FLEXIBLE MULTI-MODAL MICRO-BIOSENSOR TOWARDS ACCURATE CANCER TISSUE TARGETING DURING BIOPSY PROCESS

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ABSTRACT

We report microscale flexible biosensor with multi-modal sensing capabilities (electrical impedance, pH level and glucose concentration) and application to the sensor integrated biopsy needle towards accurate cancer tissue targeting during biopsy process. The sensor is thin enough to be integrated onto the surface of biopsy needle, which allows minimal increase of needle diameter to avoid additional tissue damage during the needle insertion. Also, the sensor shows sensing performance that can cover the physical/chemical differences between cancer and normal tissues. We expect that our sensor integrated on the biopsy needle can overcome the limitation and inaccuracy of conventional image-guided biopsy process.

INTRODUCTION

A biopsy is essential surgery during clinical process especially dealing with cancer in order to collect tissue of suspicious lesion in patients. The excised tissue is then sent to histologist in order to do histological assessment for a definitive diagnosis of diseases. The tissue extraction during biopsy procedure is mainly done by using needle called biopsy needle. Depending on types and structures of biopsy needles, biopsy is also divided into various techniques such as co-axial biopsy, fine needle aspiration, vacuum-assisted biopsy and etc [1].

During biopsy procedure, no matter which type of biopsy is used, the tissue right in front of needle tip is extracted therefore exact positioning of needle tip is important in order to extract suspicious tissue of lesion. Biopsy needle is mainly guided by using conventional medical imaging tools such as computed tomography (CT), ultrasound imaging and magnetic resonance imaging (MRI) [2]. Image contrast in medical imaging tools is enough to discriminate cancerous tissue from normal tissue and position biopsy needle at exact lesion. However, there are cases or diseases that imaging tools cannot give enough image contrast, in which extract tissue is inappropriate for histological assessment. In extreme case such as prostate cancer, all of 12 specific points of prostate were excised during biopsy procedure because prostate cancer is not normally visualized in medical imaging tools [3].

In order to overcome above-mentioned limitation of conventional image-guided biopsy procedure, there have been various efforts in both clinical and engineering ways. Some researchers have tried to solve problem by integrating sensors, such as electrical impedance sensor [4] and lead zirconate titanate (PZT) based tissue contrast sensor [5] into biopsy needle, in order to discriminate type of tissue in real-time. However, in most previous reports, there were various limitations. First, they directly used biopsy needle itself as sensor elements such as electrical electrodes or micro-machining process was essential in order to mount the sensors into biopsy needle. This process is inappropriate to apply to not only co-axial type biopsy needle but also other biopsy needles because thickness of biopsy needle is not enough to micro-machining process. Also, building various physical/chemical sensor systems in a single biopsy needle is hard because functional materials for physical/chemical sensors cannot be fabricated in previous approaches of sensor integration.

In this research, we reports novel approach of micro-biosensor integration onto surface of biopsy needle. The micro-biosensor sensors were consisted of electrical impedance, pH and glucose sensor components for multi-modal sensing capabilities in a single biopsy needle as shown in Figure 1. The multi-modal sensor components were fabricated on thin flexible polyimide substrates with thickness of 5μm in order to make conformal contact with biopsy needle and minimize increase of diameter, in which tissue damage during needle insertion was minimized. Also, we verify that performances of each sensor component were enough to cover physical/chemical differences between cancerous and normal tissues.

FABRICATION PROCESS

Preparation of Polyimide Substrates and Sensor Electrodes

Fabrication of multi-modal micro-sensor was based on a flexible polyimide substrate. First, a 6-inch handling wafer was prepared to handle polyimide substrate because the thickness of used substrate was 5μm which was hard to handle and control during fabrication process. The polyimide substrates were fabricated by spin-coating polyimide resin (Polyzen 200P, PICOMAX, Korea) on the silicon wafer and curing at 150 ˚C and 250 ˚C for 30 mins and 3 hours in a convection oven, respectively. Based on fabricated polyimide substrate, patterned gold (Au) electrodes for sensor electrodes were fabricated by using conventional microfabrication processes including lithography and e-beam deposition.

