Fabrication of Electrospun Composite Nanofibers and Their Application in Early Detection of Femtogram C- reactive protein (CRP)

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Abstract

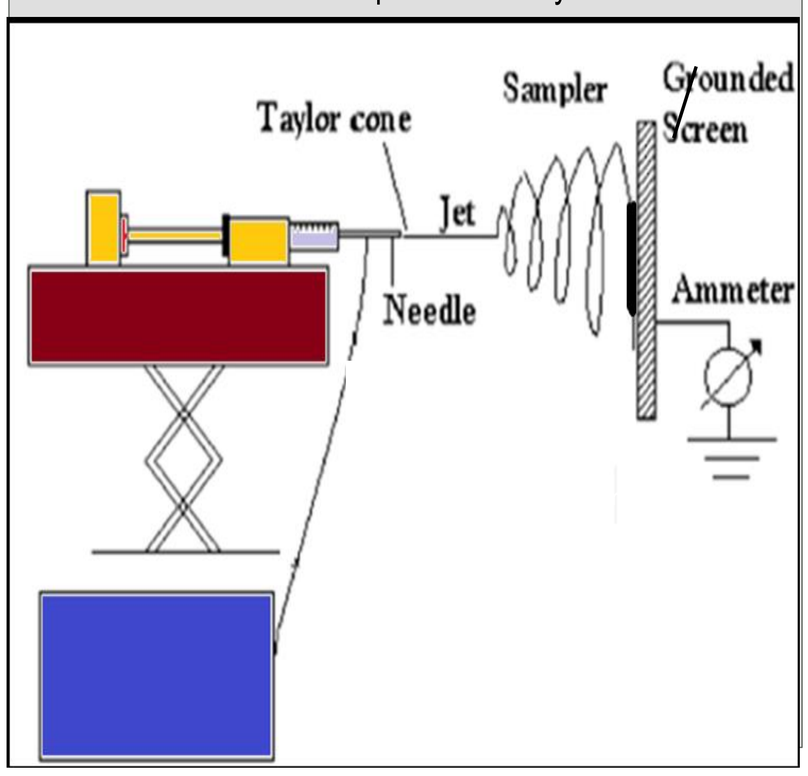
The methodology for developing a protein biosensor has been presented and it focuses on the use of carbon nanotubes and polymer nanofibers based nano composites that have been electrospun in the form of “nanowebs”. These nanowebs have been surface modified to function as sensors for detecting C-reactive protein(CRP). Detection sensitivity of CRP was achieved in the femtogram/mL regime from phosphate buffered saline solutions and in human serum. The designed biosensor is three orders more sensitive than ELISA. Sensitivity enhancement was achieved by tuning the charge transduction at the solid /liquid interface on the nanoweb matrix.

Introduction

In recent years the trend in health care has been toward developing devices that supports faster diagnosis and therapeutic turnaround time. These needs have facilitated rapid growth in research and development of point-of-care diagnostic testing (POCT). Heart disease is a major cause of death all over the world. According to “Center for Disease Control and Prevention”, heart attack is the leading cause of death in US. Every year an estimated 785,000 Americans had a new coronary attack and about 470,000 had a recurrent attack [1]. C-reactive protein (CRP) is an inflammatory cardiovascular biomarker which is over-expressed in a person susceptible of a heart disease [2] and thus rapid detection of CRP from patient serum samples may be clinically relevant in diagnosing cardiac events such as acute myocardial infarction. The novel properties of nanoscale materials such as the enhanced surface area and size based confinement of biomolecules, have the potential to increase the sensitivity of the diagnostics platforms in order to detect the protein biomarkers such as CRP at very low concentrations and give early indication of heart condition [2].

Materials and Methods

The nanoimprinting of fibers on the PCB was achieved using electrospinning method. 15kV of potential difference was applied at 10 cm distance maintaining 1.5ml/hr flow rate. The nanoweb biosensor chips comprise of a base printed circuit platform. The platform is overlaid with the nanoporous nanoweb layer, which is then encapsulated by microfluidic manifolds manufactured using polydimethylsiloxane for fluid encapsulation and confinement . We have used CRP protein in phosphate buffered saline buffer as well as 50% human serum for evaluating the sensor performance. Printed circuit board (PCB) chips comprising of gold comb shaped designs of dimensions 10 mm x 10 mm in length, finger width of 1 mm with spacing of 1 mm, as shown in Figure 2A, were manufactured with a FR4 passivation layer.



