Supporting Information:

Aptamer-based microfluidic electrochemical biosensor for monitoring cell-secreted trace cardiac biomarkers

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Experimental Methods

Materials

The 11-mercaptoundodecanoic acid (MUA), ethanol, NHS, EDC, sodium hydroxide, citric acid, potassium ferricyanide (III), and streptavidine were purchased from Sigma-Aldrich. Biotin-functionalized CK-MB antibody was used in an ELISA kit which was obtained from Mybiosource. Active human Creatine Kinase MB full length protein was purchased from Abcam and diluted by 1X Dulbecco's Phosphate Buffered Saline (DPBS) for different concentration. The DNA aptamer for human CK-MB was purchased from OTCbiotech and diluted by 1X DPBS. PDMS was purchased from Dow Corning. Teflon tubing and Tygon microbore tubing was purchased from Cole-Parmer to connect microfluidic platform. 27 G × 8 mm and 22 G × 15 mm size of stainless steel catheter coupler for connecting tubes and 22 G × 12 mm size of stainless steel catheter plug for blocking punching holes were purchased from Instech.

Fabrication of the bioreactor

A cardiac bioreactor with a total volume if approximately 1.5 ml and a 2.6 cm^2 of surface area of cell culture chamber was designed and fabricated. The layout area consisted of two polymethylmethacrylate (PMMA) personalized clamps, two layers of PDMS fluidic device, one 3-(trimethoxysilyl) propyl methacrylate (TMSPMA) treated glass and a plain PDMS layer. Molds and clamps were designed using AUTOCAD™, and then exported to Corel Draw X5 to enable the laser cutter (VLS2.30, Versa Laser Inc.) to fabricate 1.5 mm and 3 mm thick PMMA slides. PMMA masters were realized to create the chambers of the bioreactor using 1.5 mm-thick PMMA slides. The molds were attached to the bottom of a 100 mm × 15 mm Petri Dish (Fisher Scientific International Inc.) using a common double sided tape. The bioreactor clamps were fabricated by 3 mm-thick PMMA slides. The 10:1 ratio of PDMS and crosslinking agent were prepared. Once the bubbles in the PDMS mixture were removed, the PDMS layers were placed inside an oven at 80 °C for 1hr. the cured PDMS pieces were

cut according to the dimension of the glass slides (72 mm × 25 mm) and the chamber was incised and then removed from the bottom layer. A personalized 20 G needle (Becton, Dickinson and Company) was used as a puncher for the inlet/outlet holes to enable the insertion of tubing, respectively. After the bottom layer and the TMSPMA treated glass were treated with nitrogen plasma (PE-50 Plasma Etch) for 70 sec, they were irreversibly bonded together where the channel features made contact with the glass. The top and the bottom layers were sterilized by flushing 70% ethanol/DIW (Deionized water), sterile PBS and exposing to UV light for 30 min. The whole device was clamped with PMMA clamps using 6 screws as shown in Figure 3 (a). The tubing (Cole Palmer#30 PTFE) was inserted into the inlet/outlet and sealed with epoxy glue (Devcon). The system was connected using standard two stop peristaltic tubing (Analytical West, Inc) and operated in a closed loop manner at 18 μL/min of flow rate by using Cole-Parmer four channel precision micro peristaltic pump.

Human pluripotent stem cell culture and differentiation

Ventricular cardiomyocytes (CMs) generated from the human embryonic stem cell (hESC) line, ES02 (Wicell, Madison WI) were used and generated as previously described [1]. Briefly, undifferentiated hESCs were maintained in feeder-free conditions on matrigel (Corning) -coated plates using mTeSR-1 media (Stem Cell Technologies). At approximately 80% confluence, cardiogenic embryoid bodies (EB) were generated by dissociating the hESC colonies off the maintenance dish with StemPro® Accutase® (Thermo Fisher Scientific) and transferred to mTeSR-1 media containing 1 ng/mL BMP4 (Invitrogen) and Rho-kinase (ROCK) Inhibitor (10 μM; R&D Systems) on ultra-low-attachment cell culture dishes (Corning) under hypoxic condition. The following day, the media was changed to StemPro-34 complete media (Life Technologies) containing 50 μg mL^{-1} Ascorbic Acid (Sigma) and 2 mM GlutaMAX-I (Invitrogen) with the addition of 10 ng mL^{-1} BMP4 and 10 ng/mL Activin A (Invitrogen). Ninety-six hours later, the media was changed to StemPro-34 complete media with the addition of the Wnt inhibitor IWR-1 (2.5 μM) (Enzo). Cultures were maintained in a hypoxic environment (5% CO_2 and 5% O_2) until day 8 after initiation of directed differentiation, at which point the EBs were refreshed with

