Electronic Supplementary Information

Dual Action of Tyrosinase-Mesoporous Silica Nanoparticle Complex for Synergistic Tissue Adhesion

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Experimental Section

Synthesis of Mesoporous Silica Nanoparticles (MSNs)

MSNs were synthesized as previously described with slight modifications [1, 2]. We added 8 mL of cetyltrimethylammonium chloride solution (25%; Sigma-Aldrich), 80 mg of triethanolamine, and 20 mL of distilled water in a 100-mL three-neck round-bottom flask. Various amounts of mesitylene (0, 0.5, 1, or 1.5 mL; Sigma-Aldrich) were added to the reaction vessel to control the pore volume, and the vessels were labeled as MSN-1, 2, 3, and 4, respectively. The condenser was equipped with a flask, and the solution was heated at 95 °C for 1 h. Next, 1.5 mL of tetraethyl orthosilicate (TEOS, Acros) was added dropwise, and the reaction proceeded for 1 h. After cooling the reaction vessel, MSN was collected and purified via centrifugation at 8000 g for 20 min and re-dispersed with ethanol three times. To extract residual surfactant from the pore, the products were dispersed in methanol containing 1 wt% of NaCl and heated at 60 °C for 3 h on a stirring plate. After MSN was collected via centrifugation at 8000 g for 20 min, the extraction process was repeated. The final products were dispersed in ethanol to obtain a concentration of 10 mg/ml and preserved at 4 °C.

To prepare fluorescently labeled MSN, fluorescent dye-silane conjugates were prepared in advance. (3-aminopropyl)triethoxysilane (Sigma-Aldrich) and rhodamine B isothiocyanate (RITC, Sigma-Aldrich) were dissolved in ethanol to obtain final concentrations of 15 mM

and 3 mM, respectively. The mixture was shaken at room temperature overnight and preserved at 4 °C in the dark. After 50 min of addition of TEOS, 0.2 mL of dye-silane conjugates dissolved in ethanol were injected dropwise and allowed to react for 10 min. The purification and extraction of dye-conjugated MSN are described above.

Synthesis of Tyrosinase from *Streptomyces Avermitilis*

Tyrosinase from *Streptomyces avermitilis* (Ty) was synthesized and purified following previously described methods [3, 4]. Briefly, E. *coli* BL21 with tyrosinase gene was cultured overnight in Luria–Bertani broth at 37 °C and 200 rpm. Following this, 0.2 mM isopropyl β-D-1-thiogalactopyranoside and 1 mM $CuSO_4$ were added to the cell medium and cultured at 18 °C and 200 rpm for 20 h. After the cells were washed and lysed, the soluble factor was collected and purified via His-tag purification. The concentration of purified tyrosinase was quantified via BCA protein assay (ThermoFisher) following manufacturer's instructions.

Encapsulation of Tyrosinase into Mesoporous Nanoparticles

MSNs were re-dispersed in 50 mM Tris-HCl buffer (pH 7.0) before encapsulating tyrosinase. The tyrosinase solution was mixed with 30 mg/mL of MSN in Tris-HCl buffer (pH 7.0) at a specified concentration (6.25, 12.5, 25, and 50 μM). The mixture was stirred on an orbital shaker at 4 °C for 4 h. To purify T-MSN, the mixture was purified using a centrifugal concentrator (MWCO 50,000) at 4000 rpm for 10 min. The supernatant was collected to quantify the degree of Ty encapsulation. The purified T-MSN was resuspended in 50 mM Tris-HCl (pH 7.0) and frozen at -20 °C until further experimentation.

Characterization of MSN and Tyrosinase within MSN

Transmission electron microscopy (TEM) images of MSN were obtained using a JEOL EM-2010 microscope. Hydrodynamic sizes and zeta potentials were measured using a dynamic light scattering instrument (Malvern). The pore size, volume, and Brunauer–Emmett–Teller surface area of MSNs were analyzed via nitrogen adsorption/desorption using a 3Flex surface characterization analyzer (Micrometritics). Dual color fluorescence cross-correlation spectrum was obtained via fluorescence correlation spectroscopy (FCS) with a confocal microscope (LSM 780 NLO; Carl Zeiss). FITC-tagged Ty, RITC-conjugated MSN, or T-MSN (RITC-conjugated MSN loaded with FITC-Ty) dispersed in 50 mM Tris-HCl buffer was placed on a coverglass-bottom confocal dish and analyzed. The diffusion time of FITC-tagged Ty, RITC-conjugated MSN, and tyrosinase-loaded MSN was analyzed via FCS with a confocal microscope (LSM 780 NLO).

