Pharmaceutical Research, Vol. 24, No. 7, July 2007 (© 2007)
DOI: 10.1007/s11095-007-9256-x

Research Paper

Novel Microneedle Patches for Active Insulin Delivery are Efficient in Maintaining Glycaemic Control: An Initial Comparison with Subcutaneous Administration

Lina Nordquist,[1,3] Niclas Roxhed,[2] Patrick Griss,[2] and Göran Stemme[2]

Received October 30, 2006; accepted January 30, 2007; published online March 27, 2007

Purpose. Good glycaemic control is essential to minimize the risk for diabetes-induced complications. Also, compliance is likely to be higher if the procedure is simple and painless. This study was designed to validate painless intradermal delivery via a patch-like microneedle array.
Materials and Methods. Diabetes was induced by an intravenous injection of streptozotocin (50 mg/kg bw) in adult male Sprague Dawley rats. Plasma insulin and blood glucose were measured before, during and after subcutaneous or intradermal (microneedles) infusion of insulin (0.2 IU/h) under Inactin-anaesthesia.
Results. Before insulin administration, all animals displayed a pronounced hyperglycaemia (19 ± 1 mM; 359 mg/dl). Administration of insulin resulted in a reduced plasma glucose independently of administration route (subcutaneous 7.5 ± 4.2, $n = 9$, and intradermal 11 ± 1.8, $n = 9$ after 240 min), but with less errors of the mean in the intradermal group. In the intradermal group, plasma insulin was increased in all latter measurements (72 ± 22, 81 ± 34, and 87 ± 20 μIU/ml), as compared to the first measurement (26 ± 13). In the subcutaneous group, plasma insulin was elevated during the last measurement (to 154 ± 3.5 μIU/ml from 21 ± 18).
Conclusion. This study presents a novel possibility of insulin delivery that is controllable and requires minimal training. This treatment strategy could improve compliance, and thus be beneficial for patients' glycaemic control.

KEY WORDS: diabetes; insulin lispro; intradermal; microneedles; rats; transdermal.

INTRODUCTION

With the discovery of insulin diabetes management was revolutionized. Today, more than 80 years have elapsed since then. Insulin pumps and fast-acting insulin analogues have been developed, and transplantation of islets of Langerhans may soon be available for a selected few. Despite this, diabetes commonly leads to a number of mainly vascular complications (1). To avoid this, good compliance to achieve glycaemic control is mandatory. This has been demonstrated by The Diabetes Control and Complication Trial Research Group, who concluded that continuous administration with a subcutaneous insulin pump improves long-term glycaemic control in pediatric, as well as adult diabetic patients (1,2), and markedly reduces the risk of long-term complications in a cohort of 1,441 diabetic patients (1). The Diabetes Control and Complication Trial Research Group therefore recommended that most type 1 diabetic patients be treated with intensive regimens (1).

In spite of the knowledge on the pathogenesis of diabetes complications, the majority of diabetic patients today fail to achieve optimal glycaemic control even in clinical trials, and even more so in clinical practice (1). Multiple daily injections are painful and cause trauma to the skin, and so make it difficult for diabetic patients to enforce and maintain a sufficient compliance. One out of four diabetes patients taking insulin describe some anxiety regarding self-injection (3,4). The reluctance and suboptimal compliance in diabetic patients using multiple daily injection regimens, together with their fear of hypoglycaemia, are some factors that put up a barrier to achieving good glycaemic control in these patients (5). Further, since nearly 65% of patients with type 1 or type 2 diabetes are reported not to be confident in their ability to manage their disease effectively by themselves (3), a self-regulating insulin system could improve the patients' quality of life. This is further emphasized by the fact that close to 20% of diabetic children place their injections inappropriately (6). Finally, patients express a preference for discreet, non attention-drawing treatments that are easy to use (7).

During the past few years, considerable effort has been put into developing novel, more comfortable routes of insulin

[1] Department of Medical Cell Biology, Division of Integrative Physiology, Biomedical Centre, Uppsala University, Box 571, 751 23, Uppsala, Sweden.
[2] Microsystem Technology Laboratory, School of Electrical Engineering, Royal Institute of Technology, Stockholm, Sweden.
[3] To whom correspondence should be addressed. (e-mail: Lina.Nordquist@medcellbiol.uu.se)

0724-8741/07/0700-1381/0 © 2007 Springer Science + Business Media, LLC

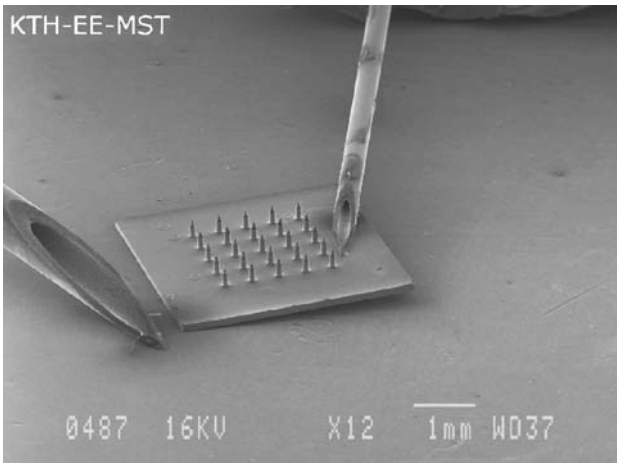

Fig. 1. Scanning Electron Microscopy (SEM) picture showing a complete microneedle chip next to conventional hypodermic needles (20G and 27G).

administration, e.g. gastrointestinal, nasal, and inhalation therapy (4,8). Gastrointestinal and nasal administrations have so far been unsuccessful, whereas inhalation therapy has been successful. However, the inhalation devices are impractical in size, and the long-term safety of inhaled formulations has not been evaluated. Insulin is known to possess a growth factor activity, and there is concern that intra-alveolar deposition of insulin could adversely affect pulmonary function (9,10). Other routes are therefore needed, without compromising with the comfort and future health of patients.

