). The average penetration force and fracture force of the MN by axial load were 0.82 ± 0.09 N (n = 3) and 5.85 ± 0.44 N (n = 3), respectively (Figure 2(c)). The penetration force was much lower than the fracture force, suggesting that this MN had sufficient mechanical strength to penetrate the skin barrier safely without fracture during SC insulin injection.

Participants

Forty subjects participated in the pain assessment as per protocol (Figure 3, Part A). The enrolled volunteers comprised 21 males and 19 females, and their ages were 41.1 ± 17.0, 34, and 23-74 years (mean ± SD, median, and min-max, respectively). Of the 40 participants in pain assessment measurements, 15 patients with T2D participated in the evaluation of the PD profile of insulin (Figure 3, panel B). The participants comprised 6 males and 9 females and their ages were 59.2 ± 13.2, 64 and 25-74 years (i.e. mean ± SD, median and min-max) respectively. Their height was 161.4 ± 9.5 cm, weight was 68.0 ± 12.3 kg, and T2D duration was 14 ± 12 years. All subjects who participated in these experiments were Koreans.

Comparative Pain and Bleeding Assessment after Insertion

Among the 40 subjects who participated in the pain as-

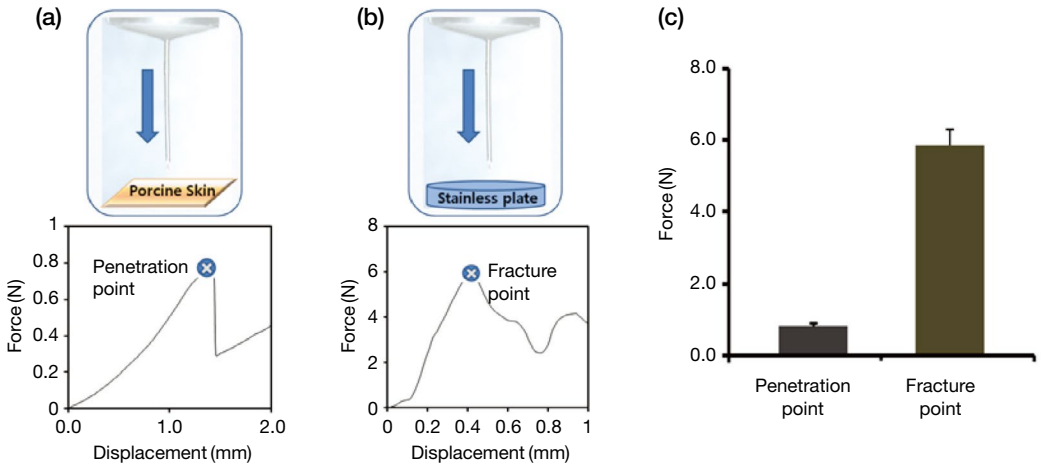

Figure 2. Mechanical characterization of the MN. (a) Penetration force was measured using porcine skin and the typical force-displacement curve of skin penetration is shown and (b) fracture force was measured by means of a stainless-steel plate by adding an axial load. (c) The porcine skin penetration force and the axial fracture force was 0.82 ± 0.09 and 5.85 ± 0.44 N (n = 3), respectively.

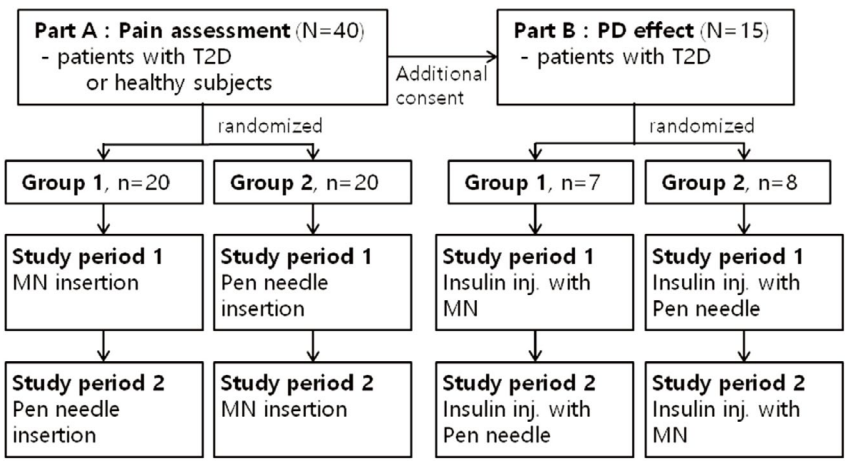

Figure 3. Study design and volunteer disposition. Pain assessment was performed on 40 healthy subjects or patients with T2D (Part A). Among them, 15 patients with T2D who signed an additional informed consent form were analysed for PD (Part B). In each Part (A and B), the subjects were randomly subdivided into two groups: one group was first tested with MN and then with a pen needle. The other group, on the contrary, was first tested with a pen needle and then with MN.

