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SUPPLEMENTARY INFORMATION

Highly-Stable Magnetically-Separable Organic-Inorganic Hybrid Microspheres for Enzyme Entrapment

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EXPERIMENTAL METHODS

1. Materials

Polyvinylpyrrolidone (PVP) (Mw ~1,300,000, Sigma-Aldrich), alginic acid sodium salt (Sigma), Calcium Chloride (Aldrich), tetramethyl orthosilicate (TMOS) (Aldrich), 3-aminopropyltriethoxysilane (APTES) (Sigma-Aldrich), glutaraldehyde (Aldrich), N, N-dimethylformamide (DMF) (99 %, Sigma-Aldrich), *p*-nitrophenyl butyrate (Aldrich), Dynabeads M-270 Amine (Invitrogen) were purchased and used without any additional purification. Esterase from *Rhyzopus oryzae* used for enzyme encapsulation was purchased from Biochemik Chemicals (Aldrich).

2. Preparation of enzyme entrapped electrospun organic microspheres

Prior to organic microspheres formation, esterase enzyme solutions were prepared at 2 mg/mL 100 mM phosphate buffer (pH 8.0). The enzyme solution mixed with $5*10^8$ number of amine functionalized magnetic beads. For enzyme coating on magnetic beads, 1.0 % of glutaraldehyde was added and incubated at 200 rpm at room temperature for 30 minutes. After the reaction esterase coated magnetic beads solution was washed three times with D.W for removal of residual enzyme solution. As organic microspheres, electrospun PVP-Alginate blended polymers were used according to normal alginate based microencapsulation. 4 wt % of PVP and 1 wt % of alginic acid salt was dissolved in D.W and then sonicated for 1 hr at 25 ^oC. The polymer solution was mixed with enzyme coated magnetic beads and then incubated for 30 min at room temperature. An aliquot from the above prepared polymer with enzyme coated magnetic beads solution was loaded into a 10 mL syringe. Electrospray was performed at 13 kV high voltage power supply (Gamma high voltage Research, ES series), with 7 cm distance between tip to 100 mM of calcium chloride solution, and a 30 gauge syringe needle fixed vertically with a flow rate set at 2.5 mL/h. The electrospun enzyme

coated magnetic beads encapsulated organic microspheres thus obtained were collected in the calcium chloride solution for short time operation. For hardening, the prepared microspheres were shaken for 3 hr at room temperature and stored at 4 ^{o}C for 1 day prior to perform silica coatings.

3. Preparation of silica coating on organic microspheres

The magnetic separable organic microspheres were coated with silica by the modified sol-gel process of 3-aminopropyltriethoxysilane (APTES) and tetramethyl orthosilicate (TMOS) to obtain the organic-inorganic hybrid microspheres. The microspheres solutions were mixed with n-hexane, then stirred for 2 min. After the addition of 3-aminopropyltriethoxysilane (APTES), additional 5 min of shaking is followed, and then tetramethyl orthosilicate (TMOS) was added to mixed solution. The volumn ratio of microsphere : n-haxnae : APTES : TMOS was 25:50:4:3. The ratio between APTES and TMOS in this study was optimized depending on the forming of silica layer coating from encapsulated enzyme stability. For stable coating, 2 hr shaking was followed and then to remove the residuals which was not coated silica on microspheres, then D.W was used continual washing for 5 times. The enzyme entrapped microspheres were stored at 4 ^{o}C before they are used.

4. Characterization of the synthesized hybrid microspheres in this study

Brightfield micrography iamge taken from Carl Zeiss LSM 510 microscope (Carl Zeiss Co. Ltd., Germany) was used to examine the microspheres. The morphology and elemental analysis of the microspheres were investigated by scanning electron microscopy (SEM;S-2360N equiped with Energy-dispersive X-ray spectroscopy (EDAX), Hitachi Co. Ltd., JAPAN). Fourier transform infrared (FT-IR) Spectrums of the microspheres were measured on Infinity gold FT-IR spectrometer (Thermomattson. Co. Ltd., USA) using ATR acessory.

