

Supporting Information

Hierarchical porous carbon from cell-assemblies of rice husk for *in vivo* applications

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1. Materials

Rice husks (*Oryza sativa* 'Koshihikari') produced in Toyama Prefecture, Japan, were used as the raw material. The precursor (50 g) was carbonized in a furnace for 1 h at 800 °C to prepare the silica-carbon composite (17 g, yield: 34%). The composite (10 g) was dissolved in 46% hydrofluoric acid solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at room temperature for 12 h to remove the silica content and to fabricate the macroporous structure. After removal of silica, the remaining porous carbon (PC) was thoroughly washed with pure water and ethanol (ca. 6 g for PC, yield: ca. 60%). Hierarchical porous carbon (HPC) was obtained by steam activation of 6.0 g PC at a flow rate of 250 mL/min for 3 h (3.3 g, yield: 56%).

2. Characterization and adsorption measurements

Surface morphology of the materials was observed with a scanning electron microscope (S-4800, Hitachi, Tokyo, Japan). Elemental analysis was carried out with an elemental analyzer system (S-4800 Hitachi, E-MAX420 Horiba). Nitrogen adsorption and desorption were measured using a BELSORP-mini 2 (BEL Japan), after drying each sample at 200 °C for 3 h under nitrogen flow. The specific surface area of each sample was determined according to the Brunauer-Emmett-Teller method at the relative pressure range from 0.01 to 0.20. The total pore volume was determined from the volume of nitrogen adsorbed at the relative pressure of 0.99. Pore size distribution by MP and BJH analysis was estimated from nitrogen desorption isotherms, and mercury porosimetry was performed using Pascal 140 and

440 (Carlo Erba) Instruments. Infrared spectra were measured with an FT-IR spectrophotometer (Avatar 360, Nicolet). The graphitic nature of the carbon materials was characterized by an X-ray diffractometer (XL Labo, MAC Science) with Cu K α radiation.

The adsorption properties of HPC, PC and activated carbon (AC) were examined using 10 mg carbon material in 40 mL aqueous solution (1 M phosphoric acid buffer, pH 7.3) containing acid green 25 (MW 623, Wako Pure Chemical Industries, Ltd., Osaka, Japan) at various concentrations (3.2 to 0.16 mM). After each carbon material was dispersed in aqueous solution, the dispersion mixture was shaken vigorously at 160 rpm for 1 h under precise temperature control at 37 °C. The materials were filtered, and concentration of guest molecules in solution at equilibrium was determined with a spectrophotometer (U-3010, Hitachi).

3. *In vivo* administration of HPC

All animal work in this study was carried out in accordance with the institutional animal protocol guidelines in place at Ina Research Inc., which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, and adheres to universal standards for animal housing and care techniques. This work was also reviewed and approved by the Animal Research Committee. Samples of 5 and 10 wt% HPC and 5 wt% AC were mixed with typical feed (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and then each feed mixture was administered to seven rats at 20 g/day per rat. The weight of the rats was measured at days 1, 3, 7 and 15. Four rats were euthanized after this experiment

to confirm that HPC did not accumulate in the alimentary canal.

Specific pathogen-free rats weighing 207–278 g (9 weeks old) were used for the following experiment. At 10 days after the left kidney was extracted, the right kidney was removed from the rats and the medication experiment was begun. The rats were randomly divided into three experimental groups (n=10 each): 1) control rats administered standard feed; 2) AC rats administered 1.0 g AC with 6 mL water per day; and 3) HPC rats administered 1.0 g HPC with 6 mL water per day. All rats were given free access to their feed and water and housed in individual cages. Blood was drawn from each rat (0.6 mL) 48 h after the start of the medication experiment.

Abnormal substances in the plasma were examined by high performance liquid chromatography (HPLC) using a Waters 2965, 2414 HPLC system. For HPLC, plasma (10 μ L) was automatically introduced to the column (ODP2 HP-4D, Shodex, Tokyo, Japan). Following elution of 1 M phosphoric acid buffer solution (pH 7.4) at a rate of 1.0 mL/min (37 $^{\circ}$ C), UV absorbance (254 nm) was detected. The fractionated peaks at 3.4 and 4.0 min were estimated as uric acid and creatinine and their peak areas were calculated by the Empower system (Waters, Massachusetts, USA).

