

Supplementary information

Surface-initiated enzymatic vinyl polymerization: synthesis of polymer-grafted silica particles using horseradish peroxidase as catalyst

Haruka Fukushima, Michinari Kohri,* Takashi Kojima, Tatsuo Taniguchi, Kyoichi Saito, Takayuki Nakahira

Experimental Section

Materials

SiO₂ particles were obtained from Nissan Chemical Industries, Ltd. Horseradish peroxidase (HRP: 450 units/ mg powder), hydrogen peroxide (H₂O₂: 30 wt%), acrylamide (AAM), and hydrogen fluoride (HF) were obtained from Wako Pure Chemical Industries, Ltd. All chemicals and solvents were of reagent grade and were used as received.

Measurements

Scanning electron microscopy (SEM) micrographs were obtained using a scanning electron microscope (JSM-6510A; JEOL) operated at 15–20 kV. Transmission electron microscopy (TEM) micrographs were obtained using a transmission electron microscope (H-7650; Hitachi). Gel permeation chromatography (GPC), using a Shodex OHpak SB-805 HQ column (Showa Denko KK) with aqueous 0.1 M NaNO₃ as a mobile phase at a flow rate of 0.4 mL/min at 40°C detected by refractive index detector. Polyethylene oxide standards with M_w/M_n of less than 1.2 are typically used to calibrate the GPC. Thermogravimetric analysis (TGA) was performed under air at heating rate of 10°C/min using thermogravimetry/ differential thermal analyzer (TG8120; Rigaku). The hydrodynamic diameter (D_h) of the particles in water was measured by dynamic light scattering (DLS) (LPA-3100; Otsuka Electronics Co. Ltd.). Shell layer thickness was calculated according to the following formula:

$$\text{Shell layer thickness} = [(D_h \text{ of SiO}_2\text{-}g\text{-PAAm}) - (D_h \text{ of SiO}_2\text{-}\beta\text{-diketone})] / 2$$

Synthesis of 3-(triethoxysilyl)propyl 3-oxobutanoate (TESPOB)

3-(Triethoxysilyl)propyl 3-oxobutanoate (TESPOB) was synthesized by modifying the procedure of Kim *et al.*¹: instead of trimethoxysilanes, triethoxysilanes were used as starting materials. One equivalent of allylacetoacetate (5.0 g, 35 mmol) and 1.25 equivalents of triethoxysilane (7.2 g, 44 mmol) were mixed together in anhydrous toluene (100 mL) under argon. Karstedt's catalyst (400 ppm) was then added into the reaction mixture. After stirring for 1 day at 60°C, the reaction mixture evaporated, affording TESPOB (10.6 g, 34.5 mmol) in a 88% yield. ¹H-NMR (400 MHz, CDCl₃): δ4.10–4.05 (t, 2H), 3.82 (m, 6H), 3.41 (s, 2H), 2.23 (s, 3H), 1.75–1.70 (m, 2H), 1.20 (t, 9H), 0.65–0.65 (m, 2H).

Preparation of silica particles bearing β-diketone groups (SiO₂-β-diketone)

To SiO₂ particles (2.0 g) in THF (50 mL) was added TESPOB (1.7 g, 5.6 mmol) in a dropwise manner at room temperature. After stirring for 24 h at 70°C, the obtained particles were separated and purified repeatedly by centrifugation (7,500 rpm, 15 min) and redispersion by water.

The grafting density of TESPOB on the SiO₂ surface was determined using equation (1).²

$$\sigma_{\beta\text{-diketone}} \left[\text{molecules}/\text{nm}^2 \right] = \frac{W_{\text{SiO}_2-\beta\text{-diketone}}}{100 - W_{\text{SiO}_2-\beta\text{-diketone}}} \times 100 - W_{\text{SiO}_2} \times \frac{6.02 \times 10^{23}}{10^{18}} \quad (1)$$

$W_{\text{SiO}_2-\beta\text{-diketone}}$, W_{SiO_2} ¹⁾: the weight loss percent of SiO₂-β-diketone and SiO₂

M : the molecular weight of organic component (144 g/mol)

S_{spe} : the specific surface area of SiO₂ particles (27 m²/g)

1) Measured by TGA.

Surface-initiated HRP-mediated polymerization

AAM (0–500 mM), HRP (25 μM), deionized water (4.0 mL), and 1 mL of a 5 wt% SiO₂-β-diketone particle solution, were placed in a sample tube. The mixture was deoxygenated by purging with argon for 15 min. To this was added aqueous H₂O₂ solution (25 μM). The mixture was incubated at room temperature with gentle stirring. After 24 h, the particles were separated and purified repeatedly by centrifugation (13,500 rpm, 15 min) and redispersion.

