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PAPER

Integrative volumetric bar-chart chip for rapid and quantitative point-of-care detection of myocardial infarction biomarkers

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Here we developed an integrated volumetric bar-chart chip (IV-Chip) technology by integration of our previous V-Chip with a fluid handling design to generate an instrument-free POC device and greatly reduce the detection time and effort. The IV-Chip test requires only 1 µL of serum separated from finger-prick blood. The serum sample and ELISA reagents are directly loaded into the device using a pipette, and a shift of the two layers of the device generates homogeneous liquid segments in the microfluidic channel. Under vacuum pressure generated by a regular syringe, the segments flow into the ELISA wells in sequence and a sandwich ELISA reaction takes place. As a result of the automated washing and reacting strategy, the IV-Chip allows rapid tests for myocardial infarction biomarkers, and turnaround time is greatly reduced to 15 min. The specificity and accuracy of quantitative multiplex detection of MI biomarkers CK-MB, troponin I and myoglobin, are 87.5 % and 95.8 %, respectively.

Introduction

Point-of-care (POC) diagnostics are performed out of a central laboratory and at the site of patient care, such as a physician's office, emergency room, or home.[1-5] A typical POC assay is affordable, specific, sensitive, portable, rapid, and user-friendly, making it suitable for use in low-resource settings.[3] Among the advantages of POC tests, the greatest is that they provide rapid diagnostic results and facilitate earlier treatment. Over the past decade, POC assays have been explosive development as a result of introduction of new technologies, including nano- and biotechnological approaches.[3-5] However, most of these methods are laborious and time-intensive, and also require cumbersome instruments and highly trained operators. Therefore, much effort is still needed to overcome these challenges.

Microfluidics has been employed in POC diagnostics to meet such challenges.[4, 6] Due to its high integrative capability,[7] microfluidics-based platforms show the potential to revolutionize POC technology in disease diagnostics. In traditional microfluidics-based approaches, sample processing, fluid handling, or signal detection require additional large instruments, which limits application to POC diagnostics.[4, 8] Valve- and equipment-free microfluidic devices would simplify the technology and make it more widely available. Although much work has focused on integrating miniaturized versions of bulky instruments into microfluidic devices, most of these devices remain as laboratory prototypes after decades of development and have limited application potential because they are too costly and complicated to execute.[9-12] At present, quantitative measurement of biomarkers remains an unmet, yet critical need in the development of POC devices for disease assessment. Recently, we developed V-Chip technology as a POC diagnostic tool to overcome this challenge by providing accurate and quantitative results.[13-15] ELISA is the clinical gold standard and is applied in our V-Chip test for detection of disease-related protein biomarkers.[16, 17] However, ELISA requires repeated washing steps and incubation with reagents to produce high signal intensity and low background. Simplifying the procedure and reducing detection time are crucial for the V-Chip and other ELISA-based POC assays.

Sequential delivery of liquid segments containing samples and ELISA reagents has been reported to generate a rapid and simple ELISA reaction.[18-20] This liquid-segment delivery system has been successfully applied in biomarker detection based on optical or electrochemical signals.[18-20] However, the system encounters two major problems. On one hand, the liquid segments are manually produced and stored in plastic tubes, which may require that operators receive additional training to generate segments of reproducible quality. On the other hand, additional instruments are needed to detect optical or electrochemical signals, which will increase device costs. Our V-Chip employs SlipChip technology,[21, 22] that has the potential to produce repeated liquid segments by

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sliding the device. Because the V-Chip allows quantitative detection of biomarkers by the naked eye, integration of the V-Chip with a fluid handling design will generate an instrument-free POC device and greatly reduce the detection time and effort.

Every year, millions of patients seek emergency care because of chest pain or other symptoms related to acute coronary syndrome, of which only 10% are subsequently confirmed to have experienced acute myocardial infarction (MI).[23] POC testing for creatine kinase (CK)-MB, myoglobin, and troponin offers rapid and accurate results and an option for patient evaluation in the emergency department. Although the American Heart Association (AHA) has recommended a turnaround time (from sample collection to results reporting) of 30 minutes for cardiac markers,[23] it is usually difficult to achieve even with the use of pneumatic tube transport and a dedicated stat laboratory. Therefore, POC detection of cardiac markers is being employed in an increasing number of health care centers.