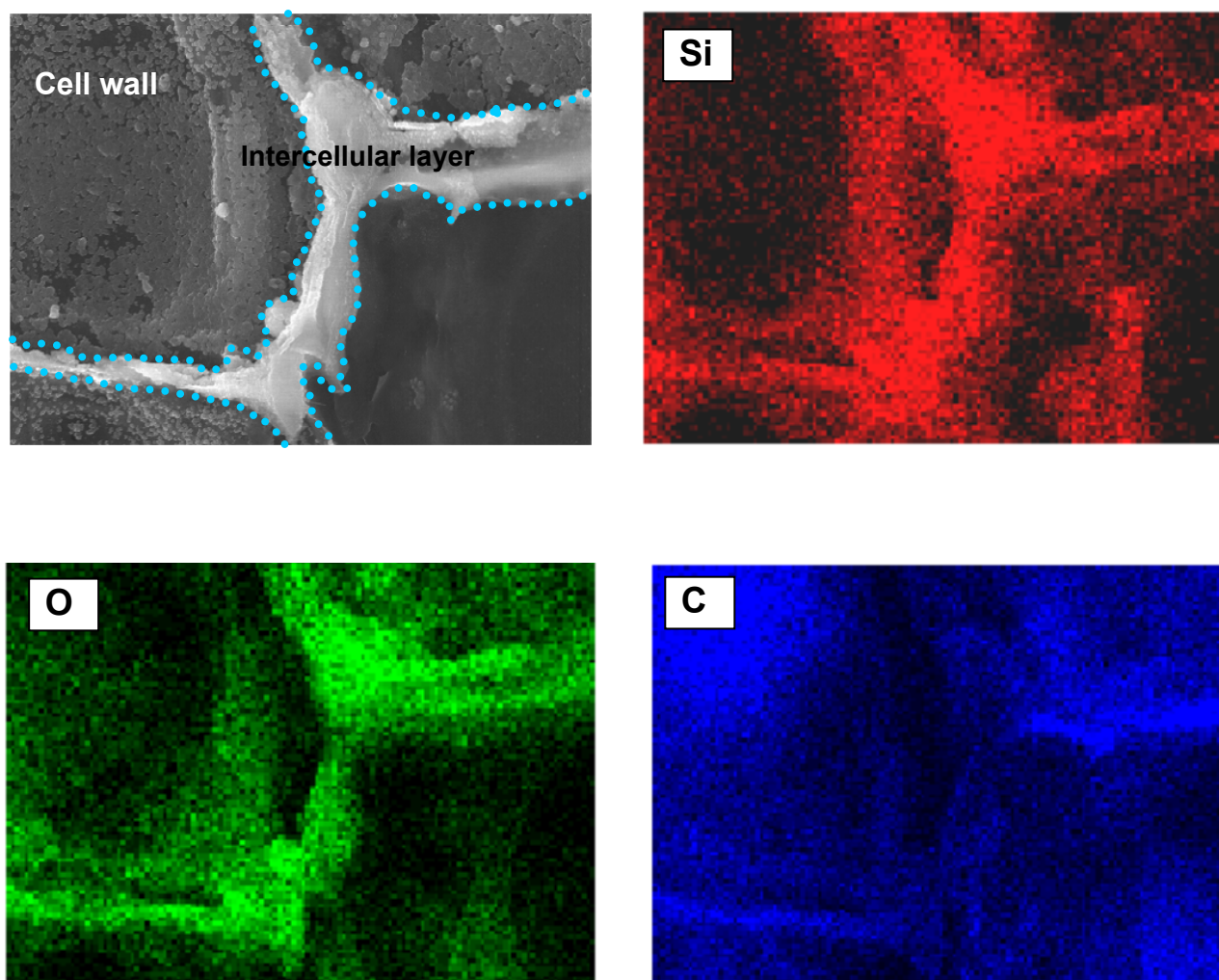


Figure S1 SEM image and elemental mapping indicating accumulation of silica on intercellular layers and cell walls of silica-carbon composite from rice husks.