Measurement of Tyrosinase Activity

The initial activity of the T-MSN complex was determined by measuring the absorbance of adducts of quinones and 3-methyl-2-benzothiazolinone hydrazine at 505 nm via UV-spectroscopy (Tecan infinite m200 pro; Switzerland) [3]. Following this, 1 mM L-tyrosine, 0.3% (w/v) gelatin (Type B; Sigma-Aldrich), and 0.25% (w/v) hyaluronic acid (Lifecore Biomedical, Chaska, MN, USA) were used as enzyme substrates. The initial velocity of the T-MSN complex was calculated using the slope of absorbance and reaction time.

Measurement of Ty-Releasing Kinetics

To determine the releasing kinetics of Ty from T-MSN, T-MSN was dispersed in 50 mM Tris-HCl buffer (pH 7.0) and stirred on an orbital shaker. After centrifuging T-MSN at 18,000 rpm for 15 min, the supernatant was collected at specific times (0.5, 2, 3, 24, 48 h). The

concentration of released Ty was determined using a BCA assay.

Preparation of Poly (Dimethylacrylamide) (PDMA) and Gelatin Substrate

Two different substrates were prepared for the adhesive test. First, the PDMA substrate was prepared as previously described [5]. Briefly, N, N-dimethyl acrylamide (DMA) was dissolved in distilled water and crosslinked using N, N′-methylene bisacrylamide (MBA) (MBA/DMA ratio: 1.0 mol %). Sodium peroxide and N, N, N′, N′-tetramethylethylenediamine were used as redox initiators. After crosslinking at 80 °C overnight, the PDMA substrate was cut into 1 cm (width) × 5 cm (length) for further experiments. Gelatin substrate was prepared following a previous method [6]. Briefly, 23% (w/v) gelatin powder was dissolved in distilled water. The gelatin solution was poured into a Petri dish and crosslinked at room temperature for 30 min. The gelatin substrate was further crosslinked at 4 °C for two days. Both substrates were incubated in distilled water overnight to prevent self-adhesion.

Quantification of Adhesive Properties on Different Substrates

The adhesion test was performed on a Universal Testing Machine (100 N of the load cell; EZ-SX STD, Shimadzu, Japan). Each substrate had dimensions of 1 cm (width) × 5 cm (length). MSN (30 mg/mL), 20 μM Ty, and T-MSN (20 μM of Ty encapsulated in 30 mg/mL of MSN) complex were added to one of the substrates, and the other substrate was put on samples. After 30 min of crosslinking, the lap-shear test was performed with 5 mm/min of the probe speed until complete separation.

In vivo Degradation Test

All animal experiments were approved by the Seoul National University Animal Care

Committee (protocol #SNU-130226-2). Fifty microlitres of MSN (30 mg/mL) or T-MSN (30 mg/mL, encapsulating 20 μM of Ty) were subcutaneously injected into 8-week-old Balb/C mice (OrientBio, Korea). Mice were sacrificed, and the liver, kidneys, spleen, and skin tissues were collected. Skin tissues were fixed with 4% (w/v) paraformaldehyde and sectioned for hematoxylin & eosin staining. To determine the silicon contents in organs and tissues, the collected mice organs were initially degraded with 5 mL of concentrated nitric acid. To fully degrade silica nanoparticles in tissue samples, nitric acid and hydrofluoric acid were added to the samples and heated in a microwave reactor. Inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900) was used to analyze the amount of silicon in digested samples. Samples with silicon below the detection limit of ICP-MS were considered zero to calculate the average amount of silicon in biological tissues.

Statistical Analysis

All data are displayed as mean ± standard deviation. Statistical significance between groups was analyzed using Student's t-test (*$p<0.05$, **$p<0.01$, ***$p<0.005$)

	Mesitylene added (ml)	BET area [m^2/g]	Total Pore volume [cm^3/g] [a]	Tyrosinase-accesible pores [cm^3/g] [a]	Pore size (nm)[b]	Average pore size (nm)[C]
MSN-1	0	257.43	0.138	**0.0167**	2.14	2.1 ± 0.8
MSN-2	0.5	266.93	0.167	**0.0204**	2.41	2.4 ± 0.9
MSN-3	1	307.33	0.308	**0.0574**	3.09	3.2 ± 0.9
MSN-4	1.5	383.85	0.395	**0.1152**	4.87	4.9 ± 3.0

Table S1. Result of gas adsorption analysis of MSNs with various pore sizes. [a]Calculated from BJH adsorption pore distribution. [b]Pore size of maximum dV/dlog(D) pore volume. [c]Measured from TEM images.

