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*Electronic Supplementary Information*

# Hepatocyte Purification Column Using Thermoresponsive Glycopolymer-Modified Silica Beads

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## S.1 Materials

*N*-Isopropylacrylamide (NIPAAm) was provided from KJ Chemicals (Tokyo, Japan). Hexane, *N,N*-dimethylformamide, potassium carbonate, chloroform, methanol, sodium hydroxide, ethanol, diethyl ether, acetone, copper(I) chloride (CuCl), formaldehyde, Tris[(2-dimethylamino)ethyl]amine ($Me_6TREN$), ethylenediamine-*N,N,N′,N′*-tetraacetic acid (EDTA), silica beads (diameter: 64–210 µm, pore diameter: 6 nm, surface area: 475 $m^2/g$; Wakosil® C-200), magnesium sulfate, toluene, and 2-propanol were purchased from Fujifilm Wako Pure Chemicals (Osaka, Japan). (Chloromethyl)phenylethyl-trimethoxysilane (CPTMS) was purchased from Gelest (Morrisville, PA, USA). Hydrazine monohydrate, lactobionic acid, and potassium phthalimido were obtained from Nacalai Tesque (Kyoto, Japan). α-Chloro-*p*-xylene was purchased from Tokyo Chemical Industry (Tokyo, Japan). Chloromethyl styrene was provided by AGC Seimi Chemical (Chigasaki, Japan). Empty columns (total volume: 1.5 mL, inner diameter: 0.6 cm, length: 56 mm; Extract-Clean) were obtained from Systech (Tokyo, Japan). *N*-*p*-vinylbenzyl-O-*β*-D-galactopyranosyl-1→4-D-guliconamide (VLA) was synthesized as described previously [S1]. HepG2 cells were obtained from the JCRB Cell Bank (Osaka, Japan). RAW264.7 cells (mouse macrophages) were obtained from the RIKEN Cell Bank (Ibaraki, Japan). Cell culture media and cell staining reagents were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

## S.2 Calculation of the amount of initiator and polymer

The carbon composition of the prepared initiator–and copolymer-modified silica beads was determined using an elemental analyzer (Unicube, Elementar, Langenselbold, Germany). The amount of modified ATRP initiator on the silica beads was calculated using the following equation:

$$\frac{\%C_C}{\%C_C(calcd) \times \left(1 - \%C_C/\%C_C(calcd)\right) \times S} \quad (1)$$

where $\%C_C$ is the increase in the carbon composition of the CPTMS-modified beads *via* silanization, $\%C_C(calcd)$ is the theoretical carbon composition of CPTMS, and $S$ is the surface area of the silica beads (475 $m^2/g$).

The amount of PVLA on the silica beads was estimated using Equation (2).

$$\frac{\%C_V}{\%C_V(calcd) \times \left(1 - \%C_V/\%C_V(calcd) - \%C_C/\%C_C(calcd)\right) \times S} \quad (2)$$

where $\%C_V$ is the increase in the carbon composition of the PVLA-modified silica beads *via* the first ATRP, and $\%C_V(calcd)$ is the carbon composition of PVLA.

The amount of modified PNIPAAm segments was estimated using the following equation:

$$(3) \quad \frac{\%C_N}{\%C_N(calcd) \times \left(1 - \%C_N/\%C_N(calcd) - \%C_V/\%C_V(calcd) - \%C_S/\right.}$$

where $\%C_N$ is the increase in the carbon composition of the PVLA-*b*-PNIPAAm-modified silica beads during the second ATRP, and $\%C_N(calcd)$ is the carbon composition of PNIPAAm.

The amount of block copolymer was calculated based on the sum of the amounts of PVLA and PNIPAAm.

**S.3 Cell culture**

Cell culture medium is described in Table S1. Cells were cultured on a 100-mm tissue culture polystyrene (TCPS) dish at 37 °C in 5% $CO_2$ using a $CO_2$ incubator (9000EX; WakenBtech, Kyoto, Japan). The cell culture medium was changed every 3 days. When the confluence was 80%, the medium was removed by aspiration. The cells were then rinsed with phosphate-buffered saline (2 mL), and trypsin solution (2.5 mL) was added to the dish. The dish was incubated for 3 min. After detaching the cells from the dish, the cell culture medium (2.5 mL) was added to the dish, and the cell suspension was collected in a centrifuge tube. Centrifugation was performed at 1500 rpm for 3 min. The supernatant was removed, and the medium was added to the cells. Then, 10 μL of the cell suspension was collected, and the cells in the cell suspension were counted. The cells were seeded at $5.0 \times 10^4$ cells/cm$^2$ in a 100-mm TCPS dish for passage culture.

**Table S1** Cell culture media

| Cells | Culture media [a)] | Additives [b)] |
| --- | --- | --- |

| | | |
|---|---|---|
| HepG2 | DMEM, high glucose, pyruvate | FBS (10%)<br>NEAA (1%)<br>Penicillin-Streptomycin (1%) |
| RAW264.7 | DMEM, high glucose, pyruvate | FBS (10%)<br>NEAA (1%)<br>Penicillin-Streptomycin (1%) |

a) The volume of the cell culture medium was 500 mL. b) Additives were added to 500 mL of the cell culture medium.

