M2-016 Design and Initial Fabrication of Microelectrode for DNA Sensor from Polymer-Carbon Nanotubes Composite

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ABSTRACT

Here we present the initial investigation on microfabrication of DNA sensor based on electrical detection. We introduced a novel material on detecting DNA hybridization event using polymer-carbon nanotubes (polymer-CNT) composite as the electrode. Moreover, we did a proof of concept on the functionality of the electrodes which covers the material design and micro-fabrication trial. As a first approach, we tested the polymer-CNT film to sense cell adhesion by monitoring its impedance changing.

Keywords: microfabrication, DNA sensor, polymer-CNT composite

1. Introduction

The DNA conventional technology utilizes lasers, optics, and fluorescent labels are bulky and ill-suited for use in a portable and diagnostic for small-scale detection [1]. We investigated a technology which are label-free and allow the miniaturization of the device. Eliminating labels saves time and expenses. Moreover, purely electronic readout is desirable because it requires relatively little space and power [2]. Therefore, we are developing a novel material for electrode from polymer composite that allow us to immobilized the DNA directly on the electrodes.

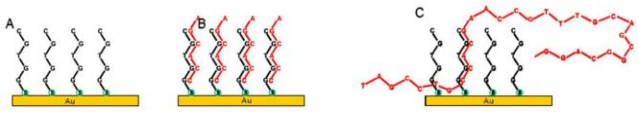

Figure 1. DNA hybridization event: (A) oligonucleotide-probe forms on gold electrode; (B) hybridization of the complementary oligonucleotides (target); (C) specific binding of a long DNA molecule through hybridization

The technical principal of DNA recognition is similar to that developed by Guiducci et.al. He performed capacitance measurements with gold electrodes, functionalised electrodes (contained oligonucleotides SAM) and functionalised electrodes that has been exposed to DNA. The device has shown high specificity for DNA hybridization detection and good reproducibility. Furthermore, Guiducci also verified the reusability of the DNA biosensor [3].

Nowadays, researchers are developing non-metal electrodes to avoid degradation in biological solution. Moreno-Hagelsieb et.al attempted to measure the capacitance change between interdigitated micro-electrodes by using metal oxides such as Al_2O_3. The electrodes provide efficient protection and adequate surface for bio-functionalization and DNA binding [4,5].

We are developing polymer conductive electrodes for sensor which were made from polymer-carbon nanotube composite. Polymers have several properties such as lightness, resistance to corrosion, ease of processing, and low cost production. Furthermore, carbon nanotubes (CNT) have tremendous properties such as extreme high strength, high electric and thermal conductivity offer advantages over other nano-fillers [6]. By dispersing CNT in polymer matrix, polymer is transformed to conductive material, thus able to become micro-electrodes.

2. Materials and methods

2.1 Polymer-CNT dispersion

Firstly, 40% g/ml polymer solution is prepared by dissolving PMMA (Sigma Aldrich) in a solvent. Then, certain amount of single wall carbon nanotubes (Sigma Aldrich) is dispersed in a solvent for 5 minutes using 10W sonic tube. Finally, polymer solution is mixed with CNT dispersion using sonic tube to obtain 5 - 0.1% weight of CNT in PMMA solution.

2.2 Microfabrication trial

We used Pressure Assisted Microfabrication (PAM) to fabricate microelectrode. PAM system, initially developed at University of Pisa, adapted a rapid prototyping technique to deposit polymer-CNT dispersion on a certain surface. The system, illustrated in Fig. 1, consists of a microsyringe with a 100 µm glass needle. The polymer-CNT dispersion was placed inside the microsyringe and extrude on the tip by the application of filtered compressed air.

