Centrifugal Micropipette-Tip with Pressure Signal Readout for Quantitative Portable Detection of Myoglobin

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Experimental

Reagents and materials

Ficoll-Paque PLUS was obtained from GE Healthcare (Pennsylvania, USA). Myoglobin (Myo) was from Hytest (Turku, Finland). Detection antibody against Myo labeled with Pt nanoparticles (DA-Pts), hand-held pressure meter (13.5*2.5*1.8 cm, detection range: 0-3000 Kpa, accuracy: 0.01 Kpa) and 8-well gas-tight chamber were obtained from Xiamen Passtech Co. Ltd (Xiamen, China). Capture antibody (anti-Myo) was purchased from Boster Biological Technology Co. Ltd (Wuhan, China). Biotin N-hydroxysuccinimide ester (BNHS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Dynabeads® M-280 Streptavidin was purchased from Life Technology (USA). Other reagents were obtained from Sinopharm Chemical Reagent (Shanghai, China). Micropipette-tips that are usually for gel loading, but used here for fabrication of centrifugal tip were purchased from Thermo Scientific Company (QSP 010-Q). Before using the tip for centrifugation, we sealed the end of the micropipette-tip (inner orifice diameter about 310 μm) within minutes with epoxy glue purchased at a local supermarket. The centrifuge (5424) was obtained from Eppendorf (German).

Preparation of detection antibody-beads (CA-beads)

Twenty μL of 5.1 mg/mL anti-Myo and 190 μL 0.1 M $NaHCO_3$ buffer (pH 8.4) are mixed with 3 μL of 10 mg/mL BNHS (dissolved in DMF) on a rotator for 2 hour at room temperature. After incubation, excess reagents are removed via a 3 kDa ultra centrifugal filter. The prepared biotinylated anti-Myo is then incubated with 100 mg Dynabeads at room temperature for 30 min to form CA-beads. Afterwards, CA-beads are washed 3 times with PBS and stored at 4°C in PBS buffer with final anti-Myo concentration of 1 mg/mL (calculated based on the added mass of anti-Myo).

Experimental procedure

The entire simple-step ELISA procedure is conducted in a centrifugal

micropipette-tip, and the total assay is completed within 35 min for 8 samples simultaneously. After loading 4.5 uL Ficoll, the sample (1 uL of Myo protein target or 3.3 uL of real serum) and ELISA reagents (3.1 uL CA-beads, 3.6 uL DA-Pts) and 2.3 uL PBST with 0.5% BSA buffer for Myo protein target (without buffer for real serum) are loaded and mixed in a liquid droplet above the Ficoll medium, which is separated from Ficoll by the air. After 15 min incubation at room temperature, 8 tips are put into 8 centrifuge tubes, then spun at 2500 rpm for 150 s in the centrifuge rotor. Following centrifugation, the beads are concentrated in the bottom of tip. Then the beads are removed by cutting the bottom of the tip and added to 30% H_2O_2 (100 uL) in an 8-well gas-tight chamber for 15 min gas generation. Due to the presence of DA-Pts on the assay beads, the substrate H_2O_2 is decomposed into $H_2O(l)$ and $O_2(g)$, increasing the pressure, which is measured by a portable pressure meter. In this way, the increase of pressure is directly related to the Myo concentration.

Clinical testing and statistics analysis.

Nine clinical samples from the first affiliated hospital of Xiamen University with various concentrations of Myo were detected by centrifugal micropipette-tip system. A series of concentrations of Myo was spiked into a normal sample (10.2 ng/mL measured by chemiluminescent microparticle immunoassay (CMIA) performed at the hospital) for the calibration curve. The final concentration of serum was 30% because of adding into ELISA reaction solution (CA-beads, DA-Pts). The calibration curve in serum was obtained under the same experimental condition as above experimental procedure. We calculated the correlation coefficient of the results by the centrifugal micropipette-tip method and CMIA (Architect) method for Myo detection.

Altman-Bland analysis by MedCalc software was used to compare the two measurement techniques of Myo detection. In this graphical method, the differences between the two results are plotted versus the average of the two techniques. The differences between two methods are plotted against CMIA method, which is a reference method in hospital. Horizontal lines were drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference plus and minus

1.96 times the standard deviation of the differences.

