

Expert Opinion

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Two-photon polymerization of microneedles for transdermal drug delivery

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Importance of the field: Microneedles are small-scale devices that are finding use for transdermal delivery of protein-based pharmacologic agents and nucleic acid-based pharmacologic agents; however, microneedles prepared using conventional microelectronics-based technologies have several shortcomings, which have limited translation of these devices into widespread clinical use.

Areas covered in this review: Two-photon polymerization is a laser-based rapid prototyping technique that has been used recently for direct fabrication of hollow microneedles with a wide variety of geometries. In addition, an indirect rapid prototyping method that involves two-photon polymerization and polydimethyl siloxane micromolding has been used for fabrication of solid microneedles with exceptional mechanical properties.

What the reader will gain: In this review, the use of two-photon polymerization for fabricating in-plane and out-of-plane hollow microneedle arrays is described. The use of two-photon polymerization-micromolding for fabrication of solid microneedles is also reviewed. In addition, fabrication of microneedles with antimicrobial properties is discussed; antimicrobial microneedles may reduce the risk of infection associated with the formation of channels through the stratum corneum.

Take home message: It is anticipated that the use of two-photon polymerization as well as two-photon polymerization-micromolding for fabrication of microneedles and other microstructured drug delivery devices will increase over the coming years.

Keywords: drug delivery, microneedle, rapid prototyping, transdermal, two-photon polymerization

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1. Overview of the market

Nucleic acid- and protein-based pharmacologic agents are finding greater use in the treatment of cancer, diabetes mellitus and other chronic medical conditions. Oral, sublingual, or rectal delivery are the most appealing routes of administration because doses can be administered by the patient. In addition, no painful or complicated techniques are necessary [1]. Unfortunately, many nucleic acid- and protein-based pharmacologic agents cannot be administered in enteral form owing to pH-driven molecular degradation or first-pass metabolism by the liver [2]. Transdermal patches have found increasing use in recent years for delivery of hormones (e.g., estrogen), analgesic agents (e.g., fentanyl), as well as various other pharmacologic agents; however, charged, polar, or large pharmacological agents cannot be delivered using transdermal patches because these agents cannot pass through an intact stratum

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Article highlights.

- Microneedles are miniaturized lancet-, thorn-, or hypodermic needle-shaped devices that have at least one dimension < 500 µm; these devices may be used to create pores in the stratum corneum layer of the epidermis.
- Over the past few decades, microneedles have been prepared using technologies that originated in the microelectronics and glassware industries.
- Numerous factors have limited the commercial viability of microneedles prepared using microelectronics-based fabrication techniques.
- Two-photon polymerization uses ultrashort laser pulses for selective polymerization of photosensitive resins into complex microscale and nanoscale structures.
- The first microneedles directly produced by two-photon polymerization were made from Ormocer (organically modified ceramic) materials.
- Microneedles with complex geometries, including rocket-like and mosquito-fascicle-like shapes, may be fabricated using two-photon polymerization.
- Ormocer microneedles fabricated using two-photon polymerization have been used for delivery of fluorescein-conjugated biotin and PEG-amine quantum dot solutions to cadaveric porcine skin.
- Two-photon polymerization followed by subsequent molding steps may be utilized for creating solid microneedles as well as solid microneedle arrays.
- Antimicrobial microneedles may be fabricated out of composite materials that contain biocompatible polymers such as polyethylene glycol as well as conventional antimicrobial agents such as gentamicin sulfate.
- Antimicrobial microneedles may also be prepared by coating microneedles with thin films of antimicrobial materials such as silver.
- Two-photon polymerization may also be used to fabricate a wide variety of microstructured and nanostructured medical devices; for example, two-photon polymerization has been used to create patient-specific medical prostheses as well as scaffolds for tissue engineering.
- Two-photon polymerization is a new rapid prototyping technique that may be used to fabricate microneedles as well as microneedle arrays with a wider range of geometries than reactive ion etching, lithography-electroforming replication, or other conventional microelectronics-based technologies.

This box summarizes key points contained in the article.

corneum [3-5]. The stratum corneum is the outermost 15-µm-thick layer of the epidermis, which contains non-viable keratinized cells and serves as a barrier to pharmacologic agent transport [6]. Transport of pharmacologic agents through the stratum corneum may be enhanced through the use of chemical penetration enhancers, electroporation, iontophoresis and sonophoresis; however, these techniques have not seen widespread clinical use owing to issues such as tissue irritation or limited effectiveness [1,7].

Nucleic acid- and protein-based agents are commonly delivered to subcutaneous tissues, muscle tissues and veins by means of subcutaneous, intramuscular and intravenous

injections; these routes of administration avoid diffusion- and enzyme-related barriers. For example, insulin is a 51 amino acid residue, two-chain protein hormone that promotes glucose storage in the liver as well as in other body locations. Insulin cannot be orally delivered because it is either degraded by proteolytic enzymes in the gastrointestinal tract or metabolized in the liver. In addition, insulin also cannot be delivered using transdermal patches because it has a relatively high molecular mass (5808 Da). Insulin is commonly delivered by means of subcutaneous injection [8,9]. However, this route of administration is associated with trauma at the injection site as well as pain.

Microneedles are miniaturized lancet-, thorn-, or hypodermic needle-shaped devices that have at least one dimension < 500 µm; these devices may be used to create pores in the stratum corneum layer of the epidermis. By creating pores in the 15-µm-thick stratum corneum layer, delivery of pharmacologic agents into deeper layers of the epidermis and the papillary dermis is enabled [10,11]. Capillaries in the papillary dermis facilitate systemic absorption of pharmacologic agents. Pain to the patient may be minimized if microneedles do not interact with Meissner's corpuscles, Pacinian corpuscles and nerve endings located within the dermis [13]. Trauma and tissue damage at the injection site are also minimized owing to the small size of these devices; this property is especially appealing for patients with diabetes and other medical conditions who require frequent injections [14]. In addition, no specialized medical training or supervision is required for use of microneedles. Microneedles have been shown to provide steady and rapid reductions in blood glucose levels in diabetic hairless rats; subcutaneous injection and microneedle delivery provide similar reductions in blood glucose levels [15].

Microneedle-based drug delivery mechanisms fall into three categories: i) applying a microneedle to the skin in conjunction with another transdermal drug delivery technique (e.g., a transdermal patch); ii) coating a solid microneedle with a pharmacologic agent; and iii) infusing a liquid pharmacologic agent through the bore of a hollow microneedle. Pore formation and topical application have been performed to deliver some pharmacologic agents, including oligonucleotides, nucleic acids and quantum dots [16-18]. Coated microneedles have been used for several classes of pharmacologic agents, including barium sulfate, bovine serum albumin, calcein, desmopressin and plasmid deoxyribonucleic acid [19,20]. In coated microneedle devices, the total amount of pharmacologic agent that can be delivered is determined by the surface area of the needle/patch assembly [21]. As transport is controlled by diffusion, complex dosing (e.g., pulsatile dosing) is not possible. Microneedles with hollow bores may enable pressure-based delivery or diffusion-based delivery of pharmacologic agents to be maintained over an extended period of time. Devices containing microneedles and microfluidic pump systems may be used for controlled dosing of pharmacologic agents [5,22-25]. In addition, microneedles may be used for both drug delivery and fluid extraction by reversing the

direction of fluid transport. For example, biosensors containing hollow microneedle patches may be used for blood sampling [22,24]. These microneedle-based sensors are being considered for monitoring of chronic medical conditions that require frequent blood sampling, including monitoring of blood glucose levels in diabetes mellitus patients [6,23]. Microneedles may be integrated with micropumps and biosensors to provide blood sampling as well as drug delivery for treatment of chronic disease. For example, patients with diabetes mellitus may be treated using transdermal 'artificial pancreas' devices that provide continual blood glucose monitoring and insulin delivery; maintenance of blood glucose levels within the narrow euglycemic range would minimize ophthalmic, cardiovascular, nephrologic and neurologic complications for these patients.

Microneedles have unique advantages for delivery of vaccines associated with high cost and limited availability, including vaccines for influenza viruses. Clinical studies by Belshe *et al.* and Kenney *et al.* have shown that intradermal administration of influenza vaccine enables use of reduced doses in comparison with conventional intramuscular administration [26,27]. For example, Belshe *et al.* demonstrated that intradermal injection provides vigorous antibody responses with reduced doses for individuals between 18 and 60 years of age; serum antibody responses for individuals who received intradermal injections (dose = 6 µg of hemagglutinin for each antigen) and individuals who received intramuscular injections (dose = 15 µg of hemagglutinin for each antigen) did not significantly differ [26]. Kenney *et al.* showed that intradermal administration of influenza vaccine (dose ≥ 3 µg of hemagglutinin per strain) provided seroprotection and seroconversion rates that were similar to or better than those provided by intramuscular administration (dose ≥ 15 µg of hemagglutinin per strain) [27]. The use of reduced doses, which is known as dose sparing, may enable the number of vaccine doses that are provided to a population using a given amount of vaccine to be maximized. Kim *et al.* attributed the improved immunogenic response provided by intradermal administration to antigen-presenting cell (e.g., dendritic cell) interactions with local lymph nodes [28].