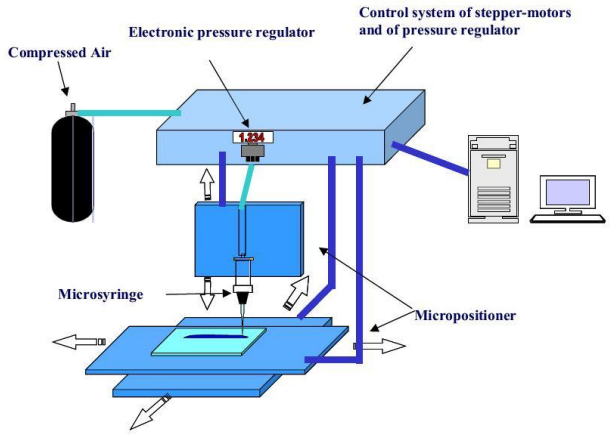

Figure 2. Schematic of fabrication method

The control software was developed in C with a userfriendly graphic interface that allows a wide range of patterns with a well-defined geometry to be designed and deposited.

2.3 Mechanical properties measurement

We prepared films from the polymer-CNT dispersion using spinning machine under 1500 rpm. Furthermore, mechanical properties of the polymer-CNT film were measured using an isotonic transducer (Ugo Basile).

Figure 3.
The preparation of mechanical properties (stress-strain) measurement of polymer film (dimension of 2 cm x 0.5 cm width).

Thermogravimetry analysis and microscope image were also performed to give information on film morphology.

2.4 Electrical properties measurement

Impedance value of polymer-CNT films is measured by Agilent RCL-meter type E4980. Figure 4 depicts the preparation of polymer-CNT films to be seeded with certain amount of cells (endothelial cells from human umbilical cords).

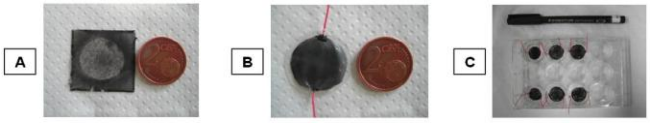

Figure 4. Preparation of polymer-CNT film: (A) after spin coated; (B) connected to wires; and (C) placed on the multi-well bioreactor

Theoretically, after 6-12 hours, cells will be adhered on the polymer film and change the electrical properties. Therefore, we monitor the impedance value to demonstrate the impedance changing by cell adhesion. We also established a control group which consisted of films without the existence of cells. Nevertheless, we also did impedance monitoring before cells seeding for 24 hours.

The confirmation of cell adhesion event is done by comparing films from both treated and control group. The cells was dyed and the film was washed hence we can observe coloured cells on the surface.

3. Result and Discussion

3.1 Material development

Material selection plays important role in the stage of this research. Although CNT is known to have tremendous conducting property, it also has limitation in dispersing property. Many results have been conducted to create a good CNT dispersion in polymer matrix [7,8].

An analytical model by Deng et.al shows the predictions of the effective electrical conductivities of CNT composites compared with the observed data in [9]. The result show that PMMA can be a good host for the purpose of the DNA sensor. Furthermore PVC is soluble in water, hence PMMA is a better matrix for the CNT.

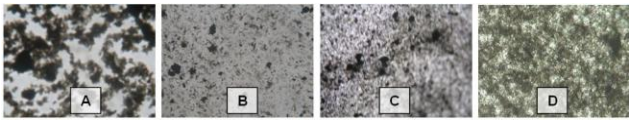

Figure 5. CNT-PMMA weight percentage (A) 5%; (B) 2%; (C) 1%; and (D) 0.15%

Figure 5 shows the dispersion of CNT in PMMA matrix with various concentration weight. Picture 2A shows that the polymer matrix is not big enough to be dwelled by the filler. Therefore, CNT particles tend to form a massive agglomeration. On the other hand, picture 2D shows that CNT agglomeration was minimized significantly. It might be concluded that the concentration of 0.15% CNT is the optimum value.

