MINI SENSING CHIP FOR POINT-OF-CARE ACUTE MYOCARDIAL INFARCTION DIAGNOSIS UTILIZING MICRO-ELECTRO-MECHANICAL SYSTEM AND NANO-TECHNOLOGY

Jianting Wang [a], Bin Hong[a], Junhai Kai[b], Jungyoup Han[b], Zhiwei Zou[b], Chong H. Ahn[b], and Kyung A. Kang[a]

Abstract: A rapid and accurate diagnosis of acute myocardial infarction (AMI) is crucial for saving lives. For this purpose, we have been developing a rapid, automatic, point-of-care, biosensing system for simultaneous four cardiac marker quantification. This system performs a fluorophore mediated immuno-sensing on optical fibers. To improve the sensitivity of the sensor, novel nanoparticle reagents enhancing fluorescence were implemented. Micro-electro-mechanical system (MEMS) technology was applied in the sensing chip development and automatic sensing operation was implemented to ensure a reliable and user-friendly assay. The resulting system is a point-of-care, automatic four cardiac marker sensing system with a 2 x 2.5cm sensing chip. An assay requires a 200 μL plasma sample and 15-minute assay time.

1. INTRODUCTION

Acute myocardial infarction (AMI) is the world's leading cause of morbidity and mortality. According to the latest data by the American Heart Association (AHA),[1] in 2004, 7.9 million cases of myocardial infarction were reported in the US, including 865,000 new and recurrent cases and 157,600 deaths. AMI is also a disease with a high rate of misdiagnosis due to the low sensitivity of the current diagnostic tools used in the emergency room.[2,3] A better method for AMI diagnosis is to detect the elevation of cardiac markers in blood plasma.[4] Currently, the cardiac markers are usually quantified

[a] Jianting Wang, Bin Hong, and Kyung A. Kang, Department of Chemical Engineering, University of Louisville, Louisville, KY 40292. [b] Junhai Kai, Jungyoup Han, Zhiwei Zou, and Chong H. Ahn, Department of Electrical and Computer Engineering and Computer Science, University of Cincinnati, Cincinnati, OH 45221.

P. Liss et al. (eds.), *Oxygen Transport to Tissue XXX*, DOI 10.1007/978-0-387-85998-9_16,

in a central laboratory, and it often takes hours from when it is ordered till the results are received. Therefore, a rapid and accurate point-of-care (POC) system is urgently needed.

Our fiber-optic, fluorophore-mediated, immuno-sensing system has demonstrated accurate, sensitive, rapid, and reliable simultaneous quantification of various biomarkers.[5,6] For the cardiac marker sensing, we have selected the following four markers: B-type natriuretic peptide (BNP) and C-reactive protein (CRP) are crucial markers for the diagnosis of congestive heart failure and acute coronary syndromes.[7,8] Myoglobin (MG) and cardiac troponin I (cTnI) are important markers for early diagnosis of a heart attack.[9] Based on the assessment of the clinical results, we have previously determined the clinically important sensing range of the marker[10,11,12] to be 26~260 pM for BNP; 30~300 pM for cTnI; 4~40 nM for MG and 5.6~56 nM for CRP. Hong et al. have successfully quantified the above four cardiac markers with four 3 cm sensors[10].

Our effort has been focused on both the sensitivity enhancement and sensor size reduction to develop a POC, AMI diagnostic system. Reduction of sensor size can usually reduce the sample and reagents volume (i.e., assay cost reduction) and improve portability. Smaller sensor size may, however, lower the sensitivity due to the less sensing surface. For BNP and cTnI, their concentrations in plasma at an early disease stage are only tens of pico molar level, requiring extremely high sensitivity. Since our sensors are mediated by fluorophore, enhancing the fluorescence can improve the sensitivity. Some nanometal particles have high-density surface plasmon polariton fields. Lone pair electrons of a fluorophore, often participating in the fluorescence self-quenching, can be transferred to the plasmon field, resulting in artificial fluorescence enhancement[13,14]. Some biocompatible solvents were also found to enhance the fluorescence significantly, possibly via effective dipole coupling between the fluorophore and the solvent molecule, resulting in an increase in the ennergy gap between the excited and the ground states[13,14]. Our research group has implemented nanomatal particles and sensor compatible organic solvents to the sensitivity[13,14]. Among nanometal particles and solvents, 5 nm-nanogold particles (5nm-NGP) dispersed in 1-butanol was found to be a very effective enhancer. MEMS technique was also incorporated for our micro sensing chip development and for a reliable and automated sensor operation.