Alarcon *et al.* demonstrated intradermal delivery administration of influenza vaccines using microneedles; several vaccines, including a whole inactivated influenza virus, a plasmid DNA encoding the influenza virus hemagglutinin, and a trivalent split-virion human vaccine, were examined using a rodent model [29]. They showed that microneedle delivery of the trivalent split-virion human vaccine for the H1N1 strain of the influenza virus provided at least 10-fold dose sparing compared with conventional intramuscular delivery. Zhu *et al.* examined solid metal microneedle arrays that were coated with inactivated influenza virus A using a rodent model [30]. They demonstrated that microneedle administration provided antibody responses similar to conventional intramuscular administration against influenza virus. Kim *et al.* evaluated delivery of trehalose-stabilized influenza vaccine using a microneedle patch in a rodent model [28]. They showed

that microneedle vaccination was better than intramuscular vaccination for imparting protective immunity. In addition, the trehalose-stabilized microneedle vaccine elicited a stronger antibody response than the conventional unstabilized vaccine. Ding *et al.* demonstrated the use of cholera toxin and diphtheria toxoid as adjuvants for delivery of influenza subunit vaccine through microneedle array-treated skin in a rodent model. In a recent clinical study [31], Van Damme *et al.* carried out a prospective, randomized trial involving healthy adults to compare the immunogenicity of a low-dose intradermal influenza vaccine delivered by means of a microneedle device with that of a conventional intramuscular influenza vaccine delivered by means of a conventional hypodermic needle [32]. They showed that low-dose intradermal vaccination provided immunogenic responses similar to full-dose intramuscular vaccination.

Several investigators, including Bal *et al.*, Haq *et al.* and Sivamani *et al.*, have examined clinical use of microneedles [33-35]. Haq *et al.* compared the sensory response to skin penetration by 180 µm wet-etch silicon microneedles, 280 µm wet-etch silicon microneedles, and hypodermic needles [33]. Pain intensity was evaluated using a visual analogue scale, sensory perception was evaluated using an adapted McGill Pain Questionnaire, and skin penetration was evaluated using external staining as well as transepidermal water loss measurements. They showed that application of microneedles was associated with less pain than application of hypodermic needles. In addition, they demonstrated that resealing of channels created by microneedle penetration took place within 8 – 24 h. Bal *et al.* examined transepidermal water loss, skin redness, blood flow and pain sensation on ventral forearm skin after microneedle penetration [34]. A positive correlation between microneedle length and several parameters, including transepidermal water loss, skin redness and blood flow, was observed. Microneedles were shown to disrupt the stratum corneum barrier layer with an absence of pain. Minimal skin irritation was noted after microneedle penetration; however, the duration of skin irritation did not exceed 2 h. Sivamani *et al.* described the use of symmetric and pointed microneedle arrays for injection of methyl nicotinate into the volar forearm skin of human volunteers [35]. Microneedle penetration was associated with a pressure sensation; on the other hand, no pain was observed. They demonstrated that microneedle arrays were effective in enhancing methyl nicotinate delivery through the stratum corneum.

Over the past few decades, microneedles have been prepared using technologies that originated in the microelectronics and glassware industries. Many of these techniques have inherent limitations in terms of fabricating microneedles that have large wall thicknesses as well as other fracture-resistant geometries. The concept of using miniaturized devices for transdermal drug delivery was originally developed ~ 50 years ago. In 1959, Wagner described the concept of using microneedle devices for transdermal drug delivery [36]. Methodologies for the preparation of glass microneedles by hand pulling glass

pipettes with a micropipette puller have been widely available for several decades (Figure 1C) [37,38]. These devices have been generally utilized in laboratory-based applications because they show poor fracture properties. In addition, preparation of glass microneedles by hand is an expensive, time-consuming process [38]. Approximately one decade ago, Prausnitz began utilizing processes that originated in the microelectronics industry in order to fabricate microneedles for transdermal drug delivery [11]. For example, Henry *et al.* described fabrication of silicon microneedle arrays by means of reactive ion etching [12]. Since that time, silicon microneedles have been fabricated by Shikida *et al.*, Wilke *et al.*, Cormier *et al.*, Griss and Stemme, Roxhed *et al.*, Gardeniers *et al.*, Ji *et al.* and Stoeber and Liepmann using microelectronics-based fabrication techniques such as wet etching, reactive ion etching and deep reactive ion etching [19,39-46]. Images of microneedles produced by etching of silicon are shown in Figure 1A, B. Wet etching is an isotropic process that involves selective chemical etching of silicon by means of a photo-patterned mask [39-41]. The geometries of microneedles prepared using etching processes are limited to radially symmetrical structures; a wide variety of simple geometric shapes such as pyramids, cones and cylinders may be fabricated [42-46]. In addition, these techniques provide poor control over microneedle aspect ratio. Furthermore, reactive ion etching and deep reactive ion etching processes are line-of-sight methods that cannot create complex shapes, including overhanging structures [42-46].

Numerous factors have limited the commercial viability of microneedles prepared using microelectronics-based fabrication techniques. For example, processing of microneedles using reactive ion etching involves high costs [47]. Silicon etching requires the use of costly cleanroom facilities, expensive equipment, as well as hazardous waste services for handling toxic chemicals [48]. Reactive ion etching techniques are commonly used for processing silicon as well as other semiconductor materials. Studies involving monolayer-forming Caco-2 cells of human origin have shown that silicon, specifically silicon doped with p-type materials, is fully biocompatible [49]. However, single crystal silicon as well as other brittle materials commonly used in microelectronics-based fabrication techniques show low fracture toughness, absence of ductility and low strain tolerance; large variations in fracture strength are commonly observed in these materials [50-52]. For example, silicon is brittle at room temperature; at low temperatures, crack tips in silicon propagate in the absence of dislocations [48]. The mechanical properties of silicon as well as other brittle materials commonly used in microelectronics-based fabrication techniques are affected by surface roughness as well as other surface defects (e.g., etching damage).

Another microelectronics-based fabrication technique that has been used for preparing microneedles involves the use of photolithography in combination with patterned photoresist masks. In this technique, a photoresist is patterned onto the surface; unprotected material is subsequently removed using X-ray exposure or other methods [53,54]. For example,

Moon *et al.* used a deep X-ray mask in combination with vertical deep X-ray exposure as well as inclined deep X-ray exposure for fabrication of microneedles containing liquid conduits out of polymethylmethacrylate, a polymer that is commonly used in ophthalmology, dentistry and orthopedic surgery. Microneedles with base areas between 190 and 300 μm , and tip angles between $15^\circ - 20^\circ$ were fabricated by altering the inclined angle as well as the mask-substrate gap. Masks for microneedles with 70 – 100 μm conduit diameters, 400 – 600 μm lengths, as well as a variety of shapes, including arrow-like, rounded and triangular geometries, were fabricated. Although this method allows microneedles to be fabricated using a wide variety of materials, several shortcomings remain. Photolithography-based microfabrication techniques such as deep X-ray lithography require costly processing equipment as well as cleanroom facilities. In addition, material is only removed along the 'line of sight' of the etchant; this parameter places a significant constraint on the microneedle geometry. Geometrical parameters, including radius of curvature and length, are limited by the aspect ratio of the deep reactive ion etching step. Overhangs and other complex features are difficult to produce. In addition, spacing between microneedles is restricted by the incidence angle of the etchant [53].

Martanto *et al.* and Gill and co-workers have described fabrication of solid metallic microneedles arrays through infrared laser ablation of two-dimensional patterns. In their study, an infrared laser was used to ablate a two-dimensional pattern corresponding to the needle shape on a stainless steel sheet. The sheet was subsequently bent at an angle of 90° out of the plane of the sheet to create a three-dimensional structure. Martanto *et al.* described fabrication of 105 needle arrays containing 1-mm-long needles from stainless steel sheets using this technique [20,55-58]. There are constraints on the geometries of microneedles fabricated by means of laser ablation, because this method involves processing of microneedles in two dimensions. The third dimension is determined by the thickness of the planar sheet. Microneedle width can be readily varied. In addition, the walls of these microneedles always have a vertical orientation. In addition, planar laser cutting is at present limited to fabrication of solid microneedles.

Electroplating is another technique that is commonly used to fabricate metallic microneedles. In electroplating, wires or structures created by one of the above-mentioned techniques serve as templates. A template is coated with a thick metal layer by means of electroplating, thereby forming a metal microneedle-shaped shell (Figure 1D). Unlike planar laser cutting, electroplating may be used for fabrication of hollow microneedles. One method to produce microneedles by electroplating involves electroplating a wire and then chemically removing the wire [22,23]. The former position of the wire has become the channel of the microneedle. For example, Kobayashi *et al.* electroplated platinum onto a Al-1% Si wire and subsequently dissolved the wire in 20 wt% NaOH

