INTEGRATED AND WEARABLE EARRING AS BIOSENSOR FOR TROPONIN DETECTION

ABSTRACT
In many cases of acute myocardial infarction (AMI), patients do not experience any symptoms until serious damage has been done to the heart. In order to administer a more immediate response, this paper presents the concept of an automated drug delivery system that is activated when Troponin I is detected. This paper proposes the use of an integrated silicon wire biosensor to detect the biomarker for cardiac damage. Upon detection, it sends a signal to the drug delivery system to release the thrombolytic drug. A functionalized silicon wire exposed to the interstitial fluids in the earlobe is used. The conductance of the silicon wire is monitored, and any significant change will trigger a response. This device is worn as an earring and has a lot of potential to be used as a biosensor for other types of biomarkers.

INTRODUCTION
Acute Myocardial Infarction
Acute myocardial infarction, or more commonly known as heart attack, is a common disease with serious consequences in mortality, morbidity and cost to the society. Each year in the United States alone, more than 1.2 million have heart attack and many of them die. It happens when the heart is not receiving oxygen as a result a coronary heart disease (CHD) that obstructs blood flow. Some of the preventive measures taken include advising the patient is to act quickly and get help as soon as symptoms like chest pain or shortness of breath occurs.

However, these symptoms tend to be overlooked as temporary discomfort and thus, many patients delay the treatment. Studies have shown that the median time from the onset of symptoms to seeking medical care ranges from 2 to 6 hours. This is way beyond the optimal benefit derived during the first hour from symptom onset (1). Even though new treatments continue to emerge, the greatest challenge lies in effectively implementing preventive actions in high-risk individuals and to expand delivery of acute treatment in a timely fashion for all eligible patients (2).

Smart Drug Delivery System
The key motivation of the smart drug delivery system proposed aims to deliver medical care within the shortest time possible. When cardiac biomarker is detected by the biosensor, drug is immediately delivered
to prevent further blockage as summarized (Figure 1). It is believed that this will be more efficient and provide an immediate response as compared to waiting for the onset of any physical symptoms. The patient and his physician will be informed, and the patient will seek medical attention immediately for further treatment. As recently as this year, Micheli et al. proposed an implantable device that claims to monitor blood for various antigens (3). However, it faces many practical issues and making it commercially available might take many more years.

The proposed smart drug delivery system is a low cost and disposable set of device that is applicable for one time use only. Consisting of a conductometric based (4) biosensor that is worn as an earring and a drug delivery system that is worn as a wristband, the set of device is carefully designed so that high risk patients can wear them without prejudice and having to face any inconvenience. The biosensor is worn as an earring (Figure 2) to test for the presence of cardiac biomarker from the interstitial fluid in the earlobe. Upon positive detection, a radio signal is emitted to the wristband and the drug will be delivered. It is also envisioned that there will be some form of indication in the form of LED notice or wireless communication but this will be beyond the scope of this paper.

Integrated and Wearable Biosensor Earring
This paper will focus on the fabrication and functionalization of the earring (Figure 2A) worn on the earlobe (Figure 2B) as a biosensor. The 2 key components of the earring are the 2 silicon wires which are exposed to the interstitial fluids (Figure 2C) and the 2 casings which hold the electrical parts of the earrings (Figure 2D). The LR44 battery, silicon wafer, radio transmitter and wiring are housed inside the casing. A switch and an LED light indicator can be added onto the casing (not shown). While it is foreseen that the device can be functionalize to detect a range of cardiac biomarker, this paper aims to show how Troponin I can be detected. This is based on the argument that Troponin I is a strong biomarker for cardiac damage (5).
Integrated and Wearable Drug Delivery Wristband

The second component of the integrated smart drug delivery system is the wristband (Figure 3A) that is worn on the wrist. Upon receiving a positive signal from the biosensor, the switch releases the compressed spring and this results in the automated compression of the microneedles into the epidermis of the skin. Drug is slowly delivered from the drug reservoir (Figure 3B), through the microneedles and into the interstitial fluid of the patient’s body.