It has been suggested that the ideal POC test for cardiac markers should exhibit the same sensitivity and specificity as conventional laboratory testing.[23, 24] POC multiplex testing shows the potential to meet such a standard by simultaneous detection of several biomarkers. Here, we developed an integrative volumetric bar-chart chip (IV-Chip) device for POC detection of biomarkers of MI. The IV-Chip test requires only 1 µL of serum, which can be separated from finger-prick blood. The serum sample and ELISA reagents are directly loaded into the device using a pipette, and a sliding generates homogeneous liquid segments in the microfluidic channel. Under vacuum pressure generated by a pipette, the segments flow into the ELISA wells in the sequence in which they were loaded and a sandwich ELISA reaction takes place. As a result of the liquid-based delivery strategy, the IV-Chip allows rapid tests for MI biomarkers, and turnaround time can be reduced to 15 min, which meets and exceeds the AHA standard. The specificity and accuracy of quantitative multiplex detection of MI biomarkers CK-MB, myoglobin, and troponin I are 87.5% and 95.8 %, respectively.

Experimental

IV-Chip preparation

All devices were designed as computer graphics using AutoCAD software and then printed out as transparency photomasks by CAD/Art Services (Bandon, OR) with 10-µm resolution. The IV-Chip was produced using standard photolithography and wet etching processes, as previously described.[13, 14] The masks, dimensions of each unit, and assembled IV-Chip are shown in Fig.s S1, S2, and S3, respectively. The standard lift-off method was used to deposit the platinum films in the wells of the bottom plates. The wells of the third lane of the bottom plate were sputter-coated with 2 nm chromium followed by 20 nm platinum/palladium (80:20) using a Cressington Sputter Coater 208 HR (Ted Pella, Redding, CA) (Fig. S4). Access holes were made using a diamond drill of diameter 0.031 inch.

For surface modification, the glass plates were acid-cleaned in piranha solution (H_2SO_4:H_2O_2=7:3) for 1 h and rinsed with Millipore water. After oxygen plasma treatment, each plate was salinized with tridecafluoro-1, 1, 2, 2-tetrahydrooctyl-1-trichlorosilane to form a hydrophobic coating, which can help to seal the two glasses tightly after applying the following oil of FC-70. To assemble the V-Chip, Fluorinert liquid FC-70 served as a lubricant to prevent formation of air pockets during device operation. The reagent or sample channels were treated with piranha solution to make hydrophilic before loading the reagents or samples. After each test, the IV-Chip could be disassembled, cleaned, and reused, which greatly reduced the assay cost.

Preparation and conjugation of platinum nanoparticles (PtNPs)

PtNPs with an average diameter of 30 nm were prepared as previously described.[154] PtNPs were characterized by scanning electron microscopy (SEM) and dynamic light scattering. For the preparation of antibody-conjugated PtNPs, 10 µL 1 mg mL^{-1} monoclonal antibody were mixed with 500 µL 1 mg mL^{-1} PtNPs in PBS, pH 6.5, and maintained at 4°C overnight. BSA was added to a final concentration of 1% to block the PtNP surface, and unconjugated antibody was removed by dialysis. Finally, the antibody-conjugated PtNPs were suspended in 500 µL PBS, pH 7.2, containing 0.1% Triton X-100, 5% sucrose, and 1% BSA.

Immobilizations of capture antibodies in ELISA wells

ELISA wells were cleaned by applying drops of piranha solution and incubating for 1 h, followed by rinsing with Millipore water and drying with nitrogen gas. Then 1% (3-glycidoxypropyl) trimethoxysilane in toluene was added to each well using a pipette. The solution was left in each well for 30 min, and then rinsed with fresh toluene to remove extra 3-GPS molecules. Glass plates were then dried using dry nitrogen gas and baked at 120°C for 30 min. Epoxy groups were covalently attached to the surface of each well, and capture antibodies were covalently immobilized in the ELISA wells by reaction with the epoxy groups. Capture antibodies were carefully added to each well and plates were incubated at 4°C overnight. Wells were washed with 1% BSA several times to prevent nonspecific binding. The wet etching process produces a rough surface, which increases the efficiency of antibody coating.

