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Fiber-optic Immuno-biosensor for Rapid and Accurate Detection of Nerve Growth Factor in Human Blood

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Abstract— **An accurate and rapid assay of cardiac nerve growth factor (NGF) levels in blood can provide physicians with critical information regarding myocardial injury and neural remodeling in cardiac tissues to identify patients at risk of impending heart attack, thereby enabling them to receive appropriate lifesaving treatment more quickly. Currently used assay methods, such as enzyme-linked immunosorbent assay (ELISA), are usually time-consuming (hours to days), expensive and technically complicated. In this paper, we described the development and clinical study of a rapid and sensitive method for detection and quantification of NGF in human blood plasma. This method utilizes a fiber-optic, immuno-biosensing system which performs a fluorophore-mediated sandwich immunoassay on the surface of an optical fiber. Physiological concentrations of NGF could be quantified in both buffer and human blood plasma samples within 5 minutes. The NGF concentrations determined by the fiber-optic sensor were comparable to those by the gold standard, ELISA. Preliminary study of NGF assay in cardiac patient plasma samples showed a great potential of the fiber-optic sensor as a rapid diagnostic and prognostic tool in clinical applications.**

I. INTRODUCTION

Cardiac nerve sprouting and sympathetic hyperinnervation were demonstrated to play important roles in arrhythmogenesis and sudden cardiac death after myocardial infarction (MI) [1-4]. It was reported that a rapid and persistent up-regulation of nerve growth factor (NGF) expression, along with growth associated protein 43 (GAP43), at the infracted site underlies the mechanisms of cardiac nerve sprouting after MI [5]. Therefore, NGF levels in blood can be used as an indicative of cardiac nerve sprouting. Moreover, monitoring of NGF profile in cardiac patients is very valuable for diagnosis and prognosis of potential arrhythmia and cardiac sudden death which accounts for 300,000 to 400,000 deaths annually in the US [6-7].

NGF is a neurotrophin that supports the survival and differentiation of sympathetic neurons and enhances target innervation [8-9]. NGF also regulates the synthesis of neurofilament and tubulin proteins, modulates synaptic transmission between sympathetic neurons and cardiac myocytes, and increases the half-life GAP43 [10]. The physiological NGF concentration in normal subjects is in the order of 10 ng/ml. Peripheral nerve injury results in increased local NGF expression with the concentration as high as 200 ng/ml. Therefore, the target sensing range for the NGF sensor development was determined to be 1~200 ng/ml.

Due to the extremely low sensing levels, the assay method needs to be highly sensitive. In addition, the presence of other structurally similar biomolecules in blood samples requires a high specific assay. Many of the tests currently used for determination of NGF involves enzyme-linked immunosorbent assay (ELISA). Although very accurate, it is time-consuming (hours to days), expensive, and technically complicated. Most of the patient samples have to be sent to the hospital central labs for NGF analysis. The need for an accurate, rapid, and cost-effective disease diagnosis and prognosis has been the motivation for developing a fluorophore-mediated, fiber-optic immuno-biosensing system. Compared to the conventional analytical methods such as DNA analysis, polymerase chain reaction, high performance liquid chromatography, and ELISA, the immuno-optical biosensor has the advantages of rapid response time, user-friendliness, and cost-effectiveness [11]. By performing a fluorophore mediated sandwich immunoassay within the evanescent wave field on the surface of an optical fiber [12], this technology has been a successful story for rapid clinical diagnostics [13-16]. A simultaneous assay of four cardiac-specific biomarkers in blood plasma can be completed within 10 minutes to provide physicians with critical information for rapid diagnosis and prognosis of cardiovascular diseases [17]. Here we describe the development of the fiber-optic immuno-biosensing system for the quantification of NGF concentrations in blood plasma of clinical samples. This assay is rapid (~ 5 minutes), user-friendly, and is comparable in sensitivity and accuracy to standard ELISA methods.

II. METHODOLOGY

A. Materials

Human nerve growth factor (NGF) and pooled control human plasma were purchased from Sigma (St. Louis, MO) and two different murine, monoclonal IgG against human NGF, from Exalpha Biologicals (Watertown, MA). ELISA

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kits for NGF assay were from Promega (Madison, WI). Alexa Fluor 647 reactive dye (AF647; the maximum excitation and emission at 650 and 668 nm, respectively) for conjugation with the second antibody (AF647-2° Mab) was obtained from Molecular Probes (Eugene, OR). Plastic waveguides (600 μm core diameter, 3 cm long) were purchased from Research International, Inc. (Monroe, WA).

