Transdermal Delivery of Insulin Using Microneedles *in Vivo*

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Purpose. The purpose of this study was to design and fabricate arrays of solid microneedles and insert them into the skin of diabetic hairless rats for transdermal delivery of insulin to lower blood glucose level.
Methods. Arrays containing 105 microneedles were laser-cut from stainless steel metal sheets and inserted into the skin of anesthetized hairless rats with streptozotocin-induced diabetes. During and after microneedle treatment, an insulin solution (100 or 500 U/ml) was placed in contact with the skin for 4 h. Microneedles were removed 10 s, 10 min, or 4 h after initiating transdermal insulin delivery. Blood glucose levels were measured electrochemically every 30 min. Plasma insulin concentration was determined by radioimmunoassay at the end of most experiments.
Results. Arrays of microneedles were fabricated and demonstrated to insert fully into hairless rat skin *in vivo*. Microneedles increased skin permeability to insulin, which rapidly and steadily reduced blood glucose levels to an extent similar to 0.05–0.5 U insulin injected subcutaneously. Plasma insulin concentrations were directly measured to be 0.5–7.4 ng/ml. Higher donor solution insulin concentration, shorter insertion time, and fewer repeated insertions resulted in larger drops in blood glucose level and larger plasma insulin concentrations.
Conclusions. Solid metal microneedles are capable of increasing transdermal insulin delivery and lowering blood glucose levels by as much as 80% in diabetic hairless rats *in vivo*.

KEY WORDS: diabetes; microelectromechanical systems (MEMS); microfabrication; skin; transdermal drug delivery.

INTRODUCTION

The development of sophisticated new medicines has challenged the pharmaceutical community to develop new delivery methods that overcome the problems of poor absorption and enzymatic degradation of drugs encountered during oral delivery. Transdermal drug delivery is an appealing alternative that offers good patient compliance and the possibility of controlled release over time while avoiding possible degradation due to the gastrointestinal tract or first-pass liver effects (1,2). Despite these advantages, transdermal drug delivery is severely limited by the low permeability of skin caused mainly by stratum corneum, the skin's outermost layer.

Skin permeability can be increased through the use of chemical enhancers (3), electrical enhancers via iontophoresis (4) or electroporation (5), ultrasonic enhancers (6), and other approaches. These methods share a common goal to permeabilize the skin by creating nanometer-scale disruptions of stratum corneum structure. Despite progress using these techniques, it remains a significant challenge to deliver macromolecules into the skin.

A novel approach to increase transdermal transport involves the use of microscopic needles that pierce the skin and create micrometer-scale openings. Though still extremely small on a clinical level, channels of micrometer dimensions are much larger than macromolecules and thereby should dramatically increase skin permeability to large drug molecules. Microneedles can be fabricated for this application using microelectromechanical systems (MEMS)-based technology from the microelectronics industry (7).

Our previous work has demonstrated that microneedles are capable of piercing human skin (8) and increasing skin permeability by orders of magnitude to small molecules (9) and proteins (10) *in vitro*. Moreover, insertion of microneedles into human subjects is reported as painless (11). Injection into chicken thigh *in vitro* using microneedles was shown by Stoeber and Liepmann (12). Using hairless guinea pig skin *in vivo*, Lin *et al.* (13) studied the use of microneedles to deliver oligonucleotides with and without the addition of iontophoresis. Vaccine delivery has also received attention using antigen-coated microneedles, where Matriano *et al.* (14) showed increased antibody titers to ovalbumin in hairless guinea pigs and Mikszta *et al.* (15) demonstrated enhanced immune response to a DNA vaccine in mice.

Building off these previous studies, we sought to investigate the use of microneedles to deliver a therapeutic protein and study the pharmacodynamic response *in vivo*. We therefore used microfabricated needle arrays to deliver insulin to diabetic hairless rats and measured insulin delivery and resulting changes in blood glucose levels. We selected insulin as a model drug because of its clinical relevance and great difficulty to deliver across intact skin (16). Diabetes mellitus is one of the leading lethal diseases in the United States and worldwide and is often treated by hypodermic injection of insulin. Due to patient discomfort that leads to poor patient compliance, alternative methods to administer insulin are of great interest.