Recently, though, attention has been drawn to the possibility of using a patch-needle hybrid to deliver insulin. These hybrids consist of short, micrometer-scale needles, and hopes are they can be used for drug delivery (11,12), allowing a drug to diffuse to the rich capillary bed of the dermis for uptake and subsequent systemic distribution in the blood stream. Since these needles would be inserted no deeper than the outmost, non-innervated layer of the skin, this technique would allow painless delivery (13). If sufficient bioavailability could be obtained using this route of administration, one could achieve the advantages of subcutaneous drug delivery, but in a non attention drawing and minimally invasive manner. Since studies report needle size and fear of pain as two major reasons for injection anxiety (4), such a device could improve patient acceptance, and the development of a "controlled release"-design could further prevent long-term complications.

This *in vivo* study was designed to quantitatively elucidate a novel, painless route of insulin administration; active, intradermal delivery to diabetic animals with an integrated patch-like microneedle system.

MATERIALS AND METHODS

Animals and induction of diabetes. The study was performed on 61 age-matched, anaesthetized male Sprague-Dawley rats (M&B, Ry, Denmark). The rats were allowed free access to water and standard rat chow (R36, Ewos, Södertälje; Sweden, containing 0.3% sodium, 0.8% potassium, and 21% protein) throughout the study. Two weeks prior to the experiment, the rats were made diabetic by means of a single intravenous injection of streptozotocin (STZ, Sigma-Aldrich, St Louis, Mo, USA, 50 mg/kg BW) (14) dissolved in 0.2 ml saline, resulting in blood glucose above 18 mM (340 mg/dl) within 3 days. After induction of diabetes, the rats were treated with a daily subcutaneous injection of long-acting insulin (6 AM, Insulatard, Novo Nordisk, Hjørring, Denmark; 5 IU · kg BW^{-1} · day^{-1}; 1 IU/ml (international unit) equals 6.95 nM). Body weight and blood glucose concentrations were monitored every second day (6 AM) throughout the entire experimental period, in order to evaluate the degree of hyperglycaemia and severity of diabetes. Blood samples were obtained from the cut tips of the tail and analysed by a glucose oxidase method (15–20 µl, Precision QID, MediSense, Bedford MA, USA). Animals were excluded if they developed a weight loss of more than 10%, or if the blood glucose concentrations exceeded 30 mM (560 mg/dl) on more than two consecutive days. All experiments were approved by the Uppsala Ethical Committee for Animal Experiments and were performed in accordance with national and European guidelines for the care and use of laboratory animals.

General surgical procedure. Thirteen to 15 days after the induction of diabetes, the animals were anaesthetized with an intraperitoneal injection of Inactin (sodium-5-sec-butyl-5-ethyl-2-thiobarbiturate; Sigma Chemical Co., St Louis, MO, USA; 100 mg/kg). The anaesthetized rats were placed on a servo-controlled heating pad in order to maintain a core temperature of 37.5 °C, and tracheotomised to facilitate spontaneous breathing. The right femoral artery was catheterized for blood pressure monitoring and for withdrawal of blood samples, and the right femoral vein was catheterized for substance administration and infusion of saline 10 ml · kg BW^{-1} h^{-1}. The urinary bladder was catheterized for drainage of urine.

Microneedle-based infusion patch. Insulin was administered by a novel intradermal patch system featuring miniaturized, micro-fabricated hollow needles (Fig. 1) and a small electrically controlled drug dispenser. The microneedles are 400 µm long and made to only penetrate the outermost skin layers and without reaching the nerve receptors in the skin. As a consequence the administration form is painless (13). Also, due to the shallow penetration and low flow rates, it is

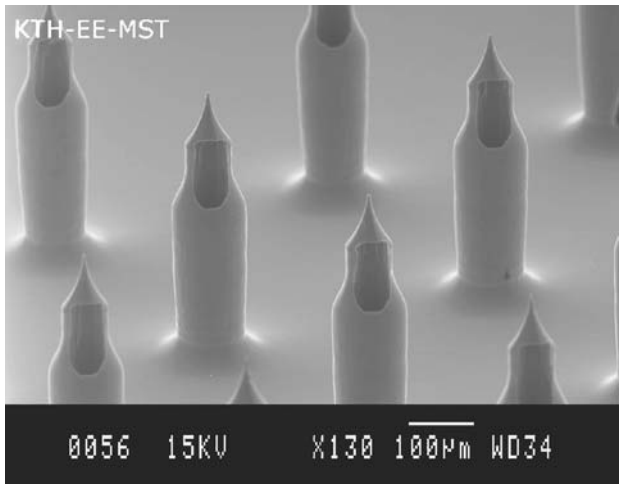

Fig. 2. Scanning Electron Microscopy (SEM) picture showing a close-up on several microneedles.

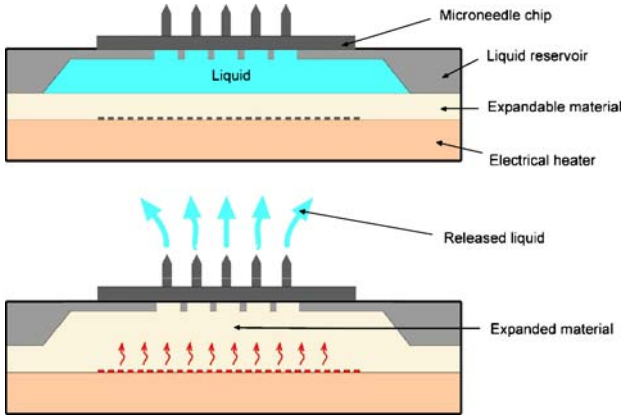

Fig. 3. Schematic view of the dispenser's principle of operation. When a voltage is supplied to the heater the expandable material expands into the liquid reservoir, consequently ejecting the liquid through the hollow microneedles.

highly unlikely for leakage into the hypodermal space to compromise the delivery with improper positioning etcetera. The microneedles are fabricated by plasma etching of monocrystalline silicon using batch processing techniques from the MEMS (MicroElectroMechanical Systems) and microelectronics industry (15). The needles are organized on 4 mm × 4 mm chips with 21 hollow needles (and four non-hollow needles for fabrication yield reasons). Fig. 2 shows a scanning electron microscopy image of the microneedles. The fabrication process, as well as the microneedles have been previously described (16).