Assembly and operation of IV-Chip

The IV-Chip was assembled as previously described[13]. Five microliters of Fluorinert liquid FC-70 (Hampton Research) were added to the top plate of the device positioned with the patterns facing up, and a blank glass slide was pressed down on the plate to spread the oil. After the oil was completely spread, the blank slide was removed and the device plate was spun at 2000 rpm for 20 s to remove extra FC-70. Then the device, consisting of the top and bottom plates, was assembled, and an ultrathin FC-70 oil layer was formed between the two glass plates. The oil layer serves as a lubricant and renders the device airtight.

The operation of IV-Chip is divided into five steps. In the first step, the chip is in the “Loading S” position. In this position, a pipette tip containing the sample or reagent is inserted into the inlet holes of the fluidic path in each loading channel, and the solution is loaded into the channels by manually pushing the pipette. In the second step, the top plate of the IV-Chip is slid in a horizontal direction against the bottom plate to produce liquid segments, connecting the channel containing liquid segments to the zigzag ELISA channel. In this position, both ends of the fluidic path

are in contact with air. In the third step, red ink and H_2O_2 are loaded into the IV-Chip. In the fourth step, vacuum pressure is applied using a pipette to gradually draw the fluid segments into the ELISA channel in the sequence in which they were loaded. To test the results under different negative pressures, a pressure gauge was applied to control the different pressures. In the fifth step, the top plate of IV-Chip is slid in an oblique direction against the bottom plate to read out the results. The "N"-shaped channel is converted to a "Z"-shaped channel in the detection zone and the H_2O_2 diffuses into ELISA wells and reacts with the bound PtNPs to generate oxygen gas. Results are read out in the form of the distance travelled by the columns of red ink (ink bar-chart) after 6 min. The results are recorded with a camera and the farthest limits of ink advancement are marked for quantitation.

ELISA in IV-Chip

For tests of antibody cross-reactivity, PBS was spiked with 250 ngmL^{-1} CK-MB, 50 ng mL^{-1} TnI, or 1000 ngmL^{-1} myoglobin, and 1 µL solution was loaded in the sample channel. The cocktail of 50 µg mL^{-1}PtNP-antibody was loaded in the PtNP probe channel, wash buffer was loaded in the wash buffer channel, and the device was ready for operation.

To generate the calibration curve, serum was spiked with CK-MB (0, 0.5, 5, 50, 100, 250, 500 ng mL^{-1}), TnI (0, 1, 10, 25, 50, 75, 100 ng mL^{-1}), or myoglobin (0, 20, 200, 500, 1000, 1500, 2000 ng mL^{-1}), and 1 µL spiked serum was loaded in the sample channel. PtNP-antibody cocktail and wash buffer were loaded as described above, and the device was ready for operation.

For detection of biomarkers in serum, 1 µL serum from patients or healthy volunteers was loaded in the sample channel. PtNP-antibody cocktail and wash buffer were loaded as described above, and the device was ready for operation.

Loading of ink and H_2O_2

Two microliters of red ink and 35% H_2O_2 were loaded into their respective fluidic paths from the right inlet. The solutions filled the fluidic path due to the hydrophilic interaction between the solutions and the glass surface.

Serum collection and clinical assay

Sera were collected at Houston Methodist Hospital from leftover samples after clinical testing. The study is approved by the institutional IRB committee. During the clinical sample collection, the MI patients and healthy controls have been classified using the standard clinical methods.

Data analysis

All serum samples were tested in triplicate, and the results are showing as mean ±standard error of the mean. Receiver operating characteristic (ROC) curves for each biomarker and the three biomarkers together were created by plotting sensitivity versus (1 – specificity). The values of area under the curve (AUC) were calculated using the trapezoidal rule. Detection sensitivity, specificity and accuracy were computed using standard formulas. Statistical analysis was performed using Graphpad Prism.

