Horseradish peroxidase-catalyzed hydrogelation consuming enzyme-produced hydrogen peroxide in the presence of reducing sugars

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1. Estimation of sugar reducing power

*Reagent preparation:* Copper sulfate (CuSO$_4$ $\cdot$ 5H$_2$O, 15g) was dissolved in 100 mL distilled water (Somogyi’s reagent A). Anhydrous sodium carbonate (Na$_2$CO$_3$, 2.5 g), Rochelle salt (COOKCHOH $\cdot$ CHOHOONa $\cdot$ 4 H$_2$O, 2.5 g), sodium bicarbonate (NaHCO$_3$, 2.0 g) and anhydrous sodium sulfate (Na$_2$SO$_4$, 2.0 g) were dissolved in 100 mL distilled water (Somogyi’s reagent B). Somogyi’s reagent A (1 mL) and B (24 mL) were mixed right before use (Somogyi’s reagent C). Ammonium molybdate ((NH$_4$)$_6$Mo$_7$O$_{24}$ $\cdot$ 4H$_2$O, 5 g) was dissolved in 80 mL distilled water. Then, sulfuric acid (Conc. H$_2$SO$_4$, 4.2 mL) was added slowly. Thereafter, sodium arsenate (Na$_2$HAsO$_4$ $\cdot$ 7H$_2$O, 0.6 g in 5 mL distilled water) was added and the total volume was fixed at 100 mL (Nelson’s reagent). The resultant mixture stored in a brown bottle at 37°C. Glucose, galactose and mannose were dissolved in distilled water.

*Procedure:* Different concentrations of each reducing sugars (0-176 μg/mL) were added in test tubes and made up to 1 mL. Somogyi’s reagent C (1 mL) was added to each tube, and the tubes were kept for 20 min in a boiling water bath. After cooling the tubes to room temperature, Nelson’s reagent (1 mL) was added with gentle mixing and rested at 37°C for 5 min. Then, the absorbance at 660 nm was measured.
Fig. S1 Calibration curves of reducing sugars determined by the Somogyi-Nelson method. The data are mean values (n=3) with their standard deviations. Photographs in the graph show the color differences of reaction solutions at 88 μg/mL.
2. Harvesting cell sheets

After optimal culture times, hydrogels were treated with medium containing hyaluronidase (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 100 units/mL). Detached cell sheets were transferred to fresh cell culture dishes and incubated at 37°C.

Fig. S2 Photographs of 10T1/2 cell-layers detached from hydrogels after transfer to fresh cell culture dishes. Scale bars: 200 μm. Arrows in photographs indicate cell attachments to culture dishes.
3. Effect of HRP concentration on mechanical property of HA-Ph hydrogel

**Fig. S3** Effects of HRP concentrations on Young’s modulus of the resultant hydrogels obtained from HA-Ph (0.75 w/v%) and (A) galactose (44 mg/mL), or (B) mannose (44 mg/mL). The data are mean values ($n=4$) with their standard deviations.
4. Effect of sugar type on mechanical property of HA-Ph + Gelatin-Ph hydrogel

**Fig. S4** Effects of sugar type on Young’s modulus of the resultant hydrogels obtained from HA-Ph (0.75 w/v%), gelatin-Ph (0.3 w/v%), HRP (800 units/mL) and reducing sugars (88 mg/mL).

The data are mean values (n=4) with their standard deviations.