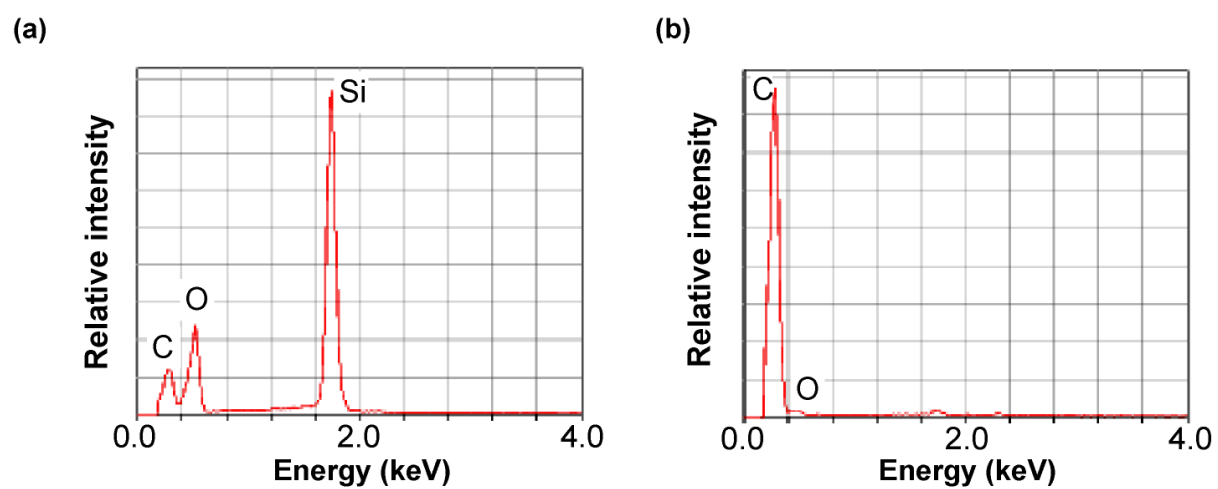


Figure S2 EDX spectra of carbon-silica composite (a) and porous carbon (b) after HF treatment.

