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Gold-coated carbon nanotube electrode arrays: Immunosensors for impedimetric detection of bone biomarkers

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ABSTRACT

C-terminal telopeptide (cTx), a fragment generated during collagen degradation, is a key biomarker of bone resorption during the bone remodeling process. The presence of varying levels of cTx in the bloodstream can hence be indicative of abnormal bone metabolism. This study focuses on the development of an immunosensor utilizing carbon nanotube (CNT) electrodes coated with gold nanoparticles for the detection of cTx, which could ultimately lead to the development of an inexpensive and rapid point-of-care (POC) tool for bone metabolism detection and prognostics. Electrochemical impedance spectroscopy (EIS) was implemented to monitor and detect the antigen–antibody binding events occurring on the surface of the gold-deposited CNT electrode. Type I cTx was used as the model protein to test the developed sensor. The sensor was accordingly characterized at various stages of development for evaluation of the optimal sensor performance. The biosensor could detect cTx levels as low as 0.05 ng/mL. The feasibility of the sensor for point-of-care (POC) applications was further demonstrated by determining the single frequency showing maximum changes in impedance, which was determined to be 18.75 Hz.

1. Introduction

Millions of individuals suffer from various forms of musculoskeletal disorders such as bone carcinoma or osteoporosis as a result of poor bone health. When these bone diseases are not treated efficiently in a timely fashion, bone health can deteriorate leading to further complications and possible fatality. 44 million Americans currently suffer from low bone density or osteoporosis, and 24% of hip fracture patients older than age 50 suffer fatalities a year after injury (Burge et al., 2007). The annual cost arising from musculoskeletal disorders alone in the United States is approximately $850 billion and the costs associated with osteoporosis related fractures exclusively are projected to reach $25 billion by 2025 (National Osteoporosis Foundation, 2012). Hence, in light of the increasing trend in the geriatric population and the escalating costs of healthcare, it is critical to detect and monitor any changes in bone modeling and remodeling for effective treatment of bone diseases during the developmental stage and preserving ambulatory functions.

Bone health is characterized by the adaptation of bone to external loading through modeling and remodeling metabolic processes via various cellular mechanisms. These processes influence the bone's mechanical structural integrity and strength by modifying the size, shape, and the micro- and macro-architecture of bone (Seeman and Delmas, 2006; Ryouji et al., 2000). During bone metabolism, bone turnover markers are released into the bloodstream and in the urine, thus serving as an indicative marker of the bone modeling and remodeling process. Therefore, changes in the bone metabolism during the bone resorption and bone formation process will be reflected in the bone turnover marker

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levels. Bone turnover markers can be particularly useful in understanding bone physiology, assessing facture risks (particularly wherein bone resorption occurs faster than bone formation), and more importantly, determining the response of bone to treatment (Burgeson, 1998; Okuno et al., 2005; Rosenquist et al., 1998).

During the remodeling process of bone, type I collagen is degraded and small peptide fragments are secreted into the blood stream and urine, including the bone resorption marker C-terminal telopeptide (cTx) (Srivastava et al., 2001). Bone turnover markers such as cTx are being studied as clinical research tools, but are not measured frequently in the same patient due to the variation in the levels from day to day or even across a given day. Moreover, the current method of detection of bone turnover markers is based upon enzyme-linked immunosorbent assay (ELISA) which is time consuming, expensive, and more importantly, involves several steps for incubation, antibody binding, and fluorescence measurement (Ross, 1999; Srivastava et al., 2001). Therefore, it would be more useful and beneficial to develop an electrochemical POC biosensor that can quantitatively determine the bone turnover markers in a timely and inexpensive fashion. This development can be achieved by exploiting electrochemical impedance spectroscopy (EIS) for the detection of bone turnover makers. EIS is a label-free operation that does not require secondary labeling, and the impedance based technique is extremely sensitive to interfacial binding events occurring at the probe surface. This is especially critical when developing sensitive detection platforms that are immune to bulk processes occurring in the sample matrix, thus making an impedimetric electronic biosensor particularly advantageous (Daniels and Pourmand, 2007; Katz and Willner, 2003).