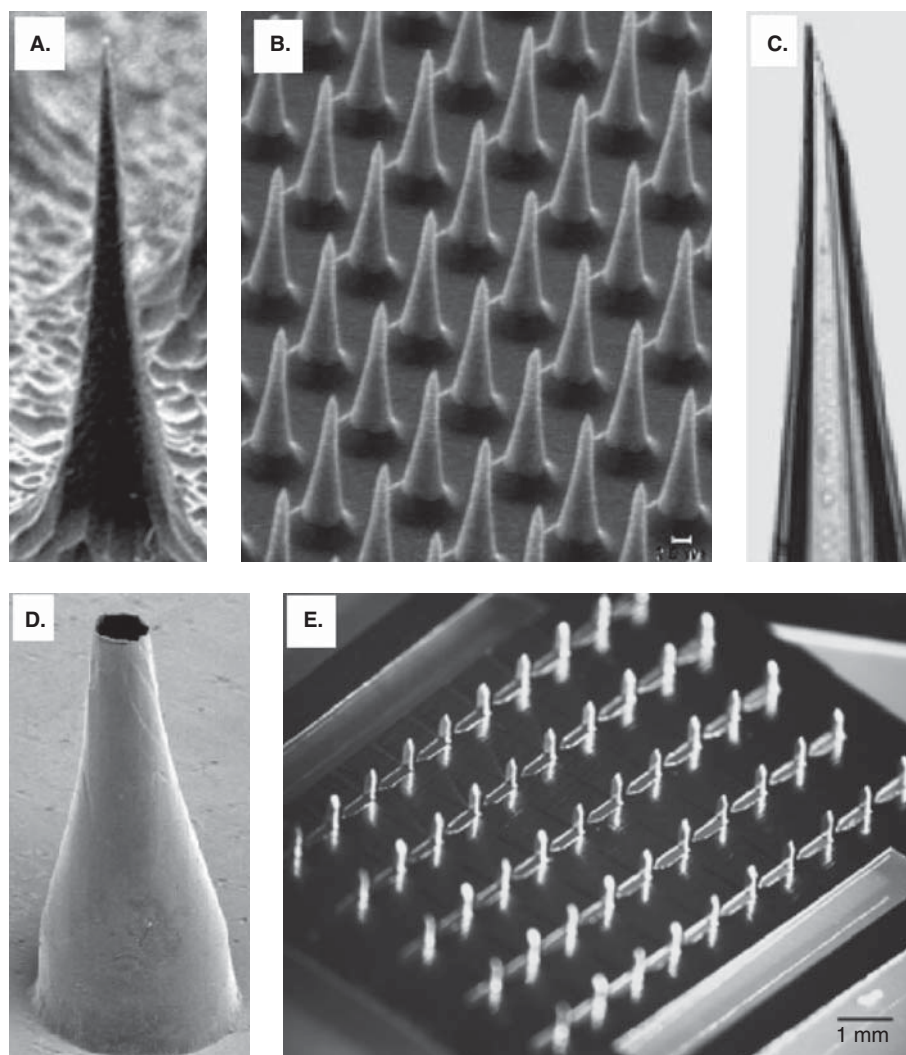


Figure 1. **A.** Solid silicon microneedle fabricated by reactive ion etching. **B.** Solid silicon microneedle array fabricated by reactive ion etching. **C.** Hollow glass microneedle fabricated using a micropipette puller. **D.** Hollow metal microneedle fabricated by electroplating a master structure. **E.** Solid metal microneedles fabricated by laser machining and bending a metal sheet.

A – D reproduced with permission from [47].

E reproduced with permission from [20].

solution; a platinum microneedle with a 50 μm inner diameter was fabricated using this technique [22]. Electroplating provides good control over the overall microneedle geometry as well as the channel geometry. This method provides little control over the angle of the microneedle wall; for example, the microneedle wall cannot easily be varied over the length of the microneedle.

Multiple-step electroplating processes have been utilized by several investigators, including Shikida *et al.*, McAllister *et al.*, Kim and Lee and Chandrasekaran *et al.*, for preparing microneedles [40,47,59,60]. An electroplating-based process provides better control over overall microneedle geometry than other microneedle fabrication processes. For example, Chandrasekaran *et al.* utilized a surface micromachining process for fabricating gold, nickel, palladium and

palladium-cobalt microneedles on silicon, plastic and glass substrates. In the first step, they deposited three metal coatings on the substrate, including: a coating to promote adhesion between the substrate and the electroplating seed layer; an electroplating seed layer; and a coating to prevent contamination or oxidation of the electroplating seed layer. A photoresist coating was spin-coated above these seed layers. The photoresist coating was subsequently patterned; this patterned surface served as a micromold of the microneedle shaft bottom wall. Gold, nickel, palladium, or palladium-cobalt coatings were subsequently electroplated into the bottom wall micromold; nickel was coated with gold to reduce interactions between nickel and biological tissues. A photoresist coating was subsequently deposited onto the structure and patterned; this structure defined the microneedle

inner lumen. A gold coating was sputtered onto the photoresist coating; this coating served as the electroplating seed layer during fabrication of the top shell. A photoresist coating was spin-coated and patterned on the surface of this structure; this coating was used to prepare microneedle shaft top walls. A palladium coating was electroplated onto this structure; this material served as the microneedle top wall. The photoresist coatings were removed in acetone. Many microneedles produced by means of electroplating have thin walls and relatively poor fracture resistance. For example, palladium microneedles produced by electroplating with 20° taper angles showed a 50% tip failure rate [61].

Several investigators have utilized template structures to fabricate molds; these molds are used for casting of microneedles. Molds have been made from several materials, including metals, hydrolytic polymers, silicon and silicone elastomer [47,54,62-67]. Casting allows fabrication of microneedles that are not compatible with direct microfabrication techniques. In addition, casting is a relatively rapid process, which is compatible with many conventional manufacturing protocols [62]. Casting appears to be the most commercially viable technique for microneedle fabrication; however, appropriate technologies for producing master structures need further development.

Despite these shortcomings, microneedles fabricated using microelectronics-derived processes are now being translated into clinical use by several companies. One company (Zosano Pharma™, Fremont, CA, USA) recently finalized Phase II clinical trials of a microneedle device for delivery of parathyroid hormone, which is used as a treatment for osteoporosis [68]. Another company (Debiotech SA, Lausanne, Switzerland) has developed a microneedle drug delivery platform (Nanoject) that they have licensed for use. Potential uses of their device include delivery of pharmacologic agents, intradermal delivery of vaccines, and biological sampling of fluids [69]. A large medical device manufacturer (Becton Dickinson, Franklin Lakes, NJ, USA) has examined extensively the use of microneedles for transdermal drug delivery; they have performed preclinical and clinical activities involving microneedle delivery of > 30 pharmacologic agents [70]. For example, Mikszta *et al.* utilized microneedles for intradermal delivery of a recombinant *Bacillus anthracis* protective antigen to rabbit and mouse models [71]. Microneedle-mediated intradermal delivery resulted in up to 90% seroconversion following a single dose; on the other hand, intramuscular injection resulted in 20% seroconversion. They demonstrated that the microneedle-treated animals developed complete protection against aerosol-administered *B. anthracis* spores. A major vaccine manufacturer (Sanofi Pasteur, Paris, France) is evaluating intradermal delivery of vaccines using the Soluvia™ microneedle-based microinjection system (Becton Dickinson, Franklin Lakes, NJ, USA) [72]. The European Commission has recently provided this company with approval for intradermal delivery of seasonal influenza vaccine in European Union territory [73]. Another company (Nanopass Technologies Ltd, Nes Ziona, Israel) has developed

a microneedle-based H1N1 vaccination system that they claim will 'dramatically enhance both the effectiveness and supply of pandemic flu vaccines'; they recently completed a clinical trial involving microneedle delivery of influenza vaccine [74]. They demonstrated that low doses of intradermally administered influenza vaccine provided immunogenic responses similar to full doses of intramuscularly administered influenza vaccine. Several patents that describe microneedle fabrication technologies, microneedle designs and microneedle delivery mechanisms have been granted [75-86].

2. How the technology works

One promising method for microneedle fabrication that has developed in recent years is a rapid prototyping process known as two-photon polymerization. The term rapid prototyping is used to describe additive, layer-by-layer processing of three-dimensional structures from solid, liquid, or powder precursors. The advantages and disadvantages of two-photon polymerization as well as other microneedle fabrication techniques are given in Table 1. It should be noted that two-photon polymerization is compatible with many photosensitive resins, which are widely available and may be obtained at low cost. In addition, two-photon polymerization can be performed in conventional facilities. For example, two-photon polymerization can potentially be set up in a conventional clinical environment (e.g., an out-patient medical office) for fabrication of patient-specific drug delivery devices that conform to a given patient's anatomy and medical condition. On the other hand, many conventional microneedle fabrication techniques require the use of cleanroom facilities; the energy consumption of cleanroom facilities may run as high as 10,200 kW/m² [87]. Finally, the two-photon polymerization processing rate is suitable for scaling up to high-rate commercial manufacturing.

Two-photon polymerization utilizes ultrashort laser pulses for selective polymerization of photosensitive resins into complex microscale and nanoscale structures. Polymerization of the photosensitive resin is initiated by a process known as two-photon absorption. Nearly simultaneous absorption of two photons from a near-infrared femtosecond laser source creates what is known as a virtual state for several femtoseconds. This electronic excitation is analogous to that of a single photon with a higher energy [88]. The process is called non-degenerate two-photon absorption when the two photons are of different wavelengths. The process is called degenerate two-photon absorption when the two photons are of identical wavelengths [89]. Two-photon absorption provides a nonlinear energy distribution, which is centered at the focal point of the laser. Owing to the nonlinear energy distribution of two-photon absorption, negligible absorption occurs except in the immediate vicinity of the focal volume of the light beam.

Lee *et al.* have examined the physical basis of two-photon polymerization [90]. The energy distribution observed in two-photon absorption is a Gaussian shape, which shows

Table 1. Advantages and disadvantages of microneedle production techniques.

Production Technique	Advantages	Disadvantages
Reactive ion etching	High resolution	Requires cleanroom, expensive, low throughput, limited to silicon, geometry restrictions
Photolithography	High resolution	Requires cleanroom, expensive, geometry restrictions
Cutting and bending	Low cost, cleanroom not required, strong materials	Only have control of two dimensions, only solid microneedles
Two-photon polymerization	Cleanroom not required, good geometry control, scalable resolution	Limited to photosensitive materials
Electroplating	Low cost, high throughput	Geometry controlled by master structure, biocompatibility concerns, poor mechanical properties due to thin walls
Casting	Low cost, high throughput, compatible with many materials	Geometry controlled by master structure

asymmetry in the planes of voxel width and voxel length (Figure 2A). In two-photon polymerization, the photon energy is absorbed by photoinitiator molecules within the photosensitive resin. On absorption of photon energy above a given threshold, the photoinitiator molecules release free radicals. These molecules make possible free radical polymerization of monomers within the photosensitive resin. Formation of a polymerization voxel occurs at locations where the photon energy is above the threshold value. Unpolymerized material is subsequently washed away using an appropriate solution. One advantage of the two-photon polymerization process over conventional stereolithography processes is that two-photon polymerization allows for three-dimensional processing of photosensitive resins; photosensitive resins used for two-photon polymerization are transparent to near-infrared light. On the other hand, conventional single-photon stereolithography processes involve polymerization of material at the surface of the photosensitive resin [88,90].