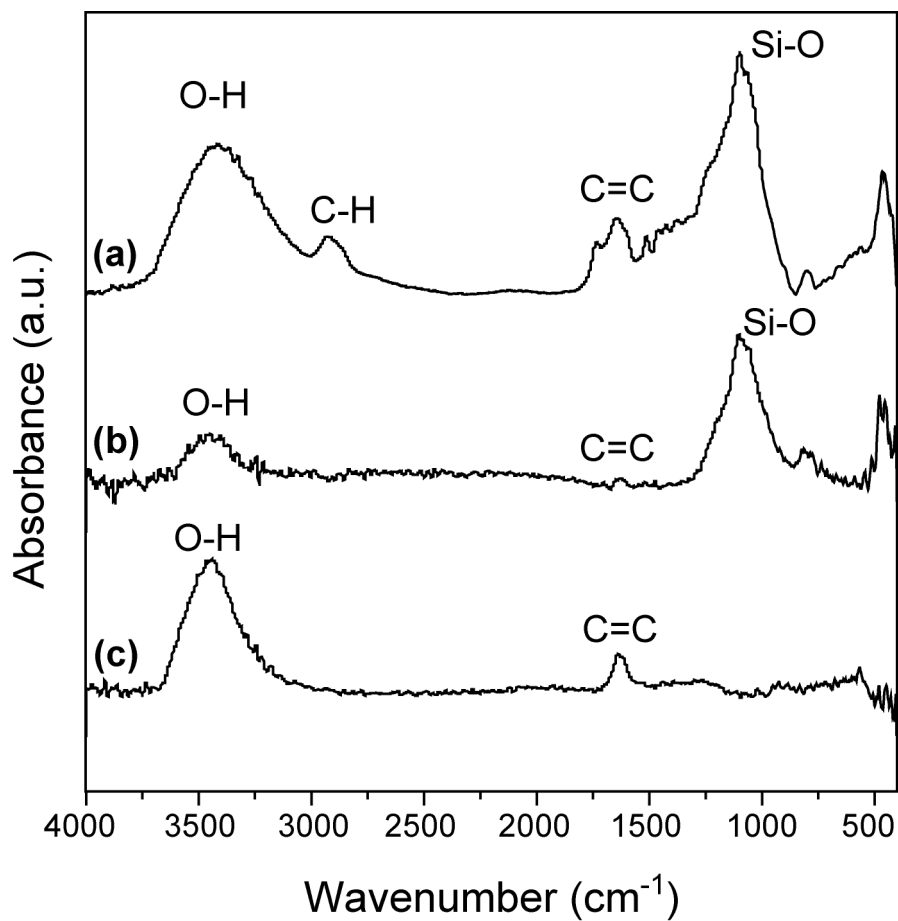


Figure S3 FT-IR spectra of rice husk (a), silica-carbon composite (b), and porous carbon (c).

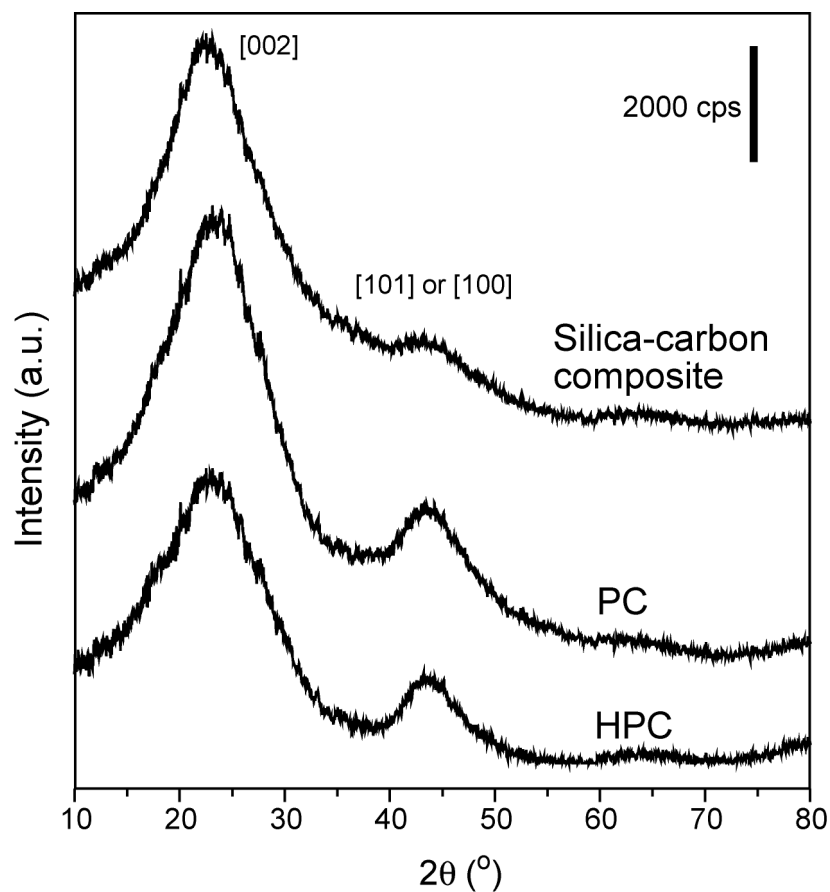


Figure S4 X-ray diffraction patterns for silica-carbon composite, porous carbon (PC) and hierarchical porous carbon (HPC). All samples indicate two broad diffraction peaks located at $2\theta = 22^\circ$ and 43° , corresponding to (002) and (101) or (100) diffractions of the graphitic structure, respectively.

