Supplementary information

Enzyme immobilization on 3D-printed reactor for aldehyde oxidation to carboxylic acid under mild conditions

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1. Experimental procedure

1.1 General

Commercial grade solvents, reagents, and chemicals for cell cultivation, enzyme purification, immobilization, and activity assay were purchased from Nacalai Tesque (Japan), Fujifilm Wako Pure Chemical (Japan), Sigma Aldrich (USA), and Tokyo Chemical Industry (Japan), and used without purification. *Gc*ALDH wild type was prepared according to the previous procedure.[1] The protein concentration was measured by Bradford method[2] using the reagent purchased from Bio-Rad (USA). Dopamine hydrochloride was purchased from Merck (USA). Glutaraldehyde (ca. 50% in water, ca. 5.6 mol/L) was purchased from Tokyo Chemical Industry (Japan). Poly(ethyleneimine) solution (average Mn ~ 60,000 by GPC, average Mw ~ 750,000 by LS, 50 wt.% in H_2O) was purchased from Sigma Aldrich (Japan). Reactors were fabricated using a 3D-printer (Value3D MagiX MF-2200D equipped with tip diameter 0.4 mm MF-2200D printhead), 3D-printing filament (Value3D MagiX material PP filament 1.75 mm 500 g reel), and 3D stage sealant ATP-101 from Mutoh (Japan). Ultraviolet-visible spectroscopic analysis was conducted in a UV-1900-UV-Visible spectrophotometer from Shimadzu (Japan). Scanning electron microscope (SEM) analysis was conducted using Bench-top Scanning Electron Microscope proX from Phenom-World (Netherlands).

1.2 Fabrication of 3D-printed reactor

The reactor (inside diameter: 2.4 cm, inside height: 0.8 cm) was designed by CAD programs (Autodesk Tinkercad and Autodesk Fusion 360) and was fabricated by the 3D-printer with nozzle temperature 235 ℃ and bed temperature 95-100 ℃.

1.3 Surface functionalization of the 3D-printed reactor

The part of the inside surface of the reactor (6.2 cm^2) was functionalized using a similar method to the previous studies.[3–5] All procedure was conducted at room temperature. The 3D-printed reactor was washed with excess acetone and water, dried, treated with dopamine (DA, 2 mg/mL, 1.0 mL) in Tris-HCl buffer (10 mM, pH 8.5) for 24 h, washed with distilled water, treated with glutaraldehyde (GA, 25% v/v, 1.0 mL) in distilled water for 3 h, washed with distilled water, treated with polyethyleneimine (PEI, 5% w/v, 1.0 mL) in distilled water for 2 h, washed with distilled water, treated with additional GA (25% v/v, 1.0 mL) in distilled water for >18 h, and washed with distilled water. The above procedures were repeated using different concentrations of DA (1 mg/mL, 4 mg/mL, 8 mg/mL), GA (1%, 12.5%, 50%), PEI (1%, 10%, 20%) or additional GA (1%, 12.5%).

1.4 *Gc*ALDH immobilization

Enzyme solution (1 mg/mL, 1.0 mL) was incubated in the reactors ((1) untreated, (2) PDA coated, (3) PDA and GA coated, (4) PDA, GA, and PEI coated, and (5) PDA, GA, PEI, and additional GA coated reactors) at 4 ℃ for >18 h. The solution with unbound enzyme was removed from the reactor, and measured protein concentration by Bradford method.[2] The reactors were washed with NaCl (0.85%) at 4 ℃ for 2 h. The above procedures were repeated using enzyme solutions of 0.25 mg/mL, 0.50 mg/mL, and 1.5 mg/mL and PDA/GA coated reactor.

1.5 Enzyme activity assay

1.5.1 Free enzyme

All solutions used for the assay were placed in an oven at 37 ºC for >15 min. Benzaldehyde solution (10 mM, 500 μl) in HEPES-NaOH buffer (pH 7.2, 100 mM) and HEPES-NaOH buffer (pH 7.2, 100 mM, 470 μl) were added to a UV cell. Then, an enzyme solution (0.5 mg/ml, 20 μl) and NAD^+ solution (10 mM, 10 μl) were added. The absorbance at 340 nm was recorded after 1, 2, and 3 min at 37 ºC. One unit of enzyme is defined as μmol of NADH produced in 1 min under the above conditions. All measurements were duplicated.