The desired structure is produced by selective polymerization of material along a laser trace, which is moved in three dimensions within the resin (Figure 2B) [88]. A computer-aided design (CAD) model of the desired structure is initially sliced into layers. Each layer is subsequently written in the photosensitive resin by rastering the laser path in order to fill in the contour of each layer (Figure 2C). Customized software for controlling the laser path may be used to fabricate complicated structures such as circles or spirals [91]. Processing of the photosensitive resin involves one of the following mechanisms: moving the photosensitive resin while keeping the laser stationary; or moving the laser pulses within the resin using a galvano-scanner [92-94]. Galvano-scanners may be used to direct laser-material interaction in two dimensions; movement of the substrate may be used to adjust the depth of laser-material interaction within the resin. The numerical aperture of the objective limits the overall dimensions of structures fabricated using a galvano-scanner. A piezoelectric stage provides a larger travel range; however, writing with a galvano-scanner is more rapid as well as more accurate than

writing with a piezoelectric stage. A system containing a galvano-scanner and piezoelectric stages provides the advantages of both techniques. For example, a galvano-scanner may be used to fabricate a single microneedle whereas a piezoelectric stage may be used to translate a surface in two dimensions for fabrication of microneedle arrays [10,92].

Two-photon polymerization allows for polymerization within volumes that are smaller than the laser wavelength [94-97]. Structures with <100 nm features have been fabricated by: i) using photoinitiators that show high initiating efficiency; ii) controlling the incident laser power; and iii) modifying the laser focus scan speed [98-100]. Other factors that determine minimum feature size include laser focus spot size, laser wavelength and numerical aperture [90]. The polymerization threshold energy may be modified through selection of an appropriate photoinitiator; the other parameters may be used to alter the height of the energy distribution and the width of the energy distribution. As focusing is performed by passing the laser pulses through an objective, the resolution of two-photon polymerization is readily scalable through selection of an appropriate objective. For example, a $\times 5$ objective may be used to prepare voxels with single-digit micrometer diameters [94]. As there is an inverse correlation between fabrication time and voxel size for a given structure, scalable processing may be used to maximize processing efficiency and minimize processing costs.

Microneedles for drug delivery must be >100 μm in length because the stratum corneum is flexible. In addition, the thickness of the stratum corneum varies with age, location and skin condition [101]. Longer (>700 μm) microneedles may be used for withdrawal of blood from dermal blood vessels. Several studies have examined the relationships between microneedle geometry and fracture properties. For example, Davis *et al.* demonstrated that the interfacial area of the microneedle tip and the microneedle insertion force had a linear relationship [102]. They showed that the microneedle wall thickness was directly related to the microneedle fracture force. In addition, they demonstrated that the microneedle

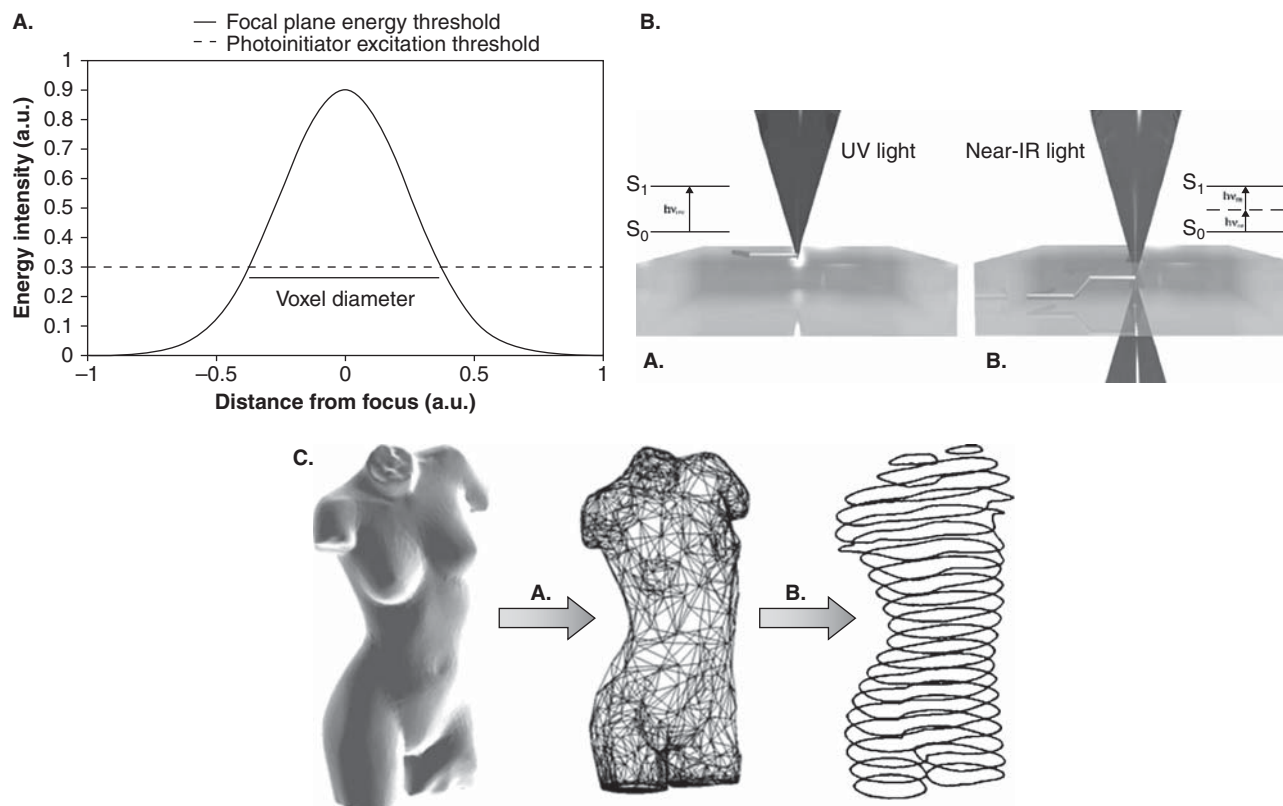


Figure 2. **A.** Schematic of the two-photon polymerization process. The energy distribution is a Gaussian shape. The radius of the polymerization voxel corresponds to the position at which the energy intensity exceeds the excitation threshold of the photoinitiator. **B.** Single-photon versus two-photon polymerization. Single-photon polymerization is limited to the surface of a given material. On the other hand, two-photon polymerization may occur within a given material. **C.** Schematic of the rapid prototyping process. In the first step, a computer-aided design program is used to prepare a STL format file. The STL format file is subsequently used to prepare a layer-by-layer contour of the structure. The structure is fabricated by rastering the laser in order to fill the area contained within the contours.

wall angle was directly related to the microneedle fracture force. They also demonstrated that the ratio of fracture force to insertion force, also known as the safety margin, increased as the microneedle tip radius was reduced. Aoyagi *et al.* also found that lower microneedle penetration forces were associated with smaller tip angles [65]. In addition, Moon *et al.* suggested that microneedles must have high aspect ratio shafts, sharp as well as tapered tips, small surface areas and out-of-plane structures in order to meet structural requirements [53]. Griss and Stemme have shown that out-of-plane microneedles, which contain flow channels that are positioned off-center with respect to the needle tip, do not suffer from obstructions caused by loose skin [103]. Two-photon polymerization is commonly used for fabricating arrays of microneedles. Microneedle arrays are favored over solitary microneedles because they provide a larger surface area over which a pharmacologic agent may be delivered or a biological fluid may be extracted. For example, microneedle arrays offer a greater probability of directly reaching blood vessels in the dermis for extraction of biological fluids. In addition, microneedle arrays provide redundancy in case individual needles are fractured or obstructed during handling or insertion [104].

Finally, microneedle arrays are also less susceptible to fracture if they are exposed to shear forces because the forces are distributed over a wider area [103].

3. Two-photon polymerization of microneedles

To be compatible with two-photon polymerization, a material must fulfill two requirements. First, the material must be capable of being polymerized by means of two-photon absorption either in unmodified form or with the assistance of a photoinitiator. Second, the material must be transparent to the wavelength of the laser [6]. Fortunately, many materials satisfy these requirements. Materials that have been shown to be compatible with two-photon polymerization include but are not limited to SU-8 photoresist [100], methacryloxypropyl trimethoxysilane-zirconium propoxide copolymer [91], organically modified ceramic materials [1,78,80,81,75,92], ethoxylated (6) trimethylolpropane triacrylate [89], polyethylene glycol diacrylate [72,89], as well as other acrylate- and methacrylate-based polymeric materials [6].

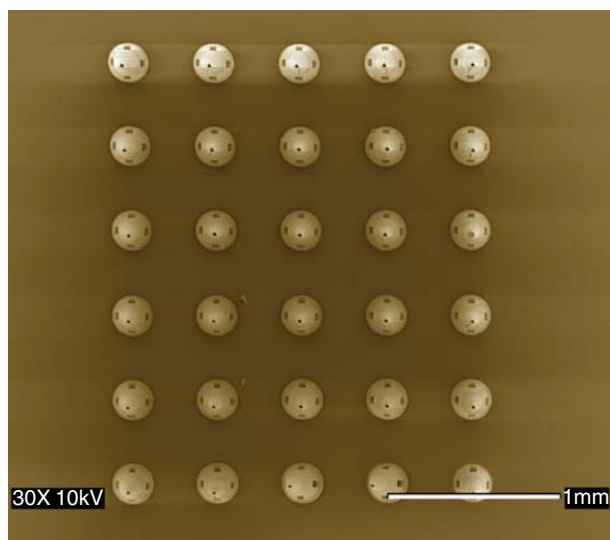


Figure 3. Scanning electron micrograph of microneedle array fabricated out of Ormocer organically modified ceramic material using two-photon polymerization.

Reproduced with permission from [111].