sessment studies, six individuals withdrew from the study owing to protocol deviation during the procedure. Because the length of the needle is important for determining the pain, the MN and pen needle were inserted into the lower abdomen to a depth of 4 mm, corresponding to the total needle length. The analysis of the degree of pain was performed on 34 subjects, except for the six subjects who deviated from the protocol during the treatment.

After injection using the pen needle, the degree of pain was 2.1 ± 1.9, 1.6, and 0.2-6 (mean ± SD, median, and min-max, respectively). On the other hand, when insulin was administered with MN, the degree of pain was 1.3 ± 1.4, 0.6, and 0-4. The mean and median pain score differences between the MN and pen needle were 0.8 and 1.0, respectively. Compared to the pen needle, the pain decreased significantly when the MN was employed (Figure 4). In general, 0 points, 2 points, and 4 points on the Numerical Rating Scale (NRS, Supplementary Material) indicate no pain, mild pain, and moderate pain, respectively. According to this NRS scale, the pen needle induced mild pain, and the MN showed the range between mild and no pain. Although the difference of pain score by NRS was 0.125-0.153 in the comparison of 32-gauge and 30-gauge needles, this difference was statistically significant and subject preferred the

less-pain needle doubled[29]. Because patients with T2D are still reluctant to use a pen needle even only mild pain is associated with its use[30], the pain reduction afforded by the MN has to be significant in order to reach widespread acceptability. Although the MN showed the pain range between mild and no pain, the median value of the MN was 0.6, which was close to no pain. This reduction in the pain score is expected to attract patients to choose MN injection.

In addition, bleeding at the injection site was less with the MN compared to the pen needle. Among 34 injections, bleeding was not observed with the MN, whereas 3 bleeding events and 31 no bleeding were observed with the pen needle. Although a small amount of bleeding at the injection site is normal when insulin is injected and does not usually cause major problems, this bleeding, which is usually caused when the needle damages a blood vessel, may cause bruising at the injection site.

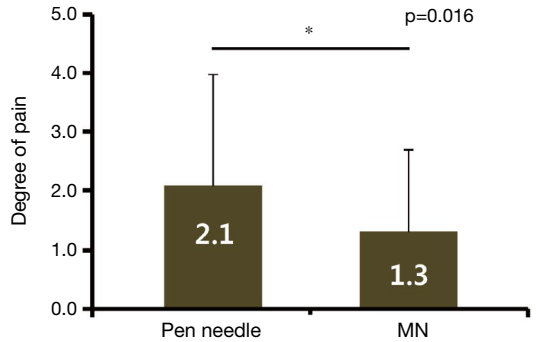

Figure 4. Comparative pain assessment, the degree of pain with the pen needle insertion and MN insertion was 2.1 ± 1.9 and 1.3 ± 1.4 (mean ± SD), respectively.

Therefore, the MN, with its ability to cause only minimal bruising and lesser pain, is expected to overcome the reluctance of patients for undertaking insulin treatment.

Comparison of PD Properties of Insulin Administration

In 15 diabetic patients, insulin was administered at 0.1 U/(kg body weight), and the blood glucose-lowering effect was evaluated for 120 min. Four cases of hypoglycaemia, two in the pen needle group and two in the MN group occurred 1.0 or 1.5 h after injections. No other significant event occurred during injections, and noticeable injection site damage was not observed. The final analysis of PD included all 15 subjects except for the patient whose blood sample showed increased blood glucose because of the administered glucose for improvement of hypoglycaemic symptoms.

