Integrated carbon fiber electrodes within hollow polymer microneedles for transdermal electrochemical sensing

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In this study, carbon fiber electrodes were incorporated within a hollow microneedle array, which was fabricated using a digital micromirror device-based stereolithography instrument. Cell proliferation on the acrylate-based polymer used in microneedle fabrication was examined with human dermal fibroblasts and neonatal human epidermal keratinocytes. Studies involving full-thickness cadaveric porcine skin and trypan blue dye demonstrated that the hollow microneedles remained intact after puncturing the outermost layer of cadaveric porcine skin. The carbon fibers underwent chemical modification in order to enable detection of hydrogen peroxide and ascorbic acid; electrochemical measurements were demonstrated using integrated electrode-hollow microneedle devices. © 2011 American Institute of Physics. [doi:10.1063/1.3569945]

I. INTRODUCTION

In vivo sensors may be used to provide real-time detection of physiological processes, such as monitoring of neurotransmitters, medically relevant molecules, cancer biomarkers, and pathogenic microorganisms.¹ Devices for monitoring physiologically relevant compounds such as acetylcholine, dopamine, glutamate, glucose, lactate, and pyruvate have been described in the literature.² Stett *et al.* described the use of a microelectrode array for evaluating physiologic and pathologic activity within a tissue; in particular, they considered the use of biosensors for drug discovery as well as drug screening.³ Electrochemical biosensors provide several advantages for point-of-care monitors, including small sensor size, low cost, simplicity, rapid response, and sensitivity; in addition, electrochemical methods may enable simultaneous monitoring of several biomarkers.^{1,4,5} For example, Wang *et al.* developed disposable electrodes by means of screen-printing, which contained carbon inks with sol-gel-derived enzymes.^{4,5} Disposable glucose oxidase electrodes have been very important in the development and clinical translation of hand-held diagnostic devices, which are used for patient self-management of diabetes.

As described by Mastrototaro and Newman *et al.*, many conventional monitoring methods utilize macroscale systems; these systems are associated with several shortcomings, including transfer of fluids between devices, painful device-tissue interactions, and large sample volumes.^{6,7} An ideal sensor should exhibit several properties, including capability of monitoring a variety of biological markers, compatibility with small sample volumes, and minimal tissue damage. An approach for achieving real-time, minimally invasive point-of-care sensing would be the realiza-

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tion of a device that is capable of obtaining biological samples (e.g., interstitial fluid) through the skin while protecting the sensing transducer from biofouling elements.^{8,9}

Hollow microneedles have recently been developed for point-of-care transdermal biosensing and drug delivery.¹⁰ McAllister *et al.* described microneedles as small-scale projections containing either hollow bores or solid bores; these devices exhibit features between 1 and 1000 μm .¹¹ McAllister *et al.* described the use of microneedles for minimizing tissue trauma and pain.¹² Gill *et al.* demonstrated that microneedles with lengths between 480 and 1450 μm were associated with less pain in normal human volunteers than conventional 26-gauge hypodermic needles; this result is attributed to the fact that microneedles have less contact with Pacinian corpuscles, Meissner's corpuscles, and nerve endings located within the dermis than conventional hypodermic needles.^{9,13} Hollow microneedles are commonly used to create pores in the stratum corneum layer of the epidermis, enabling controlled delivery of one or more pharmacologic agents to deeper layers of the epidermis or to the papillary dermis.^{12,13} This approach is particularly useful for delivery of nucleic acid-based agents (e.g., gene delivery agents) and protein-based agents (e.g., insulin); these agents are currently administered using hypodermic needles.^{11,12}