MATERIALS AND METHODS

Microneedle Fabrication

Arrays of solid microneedles were fabricated by cutting needle structures from stainless steel sheets (SS 304, 75-μm thick; McMaster-Carr, Atlanta, GA, USA) using an infrared laser (Resonetics Maestro, Nashua, NH, USA). Initially, the shape and orientation of the arrays were drafted in a CAD file (AutoCAD; Autodesk, Cupertino, CA, USA), which was used by the laser-control software. The laser beam traced the desired shape of the needle, which ablated the metal sheet and created the needles in the plane of the sheet. The laser was operated at 1000 Hz at an energy density of 20 J/cm^2 and required approximately 4 min to cut an array. The metal sheet with needles on it was cleaned in hot water (Alconox, White Plains, NY, USA) and rinsed with DI water. Each needle was then manually bent at 90° out of the plane of the sheet. The

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needles were electropolished in a bath containing a 6:3:1 mixture by volume of glycerin, phosphoric acid, and water (Fisher Scientific, Atlanta, GA, USA) to remove debris (17,18). This electropolishing process reduced the needle thickness to 50 μm.

Diabetic Animal Model

To generate a diabetic animal model, Sprague-Dawley hairless rats (male, 250–350 g, Charles River Laboratories, Wilmington, MA, USA) were injected with 100 mg/kg streptozotocin (Sigma, St. Louis, MO, USA) in sterile Dulbecco's phosphate-buffered saline without Ca or Mg (PBS, pH 7.4; Mediatech, Herndon, VA, USA) by tail vein injection. During the next day, diabetes developed due to destruction of pancreatic islet cells by streptozotocin (19). The use of hairless rats in this study was approved by the Georgia Tech IACUC and adhered to the NIH "Principles of Laboratory Animal Care."

The next day, successful induction of diabetes was verified by checking that blood glucose levels were at least 300 mg/dl (Accu-Chek Compact; Roche Diagnostics, Indianapolis, IN, USA). Diabetic rats were then anesthetized by intraperitoneal injection of 1300 mg/kg urethane (Sigma) at a concentration of 200 mg/ml in PBS. Blood glucose measurements were obtained using blood samples collected by lateral tail vein laceration. After establishing a stable baseline blood glucose level between 300 and 475 mg/dl with less than 20% variation during the course of approximately 1 h, the experiments were started.

Insulin Delivery Experiments

To test the ability of microneedles to increase skin permeability to insulin, a microneedle array was inserted into the skin at a site on the rat's lower back using a pneumatically driven insertion device (Bionic Technologies, Salt Lake City, UT, USA). This device was developed to minimize damage during insertion of microelectrode arrays into neural tissue and adapted for use on skin (20). Previous studies of microneedles for transdermal drug delivery have used similar insertion devices (14). In some cases, the needle array was repeatedly inserted (for 10 s) and removed, five times at the same site. A flanged glass chamber was then adhered to the skin around the array using cyanoacrylate glue and filled with a solution containing Humulin-R insulin (Eli Lilly, Indianapolis, IN, USA) at a concentration of 100 U/ml unless otherwise noted. Microneedles were removed using forceps 10 s, 10 min, or 4 h after adding the insulin solution. The insulin solution was kept in the chamber in contact with the rat's skin for 4 h in all experiments. Blood glucose measurements were made every 30 min by lateral tail vein laceration (Accu-Chek Compact).