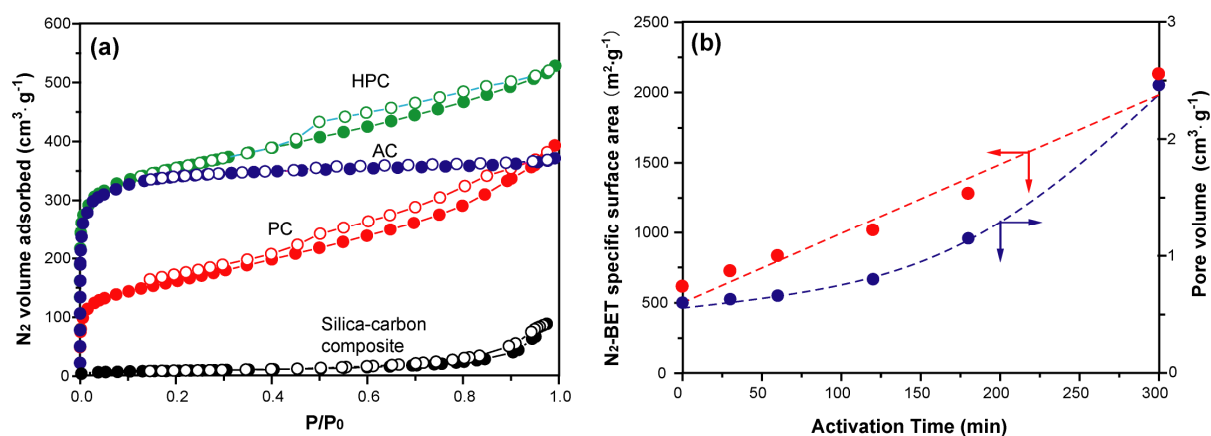


Figure. S5 Nitrogen adsorption and desorption isotherms of HPC (after 3 h of steam activation), porous carbon, silica-carbon composite, and activated carbon (a), and the relationship between pore volume, surface area and activation time (b).

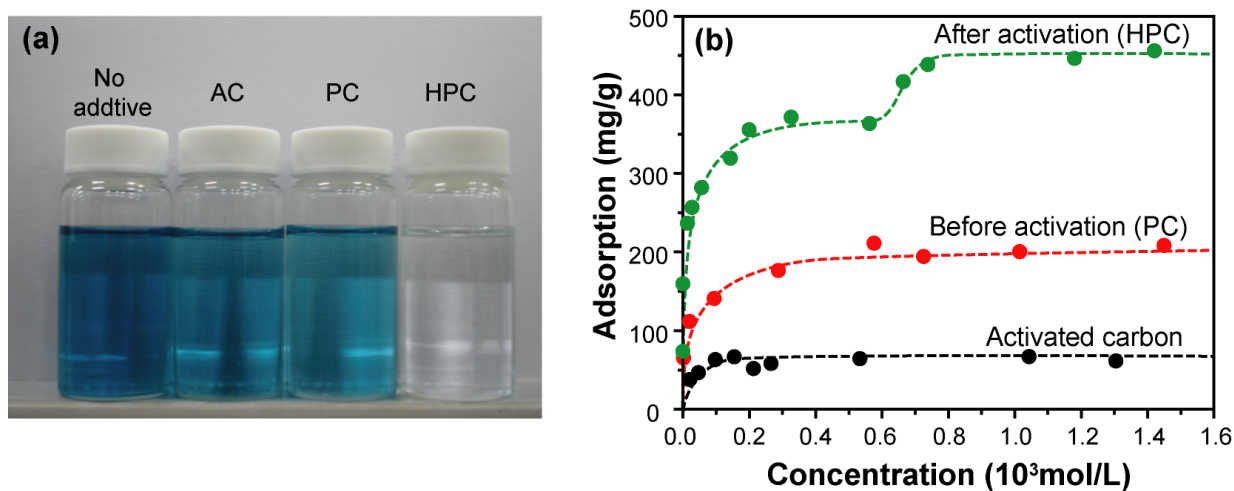


Figure S6 Representative samples of aqueous solutions containing acid green 25 after adsorption measurements (a), and adsorption isotherms of acid green 25 before and after activation (b).

