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Self-dissolving micropile array tips for percutaneous administration of insulin

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Abstract Two kinds of insulin were loaded into self-dissolving micropile array tip (following tip). Fully-loaded tip (f-tip) and partially-loaded tip (p-tip) were prepared using chondroitin sulfate for the percutaneous administration of insulin. One hundred micropiles were constructed on a 1.0 × 1.0 cm tip. The mean length of the micropile in a tip were 483.4 ± 4.7 μm for the f-tip and 492.6 ± 2.4 μm for the p-tip. The insulin content of the p-tip was 28.5% of that of the f-tip. The pharmacological efficiency of insulin loaded tip was evaluated in rat experiments by measuring their hypoglycemic effects. The maximum hypoglycemic effect of insulin was observed at 1.7 ± 0.2 h for the f-tip and 1.5 ± 0.2 h for the p-tip. Good dose-dependency was observed for the plasma glucose level vs. time curves. These results suggest the usefulness of p-tip as a percutaneous DDS of insulin.

1 Introduction

Recent advances in microfabrication technology have made it possible to prepare micropiles that have potential as a novel transdermal drug delivery system (TDDS). Since the first publication by Henry et al. in 1998 [1], microfabrication techniques for the production of silicon, metal, glass, and polymer microneedle arrays with micrometer dimensions have been reported [2–5]. The microneedles are either solid or hollow and have a geometrical shape. Microneedle TDDS is roughly defined as a micron sized needle through which a drug is percutaneously administered. Microneedle TDDS are classified as follows [6]: (1) extremely small needles through which drug solutions can be injected into the skin, (2) metallic and/or silastic micropiles of which surface is coated with a drug, and (3) metallic and/or silastic micropiles which allow micropores to be made in the skin and drug solution is applied after removing the micropiles. To understand the functions of micropiles in detail, the physiology of the skin must be considered. Human skin is made of three layers, i.e., the stratum corneum (SC), epidermis and dermis. The SC is the outer layer of the skin, which has a thickness of 10–15 μm and is a dead tissue. The SC demonstrates strong primary barrier function against exogenous compounds including drugs. The second barrier is the viable epidermis (50–100 μm), which contains tissue-like living cells. However, there are no blood vessels in the epidermis. Deeper still, there are blood capillaries in the dermis, which forms the bulk of skin volume and contains living cells nerves. If the micropiles are inserted deeply into the dermis, bleeding and pain occurs. Therefore, micropiles were designed that were not harmful to the dermis. When microneedle arrays are inserted into the skin, microconduits are created for the penetration of drug across the SC. Once a drug penetrates the SC, it can diffuse rapidly through the deeper tissue and permeate into the underlying capillaries for systemic absorption [7].

Recently, micropiles of 200–800 μm in length have been prepared. However, when their length is shorter than 300 μm, the diameter of the basement becomes less than 200 μm, which results in friability of the micropiles themselves. Therefore, even in the cases of metallic micropiles, the length was designed to be at least 500 μm [8]. When micropiles with a length of 500 μm are applied to

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the skin and pressed using a finger with a pressure of approximately 0.35–0.5 N, the whole micropile is not inserted into the skin [9]. Therefore, when a drug is added to whole micropiles, i.e., from the top to the bottom of the micropiles, a considerable amount of the formulated drug is not absorbed into the skin, and as a result low bioavailability (BA) occurs.

To clarify the contribution of the amount of drug added to the top of the micropile, two kinds of self-dissolving micropile array tips (tips) were prepared. One was fully-loaded tip (f-tip) in which the drug was added from the top to the bottom of the micropile in the tip. The other one was partially-loaded tip (p-tip) in which the drug was added to the top portion of the micropile. We prepared two kinds of insulin loaded tips, and biopharmaceutical and pharmacodynamic experiments were performed to evaluate their effects in rats.

2 Materials and methods

2.1 Materials

Insulin (28.7 IU/mg) was obtained from Sigma-Aldrich Corp. (USA). Evans blue (EB)and Glucose CII-Test kits were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Chondroitin sulfate was obtained from Nacalai Tesque Co. Ltd. (Kyoto, Japan). Male Wistar rats and standard solid commercial food meals (Labo Diet®) were purchased from Nippon SLC Co., Ltd. (Hamamatsu, Japan). All other reagents were of an analytical-reagent grade and were used as received.

