ACTIVE MICRONEEDLES WITH INTEGRATED FUNCTIONALITY

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ABSTRACT

The focus of this work is the design, fabrication, and characterization of a new class of biomedical micro systems, the active microneedle. Active microneedle systems represent an advancement over current microneedle technologies through the integration of additional functionality (e.g. biochemical sensing, mechanical, etc.). The active microneedles described in this paper include the following additional functionality: integration of multiple inlet/output ports, multiple lumens (flow channels), and bioluminescence based biosensors for monitoring metabolic levels (e.g. creatine and glucose).

INTRODUCTION

Both hollow and solid microneedles and microneedle arrays have been demonstrated by a number of research groups over the past few years. Initially, only microneedles made of solid silicon had been realized [1]. Najafi and Wise developed these early microneedles that were used as neural microprobes. Recently, solid silicon microneedles fabricated by Henry et. al. were formed using reactive ion etching [2]. The resulting planar needle array consisted of 20x20 needles for increasing the skin permeability to drugs. Lin, Pisano, and Muller were first to present hollow silicon processed microneedles [3,4]. These microneedles were fabricated on silicon substrate and used silicon nitride to fully enclose the channels. Later, Chen and Wise [5] presented hollow silicon microneedles for neural recording and drug delivery. These two approaches utilize a sacrificial processing technique that consumes the substrate on the devices are built. In contrast, Talbot and Pisano [6] have incorporated a polysilicon micromolding process to develop hollow microneedles. These hollow microneedles are fabricated by micromolding polysilicon (polymolding) in silicon micromolds.

Previously, we reported micromachined needles and needle arrays of hollow metallic needles for minimally invasive drug delivery and biofluid extraction [7,8]. These microneedles have the capability to deliver drugs subcutaneously while minimizing the pain inflicted to patients. Recent advancements in

the design and fabrication of hollow metallic microneedles has led to improved fluid handling capabilities, as well as, increased functionality. Recently, McAllister et al. [9] have reported hollow NiFe microneedle arrays.

The paper presents the design, fabrication, and characterization of a hollow metallic active microneedle. Figure 1 is a schematic representation of the described microneedle with an integrated biosensor. The active microneedle includes design features such as tapered needle tips; multiple output ports on the back and front of each needle; multiple lumens and multiple input ports, Figure 2, and bioluminescence based biosensors for monitoring metabolic levels.

FABRICATION AND PACKAGING

The micromachined multiple output port needles are fabricated using extensions of previously reported micromachining technologies [10,11,12]. The fabrication process is low temperature and is compatible with integrated circuit (IC) technology as a post process. Initially, a metal system of adhesion layers and an electroplating seed layer are electron beam evaporated onto a silicon wafer. Using standard thick photoresist micromolding techniques, this metal layer is patterned and 20µm of palladium is electroplated to form the solid layer or bottom wall of the microneedle [13]. The use of palladium as a structural material provides high mechanical strength and durability, as well as, biocompatibility for use in biomedical applications [14,15,16]. Next, 20µm of commercially available thick photoresist, AZ4620, is deposited and patterned into sacrificial structures. These sacrificial structures serve to precisely define the inner dimension of each microneedle. 800Å of gold is sputter deposited onto the sacrificial photoresist structures to act as an electroplating seed layer for top shell electroplating. Next, a 20µm layer of palladium is electroplated to form the top and side walls (hollow layer) of the microneedle. The sacrificial thick photoresist is then removed

Figure 1. Schematic representation of a micromachined multiple output port needle with an integrated biosensor.

Figure 2. Schematic representation of multi-lumen design for the active microneedles.

using a sequential rinse in acetone, isopropanol, and de-ionized water, producing the hollow metallic microneedles. In the final step, the microneedles are released from the silicon substrate by first removing the chromium layer to expose the copper seed layer. Then the copper seed layer is etched away from underneath the needles by placing the wafer in a solution of ammonium hydroxide saturated with cupric sulfate. The solid silicon substrate is not consumed in this process and may be re-used. Using the given process offers the flexibility to integrate many additional functions directly onto the microneedle. Additionally, the basic process allows for great latitude in the routing and branching of the fluid

