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Microneedle: Various Techniques of Fabrications and Evaluations

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Abstract: Microneedles find widespread use; researchers must perfect the techniques for optimally inserting them into the skin, and complete the integration of microneedles into a full diagnostic, monitoring or drug delivery system. Microneedles are expected to be less painful than conventional hypodermic needles because they are too small to significantly stimulate nerve endings. A painless "microneedle" that mimics the way a female mosquito sucks blood has been built by engineers in India and Japan. The needle could be used to draw blood, inject drugs, and as a glucose-level monitor for diabetics. The needle is also strong enough to penetrate as far as 3 millimetres into skin and reach capillary blood vessels. Its size compared to earlier models also means that surface tension effects are exploited further, and the same capillary flow that draws water up into trees helps draw blood into the microneedle.
Key word: Microneedle

Introduction:

Joint collaboration between the Indian Institute of Technology Kharagpur and Tokai University of Japan has resulted in a new hypodermic microneedle, which does not come with an iota of pain. This is due to the fact that it was designed after a mosquito's unique micro-electro-mechanical based suction system. This new design has a diameter of 60 microns, which is way smaller than a conventional needle that currently stands at 900 microns, and is hoped to be developed further for use in glucose monitoring, blood draws, insulin pumps and other drug delivery devices[1, 2, 3].

Proboscis-Mimicking Microneedle For Drug Delivery:

Precise control over the fluidic transport and the ability to scale down the analysis to very small volumes of liquid are among the most attractive capabilities of these novel health care approaches. "Such concepts provide excellent promises in revolutionizing health care protocols for the future, with the possibilities of developing substantially improved and patient-friendly health monitoring systems." The needle has been designed to mimic a mosquito's proboscis in dimensions, the manner that suction is created and rate of flow. As it has an external diameter of only 60µm, as opposed to 900µm for conventional syringes, the microneedle is said to be painless. Microneedles with similar dimensions have been created previously but have primarily been fabricated from silicon dioxide that rendered them brittle making them liable to snap, which could potentially cause a blood clot. This latest model in the needle's development is crafted from titanium and related alloys, giving it the strength needed to administer therapeutics without the risk of snapping[7, 8].

Microneedle fabrication:

In general terms, make needles that:

- Go into skin easily
- Deliver drugs effectively
- Don't hurt
- Are biocompatible

The needles need to:

Withstand typical handling
Deliver controlled amount of drug at specific rate
Deliver to precise depth in body
Withstand insertion without buckling, fracture, or delamination [2]

Materials

Needles have been made from:

Glass

Silicon

Metal—stainless steel, solid or coat of gold over Ni, Pd or Pd-Co, and Pt.

Biodegradable polymers, if a tip snaps off while inserted, it will easily biodegrade.

Microneedle fabrication:

The needle fabrication process involved four steps. First, arrays of microneedles made of SU-8 epoxy photo resist were fabricated by patterning SU-8 onto glass substrates and defining needle shape by lithography. Then, the tips of the needles were sharpened using reactive ion etching. The next step involved laser drilling holes through the microneedles and base substrate oriented off-center, but parallel to the Microneedle axis. This created holes that serve as the micro fluidic needle bores for injection or infusion, which terminate in side-opening holes along the needle shaft below the needle tip. Finally, the needle arrays were coated with nickel by electroplating to increase their mechanical strength.[3]

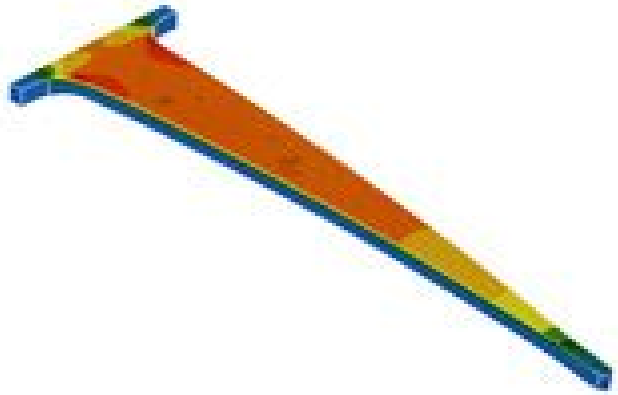
Microneedle Technology

High Strength Structural Silicon Microneedles: Miniature - Roughly the cross-section of a human hair.