5. Enzyme activity and stability measurement

The bioactivity of the enzyme entrapped organic-inorganic hybrid microspheres were checked by monitoring the production of *p*-nitrophenol from the hydrolysis of p-nitrophenyl butyrate dissolved in N, N-dimethylformamide. In brief, 4.95 mL of 100 mM phosphate buffer (pH 6.5) containing 50 μL of 50 mM of *p*-nitrophenyl butyrate dissolved in N, N-dimethylformamide was prepared as a substrate. The entrapped esterase was dispersed in above substrate solution and was shaken at 200 rpm. After short reaction, initial activity was calculated from A400 nm vs time, and the absorbance was equated with the concentration of *p*-nitrophenol. For enzyme stability experiments, after measuring the activity, entrapped esterase in organic-inorganic hybrid microspheres was washed with D.W water three times for next measurements and stored at room temperature in D.W water. All measurements were performed in triplicate for standard deviator. One unit (U) of esterase activity is defined as the amount of enzyme releasing 1 μmol *p*-nitrophenol per minute under the assay conditions. All samples

were performed in triplicate for error analysis. The standard deviations for the results are shown as error bars within the graphs.

SUPPORTING DATA

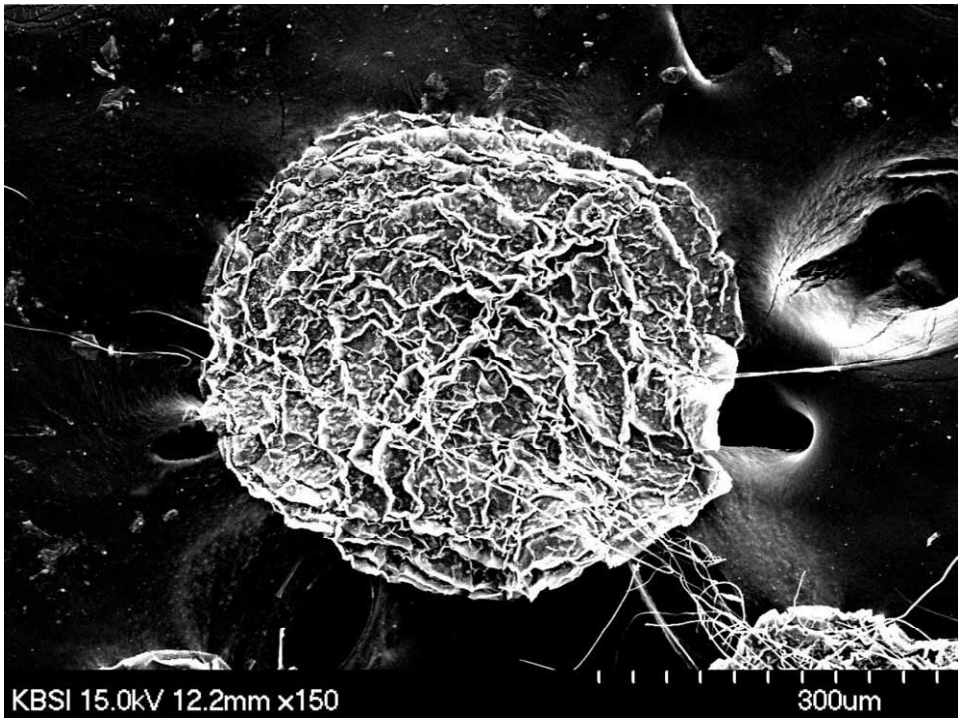
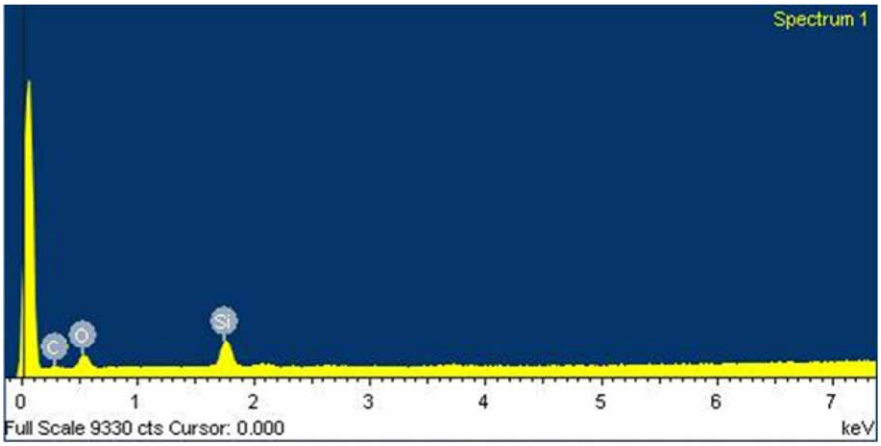