BIOLUMINESCENCE

Bioluminescence-based analysis is 100 to 1000 times more sensitive than conventional chromogenic (absorbance) measurements and is accurate over a five or more, orders of magnitude concentration range [17]. Firefly bioluminescence occurs by enzyme-catalyzed oxidation of luciferin utilizing adenosine triphosphate (ATP) [18]. Bacteria bioluminescence is closely coupled to nicotinamide adenine dinucleotide hydride (NADH). Since most of biochemistry depends on ATP and/or NADH, nearly all metabolic reactions can be monitored by bioluminescence via one or more enzymes catalyzed and linked reactions [17,18]. During the production or consumption of a metabolite of interest, enzymes linked reactions will cause the production or consumption of ATP (or NADH). Detectable light is then produced via the following reaction (for ATP):

The change in light intensity will be stoichiometrically proportional to the time changing concentration of ATP (and thus proportional to the metabolite of interest). A photodiode or charge-coupled device (CCD) can be used for detection. Depending on the instrument used to detect the luminescence, nanomolar, picomolar, and even femtomolar analyses are possible [17]. With increased sensitivity, smaller amounts of sample fluid are needed for accurate analysis. 0.05- μL sample size that can be drawn by micromachined needles can painlessly access small amounts of body fluid that can be analyzed via bioluminescence.

The transmittance of light through a media across length λ is defined by Beer's law, $T = 10^{-\text{Absorbance}}$, where $\text{Absorbance} = \epsilon_{\lambda} C_B (\lambda-x)$ and ϵ_{λ} is the molar absorption coefficient in $\text{dm}^3/\text{molEcm}$ and C_B is the concentration in mole/dm^3 [19]. If the light is generated within a homogeneous media, the transmitting light is integrated along the length it travels and is then defined as:

$$T_{\text{Total}(\lambda)} = \int_0^{\lambda} 10^{-\epsilon_{\lambda} C_B (\lambda-x)} dx = \frac{(1 - 10^{-\lambda})}{\ln 10}$$

where $\epsilon_{\lambda} C_B$ is assumed to be 1 for simplicity. For a pyramid (which is the shape of the etched wells in the <100> Si wafers) with a peak of length λ , the transmittance is integrated through the viewing area and is equal to $T_{\text{Total}(\lambda)} E a^2/3$. The assumption is that only the light being transmitted toward the viewing window is integrated and light traveling away from the viewing window is absorbed by the substrate. (Light a 560 nm is actually absorbed by crystallized Si). Thus, linear correlation of a volume verses integrated luminosity plot, for a given viewing window, should indicate that transmittance is proportional to signal intensity (averaged CCD counts over the viewing area). A flat bottom well with a reflective surface will have the transmittance of $T_{\text{Total}(2\lambda)}$, and will be about 2 time larger than $T_{\text{Total}(\lambda)}$ for $\lambda \ll 1\text{-cm}$ (0.005 – 0.025-cm in this experiment). Thus, the slope of a linear fit of volume verses integrated luminosity plot, for a reflective surface of a given viewing window, should be about 2 times that of the slope for a non-reflective surface due to the increased transmittance.

RESULTS

Fabrication

The needle arrays fabricated to date include those electroformed from low-stress nickel sulphamate, gold cyanide, and palladium electroplating solutions [20,21]. Individual hollow metallic micromachined needles with multiple output ports fabricated to date have an inner cross-sectional area of $140 \times 21 \mu\text{m}^2$ (W×H) with outer dimensions of $200 \times 60 \mu\text{m}^2$. A micromachined needle fabricated on silicon and released from its substrate is shown in Figure 3. The needle shaft dimensions are $200 \mu\text{m}$ wide and $60 \mu\text{m}$ thick while the tip dimensions are less than $15 \times 15 \mu\text{m}^2$. The length of the tapered portion of the needle shaft is 1.0 mm and the distance from the tip to the first output port is approximately $300 \mu\text{m}$. The total length of the microneedle is 6.0 mm with input port inner dimensions of $140 \mu\text{m}$ wide and $21 \mu\text{m}$ high. The wall thickness of the needle is approximately $20 \mu\text{m}$ and the microneedle output ports are on the top and bottom with dimensions of $30 \mu\text{m}^2$. The output ports are separated by $30 \mu\text{m}$ and there are nine ports on top and 12 ports on the bottom. Bioluminescent detection of the biosensor output is performed at the output ports of the microneedle. Biosensing reagents are drawn into and dried down onto the inner walls of the hollow microneedle.