Carbon nanotubes (CNTs) have gained considerable attention amongst the various materials used in the development of biosensors due to their exceptional mechanical, electrical and surface properties (Ahammad et al., 2009; Ajayan, 1999; Saito et al., 2009; Tasis et al., 2006). The facile bottom–up approach to grow them is particularly well suited as the growth conditions may be modified to achieve specific properties to fit and meet the needs for sensor integration (Chakrabarti et al., 2007; Landis et al., 2010; Yun et al., 2006). Studies have also been performed to understand the influence of the nanotube side walls, tips and their size, on their electron transfer kinetics (Gong et. al., 2008; Siddiqui et al., 2010). While understanding these parameters is critical from the standpoint of signal measurement, stable allocation of bioprobes on CNTs using suitable coupling chemistries is a requirement for selective and sensitive recognition of target analytes. Several surface chemistries have been studied in great detail involving the use of side-wall/end-tip functionalization of CNTs for bioconjugation purposes (Gao and Kyratzis, 2008). However, among all the different metals used for surface functionalization, gold is better-known for its superior biocompatibility and ability to allow for stable immobilization of biomolecules either via self-assembled monolayers (SAMs) or direct physisorption (Love et al., 2005) on its reactive surface. Recently, architectures combining gold and CNTs have garnered considerable attention as they allow for the integration of their individual unique properties to develop potentially more versatile systems. It has been shown that owing to its high conductivity, electro-deposition of gold on CNT tips also allow for better control of electrode current density (Yun et al., 2007, Yun et. al., 2008). These studies laid the framework for the present work to explore such CNT-gold based architectures and their functionalization to better detect specific biomarkers involving bone turnover.

Therefore, the objective of this study is to develop an electronic label-free carbon nanotube-based biosensor for c-terminal telopeptide detection using EIS. Extra-long vertically aligned CNT (VACNT) posts were utilized to prepare the conducting electrodes, and were further electrodeposited with gold nanoparticles on their tips. The vertical alignment of CNTs allowed for controlled exposure of the area utilized for electrode/biosensor testing, which in turns controls the electrode current density, thus enhancing reproducibility and precision. The addition of gold to the biosensing system eliminated the need for CNT functionalization for biosensing component attachment due to the biocompatibility and protein immobilization properties of gold. The CNT electrode preparation, gold deposition conditions and the chemistries used for bio-conjugation were accordingly optimized. The immobilization of cTx antibody on the gold modified CNT electrode enabled the development of cTx telopeptide biosensor. In order to demonstrate feasibility of the sensor developed for point-of-care (POC) applications, the frequency most sensitive to changes in the impedance following the antigen–antibody interaction was determined and then used for the detection of the various marker concentrations by measuring the changes in impedance at a single frequency. Furthermore, this single frequency testing of various concentrations of cTx in the presence of protein interference was also demonstrated as described in the current work.

2. Materials and methods

2.1. Materials

Non-conducting Epicure epoxy resin and silicon carbide papers of the desired grit size were purchased from Buehler (Lake Bluff, IL). Silver epoxy paste was purchased from AI Technology, Inc. (Princeton, NJ). Hydrogen tetrachloroaurate was purchased from Sigma Aldrich (St. Louis, MO). Phosphate buffer solution (10 mM PBS) was purchased from the Lonza Group Ltd. (Walkersville, MD). Potassium ferrocyanide trihydrate and potassium ferricyanide was purchased respectively, from Fisher Scientific, Inc. (Fair Lawn, NJ). Neutravidin and bovine serum albumin (BSA) was purchased from Thermo Fisher Scientific (Rockford, IL). Biotinylated C-terminal telopeptide antibody and the human C-terminal telopeptide antigen were obtained from Serum CrossLaps ELISA Kit were purchased from Immunodiagnostic Systems (Scottsdale, AZ). Dulbecco's Eagle Medium containing (DMEM) 4.5 g/L glucose, L-glutamine, and sodium pyruvate was purchased from Cellgro (Corning Life Sciences, Manassas, VA). Millipore Deionized (DI) water with a resistivity of 18.2 MΩ cm was used for all rinsing purposes.

2.2. Fabrication of gold deposited VACNT electrodes

Vertically aligned carbon nanotube posts (VACNTs) were grown on Si-wafers using thermal chemical vapor deposition (CVD) process as described in the earlier reports (Chakrabarti et al., 2007; Landis et al., 2010; Yun et al., 2006). The annealed posts were peeled individually from the silicon wafer, mounted in non-conducting epoxy, and degassed for at least 30 min to remove any bubbles. The individually mounted VACNT posts were then allowed to cure under room temperature conditions for approximately 8 h. Following this, the epoxy mounted posts were polished on one end to 50 μm using silicon carbide polishing paper. Electrical contact of the exposed nanotubes with copper wire was made using a silver epoxy paste. Non-conducting epoxy was again employed to insulate the region of contact between VACNTs and copper wire following which; it was cured for approximately 8 h under room temperature conditions. The epoxy was then polished to 50 nm to expose the other end of the VACNTs. The electrodes were cleaned ultrasonically thrice in absolute ethanol and DI water for 2 min each, in between the polishing steps. The electrodes were then subsequently rinsed in DI water and dried. Gold was electrodeposited using 0.08 M hydrogen tetrachloroaurate