This paper reports the results of the further reduction in the sensing chip size and its performance in quantifying four cardiac markers in plasma samples.

2. MATERIALS, INSTRUMENTS AND METHODS

Sensor preparation and assay protocol

For the cardiac markers and their respective monoclonal antibodies, BNP was purchased from Bachem (Torrance, CA) and monoclonal IgG against human BNP was from Strategic Biosolutions (Newark, DE). cTnI, MG, and CRP, and their antibodies were obtained from Fitzgerald Industries (Concord, MA). Plasma samples with cardiac markers were prepared by adding a known amount of cardiac markers to the human plasma.[10]

Four cardiac marker biosensors were constructed, following the protocol established by Tang et al.[12] The quartz fiber (Research International, Inc., Monroe, WA) was immobilized with streptavidin (Sigma/Aldrich, St. Louis, MO) and the monoclonal antibody (1^{o}Mab) against the respective marker is conjugated with biotin

(Sigma/Aldrich) and immobilized on the surface of fiber *via* streptavidin-biotin bond and the sensor is encased in a micro-sensing chamber. The fluorophore Alexa Fluor® 647 (AF647; max. excitation/emission wavelengths, 649/666 nm) was from Invitrogen (Carlsbad, CA). The second monoclonal antibody (2^{o}Mab) was conjugated with AF647 following the manufacture's instruction.

The cardiac marker assay proceeded as follows: a sample was introduced into the sensing chamber and incubated for 3 min at a rate of 1.2 cm/sec. Once the target marker binds specifically to the 1^{o}Mab on the sensor surface, the fiber was washed with phosphate buffered saline with 0.1% Tween 20 (PBST, pH 7.4) for 1 minute to remove the unbound molecules in the sample. Then the fluorescence was measured (baseline). The fluorometer (Analyte 2000™, Research International, Monroe, WA) has four sensing ports. AF647 tagged 2^{o}Mab, at a concentration of 10 μg/ml, was then applied to the chamber and incubated for 4 minutes to react with the bound analyte. The sensor was washed with PBST for 1 minute and another fluorescence reading was taken. The signal difference between this signal and the baseline subtracted by the negative control is the signal intensity of the sample.

For the fluorescence enhancing, 5nm-NGP coated with tannic acid was purchased from Ted Pella (Redding, CA). 1-butanol was purchased from Sigma/Aldrich. For the sensing with a nanogold particle reagent (NGPR), for the baseline, the NGPR was applied before the sample incubation. For the signal of the sample, it was applied after the second antibody was reacted with the analyte on the sensor surface.

In this paper, the enhancement is defined as the fluorescence signal increase due to the NGPR divided by the fluorescence from the sensor without NGPR.

Microfluidic system and automated sensing operation

The sensing chip and microchannel network of the sensing unit were microfabricated as described by Sohn et al.[15] and Hong et al[16]. The system includes a polycarbonate mother board with imbedded network of the microchannels; a four-channel, plastic sensing module; a micro-solenoid pump (12 v, 50 μL per stroke, 2 W); seven micro-solenoid valves (12 v, 280 mW, Lee Co.; Westbrook, CT); a data acquisition (DAQ) card (USB-6008, 8 inputs, 12 bits, 10000 samples/s, multifunctional I/O, National Instruments; Austin, TX); and a drive circuit with a power plug, a power switch, and a power LED.

3. RESULTS AND DISCUSSION

Reduction of BNP sensor size

As previously stated, here, we have attempted to reduce the sensor size from 3 to 1.5 cm. BNP sensing was tested first since BNP has the lowest sensing range among the four cardiac markers (see Introduction).

Figure 1 shows the performance of the BNP sensors at (a) 3 and (b) 1.5 cm, with and without NGPR. In terms of the signal intensity enhancement by NGPR, the signals were enhanced by 4 and 3 times for 3 cm and 1.5 cm sensor, respectively. For 1.5 cm the signal without NGPR was very low in the entire sensing range. Although the signal for the 1.5 cm sensor with NGPR was approximately only 40% that of the 3 cm sensor, the

correlation coefficient was 0.96 (0.95 for 3 cm sensor), and the signal to noise ratio (S/N) was 4.1 (3.8 for 3 cm sensor), showing that the performance of the 1.5 cm sensor was still satisfactory, but with less sample and reagent.