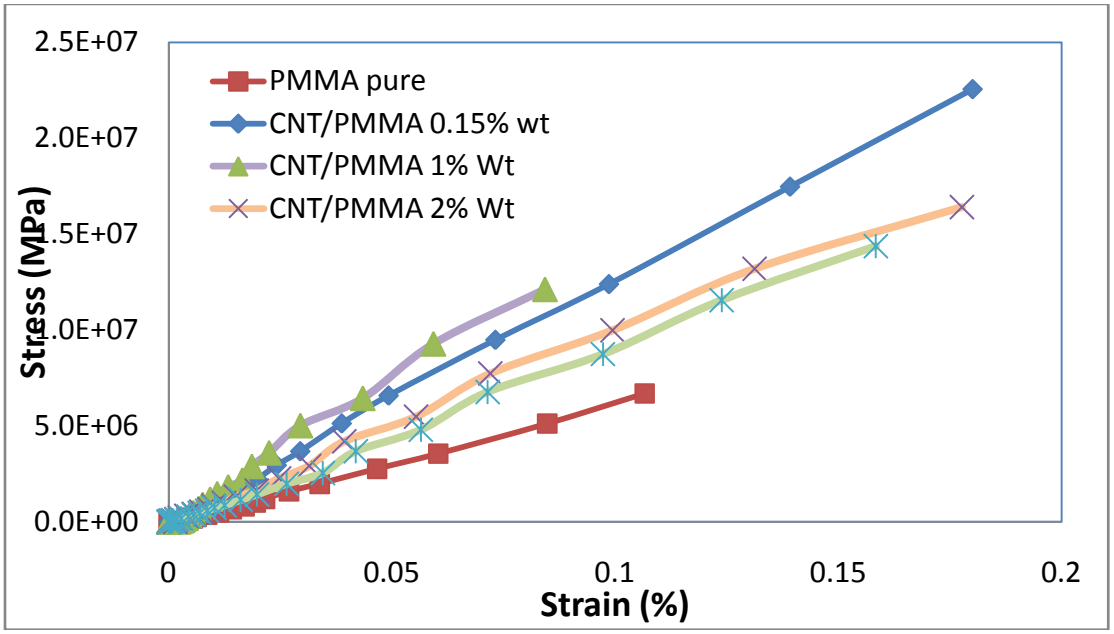

Figure 6. Stress-strain diagram of polymer-CNT film

Figure 6 shows the result of stress-strain measurement of various weight concentration of CNT in PMMA. The 0.15% CNT film has highest yield stress which almost 5 times higher than the PMMA film without CNT filler.

The termogravimetry of 0.15% CNT/PMMA shows no significant difference compare with PMMA pure. This might be caused by a very low concentration of CNT in the PMMA matrix.

3.2 Microfabrication trial

We use Pressure Assisted Microfabrication (PAM) system to fabricate the electrode with certain structures. The design are aimed to have capacitive structure. Therefore, we program the PAM software to create the polymer deposition in parallel, interdigitated and complex pattern.

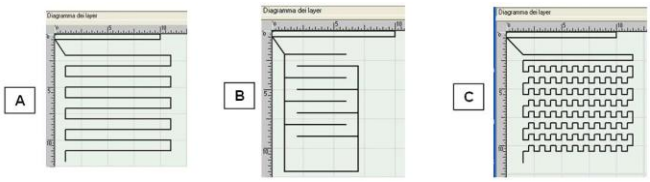

Figure 7. Capacitive electrode designs: (A) parallel; (B) interdigitated; (C) complex structure (in millimeter scale)

Figure 7 shows the PAM software window to design structure of electrodes. We use a constant value for air pressure and velocity of x-y plane.

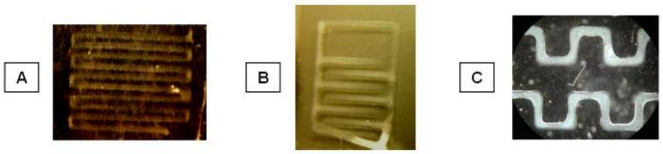

Figure 8. Realized electrode structure

The resolution of the electrode was achieved at 264±25 µm by using a syringe with diameter of 150 µm. The pressure was kept at 10 mmHg and the velocity of x-y plane was at 2.5 mm/s.

3.3 Electrical properties measurement

Firstly we measured the polymer-CNT film without the existence of any cells. Later, we seed 400.000 cells/ml on the film inside the multiwall bioreactor. A frequency range of 10 kHz-2MHz was introduced to investigate the frequency response of the polymer film. Furthermore, we measured the impedance of polymer film in 0, 3, 6 and 18 hours after the cells seeding.