The first microneedles directly produced by two-photon polymerization were made from Ormocer[®] (organically modified ceramic) materials [10]. These amorphous materials were originally prepared by the Fraunhofer-Institut für Silicatforschung (Wurzburg, Germany) using sol-gel processes. Ormocer materials are prepared from urethane- and thioether (meth)-acrylate alkoxy silane precursors. The inorganic components in Ormocer materials crosslink and form networks through condensation of organically modified alkoxy silane groups [105]. The organic components in Ormocer materials crosslink through light-initiated processes as well as other processes [105]. Interactions between the ceramic and polymer components prevent separation of Ormocer material into separate phases. X-ray diffraction confirmed that Ormocer material fabricated with two-photon polymerization shows an amorphous structure [10]. In addition, these interactions provide Ormocer materials with chemical and thermal stability. Ormocer materials remain stable to 350°C in an oxygen atmosphere and 415°C in a nitrogen atmosphere [106]. The mechanical properties for Ormocer materials may be varied between those of ceramics and those of polymers by varying several parameters, including the amount of organic network modifying elements, the amount of inorganic network modifying elements, the spacer length that connects the organic crosslinking sites and the inorganic crosslinking sites, and the concentration of fillers. In addition, Ormocer materials show less shrinkage (~ 2%) than other sol-gel materials because of the stability of the inorganic network and the inclusion of fillers [107]. Many other materials prepared by sol-gel processing show high (>20%) volume shrinkage during curing, network densifying, or solvent removal steps; these processes may lead to significant mechanical stresses, cracks, as well as

other defects. Ormocer-based matrix components (Definite[™]) and Ormocer-based light-curable dental composites (Admira[™]) are utilized in restorative dentistry. Carboxy-modified Ormocer materials are also utilized as matrix materials in light-curable glass ionomer cements [107].

In vitro assays, such as the 3{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide (MTT) assay, have been used to examine cell growth on Ormocer materials [108,109]. Live/dead assays demonstrated that polymerized Ormocer surfaces do not significantly impair cell viability or growth rates for B35 neuroblast-like cells and HT1080 epithelial-like cells [10]. In addition, the MTT assay was used to examine cell proliferation on two organically modified ceramic materials, Ormocore and Ormocer US-S4 [92,110]. This assay involves the reduction of a yellow tetrazolium salt (MTT) to a purple formazan dye by mitochondrial succinic dehydrogenase. Both materials were shown to support human epidermal keratinocyte growth. No significant differences between human epidermal keratinocyte growth on organically modified ceramic surfaces and on control surfaces were noted. These results suggest that Ormocore and Ormocer US-S4 materials processed using two-photon polymerization do not impair human epidermal keratinocyte viability or growth.

Nanoindentation of Ormocer US-S4 material provided hardness and Young's modulus values of ~ 90 and ~ 2100 MPa, respectively [110]. Considerable viscoelastic/plastic creep as well as viscoelastic recovery behavior were observed during testing. Park *et al.* suggested that microneedles fabricated using materials with Young's modulus values greater than ~ 1 GPa show fracture forces that exceed microneedle insertion forces [64,102]. Their work also suggests that microneedles fabricated using materials with higher Young's modulus values may provide larger microneedle failure forces. The high hardness and modulus values observed in Ormocer US-S4 material may be attributed to the strong interlinkages between ceramic and polymer components in the material.

Figure 3 contains a scanning electron micrograph of a microneedle array fabricated out of Ormocer organically modified ceramic material by means of two-photon polymerization [111]. The microneedles within this array show excellent microneedle-to-microneedle uniformity. Doraiswamy *et al.* fabricated a 10 × 10 hollow microneedle array out of Ormocer US-S4 material using two-photon polymerization [10]. The microneedles in this array demonstrated base diameters of 200 μm. The microneedle walls tapered linearly to sharp tips over needle lengths of 750 μm. These microneedles demonstrated much larger wall thicknesses than thin-walled microneedles created using conventional lithography-based techniques. Compression testing of the microneedles against porcine skin showed that the microneedles remained intact and did not show fracture after microneedle-skin interaction. Compression testing of Ormocer microneedles against polytetrafluoroethylene surfaces demonstrated that Ormocer microneedles undergo elastic and plastic deformation; the nonlinear stress-strain behavior of the devices

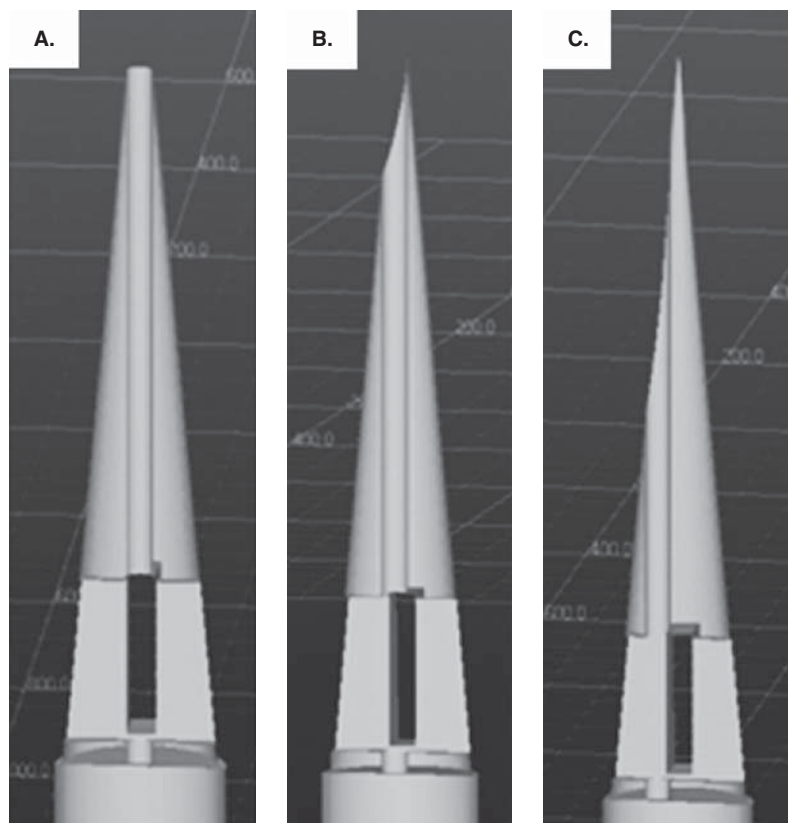


Figure 4. Computer-aided design diagrams of hollow Ormocer microneedles with (A) 0 μm , (B) 1.4 μm and (C) 20.4 μm needle center-pore displacement.

Reproduced with permission from [92].

could be attributed to the presence of the methacrylate groups in the Ormocer US-S4 material. The flexibility of the two-photon polymerization process enables the geometry, size and mechanical properties of microneedles to be readily modified. Ovsianikov *et al.* fabricated in-plane hollow microneedle arrays and out-of-plane hollow microneedle arrays (microneedle length = 800 μm , microneedle base diameter = 150 – 300 μm) with various aspect ratios out of Ormocer US-S4 material using two-photon polymerization [92]. Off-center microneedles were created by adjusting the position of the bore relative to the central axis. The aspect ratio was altered by varying the diameter of the microneedle base. The diameter of the channel was maintained at a constant value. Figure 4A – C contains computer-aided design images of microneedles with 0, 1.4 and 20.4 μm bore-center displacement values, respectively. Figure 5A – C contains scanning electron micrographs of Ormocer microneedles with 0, 1.4 and 20.4 μm bore-center displacement values, respectively. The microneedles were tapered linearly from the base to the tip. The length of the microneedle (800 μm) would allow use for withdrawal of biological fluids as well as delivery of pharmacologic agents. Ripple-like features were observed on the microneedle walls. These features were

attributed to layer-by-layer processing of the structures. The ripple-like features may be eliminated by increasing the number of the layers used for microneedle fabrication. Compression testing of several in-plane and out-of-plane hollow Ormocer microneedles against porcine skin showed that these microneedle arrays were able to pierce porcine adipose tissue without fracture. Compression testing of in-plane and out-of-plane hollow Ormocer microneedles arrays against porcine skin showed that the microneedle arrays were able to pierce porcine adipose tissue without fracture. The tips of the microneedles showed bending during compression; elastic behavior was attributed to the presence of methacrylate groups in the Ormocer US-S4 material. Compression testing of in-plane and out-of-plane hollow microneedles against polytetrafluoroethylene surfaces revealed that higher load values were required to fracture microneedles with larger base diameter values. In addition, off-center microneedles showed sharp needle tips and low penetration threshold values. Doraiswamy *et al.* also used two-photon polymerization to fabricate Ormocer microneedles with 3:1, 3.5:1 and 4:1 aspect ratios [110]. These microneedles were fabricated by varying the microneedle length while maintaining the base diameter of 150 μm as well as the inner channel diameter.

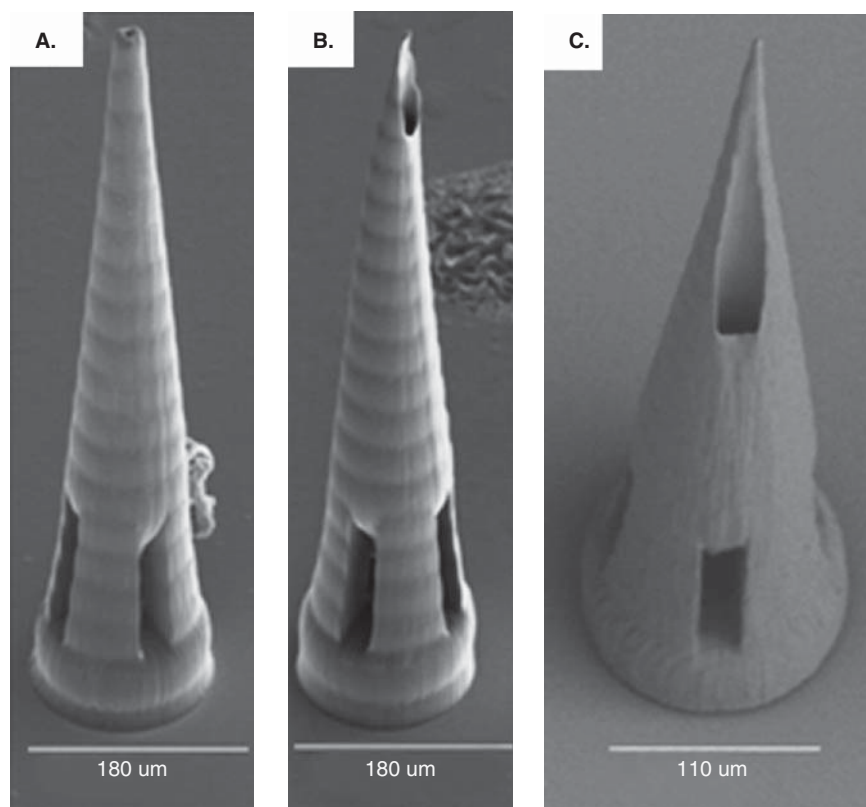


Figure 5. Scanning electron micrographs of hollow Ormocer microneedles with (A) 0 μm , (B) 1.4 μm and (C) 20.4 μm needle center-pore displacement.