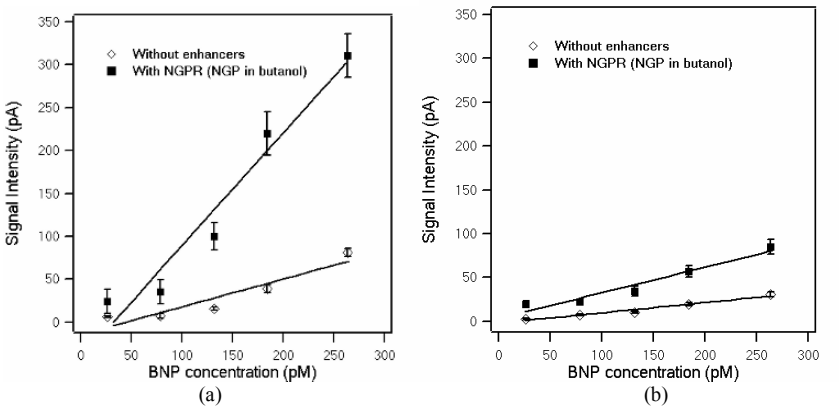

Figure 1. Performance of BNP sensor at sensor sizes of (a) 3 cm and (b) 1.5 cm with and without NGPR.

Four cardiac marker sensing by the mini-sensor

Since the sensing performance of the 1.5 cm BNP sensor was satisfactory, 1.5 cm was then tested for all four cardiac markers in a micro sensing chip. The size of the chip for four cardiac marker sensing was reduced from 4 x 4 cm (for 3 cm sensors) to 2 x 2.5 cm (for 1.5 cm sensors). A schematic diagram of the chip is shown in figure 2. As previously shown in Hong et al.[10], on the inner surface of the microchannel, bumps/baffles were microfabricated to create local turbulence, facilitating the analyte transport to the sensor surface better. The mini sensing chip requires a sample size of only 200 µl, and therefore, only 0.5 ml of a blood sample is needed for an assay.

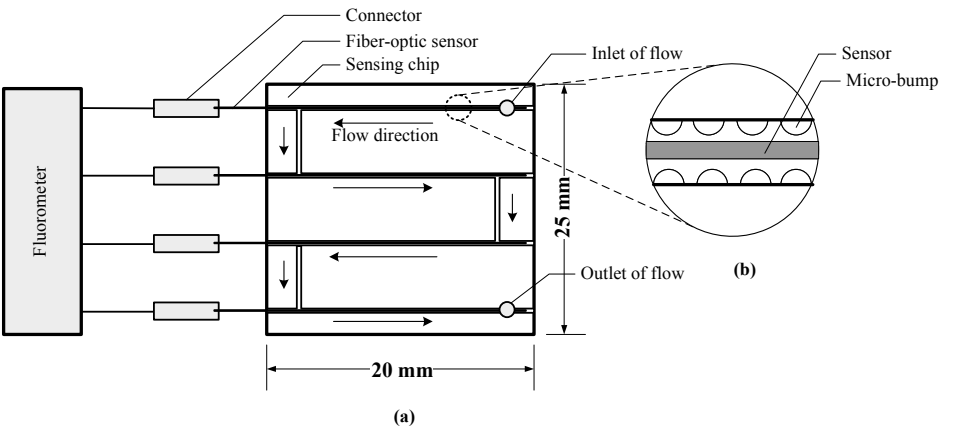

Figure 2. (a) Schematic diagram of mini-sensing chip and (b) Enlarged schematic diagram of a sensing chamber

For this study, the sample was the mixture of four cardiac markers in their sensing ranges in the human plasma (Fig. 3). For BNP and cTnI, the signal range without using NGPR was only 3~30 pA. With NGPR, the signal was enhanced by 3 and 4 times. The S/N ratios were 4.1 and 4.5, and the correlation coefficients were 0.96 and 0.97, respectively. For MG and CRP, the signal ranges without enhancer were as high as 4~105 pA and 90~400 pA, respectively. Nevertheless, since the four sensors need to be done simultaneously with a sample, NGPR was applied also to these two sensors. The signals were enhanced by 5 and 2 times and S/N were 5.2 and 6.7, respectively.

All four 1.5 cm sensors showed linear relationships between the marker concentration and the signal intensity, with correlation coefficients above 0.95.