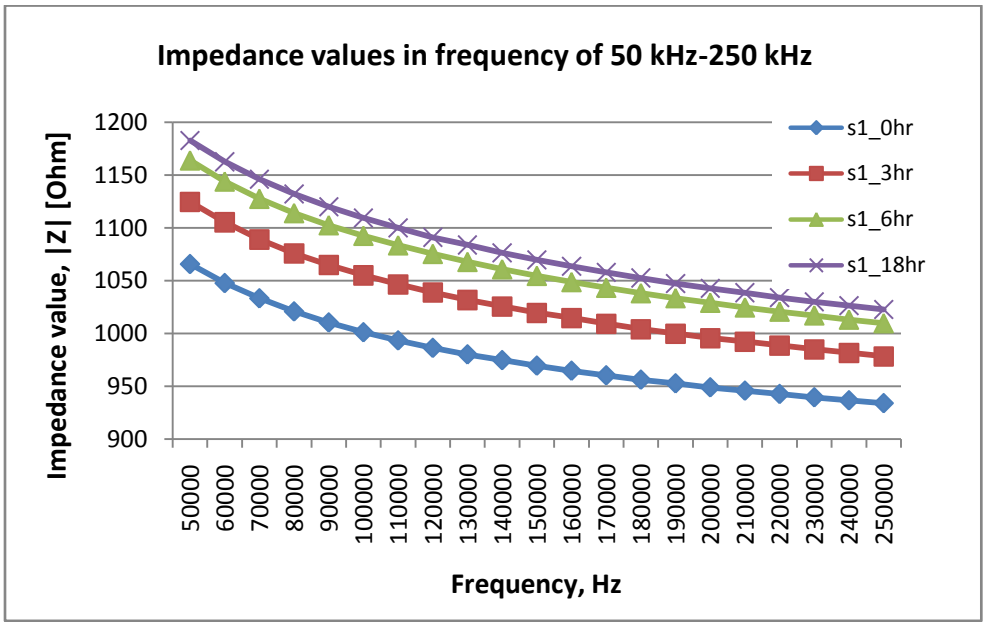

Figure 9. Extracted result of impedance measurement in a frequency of 50 kHz-250 kHz.

The result from frequency sweep measurement (10 kHz-2MHz) shows that the film has capacitive properties (down trend over the frequency). It might be caused by the introduction of aqueous media of cells on the polymer surface. Furthermore, it also showed the highest response in the range of 50-250 kHz. Later, we extracted impedance values from this frequency to give clearer comparation between values overtime (figure 9).

Figure 10 depicts the average values of impedance changing of polymer film during the observation time.

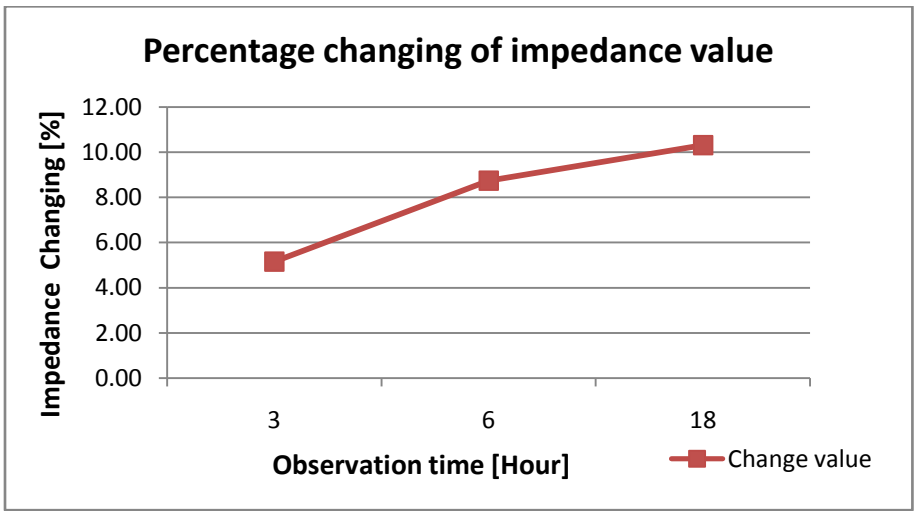

Figure 10. Average impedance changing of polymer film

Figure 11 gave the result of impedance changing of polymer film with and without the cells adhesion. It has shown that polymer-CNT films impedance were modified in range of 10±3% after 18 hours. On the other hand, polymer film without cell adhesion had a relatively stable impedance changing.