Results and discussion

The detection strategy is shown in Fig. 1a. A serum sample from an MI patient can be directly loaded in the IV-Chip for POC diagnostics, and levels of biomarkers can be readout quantitatively as a bar-chart. Fig. 1b schematically illustrates the principle underlying the function of the IV-Chip and its fluidic paths, which

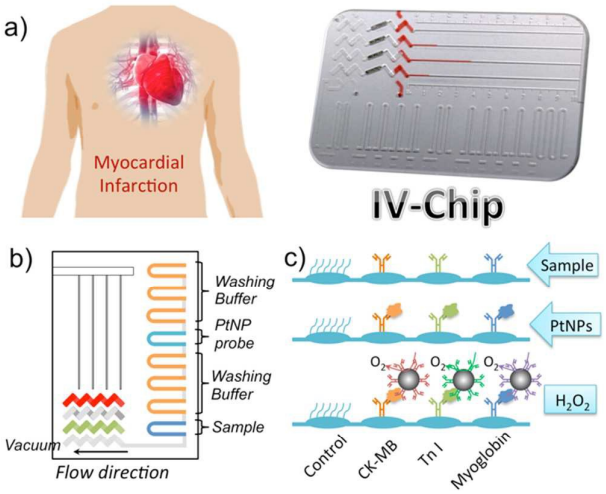

Fig. 1. POC detection of myocardial infarction (MI) using integrative volumetric bar-chart chip (IV-Chip). (a) Patient serum prepared from patient blood is loaded into IV-Chip for rapid and quantitative detection of biomarkers of MI. (b) Scheme shows principle underlying IV-Chip function. Sample, washing buffer, and PtNP probe are preloaded in the chip and form discontinuous segments separated by air spaces in the microfluidic channel after sliding of the top plate of the device in a horizontal and oblique direction against the bottom plate. Segments of sample and reagents pass over a series of four detection wells when vacuum is applied to generate fluid flow. The arrow indicates direction of the flow. (c) Schematic illustration of biochemical reactions in detection wells upon addition of indicated reagents. Standard sandwich ELISA is used to introduce antibody-conjugated PtNPs into the wells. PtNPs react with H_2O_2 to generate O_2, which is quantitatively readout on the IV-Chip.

integrate our previous V-Chip and liquid-delivery designs. Homogenous liquid segments consisting of sample solution, washing buffer, platinum nanoparticle (PtNP) probe solution, and washing buffer (shown on the right side of Fig. 1b) flow into the zigzag ELISA channel in that order when vacuum pressure is introduced at the bottom left inlet.

The wells in the ELISA channels are coated with capture antibodies for CK-MB, troponin I and myoglobin; these biomarkers in patient serum will be captured at specific locations when the sample segment flows through the wells. As shown in Fig. 1c, four individual wells are used for negative control and detection of CK-MB, troponin I and myoglobin. After sample has passed through the channel, liquid segments containing washing buffer flow through to remove unbound biomarkers and reduce nonspecific binding.

Antibody-conjugated PtNPs then flow into the ELISA wells and specifically bind to the biomarkers in sandwich-like structures. PtNPs have greater stability and catalytic activity and longer duration than catalase，which can be conjugated to probe antibodies to react with hydrogen peroxide (H_2O_2). This reaction generates oxygen gas, which propels red ink in the IV-Chip to generate a quantitative bar-chart readout.[15] The PtNP-antibody (PtNP-Ab) conjugates show high stability even after storage at 4 ^{o}C for 30 days (Fig. S6, S7, S8). The distance travelled by the red ink in the bar-chart readout corresponds to PtNP concentration when the IV-Chip is loaded with PtNPs at a uniform concentration or in a gradient generated by diffusion (Fig. S9, S10, S11). Finally, liquid segments containing washing buffer flow through the wells to remove nonspecifically bound PtNPs and reduce the background noise.