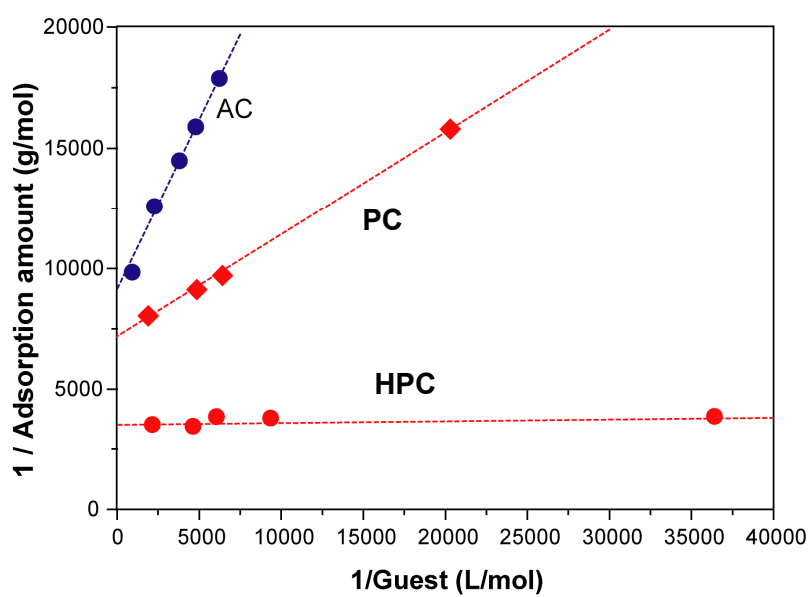


Figure S7 Langmuir plot of hierarchical porous (HPC), porous carbon (PC), and activated carbon (AC).

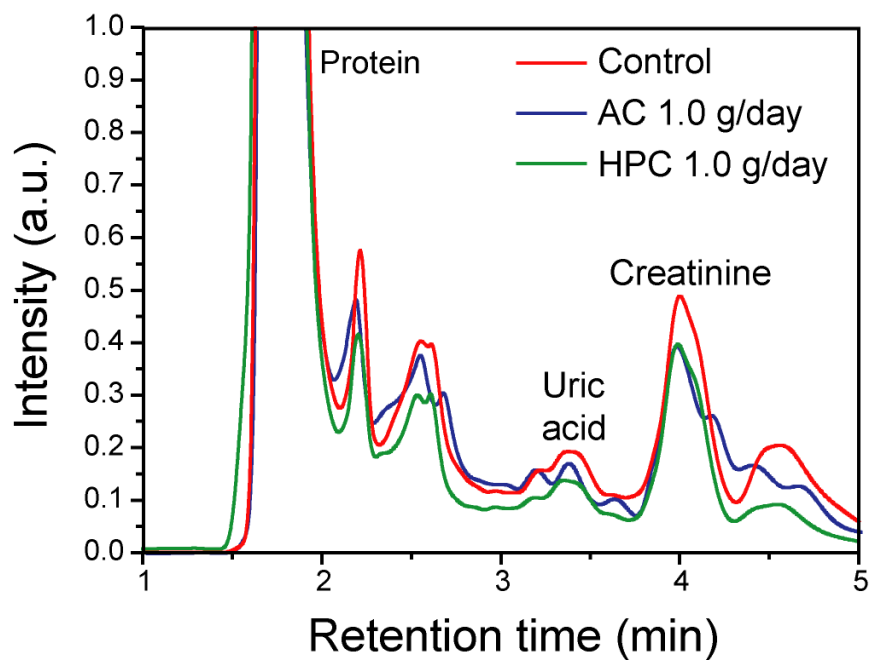


Figure S8 Typical HPLC diagrams of blood plasma of pneumonia renal failure rats administered with feed mixtures containing 5% hierarchical porous carbon (HPC) and 5% activated carbon (AC) compared to feed with no additive after 48 h.