After the 4-h insulin delivery period, the insulin solution was removed from the skin. In some cases, the skin was cleaned with DI water and blood glucose measurements were continued every 30 min for another 4 h. In most cases, 0.2–0.5 ml of blood was collected using intracardiac puncture or orbital puncture immediately before euthanasia using 0.22 ml/kg Beuthanasia (Schering-Plough Animal Health Corporation, Omaha, NE, USA). These blood samples were centrifuged at 2040 × *g* (Eppendorf Centrifuge 5415 C, Westbury, NY, USA) for 5 min to collect plasma, which was then stored at −70°C until analysis using a human insulin-specific radioimmunoassay (Linco Research, St. Charles, MO, USA) to determine plasma levels of insulin delivered to the rats. Because the assay was specific to human insulin, it measured only the exogenous insulin delivered to the rat and not the rat's endogenous insulin.

As a negative control experiment, the same protocol was followed, except no microneedles were applied to the skin (i.e., insulin solution was placed passively on the skin for 4 h). As positive control experiments, 50 μl of Humulin-R insulin diluted with PBS to different concentrations (1.0 U/ml, 10 U/ml, and 30 U/ml) was administered subcutaneously with an insulin syringe and hypodermic needle (29G U-100; Terumo Medical, Elkton, MD, USA).

To facilitate imaging needle penetration pathways within skin, additional experiments were performed in which a solution of blue dye (Tissue Marking Dye; Shandon, Pittsburg, PA, USA) was placed onto the skin instead of insulin and a 10-s microneedle insertion time was used. After 2 min, the dye was washed off the skin surface, and a skin biopsy was obtained with an 8-mm punch (Tru-Punch; Sklar Instruments, West Chester, PA, USA) around the microneedle insertion site and imaged using bright-field microscopy (Leica DC 300; Leica Microsystems, Bannockburn, IL, USA). In other experiments, microneedles were inserted into skin, and a biopsy was taken with the needles remaining in the skin. These samples were fixed using 10% neutral buffered formalin for at least 24 h, dehydrated using ethanol and xylene, and incubated in methyl methacrylate infiltration solution (Fisher, Suwanee, GA, USA) for 72 h before being embedded in methyl methacrylate and submerged in water for 48 h (21). Samples were coarse-trimmed (Isomet 1000 Precision Saw; Buehler, Lake Bluff, IL, USA) and then sectioned into 200-μm strips (Ecomet 3 Variable Speed Grinder; Buehler) before surface staining for 10 s at 55°C (Sanderson's Rapid Bone; Surgipath Medical Industries, Richmond, IL, USA) and examination by bright-field microscopy.

Statistical Analysis

At each condition and time point tested, replicate blood glucose samples from at least three different rats were measured, from which the mean and standard deviation were calculated. A two-tailed Student's *t* test ($\alpha = 0.05$) was performed when comparing two experimental conditions. When comparing three or more experimental conditions, a one-way analysis of variance (ANOVA $\alpha = 0.05$) was performed. A two-way analysis of variance (ANOVA $\alpha = 0.05$) was performed when comparing two factors. In all cases, a value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Fabrication and Characterization of Microneedles

To develop a minimally invasive means to administer insulin across the skin and offer the possibility to continuously control the delivery rate, we designed and fabricated arrays of solid microneedles. These needles were made by laser-cutting needle structures from stainless steel sheets and bending the needles out of the sheet into an array containing 7 rows of 15

needles each, for a total of 105 needles. As shown in Fig. 1, each needle measured 50 μm by 200 μm in width at the base, and tapered over a 1000-μm length to a sharp tip with 20° angle. The dark region visible behind each needle is the hole in the stainless steel sheet out of which each needle was bent. Figure 1 also shows a 27-gauge hypodermic needle for size comparison.

We used relatively long (i.e., 1 mm) microneedles in this study to facilitate easy insertion into the highly deformable skin of a rat, although shorter needles can also be inserted (10,13,14). Even though human skin is thicker, shorter needles may insert into human skin because it is less deformable on many parts of the body. Ongoing studies suggest that arrays of needles as large as 1 mm in length can be designed to avoid pain (data not shown).