EXPERIMENTAL SECTION

Materials

The wearable earring consist of several components that requires precise fabrication and functionalization. Silicon was used to fabricate both the wire and substrate. Monoclonal antibodies for cardiac Troponin I (cTnI) were functionalized onto the silicon wire to detect cTnI. PBS was used as washing and working buffer solution. Heparin was added onto the silicon wire as an anticoagulant.
In addition, a LR44 battery is used to induce a small current through the earring. An instrumentation amplifier on a silicon wafer monitors the conductance across the silicon wire and a wireless radio transmitter sends a signal when change is detected.

Fabrication
The earring is composed of 2 components that are fabricated separately. The first component is the set of copper casing that houses the electrical circuitry of the earring (shown in Figure 2A as the 2 yellow cylindrical objects). It can be easily be manufactured commercially and does not require specialized bioMEMS fabrication steps.

The second component is the silicon wires connected to the silicon wafer, which were fabricated using lithography and chemical vapor deposition (CVD) (Figure 4). Firstly, the silicon substrate is patterned using a photoresist and then etched away via wet etching. In the next step, phosphosilicate glass (PSG) as a sacrificial layer is deposited over silicon substrate using CVD. Thirdly, silicon is deposited over PSG using CVD. The last step involves using Hydrogen Fluoride (HF) to selectively etch away the PSG layer in order to obtain the patterned substrate.

Even though there is a variety of materials available to construct these wire, silicon was primarily chosen because of the extent of research conducted using it. The various materials of nanowire commonly used were compared against some of the important criterion listed for a biosensor (Table 1). Silicon was eventually chosen as the material choice for the wire because of its low cost and considerable mechanical strength.

<table>
<thead>
<tr>
<th>Properties/Conductors</th>
<th>Nickel</th>
<th>Platinum</th>
<th>Gold</th>
<th>Silicon</th>
<th>PANI (6, 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per mass (USD/LB)</td>
<td>6.13</td>
<td>25,000</td>
<td>16,000</td>
<td>0.77</td>
<td>15,000</td>
</tr>
<tr>
<td>Tensile strength (GPa)</td>
<td>200</td>
<td>168</td>
<td>79</td>
<td>150</td>
<td>2</td>
</tr>
<tr>
<td>Biocompatibility</td>
<td>Moderate</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Electrical Resistivity (Ω·m)</td>
<td>6.93E-08</td>
<td>1.05E-07</td>
<td>2.21E-08</td>
<td>1.00E+03</td>
<td>1.59E+08</td>
</tr>
<tr>
<td>Electrical Conductivity (S/m)</td>
<td>1.44E+07</td>
<td>9.52E+06</td>
<td>4.52E+07</td>
<td>1.00E-03</td>
<td>6.28E-09</td>
</tr>
</tbody>
</table>
Functionalization
The fabricated silicon wire was functionalized with monoclonal antibodies in order to detect the cardiac biomarker through surface immobilization method using EDC/NHS solution (8). The solution helps to form covalent bond between silicon and the mobilized antibodies. After washing the functionalized silicon wire with PBS and deionized water, heparin was covalently immobilized on the available silicon surface using carbodiimide-based immobilization. A (3-aminopropyl) trimethoxysilane (APTMS) self-assembled monolayer (SAM) or multilayer was first coated onto the silicon surface as the bridging layer, and heparin was then attached to the surface in the presence of water-soluble carbodiimide (9).

Detection
The detection of cardiac biomarker was carried out using the conductometric sensing method by measuring the conductance change of the silicon wire based on the relationship between conductance and resistance of the silicon wire (Equation 1). Unlike conventional methods using nanowires (10, 11), this paper proposes a design using silicon wire within the micro range so that fabrication cost can be kept low.