StemPro-34 complete media and transferred into a normoxic 5% CO_2 incubator for the remainder of the culture period, with half media changes every 2 days until dissociation for downstream experiments.

Characterization of functionalized microelectrodes

To characterize the surface of the microfabricated Au electrodes, atomic force microscopy (AFM, Asylum Research model MFP-3D) was employed to determine the surface roughness of the microelectrodes used in the experiments.

Live/Dead assay

Viability of the cells in the cardiac construct was stablished using a live/dead viability/cytotoxicity kit for mammalian cells (Life technologies). In short, an ethidium homodimer (EthD-1) and calcein AM solution in phenol red free DMEM media (life technologies) with concentrations of 2 μl ml^{-1} and 0.5 μl ml^{-1}, respectively was prepared. Samples were washed twice with warm PBS, followed by 15 min incubation with sample assay in a 37°C incubator. After that, the samples were washed three times in phenol red free DMEM media. Cell viability was monitored using a fluorescence plate reader (Nikon, TE 2000).

Supporting data

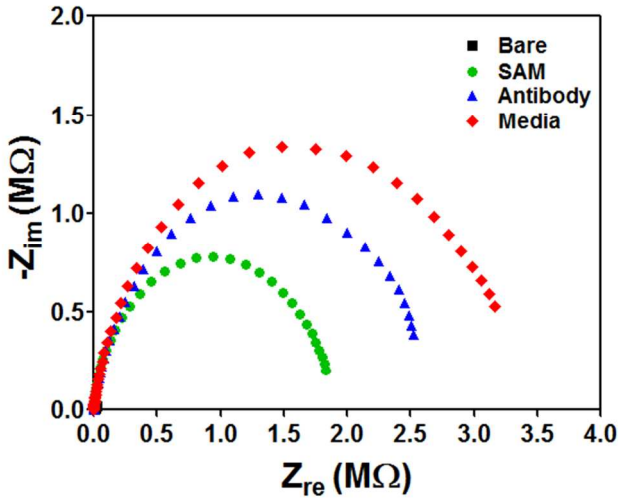

Figure S1: Nyquist curves obtained after immobilization of CK-MB antibody onto a streptavidin/SAM-functionalized microelectrode.

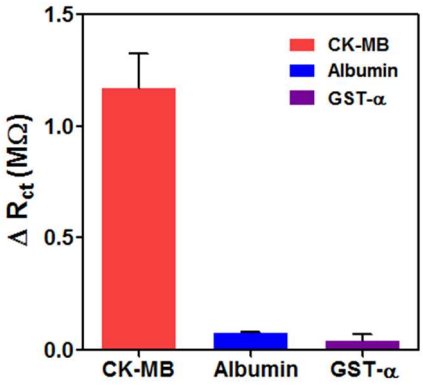

Figure S2. Measurements showing the selectivity of the CK-MB biosensor. A CK-MB aptamer-functionalized biosensor was exposed 10 ng mL^{-1} GST-α, 10 ng mL^{-1} albumin, and 10 ng mL^{-1} CK-MB respectively, where a higher signal was obtained after exposure to 10 ng/mL CK-MB as expected.

(1) Weng, Z.; Kong, C.-W.; Ren, L.; Karakikes, I.; Geng, L.; He, J.; Chow, M. Z. Y.; Mok, C. F.; Keung, W.; Chow, H. *Stem cells and development* **2014**, *23*, 1704-1716.