The drug dispenser, fabricated using similar microfabrication techniques, is designed to store and dispense a drug volume at a certain flow-rate. Its working principle is based on a thermally expandable silicone material which expands into a liquid reservoir and thereby causing the liquid to move. The expansion rate and thus the flow of the liquid, is controlled by the voltage supplied to the dispenser. A characterization of the dispenser at stable temperature conditions has been made earlier, cf. (17). Fig. 3 shows a schematic view of the dispenser's working principle. In the tested devices, dispensers with a drug reservoir of 12 µl and outer dimensions of 10 mm × 10 mm × 1.5 mm were used. Each dispenser was equipped with a microneedle chip incorporated using adhesives. The study recently performed by Roxhed et al (17) gives a more comprehensive description of the drug dispenser. Fig. 4 shows an assembled patch system with microneedles and drug dispenser.

Doses and preparation. Microneedle-based patch systems were filled with the synthetic insulin analogue Lispro (18) insulin (Humalog, 100 IU/ml, Eli Lilly and Company, Indianapolis, IN, USA, (RIA specificity 100 %)) to avoid measurement cross-reactivity with human (RIA specificity <0.5 %) or endogenous rat insulin (RIA specificity <0.5 %). On all rats, the fur coat was removed with potassium thioglycolate (Veet creme, Adaco AB, Stockholm, Sweden) from a limited area of the rats thorax, and before starting an experiment, the administration device was attached to this dry, hairless area of the skin and fixated by a piece of cellartape. In groups D-iv and D-sc, a hypodermic needle was connected to a syringe pump. In groups D-forced-low, D-forced-high, and D-diff, a microneedle-based infusion patch was gently attached to the skin using moderate finger force and fixated with a piece of surgical tape across the device. The device was then connected to an external power supply through pre-mounted thin electrical leads. Before and after each experiment, the device was weighed to ensure proper flow rates. In group D-puncture, the rat's skin was pierced once using a microneedle chip mounted on a staff, after which 15 µl of Lispro insulin was placed on the pierced skin area. To prevent evaporation of the insulin, a piece of Parafilm M laboratory film (Nesco, Heraco AB, Holmsund, Sweden) was placed on top of the liquid.

Experimental protocol. After the initial surgery and a 45-min recovery period, glucose and insulin base-line values were recorded. Thereafter, in all groups except D-c, plasma insulin and glucose were recorded while lispro insulin (100 IU/ml, Eli Lilly Sweden AB, Stockholm, Sweden) was supplied to all groups for a total of 180 min. In the intravenous group (D-iv), the insulin concentration was adjusted to avoid severe hypoglycaemia. After ending administration, plasma insulin and blood glucose values were recorded for another 60 min. Throughout the experiment, all animals were given an intravenous infusion of saline solution (10 ml kg $BW^{-1} \cdot h^{-1}$ to diabetic animals) to compensate for fluid loss (Fig. 5).

Experimental groups. Seven groups of diabetic animals were studied before and after drug administration:

Intravenous saline solution only (D-c, $n = 9$)

Intravenous insulin infusion (D-iv; 0.14 IU/h, 70 IU/ml, $n = 7$)

Subcutaneous insulin infusion, (D-sc; 0.20 IU/h, 100 IU/ml, $n = 9$)

Microneedle-aided intradermal low-rate insulin infusion (D-forced-low; 0.20 IU/h, 100 IU/ml, $n = 9$)

Microneedle-aided intradermal high-rate insulin infusion (D-forced-high; 0.40 IU/h, 100 IU/ml, $n = 9$)

Microneedle-aided intradermal passive insulin diffusion (D-diff; 100 IU/ml, $n = 9$).

Insulin on microneedle-penetrated skin (D-puncture; 100 IU/ml, n = 9).

Data collection. In each experimental group, blood pressure was continuously measured by connecting the

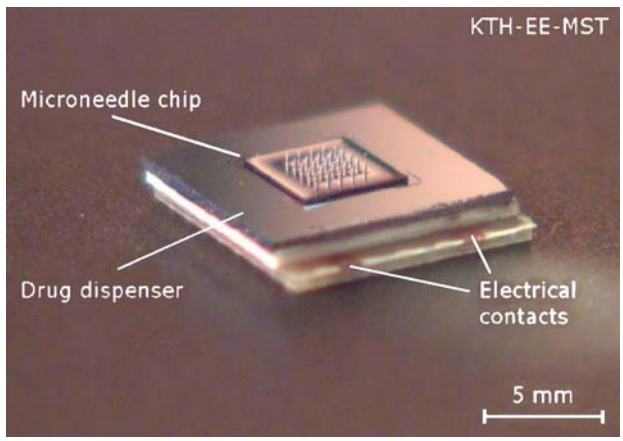

Fig. 4. Photograph of an assembled drug delivery patch. The unit can store and dispense 12 µl of liquid.