Using 2 silicon wire with a length of 0.005m each and radius of 0.001m in order to form a closed circuit, its conductance and resistance value is calculated to be as shown.

\[ G = \frac{I}{V} = \frac{1}{R} \quad \text{(Equation 1)} \]

\[ R = \frac{\rho l}{A} \quad \text{(Equation 2)} \]

For \( \rho = 10000 \, \Omega \cdot m, l = 0.01 m, A = \pi(0.001)^2 \); \( R = 3.183 \times 10^6, G = 3.142 \times 10^{-7} \, \Omega^{-1} \)

Using a 1.5V LR44 battery to supply a small current through the silicon wire, and assuming that the resistance of the other components of the circuit is much smaller than the silicon wire, the current flowing through the circuit is calculated (Equation 3).

\[ V = RI \quad \text{(Equation 3)} \]

For \( V = 1.5V, I = 4.713 \times 10^{-7} \, A \)

\[ \frac{1 \, Ah}{4.713 \times 10^{-7} \, A} = 2120000 \, hours = 242 \, years \quad \text{(Equation 4)} \]

In an alkaline medium, it is assumed that an increase in antibody-antigen complex is directly proportional to a linear increase in conductance. In a normal human being, there will be no complex formed and the registered conductance will be the value calculated as above. A diagnostic limit of 0.04 ng/ml and above of cTnI in the blood is an indication of AMI onset (12). However, in most cases, the concentration of cTnI is much higher than this value. Thus, by quantifying conductance change due to the presence of cTnI in the blood which will bind to the antibodies, the instrumentation amplifier can be configured to set off a positive signal once that happens.

A standard 1.5V LR44 battery has an electric charge of 1 Ah, and base on the current that the device draws, it can be expected to last for a period of more than 200 years (Equation 4), which makes the device long lasting as long as it is not activated.
Feedback
When conductance change across the silicon wire exceeds the threshold value, it is an indication that antigen-antibody complexes have formed and cTnI concentration is beyond the clinical limit. Thus, the instrumentation amplifier (Figure 2D, red box) sets off a positive signal to close the field-effect transistor switch (Figure 2D, yellow box)

Connected in series is the radio transmitter (Figure 2D, green box), which forms an open loop with the battery. Once activated, the switch at the field-effect transistor is closed to form a close loop with the battery. As current flows through the radio transmitter, radio waves will be transmitted to the drug delivery system for drug release. At the same time, the user and his medical doctor will be informed of troponin detection.

RESULTS AND DISCUSSION
Detection of Cardiac Biomarker
Detection of the cardiac biomarker is based on the conductance change of the silicon wire. When cTnI is released into the blood as a result of heart damage, they will diffuse through the blood capillaries and into the interstitial fluid. Since the device is worn as an earring, the immobilized antibodies functionalized on the silicon wire will be exposed to the antigen. It will bind with the antigen in a reversible reaction (Equation 5). However, several concerns regarding this method need to be addressed beforehand.

The working principle assumes that the diffusion of cTnI from the blood capillaries into the interstitial fluid has high diffusion rate in the earlobe. While not common, technique for sampling capillary blood in the earlobe were described over 2 decades ago and has been found to be a reliable substitute for arterial sampling in routine clinical practice (13). Thus, this paper aims to show that the concentration of cTnI in the blood is approximately the same as in the interstitial fluid.

Any puncture on the skin will produce a hemostasis response by the body. Such a biological response is a common problem faced in the field of implantable biosensor (14). In order to prevent platelet adhesion to the surface of the silicon wire and the closure of the hole, heparin is added in the functionalization process of the silicon earring as an anticoagulant. Given many successful examples of utilizing co-immobilization to prevent fibrin “clot” (15, 16), investigation will be conducted to evaluate the success on the earring and the period of validity before heparin concentration is depleted.

Reaction Kinetics
Steps are taken to simplify the reaction kinetics of the 2nd order reversible reaction so that it will be more easily quantifiable for all different k values (17).

