A HEXAGONAL MULTIPOINT PH AND TEMPERATURE SENSING NEEDLE FOR 3D PIG HEART MUSCLE PH MONITORING WITH TEMPERATURE COMPENSATION

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ABSTRACT

The paper presents a hexagonal silicon microneedle with an outer diameter of 0.7mm same size as the medical needle of 22G, where an Au/Cu-based resistive temperature probe is back-to-back, flip-chip bonded with a (IrOx)/AgCl-based pH probe using SU-8 as the bonding layer. The microneedle containing two sets of temperature and pH sensors can exhibit a linear sensitivity of $0.0298\Omega/^{\circ}\text{C}$ and -58mV/pH respectively and sustain up to $\sim 3.8\text{Nt}$ compressive load before buckling failure that is mechanically robust enough to penetrate myocardium. Owing to the reduction of process complexity by separating probe fabrications, the new needle can have at least 10% overall improvement in comparison with the prior arts in terms of the probe size and sensitivity and 220% improvement in terms of buckling strength. In addition, a 3D pH measurement of a pig heart has been performed with temperature compensation in this work.

INTRODUCTION

Small form factor is the key feature in the next generation biomedical instruments which facilitate the new exploratory in the signal detection down to the tissue or even cell level [1-3]. For instance, Ivorra et al. developed a minimally invasive needle shaped probe to measure the electrical impedance of living tissues of rat kidneys for the study of the extracellular medium and found the appearance of 65% impedance change while the kidney was kept in an ischemia period of 45 minutes [1]. Chan et al. demonstrated a polycrystalline diamond neural probe equipped with both electrical and electrochemical recording abilities can efficiently detect Norepinephrine in vitro [2]. Zheng et al. employed a silicon-nanowire field-effect transistor array for multiplexed real-time monitoring of protein markers and telomerase activity with high sensitivity and selectivity activity in clinical samples, which disclosed the potential application of the platform for diagnosis and treatment of cancer and other complex diseases [3].

On the other hand, recent researches in 3-D measurement has paved a way to help us understand the biosignal distribution and thus the development of appropriate therapies in a biological system. Nanoscale devices, such as nanowire or nanotube FET probes, 3-D macroporous nanoelectronic scaffolds etc. have been successfully developed for intracellular electrical recording of action potentials from single live cells to tissues as well as 3-D neural activity mapping for the fundamental biophysical studies and medical monitoring and intervention [4]. The establishment of multicellular 3D aggregation in vitro model based on the recorded electrical potential and impedance using the combination of the bio-hybrid sensors like integrated electrode arrays with a self-assembling peptide nanofiber scaffold for mimicking the cellular microenvironment in a living organism has become a critical research task for determining the effectiveness of drugs and therapies [5,6].

Prior studies have shown the heart sarcolemma failure can be detected via the change of the pH value of heart muscle, resulting from the massive influx of calcium into the muscle cells during

hypoxia [7]. Cardiovascular surgeon could have a lower surgery risk as long as there is a minimally invasive probe can be inserted in the right or left ventricular anterior wall for fixed-point temperature and pH sensing. Previously, we successfully developed a silicon probe integrated with multiple Au/Cu-based resistance temperature detectors (RTDs) and three (IrOx)/AgCl-based pH meters for heart muscle monitoring [8,9]. The integrated sensing probe could detect the pH variance at different sites to show the real-time status of muscle like decomposition and related germs breeding and $\sim 3^{\circ}\text{C}$ difference between the outer and inner parts of the pig heart, which is an important factor in heart surgery.

Nevertheless, bulky needle size and handling difficulty resulting from tens of interconnects and asymmetrical needle shape, respectively, still prevent the probe from the translation of clinical advances into practice. This paper will demonstrate a new probe design with simpler fabrication processes by bonding two micromachined trapezoidal probes to form a robust hexagon-shaped needle where the space for sensor feedthroughs and process-sensitive materials can be effectively reduced and protected for better sensing performance.

PROBE DESIGN AND FABRICATION

In the prior probe design, a total of six sensors, three for each temperature and pH sensor respectively, are distributed in a silicon shank expected to detect the signals at three different depth of myocardium as a result of a 1.1mm wide probe where sixteen electrical interconnects are distributed. For realizing minimal wound that can recover without being sutured and having a good surgical field that requires the probe to be placed in the back site of heart limited by chest cavity during open chest surgery, it is necessary to resize the probe. Fig. 1 shows the new cardiac sensing probe whose shank width and length have been reduced from 1.1 to 0.7mm and 8 to 6mm, respectively, in comparison with that of the prior one. The newest needle comprises of two silicon probes back-to-back flip-bonded to form a hexagonal type needle where pH and thermal sensors are placed at two sides of the probe shank, respectively, which can also effect resolve the indispensable space resulting from the interconnects.

Fig. 1. A micrograph of two different cardiac sensing probes: prior design (left [9]) and the new design (right).