Fig. S1 SEM Image of organic-inorganic hybrid microspheres



Element	Weight %	Atomic %
C	31.46	40.54
O	52.11	50.41
Si	16.43	9.05
Totals	100.00	

Fig. S2 EDX Spectrum of organic-inorganic hybrid microspheres

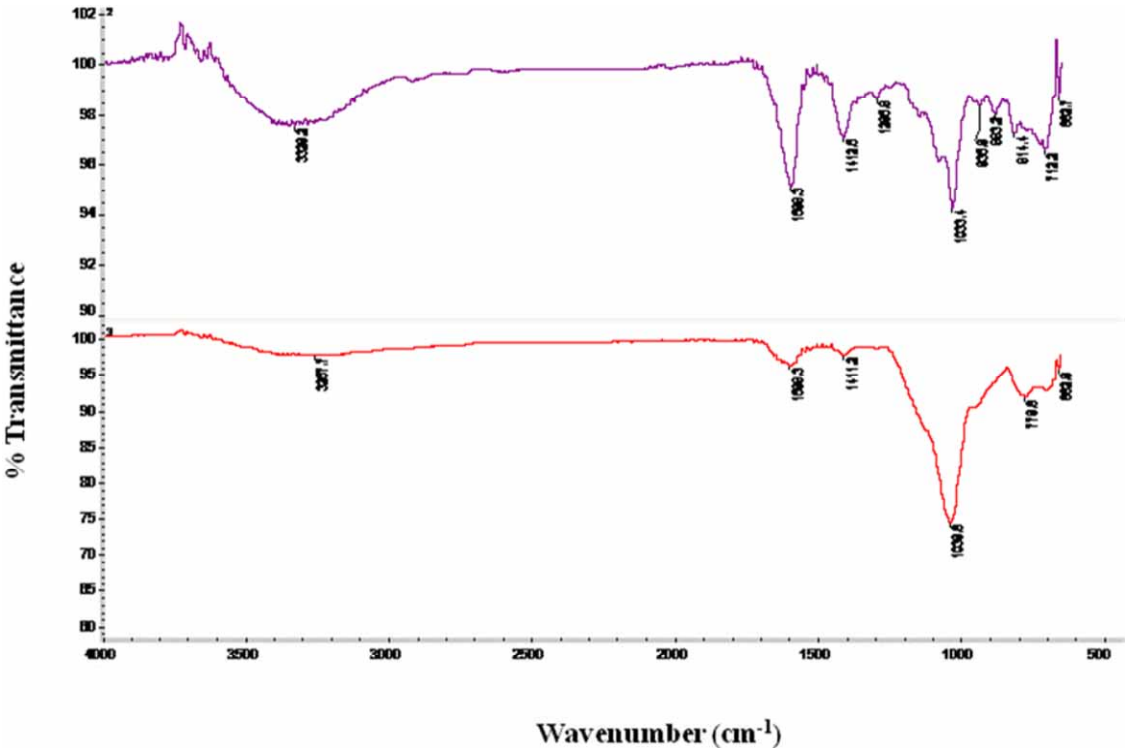


Fig. S3 FT-IR spectrum of organic microspheres and organic-inorganic hybrid microspheres