Blood glucose gradually decreased after an insulin injection for 120 min in both groups (MN and pen needle injection), and no significant differences were observed at each time point during treatment periods (Figure 5(a)). Furthermore, the blood glucose area under the curve (AUC) during minutes 0-120 post-injection was very similar between the MN injection group and the pen needle injection group, implying that there was no statistically significant difference between the two needles (Figure 5(b)).

Maintaining the existing PD profiles with the development of new medical devices is important because of both clinical regulatory issues and cost considerations. The similarity of the insulin PD profile with the use of the MN as compared to the pen needle indicated that a large-scale clinical study is not required to confirm the efficacy and safety, which can incur a huge develop-

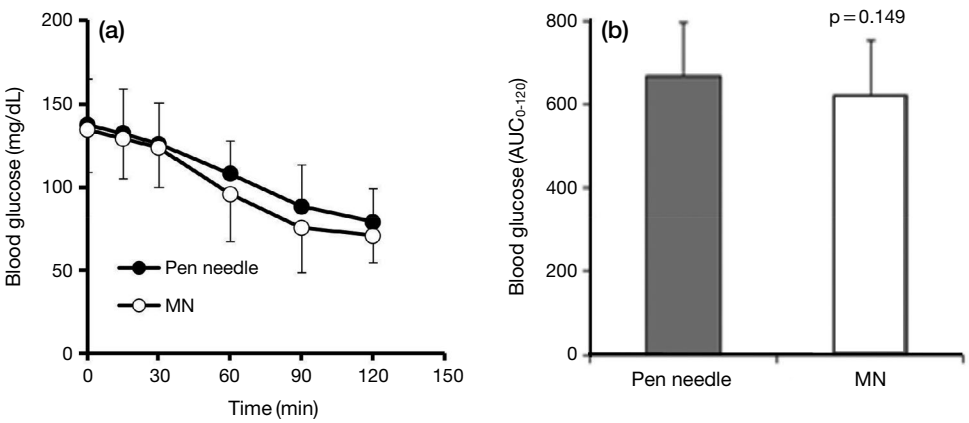

Figure 5. The PD profile of glucose in patients with T2D after insulin injection via (●) a pen needle and (○) MN. (a) The curve of blood glucose concentration versus time. (b) Glucose AUC. (b) AUCs of blood glucose changes in the time slot 'minutes 0-120'. No significant differences were observed between the two needles. All the data represent average values and are expressed as mean ± SD.

ment cost and act as a new barrier. The MN evaluated in this study is expected to be more acceptable to patients with diabetes because of low pain and similar blood glucose-lowering effects as compared to the conventional pen needle.

Conclusion

In this study, we conducted a comparative pain assessment and a PD assay of insulin injected with an MN versus a conventional pen needle. In the pain assessment assay, the injection-related pain significantly diminished to the range of mild to no pain when the MN was used. Furthermore, bleeding at the injection site was less when the MN was employed, suggesting that the probability of damaging blood vessels was much smaller. In the PD assay, no significant differences were observed in the blood glucose-lowering effect between the conventional pen needle and MN. The MN is expected to be a substitute for conventional hypodermic pen needles and to improve the quality of life of patients by significantly reducing pain during insulin treatment.

Materials and Methods

Fabrication of the Hollow MN

The MN was manufactured by the electroplating on the surface of the SU-8 mould[31]. Briefly, a flat glass panel was spin-coated with the SU-8 2050 polymer (Microchem, USA) at 1,500 rpm and placed on a 100°C hot plate for 1 h. A blunt, 27-gauge (G) needle made of stainless steel was brought into contact with spin-coated SU-8, and reverse drawing lithography was performed at a drawing rate of 1 mm s^{-1} for 5 s. The polymer stretched to the desired length was left to harden for 15 min at room temperature. The polymer bridge between the blunt needle and a flat glass panel was separated by additional drawing and produced the solid MN mould. Tollens' reagent was deposited on the solid MN moulds for seeding an electroplating layer. Nickel electroplating was carried out in the bath of an electroplating device at a temperature of 60°C and a current density of 1 A dm^{-2}. The hollow metallic MN array was released from the micro-glass panel by elimination of the photoresist MN mould by means of a SU-8 remover. Then, the biocompatible surface of the MN was created by parylene coating. Finally, the complete MN was mounted onto the syringe connector via an epoxy resin adhesive (Araldite 506 epoxy resin, Sigma-Aldrich, USA).