A typical anti-cardiac Troponin I antibody from Millipore™ has an affinity constant of $8 \times 10^{10}$. The definition of affinity constant is the reaction constant of the forward reaction divided by the reaction constant of the backward reaction. Thus, the reaction kinetics of this reaction can be treated as a 2nd order kinetic reaction (Equation 6).

$$cTnI\ (Ag) + cTnImAbs\ (Ab) \leftrightarrow Complex(ABAg) \ (Equation\ 5)$$
\[
\frac{d[AbAg]}{dt} = k_f[Ag][Ab] - k_b[AbAg] \quad (Equation \ 6)
\]

However, in order to simplify the equation, a few assumptions will be made. First of all, the reaction constant of the forward reaction is much greater than the reaction constant of the backward reaction, which can be given an arbitrary value of 1 in this case. Thus, the reaction constant of the forward reaction is the same as the affinity constant. The second assumption to be made is that the concentration of the antibody is much greater than the antigen. As a result, the binding of the antigen will not significantly reduce the concentration of the antibody and the concentration of the antibody can be assumed to take on a constant value, K. With these 2 assumptions, the rate of complex formation can be approximated to a 1st order reaction (Equation 7).

It is generally known that cTnl has isoelectric points lower than the alkaline interstitial fluids and therefore the net charges of these target proteins are negative. Base on the biosensing experiments from previous studies, it was speculated that the negative charge of the target proteins resulted in carrier accumulation on the silicon wire and consequently an increase in conductance (18). By assuming that the formation of the complex is directly proportional to the increase in conductance, the conductance at any time will be similar to the 1st order reaction equation of the complex (Equation 8).

\[
\frac{d[AbAg]}{dt} = KK_f[Ag] = Kk_f([Ag]_0 - [AbAg]) \quad (Equation \ 7)
\]

\[
[AbAg] = [Ag]_0 - De^{-Kk_f t} \quad (Equation \ 8), \text{ where } [Ag]_0 = 0.04, D = 0.04 \text{ and } Kk_f = 8000
\]

Change in Conductance

To quantify the change in conductance, it is first necessary to conduct an experiment using the diagnostic limit of troponin, and measure the change in conductance change as time approaches infinity. In the above graph, a plot is done with different k values. These k values depend on the specific antigen and antibody reaction kinetics.

Using the k value of a typical anti-cardiac Troponin I antibody from Millipore™ as reference, it is possible to predict the different time taken for antibody from different sources, or for different antigen detection. For example, this experiment can be extended to detect other cardiac biomarkers like myoglobin (Myo), creatine kinase-MB (CK-MB), and b-type natriuretic peptide (BNP) (19). Figure 5A shows how the change of conductance over time can be predicted as long as the k values of these reactions with reference to affinity constant of cTnl are known. This is based on the assumption that they all have the same effect on the conductivity change of the silicon wire.
Sensitivity of Silicon Wire

In order to quantify the sensitivity of the experiment using different materials of wire, it is necessary to conduct a second experiment and measure the change in conductance due a change in concentration of the antigen. While silicon was chosen in this paper, it might not have the highest sensitivity. By measuring the conductivity of the various metals compared in Table 1, we will be better able to determine the right choice of wire and balance the tradeoff that come with each type of wire. A desired sensitive material will reflect the greatest increase in conductance per unit increase in complex formation. This is will give the highest signal to noise ratio and minimize the effect of interference from external sources.

It is also important to note that these experiments should be conducted within the linear range of interest (20, 21). Through further experiments, this paper aims to prove that the above is the true and the use of silicon as a material choice is appropriate.

CONCLUSION

Advancement in science and technology has led to significant improvement in biosensor for early detection of diseases and illnesses. However, the complex design of some of these biosensor makes it hard to downscale the device into a wearable product. Manufacturing and costs are other factors overlooked as they are more often used by research scientists (22). This paper presents a novel idea of a low cost and integrated system that aims to be affordable for the masses. Utilizing fundamental concepts and simplistic design, it is hoped that the device can be expanded to become a tool for early detection of other illnesses. With appropriate testing and quantification of parameters in these different application, this device appears to be a promising alternative to conventional methods like the enzyme-linked immunosorbent assay (ELISA) for medical diagnosis.
REFERENCES