The IV-Chip employs SlipChip technology[21] and was assembled as previously described.[13] Silicon oil was used as a lubricant and to render the device airtight. As shown in Fig. 2a, the IV-Chip workflow consists of five steps. In the first step, sample, washing buffer, and PtNP probes are loaded in specific fluidic channels. In field applications, washing buffer and PtNP probes are preloaded in the chip, which is very convenient for remote application of IV-Chip. Because the sample channel requires less than 1 µL solution, the IV-Chip is suitable for serum obtained by finger prick or for rare clinical samples.[3] Additionally, assaying finger-prick whole blood will become available after a future integration of on-chip blood separator. In the second step, each sample and reagent is loaded in an independent channel. The top plate of the device is then slid in a horizontal and oblique direction against the bottom plate, producing liquid segments in the individual channels and rendering the channels containing the segments continuous with the zigzag ELISA channel. This is shown at higher power in Fig. 2b and 2c. Segment lengths are 52 mm for sample and PtNP probe and 27 mm for washing buffer (Fig. 2d). Individual segments of sample/PtNP probe and washing buffer are separated by air bubbles 3.3 mm in length, and individual washing buffer segments are separated by 1.3-mm bubbles. In the third step, red ink and H_2O_2 are loaded in the specific channel, and in the fourth step, vacuum pressure is applied to draw the fluid segments into the ELISA channel (Movie S1). Negative pressure produces more stable fluid flow and less contamination between each channel than positive pressure in the IV-Chip (Fig. S12, S13). A pipette or syringe produces sufficient vacuum pressure at the end of the ELISA channel to gradually draw the flow of fluid segments into the channel. Though we found that the rear fluid went through the wells faster than the front fluid due to the decrement of resistance, it had little influence to the result.

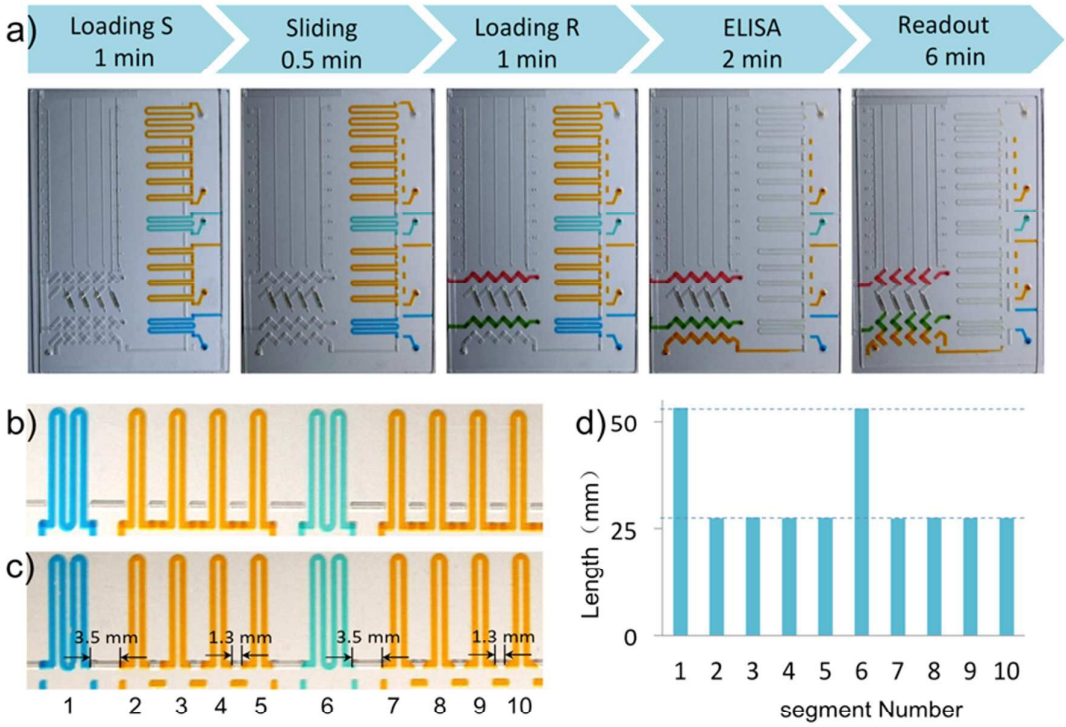

Fig. 2. Principle underlying operation of IV-Chip. (a) Complete IV-Chip assay includes five steps; these are loading samples, washing buffer and PtNP probe (Loading S), sliding of the top plate of the device in a horizontal direction against the bottom plate, loading red ink and H_2O_2 (Loading R), ELISA, and readout. Dark blue, sample; orange, washing buffer; light blue, PtNP probe; red, red ink; green, H_2O_2. (b, c) Higher magnification images of the fluid handling zone before and after sliding of the plates. Liquid segments (as indicated by numbers) are formed after the sliding. Lengths of the air bubbles between segments are shown in (c). (d) Length of each liquid-segment corresponding to C in the microfluidic channel after sliding of the plates.