After making microneedles, we tested their ability to insert into hairless rat skin *in vivo*. We found that insertion of this large array of needles by hand was difficult. However, with the aid of a high-velocity insertion device designed specifically for microneedle insertion, microneedles readily penetrated into the skin. As shown in Fig. 2, microneedles were inserted to their full length into the skin, piercing across the epidermis and terminating within the dermis. In Fig. 2, the black material corresponds to the stainless steel needles, the lightly stained region adjacent to the needle array base is the epidermis, the darkly stained tissue below that is dermis, and the light tissue at the bottom of the image is subcutaneous tissue.

Closer examination of Fig. 2 indicates that some skin deformation occurred around each needle, as shown by the epidermal surface dimpled downward along the needle shaft (especially evident in the inset image). Due to the angle of tissue sectioning, the left portion of the needle array has a continuous base, whereas the right portion shows the holes in the base out of which each needle was bent. To supplement the side view shown in the main image, the inset image displays a section rotated by 90° to show the front view of a needle.

To qualitatively assess the ability of microneedles to increase transdermal transport, a needle array was inserted and then removed from hairless rat skin *in vivo*, after which a dye solution was placed on the skin and then wiped off to stain the sites of needle penetration. Figure 3 provides an image of the skin surface, which indicates that essentially all microneedles were inserted into the skin to create pathways for transdermal transport.

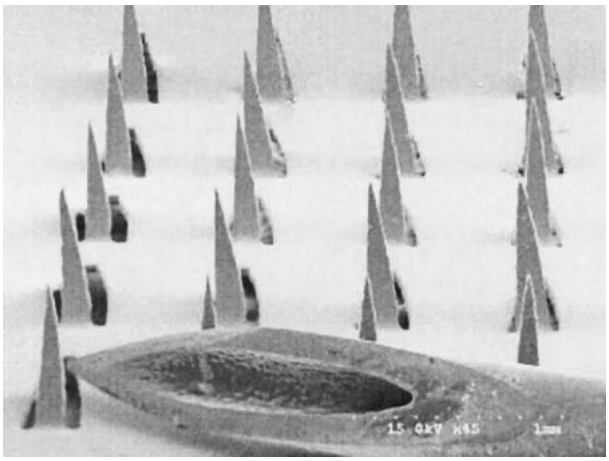

Fig. 1. Scanning electron micrograph of solid metal microneedles shown next to the tip of a 27-gauge hypodermic needle. The complete microneedle array contains 105 needles, each measuring 1000 μm in length, 50 μm by 200 μm in cross section at the base, and tapering to a sharp tip with an angle of 20°.

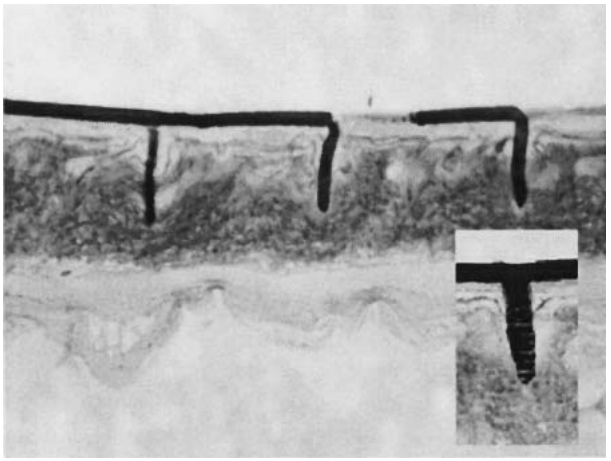

Fig. 2. Cross section of an array of microneedles inserted into rat skin *in vivo* and imaged by light microscopy after biopsy, fixation, and staining. The dark structures are the needles, which pierce vertically into the skin, and the base plate of the array, which is aligned horizontally along the skin surface. A portion of the base plate is missing on the right side, which corresponds to the hole in the base plate formed after cutting and bending the needle out at 90° (see text). The lightly stained tissue corresponds to the epidermis, and the thicker, darkly stained tissue below corresponds to the dermis. The inset shows a side view of a single microneedle sectioned at an angle rotated 90° relative to the main image. This figure shows that the microneedles inserted to their full length into the skin.