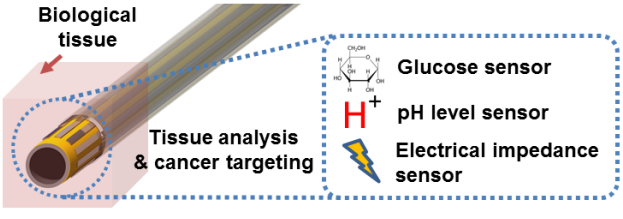

Figure 1: Schematic image of proposed flexible multi-modal biosensor integrated biopsy needle

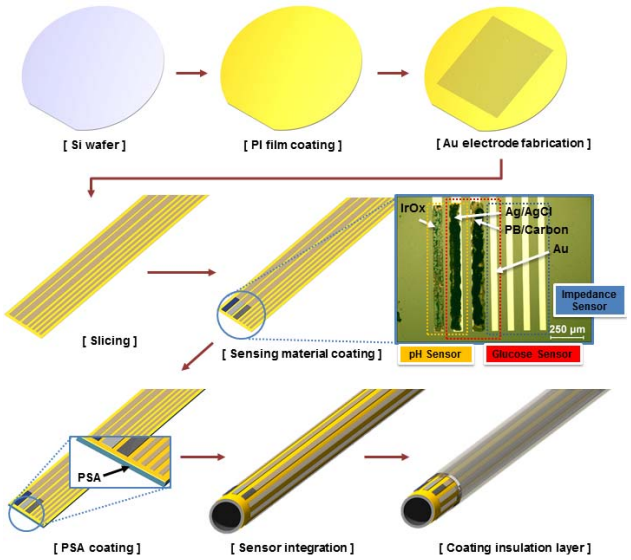

Figure 2: Schematic image of fabrication process of flexible multi-modal sensor and sensor integrated biopsy needle.

Fabrication Process of Multi-modal Biosensor Components

First, the sensor component for electrical impedance sensing is only consisted of electrically conducting electrodes. Therefore, bare Au electrodes were directly used as sensor electrodes for electrical impedance measurement.

In case of pH sensor, previous reported iridium oxide layer was utilized as a working electrode in pH sensor. Among various fabrication methods of iridium oxide, sol-gel deposition based iridium oxide fabrication was used by following Nguyen's work [6]. The advantages of sol-gel deposition of iridium oxide were simple and compatible with polymer substrate. First, SU-8 (MicroChem, USA) based masking layer for iridium oxide layer was fabricated by lithography process. After that, 0.2μL of iridium oxide sol-gel solution containing ethanol (C_2H_5OH, Samchun Chemical, Korea), acetic acid (CH_3COOH, Junsei Chemical, Japan) and iridium chloride ($IrCl_4 \cdot 2H_2O$, Sigma-Aldrich, USA) was dropped 5 times at the SU-8 openings of sensor electrodes. During each deposition of sol-gel solution, substrates was placed on hotplate at 120˚C in order to vaporize solvent of sol-gel solution. Then, SU-8 based masking layer was easily delaminated from polyimide substrate by utilizing poor adhesion between polyimide and SU-8 layer and the deposited iridium chloride was oxidized at 300˚C for 5 hours in a box furnace. Because iridium oxide based pH sensor was potentiometric sensor, additional reference electrode was needed in order to measure exact potential at iridium oxide layer. Ag/AgCl reference electrode was fabricated by using conventional screen printing process and a commercial Ag/AgCl paste (C2130102D1, Gwent Group, UK). Screen-printed Ag/AgCl was cured at 150˚C for 30 mins in a convection oven in order to evaporate solvent of the paste.

Glucose sensor was fabricated by following conventional glucose oxidase (GOx) based glucose sensor.

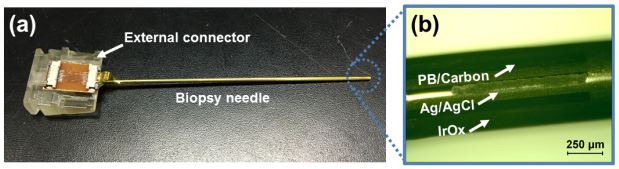

Figure 3: (a) Photograph image and (b) microscopic image of fabricated multi-modal sensor integrated biopsy needle

Carbon paste containing prussian blue (PB/Carbon, C2070424P2, Gwent Group, UK) as a catalyst of hydrogen peroxide reduction was used as working electrode and also fabricated by using screen printing process. As a reference electrode, the same Ag/AgCl electrode was utilized. Then, solution mixed with GOx (Sigma-Aldrich, USA) and Nafion® perfluorinated resin solution (Sigma-Aldrich, USA) was drop-casted on patterned carbon electrode, manually.