Materials

A pen needle (4 mm, 32G, BD, Korea) was chosen as a comparator for the MN for pain assessment and PD analysis. A disposable 1 mL syringe (BD, Korea) attached to the MN was used for the administration of insulin. Two types of Insulin, Humalog inj. (insulin lispro, 100 U/mL, Eli Lilly and Co., Korea) for the MN and Humalog KwikPen (insulin lispro, 100 U/mL, Eli Lilly and Co., Korea) for the pen needle were employed in the PD assay, respectively.

Analysis of the Mechanical Strength of the MN

The porcine skin penetration force and mechanical strength parameters of the MN, such as axial fracture force, were measured on a force measurement system (Zwick, Germany) as described in our previous study[25]. Briefly, fresh pig cadaver skin was chosen as a suitable model of human SC tissue to measure the penetration force of the MN. After each MN was connected to the holder of the force measurement system, the MNs were pressed against the fixed pig skin at a rate of 50 μm/s until they reached a pre-set displacement of 4 mm. To measure the mechanical strength of the MN, the axial load force was respectively driven against the MN at a rate of 50 μm/s by the holder of the force measurement system until it reached a pre-set maximum load of 8 N. The force was recorded as a function of the displacement seen in the MN.

Participants

Patients with T2D or healthy subjects who were over the age of 19 were recruited. To compare the degree of pain experienced during skin puncture with the MN and traditional pen needle, we recruited 40 subjects with or without diabetes, because at least 30 samples were required for achieving statistical normal distribution. Sample size determination for the PD assay was not performed to meet any specific significance and power requirements of statistical analysis. In the assay comparing the PD profile of intradermal (ID) insulin injection with the MN versus SC insulin injections via a conventional hypodermic needle, 14 subjects were analysed to identify significant differences[27]. Therefore, we recruited 15 subjects with T2D for the PD evaluation of insulin injection with the MN. Exclusion criteria for recruiting subjects included pregnancy and illiteracy (subjects who cannot read the consent form). Volunteers were free to withdraw from the study at any time.

Pain and Bleeding Assessment

The degree of pain caused by skin puncture by means

of the MN was compared with that of the pen needle in a randomized, single-blind, cross-over study. The subjects were randomized according to a random number generator software. The results of the randomization were not reported to the participants. Skin punctures were applied to the same subject in the lower abdomen using both the MN and pen needle successively. The order of injection with the two types of needle was determined by random assignment, and injection was performed by a well-trained nurse. Subjects were assessed for pain at the time of injection on the NRS: 0-10. When the degree of pain was all below 2, the modified NRS was self-assessed by subdividing the 0-2 region into 0.2 intervals. After injection, the site of insertion was visually observed for bleeding, but the amount of bleeding was not measured. Compression haemostasis was conducted for 3 min.

PD Profiles of Insulin Injection with the MN Versus Pen Needle

Randomization of volunteers and single-blinding were performed as in pain assessment. Insulin was administered on a cross-over basis. Volunteers in each of the two parallel groups received a single 0.1 U/kg dose of insulin in the lower abdomen using the MN and pen needle in a random order. To measure serum glucose concentration, intravenous blood was collected for 120 min at intervals of 15 min for 30 min after administration and at intervals of 30 min thereafter. The second dose was given after a resting period of at least 20 h following the first dose. Blood sampling for serum glucose measurement was performed as in the procedure for the first dose. If hypoglycaemic symptoms developed during the study, we immediately stopped the study and administered 100 mL of 20% glucose intravenously.

Statistical Analysis

Wilcoxon signed-rank test was conducted to compare the intensity of pain represented by NRS score between the pen needle and MN. A difference with a p value <0.05 was considered statistically significant. This statistical analysis was performed using Minitab 17 software. The PD profile assessment was performed based on a comparison of the AUC of blood glucose levels for 120 min after insulin administration and by Student's *t* test. A difference with a p value <0.05 was considered statistically significant. This statistical analysis was performed with SPSS software, version 23.0 for Windows.

Ethics Approval

This study was reviewed and approved by the Institutional Review Board (IRB), Yonsei Severance Hospital (IRB No. 1-2017-0042), and all volunteers provided written informed consent. All procedures performed in this study involving human participants were in compliance with the ethical standards and with the tenets of the Declaration of Helsinki.

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