2.2 Preparation of insulin loaded tips

A dense solution of drug glue was prepared by adding 150 µl of insulin solution, at 100 or 50 µg/ml, to a mixture of 60 mg of chondroitin sulfate and 0.45 mg of EB, and were mixed well under room temperature. After the drug glue had been degassed under reduced pressure, it was dispensed into a mold made of silicon resin containing 100 inverted cone shaped wells in a 10 by 10 matrix with an area of 1.0 cm^2. Each well had a depth of 500 µm and a diameter of 300 µm at its base. The mold was covered with a 300 g steel plate, and the wells were filled with drug glue. Thirty minutes later, the plate was removed, and the drug glue that remained on the surface of the mold was removed by a squeegee process. Glue made of 30 mg of chondroitin sulfate and 30 ml of distilled water was painted over the mold and dried under the pressure of the stainless plate for 3 h. Thereafter, the plate was removed, and f-tip were obtained by detaching them with a supporting material. To prepare p-tip as shown in Fig. 1, the same mold was used. At first, degassed drug glue was dispensed into the wells and centrifuged for 3 min at 3,000 rpm (Kubota 1700, Tokyo, Japan). The drug glue that remained on the surface of the mold was removed by a squeegee process before being centrifuged for 30 min at 3,000 rpm. Thereafter, the plate was removed and p-tip were obtained by detaching them with a supporting material. The two types of tips are shown in Fig. 2. The lengths of the micropiles in tips was approximately 500 µm, and the diameter of the base was approximately 300 µm. The insulin contents of both tips were 1.73 ± 0.17 IU for the p-tip and 6.08 ± 0.42 IU for the f-tip. Those tips were designed for manual insertion into the skin by finger application. For the evaluation of the dose dependency of p-tip on hypoglycemia in rats, another two p-tips containing different amounts of insulin were prepared as described above. The insulin contents of these p-tips were 0.70 ± 0.06 and 0.37 ± 0.03 IU, respectively.

2.3 Microscopic observation of insulin loaded tip

Tips containing both insulin and EB was observed using a VH-5500 digital video microscope (Keyence Co. Ltd, Osaka, Japan) under normal light.

2.4 Insulin contents in tips

Insulin was extracted from a tip with a 5.0 ml aliquot of phosphate buffer, at pH 7.4, for 10 min. The obtained extract was centrifuged at 13,000 rpm for 5 min at 8°C using a Kubota 1700 centrifuge. The supernatant was separated from the debris and was stored in a deep freezer at −80°C until analysis.

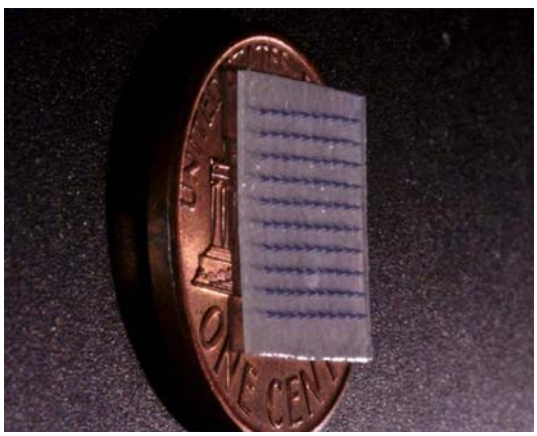

Fig. 1 An image of the partially-loaded self-dissolving micropile array tip (p-tip) used in this study