Temperature and pH Sensors

The temperature sensor is designed with a serpentine-shaped resistance temperature detector (RTD) made of electroplated Cu with Au same as the prior work because of high temperature coefficient, $TCR \sim 4.29 \times 10^{-3}/^{\circ}\text{C}$, good linear dependence of resistivity from -100 to 260°C , and compatible with CMOS backend process. Besides, Au surface on the electrical interconnects can exhibit good biocompatibility and be easily applied with any flexible electrical feedthrough via solder bonding or metal-metal thermal-compressive bonding for in-situ monitoring heart muscle. In the temperature sensing probe, there are only two sensing sensors spaced with 5mm apart for myocardium temperature monitoring at different depth. As for the pH probe, 600mm x 200mm silver-silver chloride (Ag/AgCl) and Iridium oxide (IrO_x) pads are used for pH reference and sensing electrode, respectively. Nernstian equation listed as follows show the pH sensitivity will be -59mV/pH at 25°C if the sensing film electrodes are with space charges:

$$E = E^0 - 2.303 \frac{RT}{F} \text{pH} = E^0 - 0.05916 \text{pH} \quad (1)$$

where F is Faraday's constant with a value about 96500 C/mole, R is the value of gas constant 8.314 J/mole K, and E^0 is the standard potential with a value of 577mV for AgCl. The pH value is measured by open circuit method.

Microneedle Fabrication

Fig. 2 shows the process flow for the cardiac sensing microneedle fabrication using two 250 μm thick Si(100) substrates, one for temperature probe and the other for pH probe. Firstly, thermal oxide (6000 $\text{\AA}$) and LPCVD nitride layer (7000 $\text{\AA}$) are deposited and photo-patterned using PR AZ4620 and reactive ion etcher on the silicon substrates as electrical insulation and mask layer for KOH silicon anisotropic etching (Fig. 1(a)). Secondly, sputtering and photo -patterning a layer of Ti/Cu/50nm/100nm on the front side of the substrate as a seed layer for electroplating 1 μm thick Cu patterned as sensor parts and covered with electroless-plated Ni(0.1 μm)/Au (0.4 μm) (Fig. 1(b) or Fig. 1(d)). The Au layer can be used for the prevention of copper oxidation. So far, both temperature and pH probes have been proceeded in common. The temperature probe will be realized after the seed layer removal by Cu etchant and buffer HF solution and then KOH etching (Fig. 1(c)).

For the pH probe fabrication, the aforementioned substrate is selectively electroplated with 1.5 μm thick Ag pads, which will be chlorinated with 50mM FeCl_3 solution for 20 second and soaked in a 3M KCL solution to stabilize the potential of AgCl for the realization of the on-probe Ag/AgCl reference electrode fabrication (Fig. 2(e)). The substrate is then covered with a layer of Cu to protect the as-fabricated interconnects and pads for probe patterning by KOH etching (Fig. 2(f)). After the removal of the Cu layer, the IrO_x -based working electrode is fabricated on the top of the Cu/Ni/Au pads area using the cyclic voltammetry electroplating method where the electroplating solution contains 0.75 mg of IrCl_4 dissolved in a solution of 50mL of D. I. water, 0.5mL 30% H_2O_2 , and 250 mg $\text{Na}_2\text{C}_2\text{O}_4$ and will be adjusted to have a pH value of 10.5 by adding 1M K_2CO_3 and stabilized for 2 days. The electrochemical method is conducted using a three electrode cell system, commercial Ag/AgCl as a reference electrode, platinum foil as a counter electrode, and Au pad of the pH probe as a working electrode. Adapt cyclic voltammetry method from 0V to 0.6V with respect to the AgCl electrode at the rate 20 mV/s for 280 cycles to electroplate the IrO_x film followed by bathing the substrate in a pH 7 buffer solution for 15 hrs. for potential stabilization of the IrO_x .

Fig. 2. Process flow for the cardiac sensing probe fabrication.

Once two sensor probes are fabricated separately, the probes will be back-to-back flip-chip bonded using SU-8 at 150°C for 3min. to form the hexagonal multipoint pH and temperature sensing needle.

RESULTS AND DISCUSSION

Figure 3 shows the as-fabricated hexagonal microneedle comprising of two sets of RTDs and pH meters (Fig. 1(a) and (b)). Figure 1(c) and (d) show close views on the shank and tip, the RTD, and the pH meter of the microneedle. Fig. 4 shows the characterization results from one pair of the RTDs and pH meters on the hexagonal microneedle whose sensitivities are $0.0298\Omega/^{\circ}\text{C}$ and -58mV/pH i.e. $\sim 10\%$ performance improvement than the prior art mainly due to process simplicity. Besides, excellent linear dependence of the resistivity on temperature and the open circuit potential (OCP) and pH values respectively have been accomplished with higher than 99% correlation. The measured titration cycle (Fig. 4(b)) shows the pH sensor can exhibit excellent reversibility.

