Supporting Information for

Highly Sensitive Immunoassay for the Diagnosis of Acute Myocardial Infarction Using Silica Spheres Encapsulating a Quantum Dot Layer

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Experimental Section

Materials

For the synthesis of SQS, Octadecylamine (ODA)-protected CdSe/ZnS core/shell quantum dot (QD-ODA) was purchased from Nanosquare Inc. (Seoul, Korea). Silica sphere (10% in water by weight/volume) was obtained from Polysciences, Inc. (Warrington, PA, USA). Ethanol (99.9+%, Burdick & Jackson®, Morristown, NJ, USA), NH_4OH (28.0–30.0%, Junsei Chemical Co., Ltd., Tokyo, Japan), and NaOH (93%, Showa Chemical Co., Ltd., Tokyo, Japan) were used as purchased. Tetraethylorthosilicate (TEOS, 98%), methanol (≥ 99.8%), 3-(aminopropyl)trimethoxysilane (APTMS, 97%), and 3-mercaptopropionic acid (MPA, 99%) were purchased from Sigma Aldrich® Co., LLC. (St. Louis, MO, USA).

For the immunoassay, triple purified water (resistivity < 18.3 MΩ•cm) was prepared using Millipore Synergy (Molsheim, France). The peptide standard, NpY, was purchased from Phoenix Pharmaceuticals and SubP was purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade organic solvents were purchased from J.T. Baker (Phillipsburg, NJ, USA). Glutaraldehyde, bovine serum albumin (BSA), phosphate buffered saline (PBS), Tween-20, and sulfuric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The SubP monoclonal Ab (mAb) and tetramethylbenzidine (TMB) were purchased from Biorbyt (Cambridge, UK), and SurModics (Eden Prairie, MN, USA), respectively. The SubP polyclonal antibody and horseradish peroxidase- (HRP-) conjugated immunoglobulin G (IgG) was obtained from Abclon (Seoul, Korea) after immunizing rabbits.

Preparation of Highly Photoluminescent SQS

The purchased silica particle solution [20 mL, 5.82% (w/v) in water, 7.11×10^{-7} M (Molar concentration of silica particles was calculated using silica density (2.0 g/cm^3) provided by vendor and average diameter (50.7 ± 4.2 nm, n > 100) obtained from TEM images)] was centrifuged after addition of 20 mL of ethanol and the solid was dispersed in 40 mL of methanol. After adding APTMS (~5% of silica), the solution was refluxed overnight. After centrifugation, the NH_2-functionalized silica was washed twice with ethanol and then dispersed in 23.2 mL of ethanol as a stock solution (6.13×10^{-7} M). One mL of the stock solution was centrifuged and the solid was dispersed in deionized water (DW). The pH was adjusted to ~4 to yield 5 mL of solution S (1.23×10^{-7} M). The carboxy-terminated QD solution was freshly prepared from purchased QDs (1.0×10^{-5} M, 4 mL) in $CHCl_3$ by stirring with 0.1 M MPA containing 0.12 M NaOH in methanol (1.6 mL) for 15 h. To this solution, 4 mL of DW was added and the resultant QD-MPA in the water layer was transferred to a centrifuge tube. A 4:1 mixture (16 mL) of ethyl acetate and methanol was added and centrifuged. The solid was dispersed in DW and the solution was adjusted to pH~10 to yield solution Q (1.0×10^{-6} M, 40 mL). Solution S (1.23×10^{-7} M, 3.75 mL) at pH~4 was added dropwise slowly into 30 mL of solution Q at pH~10 with gentle shaking for self-assembly of Qs on S. This solution was centrifuged and the solid SQ assembly was dispersed in two sets of ethanol (300 mL) for encapsulation with SiO_2. To each set, DW (1.8 mL), NH_4OH (1.2 mL), and TEOS (0.3 mL) was added and the solution was stirred for 3 h. After the first 1 h, the solution was sonicated every 30 min for 1 min to avoid aggregation between intermediate SQSs. The solid SQS was separated and washed with ethanol three times via centrifugation.

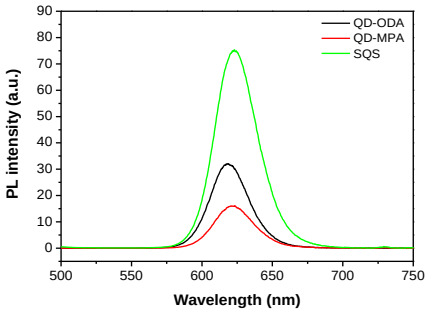
Finally, the combined SQS solid was dispersed in 40 mL of ethanol ([QD] ~7.5×10^{-7} M, [SQS] ~1.15×10^{-8} M).

Table S1. Characteristics of healthy controls and subjects with acute myocardial infarction (AMI) for marker validation

	Control	**AMI**
Age (years old)	53 ± 13	60 ± 14
Number of patients (male/female)	30 (13/17)	16 (12/4)
Number of hypertensive patients	3	8

Figure S1 (a) PL spectra of QD-MPA, QD-ODA, and SQS and (b) TEM image of SQS prepared without sonication.

(a)



(b)