Reduced Blood Glucose Levels

We next tested the ability of microneedles to increase transdermal delivery of insulin to diabetic hairless rats. In these initial experiments, insulin was delivered for 4 h during and after a 10-min microneedle treatment. Then, insulin was removed from the skin, and blood glucose levels were monitored for another 4 h. Figure 4 shows that blood glucose dropped rapidly and continuously during the 4-h insulin delivery period and eventually stabilized after insulin was removed (black symbols), which is significantly different from the negative control rats (X symbols), in which insulin was applied to the rat's skin without microneedles (ANOVA; $p < 0.0001$). In comparison to positive control experiments using subcutaneous injection with a hypodermic needle (white symbols), changes in blood glucose level induced by microneedles were greater than those caused by injection of 0.05 U but less than 0.5 or 1.5 U of insulin. This indicates that microneedles can increase skin permeability in a long-lived manner to deliver physiologically relevant amounts of pharmacologically active insulin.

To understand further the effects of microneedles on transdermal insulin delivery, we examined the effect of delivery conditions on insulin delivery rate by varying three parameters: insulin concentration, needle insertion time, and

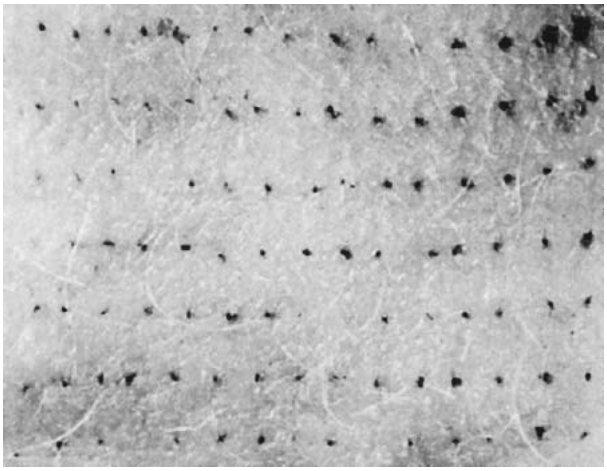

Fig. 3. Surface of hairless rat skin after insertion and removal of microneedles *in vivo* followed by topical staining with a tissue-marking dye, as shown by light microscopy. Each stained spot corresponds to the site of microneedle penetration into the skin.

number of insertions. Figure 5A shows the effect of varying insulin donor solution concentration, where higher insulin concentration (500 U/ml) reduced blood glucose levels to a greater extent than lower insulin concentration (100 U/ml) (ANOVA; $p < 0.05$), which is consistent with the expected dose-response relationship.

The effect of inserting microneedle arrays into the skin for different amounts of time is shown in Fig. 5B. Microneedles inserted and then removed after just 10 s (followed by 4 h of insulin delivery across the skin) yielded larger drops in blood glucose level compared to that of removing the needles after 10 min, which in turn yielded larger drops in blood glucose level compared to that of leaving the needles in the skin for the full 4 h (ANOVA; $p < 0.0001$). The observation that shorter insertion times are advantageous suggests applications might best involve a brief pretreatment with microneedles followed by extended delivery across permeabilized skin. The reduced delivery caused at longer insertion times could be explained by the presence of needles blocking holes created in skin; by an unfavorable interaction (e.g., insulin aggregation) between needles and insulin (10); or other effects.

As shown in Fig. 5C, the number of needle insertions also affected blood glucose levels. Inserting the needles multiple times (5 times) led to smaller reductions in blood glucose levels compared to that of a single insertion prior to insulin delivery (ANOVA; $p < 0.001$). The observation that repeated insertions resulted in smaller changes in blood glucose level could be explained by local damage to the skin caused by multiple insertions that altered insulin clearance and capillary uptake in the skin. Additional studies are needed to validate this hypothesis.