B. Methods

Pooled control plasmas (human) were reconstituted and fast frozen and stored at -20˚C, according to the manufacturer's instructions. Immediately prior to use, aliquots were thawed at room temperature and centrifuged at 2000 × g for 20 minutes to remove cryoprecipitate. Known amounts of NGF in the target sensing range (1-200 ng/ml) were spiked into the pooled control plasmas to generate the standard curve for NGF sensing in physiological plasma samples.

The development of the fiber-optic NGF sensor was based on the sensing mechanism previously described for the anticoagulants and cardiac markers sensors [18-21]. Instead of quartz optic fiber, plastic waveguide was used as the sensing platform because it was more cost-effective and easier for the sensor preparation. Briefly, the NGF sensor preparation was started with coating the first anti-NGF on the plastic waveguide by incubation in the antibody solution (100 μg/ml) at 4˚C overnight. The coated antibody was used to specifically capture the target analyte, NGF molecules, in samples for detection. The antibody-coated waveguides were then assembled into the sample incubation cuvette and connected to the fluorometer, Analyte 2000™ (Research International; Monroe, WA) through waveguide adapters, following the manufacturer's instructions. The fiber-optic sensing system performs a fluorophore mediated sandwich immunoassay within the evanescent wave field on the surface of an optical fiber [12]. During an assay, the procedures were performed as described by Spiker, et al. and Spiker and Kang, unless otherwise specified [18,19]. The assay procedures are briefly described as follows:

- ♦ Step 1. Sample injection and incubation;
- ♦ Step 2. Washing the sensor to remove excessive molecules;
- ♦ Step 3. AF647-2° Mab-NGF injection and incubation;
- ♦ Step 4. Washing the sensor to remove excessive AF647-2° Mab;
- ♦ Step 5. Regenerating the sensor using the regeneration buffer for a next assay cycle.

At the end of steps 2 and 4, readings were taken using the Analyte 2000™, where optical signal is converted to photocurrent (pA). The difference in the signal intensity between these two steps (ΔpA) is a direct measure of the fluorescence produced by the 1° Mab/NGF/AF647-2° Mab complex on the sensor surface, which is correlated with the target NGF concentration in the sample. Currently, the total sample volume for an assay is approximately 400 μl and the assay time was approximately 5 minutes.

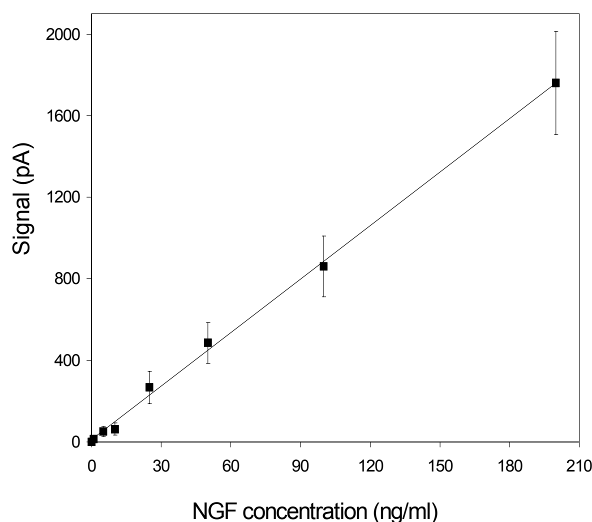

Fig. 1. Quantification of NGF sensing in buffer (experimental conditions: NGF between 1 and 200 ng/ml spiked in PBS buffers; 1 and 2 min incubation for the sample and second antibody).

III. Results and Discussion

It should be noted that each data point is presented with the mean value and standard deviation of multiple measurements (> 3) on at least three different NGF sensors.

A. NGF Quantification in Buffer

A feasibility study was performed with NGF at various concentrations in the target sensing range of 1-200ng/ml spiked in PBS buffer. Fig. 1 shows the signal intensity as a function of the NGF concentration. The incubation times for the sample and the AF647-2° Mab were 1 and 2 minutes, respectively. The signal intensity was in linear relationship with the NGF concentrations in the sensing range (r^2=0.9981), at an average signal-to-noise ratio (S/N) of 6.

B. Standard Curve of NGF Quantification in Human Blood Plasma

To evaluate NGF concentrations in cardiac patients, a standard curve was constructed by spiking known amounts of NGF in pooled control plasmas. This standard curve, against which the signal intensities from patient sample assays are compared, will be used to extrapolate the unknown NGF concentrations in patients. As shown in Fig. 2, the signal intensity is linear with the NGF concentrations in the target sensing range (r^2=0.9983). This curve does not intersect the ordinate at the origin, indicating a presence of the endogenous NGF in low concentrations in normal plasma samples. Compared to NGF in buffers, the signal intensity was decreased by approximately 70%. One of the main causes is the reduced analyte mass transport rate to the