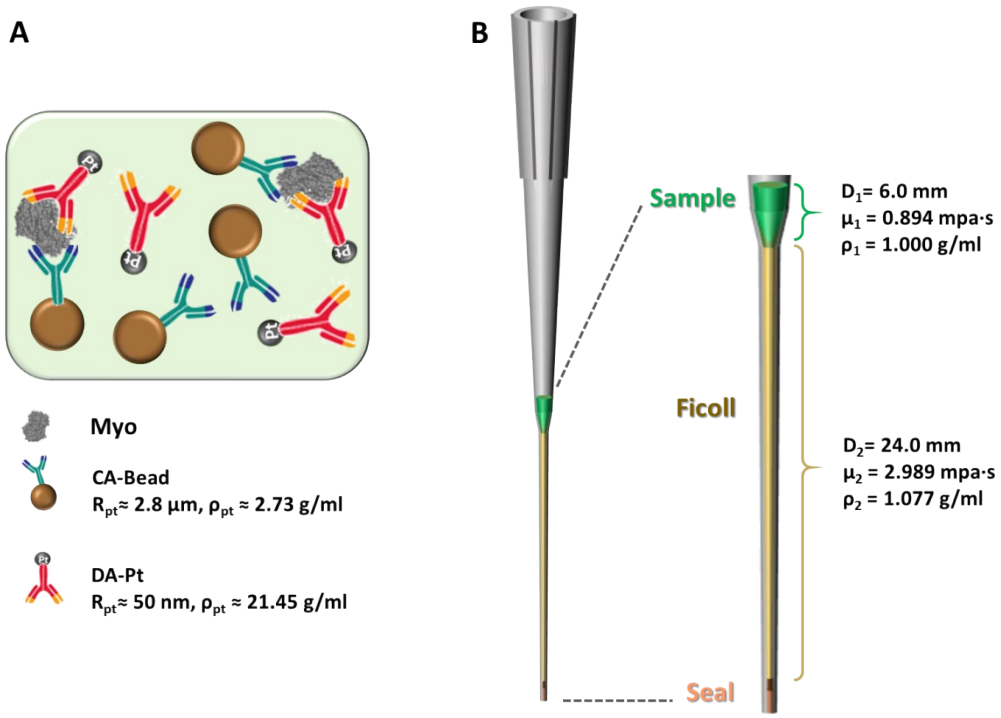

Figure S1 Bead sedimentation immunoassay principles. (A) Sample mixed with detection solution containing CA-beads and DA-Pts, allowing formation of capture antibody-antigen-detection antibody sandwich complexes. The average particle radius and density of CA-bead are from product introduction. The average particle radius of DA-Pt is estimated from the TEM result, and the density of DA-Pt is evaluated based on Pt element. (B) Separation by sedimentation rate. The reaction solution is layered on pre-loaded density media in the centrifugal micropipette-tip whose bottom was pre-sealed by epoxy glue. The fluid viscosities are measured by viscometer. The density of Ficoll is provided by product introduction.

$$v = \frac{2}{9} \cdot \frac{(\rho_p - \rho_l) \cdot g \cdot R_p^2}{\mu} \quad (Eq\ 1.)$$

$$T_{b1} + T_{b2} \leq T_{Pt2} \quad (Eq\ 2.)$$

$$\frac{D_1}{v_{b1}} + \frac{D_2}{v_{b2}} \leq \frac{D_2}{v_{Pt2}}$$

$$\frac{9}{2}\cdot\frac{D_1\cdot\mu_1}{(\rho_b-\rho_1)\cdot g\cdot R_b^2}+\frac{9}{2}\cdot\frac{D_2\cdot\mu_2}{(\rho_b-\rho_2)\cdot g\cdot R_b^2}\leq\frac{9}{2}\cdot\frac{D_2\cdot\mu_2}{(\rho_{Pt}-\rho_2)\cdot g\cdot R_{Pt}^2}(Eq\ 3.)$$

$$\frac{R_b^2}{R_{Pt}^2}\geq\frac{D_1\cdot\mu_1}{D_2\cdot\mu_2}\cdot\frac{\rho_{Pt}-\rho_2}{\rho_b-\rho_1}+\frac{\rho_{Pt}-\rho_2}{\rho_b-\rho_2}\approx 14.77$$

$$\frac{R_b}{R_{Pt}}\geq 3.8$$

Sedimentation fundamentals: Eq 1. and Eq 2 are the sedimentation rate and time of spherical particles. Effective separation is achieved when CA-beads reach the bottom of the centrifugal micropipette-tip, while but the DA-Pts do not, leading to Eq 2. Substitution of actual values (Fig S1) into Eq 3 yields a sizing requirement $\frac{R_b}{R_{Pt}}\geq 3.8$ for effectively separation.

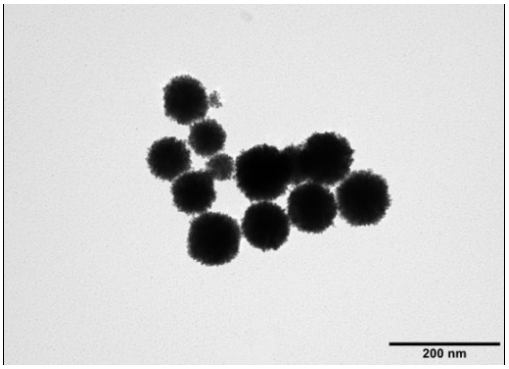

Figure S2 A TEM image of DA-Pt.

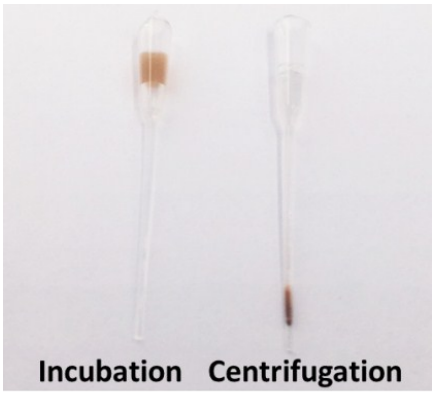

Figure S3 Real picture of the centrifugal micropipette-tip in the incubation step and after centrifugation.