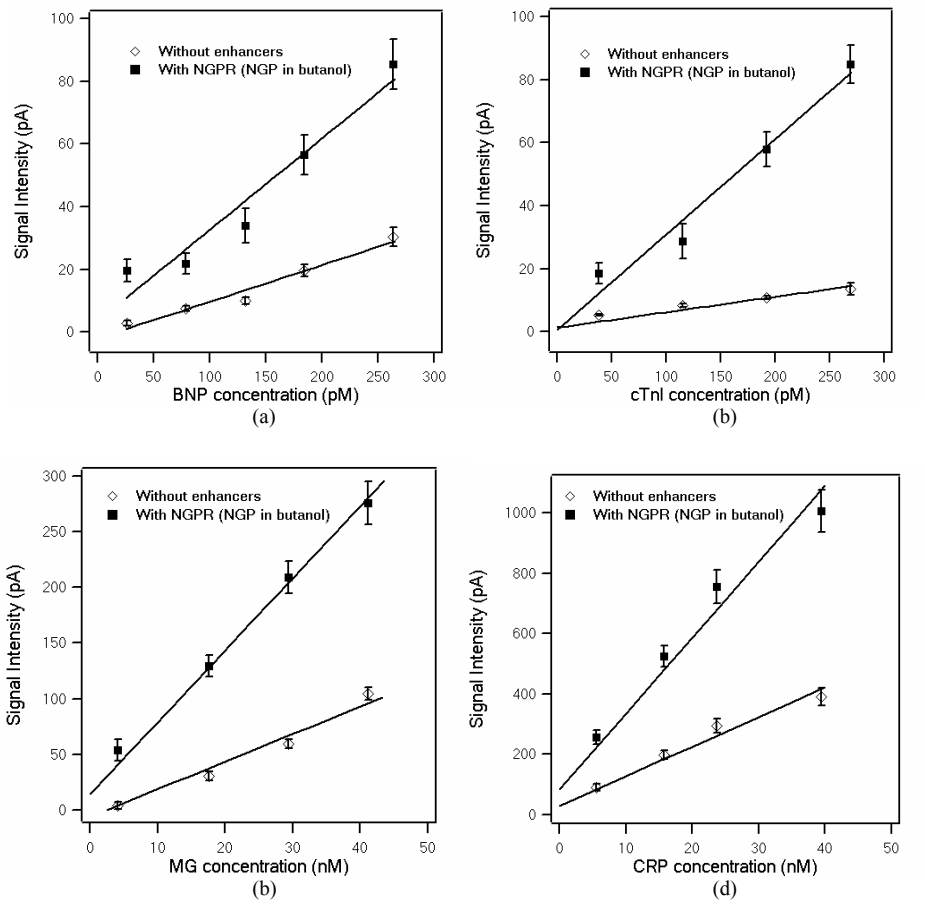

Figure 3. Sensing performance of 1.5 cm (a) BNP, (b) cTnI, (c) MG, and (d) CRP sensors with and without NGPR

Futuristic portable all-in-one device

In the near future, our current sensing system[16] of fluorometer, microfluidic flow control unit, display monitor and input keyboard may be put into a highly portable, all-in-one device at a size of 30 x 20 x 15 cm (Fig. 4). With disposable sensing chip and sample and reagent containers, a four cardiac marker sensing may be performed in the emergency room or even in an emergency-medical-service vehicle within 15 min.

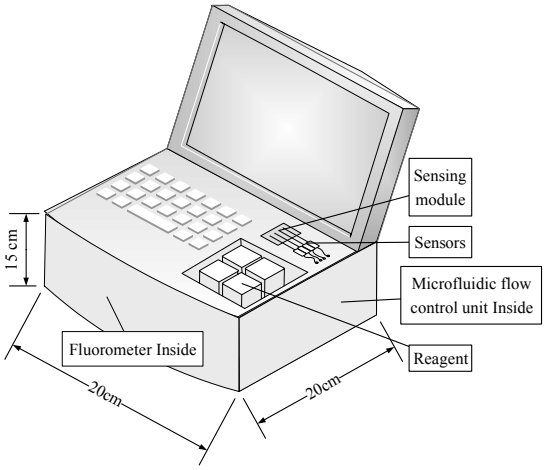

Figure 4. Schematic design diagram of all-in-one four cardiac marker sensing device.