To date, the majority of microneedle research activities have involved the use of microneedles as transdermal drug delivery devices. Microneedles have also been considered for use in micro-total analysis systems, which may be used for evaluation of biological samples; microneedle-based monitors provide simplified acquisition and transfer of biological samples to the electrode, reduced patient pain, reduced energy consumption, and smaller device dimensions than conventional monitors.^{14,15} Suzuki *et al.* utilized micromachining for fabrication of a disposable microanalysis device, which contained a microscopic needle, a sampling (pumping) mechanism, and a micro-glucose sensor; they demonstrated sensor responses to glucose solutions with concentrations up to 8 mM and 90% response times of 30–40 s.¹⁶ Zimmermann *et al.* described the fabrication of a device containing an enzyme-based glucose sensor, a porous silicon dialysis membrane, and hollow out-of-plane microneedles; they introduced interstitial fluid to the microneedles and observed a significant sensor response.¹⁷ Mukerjee *et al.* utilized a device containing a hollow microneedle array and microfluidic channels for measuring the glucose concentration of interstitial fluid, which was extracted from human skin.¹⁸ Tsuchiya *et al.* described the development of a blood sampling device, which consisted of titanium microneedle with dimensions similar to those of a female mosquito labrum, a shape memory alloy actuator for skin penetration, and a glucose oxidase-based biosensor; they demonstrated that this pumping system was capable of extracting 2 $\mu\text{l}/\text{min}$ of whole blood.¹⁹ Zahn *et al.* noted that large molecular weight compounds (e.g., large molecular weight proteins) have negative effects on sensor function, including altering signal stability and disrupting enzyme function; they discussed the use of a microdialysis microneedle for filtering glucose and other small molecule weight compounds from biological samples.¹⁵ It should be noted that hollow microneedle-based biosensors commonly require the use of pumping systems for sample acquisition and complex microfluidic channels for sample calibration; furthermore, hazardous chemicals may be required for biosensor calibration.^{17,18}

As described by McAllister *et al.*, early microneedle fabrication methods were adopted from microelectronics industry-based processes, including lithography-based processes.²⁰ Due to its compatibility with conventional microfabrication processes, microneedles were initially fabricated out of silicon. For example, Henry *et al.* utilized reactive ion etching to fabricate 150 μm long solid microneedles out of silicon.²¹ Chun *et al.* used Bosch deep reactive ion etching to fabricate holes on a silicon substrate; hollow microcapillary arrays were subsequently fabricated on the substrate.²² Mukerjee *et al.* described machining a hollow microneedle array with microfluidic channels out of single crystal silicon.¹⁸ Kim *et al.* fabricated a hollow metallic microneedle array using a multiple step process, which involved backside exposure of SU-8 and conformal electroplating of nickel.²³ Park *et al.* prepared microneedles out of biodegradable polymers, including polyglycolic acid and polylactic acid, using either electromechanical masking/etching and polydimethylsiloxane (PDMS) micromolding or *in situ* lens-based lithography and PDMS micromolding.^{12,24} Pérennès *et al.* demonstrated fabrication of hollow microneedle arrays out of polymethylmethacrylate using a multiple step process, which utilized a double deep x-ray lithog-

raphy, electrodeposition, and molding.²⁵ Wang *et al.* described the use of conventional drawn glass micropipette methods for preparing hollow microneedles out of borosilicate glass.²⁶

Many conventional microneedle fabrication processes are not compatible with preparation of large numbers of devices due to the fact that these processes involve complex, multiple step procedures and/or long fabrication times. In order to overcome these obstacles, rapid prototyping methods have recently been utilized for microneedle fabrication. Rapid prototyping, also known as solid freeform fabrication or layer manufacturing, involves building a structure in an additive manner by means of layer-by-layer, selective joining of material.^{27–29} Fabrication of the structure is commonly directed by a computer-aided design drawing. In addition, this approach is compatible with low processing costs and high yield; furthermore, a specialized processing environment (e.g., a clean room) is not required.³⁰ Stereolithography is one rapid prototyping method that is associated with low processing costs as well as short processing times; Tse *et al.* demonstrated use of stereolithography for fabricating high aspect ratio three-dimensional microscale structures.³⁰ Matsuda and Mizutani used a stereolithography apparatus with an ultraviolet light pen to create microneedles out of acrylate-endcapped poly(epsilon-caprolactone-co-trimethylene carbonate) materials.³¹ Microneedles loaded with indomethacin, an anti-inflammatory pharmacologic agent, were shown to minimize inflammation in a murine model. Zhang *et al.* demonstrated that microstereolithography, a process that is capable of creating structures with 1.2 μm resolution, may be used to prepare microscale channels, microcones, and other three-dimensional structures.³² Choi *et al.* utilized microstereolithography to create microneedle arrays.³³ Two photon polymerization is another rapid prototyping technology that has been used for microneedle fabrication. In two photon polymerization, selective polymerization of a photosensitive resin is obtained by means of nearly simultaneous absorption of ultrashort laser pulses; the voxel size is dependent on several parameters, including laser power, sensitivity of the resin, and numerical aperture of the objective lens.¹⁰ Ovsianikov *et al.* recently demonstrated fabrication of poly(ethylene glycol) diacrylate structures with 200 nm features by means of two photon polymerization.³⁴ Doraiswamy *et al.* demonstrated fabrication of a microneedle array out of organically modified ceramic (Ormocer[®]) material using two photon polymerization.³⁵ Ovsianikov *et al.* used two photon polymerization to create in-plane microneedles and out-of-plane hollow microneedles.³⁶ More recently, Gittard *et al.* fabricated microneedle arrays out of an acrylate-based polymer using two photon polymerization.³⁷