Figure 3. SEM of a multi-lumen, multiple output port micro needle. Shaft dimensions are $200 \mu\text{m}$ wide and $60 \mu\text{m}$ thick. Tip dimensions are less than $15 \mu\text{m} \times 15 \mu\text{m}$. Output ports are $30 \mu\text{m}^2$.

Figure 4. Photograph of packaged microneedle connected to standard 5cc syringe as compared to a penny. **Insert:** Close-up of packaged microneedle.

The completed micromachined needle structures were packaged into a standard luer-lock fitting using a polymeric medical-grade UV-curable adhesive (3321, Loctite Corp.). This interface between the microneedle and syringe consists of a simple female luer-to-1/16" barb adapter manufactured by Western Analytical (part# p-857x). Figure 4 is a photograph of a packaged microneedle connected to a standard 5cc syringe as compared to a penny. The insert of Figure 4 shows a close-up of the packaged microneedle that has been secured with UV epoxy on the barbed end of the interface. The UV curable epoxy was found to permanently affix the microneedle to the interface while providing a leak resistant seal. Fluid flow experiments were performed on five packaged microneedles at pressures ranging from 1psi to 70psi [9].

Bioluminescence Studies

In order to determine the feasibility and the physical limitations of using bioluminescence as a means for highly sensitive analyte measurement of small sample volumes, μ -reaction chambers (μ RCs) were fabricated on silicon wafers using KOH anisotropic wet etching. An ATP firefly luciferase/luciferin solution was placed in the μ RCs and observed through a close up lens with a CCD. The integrated CCD signal was recorded and compared with well size and depth. The attenuation of the CCD signal was also observed for wafers coated with titanium (500Å) followed by chromium (1500 Å).

A 5-mL firefly luciferase/luciferin solution consisted of 1.25-mg/mL bovine serum albumin (Sigma - reconstituted into the solution. Used for coating the glass vial to prevent denaturing of the luciferase), 1.25-mM ethylene diaminetetra acetic acid (Sigma), 12.5-mM Mg^{++} (Sigma - from $MgSO_4$), 1.84- μ M firefly luciferase (Promega), and 1.25-mM luciferin (Biosynth) in a 1.25mM glycyl-glycine buffer. This mixture was able to maintain 90% activity for about 20-hrs when stored in the dark. A 5-mM ATP, glycyl-glycine buffer solution was also prepared. 20- μ L of the firefly luciferase/luciferin solution was pipetted into 5- μ L of the ATP solution that resulted in a 1.0-mM ATP mixture, saturated

Figure 5. 20-sec integration of bioluminescence for the wafer that was etched 242.1- μ m and coated with Ti/Cr. The μ RCs seen here are the 750, 500, 400, 300, 250, and 200- μ m wide squares.

μ RC Width (μ m)	P value from T-test between Intensity/Volume Slopes of Non-Reflective μ RCs and Reflective μ RCs	Ratio of Intensity/Volume Slopes for Reflective and Non-Reflective Substrates
750	0.00001	3.02
500	0.002	2.37
400	0.004	4.94
300	0.005	5.11
250	0.36	2.87
200	0.030	1.07
150	0.72	0.88
100	0.55	0.21

Table 1. Statistical comparison of the intensity/volume slope relationships for reflective vs. non-reflective surfaces of the same viewing window size.

Figure 6. Average CCD counts from the 20-sec integrated CCD reading for different sample volumes. Data was plotted in sets for the same viewing area (or μ RC square width). Data was also separated according for Ti/Cr reflective substrates and plain, non-reflective, substrates. For each set of data (wells with same width and coating), the increasing volume occurs from the increased etch depths 250- μ m, 100- μ m and 50- μ m. Data from the different etch depths are pointed out for the 750- μ m wide wells as an example. The higher intensity values occur for the deeper wells. The intensity/volume slope is greater for Ti/Cr reflective substrates than for plain substrates.