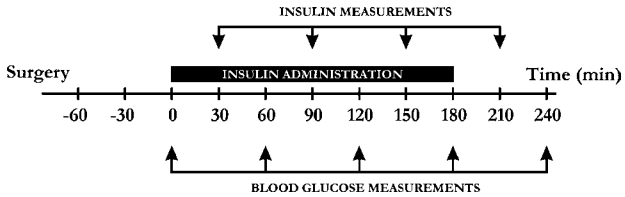

Fig. 5. Time line of the experimental procedure.

arterial catheter to a polygraph (Model 7D Polygraph, Grass Instrument Co., Quincy, MA., USA). Blood clotting in the catheter was prevented by addition of heparin (50 IU/ml). Every 60 min, blood samples were obtained from the cut tip of the tails and analysed for glucose concentration by a glucose oxidase method (15–20 μl, Precision QID, MediSense, Bedford MA, USA). Every 60 min, a blood sample was obtained, and the plasma insulin lispro concentration was specifically determined using a ^{125}I-monoiodinated, competitive-binding radioimmunoassay analysis of plasma lispro insulin content (Lispro insulin RIA kit, Linco Research, MO, USA).

Statistical evaluation. All statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA., USA). Descriptive statistics are presented as mean values ± SEM. Multiple data sets between groups were analysed with analyses of variance (ANOVA) followed by Tukey's post hoc test when appropriate. Multiple data sets within groups were analysed with ANOVA followed by Dunnet's post hoc test when appropriate. When analysing two data sets, unpaired or paired Student's *t*-tests were applied for comparisons between or within the same group, respectively. For all comparisons, $P < 0.05$ was considered statistically significant.

RESULTS

All animals displayed a pronounced elevation of blood glucose concentration (19.1 ± 0.5 mM, $n = 61$ as compared to baseline values 5.7 ± 0.1; 1 mM glucose converts to 18.8 mg/dl) in the present study and had an average weight of 277 ± 4 g (average increase 9% as compared with pre-diabetic weights). Mean arterial pressure and hematocrit remained unaffected throughout the experiments in all investigated groups (data not shown).

Insulin concentrations. Plasma insulin lispro concentrations were measured after 30, 90, 150, and 210 min of infusion (Table I, Figs. 6 and 7). In the microneedle-infused group with a low-rate delivery rate (D-forced-low), insulin concentrations were increased from 90 min and onwards, as compared to the first measurement. In the group with the high-rate infusion (D-forced-high), insulin concentrations were increased at 150 and 210 min, as compared to the first measurement. In the microneedle-infused group with a high-rate delivery rate (D-forced-high), there was a 90% increase in plasma insulin concentration after 210 min as compared to the group with the low-rate infusion, but otherwise no differences between the groups were seen. In the subcutaneously infused animals (D-sc), insulin concentrations were elevated in the last measurement. Animals receiving an intravenous infusion (D-iv), did not display any changes in insulin concentrations between measurements. This was also the case in the passively microneedle-infused group (D-diff) and the pierced skin group (D-puncture).

Blood glucose concentrations. Blood glucose was measured at baseline, and after 60, 120, 180, and 240 min of infusion (Table II, Figs. 8 and 9, 1 mM glucose converts to 18.8 mg/dl). At baseline, all animals displayed a pronounced hyperglycaemia (19.1 ± 0.5 mM; 359 mg/dl). The elevation persisted during infusion of saline (D-c) until the last measurement at 240 min. After 60 min, blood glucose was lowered only in the intravenously infused group (D-iv) and the microneedle-infused group with a low-rate delivery rate (D-forced-low). Lispro insulin infused subcutaneously (D-sc) or intradermally with a high-rate delivery (D-forced-high) resulted in reduced plasma glucose after 120 min, as did passive diffusion through microneedle patches (D-diff). In the pierced skin group (D-puncture) blood glucose was lowered only in the two last measurement periods, i.e., after 180 min.

There was no difference between groups in blood glucose before the experiment. The intravenously infused group (D-iv) and the microneedle-infused group with a low-rate delivery rate (D-forced-low) differed from time control (D-c) in all measurements except baseline. The group infused intradermally with a high-rate delivery (D-forced-high), the group infused subcutaneously (D-sc), and the group receiving insulin lispro passively through the microneedles (D-diff) differed from time control (D-c) after 120 min. The pierced skin group (D-puncture) did not at all differ in blood glucose from time controls (D-c) during the experiment.

DISCUSSION

All results taken together, it is not unlikely that patch-like microneedles could be used to actively administer insulin in a manner comparable to standard, subcutaneous treat-

Table I. Mean Insulin Lispro Concentrations (μIU/ml) in Diabetic Animals During Microneedle-Aided Intradermal Low-Rate Insulin Infusion, (D-forced-low; 0.20 IU/h, $n = 9$), Microneedle-Aided Intradermal High-Rate Insulin Infusion, (D-forced-high; 0.40 IU/h, $n = 9$), Subcutaneous Insulin Infusion, (D-sc; 0.20 IU/h, $n = 9$), Microneedle-Aided Intradermal Passive Insulin Diffusion (D-diff; $n = 9$), Insulin Administered on Microneedle-Penetrated Skin (D-puncture; $n = 9$), and Intravenous Insulin Infusion (D-iv; 0.14 IU/h, $n = 7$)

Time (min)	D-forced-low	D-forced-high	D-sc	D-diff	D-puncture	D-iv
30	26 ± 14	57 ± 20	21 ± 18	50 ± 49	43 ± 18	121 ± 35
90	73 ± 27*	48 ± 11	50 ± 27	35 ± 25	96 ± 33	129 ± 25
150	81 ± 34*	113 ± 29*	41 ± 15	9 ± 4	39 ± 22	215 ± 43
210	87 ± 20*	167 ± 15*	154 ± 35*	44 ± 20	64 ± 17	196 ± 20*

* Denotes $P < 0.05$ when comparing with 30-min values within the same group.