A rapid prototyping approach involving use of a digital micromirror device (DMD[™]), which was originally developed by Texas Instruments, as a dynamic mask was used for selective polymerization of a photosensitive acrylate-based polymer resin into a microneedle device. In recent years, several investigators have utilized digital micromirror device-based stereolithography to fabricate small-scale devices. For example, Sun *et al.* used a digital micromirror device-based stereolithography system to prepare three-dimensional microscale structures, including a matrix and a microspring array with minimum feature size of 0.6 μm .³⁸ Gittard *et al.* utilized digital micromirror device-based stereolithography to prepare scaphoid and lunate bone prostheses from computed tomography data; the prostheses exhibited $\sim 50\text{-mm}$ -thick layers.³⁹ In this study, the minimum compressive force to obtain fracture was 1248 N for the lunate prosthesis and 1360 N for the scaphoid prosthesis. Han *et al.* used digital micromirror device-based stereolithography to create multilayered scaffolds out of a resin containing poly(ethylene glycol) diacrylate and fibronectin; attachment of murine marrow-derived progenitor cells to fibronectin-containing scaffolds was demonstrated.⁴⁰ Choi *et al.* prepared tissue engineered scaffolds with $\sim 100\text{ }\mu\text{m}$ interconnected pores out of poly(propylene fumarate) by means of digital micromirror device-based stereolithography.⁴¹ In more recent work, Choi *et al.* demonstrated processing of structures containing multiple materials using digital micromirror device-based stereolithography; these materials have potential use as scaffolds in tissue engineering.⁴²

A variety of materials have been used for microneedle fabrication, including silicon, glass, metal (e.g., stainless steel and nickel), and resorbable polymers (e.g., polyglycolic acid and polylactic acid).^{11,24,26,43} A commercially obtained acrylate-based polymer, e-Shell 200, was utilized for microneedle fabrication. The material is a class-IIa biocompatible, water-resistant material; it

has been used in thin-walled hearing aid shells, solid microneedle arrays, as well as nonmedical applications.^{44,45} Information provided by the manufacturer (Envisiontec, Gladbeck, Germany) shows that e-Shell 200 contains 0.5%–1.5% weight phenylbis(2,4,6 trimethylbenzoyl)-phosphine oxide photoinitiator, 15%–30% weight propylated (2) neopentyl glycoldiacrylate, and 60%–80% weight urethane dimethacrylate. Energy-dispersive x-ray spectroscopy indicated that e-Shell 200 contains carbon, oxygen, and titanium; these elements are known to possess excellent biocompatibility.³⁹ According to technical data supplied by the manufacturer, e-Shell 200 exhibits a water absorption value of 0.12% (D570-98 test method) and a glass transition temperature of 109 °C (E1545-00 test method). It exhibits tensile strength of 57.8 MPa (D638M test method), flexural strength of 103 MPa (D790M test method), and elongation at yield of 3.2% (D638M test method). Gittard *et al.* showed using nanoindentation that e-Shell 200 exhibits hardness and Young's modulus values of 93.8 ± 7.25 and 3050 ± 90 MPa, respectively.³⁹ Work by Park *et al.* suggests that microneedles fabricated out of materials with Young's modulus values greater than ~ 1 GPa possess fracture forces that exceed skin insertion forces.²⁴