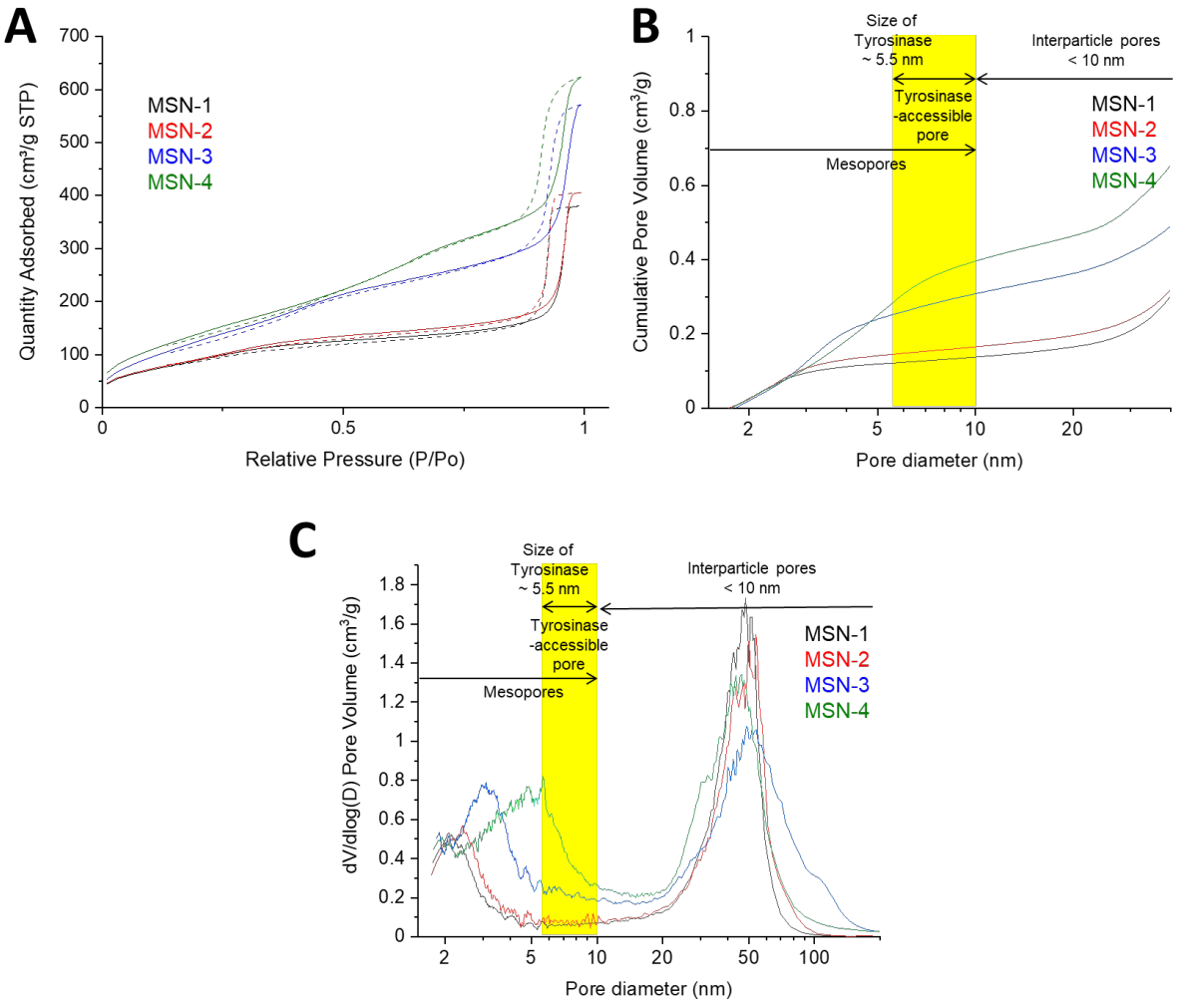

Figure S1. Gas adsorption analysis of MSN. (A) Adsorption and desorption curves of nitrogen depending on the pressure. (B) Cumulative pore volumes depending on the pore diameter. (C) Distribution of pore volume. Pores estimated over 10 nm are considered interparticle pores. The region highlighted in yellow is the pore size between 5.5 and 10 nm, where tyrosinase can be accessible.