Capillary Flow - The microneedle draws blood (or other liquid) flow by capillary force into the disposable microchip.

No Pain. BIG Gain - The miniature microneedle has been proven in clinical trials to be pain free.

Strong - Kumetrix single crystal silicon needles are stronger than steel, and will not break during skin penetration.



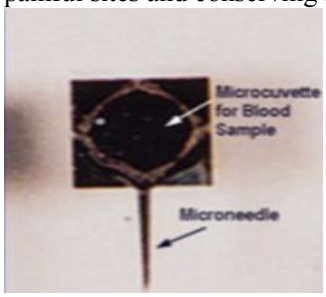
Finite element analysis is used to design the silicon microneedle

Reverse Engineered From Nature:

The silicon microneedle was engineered to mimic the painless bite of the mosquito. In the majority of mosquito bites, the target is unaware of being bitten. The bite itself typically causes no pain – irritation, redness, and swelling are caused by enzymes injected by the mosquito to thin the blood. Kumetrix worked with a leading entomologist at the National Institutes of Health (NIH) to design the product.

The process to make a silicon microneedle is identical to the manufacturing of computer chips, except that the resultant silicon micro-device performs a mechanical function, rather than an electrical one. Chips that do mechanical work are called micro-electro-mechanical systems or MEMS.

Unlike conventional finger lancets or heel sticks, Kumetrix has integrated on-chip internal micro fluidics and assay capability with the microneedle into a single device. This allows for a one-step assay to be performed by the user, without the need for blood transfer. The microneedle chip takes a much smaller blood sample, allowing testing to be done on the arm or other less painful sites and conserving a neonate's limited blood.



Different types of microneedles:

Hollow Metal Microneedles for Insulin Delivery to Diabetic Rats: The goal of this study was to design, fabricate, and test arrays of hollow microneedles for minimally invasive and continuous delivery of insulin in vivo. As a simple, robust fabrication method suitable for inexpensive mass production, we developed a modified-LIGA process to micro machine molds out of polyethylene terephthalate using an ultraviolet laser, coated those molds with nickel by electro deposition onto a sputter-deposited seed layer, and released the resulting metal microneedle arrays by selectively etching the polymer mold. Mechanical testing showed that these microneedles were sufficiently strong to pierce living skin without breaking. Arrays containing 16 microneedles measuring 500 /spl mu/m in length with a 75 /spl mu/m tip diameter were then inserted into the skin of anesthetized, diabetic, hairless rats. Insulin delivery through microneedles caused blood glucose levels to drop steadily to 47% of pretreatment values over a 4-h insulin delivery period and was then approximately constant over a 4-h post delivery-monitoring period. Direct measurement of plasma insulin levels showed a peak value of 0.43 mg/ml. Together, these data suggest that microneedles can be fabricated and used for in vivo insulin delivery.

Microfabricated Needles for Transdermal Delivery of Macromolecules and Nanoparticles: Fabrication Methods And Transport Studies: Arrays of micrometer-scale needles could be used to deliver drugs, proteins, and particles across skin in a minimally invasive manner. We therefore developed micro fabrication techniques for silicon, metal and biodegradable polymer micro needle arrays having solid and hollow bores with tapered and beveled tips and feature sizes from 1 to 1,000 µm. When solid microneedles were used, skin permeability was increased *in vitro* by orders of magnitude for macromolecules and particles up to 50 nm in radius. Intracellular delivery of molecules into viable cells was also achieved with high efficiency. Hollow microneedles permitted flow of micro liter quantities into

skin *in vivo*, including microinjection of insulin to reduce blood glucose levels in diabetic rats.
Micro fabricated silicon microneedles for nonviral cutaneous gene delivery
The skin represents an accessible somatic tissue for therapeutic gene transfer. The superficial lipophilic layer of the skin, the stratum corneum, however, constitutes a major obstacle to the cutaneous delivery of charged macromolecules such as DNA.
Objectives to determine whether silicon-based microneedles, microfabricated via a novel isotropic etching/BOSCH reaction process, could generate micro channels in the skin of sufficient dimensions to facilitate access of lipid: polycation: pDNA (LPD) nonviral gene therapy vectors.