In this study, we report on the use of a digital micromirror device-stereolithography instrument to fabricate hollow microneedles and the integration of carbon fiber electrodes within the bores of these hollow microneedles. Carbon fiber-containing materials have been considered for use in a variety of medical device applications, including joint prostheses, spinal surgery devices, and tumor surgery devices.⁴⁶ Wolter described carbon fibers as well as carbon fiber microparticles as having excellent biocompatibility.⁴⁷ It should be noted that fragmentation of carbon fibers remains a source of concern. Neugebauer *et al.* showed that carbon fiber fragments were associated with a small amount of fibrosis as well as a foreign body giant cell reaction; it should be noted that inflammation, tissue necrosis, and toxic effects on tissues were not associated with carbon fiber fragments.⁴⁸ Mendes *et al.* suggest that carbon fibers are biocompatible as long as the fiber structure is preserved.⁴⁹ The carbon fibers were chemically modified to enable detection of two medically significant molecules, hydrogen peroxide and ascorbic acid. Electrochemical characterization was performed on the chemically modified electrode-hollow microneedle devices. Hydrogen peroxide (H_2O_2) is a reactive oxygen species that is monitored in many common enzyme-based electrochemical sensors.^{5,50} For example, most glucose sensors contain immobilized glucose oxidase. Hydrogen peroxide and gluconolactone are produced in reactions between glucose and glucose oxidase; monitoring of released hydrogen peroxide is used for quantification of glucose. Monitoring of released hydrogen peroxide may also be used for quantification of glutamate in brain dialysate; hydrogen peroxide is produced in reactions between glutamate and glutamate oxidase.^{1,51} Glutamate is an excitatory neurotransmitter, which has been linked with aggressive activity.^{1,52} Ascorbic acid has recently been described as an indicator of oxidative stress that is experienced by cells.⁵³ Harrison *et al.* noted that ascorbic acid deficiency is associated with obstetric complications, such as preterm labor and premature rupture of the membranes.⁵⁴ Deficient levels of ascorbic acid ($<14.1 \mu\text{M}$) were noted in 6% of individuals in a sample of 200 lactating women; no symptoms of clinical deficiency (e.g., scurvy) were noted in these individuals.⁵⁵ Assessment of ascorbic acid levels may be useful for detecting clinically silent ascorbic acid deficiencies in individuals, particularly nursing mothers. Downard *et al.* described covalent modification of glassy carbon fiber microelectrodes and glassy carbon electrodes with P-phenylacetate groups; these electrodes exhibit excellent resolution between ascorbic acid and dopamine.⁵⁶ We anticipate that integrated electrode-hollow microneedle devices fabricated using this approach may be used to provide real-time detection of a wide variety of physiologically relevant biomarkers.

II. MATERIALS AND METHODS

Proliferation of human dermal fibroblasts and neonatal human epidermal keratinocytes on e-Shell 200 surfaces was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) assay, which involves reduction of a yellow tetrazolium salt to a purple formazan dye by mitochondrial succinic dehydrogenase.⁵⁷ In this study, e-Shell 200 wafers (diameter=15 mm, thickness=2 mm) were compared against glass cover slips (diameter

FIG. 1. Computer-aided design drawing of the microneedle array that was fabricated using the digital micromirror device-based stereolithography instrument.

= 15 mm). The cover slips and e-Shell 200 wafers were rinsed and sterilized in two 30 min rinses of 70% ethanol; the materials were subsequently rinsed in sterile de-ionized water. The e-Shell 200 wafers were placed in sterile Petri dishes in a laminar flow cabinet and sterilized with ultraviolet B light; both surfaces were exposed to ultraviolet B light. The materials were rotated 90° after a minimum of 2 h light exposure. Polymers were transferred to sterile 24-well culture plates, rinsed twice in sterile Hank's balanced salt solution, and once in the appropriate cell culture medium. The e-Shell 200 wafers were placed in 2 ml of the appropriate cell culture medium and held in the incubator until seeded.