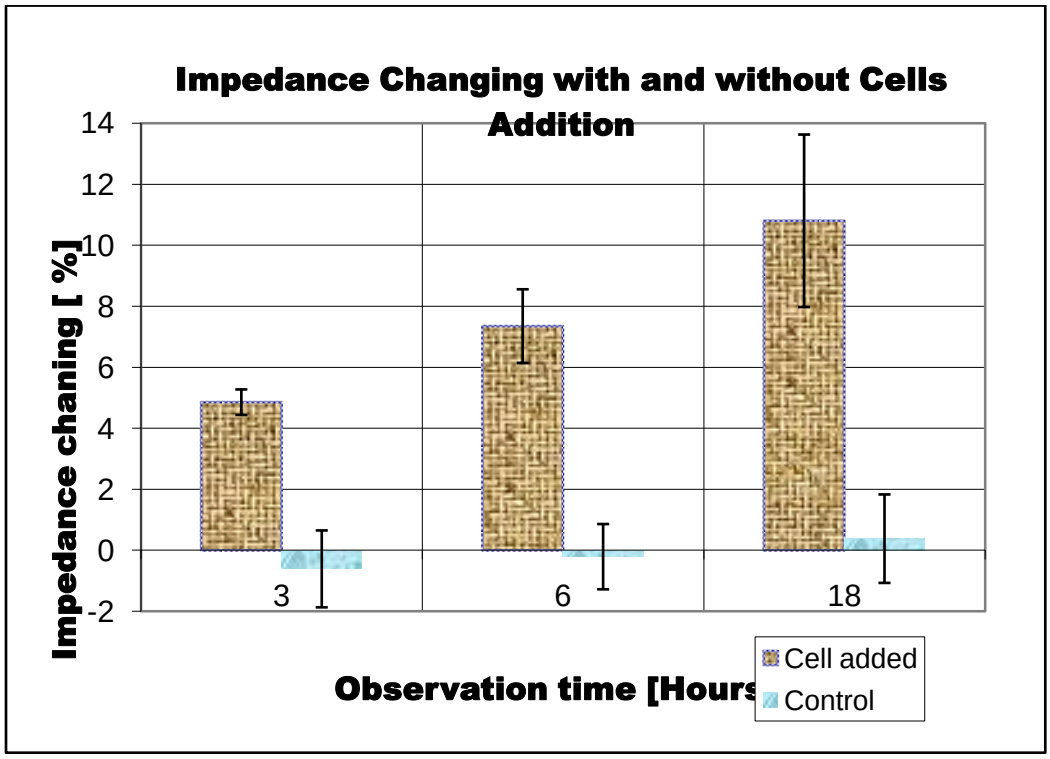

Figure 11. Impedance changing of polymer film with and without cells addition.

We also performed microscope observation to confirm the adhesion event of cells on the polymer films.

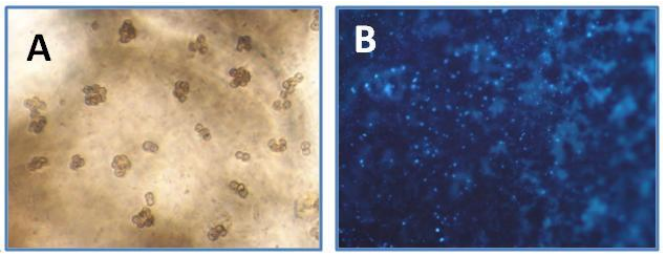

Figure 12. Polymer-CNT film with cells addition (A) after washed and (B) with dyed cells.

Figure 12a confirmed that the cells were adhered on the polymer surface and remain attached after the washing process. Figure 12b presented dyed cells observed under fluorescence filter microscope. Those white dots indicated the cells that adhered on the surface of polymer-CNT films.

4. Conclusion

1. Composition of CNT:polymer in dispersion found to be optimum in level of 0.15% weight.
2. Resolution of the electrode is achieved at ±250μm by Pressure Asissted Microfabrication system.
3. Electrical monitoring has been performed to be able to detect cell adhesion by several limitation such as low reproducibility of film composite.

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