To measure the molecular weight of the graft polymer, PAAM chains were cleaved from the SiO₂ surface. SiO₂-g-PAAM particles were dispersed in H₂O, to which 48 wt% HF aqueous solution (final percentage: 10 wt%) was added, and the solution was allowed to stand at room temperature overnight. The obtained PAAM chains were purified and concentrated using an Amicon Ultra 10 kDa centrifuge tubes (Millipore Co.), and then lyophilized. Molecular weights of grafted polymers were determined by GPC analysis. These results coupled with TG analysis, lead to the determination of the grafting density of grafted PAAM and of the initiator efficiency using equation (2).² The number average molecular weight (M_n) was used as a molecular weight of the PAAM.

$$\sigma_{\text{PAAM}} \left[\text{chains}/\text{nm}^2 \right] = \frac{W_{\text{SiO}_2\text{-g-PAAM}}}{100 - W_{\text{SiO}_2\text{-g-PAAM}}} \times 100 - W_{\text{SiO}_2} \times \frac{6.02 \times 10^{23}}{10^{18}} \quad (2)$$

$W_{\text{SiO}_2\text{-g-PAAM}}$, W_{SiO_2} ¹⁾: the weight loss percent of SiO₂-g-PAAM and SiO₂

M_n ²⁾: the number average molecular weight of cleaved PAAM component

S_{spe} : the specific surface area of SiO₂ particles (27 m²/g)

1) Measured by TGA.

2) Measured by GPC.

References

1. R. Ganesan, H. J. Lee and J. B. Kim, *Langmuir*, 2009, **25**, 8888–8893.
2. R. Inoubli, S. Dagréou, M. H. Delville, A. Lapp, J. Peyrelasse and L. Billon, *Soft Matter*, 2007, **3**, 1014–1024.

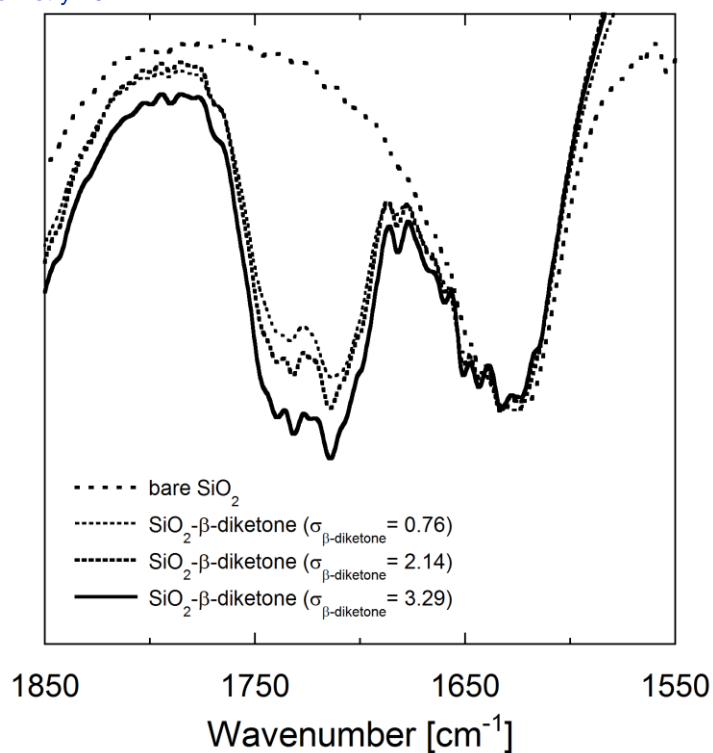


Fig. S1 IR spectra of bare SiO₂ and SiO₂-β-diketone particles.

