

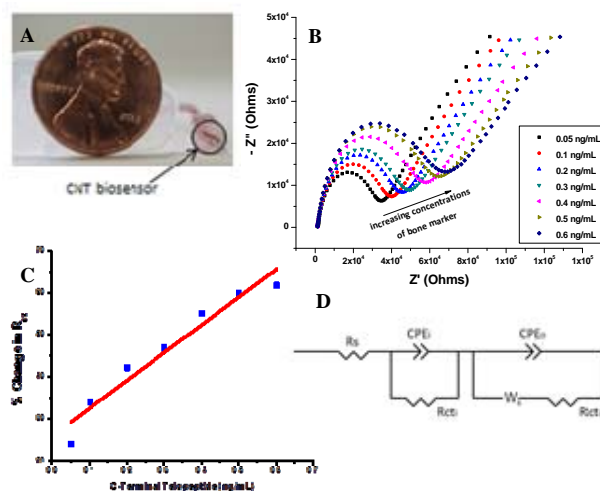
**Development of Carbon Nanotube-based Bio-impedimetric Bone Marker Sensors**  
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**Statement of Purpose:** 34 million Americans suffer from low bone mass due to chronic musculoskeletal conditions, resulting in severe long-term pain and physical disabilities. The current technology used to monitor bone mass and bone density for the detection and evaluation of abnormal bone metabolism requires the use of bulky, costly, and complex equipment with slow response to detect pathological changes in bone via imaging. Further, the necessary regular hospital visits and expenses are also inconvenient for both the patients and the hospitals. The development of impedimetric biosensors for the detection of bone formation markers would not only greatly enhance the speed of detection, but it would also allow for evaluation of pathological changes and treatment methods. Additionally, it would be considerably cheaper and offer a more convenient method for monitoring bone metabolism for both medical facilities and above all for patients. Carbon nanotubes (CNT) are particularly favorable for use in impedimetric sensor development due to their small size, high strength, high electrical and thermal conductivity, and high reactivity due to the extremely high surface area. Therefore, the purpose of this study is to develop an immunosensor for bone marker detection based on CNT array electrodes modified with an electrochemically deposited gold nanoparticle layer.

**Methods:** Multi-walled CNTs approximately 10 mm in length and 200  $\mu\text{m}$  in diameter were utilized as the material interface for the immunosensor. The nanotubes were cast in epoxy resin, mechanically polished at one end to establish electrical contact with silver epoxy paste, recast to insulate the region of contact, and then mechanically polished at the other end to expose the CNT surface. metal nanoparticles were electrochemically deposited onto the exposed surface of the CNT, and the surface was further modified through a sequence of protein adsorption and binding steps to promote c-terminal telopeptide antibody immobilization. The prepared immunosensors (Fig. 1A) were then utilized for detection of a range of human c-terminal telopeptide antigen concentrations (0.05-0.60 ng/mL). Success of surface alterations and antigen detection were tested via cyclic voltammetry or electrochemical impedance spectroscopy conducted in ferro/ferricyanide ( $\text{Fe}(\text{CN})_6^{3-/4-}$ ) in phosphate buffered saline (PBS), using silver/silver chloride as the reference electrode and platinum wire as the counter electrode.

**Results:** The electrochemical deposition of metal on the CNT-based immunosensors enhanced the electrochemical response of the CNT electrodes by reducing charge-transfer resistance in the impedance spectra and increased peak currents in the voltammograms. The CNT-based immunosensors demonstrated progressively increasing charge-transfer resistance upon treatment with progressively increasing concentrations of human c-terminal telopeptide antigen (Fig. 1B). The equivalent circuit model designed to fit the obtained Nyquist

impedance spectra is shown Fig. 1D. Differences in charge-transfer resistance between each antigen treatment were calculated using the data obtained from the equivalent circuit model, and the percent change in charge-transfer resistance was plotted against concentration, demonstrating a nearly linear trend between increasing charge-transfer resistance with increase in antigen concentration (Fig. 1C).



**Figure 1.** (A) Comparison of the size of the CNT-based immunosensor compared to a penny (B) Nyquist interpretation of impedance spectra with successive increase in c-terminal telopeptide antigen treatment (C) Linear fit of percent change in charge-transfer resistance against c-terminal telopeptide antigen concentration (D) Equivalent circuit model utilized to obtain charge-transfer resistance values ( $R_s$  = solution resistance, CPE = constant phase element,  $R_{ct}$  = charge transfer resistance,  $W_s$  = Warburg element, o = outer layer, i = inner layer)

**Conclusions:** While unmodified CNT electrodes yield functional electrodes and acceptable results, metal deposition on CNT electrodes improved the cyclic voltammetry redox peaks and charge transfer-resistance. In addition, the CNT-based immunosensors designed upon metal-coated CNT-electrodes were successful in detecting a lower range of C-terminal telopeptide concentrations in a linear pattern. The range selected for this experiment coincides with the levels of c-terminal telopeptide present in the human body, therefore demonstrating that with further experimentation and development, the presented CNT-immunosensor has the potential to be utilized for human c-terminal telopeptide antigen detection as a method for monitoring bone formation and loss. In addition, the footprint of the sensor is such that it can be universally utilized for detection of various antigens simply by changing the immobilized antibody. Future experimentation with antigen detection in human-body simulated media, further testing the sensitivity and selectivity of the sensor against other protein/antigen interference, and optimization to enhance the reproducibility of the sensor are necessary to determine the full potential of the CNT-immunosensors.