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 diabetic 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/cm² 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
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 (α = 0.05) was performed when comparing two experimental conditions. When comparing three or more experimental conditions, a one-way analysis of variance (ANOVA α = 0.05) was performed. A two-way analysis of variance (ANOVA α = 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