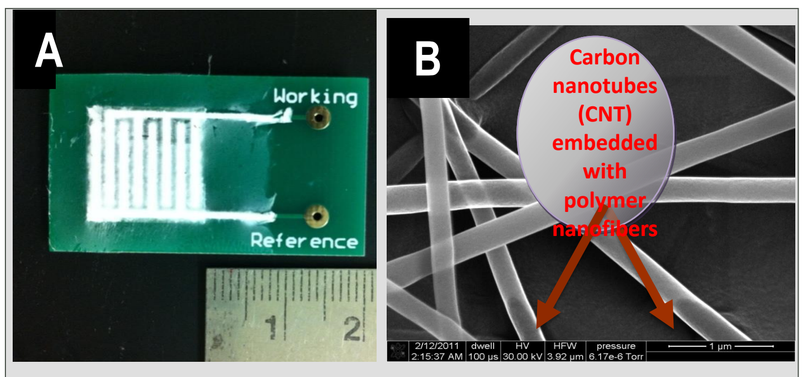


Figure 2. A) Optical image of the protein biosensor patterned with nanowebs on PCB. B) SEM image of PVA+CNT nanoweb with an average diameter 200nm.

Clean the metal surfaces with 10 mL of isopropyl alcohol (IPA) and air dry the samples for 10 minutes. Nanowebs constituted of 10 mg of CNT in 100ml of 10 % PVA were electrospun. The dimensions of the nanowebs used for the experiments were 13.2 mm x 13.2 mm. The nanoweb is encapsulated with a polydimethylsiloxane (PDMS) manifold with dimension 13 mm x 13 mm. The manifold has a groove of 5 mm depth to enable the localization of the nanoweb onto the sensor surface.

Results and Discussion

Two sets of dose response experiments were performed to test the capabilities of the nanoweb sensor platform. The first set was focused on detecting human serum-CRP (hs-CRP) when aliquoted in isotonic buffer solution, 0.15 M phosphate buffer saline (PBS). Zero dose corresponding to 0.15 M PBS was injected into the manifold and the measured impedance was considered as the baseline measurement. All the impedance measurements for different dose concentrations of CRP were normalized to this baseline measurement. Starting from the lowest dose within the range, 150 µL of CRP spiked buffer was injected into the manifold, incubated for 15 minutes and impedance measurement was taken. The change in impedance from baseline measurement was calculated and converted into percentage change of impedance from baseline readings.

The PVA sample have characteristic C-H peaks at 670cm^{-1} and 2910cm^{-1} [3], but the 670cm^{-1} peak is absent in PVA –CNT sample. The peak at 924cm^{-1} is O-H out of plane motion of the carboxylic group. At 1329cm^{-1} observes the CH_2 stretch peaks present in both samples. The –OH stretch peak is at 3290cm^{-1} and 1650 [4,5], C-C stretch is a 1420 cm-^{1}[6] and the C-OH stretch peaks are at 840cm^{-1}, 1090 cm^{-1} and 1320 cm^{-1}. A –COOH stretch peak is observed at 1710 cm^{-1} [7] was observed in composite sample but not in pure PVA samples. This difference may indicate an interaction between PVA and CNT matrix.

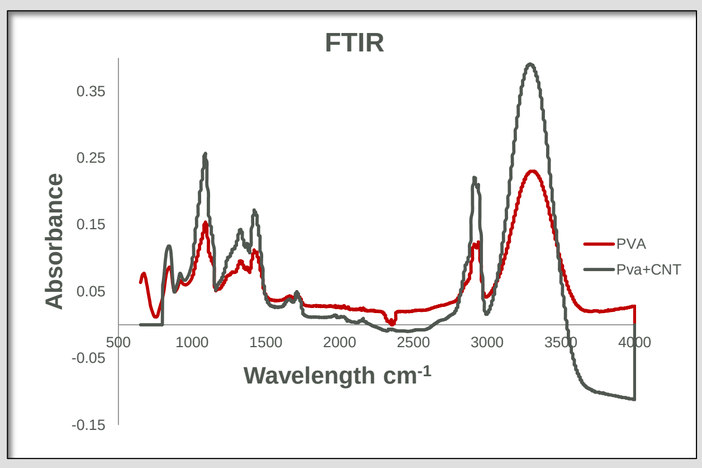


Figure 3. FTIR for PVA, PVA_CNT samples

EIS measurements were taken using the Gamry Reference 600 potentiostat (Figure 4A) with a fixed voltage of 10 mV. A range of frequencies were swept across the working and the reference terminals and the impedance was measured at each of the frequency points. Impedance values at the 100 Hz frequency was extracted using Microsoft Excel spreadsheet macros and were plottted as the change in impedance at that experimental step The percentage change in impedance for PBS varied from 38% to 60% and for human serum it varied from 45% to 70% over the dose range.