Reproduced with permission from [92].

Microneedles with complex geometries, including rocket-like and mosquito-fascicle-like shapes, may be fabricated using two-photon polymerization. For example, microneedles with a rocket-like shape consisting of a cylindrical shaft and support braces have been fabricated using two-photon polymerization (Figure 4A). This needle geometry shows relatively small tip angle; as mentioned previously, lower microneedle penetration forces are associated with smaller microneedle tip angles [61,112]. Two-photon polymerization has also been used for fabrication of microneedles with biomimetic attributes. For example, the fascicle of the mosquito has submicrometer features that enhance skin penetration. The fascicle contains several organs, including the hypopharynx, labrum (feeding tube), mandibles and maxillae. The maxillae has serrated features, which facilitate skin penetration at relatively low forces [113,114]. Aoyagi *et al.* used finite element modeling as well as experimental studies to show that the serrated tips provide enhanced stress concentrations that facilitate skin penetration [65]. An Ormocer mosquito-like microneedle was fabricated by means of two-photon polymerization [115,116]. Figure 6 shows a scanning electron micrograph of the Ormocer mosquito-like microneedle; serrated features on the microneedle tip are clearly observed.

Ormocer microneedles fabricated using two-photon polymerization have been used for delivery of fluorescein-conjugated biotin and PEG-amine quantum dot solutions to cadaveric porcine skin. Quantum dots are 2 – 10 nm diameter semiconductor nanoparticles that are composed of II–VI or III–V semiconductor materials [117]. Quantum dots demonstrate large molar extinction coefficients and high quantum yield values; as a result, these materials provide higher brightness than conventional fluorophores [118]. Quantum dots may be used to detect cancer by means of passive mechanisms (e.g., quantum dots may be preferentially retained within tumor cells) or active mechanisms (e.g., quantum dots may be conjugated with tumor-specific molecules that enable them to attach to tumor cells) [119–122]. These materials may not be administered in oral form because they may be confined within the intestine, liver, kidneys, or lungs before entering systemic circulation [123,124]. In recent work, Ormocer microneedles successfully created pores in the stratum corneum layer, which facilitated transdermal administration of PEG-amine quantum dot solution [110]. Microneedles created using two-photon polymerization have been shown to enable distribution of PEG-amine quantum dot solution to deep epidermis and dermis layers of cadaveric porcine skin after 60 min. Figure 7 contains DIC-fluorescence micrographs

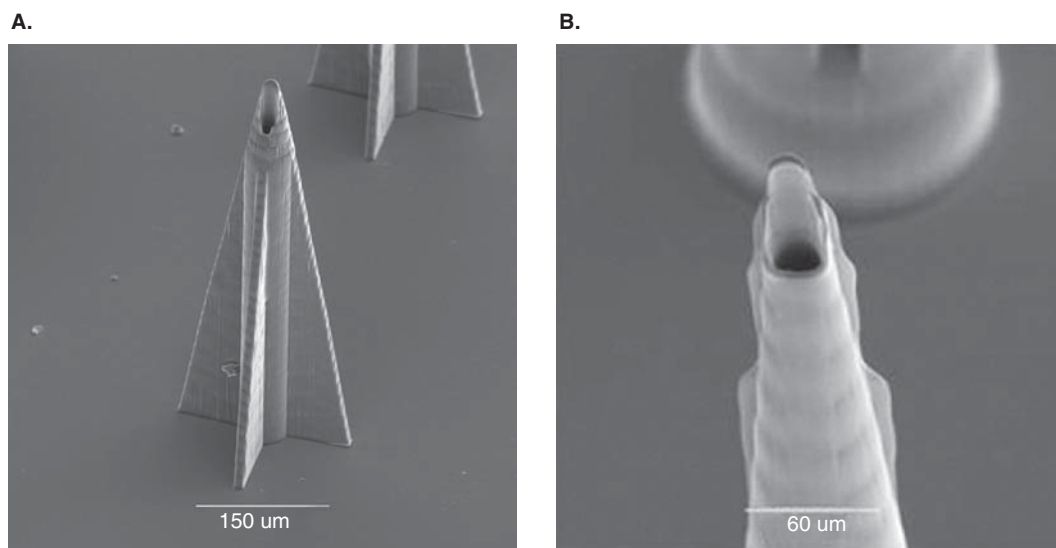


Figure 6. Scanning electron micrographs of microneedles with complex geometries. **A.** Scanning electron micrograph of an Ormocer rocket-shaped microneedle fabricated using two-photon polymerization. The small cross-sectional area minimizes the skin penetration force. **B.** Scanning electron micrograph of an Ormocer mosquito geometry microneedle fabricated using two-photon polymerization. The serrated tip provides stress concentrations, which decrease the skin penetration force.

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of porcine skin sections that were obtained 5 min after fluorescein-conjugated biotin administration and 60 min after fluorescein-conjugated biotin administration. The Ormocer microneedles enabled distribution of fluorescein-conjugated biotin to deep epidermis and dermis layers of cadaveric porcine skin after 60 min. It is anticipated that microneedles with different dimensions may provide control over transport of pharmacologic agents into specific epidermal, dermal, or subdermal layers.

Two-photon polymerization followed by subsequent molding steps may be utilized for creating solid microneedles as well as solid microneedle arrays [62,66,67]. The chemical, mechanical and physical properties of the materials used to fabricate the devices in this indirect rapid prototyping process are conserved because these materials do not undergo two-photon polymerization processing. In addition, microneedles may be fabricated out of a broader range of materials that are incompatible with two-photon polymerization. LaFratta *et al.* showed that an indirect rapid prototyping process involving photopolymerization and micromolding may be used for fabrication of three-dimensional microscale structures [125]. Gittard *et al.* recently used two-photon polymerization and polydimethyl siloxane micromolding for microneedle fabrication [62]. Two-photon polymerization was used to make a master structure, which was subsequently used to make a negative mold from poly(dimethyl siloxane). The negative mold was then used to cast microneedles out of photoreactive acrylate-based polymer (Envisiointec, Ferndale, MI, USA). The acrylate-based polymer is a water-resistant material that shows flexural strength of 2300 MPa (D790M test method), hardness of 83 Shore (D2240 test

method), tensile strength of 57.8 MPa (D638M test method), Young's modulus of 2400 GPa (D638M test method) and glass transition temperature of 109°C (E1545-00 test method); it should be noted that this material is opaque and as such may not be processed using two-photon polymerization. The solid microneedles had base diameters of 150 μm and lengths of 500 μm . The microneedle array demonstrated good microneedle-to-microneedle uniformity. In most cases, microscale features in the microneedles were consistent with dimensions described in the computer-aided design file; however, some of the microneedles showed slight deviations from the dimensions described in the computer-aided design file. For example, the tips of a small fraction of microneedles in the microneedle arrays showed truncated features. Twenty-four-hour MTT assays indicated that human epidermal keratinocyte growth on the acrylate-based polymer surfaces was similar to that on control surfaces. The 5×5 acrylate-based polymer microneedle arrays were able to withstand an axial load of 10 N without fracture; this result suggests that acrylate-based polymer microneedle arrays possess suitable compressive strength for use in transdermal drug delivery. *Ex vivo* studies demonstrated that microneedle arrays successfully penetrated human stratum corneum and epidermis. Irregular $\sim 58 \mu\text{m}$ diameter pores were observed in the human stratum corneum and epidermis. The pores created by the microneedles were shown to penetrate completely the stratum corneum layer and enter the deeper epidermal layers. The pores became smaller after microneedle removal; however, they remained open and retained capability for use as conduits for pharmacologic agents. Two-photon polymerization-icromolding represents a