Cryopreserved neonatal human epidermal keratinocytes and human dermal fibroblasts were obtained from a commercial source (Lonza, Walkersville, MD). Fibroblast growth media (FGM-2) and keratinocyte growth media (KGM-2) were also obtained from a commercial source (Lonza, Walkersville, MD). The human dermal fibroblasts and neonatal human epidermal keratinocytes were propagated in 75 cm² flasks, grown to 75% confluency, and subsequently harvested. The cells were then seeded (concentration=40 000 cells per well) in a 24-well plate on e-Shell wafers 200 (n=4), glass cover slips (n=4), and polystyrene well plates (n=4). Material rinsing and all media changes were performed by moving the test materials from one solution to the other using a forceps. The materials were placed in fresh medium after 48 h; this time point corresponded with 80% confluency for human dermal fibroblasts and neonatal human epidermal keratinocytes. MTT viability was assessed 24 h later. The materials with cells were rinsed using Hank's balanced salt solution; desorption using isopropyl alcohol and agitation were subsequently performed. Isopropyl alcohol (quantity=100 μ l) was transferred to a new 96-well plate. Absorbance was determined (λ =550 nm) with a Multiskan RC plate reader (LabSystems, Inc., Franklin, MA). The mean values for percent viability were calculated. Significant differences ($p < 0.05$) were determined using the PROC GLM Procedure (SAS 9.1 for WINDOWS) (SAS Institute, Cary, NC). When significant differences were found, then multiple comparisons were performed using Tukey's Studentized Range HSD (Honestly Significant Difference) test at $p < 0.05$ level of significance.

Arrays of hollow microneedles were fabricated from three-dimensional drawings that were created using Solidworks (Dassault Systemes S.A., Velizy, France). Support structures were fabricated from three-dimensional drawings that were created using Magics RP 13 (Materialise NV, Leuven, Belgium). In the tetrahedron-shaped microneedle design, two faces of the microneedle exhibit a vertical orientation with respect to the substrate (Fig. 1). The microneedle input dimen-

FIG. 2. Schematic showing steps used for assembly of the microneedle device.

sions included a triangular base with 1.2 mm sides, a height of 1.5 mm, and a vertical cylindrical channel (diameter=400 μm). The needles were arranged into a 2×2 square array with 2 mm intermicroneedle spacing. The substrate input dimensions included lateral dimensions of 1 cm $\times$ 1 cm and a thickness of 500 μm . Rapid prototyping of the microneedle array was performed using a Perfactory III SXGA+instrument (EnvisionTEC GmbH, Gladbeck, Germany). A 150 W halogen bulb was used as the light source for polymerization of liquid e-Shell 200 resin. Selective polymerization of the resin in the X-Y plane was achieved using DMD optics (Texas Instruments, Dallas, TX), specifically a DMD SXGA+guidance chip with 1280×1024 -pixel resolution. This instrument contains a build envelope of 90 mm $\times$ 67.5 mm. After fabrication, the e-Shell 200 microneedle array was washed in isopropanol in order to remove the unpolymerized material. Postbuilding curing was accomplished using an Otofash Post Curing System instrument (EnvisionTEC GmbH, Gladbeck, Germany). This system contains two photoflash lamps and provides light exposure over a wavelength range of 300–700 nm.

A Hitachi S-3200 (Hitachi, Tokyo, Japan) variable pressure scanning electron microscope with a Robinson backscattered electron detector was used for imaging the microneedle arrays. The microneedle arrays were coated with 60% gold-40% palladium using a Technics Hummer II instrument (Anatech, Battle Creek, MI) prior to imaging. Skin penetration testing was performed with full-thickness cadaveric porcine skin since human skin and porcine skin exhibit similar structures. Trypan blue (Mediatech, Inc., Manassas, VA), a toluidine-based dye, was used to assess the transdermal drug delivery functionality of the hollow microneedle arrays.^{24,43,58} Cadaveric full-thickness weanling Yorkshire/Landrace skin was stored at 3 °C until testing was performed. Hollow microneedle arrays were inserted into full-thickness porcine skin. After removal of the arrays, trypan blue was applied to the insertion site; the site was subsequently washed with isopropanol swabs. The trypan blue-treated skin was subsequently imaged using optical microscopy. Images of a microneedle device before and after insertion into porcine skin were obtained using optical microscopy.

The completed microneedle device was comprised of two layers (Fig. 2). The upper layer consisted of the microneedle array; the lower layer provided support for the carbon fibers and facilitated the alignment of the carbon fibers to the microneedle array. The support component was fabricated from a 1.6 mm thick poly-(methylmethacrylate) (PMMA) piece. An array of holes was laser drilled through the PMMA piece using a Model PLS instrument, consisting of a 6.75 60 W CO_2 laser and a computer-controlled XY stage (Universal Laser Systems, Scottsdale, AZ) [Fig. 2(a)]. The holes were placed in a square pattern with 2 mm spacing. Using a Model HPDFO high

FIG. 3. Optical images of (a) an array of carbon fiber electrodes (scale bar=1 mm) and (b) a single carbon fiber electrode (scale bar=100 μm).