Increased Plasma Insulin Concentration

To supplement blood glucose measurements that indirectly assess the ability of microneedles to deliver insulin across skin, we directly measured plasma insulin concentration using a radioimmunoassay specific for the human insulin delivered (as opposed to endogenous rat insulin). Consistent with the observation that increased insulin concentration in the donor solution had a greater effect on blood glucose levels (Fig. 5A), it also resulted in higher plasma insulin concentration (Fig. 6A). The 5-fold increase in donor insulin concentration resulted in approximately a 10-fold increase in plasma insulin concentration. Figure 6B shows that microneedle insertion time also influenced plasma insulin levels. Consistent with blood glucose measurements (Fig. 5B), shorter needle insertion times resulted in larger plasma insulin levels.

The total insulin dose delivered can be estimated from these plasma insulin measurements. Based on an aqueous compartment volume of distribution in rats of 0.4 L/kg (22,23), the total insulin dose delivered was 1.6–4.1 mU (0.06–0.15 μg) using the 100 U/ml insulin formulation (Fig. 6B). In contrast, Fig. 4 shows that microneedles lowered blood glucose levels to an extent bounded by subcutaneous injection of 0.05–0.5 U insulin. This discrepancy could be explained by slower absorption or lower bioavailability of insulin injected subcutaneously compared to insulin delivered into the skin using microneedles.

Implications for Transdermal Drug Delivery

The ability of microneedles to create pathways across skin for delivery of insulin has potential significance for transdermal delivery of macromolecules. Using a relatively simple procedure, this study showed that microneedles are capable of delivering physiologically relevant amounts of biologically active insulin *in vivo* with no apparent lag time to onset of

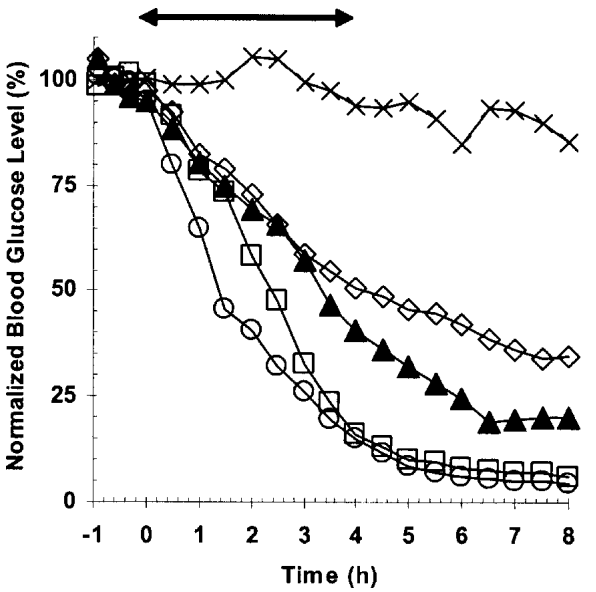

Fig. 4. Changes in blood glucose level in diabetic, hairless rats after insulin delivery using microneedles (▲), subcutaneous hypodermic injection of 0.05 U (◇), 0.5 U (□), or 1.5 U (○) of insulin, or passive delivery across untreated skin (×). Microneedles were inserted into skin for 10 min and then removed. Insulin solution was applied to the skin immediately after microneedle insertion and left on the skin for 4 h (as shown by arrow). Subcutaneous injections took a few seconds to perform. The pharmacodynamic effect of insulin delivery by microneedles was bounded by that of 0.05–0.5 U injected subcutaneously. Data are expressed as mean values ($n \geq 3$) with average standard deviation associated with each data point of 14%. Blood glucose levels have been normalized relative to average pretreatment levels.

