Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information (ESI)

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 ($CuSO_4 \cdot 5H_2O$, 15g) was dissolved in 100 mL distilled water (Somogyi's reagent A). Anhydrous sodium carbonate (Na_2CO_3, 2.5 g), Rochelle salt (COOKCHOH · CHOHCOONa · 4 H_2O, 2.5 g), sodium bicarbonate ($NaHCO_3$, 2.0 g) and anhydrous sodium sulfate (Na_2SO_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 ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 5 g) was dissolved in 80 mL distilled water. Then, sulfuric acid (Conc. H_2SO_4, 4.2 mL) was added slowly. Thereafter, sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, 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.

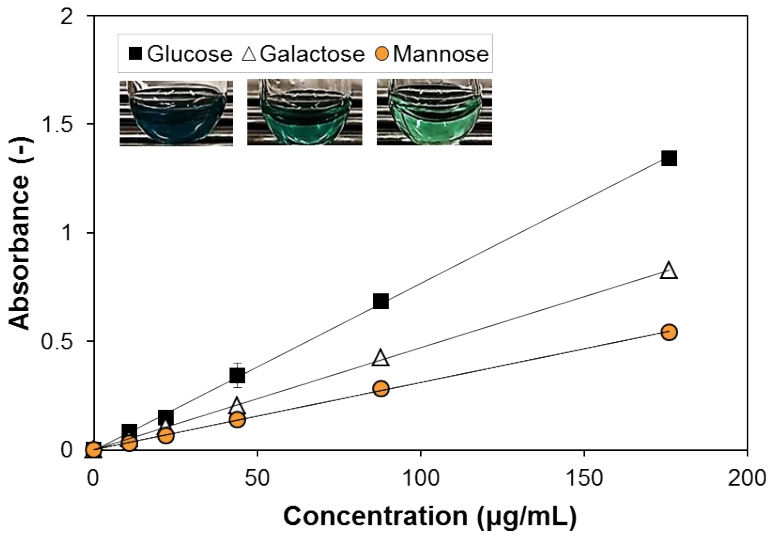


Fig. S1 Calibration curves of reducing sugars determined by the Somogyi-Nelson method. The data are mean values (*n*=3) with their standard deviations. Photographs in the graph show the color differences of reaction solutions at 88 μg/mL.

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.