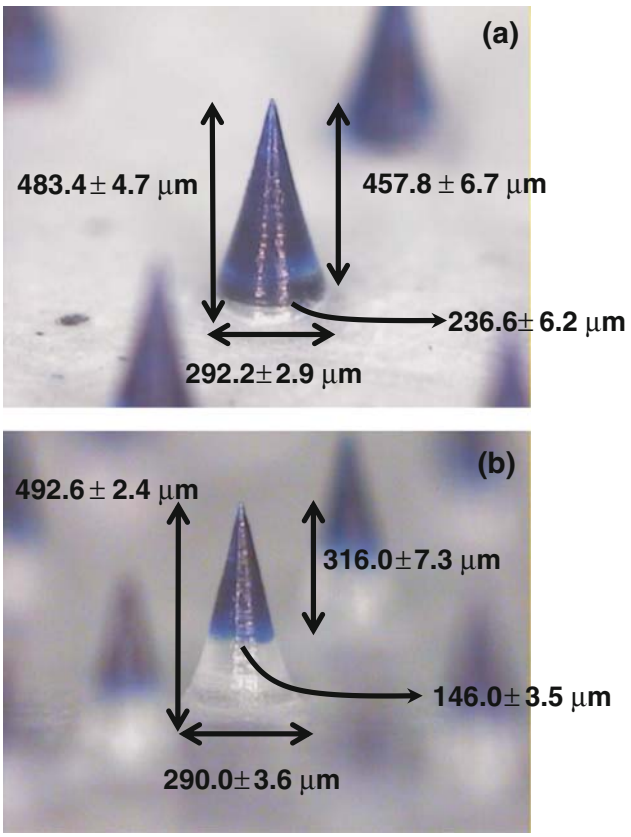

Fig. 2 Microscopic photographs of a micropile in the insulin loaded tips before administration to rats. **a** fully-loaded tip (f-tip) and **b** partially-loaded tip (p-tip)

2.5 Analytical method of insulin

One hundred microliters of the insulin sample were injected onto a HPLC system (Shimadzu LC-10A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10A) and a NUCLEOSIL 5C18, 4.6 × 150 mm reversed phase column (Chemco Scientific Co. Ltd. Osaka, Japan). The mobile phase consisted of 0.2 M anhydrous sodium sulfate adjusted to pH 2.3 with *ortho* phosphoric acid and acetonitrile (74:26, v/v). The flow rate was 1.5 ml/min, and the column temperature was 36°C. The detection wavelength of insulin was 214 nm as described previously [10].

2.6 Pharmacodynamic study after the percutaneous administration of insulin loaded tip to rats

Male Wistar rats, with a mean weight of 338 ± 24 g (mean ± SD), were fasted for 12–16 h but had ad libitum access to water. They were anesthetized with an intraperitoneal injection of sodium pentobarbital at 50 mg/kg. One group consisted of three to four rats. Five minutes before the administration, 200 μl of blank blood sample was obtained from the left jugular vein using a heparinized syringe. After the hair of the abdominal skin had been removed, the insulin loaded tip was pressed into the skin for 3 min. At 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 h after the administration, blood samples were collected from the right jugular vein using a heparinized syringe. For the subcutaneous (sc) injection of insulin, 1.0 IU/kg, insulin solution was prepared by dissolving insulin sodium salt in phosphate buffered saline (PBS) and injected into the right jugular vein, after blank blood samples had been obtained from the left jugular vein. Thereafter, blood samples were collected at 0.5, 1, 2, 3, 4, and 5 h from the left jugular vein. In each sampling period, 0.15 ml of the blood sample was obtained with a heparinized syringe. By centrifuging at 13,000 rpm for 15 min at 8°C, 40 μl of the plasma samples were obtained. All these plasma samples were immediately frozen in a deep freezer at −80°C until the analysis. All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Kyoto Pharmaceutical University.

2.7 Plasma glucose level assay

The plasma glucose level was determined using a glucose CII-Test kit. The post-dose plasma glucose levels are expressed as a percentage of the initial level. The percentage change in the plasma glucose level was defined as the percentage of the post-dose levels subtracted from 100. The cumulative percentage change in the plasma glucose level was calculated using the linear trapezoidal rule up to the last measured plasma glucose level of the percentage change *vs.* time curves (AAC) for 0–5 h.

2.8 Pharmacokinetic analysis

The relative pharmacological availability (RPA) of insulin was calculated using the following equation:

$$\mathrm{RPA}(\%) = \left(\mathrm{AAC}_{\mathrm{per}} \cdot \mathrm{Dose}_{\mathrm{sc}}\right) / \left(\mathrm{AAC}_{\mathrm{sc}} \cdot \mathrm{Dose}_{\mathrm{per}}\right) \times 100 \quad (1)$$

where $\mathrm{AAC}_{\mathrm{per}}$ and $\mathrm{AAC}_{\mathrm{sc}}$ are the plasma glucose levels of the percentage change vs. time curves after percutaneous and sc administration of insulin at different doses ($\mathrm{Dose}_{\mathrm{per}}$ and $\mathrm{Dose}_{\mathrm{sc}}$). The time, T_{min}, at which the plasma glucose level reached its minimum level, C_{min}, was determined from the authentic plasma glucose concentration vs. time data.

