Supplementary Material

Development and Comparison of Two Immuno-disaggregation Based Bioassays for Cell Secretome Analysis

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This document contains the following supplementary information:

Size Distribution of Conjugated Microparticles.

Stability of Microparticle Aggregates.

Kinetics of Microparticle Aggregation.

Measurement of VEGF Protein from Human Mesenchymal Stem Cell Secretome.

Size Distribution of Conjugated Microparticles. The size distribution of MPs before and after biotinylated VEGF antibody or protein conjugation was measured with an AccuSizer™ 780 Optical Particle Sizer (Particle Sizing Systems, Port Richey, FL, USA). Our results showed that the MPs have a wide distribution of particle size ranging from ~ 1.5 µm to 10 µm (Figure S1). The mean diameters of MPs before conjugation, MPs after VEGF antibody conjugation, and MPs after VEGF protein conjugation were measured as 2.741 ± 0.012 µm, 2.788 ± 0.017 µm, and 2.787 ± 0.014 µm, respectively. The results were further confirmed by observing the MPs using a light microscope.

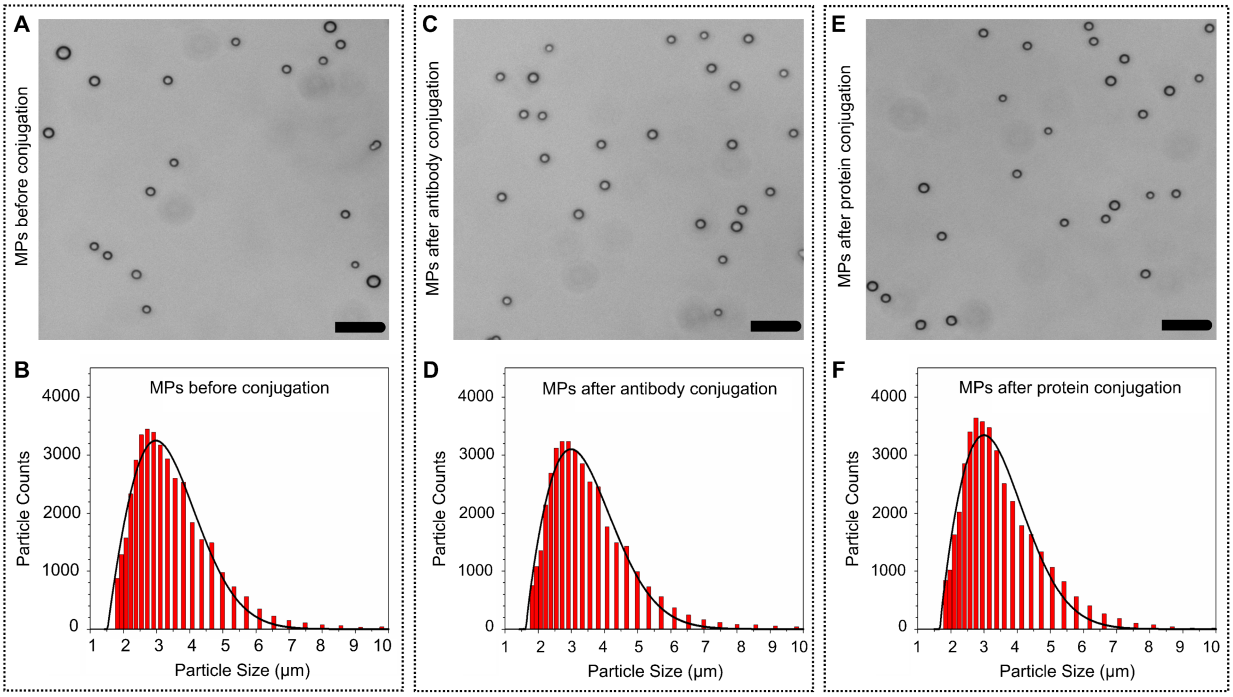

Figure S1: Size distribution of MPs. (A, B) MPs before conjugation. (C, D) MPs after biotinylated VEGF antibody conjugation. (E, F) MPs after biotinylated VEGF protein conjugation. One way analysis of variance (ANOVA) statistical test showed no significant difference in the size distribution of MPs before and after biotinylated antibody or protein conjugation. Scale bar: 20 µm.

Stability of Microparticle Aggregates. After forming MP aggregates, we checked the stability of the formed MP aggregates. To confirm the status of MP aggregates over time, we performed the following experiments. Using both immuno-disaggregation bioassays, the negative control groups (maximum MP aggregates) were prepared following the protocols described in the Materials and Methods section. We compared the average aggregation volume (avg AV) of MPs immediately once the aggregates were formed (0 h) and after incubating the MP aggregates at 2-8°C for 4 h. For the immuno-disaggregation bioassay using antibody-conjugated MPs, the avg AVs were 40.75 μm^3 at 0 h and 40.70 μm^3 at 4 h (Figure S2). For the immuno-disaggregation bioassay using protein-conjugated MPs, the avg AVs were 27.41 μm^3 at 0 h and 26.2 μm^3 at 4 h (Figure S2).