Fig. 3. As-fabricated hexagon shaped multipoint pH and temperature sensing probe, (a) RTD side, (b) pH meter sides, (c) probe tip and shank, (d) enlarged view on the resistive temperature sensor (top) and enlarged view on the pH meter (down).

Fig. 4. (a) Resistance vs. temperature from one of RTDs, TCR of the Cu/Ni/Au ($1\mu\text{m}/0.1\mu\text{m}/0.4\mu\text{m}$) $\sim 4.79 \times 10^{-3}/^\circ\text{C}$ with linearity of 99.1%. (b) The measured titration cycle, and (c) The OCPs from one of pH meters measured by dipping the probe in pH 2, 4, 6, 7, 8 and 10 buffer solutions, the meter sensitivity is -58 mV/pH with linearity of 99.5%.

Fig. 5 shows the measured maximum compressive strength of the microneedle, i.e. before buckling failure, about 2.2X improvement, i.e. from 1.7 to 3.8Nt, in comparison with that of a single trapezoidal probe. Because the needle is broken as two parts and the failure interface does not happen in the SU-8 bonding layer, the maximum load to cause the probe to be in a state of unstable equilibrium, i.e. failure, can be estimated by the equation as follows:

$$F = \frac{\pi^2 EI}{(KL)^2} \quad (2)$$

where F : maximum load, E : modulus of elasticity, I = area moment of inertia of the cross section of the probe, L = unsupported length of column, and K : column effective length factor. Since the maximum

load is proportional to the area moment of inertia of the cross section, the 2.2X improvement is lower than the theoretical calculation. It could be attributed to uneven load applied on the probe during the test. Further investigation is required to understand the failure mechanism.

Fig. 6 shows the multipoint temperature measurement in the pig heart kept at 5°C in a refrigerator for 2 hours and then tested @ 24°C . With a good temperature match between the RTDs and the commercial sensors, $\sim 3^\circ\text{C}$ difference detected at two points, 0.5 and 1 cm underneath the pig heart surface respectively, indicates that temperature compensation is required for the pH detection in thick muscle. In addition, according to Nernstian relation (Eq. (1)), the pH value is correlated with environmental temperature. Fig. 7 shows the OPV measurement under different temperature where the pH values are calibrated using a commercial pH probe. The slope of the regression lines increases with increasing temperature. Thus, temperature compensation is required for following pH monitoring of pig heart muscle owing to the temperature difference within the pig muscle. Fig. 8 shows a real-time 3D pH measurement in a pig heart. A pH profile at four different positions (A, B, C and D) with different time, $t = 0, 2$, and 4 hours shows that the pH variance and difference at the same depth and in the inner and outer sides of the heart will become smaller after with temperature compensation. The 3D profile also shows the status of heart muscle will be similar at the positions with the same depth and could be different with muscle depth. Meanwhile, it is found that the detected pH values are larger at the positions close to the heart surface, which is consistent with the prior result. The higher pH value can be attributed to the decomposition of glycogen accompanied by the generation of amino acid and peptide at a higher temperature.

The hexagonal microneedle can be also accomplished using double-side KOH silicon etching instead of back-to-back flip-chip bonding process. Double side etching process can make the sensing needle with better structure rigidity due to the lack of bonding interface in between two probes. However, process compatibility and manufacture yield should be taken into consideration. A passivation layer is required to protect the working and sensing electrodes of the pH probe in the KOH etching and can be patterned without causing any detrimental effects on the IrOx electrode after the KOH etching.

Fig. 5. Maximum compressive strength characterization (Inset). There are total 12 samples tested (6 for each case).

Fig. 6. A multipoint temperature measurement in a pig heart using commercial temperature sensors and the as-fabricated needles: (a) the measurement setup and (b) the temperature difference of $\sim 3^\circ\text{C}$ detected at two points, which are located 0.5cm and 1 cm, respectively, underneath the pig heart surface.

Fig. 7. The OPV measurement under different temperature.

Fig. 8. Multipoint pH measurement in a pig heart: the pH profile at four different positions (A, B, C and D): the upper/lower parts are without/with temperature compensation, respectively.

CONCLUSION

In conclusion, we have successfully demonstrated a hexagonal silicon microneedle with the size of 22G. The microneedle comprises of two sets of temperature and pH sensors which can provide the functionality of multipoint sensing and sustain up to $\sim 3.8\text{Nt}$ compressive load before buckling failure that is mechanically robust enough to penetrate myocardium. Owing to the reduction of process complexity by separating probe fabrications, the new microneedle can exhibit better electrical and mechanical characteristics in comparison with the prior work. In

addition, a 3D pH measurement of a pig heart has been performed with temperature compensation, which shows smaller pH variance and difference at the same depth and in the inner and outer sides of the heart. We think the microneedle can facilitate correct multi-point pH detection potential for enhancing the successful rate of heart transplant surgery.

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