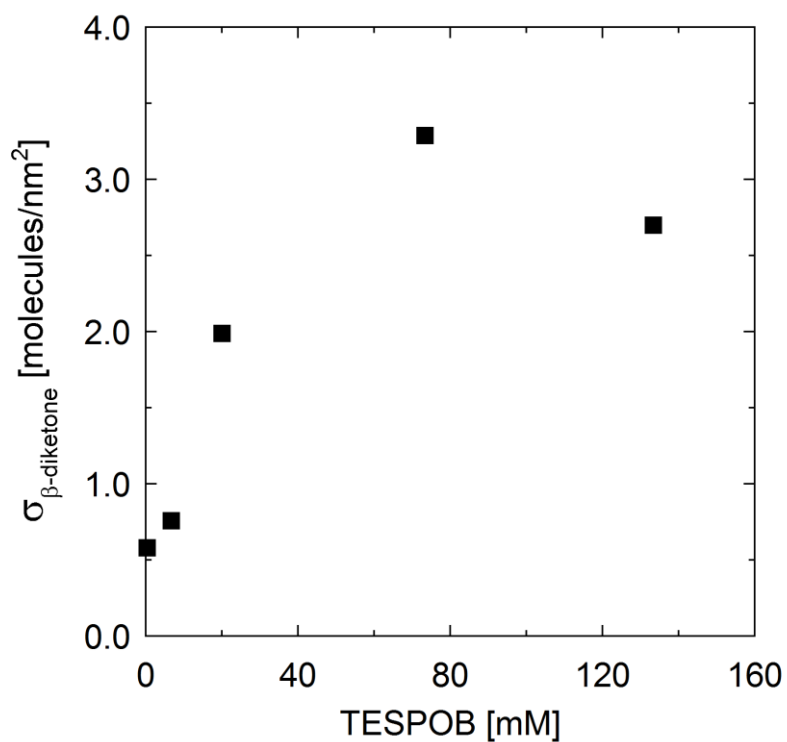


Fig. S2 Initiator site graft densities of SiO₂-β-diketone particles ($\sigma_{\beta\text{-diketone}}$) as a function of TESPOB concentration employed for immobilization.

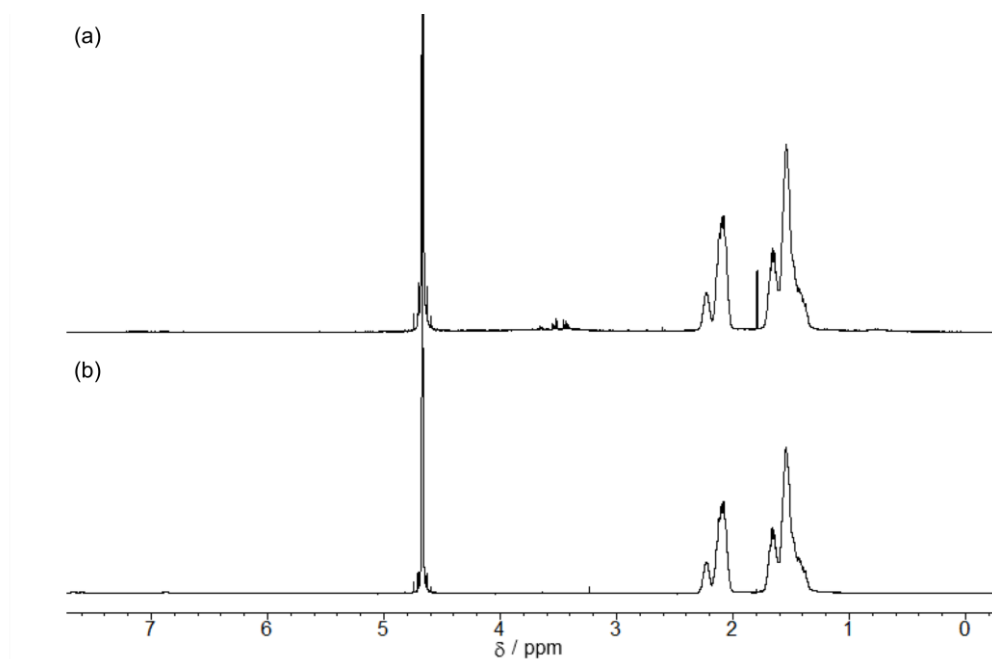


Fig. S3 $^1\text{H-NMR}$ spectra of (a) cleaved polymer from $\text{SiO}_2\text{-g-PAAm}$ using HF treatment and (b) PAAm in D_2O . PAAm was prepared by solution polymerization.

Table S1. Condition of HRP-mediated polymerization using Eaa as an initiator and the conversion and the number average of molecular weight of PAAm

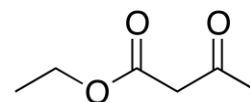
Entry	Eaa[mM]	Yield ^{a)} [%]	α ([H ₂ O ₂]/[HRP])	M_n^b [g/mol]	M_w/M_n^b
1	0	n.d. ^{c)}	n.d. ^{c)}	n.d. ^{c)}	n.d. ^{c)}
2	10	81	100	380,000	2.1
3	50	85	100	183,000	2.8
4	100	84	100	127,000	2.5
5	200	84	100	108,000	2.6

[AAm]: 1 M, [Eaa]: 10 mM, [HRP]: 0.025 mM, water: 2 mL, 30°C, 24 h.

^{a)} Measured by the weight method.

^{b)} Measured by GPC.

^{c)} Not determined.



ethyl acetoacetate (Eaa)