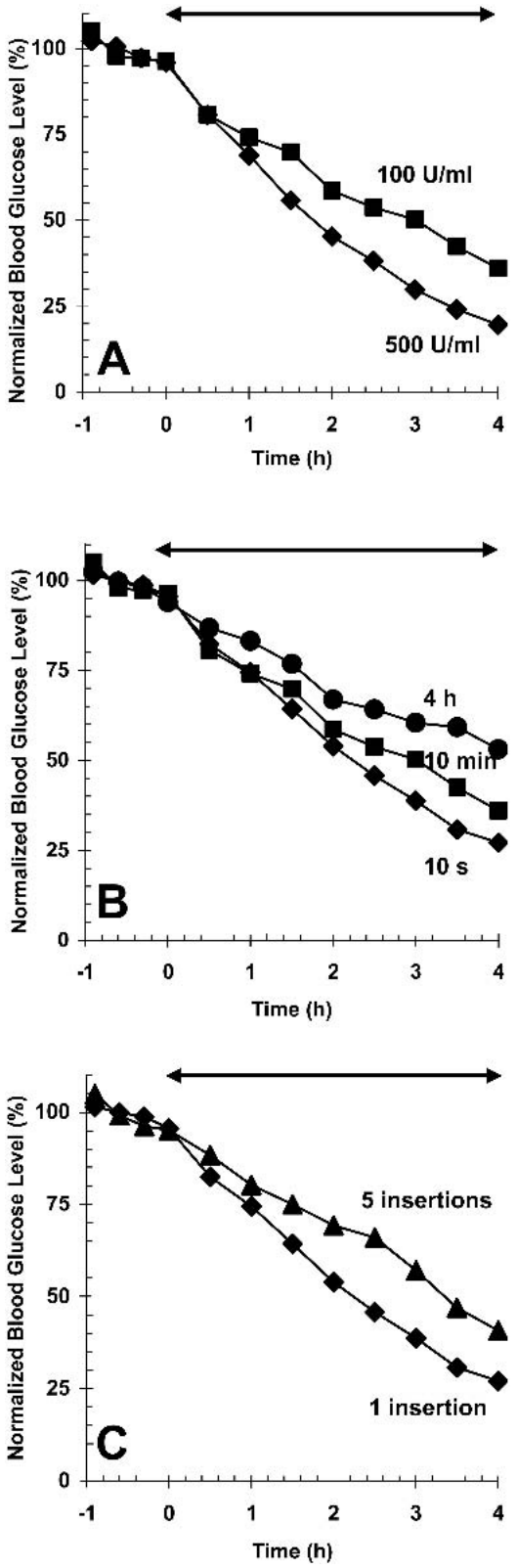

Fig. 5. The effects of insulin donor concentration, needle insertion time, and number of insertions on changes in blood glucose level in diabetic, hairless rats. (A) Higher insulin concentration [500 U/ml (♦)] in the donor solution reduced blood glucose level to a greater extent than lower insulin concentration [100 U/ml (■)] during delivery using microneedles that were inserted into skin for 10 min and then removed. (B) To vary insertion time, microneedles were inserted into skin and then removed after 10 s (♦), 10 min (■), or 4 h (●). Leaving needles inserted for longer times led to smaller reductions in blood glucose level. (C) Microneedles were inserted into skin and then removed after 10 s for the single insertion experiment (♦), whereas microneedles were repeatedly inserted (for 10 s) and removed five times at the same site for the multiple insertion experiment (▲). Inserting the needles multiple times led to smaller reductions in blood glucose level. Data are expressed as mean values ($n \geq 3$) with average standard deviation associated with each data point of 15%, 12%, and 14% for (A), (B), and (C), respectively. Blood glucose levels have been normalized relative to average pretreatment levels. In all cases, blood glucose levels were significantly different from control rats exposed to insulin without microneedles (Fig. 4). In all experiments, insulin remained in contact with the skin for 4 h (as shown by arrows).

action. As a convenient and effective procedure, this study indicated that the skin could be briefly pretreated with microneedles and followed by application of a drug formulation to the treated site for many hours of transdermal delivery. When considered in combination with previous *in vitro* studies showing transdermal delivery of compounds covering a broad range of molecular sizes (10) and *in vivo* studies showing delivery of oligonucleotides (13), as well as protein and DNA antigens (13,14), microneedles appear to be a broadly applicable technology for transdermal delivery.