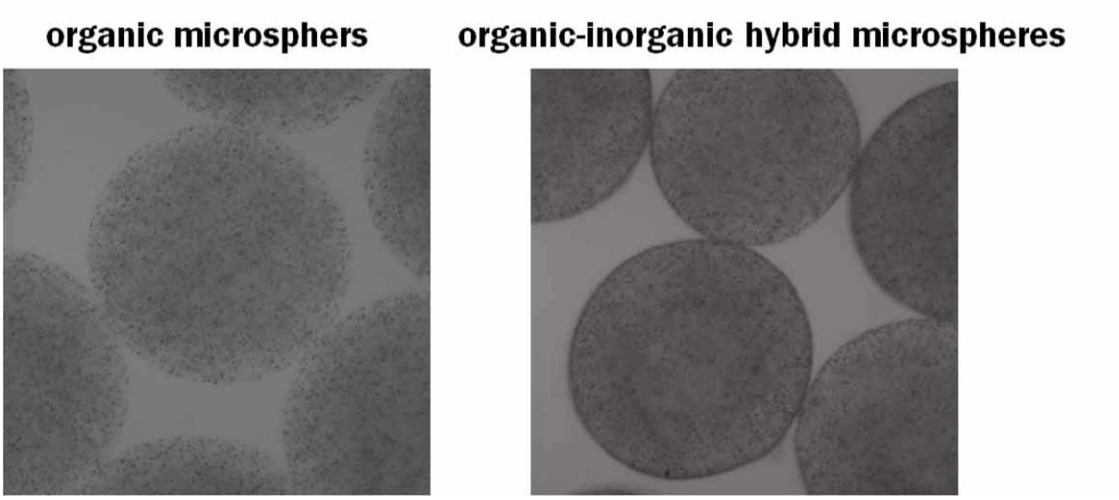


Fig. S4 Microscope Image of magnetic separable organic-inorganic hybrid microspheres and magnetic separable organic microspheres

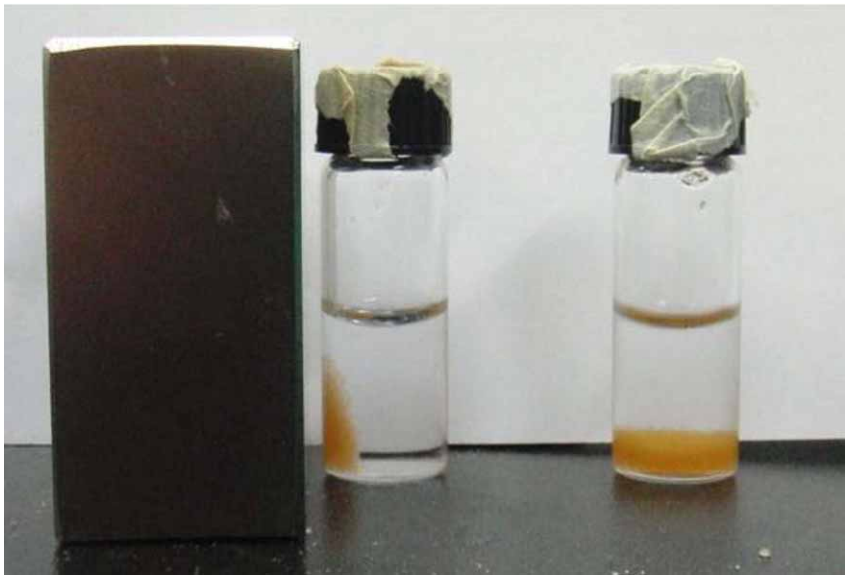


Fig. S5 Digital image of magnetic separable organic-inorganic hybrid microspheres with magnet separation

	free esterase	esterase entrapped in magnetic separable organic-inorganic hybrid microsphers
Km (mM)	**0.3847**	**0.3526**
Vmax (mM/min)	**0.1364**	**0.0184**

Table S1 Summary of the Lineweaver–Burke plot for the free esterase, esterase entrapped in magnetic separable organic-inorganic hybrid microspheres