1.5.2 Immobilized enzyme

All solutions used for the assay and the reactor with immobilized enzyme were placed in an oven at 37 ºC for >15 min. Benzaldehyde solution (10 mM, 550 μ) in HEPES-NaOH buffer (pH 7.2, 100 mM), HEPES-NaOH buffer (pH 7.2, 100 mM, 539 μL), and NAD^+ solution (10 mM, 11 μL) were added to the reactor and kept at 37 ºC for 5 min. Then, 1.0 mL of the reaction mixture was taken, and the absorbance at 340 nm was measured to determine the enzyme activity. All measurements were duplicated. The activity of PDA/GA/*Gc*ALDH reactor was 5.8 ± 1.9 mmol・min^{-1}・(m^2(reactor surface area))$^{-1}$.

1.6 Characterization of the immobilized *Gc*ALDH on the PDA/GA reactor

The free enzyme (0.48 mg/ml, 1.0 mL) in HEPES-NaOH buffer (pH 7.2, 100 mM) was held at 50 °C for up to 180 min. An aliquot (20 mL) was withdrawn to determine the residual activities according to the method in section 1.5.1. The PDA and GA treated reactor with an immobilized enzyme (0.24 mg) was filled with HEPES-NaOH buffer (pH 7.2, 100 mM, 1.0 mL) and held at 50 °C for up to 180 min. The HEPES buffer was discarded, and residual activity was determined according to the method in section 1.5.2.

The recyclability of the immobilized *Gc*ALDH on the PDA/GA reactor was investigated by repeating the activity assay in section 1.5.2. using a recycled bioreactor up to 9 times. The bioreactor was washed with HEPES-NaOH buffer (pH 7.2, 100 mM) 3 times before being used in the next cycle.

The activity assay of the immobilized *Gc*ALDH on the PDA/GA reactor in section 1.5.2. was repeated using substrates **2a**-**15a**.