Methods:

Scanning electron microscopy was used to visualize the micro conduits created in heat-separated human epidermal sheets after application of the microneedles. Following confirmation of particle size and particle surface charge by photon correlation spectroscopy and micro electrophoresis, respectively, the diffusion of fluorescent polystyrene nanospheres and LPD complexes through heat-separated human epidermal sheets was determined *in vitro* using a Franz-type diffusion cell. *In-vitro* cell culture with quantification by flow cytometry was used to determine gene expression in human keratinocytes (HaCaT cells).

Results:

The diffusion of 100 nm diameter fluorescent polystyrene nanospheres, used as a readily quantifiable predictive model for LPD complexes, through epidermal sheets was significantly enhanced following membrane treatment with microneedles. The delivery of LPD complexes either into or through the membrane micro channels was also demonstrated. In both cases considerable interaction between the particles and the epidermal sheet was observed. *In-vitro* cell culture was used to confirm that LPD complexes mediated efficient reporter gene expression in human keratinocytes in culture when formulated at the appropriate surface charge.

Electrically Conductive Microneedle Roller: An electrically conductive microneedle roller includes stacked discs, each of which includes a plurality of radial grooves, a plurality of microneedles that are received in the radial grooves of the disc, an electrically conductive bracket that supports the stacked discs, and a handle that supports the bracket. Electric current flows to the skin via the microneedles and provides electric stimulation. The discs are assembled using UV bond thereby reducing the assembly time. The roller has enhanced service life since the microneedles do not fall off from the roller since radial grooves holding the microneedles have tapered shape.
The skin of a human being is composed of three primary layers, the epidermis, the dermis and the appendages. Among them, the dermis forming skin wrinkles is disposed under the epidermis as the lowest skin layer at a thickness of about 0.7 mm to 4 mm. Collagen in the dermis shrinks as the aging proceeds. As the result, the skin gets dry, and more skin wrinkles appear. In order to prevent wrinkles from appearing and to keep the skin tight, it is needed to induce the skin to create more collagen or to inject collagen into the skin.
When nutrients required for forming collagen like Vitamin C or peptide are spread or sprayed on the skin, only 0.3% of such nutrients pass through the skin, and the remaining 99.7% remains on the skin, dried and removed away.
The thickness of the epidermis is about 0.03 mm to 1 mm. The exposed ends of the micro needles penetrate the epidermis, thereby forming micro channels to the dermis. The nutrients can be effectively supplied to the dermis through the micro channels for sustaining the skintight or for preventing wrinkles from appearing.
Minute wounds are formed in the skin with the micro needle roller, and the skin is stimulated and creates collagen by natural curing process of the skin, thereby reproducing the skin. That is, collagen is formed by natural curing process and restores the aged skin without hurting the epidermis like laser peeling operation.
Microneedle rollers by prior art have disadvantages that they cannot supply electric stimulation, the roller unit is easy to be disassembled, the microneedles fall off from the roller, and productivity is low since it takes much time to assemble the roller unit.
Another objective of the invention is to provide a microneedle roller that has good maintainability and productivity.
In order to achieve the above objectives, the present invention provides an electrically conductive microneedle roller that includes a plurality of discs, a plurality of microneedles, a bracket and a handle.
Each of the discs comprises a first side surface, a second side surface, and an electrically conductive part. The first side surface comprises a plurality of radial grooves. The discs are stacked on one another in a way that the first side surface of one disc contacts the second side surface of the adjacent disc.
The microneedles are received in the radial grooves of the disc, and each of which has a pointed end and a base end. The pointed end protrudes beyond the outer circumference of the disc. The base end is electrically connected to the electrically conductive part of the disc.
The bracket supports the stacked discs. The bracket is electrically conductive. The handle supports the bracket.
The electrically conductive part comprises a circular plate that is provided at the center of the disc.
The handle comprises an electrically conductive wire that is connected to the bracket.
The electrically conductive part of the disc comprises a projection on one side and a recess on the other side. The projection of one electrically conductive part engages with the recess of the adjacent electrically conductive part.