Integrating Multi-modal Biosensor onto a Biopsy Needle

After sensor fabrication, each multi-modal biosensor unit was sliced into width of 2 mm. Because of poor adhesion between polyimide substrate and the surface of stainless steel biopsy needle, pressure sensitive adhesive (PSA, MD-7, Dow Corning, USA) was coated on the back side of micro-biosensor unit by bar-coating. Then, the sensor unit was directly attached to the surface of biopsy needle and the needle was insulated by commercial medical grade heat-shrink tube in order to avoid unnecessary exposure of electrodes as shown in the last step of Figure 2. Fabricated multi-modal biosensor integrated biopsy needle is shown in Figure 3. A commercial flexible printed circuit connector (FPC connector) was used in order to connect sensor electrodes to external measurement systems. As shown in Figure 3 (b), multi-modal biosensor was clearly attached to the surface of biopsy needle without delamination between the polyimide film and the biopsy needle. Also, we could confirm that there was no crucial damage to pre-fabricated functional sensing layer including iridium oxide, Ag/AgCl and PB/Carbon, during sensor integration process.

EXPERIMENTAL METHODS

In case of electrical impedance measurement, direct connection of electrical impedance sensor electrodes to LCR meter (E4980a, Keysight, USA) was enough to measure. Electrical potential at iridium oxide pH sensor, which is potentiometric sensor, was measured by using electrochemical analyzer (6016D, CH Instrument, USA). The glucose sensor was amperometric sensor and measurement was also carried out with electrochemical analyzer. However, electrical current from LCR meter during electrical impedance measurement could interfere in the electrical potential at iridium oxide and PB/carbon electrodes. Therefore, individual sensor modality was investigated separately.

We characterized performances of electrical impedance, pH and glucose sensor by measuring NaCl solution, pH buffer solution and glucose solution, respectively. Conductivities of NaCl solutions were from

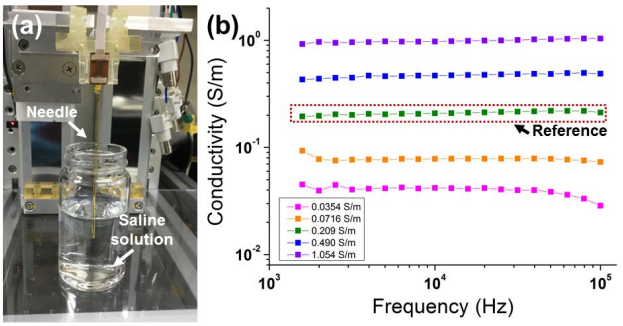

Figure 4: Impedance sensing result: (a) Photograph image of experimental setup and (b) measured conductivity of saline solutions with concentrations from 0.0354 S/m to 1.054 S/m

0.0354 S/m to 1.054 S/m which is similar to biological tissues according to previous report [7]. In case of pH buffer solution, pH range from 4 to 10 and 6.75 to 7.75 was used in order to investigate sensor performance in wide and narrow pH variation similar to difference between normal and cancerous tissue. Especially in narrow pH variation, pH variation was set to similar pH variation between cancerous and normal tissue according to previous study [8]. The ion concentration of Cl^- was maintained to 100mM in all pH buffer solution in order to maintain fixed potential at Ag/AgCl reference electrodes. The concentrations of glucose in glucose solution were from 0 mM to 16.67 mM which cover possible glucose concentrations in human body [9].

EXPERIMENTAL RESULTS

Sensor Performance of Electrical Impedance Sensor

According to Peyman's work, there is no dielectric relaxation of NaCl solutions under 1 MHz of measurement AC current [10]. Therefore, measuring electrical conductivity of NaCl solution was generally used in order to evaluate performance of probe. Measured parameter from LCR meter is conductance which is dependent of dimensional factors of measurement probe. Therefore, cell constant of probe is used in order to convert measured conductance into conductivity which is intrinsic electrical property of medium as described below equation (1) where

$$G = k \cdot \sigma \quad (1)$$

The electrical conductance measurement results of the NaCl solutions with various concentrations were shown in Figure 4. We set and measured NaCl solution with electrical conductivity of 0.209 S/m as a reference solution for cell constant calculation and measured conductances of other NaCl solution were converted into conductivities. Measured and converted conductivities of NaCl solutions were almost constant excepting for NaCl solution with conductivity of 0.0354 S/m up to 100 kHz of measurement frequency of AC current. This exception might be caused from parasitic impedance of sensor electrodes because effect of parasitic impedance became larger and dominant in media with low conductivity. This verify that fabricated electrical impedance sensor in multi-modal biosensor system could be used as tools for electrical impedance measurement however additional calibration process was also needed when tissue with low conductivity was measured.