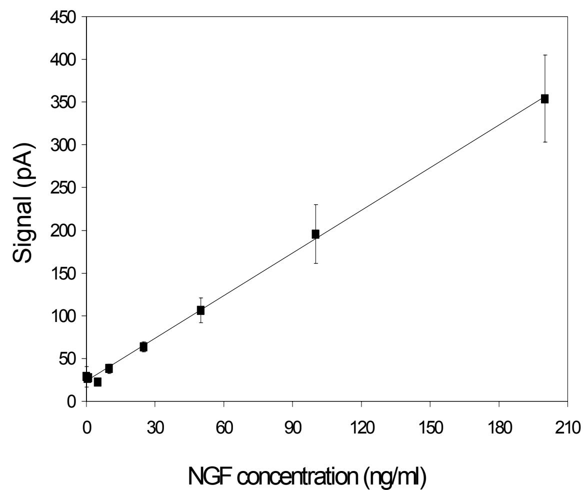

Fig. 2. Standard curve of NGF sensing in blood plasma (experimental conditions: NGF between 1 and 200 ng/ml spiked in pooled control plasmas ; 1 and 2 min incubation for the sample and second antibody).

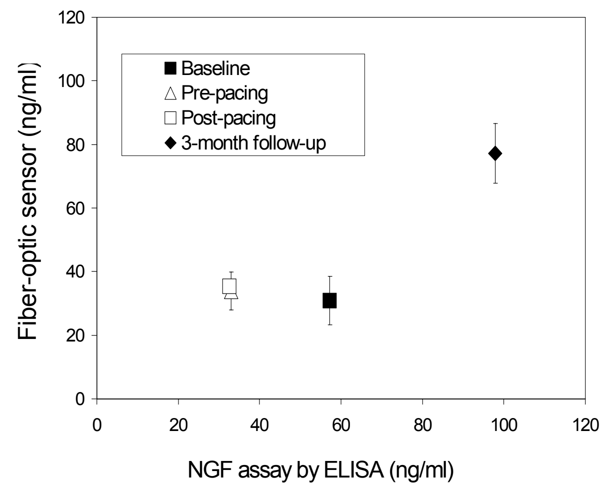

Fig. 3. Correlation between ELISA and fiber-optic sensor for patient plasma samples.

sensor surface for reaction with the capture antibody, due to the high viscosity of blood plasma. Application of convection during the incubation times can enhance the sensing performance significantly by improving the mass transport [16,20,21]. Despite the signal reduction, the NGF sensor was capable of clearly differentiating the NGF concentration in the sensing range (S/N: 6). This result shows that the NGF sensor can accurately assess the NGF concentrations in human blood samples within 5 minutes.

C. Preliminary Study of the NGF Sensor in Clinical Application

To assess the accuracy and reliability of the NGF sensor in clinical environments, a preliminary study of NGF assay in patient blood plasma was performed and results were compared with the gold standard, ELISA, for data validation.

Plasma samples were obtained from a patient with congestive heart failure who went through biventricular pacing. Samples were collected at baseline and before and after pacing from coronary sinus. A follow-up study was also performed to collect blood samples after a 3-month treatment. Each sample was assayed by both ELISA and the NGF sensor. The assay time was 5 minutes for one sample using the fiber-optic sensor and 3 days by ELISA (96 wells), following the manufacturer's protocol. At least three measurements for each sample were performed using the fiber-optic sensor and the NGF concentrations were interpolated by fitting the signal intensities to the equation determined for the standard curve in pooled control plasmas (Fig. 2). NGF concentrations assayed from the fiber-optic sensor (ordinate) showed a high degree of correlation with those from the ELISA (abscissa; Fig. 3). For both assay methods, the NGF levels in plasma samples were similar at baseline and after pacing. A significant increase in NGF concentration was recorded after 3 months, by both fiber-optic sensor and ELISA. This result shows that the NGF assay by fiber-optic sensor is comparable to ELISA, yet more rapid and user-friendly. Although still in early stage of its development as a diagnostic tool, the fiber optic sensor has been demonstrated to be very promising as an alternative assay method to ELISA for rapid and reliable risk stratification among cardiac patients by NGF quantification in blood. Additional studies are under way to more accurately evaluate the potential value of this technology in clinical environments.

IV. Conclusion

A fiber-optic, immuno-biosensor was developed to rapidly (~ 5 min) detect and quantify the NGF concentration in blood plasma samples for clinical applications, e.g. identifying patients at risk of impending heart attack. The sensor was capable of accurately assessing the NGF concentrations in the clinically significant sensing rage. The assay by the fiber-optic sensor was highly correlated with that from ELISA, demonstrating a promising potential use in clinical settings as a rapid diagnostic tool, especially in emergency room.

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