The radial groove comprises an inner end and an outer end. The outer end meets the circumference of the disc. The outer end has a tapered shape that becomes narrower toward the circumference of the disc.

Biodegradable Polymer Microneedles: Fabrication, Mechanics and Transdermal Drug Delivery: To overcome the skin's barrier properties that block transferal delivery of most drugs, arrays of microscopic needles have been micro fabricated primarily out of silicon or metal. This study addresses micro needles made of biocompatible and biodegradable polymers, which are expected to improve safety and manufacturability. To make biodegradable polymer micro needles with sharp tips, micro-electromechanical masking and etching were adapted to produce beveled- and chisel-tip micro needles and a new fabrication method was developed to produce tapered-cone micro needles using an in situ lens-based lithographic approach. To replicate micro fabricated master structures, PDMS micro molds were generated and a novel vacuum-based method was developed to fill the molds with polydactyl acid, polyglycolic acid, and their co-polymers. Mechanical testing of the resulting needles measured the force at which needles broke during axial loading and found that this failure force increased with Young's modulus of the material and needle base diameter and decreased with needle length. Failure forces were generally much larger than the forces needed to insert microneedles into skin, indicating that biodegradable polymers can have satisfactory mechanical properties for microneedles. Finally, arrays of polymer microneedles were shown to increase permeability of human cadaver skin to a low-molecular weight tracer, calcein, and a macromolecular protein, bovine serum albumin, by up to three orders of magnitude. Altogether, these results indicate that biodegradable polymer microneedles can be fabricated with an appropriate geometry and sufficient strength to insert into skin, and thereby dramatically increase transdermal transport of molecules.

Production challenges: The design uses a shape-memory alloy to drive the needle into skin and a micro-pump for delivering drugs. The latter could be used to inject insulin (or other drugs) into the patient when required.

"The working principle of this device follows on from our discovery that in a well-designed microneedle, surface tension forces may overcome resistance from friction and draw up blood with unprecedented efficiency.

Microneedle Arrays for Drug Delivery and Fluid Extraction:

The microneedle array is fabricated by employing a bi-mask technique to facilitate sharp tips, a cylindrical body and side ports. The array has advantages over previously published results including ease of fabrication and bonding, and high needle density and robustness. In addition, the microneedle comprises side ports which minimizes the potential for clogging. This microneedle array can be used for fluid extraction and drug delivery systems; e.g., biological sampling, and insulin delivery into the human body.

Microneedle Evaluation:

Microneedle systems have gained attention as having many advantages over transdermal patches and hypodermic needles. The procedure provides adequate skin permeation rates without pain or severe infection. To obtain information for designing a microneedle system, macroneedles were used instead of microneedles to investigate the effects of pretreatment of needle puncture in the skin barrier stratum corneum on in vitro skin permeation of fluorescein isothiocyanate (FITC)-dextrans (4.3, 9.6 and 42.0 kDa) (FD-4, FD-10 and FD-40). The effect of sandpaper abrasion was also investigated for comparison. Both pretreatments on the skin barrier significantly increased the skin permeation of FDs. Lactate dehydrogenase (LDH) leaching was measured after pretreatment of macroneedle and sandpaper abrasion on the skin to evaluate the skin damage by these pretreatment methods. Lower leaching of LDH was observed after macroneedle puncture than after sandpaper abrasion. Next, a parallel permeation-resistance model of the skin barrier was established. Skin permeation of FD-10 was predicted by the model as a function of the number of pores in the skin barrier. Our results suggest that needle puncture may provide a safe, efficient and controllable alternative for increasing transdermal drug delivery[5, 6].