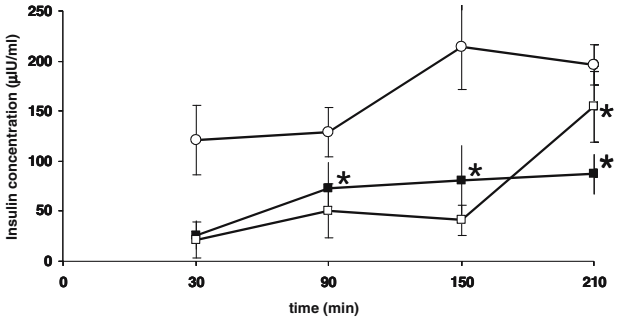

Fig. 6. The effect of route of administration of insulin lispro on insulin concentration. *Filled square* denotes microneedle-aided intradermal infusion, (D-forced-low; 2 µl/h, 100 IU/ml, $n=9$), *open square* denotes subcutaneous infusion (D-sc; 2 µl/h, 100 IU/ml, $n=9$), and *open circle* denotes intravenous infusion (D-iv; 2 µl/h, 70 IU/ml, $n=7$). *Asterisk* denotes a difference compared to the first measurement in that same group. For all measurements, $P<0.05$ was considered statistically significant.

ment. If so, the controllable, intense therapy together with an improved compliance due to the minimally invasive, painless profile of this device could prove beneficial in the prevention of long-term complications in diabetes.

Good glycaemic control is essential in order to minimize the risk for diabetes-induced complications, and this initial study shows no disadvantage using the intradermal patches as compared to the standard, subcutaneous delivery route. Subcutaneous injections are known to cause variability in dose from injection to injection (5). Intradermal delivery reduced this variability, possibly due the size of the infusion area minimizing the influence of the infusion site. The novel, forced intradermal infusion showed a significant effect on blood glucose before an effect on blood glucose was obtained with subcutaneous infusion. This could also be due to the larger infusion area of the microneedle array. Also, in accordance to the expected, with a delivery rate twice as high (D-forced-high), there was a doubling in plasma insulin concentration after 210 min as compared to the group with the low-rate infusion (D-forced-low), i.e., from 87 ± 11 to 167 ± 9 µIU/ml. Although it is likely that a doubling of the dose of insulin would be reflected in plasma concentrations, a certain extent of lag time before a drug is reflected in plasma cannot be avoided in any delivery other than intravenous administration. Therefore, plasma insulin concentrations should not be immediately affected after a change in dose. This delayed increase thus indicates that this integrated device seems to be readily controlled. It needs to be taken into consideration, however, that the dermal capillaries involved in drug uptake with these microneedles also play a major part in temperature regulation. It may therefore be wise to apply the patch-like array where temperature conditions are less varying, e.g. under clothing, to avoid fluctuations in blood flow and drug uptake.

In previous attempts at intradermal delivery, solid needles have been used to penetrate the stratum corneum and facilitate skin permeability to insulin (19,20,23,28). The needles were then used to pierce minute holes in the skin as a pretreatment before the application of the drug solution, making the insulin detectable in the systemic circulation. While dose-adjustment was not possible, and flow rates could not be controlled in this manner, these results implied intradermal delivery as a possible route of insulin administration.

Previous studies have also demonstrated fabrication and (21–23) intradermal infusion with hollow microneedles (19,20,23–26). These studies are fewer, since hollow needles are inherently weaker than their solid counterparts, making both design and insertion more difficult. In vitro results from thawed cadaver skin look promising (24), but few data have been obtained from in vivo studies on diabetic animals, and never on animals given the time to develop the skin characteristics of diabetes mellitus (27). Furthermore, previous studies have been performed on small groups of anaesthetized rats without facilitated breathing, compensation for fluid losses over time or the recording of hematocrite and blood pressure to guarantee physiologically stable conditions (28). Such factors are especially important in streptozotocin-treated animals, since their fluid losses are twice those of normoglycaemic animals, a factor that could influence plasma concentrations directly, as well as the activity of insulin-antagonists such as adrenaline (29). Also, an effect of dehydration on skin characteristics and drug uptake cannot be ruled out (30,31). Finally, there have been no reports describing an integrated system where the microneedles are attached to a drug dispenser, and there is little previous research on active microneedle delivery, where the insulin can be administered in a controllable fashion.

There were no detectable differences in blood glucose between the high- (D-forced-high) and low-rate (D-forced-low) insulin delivery groups, probably due to interindividual differences and to the well documented phenomenon of insulin resistance in diabetic rats (32–34). However, following each group over time, Figs. 7 and 9 demonstrate the advantages of forced, intradermal insulin infusion as opposed to delivery via previously penetrated pores. The penetrated curve displayed a difficult-to-maneuver, uneven concentration, whereas the forced infusion seemed to render possible a controlled distribution to the systemic circulation. Also, the penetrated infusion produced a larger standard error of the mean (SEM) in blood glucose than did the forced infusions (D-puncture; SEM = 4.0, as compared to D-forced-low; SEM = 1.8, and D-forced-high; SEM = 1.2).

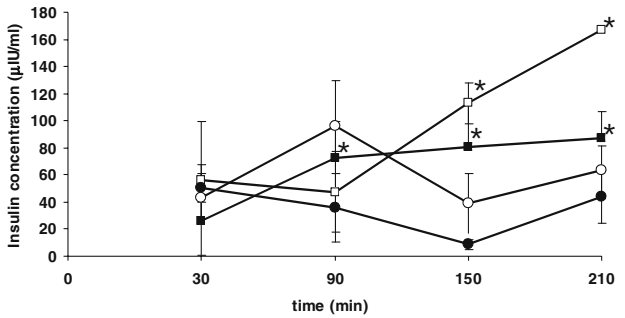

Fig. 7. The effect of rate of intradermal administration of insulin lispro on insulin concentration. *Filled square* denotes microneedle-aided low-rate intradermal infusion, (D-forced-low; 2 µl/h, 100 IU/ml, $n=9$), *open square* denotes microneedle-aided intradermal high-rate infusion, (D-forced-high; 4 µl/h, 100 IU/ml, $n=9$) , *filled circle* denotes microneedle-aided intradermal passive infusion (D-diff; 0 µl/h, 100 IU/ml, $n=9$), and *open circle* denotes rats whose skin was penetrated with microneedles, and then passively infused (D-puncture; 2 µl/h, 100 IU/ml, $n=9$). *Asterisk* denotes a difference compared to the first measurement in that same group. For all measurements, $P<0.05$ was considered statistically significant.