power density focusing optics lens (Universal Laser Systems, Scottsdale, AZ), the diameter of the hole at the exit surface was measured at $\sim 45 \mu\text{m}$. To control the carbon fiber length beyond the support surface, the support component was placed on top (exit-side down) of a well with a depth of $100 \mu\text{m}$. Carbon fiber was manually inserted into each of the holes (entrance side) and allowed to rest at the bottom of the well [Fig. 2(b)]. The fibers were secured in place with acrylic adhesive on the entrance side after the desired well depth had been achieved [Fig. 2(c)]. Figure 3 shows the optical images of an array of carbon fiber electrodes and a single carbon fiber electrode. The support layer and the microneedle layer were brought together in such a manner that the carbon fibers were positioned within the hollow shafts of the microneedles. The layers were subsequently adhered to each other. Metallic epoxy was applied to the back of the fibers in order to create the connection for the working electrode [Fig. 2(d)].

$7 \mu\text{m}$ carbon fibers (Alfa Aesar, St. Louis, MO) were activated in a KOH solution (concentration=0.1 M) at a pH of 13 and at a potential of 1.3 V for 5 min. *In situ* diazotation of 2-amino-4-nitrophenol was performed by mixing a solution of 8 mM sodium nitrite and 6 mM 2-amino-4-nitrophenol on ice for 5 min to create the corresponding diazonium salt. After 5 min, the activated carbon fibers were inserted. Two cyclic voltammetry (CV) scans were run from 0.4 to -0.8 V at 0.1 V/s to enable the electrochemical grafting of the 2-nitrophenol and subsequent reduction to the aminophenol. The carbon fibers were modified with palladium to enable the detection of hydrogen peroxide. Activated carbon fiber bundles were placed in a solution of 1 mM palladium (II) chloride; Pd was deposited by applying a potential of -0.8 V for 120 s. All electrochemical measurements were obtained using a PGSTAT12 Autolab electrochemical instrument (EcoChemie, Utrecht, The Netherlands). Data were acquired versus an Ag/AgCl reference and a Pt counterelectrode.

III. RESULTS AND DISCUSSION

24 h MTT assay data for neonatal epidermal keratinocytes and human dermal fibroblasts are shown in Figs. 4(a) and 4(b), respectively. The test materials were noted to remain intact in the cell culture media over the course of the MTT assay. The e-Shell 200 and glass materials did not float in the wells; data for each material were fairly consistent. MTT viability of neonatal human keratinocytes was significantly higher for glass than for e-Shell 200 [Fig. 4(a)]. MTT viability for human dermal fibroblasts was significantly higher for glass than for e-Shell 200 [Fig. 4(b)]. Very slight differences in viability were noted; both neonatal epidermal keratinocytes and human dermal fibroblasts showed a slight decrease on e-Shell 200. Dissimilar responses by neonatal human epidermal keratinocytes and human dermal fibroblasts were attributed to the fact that epithelial

FIG. 4. MTT viability data for cells grown on e-Shell 200 acrylate-based polymer and glass cover slip. (a) MTT viability of human epidermal keratinocytes grown on e-Shell 200 acrylate-based polymer compared to glass cover slip. A and B denote statistical differences ($p < 0.05$) between the polymer and the control. (b) MTT viability of human dermal fibroblasts grown on e-Shell 200 acrylate-based polymer compared to glass coverslip. A and B denote statistical differences ($p < 0.05$) between the polymer and the control.

cells are derived from ectoderm; on the other hand, fibroblasts are derived from mesoderm.³⁷ Consequently, these cell types have dissimilar cell receptors and responses to synthetic materials. It should be noted that e-Shell 200 only exhibited a slight but significant decrease in viability, which would not raise concerns regarding potential *in vivo* use in a transdermal medical device.