Sensor Performance of Iridium oxide based pH Sensor

Measurement result of iridium oxide based pH sensor in range of 4 to 7 and 6.75 to 7.75 was shown in Figure 5. In case of wide range of pH variation from 4 to 10 as shown in Figure 5 (a), measured electrical potential at iridium oxide was 0.0955 V, 0.1975 V and 0.325 V with respect to the reference electrode in pH 4, 7 and 10, respectively. The sensitivity of iridium oxide based pH sensor was -38.4 mV/pH and the electrical potential at iridium oxide showed highly linear response to pH variation. However, because of large pH change, stabilization of electrical potential was slow and additional stabilization time might be needed in case of large pH variation environment. However, in case of small pH variation, response time of pH sensor was less than 10 s as shown in Figure 5 (b). Also, in this case, measured sensitivity was -37 mV/pH and showed linear response to pH change. Therefore, we could confirm that fabricated iridium oxide based pH sensor performed enough to cover and discriminate pH changes between normal and cancerous tissues.

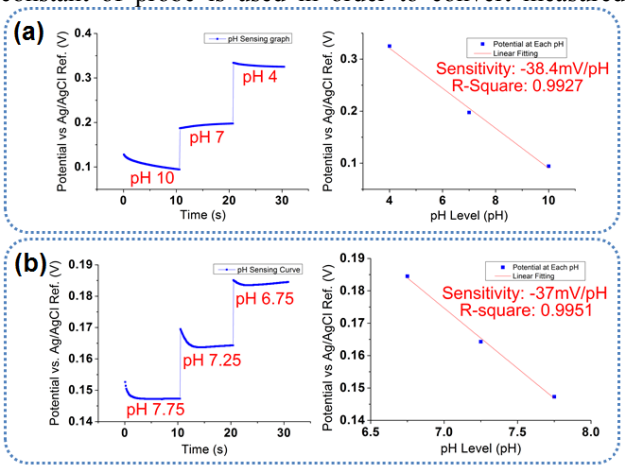

Figure 5: Potentiometry result for pH measurement: (a) measurement from pH 4 to pH 10 and its sensitivity graph; (b) measurement from pH 6.75 to pH 7.75 and its sensitivity graph

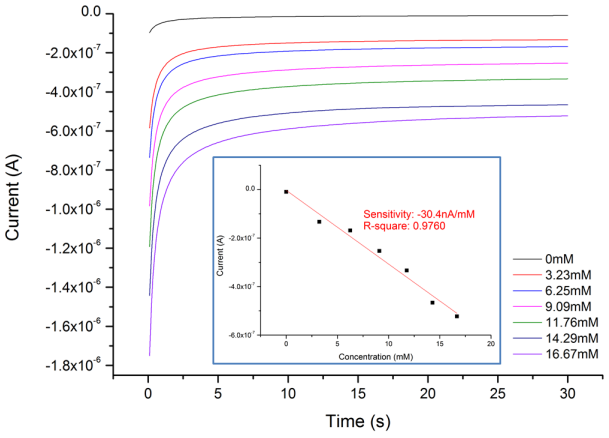

Figure 6: Amperometry result for glucose measurement of solution with concentration from 0mM to 16.67mM glucose and (inset) sensitivity graph

Sensor Performance of Glucose Sensor

Prior to measurement of glucose sensor, reduction potential of H_2O_2 which is by-product of oxidation process of glucose from GOx was measured by cyclic voltammetry and measured reduction potential was -0.1 V with respect to the Ag/AgCl reference electrode. Based on measured reduction potential, amperometry was carried out in order to measure concentrations of glucose concentrations from 0 mM to 16.67 mM as shown in Figure 6. Measured sensitivity of glucose sensor was -30.4 nA/mM and showed highly linear response to concentrations of glucose solutions. Therefore we verified that fabricated glucose sensor in multi-modal micro-biosensor could cover whole possible range of glucose concentrations in human body [9] therefore sensor could discriminate glucose concentration difference between normal and cancerous tissues.

CONCLUSION

In this research, we propose novel flexible micro-biosensor with capabilities of multi-modal sensing including electrical impedance, pH and glucose sensing. The micro-biosensor was thin enough to be attached to the surface of biopsy needle without significant increase of diameter therefore possibility of additional tissue damage was almost negligible. Also, performance of each sensor modality was demonstrated by using solutions such as NaCl solution, pH buffer solution and glucose solution and it showed that each sensor was enough to discriminate physical/chemical differences between normal and cancerous tissues.

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