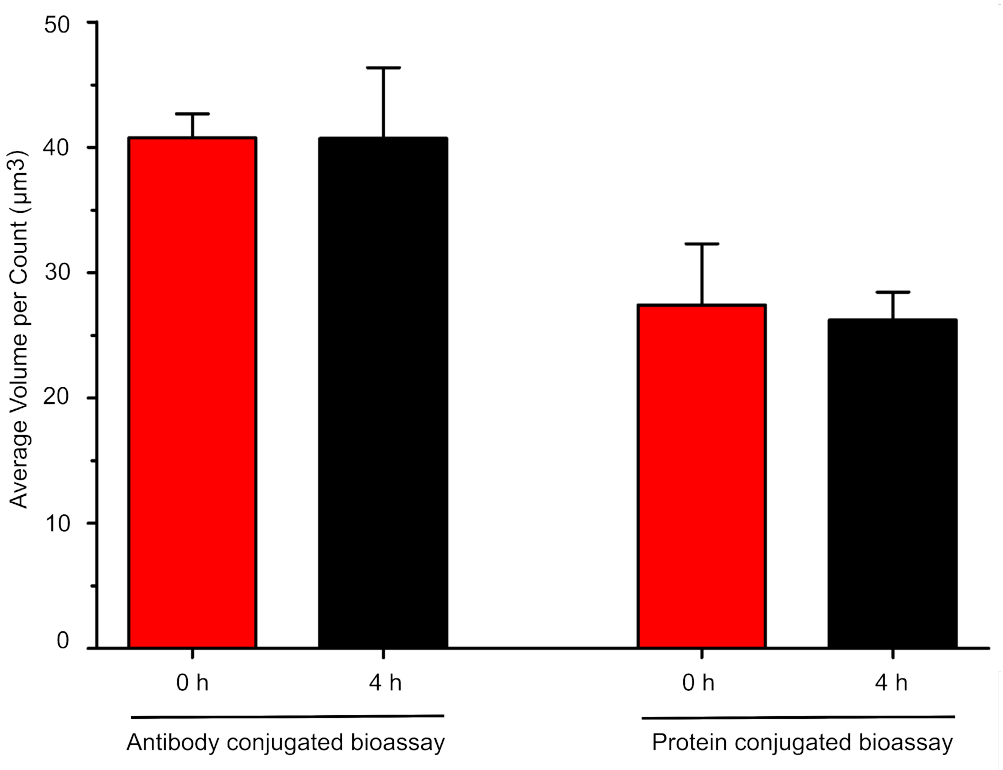

Figure S2: Average aggregation volume of negative control groups. The measurement was performed at 0 h and 4 h after forming aggregation. Student's t-test showed no statistical significant difference between 0 h and 4 h experiment groups in the corresponding bioassays.

Kinetics of Microparticle Aggregation. The following experiments were performed to assess the aggregation kinetics of MPs. For the immuno-disaggregation bioassay using antibody-conjugated MPs, the MPs were first conjugated with biotinylated VEGF antibody. To induce MP aggregation, the antibody-conjugated MPs were reacted with streptavidin protein at 25°C by rotating the samples at the speed of 6 rpm for 15 min, 30 min, 45 min, and 60 min. For the immuno-disaggregation bioassay using protein-conjugated MPs, the MPs were conjugated with biotinylated VEGF protein. To form MP aggregates, the protein-conjugated MPs were mixed with VEGF antibody at 25°C and 6 rpm rotation for 15 min, 30 min, 45 min, and 60 min. The degree of MP aggregation was measured quantitatively with an AccuSizer^{TM} 780 Optical Particle Sizer. Our results demonstrated that, in the presence of the linker, the degree of aggregation increased with longer incubation time during the aggregates formation (Figure S3). For immuno-disaggregation bioassay using antibody-conjugated MPs, the average volume change % of MP aggregates at 15 min, 30 min, 45 min, and 60 min were 121.68%, 139.45%, 161.51%, and 165.99% respectively. Similarly, for immuno-disaggregation bioassay using protein-conjugated MPs, the average volume change % of MP aggregates at 15 min, 30 min, 45 min, and 60 min were 37.12%, 50.53%, 90.41%, and 95.01% respectively.