We have tested the IV-Chip assay under different conditions of vacuum pressure (measured by a pressure gauge), and demonstrated that, for TnI, the pipette produces pressure equivalent to -0.17 KPa (Fig. S14). Our results also suggested that lower negative pressure would help to increase incubation time and obtain more sensitive readout. In the fifth step, the top plate is slid in an oblique direction against the bottom plate to generate the bar-chart readout. The "N"-shaped channel is transformed into an "Z"-shaped channel,[13] and H_2O_2 diffuses into the ELISA wells and react with the bound PtNPs to generate oxygen gas. Results can be readout by measuring the distance travelled by the red ink 6 min later. A test is complete in less than 12 min, which is much less than the recommended time of 30 min for POC diagnosis of MI.[23]

parallel assays of the three biomarkers greatly reduce replicates, thereby significantly reducing assay time and effort compared with traditional diagnostic approaches.

To obtain reliable detection results, it is critical to assess the specificity of each antibody. Because of the complex structure of proteins, some antibodies bind aberrantly to off-target proteins having structures identical or similar to that of the original antigen, leading to nonspecific cross-reactivity.[27-29] Currently, there is no universal method to assess antibody cross-reactivity and, although many such methods have been described,[30] these are technically challenging, time consuming, or do not provide information that is generally applicable beyond the conditions of the current experiment.

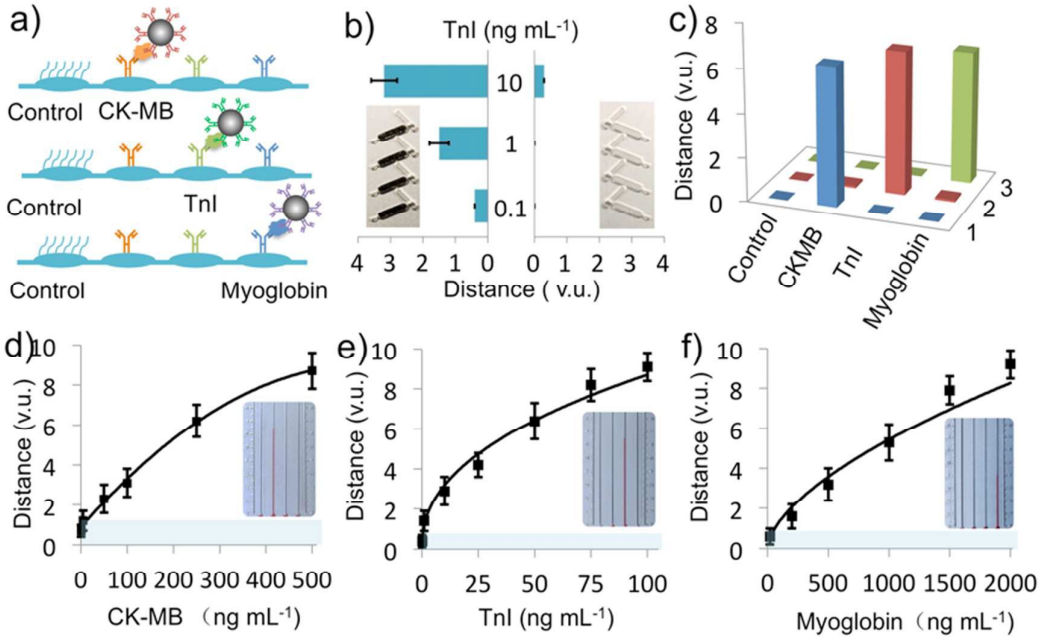

Fig. 3. Detection of spiked CK-MB, TnI, and myoglobin in serum using IV-Chip. (a) Scheme shows the position of each biomarker in IV-Chip for assessment of antibody cross-reactivity. (b) IV-Chip coated with platinum film (black wells) show 100-fold amplification of TnI signal compared with chip without platinum. (c) Results of tests of cross-reactivity. (d) Calibration curve for detection of CK-MB. Inset shows bar-chart readout for 250 ngmL^{-1} CK-MB. (e) Calibration curve for detection of TnI. Inset picture shows bar-chart readout for 50 ngml^{-1}TnI. (f) Calibration curve for detection of myoglobin. Inset shows bar-chart readout for 500 ng mL^{-1} myoglobin. v.u., IV-Chip unit.