2.9 Data analysis

All values are expressed as their mean ± SE Statistical differences were assumed to be reproducible when $P < 0.05$ (Student's unpaired *t*-test).

Table 1 Physical characteristics and insulin contents of full and partially-loaded tips

Type of tip	Diameter (μm)	Length (μm)	Length of the drug loaded space (μm)	Volume of drug loaded space ($\times 10^6$ μm^3)	Insulin content (IU)
f-tip	292.2 ± 2.9	483.4 ± 4.7	457.8 ± 6.7	6.7 ± 0.4	6.08 ± 0.42
p-tip	290.0 ± 3.6	492.6 ± 2.4	316.0 ± 7.3	1.8 ± 0.1	1.73 ± 0.17

Each value represents the mean ± SE (n = 4–5)

3 Results

3.1 Physicochemical properties of insulin loaded tip

The two types of insulin loaded tips were prepared as shown in Fig. 2, where EB was used as a marker dye. As the same mold was used to prepare them, the sizes of each micropiles in the tips were almost the same. Table 1 shows the physicochemical characteristics of the obtained tips. The mean lengths of the micropiles in f-tip and p-tip were 483.4 ± 4.7 and 492.6 ± 2.4 μm, respectively. The diameters of their basements were 292.2 ± 2.9 and 290.0 ± 3.6 μm. In the case of the p-tip, the mean length of the drug loaded space was 316.0 ± 7.3 μm from the top of the micropiles. On the other hand, the mean length of the drug loaded space in the f-tip was 457.8 ± 6.7 μm. The theoretical volumes of the drug loaded-space are shown in the fifth column of the table. The mean volume of the micropiles in the p-tip was 1.8 ± 0.1 × 10^3 μm^3, which corresponds to 26.9% of the mean volume in the f-tip (6.7 ± 0.4 × 10^3 μm^3). The mean insulin contents of the two tips were 1.73 ± 0.17 IU for p-tip and 6.08 ± 0.42 IU for f-tip. The insulin content in the p-tip was 28.5% of that in the f-tip. The insulin content of the micropiles in the tips corresponded well to the volume of the drug loading space of micropiles in the tips.

3.2 Pharmacodynamic studies of insulin loaded tips

To compare the pharmacological efficiency of two types of tips, p-tip and f-tip, were administered to the skin of rats *via* finger pressing, and the hypoglycemic effects of insulin were measured. After the administration of the insulin loaded tips, the minimum plasma glucose levels appeared at 1.0–2.0 h for both tips as shown in Fig. 3. The hypoglycemic effects were almost the same in two tips. Table 2 shows the pharmacodynamic parameter values obtained by pharmacokinetic analysis. The T_{min}s were observed at 1.7 ± 0.2 h for f-tip and 1.5 ± 0.2 h for p-tip. The mean areas above the percent change of the plasma glucose level vs. time curve (AACs) were 159.8 ± 30.6% h for f-tip and 149.7 ± 8.0% h for p-tip. There were no significant differences in the AAC between two tips. In another group of rats, insulin was sc injected at a dose of 1.0 IU/kg, and the plasma glucose level vs. time curve is also shown in Fig. 3. The mean AAC was 94.8 ± 12.3% h. By comparing the AAC obtained after the percutaneous administration of insulin loaded tips with that obtained from the sc injection of insulin solution, relative pharmacological availabilities (RPAs) were determined by the Eq. 1. When the mean insulin contents of the tips (1.73 IU for p-tip and 6.08 IU for f-tip) were used, the mean values of RPA were 9.2 ± 1.6% for f-tip and 30.7 ± 1.9% for p-tip. The RPA of p-tip was 3.3 times higher than that of f-tip. The recovered tips are shown in Fig. 4, in which the top part of the micropiles in the tip disappeared because of their dissolution and absorption into the skin. However, the blue color due to EB formulated with insulin remained in the tip. These results suggest that the formulated insulin in the tip was not completely absorbed into the rat skin. Therefore, the remaining amount of insulin in the recovered tip was measured by a HPLC method. The mean recovered amounts of insulin in the two tips were 1.14 ± 0.09 IU for p-tip and 4.91 ± 0.28 IU for f-tip, respectively. By subtracting the recovered amount of insulin from the insulin contents in the tip, the released amount of insulin was determined to be 0.57 ± 0.09 IU for p-tip and 1.12 ± 0.27 IU for f-tip. By assuming these values as the administered doses of insulin, RPAs were calculated to be 52.2 ± 12.9% for f-tip and 97.5 ± 16.5% for p-tip.