4. CONCLUSIONS

Our MEMS based sensing device with mini-sensing chips (2 x 2.5 cm) and novel nano metal reagent can accurately quantify four cardiac markers simultaneously at clinically significant concentration range within 15 min, requiring a plasma sample volume of 200 µl. The entire sensing system may be converted to be a highly portable, all-in-one, POC sensing device.

5. ACKNOWLEDGEMENTS

The authors acknowledge the financial support from Kentucky Science and Engineering Foundation (KSEF-148-502-03-55) for fluorescence enhancement studies and National Science Foundation (BES-0330075) for cardiac marker biosensing.

6. REFERENCES

1. American Heart Association (AHA), Heart disease and stroke statistics, 2007 update

2. A. Wu, F. S. Apple, W. B. Gibler, R. L. Jesse, M. M. Warshaw, R. Valdes. National Academy of Clinical Biochemistry Standards of Laboratory Practice: recommendations for the use of cardiac markers in coronary artery diseases. *Clin Chem* **45**:1104–21 (1999).
3. J. H. Pope, T. P. Aufderheide, R. Ruthazer, Missed diagnoses of acute cardiac ischemia in the emergency department. *N Engl J Med* **342**: 1163–70 (2000).
4. R. Bernard, E. Corday, H. Eliasch, Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature. *Circulation* **59**:607–9 (1979).
5. J. O. Spiker, K. A. Kang, W. Drohan, and D. F. Bruley, Protein C detection via fluorophore mediated immuno-optical biosensor. *Advances in Experimental Medicine and Biology* **428**, 621-627. (1999)
6. L. Tang and K. A. Kang. Preliminary study of simultaneous multi-anticoagulant deficiency diagnosis by a fiber optic multi-analyte biosensor. Proceedings of the 31st IOSTT Annual Meeting, Aug. 16-20, 2003, Rochester, NY.
7. A. S. Maisel, P. Krishnaswamy, H. C. Herrmann, and P. A. McCullough, Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure, *New Engl. J. Med.* **347**, 161-167 (2002).
8. M. S. Sabatine , D. A. Morrow, C. P. Cannon, and E. Braunwald, Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: Simultaneous assessment of troponin I, c-reactive protein, and b-type natriuretic peptide, *Circ.* **105**, 1760-1763 (2002).
9. F. S. Apple, R. H. Christenson, R. Valdes, A. J. Andriak, K. Mascotti, and A. H.B. Wu, Simultaneous rapid measurement of whole blood myoglobin, creatine kinase MB, and cardiac troponin I by the triage cardiac panel for detection of myocardial infarction, *Clin. Chem*. **45**(2), 199-205 (1999).
10. L. Tang, Multi-analyte, fiber-optic immuno-biosensing system for rapid disease diagnosis: model systems for anticoagulants and cardiac markers. Dissertation. Chemical Engineering, University of Louisville, Louisville, KY. (2005)
11. L. Tang and K. A. Kang, Preliminary study of simultaneous multi-anticoagulant deficiency diagnosis by a fiber optic multi-analyte biosensor. *Advances in Experimental Medicine and Biology* **566**:303-309 (2006).
12. L. Tang, Y. J. Ren, B. Hong, and K. A. Kang, A fluorophore-mediated, fiber-optic, multi-analyte, immuno-sensing system for rapid diagnosis and prognosis of cardiovascular diseases, *J. Biomed. Optics* **11**, 021011 (2006).
13. B. Hong and K. A. Kang, Biocompatible, nanogold-particle fluorescence enhancer for fluorophore mediated, optical immunosensor, *Biosens. Bioelectron*. **21**(7), 1333-1338 (2006).
14. K. A. Kang and B. Hong, Biocompatible nano-metal particle fluorescence enhancers, *Crit. Rev. Eukar. Gene Expres.* **16**(1), 45-60 (2006).
15. Y. Sohn, J. H. Kai, C. H. Ahn, Protein array patterning on Cyclic Olefin Copolymer (COC) for disposable protein chip, *Sensor Lett.* **2**, 171-174 (2005).
16. B. Hong, J. H. Kai, Y. J. Ren, J. Y. Han, Z. W. Zou, C. H. Ahn, and K. A. Kang, Higly sensitive, rapid, reliable and automatic cardiovascular disease diagnosis with nanoparticle fluorescence enhancer and MEMS, Proceeding of the 34rd ISOTT annual meeting, August 12-17, Louisville, KY, USA, (in press)