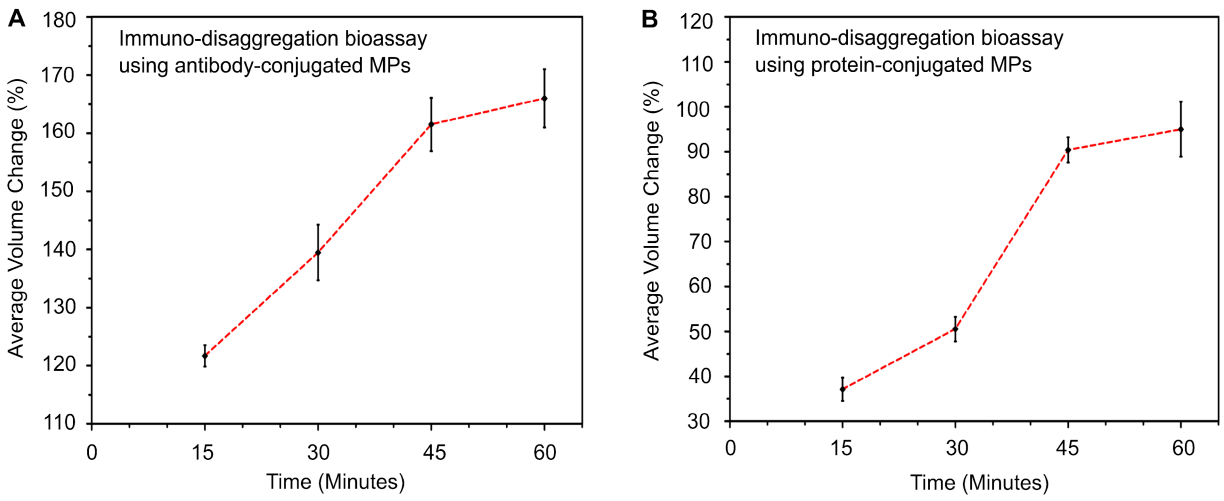

Figure S3: Kinetics of MP aggregation. (A) immuno-disaggregation bioassays using antibody-conjugated MPs. (B) immuno-disaggregation bioassays using protein-conjugated MPs. Student's t-test showed no significant difference in MP aggregates between 45 min and 60 min samples for both bioassays.

Measurement of VEGF Protein from Human Mesenchymal Stem Cell Secretome. The VEGF protein secreted by human mesenchymal stem cells (hMSCs) was measured with two detection methods: immuno-disaggregation bioassay using antibody-conjugated MPs and standard enzyme-linked immunosorbent assay (ELISA). The secretome samples were obtained by seeding hMSCs bilaterally on 600 μm decellularized porcine myocardium slices (dPMSs) as we previously described[1]. Briefly, 2 x 10^5 hMSCs were seeded on one side of dPMS and incubated in humidified cell culture incubator (37 °C; 5% CO_2) for 4 h. After incubation, dPMS was flipped to receive cells on the other side at same cell density. The cells were incubated for another 4 h before adding 1 mL of complete cell growth medium for continuing culture. Next, the cells were maintained in humidified cell culture incubator (37 °C; 5% CO_2) for 5 h, 10 h, 24

h, 72 h, and 120 h without replacing the growth medium. Subsequently, hMSC secretome was collected and centrifuged at 600 x g for 5 min at 4ºC to remove non-adherent cells. The hMSC secretome was either immediately used or stored at -20˚C until use. Following the same protocol, a negative control secretome sample was prepared using dPMS without incorporating cells. To validate our bioassay, we performed the following experiments. First, we measured the VEGF concentration of hMSC secretome with our immuno-disaggregation bioassay using antibody-conjugated MPs following the protocol described in the Materials and Methods section. Secondly, we measured the VEGF concentration of hMSC secretome using commercially available human VEGF quantikine ELISA kit (R&D systems, Minneapolis, MN, USA) following the manufacturer's recommendations. Table S1 shows the concentration of VEGF protein in hMSC secretome measured from ELISA and our bioassay. Our results demonstrated that VEGF protein detected from our bioassay were comparable to standard ELISA method. Student's t-test showed no statistical difference in the corresponding samples between the two detection methods.

	VEGF protein concentration (ng/mL)	
Incubation time (hours)	*Standard ELISA method*	*Antibody conjugated bioassay*
5	2.82 ± 0.62	2.73 ± 0.71
10	5.22 ± 1.06	5.36 ± 0.76
24	7.22 ± 0.88	7.63 ± 0.12
72	19.53 ± 0.24	18.29 ± 0.86
120	30.94 ± 3.18	30.5 ± 0.99

Table S1: Comparisons of VEGF protein concentration in hMSC secretome determined from our bioassay and ELISA method. The data are presented as mean ± SE. All experiments were performed with 3-6 independent samples.

REFERENCE

(1) KC P, Shah M, Liao J, Zhang G. Prevascularization of Decellularized Porcine Myocardial Slice for Cardiac Tissue Engineering. ACS Appl Mater Interfaces. 2017; 9 (3): 2196–2204.