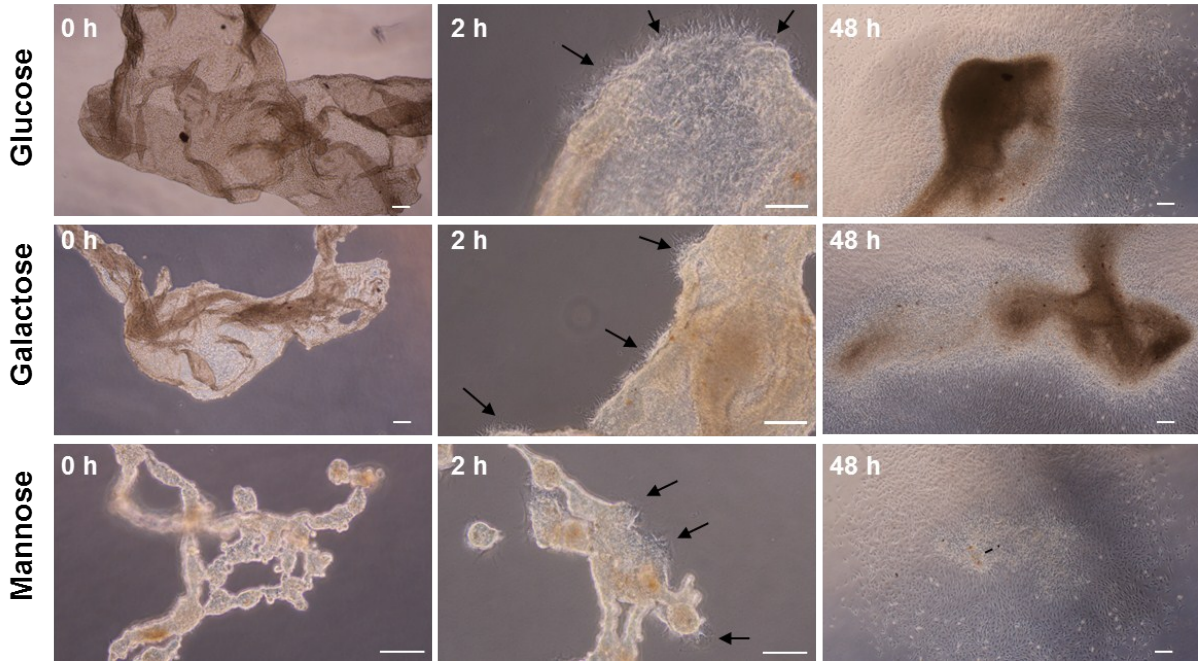


Fig. S2 Photographs of 10T1/2 cell-layers detached from hydrogels after transfer to fresh cell culture dishes. Scale bars: 200 µm. Arrows in photographs indicate cell attachments to culture dishes.

3. Effect of HRP concentration on mechanical property of HA-Ph hydrogel

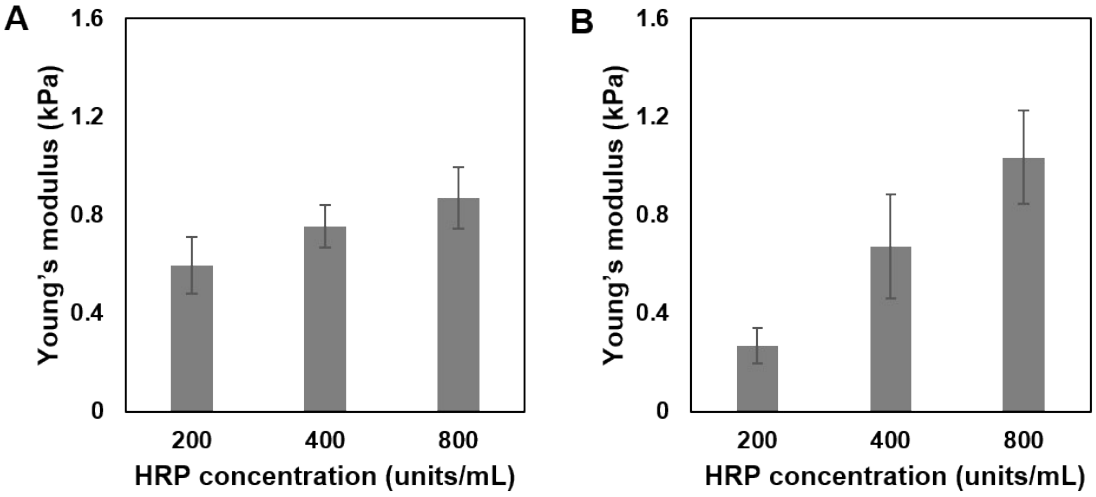
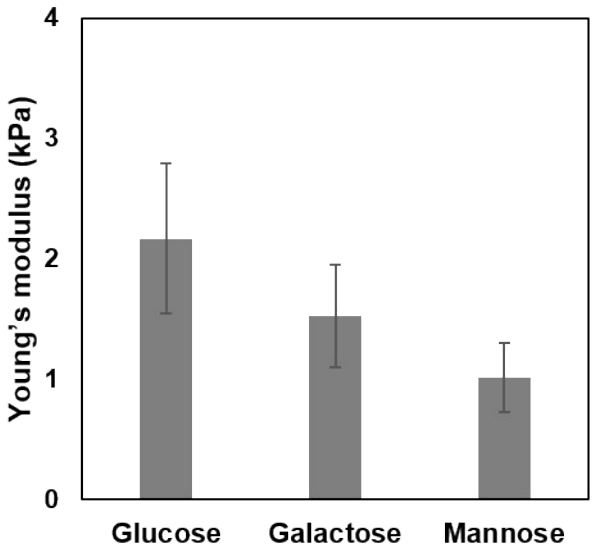


Fig. S3 Effects of HRP concentrations on Young's modulus of the resultant hydrogels obtained from HA-Ph (0.75 w/v%) and (A) galactose (44 mg/mL), or (B) mannose (44 mg/mL). The data are mean values (*n*=4) with their standard deviations.



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.