Table II. Mean Blood Glucose Concentrations (mM) in Diabetic Animals during Microneedle-Aided Intradermal Low-Rate Insulin Infusion, (D-forced-low; $n=9$), Microneedle-Aided Intradermal High-Rate Insulin Infusion, (D-forced-high; $n=9$), Subcutaneous Insulin Infusion, (D-sc; $n=9$), Microneedle-Aided Intradermal Passive Insulin Diffusion (D-diff; $n=9$), Insulin Administered on Microneedle-Penetrated Skin (D-puncture; $n=9$), Intravenous Insulin Infusion (D-iv; $n=7$), and Time Control (D-c; $n=9$)

Time (min)	D-forced-low	D-forced-high	D-sc	D-diff	D-puncture	D-iv	D-c
0	19±1	19±1	18±3	19±2	20±3	18±2	20±1
60	16±3**	17±1	16±3	17±2*	18±2	8±2*·***	20±1
120	13±2*·***	14±1*·***	15±4**	16±1**·*	17±3	4±1*·***	20±1
180	12±2*·***	12±1*·***	9±4*·***	15±1*·**	15±3*	3±0.5*·***	19±1
240	11±2*·***	9±1*·***	7±4*·***	14±1*·**	13±5*	5±1*·***	18±1*

*Denotes $P<0.05$ when comparing with 30-min values within the same group, ** $P<0.05$ when compared to vehicle-treated animals within the same period, whereas *** denotes $P<0.001$ when compared to vehicle-treated animals within the same period.

Recent results from cadaver skin suggest that, top-opened, hollow microneedles could be retracted after insertion to optimize infusion properties. Flow rate has been shown to increase more than tenfold after retraction of the needles (24,25). The explanation for this is probably that the insertion of the microneedles compacts the tissue, thus lowering the skin flow conductivity beneath the needle tips (35). Although the microneedles used in the present study have their needle openings on the side of the needles (and therefore less affected by compaction), these findings suggest insulin infusion could possibly be made further effective, was the needle array to be partially retracted after insertion. Other flow-rate increasing methods, such as adding hyaluronidase to the formulation, should also be evaluated, although such methods are associated with considerable costs, and the risk of affecting the patient's immune system must be considered.

After 80 years of insulin therapy, subcutaneous insulin injections or infusion with fast-acting insulins such as Humalog are still golden standard. However, compliance is likely to be higher if the procedure is simple and painless, as with the microneedle patch (17). The introduction of continuous insulin pumps was a step in this direction. However, insulin pump therapy does not fulfill patients' demands for a painless, discreet, and easy-to-use treatment regimen. Judging from the insulin lispro data on the microneedle-based infusion patches used in this study, a diabetic patient using 0.5–1 IU/kg insulin daily would require a patch approximately 2.5×2.5 cm, i.e. roughly the size of a 1/2 US dollar coin, were the patch is to be filled with standard insulin of 100 IU/ml and changed daily. The system is fabricated using microfabrication batch techniques which potentially allow high volume and cost-efficient production. Thus, the patch-like microneedles are a feasible option to improve glycaemic control, and reduce long-term complications.

We have shown that microneedles are a possible treatment strategy for a common, fast-acting insulin lispro. In parallel with avoiding multiple-injection regimens, this route of administration requires minimal training and attention. Further studies are needed, investigating this device further in terms of increased flow rate, biocompatibility and device safety, and to study designs with incorporated blood glucose sensing and a self-controlled insulin rate. Given this development, all insulin injections could be replaced by these microneedles. The patch-like appearance would reduce needle anxiety, as well as minimize social difficulties associated with

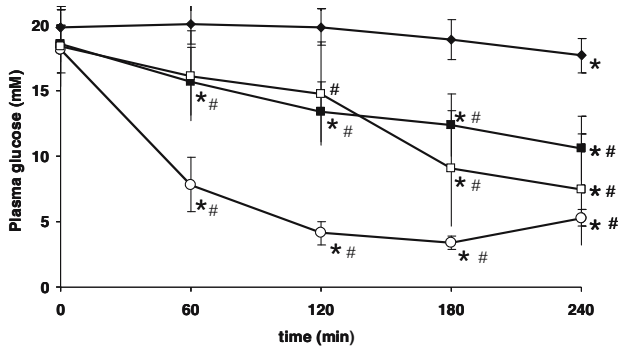

Fig. 8. The effect of route of administration of insulin lispro on blood glucose. *Filled square* denotes microneedle-aided intradermal infusion, (D-forced-low; 2 μl/h, 100 IU/ml, $n=9$), *open square* denotes subcutaneous infusion (D-sc; 2 μl/h, 100 IU/ml, $n=9$), *filled diamond* denotes time control, and *open circle* denotes intravenous infusion (D-iv; 2 μl/h, 70 IU/ml, $n=7$. *Asterisk* denotes a difference compared to the first measurement in that same group and *number sign* denotes differences when comparing to vehicle-treated animals within the same time period. For all measurements, $P<0.05$ was considered statistically significant.