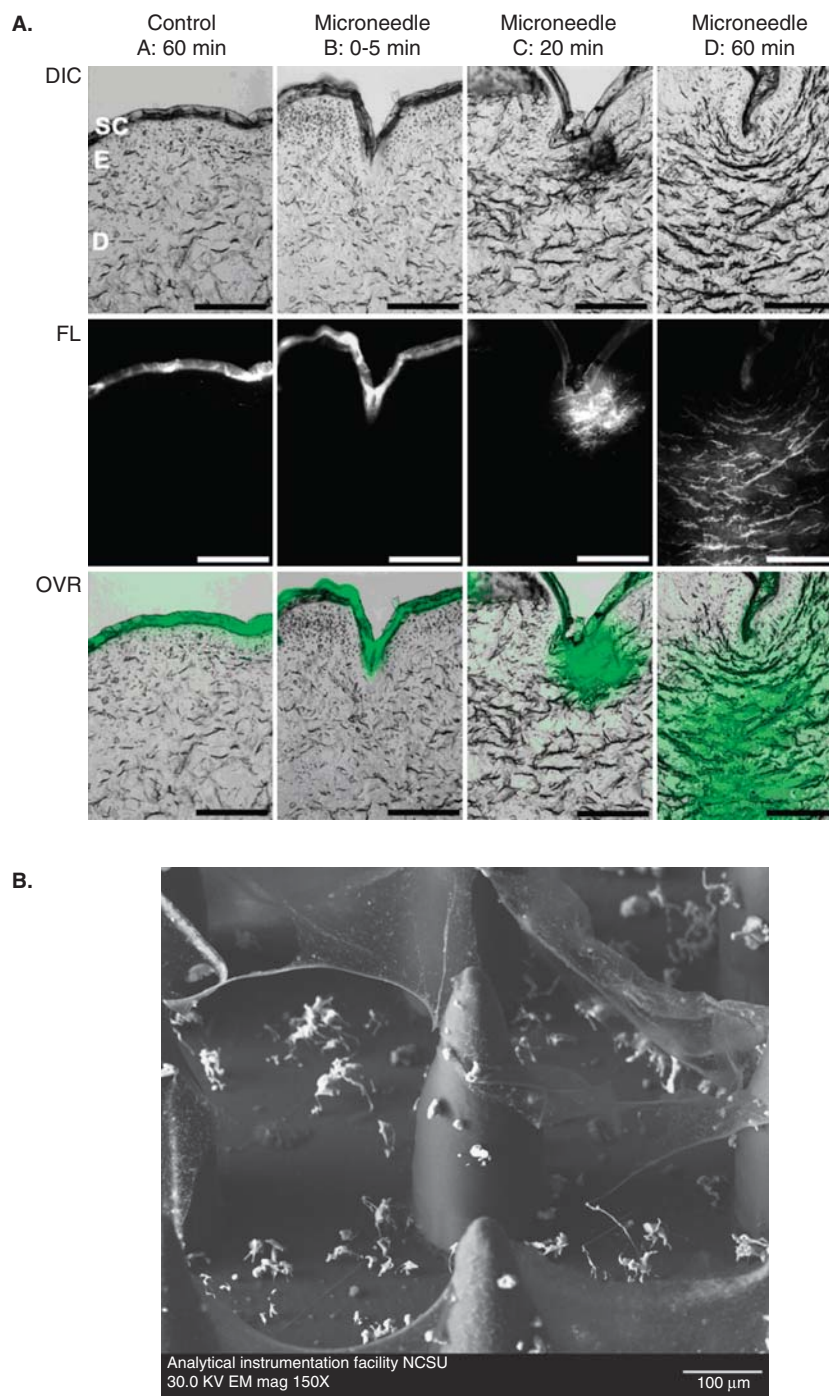


Figure 7. A. DIC-fluorescence confocal microscopy images showing administration of fluorescein-conjugated biotin solution to porcine skin. The center columns and the right column demonstrate that administration of fluorescein-conjugated biotin solution to deeper layers of porcine skin was enabled by a microscale pore, which was created by an Ormocer microneedle. The left column shows administration of fluorescein-conjugated biotin solution to porcine skin without microneedle enhancement. The topically administered fluorescein-conjugated biotin solution remained on the skin surface. Top row: DIC single-channel demonstrates stratum corneum (SC), deeper epidermis (E) and dermis (D) layers of the skin. Bottom row: DIC-fluorescence overlay (OVR) shows distribution of fluorescein-conjugated biotin solution within various skin layers. Scale bar equals 100 μ m. **B.** Scanning electron micrograph of polyethylene glycol 600 diacrylate microneedles after exposure to platelet-rich plasma. No protein aggregation and no platelet aggregation were observed on the polyethylene glycol 600 diacrylate microneedles. Small, widely scattered crystals were observed on the surface; the presence of sodium, chlorine and phosphorus in energy-dispersive X-ray spectroscopy data suggests that sodium chloride crystals precipitated from the platelet-rich plasma.

scalable approach that may be used to create solid microneedles and microneedle arrays out of a broader range of materials than two-photon polymerization.

The two-photon polymerization-micromolding process has also been used to fabricate microneedles out of polyethylene glycol diacrylate. Once polymerized, polyethylene glycol is a relatively stable material because hydrolytic degradation can take place only at crosslink points [126]. Polyethylene glycol shows inert behavior because uncharged hydrophilic groups are exposed in aqueous solutions. Miyano *et al.* demonstrated that microneedles fabricated from polyethylene glycol were able to penetrate a living human skin model; their work suggested that polyethylene glycol microneedles showed sufficient mechanical strength to be form conduits through the stratum corneum [127]. There are minimal concerns regarding fracture of polyethylene glycol microneedles during skin insertion because the devices undergo hydrolytic degradation on the skin surface. Furthermore, polyethylene glycol microneedles may be destroyed by means of boiling. Takano *et al.* recently demonstrated insertion of polyethylene glycol microneedles into a wet cultured human skin model under biaxial tension; shortening of microneedles was attributed to hydrolysis [128]. They described placement of polyethylene glycol microneedles on the skin surface for extended periods of time; hydrolysis of polyethylene glycol microneedles may allow for gradual release of pharmacologic agents. Gittard *et al.* recently fabricated polyethylene glycol diacrylate microneedle arrays using photopolymerization-micromolding, which had base diameters of 150 μm , lengths of 500 μm , tip angles of 45°, and > 10 μm tip diameters; good correspondence with the master structure was observed [66]. Truncated microneedle tips as well as voids within microneedles were observed in a small fraction of the microneedles within the microneedle arrays. These defects were attributed to incomplete degassing of the unpolymerized polyethylene glycol solution. Gittard *et al.* showed that polyethylene glycol 600 diacrylate microneedles exposed to human platelet-rich plasma did not show protein aggregation or platelet aggregation [66]. Several investigators have demonstrated that polyethylene glycol diacrylate-based materials possess nonspecific resistance to cell adhesion and protein adsorption [129-131]. The ability of polyethylene glycol surfaces to resist biological molecules is attributed to the formation of hydrogen bonds with water molecules; this process is enhanced by steric stabilization. Steric stabilization is a repulsive force associated with: i) the loss in conformational freedom of polyethylene glycol chains when proteins move closer to polyethylene glycol surfaces; and ii) osmotic interactions between proteins and polyethylene glycol surfaces [132]. These properties are appealing for microneedles, as protein fouling and cell adhesion can impede pharmacologic agent transport from microneedles to adjacent tissues.

One important factor that is limiting clinical use of microneedles and microneedle arrays is the risk of infection. The stratum corneum prevents a variety of foreign materials, including microorganisms, from entering the body.

Pathogenic microorganisms such as *Staphylococcus aureus* reside on the surface of the skin; a pore in the stratum corneum created by a microneedle could enable microorganisms to cause local or systemic infection [133-135]. For example, Birchall noted that the channels formed by microneedles through the stratum corneum could increase the risk of infection [136]. He also noted that damage to the stratum corneum is transient as well as minor in nature; however, the skin repair mechanisms in response to microneedle-fabricated pores are not completely understood. Donnelly *et al.* recently examined the movement of microorganisms across microneedle-fabricated pores by means of *in vitro* models [137]. They demonstrated that pathogenic microorganisms, including *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, adhered to microneedle arrays during insertion into excised porcine skin. It should be noted that no microorganisms entered viable epidermis layers by means of microneedle-fabricated pores. In addition, the number of microorganisms that penetrated microneedle-punctured Silescol® (Barloworld Scientific Ltd, Staffordshire, UK) membranes was an order of magnitude lower than the number of microorganisms that penetrated hypodermic needle-punctured Silescol membranes. They stated that microneedle-fabricated pores would not lead to infections under normal circumstances in immune-competent patients; however, they suggested that patient safety would be enhanced by manufacturing microneedles using sterile techniques. The risk of infection associated with microneedle-fabricated pores may also be reduced by imparting microneedles with antimicrobial properties.

Antimicrobial microneedles may be fabricated out of composite materials that contain biocompatible polymers such as polyethylene glycol as well as conventional antimicrobial agents such as gentamicin sulfate. Gentamicin is a broad spectrum antimicrobial agent that shows efficacy against Gram-negative organisms as well as some Gram-positive organisms. It is commonly used for treatment of pyoderms and other skin infections; for example, Changez *et al.* showed that gentamicin is active against *Staphylococcus* bacteria that are commonly found on the skin [138]. Gentamicin is hydrophilic material; aqueous suspensions containing polyethylene glycol and gentamicin may be readily produced [139]. Gittard *et al.* used photopolymerization-micromolding to fabricate microneedle arrays out of a photosensitive material containing polyethylene glycol 600 diacrylate, gentamicin sulfate and a photoinitiator. An agar plating assay revealed that the gentamicin-doped polyethylene glycol 600 diacrylate microneedle inhibited growth of *S. aureus* bacteria. The 26.8 mm diameter zone of inhibition indicated that gentamicin sulfate was released from the microneedle array. Acellular bubble-like structures were observed on the agar surface; these features were attributed to hydrogel degradation.

