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PRELIMINARY STUDY OF FIBER OPTIC MULTI-CARDIAC-MARKER BIOSENSING SYSTEM FOR RAPID CORONARY HEART DISEASE DIAGNOSIS AND PROGNOSIS

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1. INTRODUCTION

Several cardiac-specific biomarkers have emerged as strong and reliable risk predictors for coronary heart disease (CHD), the leading cause of death with an annual incidence of 6.4% and a mortality of 42% in the US[1]. Myoglobin (MG), although not very specific, is the first marker released after myocardial muscle cells are damaged. B-type natriuretic peptide (BNP), cardiac troponin I (cTnI), and C-reactive protein (CRP) are released later than MG, but they are specific markers for coronary events. BNP is useful for the emergency diagnosis of heart failure[2] and for the prognosis in patients with acute coronary syndromes (ACS)[3]. cTnI has become a standard marker for the detection of acute myocardial infarction (AMI)[4]. CRP is an important prognostic indicator of CHD and ACS[3]. Elevated concentrations of these cardiac markers in serum are associated with recurrent CHD events and higher death rates. Simultaneous quantification of these biomarkers allows clinicians to diagnose CHD quickly and/or to accurately design a patient care strategy[3]. A fast and reliable detection of these proteins will also help medical professionals differentiate diseases among those showing similar symptoms. For example, both AMI and pulmonary embolism cause chest pain[5].

The clinically significant sensing ranges of MG, BNP, cTnI, and CRP are extremely low (pM~nM), and therefore, assay methods for these biomarkers need to be highly sensitive. A frequently used method is enzyme linked immunosorbent assay (ELISA). Although very accurate, it is time-consuming, expensive, and technically complicated. Commercially available test kits for BNP (Biosite; San Diego, CA), cTnI (Roche; Basel, Switzerland), and CRP (Dade Behring; Deerfield, IL) can provide fast, easy, and point-of-care assays. However, they usually provide only qualitative single biomarker information and most of the assay kits are expensive. Therefore, a fiber-optic, multi-

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cardiac-marker biosensing system is currently under development in our research group, to simultaneously quantify all four markers in a rapid, accurate, cost-effective, and user-friendly way. The system performs a fluoro-mediated sandwich immunoassay within the evanescent wave field of optical fiber surface[6]. This technology has been applied for simultaneous quantification of protein C and S (anticoagulants; nM) for rapid (~5 min) deficiency diagnosis[7-10]. In this paper, as an initial effort toward realizing a multi-sensing unit, four individual BNP, cTnI, MG, and CRP sensors were developed and the results of the sensor performance and the level of their specificity are presented.

2. MATERIALS, INSTRUMENTS, AND METHODS

2.1. Materials and Instruments

Purified cTnI, MG, and CRP from human heart and respective murine, monoclonal antibodies were obtained from Fitzgerald (Concord, MA). Human BNP was purchased from Bachem (Torrance, CA) and two different murine, monoclonal anti-human BNP, from Strategic Biosolutions (Newark, DE). Alexa Fluor 647 reactive dye (AF647; the maximum excitation and emission at 650 and 668 nm, respectively) was from Molecular Probes (Eugene, OR) and human serum albumin (HSA), from Sigma (St. Louis, MO). Quartz optical fibers (600 μm core diameter) and Fluorometer, Analyte 2000^{TM} were from the Research International (Monroe, WA).

2.2. Methods

To emulate MG-, BNP-, cTnI-, or CRP-free human plasma, samples were prepared in HSA solution at 103 mg/ml-phosphate buffered saline[9]. The conjugation of AF647 with the respective second monoclonal antibody (AF647-2^{o} Mab) was performed according to manufacturer's instructions. Tapered optical fibers (3-12 cm long) were chemically treated to immobilize the first monoclonal antibodies (1^{o} Mabs), against MG, BNP, cTnI, or CRP, on the surface of respective fibers *via* avidin-biotin bridges[7, 11]. Then, the fibers were inserted into glass chambers (100-200 μl) and the two ends were hot glued to form functional sensor units[7]. Assays with static incubation (no flow) were performed as described by Spiker and Kang[7]. For the assay with convective flow, the sample and the AF647-2^{o} Mab were injected and circulated within an enclosed sensing unit at a pre-determined velocity for a pre-determined incubation period[12].

3. RESULTS AND DISCUSSION

3.1. Development of BNP and cTnI Sensors

The target sensing ranges for BNP and cTnI are 0.1~1 ng/ml (26~260 pM) and 0.7~7 ng/ml (30~300 pM), respectively. The initial sensing study was performed with a 12 cm sensor and 10 minutes each for the sample and the AF647-2^{o} Mabs incubation. With static incubation (i.e. no flow during the incubation), the signal intensity of the BNP or cTnI sensor was very low (data not shown). Our previous study results demonstrated that convective flow can improve the sensor performance significantly by increasing the analyte mass transport to the sensor surface[8, 13]. Therefore, the effect of the convective