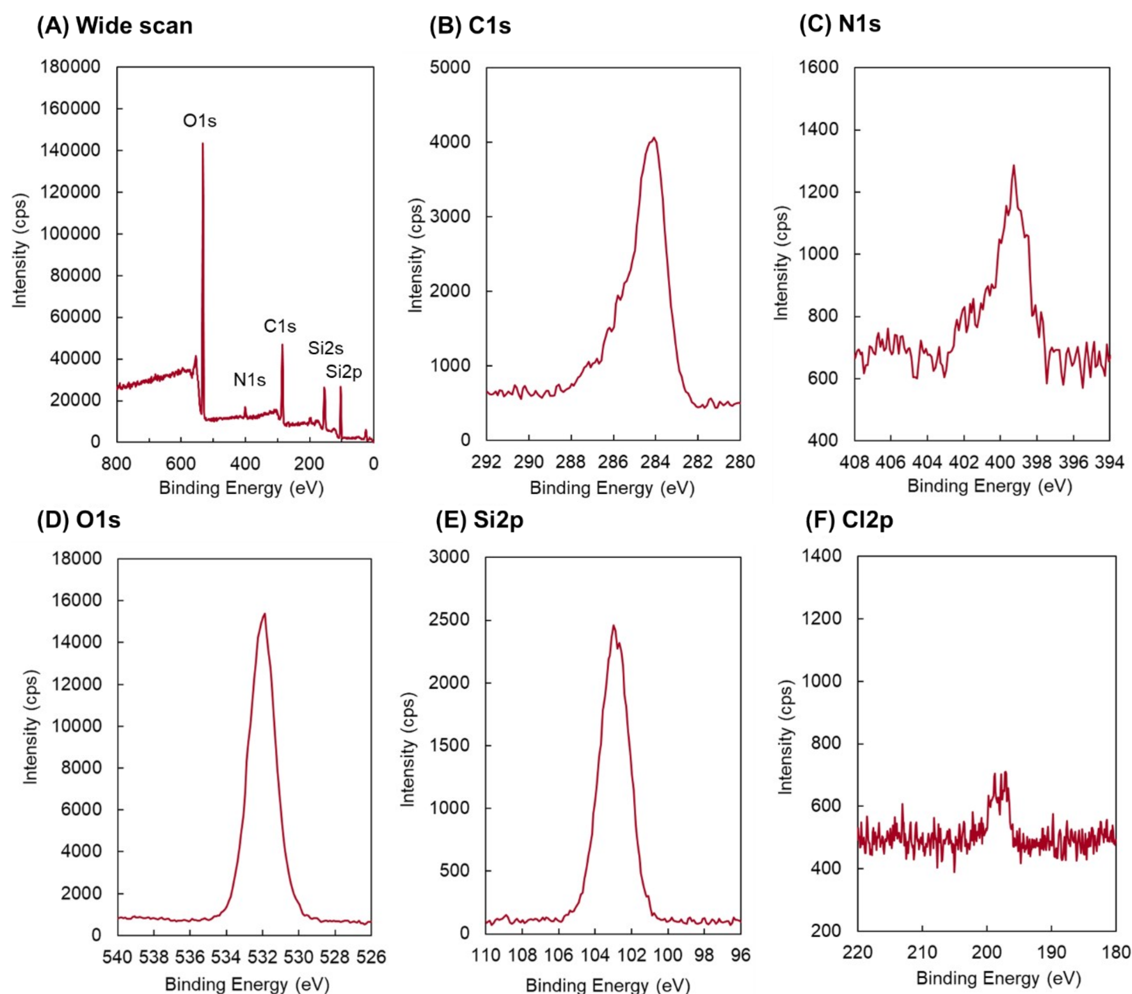


**Fig. S1** X-ray photoelectron spectroscopy spectra of the prepared mixed polymer brush-modified beads (PV50-PN1000). (A) Wide scan: (B) C1s, (C) N1s, (D) O1s, (E) Si2p, and (F) Cl2p.

**Table S3** Elemental analyses of copolymer-modified beads via X-ray photoelectron spectroscopy at a take-off angle of 90°.

| Code | Atom (%) | | | | | N/C ratio |
|---|---|---|---|---|---|---|
| | C | N | O | Si | Cl | |

| | | | | | | |
|---|---|---|---|---|---|---|
| PVLA-*b*-PNIPAAm brush modified beads (PV50-PN1000) | 34.5 | 2.6 | 38.4 | 24.1 | 0.4 | 0.075 |
| PNIPAAm [a)] | 75.0 | 12.5 | 12.5 | - | - | 0.167 |
| PVLA [a)] | 63.6 | 3.03 | 33.3 | - | - | 0.048 |
| Silica beads [a)] | - | - | 66.7 | 33.3 | - | - |
| CPTMS [a)] | 70.6 | - | 17.6 | 5.88 | 5.88 | - |

a) Theoretical atomic compositions of each polymer and silica bead

## References


S1. Kobayashi, K., Sumitomo, H. & Ina, Y. Synthesis and Functions of Polystyrene Derivatives Having Pendant Oligosaccharides. *Polym. J.* **17**, 567, (1985).