Antimicrobial microneedles may also be prepared by coating microneedles with thin films of antimicrobial materials such as silver. Silver shows broad spectrum antimicrobial properties

against viruses, fungi and bacteria (e.g., *S. aureus*); this activity is attributed to disruption of electron transport and interruption of DNA replication [140-147]. Silver also reduces inflammatory activity as well as facilitates healing in wounds. In addition, Demling and co-workers suggest that silver increases re-epithelialization compared with a conventional antimicrobial solution [148-150]. Coatings containing antimicrobial materials may be applied to microneedle surfaces using a physical vapor deposition process known as pulsed laser deposition [151]. In pulsed laser deposition, a solid target is vaporized using a high energy excimer laser; the average kinetic energies of atomic and molecular species produced by excimer laser ablation is 100 – 1000 kT; kT is equal to 26 meV at room temperature [152,153]. The high energies of the laser-ablated species serve to promote adhesion of the film as well as chemical reactions between the substrate and the film [154]. Pulsed laser deposition is ideally suited for film deposition on polymeric substrates because many coatings may be prepared at room temperature. For example, Gittard *et al.* fabricated microneedles with antimicrobial functionality using a combination of two-photon polymerization-micromolding and pulsed laser deposition [67]. In this study, pulsed laser deposition was used to deposit silver thin films onOrmocer microneedle arrays, which were fabricated using two-photon polymerization-micromolding. No pinholes or other non-uniformities were noted in the silver thin films on the silver-coated microneedles. MTT studies showed that human epidermal keratinocyte viability on the silver-coated Ormocer surfaces was similar to that on uncoated Ormocer surfaces. An agar diffusion assay was used to evaluate the antimicrobial performance of the silver-coated Ormocer microneedle array and the uncoated Ormocer microneedle array. On the plate containing the uncoated Ormocer microneedle array, *S. aureus* growth was noted directly beneath the microneedle array. On the plate containing the silver-coated Ormocer microneedle array, an absence of *S. aureus* growth beneath the microneedle array was noted. In addition, inhibited growth was noted in the agar that surrounded the microneedle array; the presence of a zone of inhibition suggests that silver was released into agar. The results of these studies suggest that the photopolymerization-micromolding process may be used alone or in combination with pulsed laser deposition to fabricate microneedles that show antimicrobial functionality.

4. Other medical applications of two-photon polymerization

Two-photon polymerization may also be used to fabricate a wide variety of microstructured and nanostructured medical devices; for example, two-photon polymerization has been used to create patient-specific medical prostheses as well as scaffolds for tissue engineering. Ovsianikov *et al.* utilized two-photon polymerization for fabrication of ossicular replacement prostheses, which are used to restore sound conduction in the middle ear [156]. Three bones in the middle ear, the malleus, the incus and the stapes, transmit sounds

from the tympanic membrane to the inner ear. Discontinuity or fixation of these bones may result in conductive hearing loss. Conventional implants are produced in several standardized shapes and sizes; however, these designs do not take into account individual patient anatomy. These prostheses are shaped during implantation procedures; the intraoperative shaping process requires extra operative time. One method for lowering the cost as well as increasing the success of ossicular replacement prostheses involves the development of patient-specific implants. For example, imaging (e.g., computed tomography or magnetic resonance imaging) and analysis software may be used to generate an implant with an appropriate geometry and size for a particular patient [155]. Two-photon polymerization enables prosthesis to be fabricated with smaller features than stereolithography, selective laser sintering, fused deposition modeling, or other rapid prototyping techniques. Ovsianikov *et al.* demonstrated fabrication of total ossicular replacement prostheses out of Ormocer US-S4 material using two-photon polymerization [156]. The prostheses were similar in design to a commercially available total ossicular replacement prosthesis (Kurz Medical, Inc., Dusslingen, Germany). Conical structures were fabricated on the head of the prosthesis; these structures were intended to reduce device migration, improve cell adhesion and decrease the likelihood of tympanic membrane perforation. The disk-shaped headplate of the prosthesis was intended to be placed under the tympanic membrane. The shaft of the prosthesis connected the footplate of the stapes with the undersurface of the tympanic membrane; this structure was intended to enable sound conduction. An Ormocer ossicular replacement prosthesis was placed on the footplate of the stapes and underneath the tympanic membrane in a commercially obtained de-frozen human head; this prosthesis was implanted and removed from the implantation site without fracture. A total ossicular replacement prosthesis fabricated using two-photon polymerization may be prepared with an appropriate geometry for a given patient. The prosthesis may be fabricated with features that: i) maintain a small angle between the stapes and the prosthesis; ii) place the prosthesis on the center of the footplate; and iii) properly align the prosthesis with respect to the surrounding structures.

Two-photon polymerization may also be used to develop artificial tissues and organs. Tissue and organ substitutes may be created by placing living cells within three-dimensional scaffolds, which guide cell development. The cell-seeded scaffolds are subsequently placed in bioreactors, which provide nutrients that enable cell proliferation within the scaffolds. The cell-seeded scaffolds are later implanted within the body, where the tissue substitutes can enable the body to resume normal function. Rapid prototyping technologies such as two-photon polymerization hold great potential for the fabrication of artificial tissues and organs with appropriate geometries and mechanical properties for a given individual. For example, Doraiswamy *et al.* fabricated

three-dimensional tissue engineering scaffolds with Lego[®]-like interlocking structures out of Ormocer US-S4 material [10]. The Lego-like interlocking tissue engineering scaffolds contained arrays of 75 µm diameter, 20 µm tall cylindrical pillar structures on both sides of a flat Ormocer chip. The cylindrical structures could facilitate layer-by-layer stacking of scaffolds for the development of three-dimensional tissues. Cells seeded on these structures were orientated along the pillar walls, and were found to increase gradually in number over time. One hundred per cent of the B35 neuroblast-like cells remained viable 48 h after placement on the Ormocer substrates. The cell interaction and cell proliferation properties of B35 neuroblast-like cells on Lego-like interlocking tissue engineering scaffolds suggest that Ormocer structures fabricated using two-photon polymerization may provide an alternative to conventional scaffolds for creating multilayered artificial tissues and organs. Proliferation of endothelial, neuroblastoma, granulosa and keratinocyte cells on scaffolds fabricated using two-photon polymerization has also been examined [10,112,157,158]. In addition, two-photon polymerization has been used to fabricate small storage capsules on metal stents; these structures may make possible localized delivery of pharmacologic agents at the implantation site [88].

5. Conclusions

Two-photon polymerization is a new rapid prototyping technique that may be used to fabricate microneedles as well as microneedle arrays with a wider range of geometries than reactive ion etching, lithography-electroforming replication, or other conventional microelectronics-based technologies. The two-photon polymerization process relies on temporal and spatial overlap of photons to achieve photopolymerization and hardening of material within highly localized and well-defined volumes. The authors' results suggest that two-photon polymerization is able to create microneedles with a wide range of geometries, including in-plane microneedles, out-of-plane microneedles, mosquito fascicle-shaped microneedles and rocket-shaped microneedles. Ormocer microneedles fabricated using two-photon polymerization penetrated cadaveric porcine adipose tissue without fracture. Penetration of the stratum corneum layer by Ormocer microneedles enabled transdermal delivery of PEG-amine quantum dot solution and fluorescein-biotin solution to the deep epidermis and dermis layers of porcine skin; microneedles were shown to enable more rapid distribution than topical administration. A two-photon polymerization-polydimethyl siloxane micro-molding process has been used to fabricate solid microneedles out of a photoreactive acrylate-based polymer, polyethylene glycol 600 diacrylate and Ormocer material; this indirect rapid prototyping process is more suitable for large-scale processing than two-photon polymerization. Solid microneedle arrays fabricated out of photoreactive acrylate-based polymer were successfully able to create pores in human stratum corneum and epidermis; these arrays were able to withstand an axial

load of 10 N without fracture. Scanning electron microscopy revealed no platelet aggregation on the surfaces of platelet-rich plasma-exposed polyethylene glycol 600 diacrylate microneedles. Antimicrobial solid microneedles were fabricated out of a photosensitive material containing polyethylene glycol 600 diacrylate, gentamicin sulfate and a photoinitiator using two-photon polymerization-micromolding. Antimicrobial solid microneedles with silver coatings were fabricated using a combination of two-photon polymerization-micromolding and pulsed laser deposition. These studies suggest that the geometry as well as the mechanical properties of microneedles may be rapidly optimized using two-photon polymerization for depth-dependent transdermal delivery of pharmacologic agents. Several other microstructured medical devices with unique geometries, including ossicular replacement prostheses and tissue engineering scaffolds, have been successfully fabricated using two-photon polymerization of Ormocer organic-inorganic hybrid materials.

6. Expert opinion

Over the next few years, microneedles will find greater use for delivery of nucleic acid- and protein-based pharmacologic agents that cannot be administered in enteral form. Over the past few decades, microneedle devices have been prepared using reactive ion etching, lithography-electroforming replication, or other microelectronics-based fabrication techniques. Several small and large companies are in the process of bringing their microneedle-based devices to market for transdermal delivery of vaccines and hormones. However, numerous factors are limiting the commercial viability of microneedles prepared using microelectronics-based fabrication techniques. In recent years, two-photon polymerization has been used to fabricate microneedles as well as microneedle arrays with a wider range of geometries than conventional microelectronics-based technologies. Perhaps the most advantageous attribute of two-photon polymerization is that it provides a high degree of control over microneedle geometry. Microneedles must not fracture during penetration into the skin, use, or removal from the skin. As mentioned earlier, many of the problems associated with microneedles prepared using conventional techniques may be attributed to the fact that these techniques have inherent limitations in terms of fabricating microneedles that have large wall thicknesses as well as other fracture-resistant geometries. In addition, two-photon polymerization is compatible with many photosensitive resins, which are widely available and may be obtained at low cost. Furthermore, two-photon polymerization can be performed in conventional facilities; for example, two-photon polymerization can potentially be set up in a conventional clinical environment (e.g., an out-patient medical office) for fabrication of patient-specific drug delivery devices that conform to a given patient's anatomy and medical condition. Several commercialization and device-related challenges will need to be considered in the coming years. For example,

techniques for creating microneedles that show a clinically acceptable degree of uniformity from batch to batch must be developed. Refinement of microneedle geometry for a given clinical application as well as a specific patient population is also necessary. In addition, optimization of the two-photon polymerization processing rate must be undertaken. It should be noted that a combination of two-photon polymerization and micromolding may be directly implemented in current manufacturing environments for large-scale processing of solid microneedles as well as microneedle arrays. Furthermore, work is needed to incorporate multiple functionalities (e.g., monitoring and treatment of multiple chronic diseases) within a single microneedle-based device. Finally, two-photon polymerization needs to be cost-competitive with conventional techniques. If these obstacles can be overcome,

two-photon polymerization of microneedles as well as other drug delivery devices may attain commercial significance over the coming decades.

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Declaration of interest

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