The biomarkers CK-MB, troponin I and myoglobin, have been approved to facilitate screening of MI patients and discharge of healthy individuals.[23-26] Because of the limited accuracy of tests of the individual biomarkers outside specific time frames, measurement of a single biomarker is not reliable for diagnosis of MI.[23, 26] It has been demonstrated that myoglobin serves as a high-sensitivity indicator of MI only in the first few hours after symptom onset. Tests of myoglobin alone also showed low specificity in MI patients with renal failure or skeletal muscle trauma.[24] CK-MB and cTnI can be detected 3 to 6 h after symptom onset. The inclusion of the three biomarkers together in a multiplex assay is believed to increase accuracy.[24] However, multiplexing of traditional assays always increases diagnostic cost significantly. With the IV-Chip, the microfluidic channels require minimal amounts of reagents, and

The IV-Chip offers the potential to rapidly and inexpensively assess antibody cross-reactivity without additional equipment. To improve analytical sensitivity of IV-Chip, 20-nm platinum films (black wells) were deposited in the wells of the third lane of the bottom plate to amplify the signals. Without amplification, less than 10 ngmL^{-1}, TnI is undetectable in serum. However, with the platinum films, sensitivity improved approximately 100-fold and the detection limit in serum reached 0.1 ngmL^{-1}, which is below the threshold of clinical diagnosis.[26] This amplified IV-Chip was used for measurement of antibody cross-reactivity. As shown in Fig. 3c, when serum containing 250, 50, or 1000 ng/mL of CK-MB, TnI or myoglobin, respectively, is applied to the IV-Chip, negligible background is seen in the control channels. Specificity was also confirmed by the fluorescence analysis of the IV-Chip assay using

FITC-conjugated antibodies (Fig. S15). Because the three detection antibodies conjugated to PtNPs are loaded together in a cocktail, these data suggest that the IV-Chip allows simple and rapid screening of antibody cross-reactivity by the naked eye. By adjusting the number of channels, the IV-Chip can assess a panel of 10 different antibodies.

Because of the high specificity of each antibody, we can measure a range of concentrations of spiked CK-MB (0.5–500 ngmL^{-1}), TnI (0.1–100 ngmL^{-1}), and myoglobin (20–2000 ngmL^{-1}) in serum using the IV-Chip (Fig. 3d, e, f), and the corresponding ink bar charts provide direct quantitative results. As the concentration of biomarker increased, the bar charts of the IV-Chip showed a near linear corresponding increase in distance travelled by the ink. In each case, the signals obtained with serum containing 0.5 ng mL^{-1} CK-MB, 0.1 ng mL^{-1}TnI, or 20 ng mL^{-1} myoglobin are greater by a factor of at least three standard deviations than background, indicating that the limit of detection resides at or below these respective concentrations. The cutoff concentrations of CK-MB, TnI, and myoglobin in serum for diagnosis of MI are 5, 0.6, and 50 ngmL^{-1}.[26] Therefore, the detection capability of the IV-Chip is well within the range of clinical relevance for diagnosing MI.