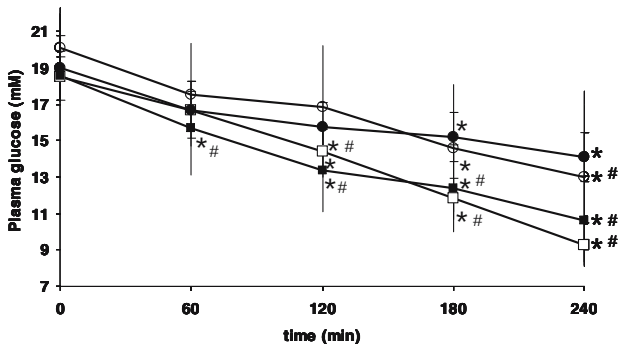

Fig. 9. The effect of rate of intradermal administration of insulin lispro on blood glucose. *Filled square* denotes microneedle-aided low-rate intradermal infusion, (D-forced-low; 2 μl/h, 100 IU/ml, $n=9$), *open square* denotes microneedle-aided intradermal high-rate infusion, (D-forced-high; 4 μl/h, 100 IU/ml, $n=9$), *filled circle* denotes microneedle-aided intradermal passive infusion (D-diff; 0 μl/h, 100 IU/ml, $n=9$), and *open circle* denotes rats whose skin was penetrated with microneedles, and then passively infused (D-puncture; 2 μl/h, 100 IU/ml, $n=9$). *Asterisk* denotes a difference compared to the first measurement in that same group. and *number sign* denotes differences when comparing to vehicle-treated non-diabetic animals within the same time period. For all measurements, $P<0.05$ was considered statistically significant.

self-injecting. Thus, this technique could prove to be useful for newly diagnosed young type 1 diabetics, as well as for the type 2 diabetics who are today delaying initiation or intensification of insulin injections. In addition, patient-reported fear of blood glucose self-testing by finger pricking (36) is possible to target with this treatment method, since the microneedles can also be used for glucose monitoring as shown by others (37).

Finally, microneedle technology has not only been studied with regard to insulin therapy (38–42). This needle design could also prove to be efficient in systemic as well as local delivery of other macromolecular drugs, such as DNA vaccines, and endocrine substances, e.g. desmopressin.

In conclusion, this study showed promising results putting the intradermal route of delivery on the map in insulin treatment. This study presents a novel possibility of insulin delivery that is controlled and patient-friendly. The findings in the present investigation indicate that microneedle arrays used as intradermal patches can be used clinically to bring about a more efficient treatment for diabetic patients.

ACKNOWLEDGEMENTS

The sharp-minded and kind-hearted help and support from Professor Leif Jansson is gratefully acknowledged. This work was funded by the Swedish Foundation for Strategic Research (SFF), the Swedish Association of Nephrology, the Petersenska Hemmet Foundation, and the Scandinavian Physiological Society.

REFERENCES

1. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N. Engl. J. Med.* **329**: 977–986 (1993).
2. N. Sulliand and B. Shashaj. Long-term benefits of continuous subcutaneous insulin infusion in children with Type 1 diabetes: a 4-year follow-up. *Diabet. Med.* **23**:900–906 (2006).
3. M. Korytkowski, L. Niskanen, and T. Asakura. FlexPen: Addressing issues of confidence and convenience in insulin delivery. *Clin. Ther.* **27**(Suppl B):S89–S100 (2005).
4. D. R. Owens, B. Zinman, and G. Bolli. Alternative routes of insulin delivery. *Diabet. Med.* **20**:886–898 (2003).
5. M. Davies. The reality of glycaemic control in insulin treated diabetes: defining the clinical challenges. *Int. J. Obes. Relat. Metab. Disord.* **28**(Suppl 2):S14–S22 (2004).
6. N. H. Birkebaek, A. Johansen, and J. Solvig. Cutis/subcutis thickness at insulin injection sites and localization of simulated insulin boluses in children with type 1 diabetes mellitus: need for individualization of injection technique? *Diabet. Med.* **15**:965–971 (1998).
7. M. Korytkowski, D. Bell, C. Jacobsen, and R. Suwannasari. A multicenter, randomized, open-label, comparative, two-period crossover trial of preference, efficacy, and safety profiles of a prefilled, disposable pen and conventional vial/syringe for insulin injection in patients with type 1 or 2 diabetes mellitus. *Clin. Ther.* **25**:2836–2848 (2003).
8. A. H. Barnett. Exubera inhaled insulin: a review. *Int. J. Clin. Pract.* **58**:394–401 (2004).
9. T. C. Woods, B. Zhang, F. Mercogliano, and S. M. Dinh. Response of the lung to pulmonary insulin dosing in the rat model and effects of changes in formulation. *Diabetes Technol. Ther.* **7**:516–524 (2005).
10. T. K. Mandal. Inhaled insulin for diabetes mellitus. *Am. J. Health Syst. Pharm.* **62**:1359–1364 (2005).
11. M. R. Prausnitz. Overcoming skin's barrier: the search for effective and user-friendly drug delivery. *Diabetes Technol. Ther.* **3**:233–236 (2001).
12. M. A. Teo, C. Shearwood, K. C. Ng, J. Lu, and S. Moochhala. *In vitro* and *in vivo* characterization of MEMS microneedles. *Biomed Microdevices* **7**:47–52 (2005).
13. S. Kaushik, A. H. Hord, D. D. Denson, D. V. McAllister, S. Smitra, M. G. Allen, and M. R. Prausnitz. Lack of pain associated with microfabricated microneedles. *Anesth. Analg.* **92**:502–504 (2001).
14. A. Junod, A. E. Lambert, W. Stauffacher, and A. E. Renold. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. *J. Clin. Invest.* **48**:2129–2139 (1969).
15. M. Madou. Fundamentals of Microfabrication, CRC, (1997).
16. N. Roxhed, P. Griss, and G. Stemme. Reliable *in-vivo* penetration and transdermal injection using ultra-sharp hollow microneedles. In *Proceedings IEEE International Conference on Solid State Sensors, Actuators, and Microsystems (Transducers)*, vol. 1, IEEE, Seoul, Korea, 2005, pp. 213–216.
17. N. Roxhed, B. Samel, L. Nordquist, P. Griss, and G. Stemme. Compact, Seamless Integration of Active Dosing and Actuation with Microneedles for Transdermal Drug Delivery. In *IEEE International Conference on Micro Electro Mechanical Systems (MEMS)*, 2006, pp. 414–417.
18. D. C. Howey, R. R. Bowsher, R. L. Brunelle, H. M. Rowe, P. F. Santa, J. Downing-Shelton, and J. R. Woodworth. [Lys(B28), Pro(B29)]-human insulin: effect of injection time on postprandial glycemia. *Clin. Pharmacol. Ther.* **58**:459–469 (1995).
19. W. Martanto, S. P. Davis, N. R. Holiday, J. Wang, H. S. Gill, and M. R. Prausnitz. Transdermal delivery of insulin using microneedles in vivo. *Pharm. Res.* **21**:947–952 (2004).
20. D. V. McAllister, P. M. Wang, S. P. Davis, J. H. Park, P. J. Canatella, M. G. Allen, and M. R. Prausnitz. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *Proc. Natl. Acad. Sci. USA* **100**:13755–13760 (2003).
21. S. P. Davis, B. J. Landis, Z. H. Adams, M. G. Allen, and M. R. Prausnitz. Insertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force. *J. Biomech.* **37**:1155–1163 (2004).
22. P. Griss and G. Stemme. Side-opened out-of-plane microneedles for microfluidic transdermal liquid transfer. *IEEE ASME J. Microelectromech. Syst.* **12**:296–301 (2003).
23. H. J. G. E. Gardeniers, R. Luttge, E. J. W. Berenschot, M. J. de Boer, S. Y. Yeshurun, M. Hefetz, R. van't Oever, and A. van den Berg. Silicon micromachined hollow microneedles for transdermal liquid transport. *IEEE ASME J. Microelectromech. Syst.* **12**:855–862 (2003).
24. P. M. Wang, M. Cornwell, J. Hill, and M. R. Prausnitz. Precise microinjection into skin using hollow microneedles. *J. Invest. Dermatol.* **126**:1080–1087 (2006).
25. W. Martanto, J. S. Moore, O. Kashlan, R. Kamath, P. M. Wang, J. M. O'Neal, and M. R. Prausnitz. Microinfusion using hollow microneedles. *Pharm. Res.* **23**:104–113 (2006).
26. R. K. Sivamani, B. Stoeber, G. C. Wu, H. Zhai, D. Liepmann, and H. Maibach. Clinical microneedle injection of methyl nicotinate: stratum corneum penetration. *Skin Res. Technol.* **11**:152–156 (2005).
27. M. Meurer, M. Stumvoll, and R. M. Szeimies. [Skin changes in diabetes mellitus]. *Hautarzt* **55**:428–435 (2004).
28. S. P. Davis, W. Martanto, M. G. Allen, and M. R. Prausnitz. Hollow metal microneedles for insulin delivery to diabetic rats. *IEEE Trans. Biomed. Eng.* **52**:909–915 (2005).
29. W. Waldhausland and P. Bratusch-Marrain. [Causes of insulin resistance in type 1 diabetes]. *Wien. Klin. Wochenschr.* **97**:359–363 (1985).
30. H. Wiig, O. Tenstad, and J. L. Bert. Effect of hydration on interstitial distribution of charged albumin in rat dermis *in vitro*. *J. Physiol.* **569**:631–641 (2005).
31. R. K. Reed, S. Lepsoe, and H. Wiig. Interstitial exclusion of albumin in rat dermis and subcutis in over- and dehydration. *Am. J. Physiol.* **257**:H1819–H1827 (1989).
32. J. Berger, C. Biswas, P. P. Vicario, H. V. Strout, R. Saperstein, and P. F. Pilch. Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting. *Nature* **340**:70–72 (1989).