The digital micromirror device-based stereolithography instrument allowed for the fabrication of approximately 200 arrays over a 3 h period. Figure 5 contains the scanning electron microscopy (SEM) images of a hollow microneedle array and a single hollow microneedle prior to incorporation of carbon fiber electrodes. Measurements obtained from scanning electron micrographs showed that the microneedles exhibited heights of $\sim 1030 \mu\text{m}$, triangular bases with side lengths of $\sim 1120 \mu\text{m}$, and bore diameters of $\sim 375 \mu\text{m}$. Good microneedle-to-microneedle uniformity was noted among the microneedles in the microneedle array. Differences between input and measured dimensions were attributed to translation of the computer-aided design drawing by the software. Digital micromirror device-based stereolithography and other rapid prototyping techniques involve tessellation, a conversion of the surface of the computer-aided design drawing into

FIG. 5. Scanning electron microscopy image of hollow microneedles prior to incorporation of carbon fiber electrodes: (a) plan view of hollow microneedle array and (b) isometric view of single hollow microneedle.

a series of polygons. This polygon series is converted into a series of cross-sectional layers, which is subsequently used for layer-by-layer fabrication of the microneedle device. It is not possible to predict how the computer-aided design drawing is manipulated by the software.

Microneedles undergo bending forces, compressive forces, shear forces, and skin resistance during skin insertion; the pressure necessary for human skin penetration exceeds 3.0×10^6 Pa.²¹ The skin penetration properties of the microneedle devices were evaluated using cadaveric porcine skin, which has been previously used as a model for assessing microneedle functionality.^{12,43} Figure 6(a) shows the porcine skin after application of the microneedle array, removal of the microneedle array, and application of trypan blue. The trypan blue spots indicate the penetration through the stratum corneum layer (outermost layer) of the epidermis by the microneedle array and the localization of Typan Blue within the microneedle-generated pores. Figures 6(b) and 6(c) show optical micrographs of hollow microneedles before insertion into porcine skin and after insertion into porcine skin, respectively. These images indicate that the microneedles remain intact after skin insertion.

The positioning of the carbon fiber electrodes within the microneedle device should (a) facilitate interactions with the biological sample and (b) minimize carbon fiber exposure to stresses associated with microneedle insertion into skin and movement at the microneedle device-skin interface. To facilitate interactions between the biological sample and the carbon fiber electrodes, the carbon fiber electrodes must be positioned at the centers of the microneedle bores. In addition, dead space between the carbon fiber electrodes and the microneedle sidewalls may allow for infiltration of the biological sample. Figure 7 contains the scanning electron microscopy images of a hollow microneedle array and a single hollow microneedle after incorporation of carbon fiber

FIG. 6. Images of microneedle array and cadaveric porcine skin after microneedle insertion. (a) Optical micrograph showing delivery of trypan blue into microneedle-fabricated pores within cadaveric porcine skin (scale bar 1=mm). (b) Optical micrograph showing hollow microneedles before insertion into cadaveric porcine skin. (c) Optical micrograph showing hollow microneedles after insertion into cadaveric porcine skin.

FIG. 7. Scanning electron microscopy images of hollow microneedles after incorporation of carbon fiber electrodes: (a) plan view of electrode-hollow microneedle array and (b) isometric view of single electrode-hollow microneedle.

electrodes. Figure 7(b) shows a scanning electron microscopy image of a single hollow microneedle after incorporation of carbon fiber electrodes; this image reveals that the carbon fiber electrodes do not extend beyond the tip of the microneedle bore. Placement of carbon fibers within the microneedle bores necessitated a precise alignment of the microneedle bores and the carbon fibers [Figs. 2(e) and 2(f)]; the positions of the laser-ablated holes in the lower layer of the microneedle device were coordinated with the positions of the microneedle bores in the upper layer of the microneedle device.

The electrochemical response of the carbon fibers within the electrode-hollow microneedle device toward 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ / 1 M KCl was evaluated. Figure 8 shows a cyclic voltammetric scan of 5 mM ferricyanide in 1 M KCl versus Ag/AgCl and Pt reference counterelectrodes, respectively, at a scan rate of 100 mV/s. Well defined oxidation/reduction waves were observed, indicating interaction between the carbon fiber electrodes and the test solution as a result of

FIG. 8. Cyclic voltammetric scan of 5 mM ferricyanide in 1 M KCl vs Ag/AgCl and Pt reference counterelectrodes, respectively, at a scan rate of 100 mV/s.

FIG. 9. Cyclic voltammetric scans of 0, 50, 100, 300, and 500 μM hydrogen peroxide (pink, black, green, blue, and red curves) vs Ag/AgCl and Pt reference counterelectrodes, respectively, at a scan rate of 100 mV/s.

permeation of the microneedle bore by the test solution. The average formal potential (E^0') for $\text{Fe}(\text{CN})_6^{3-/4-}$ was measured at 220 mV versus Ag/AgCl reference and platinum counterelectrodes, respectively. The average peak separation was $\Delta E_p = 125$ mV. These results indicate that the carbon fibers within the electrode-hollow microneedle device were capable of providing electrochemical measurements.