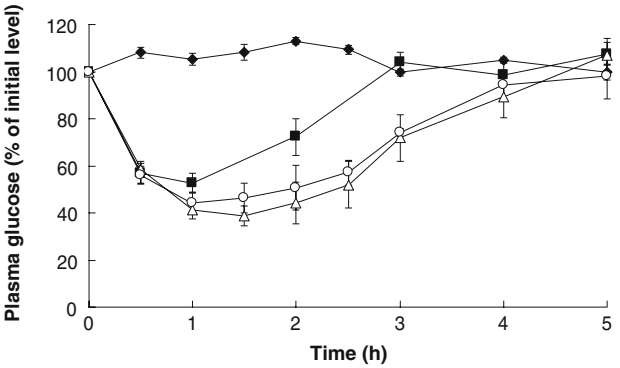

Fig. 3 Plasma glucose level-time curves after subcutaneous injection of insulin solution and percutaneous administration of f-tip and p-tip to rats. *filled square* insulin solution for sc injection (1.0 IU/kg), *open square* f-tip (6.08 ± 0.42 IU/rat), *open circle* p-tip (1.73 ± 0.17 IU/rat), *filled diamond* placebo tip. Each point represents the mean ± SE of 3 and 4 experiments

Table 2 Pharmacodynamic parameters of insulin after percutaneous administration of tips to rats

Type of tip	C_{min} (%)	T_{min} (h)	$AAC_{0\text{-}5}$	RPA (%)	Calculated RPA (%)
f-tip	37.0 ± 5.4	1.7 ± 0.2	159.8 ± 30.6	9.2 ± 1.6	52.2 ± 12.9
p-tip	37.6 ± 2.6	1.5 ± 0.2	149.8 ± 7.7	30.7 ± 1.8	97.9 ± 17.3

Calculated RPA: relative pharmacological availability calculated with authentic dose which was determined by subtracting the remaining insulin amount from insulin amount before administration

Each value represents the mean ± SE ($n = 3$–4)

C_{min} minimum glucose level, T_{min} time when minimum glucose level were determined from the plasma glucose level versus time data, $AAC_{0–5h}$ area above the plasma glucose level versus time data, *RPA* relative pharmacological availability compared to s.c. injection data

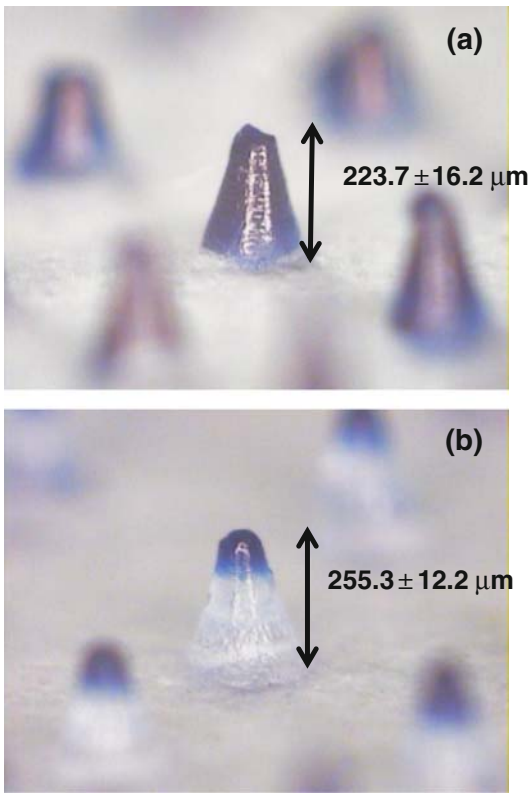

Fig. 4 Microscopic photographs of the recovered micropiles in the tips after percutaneous administrations to rats. **a** f-tip, **b** p-tip