The sensor performed as a switch, with a significant change in impedance at the 1 fg/mL dose and impedance comparable to the baseline signals for lower doses. The percentage change in the impedance over the concentration range from 1 fg/mL to 100 ng/mL has been represented. The changes to the impedance have been expressed as a percentage change from the antibody saturation. The limit of detection (LoD) in both the PBS and in the hs cases were 1 fg/mL. The regression trends in both the cases sustained at high values of 0.75 and 0.76, respectively.

The gray line with both dots is an overlay of the CRP protein tested using the gold-standard method, ELISA [8]. Data was plotted in comparison to the Nanomonitor dose range. There is a clear distinction in both the sensitivity and linear dynamic range of detection. The sensitivity with the Nanomonitor was in the femto-gram regime where as the ELISA has low picogram-per-mL detectability. Additionally, ELISA is a lab-bench based analysis versus Nanomonitor is a hand-held device methodology.

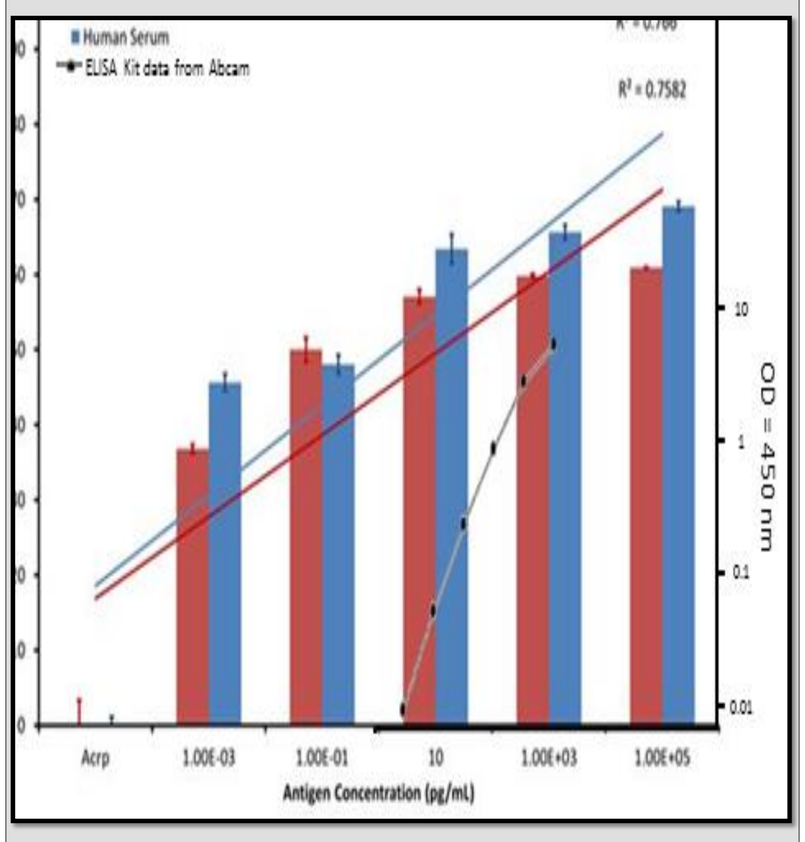


Figure 4. Graphical representation of C-reactive protein dose response in phosphate buffered saline (red bars) and human serum (blue bars) as compared to standard ELISA test (black points)

Conclusions

The CNT embedded ultrafine fibers helps to bind the target proteins in the sensor and hence sensor device achieves amplification of the measured electrochemical impedance signal associated with protein binding. The changes to the impedance were comparable in both the ionic buffer as well as in the human serum. The protein binding is significantly enhanced at the low concentrations which in turn improves the sensitivity. However, future work will be focused on the testing different protein types and biomolecule sensing at femtogram/ml concentration.

References

1) Lloyd-Jones et al. “Heart Disease and Stroke Statistics – 2011,” Circulation, Journal of American Heart Association, doi: 10.1161/CIR.0b013e3182009701
2) P.M. Ridker, Circulation 2003; 107: 363-369
3) Deschenaux Ch, A. Affolter, D. Magni, Ch. Hollenstein, P. Fayet, J. Phys. D: Appl. Phys. 32 (1999) 1876–1886.
4) S. Matveev, M. Portnyagin, C. Ballhaus, R. Brooker, C. A. Geiger, J. Petrol. 46(2005) 603–614
5) X. M. Sui, C. L. Shao, Y.C. Liu, Appl. Phys. Lett. 87 (2005) 113–115
6) D. Okuno, T. Iwase, K. Shinzawa-Itoh, S. Yoshikawa, T. Kitagawa, J. Am. Chem.Soc. 125 (2003) 7209–7218
7) P. Hellwiq, B. Rost, U. Kaiser, C. Ostermeier, H. Michel, W. Mantele, FEBS Lett. 385 (1996) 53–57
8) C-Reactive Protein ELISA Kit, www.abcam.com; product id: ab99995