Palladium-catalyzed oxidation of hydrogen peroxide on the carbon fibers within the electrode-hollow microneedle devices was also investigated. Palladium was deposited onto the carbon fibers by applying a potential of -0.8 V for 120 s in 1 mM Pd/0.5 M HCl prior to insertion into the microneedle device. Figure 9 contains cyclic voltammetric scans of 0, 50, 100, 300, and 500 μM hydrogen peroxide, represented by pink, black, green, blue, and red curves, respectively, versus Ag/AgCl and Pt reference counterelectrodes, respectively, at a scan rate of 100 mV/s. This figure shows an increase in reductive currents after additions of 0, 50, 100, 300, and 500 μM hydrogen peroxide, exhibiting a linear range of 100–500 μM and a detection limit of ~ 15 μM (based on the response of 50 μM hydrogen peroxide; $S/N=3$).

Following the examples by Civit *et al.* and Nassef *et al.*, the carbon fibers were modified with aminophenol (o-AP) groups following *in situ* diazotination and electrografting of the corresponding diazonium salt.^{59,60} Modification of the carbon fiber electrode with o-AP has been shown to result in electrocatalytic oxidation of ascorbic acid and selective oxidation of ascorbic acid in the presence of common interferents such as uric and citric acids. Uric acid is a well-known interferent in electrochemical analysis of ascorbic acid, which is attributed to the fact that uric acid and ascorbic acid possess similar oxidation potential values.⁶¹ Linear sweep voltammograms of 100 mM phosphate buffer (blank solution) and 1 mM ascorbic acid in 100 mM phosphate buffer ($\text{pH}=7$) versus Ag/AgCl and Pt reference counterelectrodes, respectively, at a scan rate of 100 mV/s are shown in Fig. 10. This result indicates that the carbon fibers within the electrode-hollow microneedle device are able to detect the ascorbate analyte with the low potential oxidation of ascorbic acid at 195 mV.

IV. CONCLUSIONS

In summary, we have demonstrated that carbon fiber electrodes integrated within hollow microneedles, which were produced using digital micromirror device-based stereolithography, can

FIG. 10. Linear sweep voltammograms of 0 mM (black) and 1 mM (red) ascorbic acid in 100 mM phosphate buffer ($pH=7$) vs Ag/AgCl and Pt reference counterelectrodes, respectively, at a scan rate of 100 mV/s.

function as electrochemical transducers. The low cost and high yield offered by the digital micromirror device-based stereolithography process suggest that it is a suitable approach for large-scale production of hollow microneedles. The rapid processing rate offered by the digital micromirror device-based stereolithography process suggests that it is an appropriate approach for tailoring microneedle design. Studies involving porcine skin demonstrated that the microneedle arrays were able to penetrate the outermost layer of the skin and that the microneedles remained intact after insertion. The MTT assay indicated that the e-Shell 200 only exhibited a slight decrease in viability in a manner that would raise concerns regarding potential *in vivo* use in a nonimplantable medical device.

Electrochemical measurements by the carbon fibers within the electrode-hollow microneedle device were demonstrated. In addition, chemical modification of the carbon fibers for selective analytes was shown; detection of hydrogen peroxide and ascorbic acid using these modified carbon fibers was demonstrated. While these experiments used outside reference and counterelectrodes, it is envisioned that selective microneedles within the microneedle arrays can be modified to integrate quasireference and counterelectrode components. It is also envisioned that a single microneedle array can include multiple functionalities, including drug delivery and monitoring of several analytes; such a device would be capable of integrated, closed-loop sensing and drug delivery. As mentioned by Wilson *et al.*, biocompatibility issues must be successfully addressed in order to achieve continuous *in vivo* monitoring.² Decreasing the microneedle length is another important parameter that must be considered in future efforts; Gill *et al.* demonstrated that microneedle length was positively correlated with pain; patient pain may be reduced by decreasing microneedle length.¹³ Use of novel stereolithography-compatible materials, including materials based on vinyl esters, may provide higher cell viability rates.⁶²

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