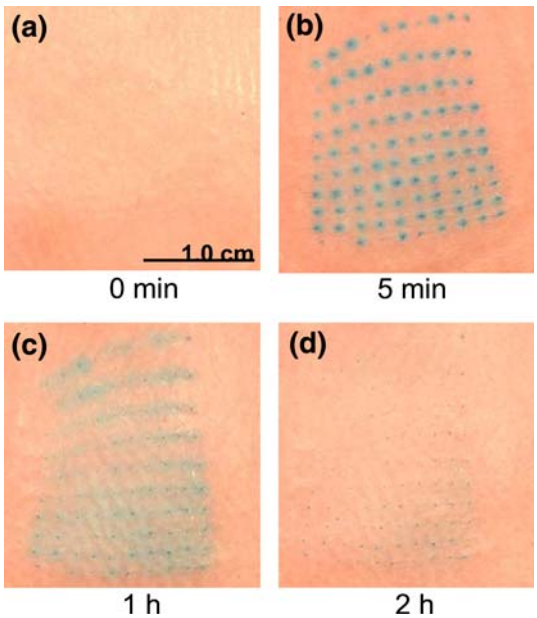

Fig. 5 Images of the rat skin before and after the administration of p-tip. **a** 0 min, **b** 5 h, **c** 1 h and **d** 2 h

Figure 5 shows the images of rat skin at 2 h after the p-tip administration. The blue color due to EB disappeared and there was no damage on the rat skin.

To study the dose-dependency of the hypoglycemic effects of insulin delivered by insulin loaded tip, two types of p-tips containing less insulin (0.70 ± 0.06 and 0.37 ± 0.03 IU) were prepared. These low insulin content in p-tip were administered to the rat skin, and pharmacodynamic studies were performed. The obtained plasma glucose level vs. time profiles are shown in Fig. 6. As shown in this figure, good dose-dependency of the plasma glucose level vs. time profile was observed in the rats. The pharmacodynamic parameter values are shown in Table 3.

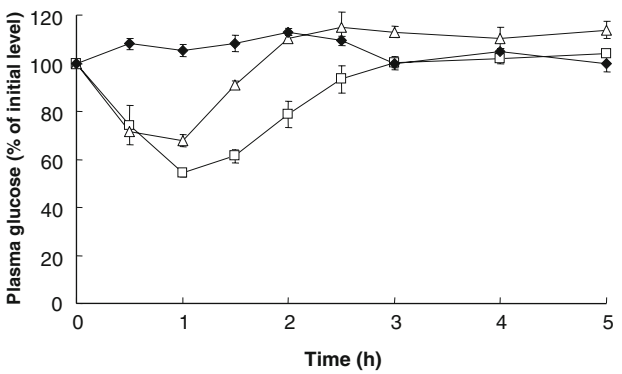

Fig. 6 Plasma glucose level-time curves after percutaneous administration of three kinds of p-tip containing different amounts of insulin to rats. *open square* median dose (0.37 ± 0.03 IU/rat), *open triangle* low dose (0.70 ± 0.06 IU/rat), *filled diamond* placebo tip. Each point represents the mean ± SE of 3 and 4 experiments

Table 3 Effect of dose on pharmacodynamic parameters of insulin after percutaneous administration of p-tips to rats

Preparation of p-tip	Insulin (IU)	C_{min} (%)	T_{min} (h)	AAC_{0-5} (%·h)	RPA (%)
Low dose	0.37 ± 0.03	67.9 ± 2.6	1.0 ± 0.0	34.3 ± 2.2	33.2 ± 1.2
Middle dose	0.70 ± 0.06	54.2 ± 1.4	1.0 ± 0.0	69.0 ± 7.2	35.2 ± 4.1

C_{min} minimum glucose level, *T_{min}* time when minimum glucose level were determined from the plasma glucose level versus time data, *AAC_{0-5h}* area above the plasma glucose level versus time data, *RPA* relative pharmacological availability compared to s.c. injection data

Each value represents the mean ± SE (n = 3–4)

There were no significant differences in either C_{min} or T_{min} between the two p-tips. On the other hand, the mean AAC_{0-5} of the lowest insulin loaded p-tips was 34.3 ± 2.2% h and the mean AAC_{0-5} of the median insulin content of p-tip was 69.0 ± 7.2% h, respectively. The AAC_{0-5} values showed good dose-dependency between these two p-tips. The RPA values of insulin delivered from the two p-tips were 33.2 ± 1.2 and 35.2 ± 4.1%, respectively.