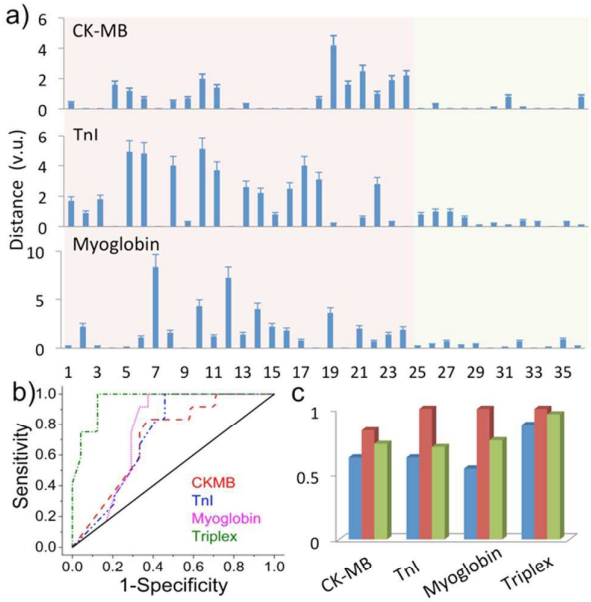

Fig. 4. Detection of MI using clinical serum samples and IV-Chip. (a) Bar-chart readouts for CK-MB, TnI, and myoglobin in serum of 24 MI patients (light red) and 12 healthy controls (light green) using IV-Chip. Values represent mean ± standard deviation of three IV-Chip measurements. v.u., IV-Chip unit. (b) Receiver operating characteristic (ROC) curves for each biomarker and multiplex test (triplex) obtained from IV-Chip results. (c) Specificity (blue), sensitivity (red), and accuracy (green) of detection of each biomarker and triplex test. Although the accuracy of detection of for a single biomarker alone was less than 80 %, when all markers were assessed together in a triplex test, accuracy reached 95.8 %.

To investigate the performance of the IV-Chip in clinical applications, we conducted a set of tests to measure the three biomarkers in clinical serum samples of patients with MI (n = 25) and healthy volunteers (n = 11; Fig. 4 and Table S1) using the IV-Chip. Fig. 4a shows the plot of bar-chart results for CK-MB, TnI, and myoglobin. Consistent with the previous report,[26] CK-MB, TnI, and myoglobin exhibit prevalence in MI patients with the specificity of 62.5 %, 62.5 % and 54.0 %, respectively. The accuracies of the individual markers in MI detection, derived from receiver operating characteristic (ROC) curves, were 72.9%, 70.5%, and 75.7%, respectively (Fig. 4c). However, upon combining the bar-chart results of all three markers, the specificity and accuracy of detection increased to 87.5% and 95.8%, respectively.

Conclusions

In summary, we have developed a POC device by integrating a volumetric bar-chart chip with a reagent delivery system. Unlike the manually produced segments employed in the previous delivery system, the IV-Chip can automatically generate several liquid segments in microfluidic channels simultaneously by simply sliding the top plate against the bottom, which renders reproducibly loads the same volumes of sample or reagents each time. Compared with our previous V-Chip technology, the operation time is greatly decreased to 15 min, which meets the AHA recommendation for turnaround time for POC detection of MI. By introducing the platinum film-based amplification design, the quantitative bar-chart can be visualized at biomarker levels lower than the clinical cutoff value. In tests of clinical samples, the IV-Chip exhibited excellent capability for diagnosis of MI by detection of the three biomarkers, with specificity and accuracy reaching 87.5% and 95.8%, respectively.

POC detection of MI offers unique benefits compared with traditional methods.[3] Compared with the previous methods, IV-Chip provides an inexpensive and quantitative strategy for POC detection of MI biomarkers.[31] In this work, the estimate total cost for each test was less than 10 dollars, which seem to be suitable for low-cost detection, given that multiple biomarkers can be assayed within one run.The AHA recommends a 30-min turnaround time for detection of cardiac markers used for evaluation of MI in the emergency department. The logistic hurdles, including transport time, sample handling, and communication of the results to the physician, make the 30-min goal a significant challenge in a central laboratory.[24] ELISA-based detection of cardiac biomarkers has been identified as a promising technology in the development of POC devices to overcome these hurdles. However, POC device design is always limited by the repeated washing steps and long incubation time required in ELISA. Thus, most of the commercial POC devices employ a lateral-flow assay, which simplifies ELISA by reducing assay steps. The semi-quantitative results and hook effect in lateral-flow assays preclude accurate measurement of biomarker levels.[32, 33] Simplification of ELISA without damage to assay accuracy is a promising approach for the development of a new generation of POC devices. Microfluidic technology allows rapid, parallel analysis of multiple samples with high-sensitivity using small volumes of samples and reagents. The study presented herein lays the foundation for rapid generation of liquid segments and reagent delivery for IV-Chip or other technologies based ELISA detection to

be widely used in clinical diagnostics, drug screening, and environmental monitoring.

Acknowledgements

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