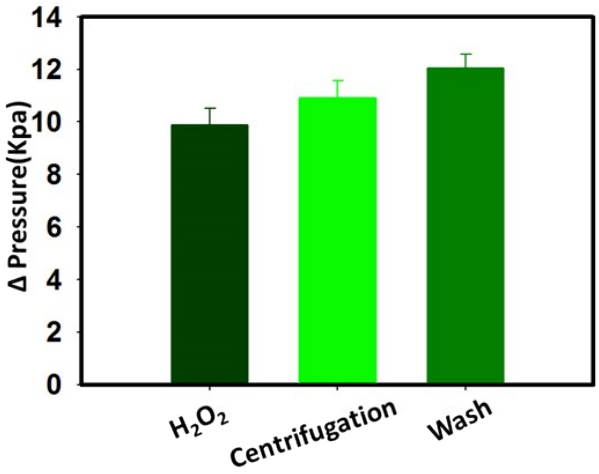

Figure S4. Comparison of the pressure values of the self-decomposition of H_2O_2, after the centrifugation wash method and after washing 3 times using magnetic separation.

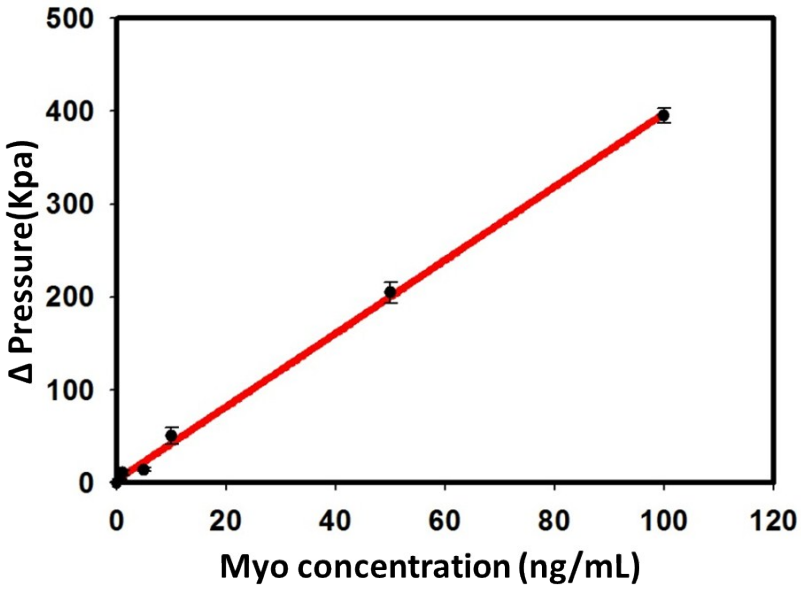

Figure S5. Linear response of Myo concentration (0, 1, 5, 10, 50, 100 ng/mL) by incubation in tubes and washing 3 times using magnetic separation in buffer solution (R^2=0.999).

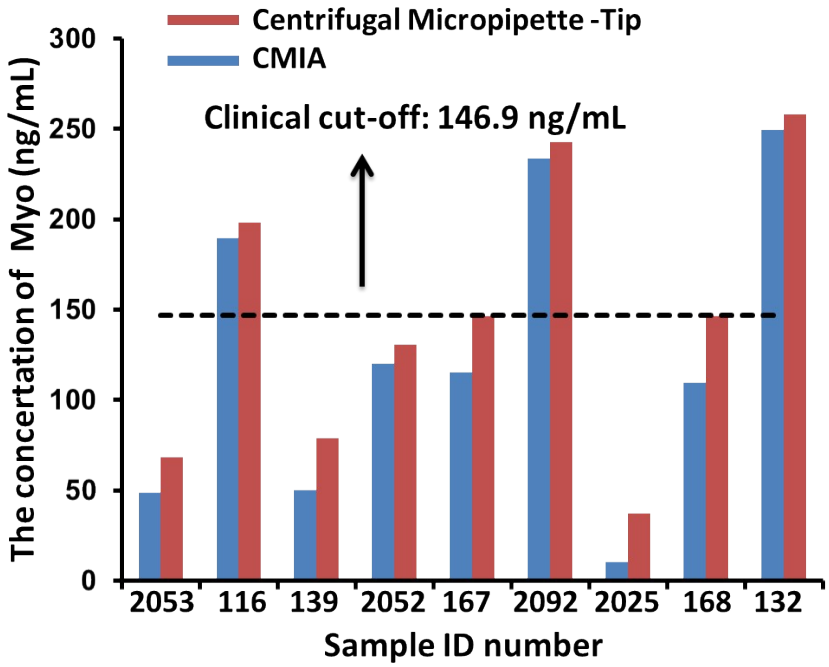

Figure S6. Comparison of centrifugal micropipette-tip and CMIA for detection of Myo in real serum samples. Samples 116, 2092, 132 are positive samples, and the others are negative samples.