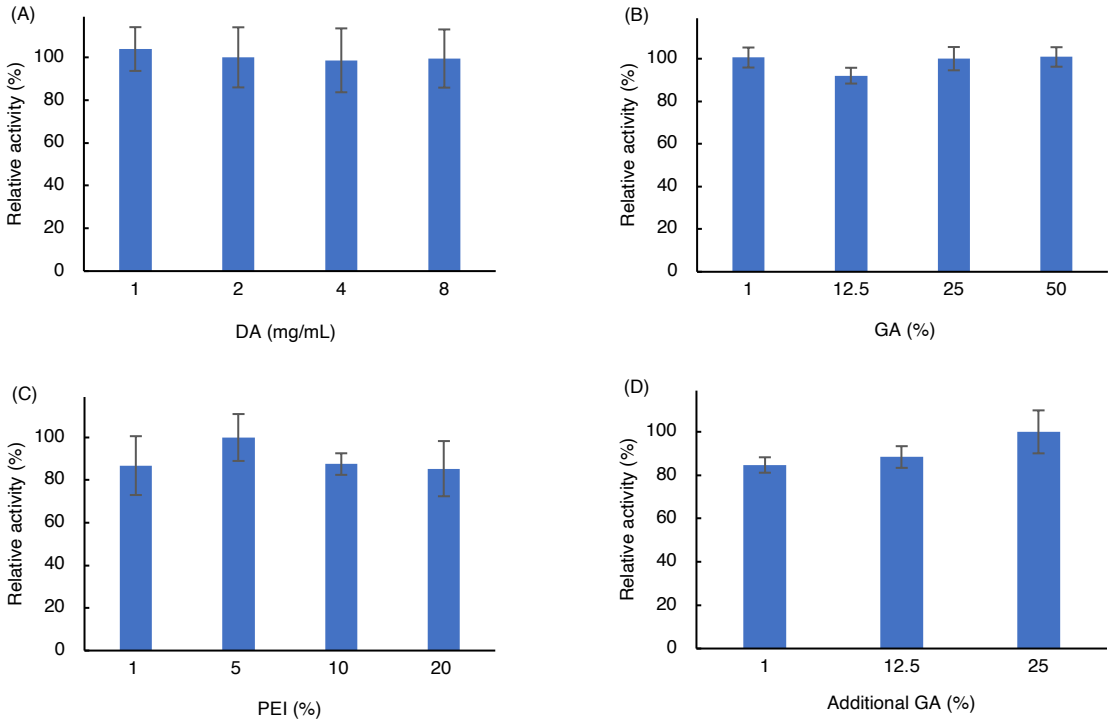

Fig. S1 Effect of the concentrations of (A) dopamine (DA), (B) glutaraldehyde (GA), (C) polyethyleneimine (PEI), and (D) additional GA on the activity of the immobilized enzyme.
The activity of the DA (2 mg/ml)/GA (25 %v/v)/PEI (5 %w/v)/GA (25 %v/v)/*Gc*ALDH was set to 100%. The experimental procedures are described in sections 1.3.-1.5. in the supporting information.

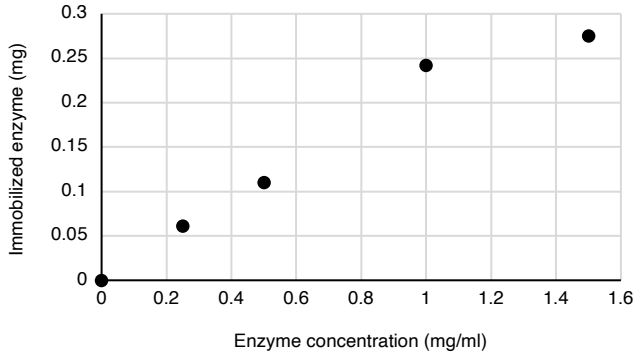

Fig. S2 Effect of the concentration of enzyme solution during the immobilization process on the amount of enzyme immobilized.

The experimental procedures to prepare the immobilized enzyme (PDA/GA/*Gc*ALDH) are described in sections 1.3. and 1.4. in the supporting information.