Of practical significance, microneedles can be fabricated using a number of different low-cost, mass-production technologies (10), including the laser-cutting approach used in this study. As a purely mechanical device that does not require electronic controls or a power supply, microneedles are expected to be less expensive, easier to use, and less likely to suffer from FDA-approval hurdles to validate manufacturing as compared to other approaches involving electrical, ultrasonic, thermal, or other energy-based mechanisms.

CONCLUSIONS

Microfabricated arrays of solid metal microneedles were capable of fully inserting into the skin of hairless rats. These microneedles increased transdermal insulin delivery and thereby lowered blood glucose levels by as much as 80% in diabetic hairless rats *in vivo*. Shorter microneedle insertion times, fewer insertions, and larger insulin concentration in the donor solution all led to more insulin delivery and larger drops in blood glucose level. For the conditions used in this study, the pharmacodynamic response to insulin delivery using microneedles showed reduction in blood glucose levels similar to subcutaneous hypodermic injection of 0.05–0.5 U insulin. Direct measurements showed blood insulin concentrations of 0.5–7.4 ng/ml. Altogether, this study shows that microneedles can be used to deliver biologically active protein *in vivo*, which suggests future uses for minimally invasive protein delivery in clinical applications.

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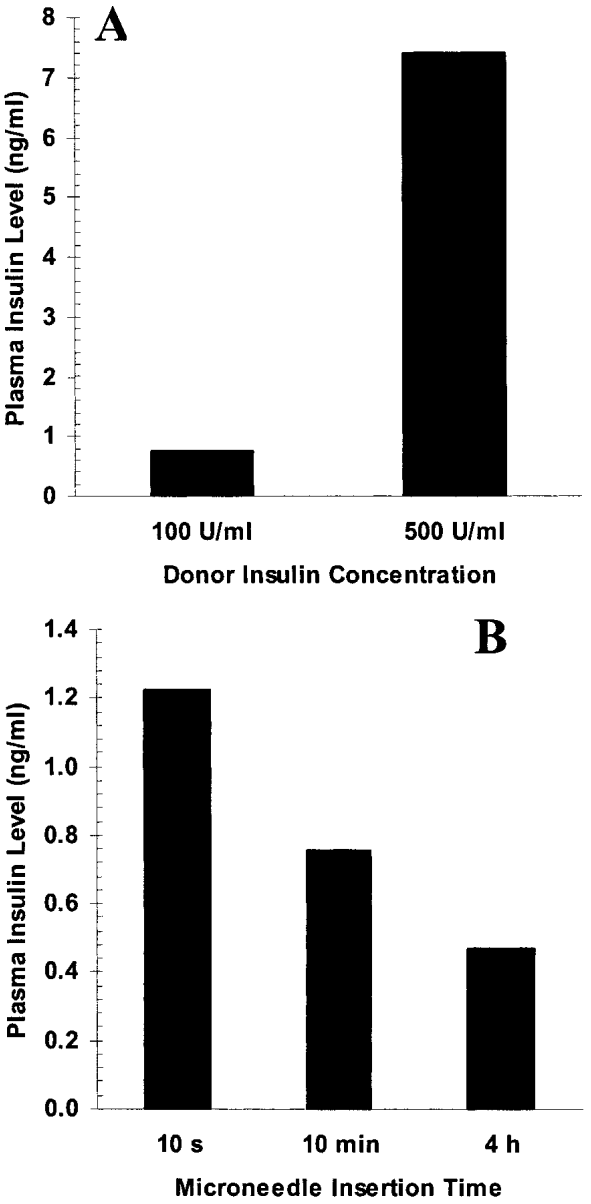

Fig. 6. Plasma insulin concentration in diabetic, hairless rats plotted vs. donor solution insulin concentration and microneedle insertion time. (A) Plasma insulin levels increased with insulin concentration in the donor solution and (B) decreased with longer microneedle insertion times. The experimental conditions were the same as in Fig. 5A and 5B for (A) and (B), respectively. Blood samples were collected to measure plasma insulin levels immediately after the 4-h insulin delivery period.

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