4 Discussion

In this study, two types of insulin loaded tips, f-tip and p-tip, were prepared. As the same mold was used to prepare both, there were no significant differences in shape or size between the two tips. After the percutaneous application of tips, almost the same portions of the top part of the micropiles in the tip were administered to the rat skin as suggested in Fig. 4. By subtracting the length of the remaining portions of the micropiles from the authentic micropiles in the tip, the lengths of the micropiles in the tip that were inserted into the rat skin was estimated. The inserted length was 259 μm for the f-tip and 237 μm for the p-tip. Therefore, the top part of micropiles in the tip, of which the mean length was approximately 250 μm, was inserted into the rat skin. The epidermal thickness was reported to be 11.58 ± 1.02 μm for rats and 22.47 ± 2.40 μm for dogs [11]. With respect to human skin, the epidermal thickness was reported to be 60.3 ± 15.0 μm [12]. Therefore, the lengths of the micropiles in the tip were far longer than the epidermal thickness. However, the tip was made of water-soluble components, i.e., chondroitin sulfate, EB, and insulin. The tip was also conical, and the tops of the micropiles in the tip were very narrow (5–10 μm). Therefore, after the insertion of the tip into the skin, hydration and dissolution were thought to occur at the top part of micripiles in the tip, and 250 μm length of micropiles in the tip were inserted into the skin, because the tip encountered environments in which the water content can be as high as 70% [13]. Although the dermal region of the skin contains both of the microcapillary and nervous systems, no bleeding occurred in our experiment after the insertion of the tip into the rat skin. Therefore, the tip was administered to the epidermis not to the dermal region.

In this study, insulin was used as a model peptide drug for the preparation of self-dissolving micropile array tip (tip). However, microneedle technology is a good system for the percutaneous absorption of both low molecular weight organic compound drugs and macromolecular drugs that have low membrane permeability. Generally, macromolecular drugs are produced by recombinant technology and their bulk price is considerably more expensive than those of organic compound drugs. Therefore, high bioavailability (BA) is required to formulate them into pharmaceutical preparations. Wermeling et al. performed a clinical phase I trial with naltrexone (NTX) micropiles [8]. Metallic micropiles, made out of stainless steel, were used to pierce the skin and micro conduits were formed in the skin. Thereafter, drugs were applied to the skin with a sponge, and the plasma NTX levels were measured. Their pharmacokinetic study suggested that plasma NTX concentration vs. time curves show less inter-subject variation than those obtained after oral administration. In the case of NTX, it undergoes first-pass metabolism during its passage through the liver. Therefore, large inter-subject variation was observed in the plasma NTX concentration vs. time profiles [14]. On the other hand, in the case of microneedle TDDS, as NTX was directly absorbed into the systemic circulation without a hepatic first-pass effect, a higher plasma NTX concentration vs. time profile with low inter-subject variation was obtained. In addition, several groups have prepared coating formulations for micropiles [15–17]. When the clinical dose of the drug is less than 100 μg, the coating system is thought to work well. However, when larger amounts of the drug are coated onto the surface of micropiles, it is difficult to insert all the loaded drug into the epidermal and dermal areas. A considerable amount of the loaded drug is thought to leak out onto the skin surface due to the pressure formed by the insertion process. The self-dissolving micropile array tip (tip) overcomes the pitfalls of coating type micropiles. However, we have succeeded in preparing tips with molded microfabrication technology. Using our method, f-tip and p-tip can be prepared and the role of p-tip was emphasized. The mean

length of the drug loaded portion from the top of the micropiles in the tip was 316.0 ± 7.3 μm. By pressing the tip with a finger, the micropiles were inserted into the rat skin, and the distal ends of the micropiles in the tip dissolved in the skin as shown in Fig. 3. After administration, the tip was recovered, and the mean remaining amount of insulin was measured to be 169.4 ± 9.7 IU, which corresponds to 80.8% of the formulated amount of insulin. In the case of p-tip, approximately 65.8% of the loaded insulin was recovered. By loading insulin more to the top part of the micropiles in the tip, the recovered amount of insulin is decreased, and the BA of insulin will be increased.

5 Conclusion

Insulin loaded self-dissolving micropile array tip (tip) were made using mold based microfabrication technology and the superiority of p-tip to f-tip was studied by pharmacological experiments in rats using insulin as a model drug. The amount of insulin loaded into the p-tip was 1/4 of that of f-tip, and the pharmacological activity did not show a significant difference between the two kinds of tips. The p-tip is superior to f-tip because less insulin is formulated to p-tip.

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