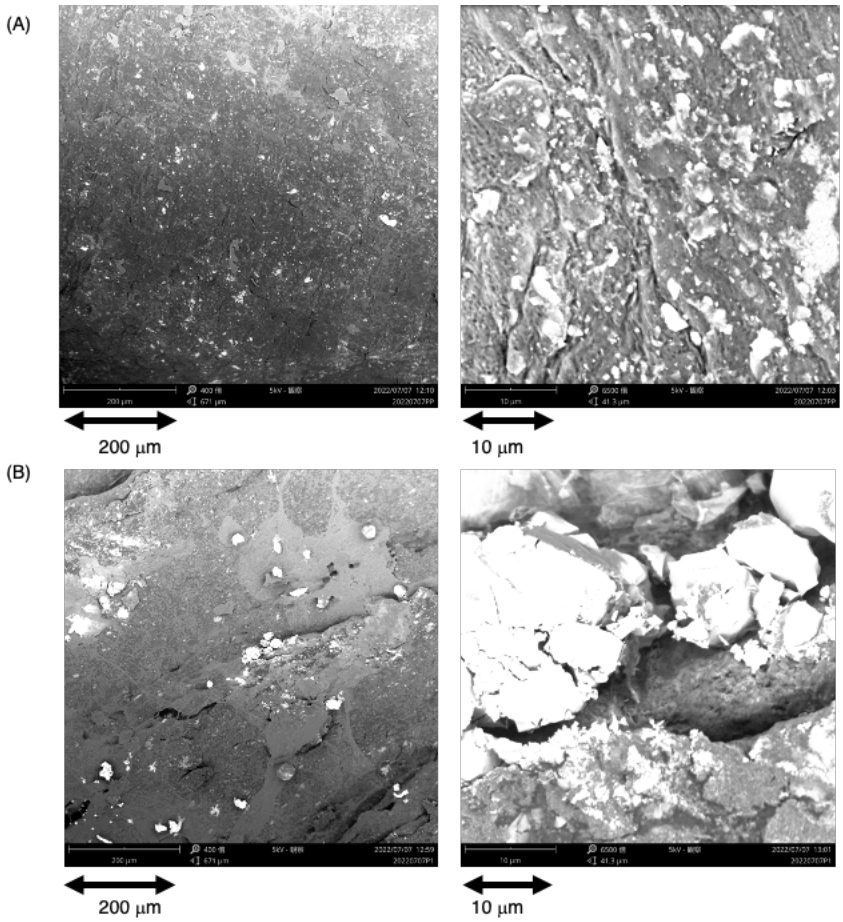

Fig. S3 SEM images of surfaces of (A) untreated PP reactor and (B) PDA, GA and *Gc*ALDH treated reactor.

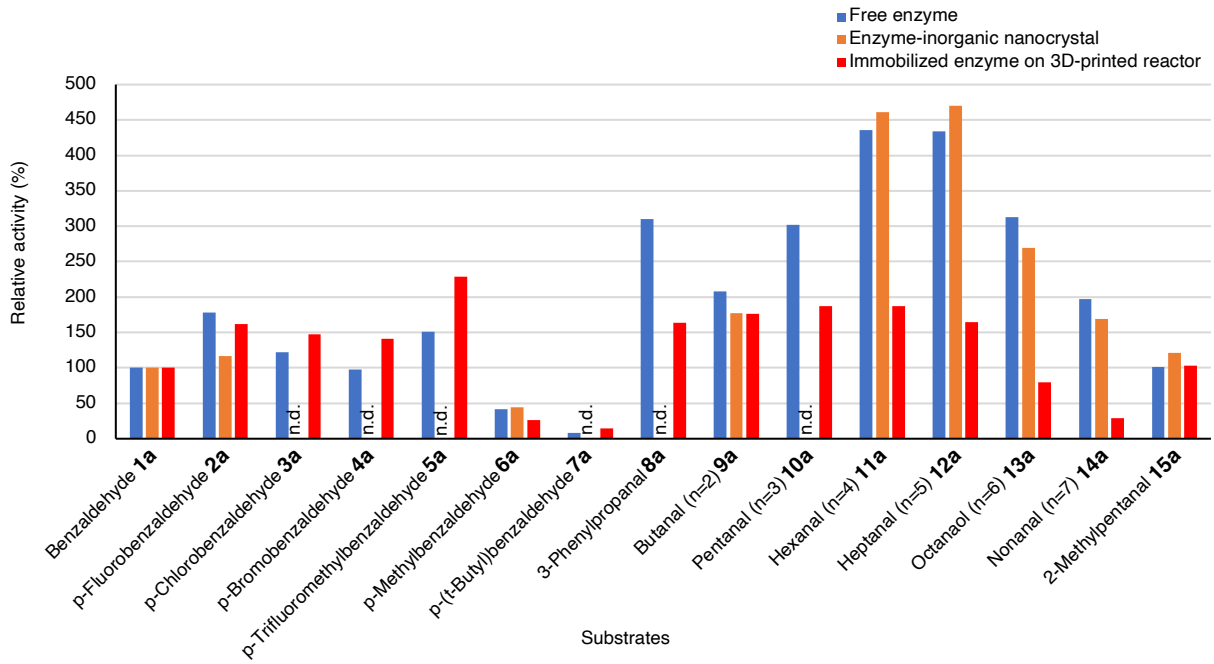

Fig. S4 Comparison of substrate specificity of free *Gc*ALDH, *Gc*ALDH-inorganic nanocrystal, and immobilized *Gc*ALDH on a 3D-printed reactor (PDA/GA/*Gc*ALDH). The enzyme activities of free, nanocrystal, and 3D-printed bioreactor toward benzaldehyde were set to 100%, respectively. The data were taken from references for free *Gc*ALDH,[1] and *Gc*ALDH-inorganic nanocrystal,[6] and from Table 1 for immobilized *Gc*ALDH on a 3D-printed reactor.

References

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