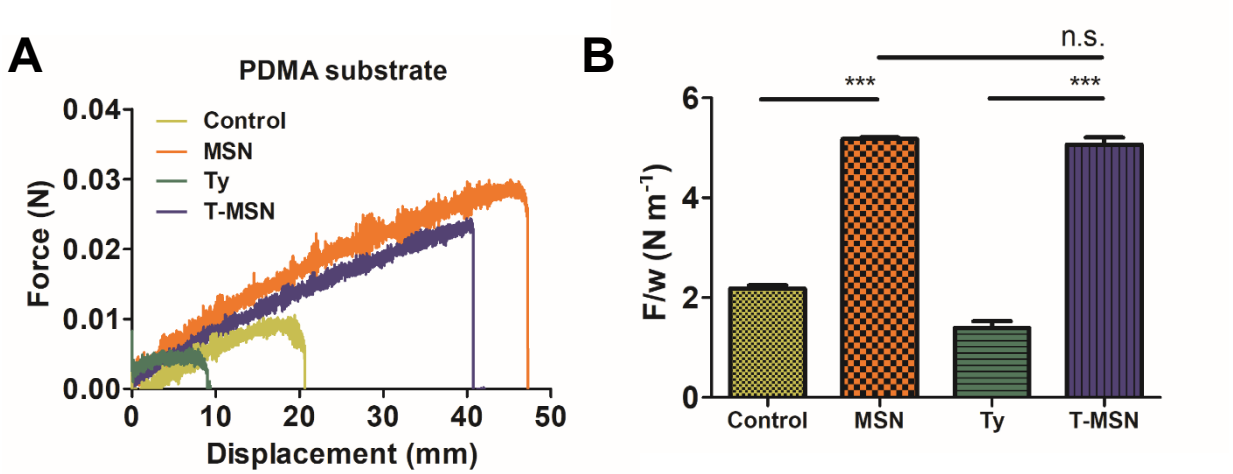

Figure S2. Adhesion properties of each sample on the PDMA substrate. (A) Stress-strain curve of adhesion test. (B) Quantification of adhesion force per width.

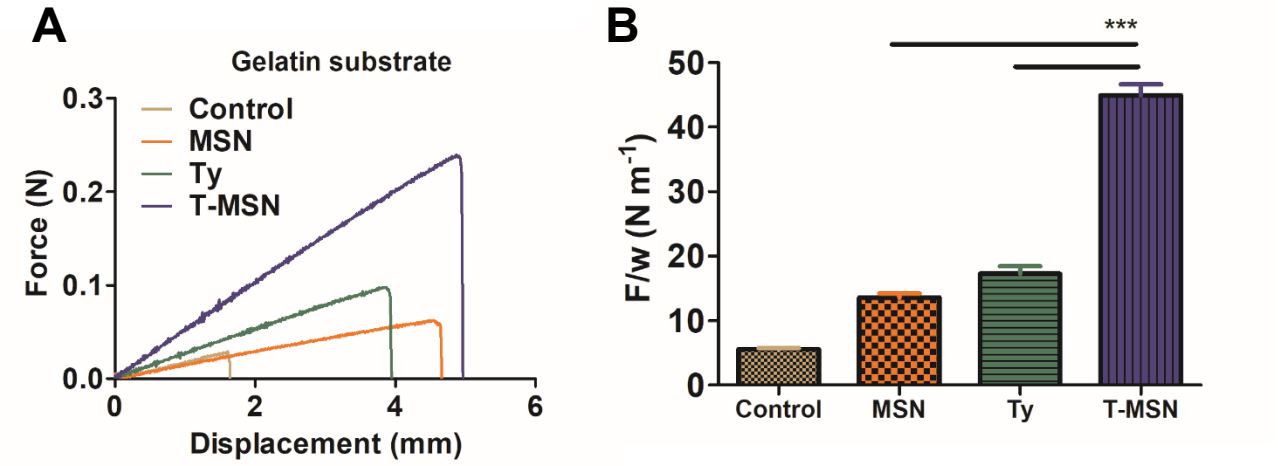

Figure S3. Adhesion properties of each sample on the gelatin substrate. (A) Stress-strain curve of adhesion test. (B) Quantification of adhesion force per width.

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