Materials

FITC-dextrans (FD-4, FD-10 and FD-40; average molecular weight, 4.3, 9.6 and 42.0 kDa, respectively) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Other reagents were of analytical grade and used without further purification.

Preparation of Macroneedles For Skin Puncture

In the needle puncture experiment in the skin barrier, a 27-gauge disposable hypodermic needle (i.d., 0.22 mm; o.d., 0.40 mm; Terumo Co., Tokyo, Japan) was used. The needle was covered with polyethylene tubing (PE-50; i.d., 0.58 mm; o.d., 0.97 mm; Hibiki, Tokyo, Japan) as a needle sheath to maintain constant insertion depth in the skin barrier, as shown in. The PE-50 cover allows only insertion of the needle tip (about 160 μm length) into the skin[38, 39, and 40]

Experimental animals

Male hairless rats (WBM/ILA-Ht, 7–9 weeks-old, body weight: 180–250 g) were purchased either from Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratories (Fukaya, Saitama, Japan). They were housed in temperature-controlled rooms (25 ± 2 °C) with a 12 h light–dark cycle (07:00–19:00 h). The rats were allowed free access to food (M.F. Oriental, Tokyo, Japan) and tap water for a week before experiments began. Every animal experiment was conducted under the guidelines of the Life Science Research Center at Josai

University[9, 10].

Pretreatment of skin

Abdominal full-thickness skin of hairless rats was excised under anesthesia by i.p. injection of sodium pentobarbital (50 mg/kg) and excess subcutaneous fat was carefully eliminated.

Puncturing with a 27-gauge hypodermic needle

The excised skin was punctured with a 27-gauge hypodermic needle with the PE-50 sheath .The number of punctures with a depth of about 160 μm was set to be 1, 3, 9 or *n* in the stratum corneum.

Abrading with sandpaper

The excised skin surface was gently abraded once with sandpaper of No. 600 (Sankyo Rikagaku Co., Okegawa, Saitama, Japan). In order to maintain consistent abrasion strength, the technique was practiced at length until an acceptable repeatability was reached.

Stripping the stratum corneum with adhesive tape

The stratum corneum of the excised skin was stripped with adhesive tape (Scotch Magic Transparent Tape®, 3M Co., Minneapolis, MN, U.S.A.) about 20 times, until the stratum corneum was entirely removed from the skin.

In vitro skin permeation study

The skin pretreated with needle puncture or sandpaper abrasion was mounted in a vertical diffusion cell with an effective diffusion area of 1.77 cm^2. Intact full-thickness skin and stripped skin were also used for comparison. A test solution (1.0 mL) containing 0.25 mM FD-4, FD-10 or FD-40 was placed on the stratum corneum side of the skin. The receiver solution was 6.0 mL of pH 7.4 phosphate buffered saline (PBS), which was maintained at 32 °C using a thermo-regulated water bath. A magnetic stirrer bar was added in the receiver compartment, which stirred at about 1200 rpm throughout the experiment. The receiver solution (0.4 mL) was withdrawn every 1 h, and the same volume of PBS was added to the receiver compartment to keep the volume constant. Every permeation-run was repeated 3–5 times[11, 12].

Conclusion:

The present study demonstrated that physical pretreatments, needle puncture and sandpaper abrasion, are capable of effective delivery of macromolecules through skin. Measurement of LDH leaching shows that needle puncture to the stratum corneum is much safer than sandpaper abrasion. These results may indirectly support the concept that microneedles provide a safe and efficient alternative for minimally invasive transdermal drug delivery. Using a parallel resistance model, skin permeability after needle puncture can be predicted as a function of pore number. Although further studies are needed concerning the resistance model, these preliminary results show a strong possibility of precise prediction.

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