33. E. Karnieli, P. J. Hissin, I. A. Simpson, L. B. Salans, and S. W. Cushman. A possible mechanism of insulin resistance in the rat adipose cell in streptozotocin-induced diabetes mellitus. Depletion of intracellular glucose transport systems. *J. Clin. Invest.* **68**:811–814 (1981).
34. G. Lisato, I. Cusin, A. Tiengo, S. Del Prato, and B. Jeanrenaud. The contribution of hyperglycaemia and hypoinsulinaemia to the insulin resistance of streptozotocin-diabetic rats. *Diabetologia* **35**:310–315 (1992).
35. W. Martanto, J. S. Moore, T. Couse, and M. R. Prausnitz. Mechanism of fluid infusion during microneedle insertion and retraction. *J. Control. Release* (2006).
36. R. J. Heine. Unlocking the opportunity of tight glycaemic control. Promise ahead: the role of inhaled insulin in clinical practice. *Diabetes Obes. Metab.***7**(Suppl 1):S19–S23 (2005).
37. E. V. Mukerjee, S. D. Collins, R. R. Isseroff, and R. L. Smith. Microneedle array for transdermal biological fluid extraction and in situ analysis. *Sens. Actuators A, Phys*. **A114**:267–275 (2004).
38. J. A. Matriano, M. Cormier, J. Johnson, W. A. Young, M. Buttery, K. Nyam, and P. E. Daddona. Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm. Res.* **19**:63–70 (2002).
39. W. Lin, M. Cormier, A. Samiee, A. Griffin, B. Johnson, C. L. Teng, G. E. Hardee, and P. E. Daddona. Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux) technology. *Pharm. Res.* **18**:1789–1793 (2001).
40. S. A. Coulman, D. Barrow, A. Anstey, C. Gateley, A. Morrissey, N. Wilke, C. Allender, K. Brain, and J. C. Birchall. Minimally invasive cutaneous delivery of macromolecules and plasmid DNA via microneedles. *Curr. Drug. Deliv.* **65**:65–75 (2006).
41. J. A. Mikszta, J. B. Alarcon, J. M. Brittingham, D. E. Sutter, R. J. Pettis, and N. G. Harvey. Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery. *Nat. Med.* **8**:415–419 (2002).
42. M. Cormier, B. Johnson, M. Ameri, K. Nyam, L. Libiran, D. D. Zhang, and P. Daddona. Transdermal delivery of desmopressin using a coated microneedle array patch system. *J. Control. Release* **97**:503–511 (2004).