with luciferase and luciferin (which means the reaction rate was at its peak). After the solution mixed, 20- μ L of it was pipetted on an area about 20 $\times$ 15-mm wide. A thin glass cover slip was placed on the solution, starting at one end and tilting the cover slip as it was laid down, so that the excess bioluminescent fluid would disperse. For high concentrations of ATP (> 8- μ M), the initial mixing of ATP with the luciferase/luciferin solution causes a peak luminescence within 3 seconds. The luminescence then tapers and levels off after 1 minute. Therefore, the light measurements for this experiment was integrated for 20 seconds, 2 minutes after the ATP and luciferase/luciferin solution were mixed, with the light intensity essentially constant. An ST6-A CCD camera, by Santa Barbara Instruments Group was fitted with an Olympus wide-angle lens and close-up ring. The experimental substrates were focused 55-mm below the lens with the aperture set at 2.8. The field of view was about 20 $\times$ 15-mm. The camera was operated at -20.00°C. 1.0-minute after the luciferase/luciferin solution was added to the ATP, a 20-sec dark field exposure was taken with the CCD. 2-min 55-sec after the ATP and luciferase/luciferin solution mixed, the CCD integrated a 20-sec exposure of the bioluminescent reaction while shrouded in a dark room. The resulting image was saved as a TIF file (Range: 0 – 400). Scion Image (based on the NIH image software) was used to determine the average and standard deviation of the pixel values for each μ RC.

Figure 5 shows an image of the 20-second integration of bioluminescence recorded by the CCD camera. One pixel of the CCD image was equal to 46.875- μ m. Actual results failed to show light emitting from the 75, 50, 25, and 10- μ m wide wells on all substrates. Figure 6 shows the average CCD counts from the 20-sec integrated CCD reading for different sample volumes. Table 1 statistically compares the intensity/volume slope relationships for reflective vs. non-reflective surfaces of the same viewing window size. The reflective wells with intensity/volume slopes that were statistically different from the intensity/volume slopes for the non-reflective surfaces ($P < 0.05$ using the T-test) were over two times greater. This follows the hypothesis that transmittance is nearly doubled for chromium (reflectivity=0.67 @ 560 nm) coated versus uncoated silicon (reflectivity=0.33 @ 560 nm) μ RCs.

The intensity/volume slope also increases as the μ RC square width decreases. This is ideal for using long, narrow microneedles as the μ RC. Table 2 statistically compares the intensity/volume slopes between reflective wells of different viewing window sizes. It shows that there is a significance increase in the intensity/volumes slopes, as the μ RC width decreases. The increase in intensity/volume is only significant down to wells that are 400- μ m wide. For smaller μ RC widths (150 – 300- μ m), the non-reflective substrates show little correlation between average CCD counts and sample volumes ($R^2 < 0.25$). The Ti/Cr reflective substrates still show some correlation ($R^2 > 0.25$ for μ RCs 250- μ m wide and wider) between intensity and volume. However, as the μ RC square width decreases (250- μ m wide and smaller), there is little difference in signal intensity values for different etch depths.

μ RC Square Widths Being Compared (μ m)	P value from T-test between Intensity/Volume Slopes μ RCs being compared	Ratio of Intensity/Volume Slopes for Smaller μ RC Widths Verses Wider μ RC Widths
500/750	0.004	1.89
400/500	0.04	1.98
300/400	0.28	1.59
250/300	0.89	1.11
200/250	0.81	1.28
150/200	0.91	0.73

Table 2. Statistical comparison of the intensity/volume slopes between reflective wells of different viewing window sizes. The intensity/volume slope ratio is the slope for the smaller window over the slope for the next larger window.

The error in the signals for wells 200- μ m wide and smaller is large enough to overlap with the background intensity. This suggests that 250- μ m that smallest well window that would produce a discernable intensity signal. However, this does not mean that narrower, deeper μ RCs like microneedles could not produce a good signal because their longer depths are able to hold a larger sample volume, which would produce a stronger signal.

CONCLUSION

Both silicon and metal electroformed micro needle technologies have been previously developed. This paper provides a basis for the next technological step in micro needle technologies through the development of active micro needles. Active micro needles include the integration of additional functionality into the basic micro needle structure. Additional functionality such as multiple lumens, multiple input and output ports, and the integration of biosensing capabilities has been shown.

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