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Electronic Supplementary Information (ESI)

Saccharides and Temperature Dual-Responsive Hydrogel Layers for Harvesting Cell Sheets

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Materials

N-isopropylacrylamide (NIPAAm, 98%, J&K Scientific Ltd) was recrystallized from hexane prior to use. Methylene bisacrylamide (MBAAm, Alfa Aesar) and acrylamide (AAm, Shanghai Reagent General Factory) were recrystallized from acetone. Ammonium persulfate (APS, Richu Bioscience) and *N,N,N',N'*-tetramethylethylenediamine (TEMED, Alfa Aesar) were used as received.Acryloyl chloride (99%, Alfa Aesar), 3-aminophenylboronic acid (PBA, Alfa Aesar), a-D(+)-fructose and a-D(+)-glucose (97%, J&K) were used as received, 3-acrylamidophenylboronic acid (APBA) was synthesized according previously reported method.[1] 0.25 % Trypsin/EDTA solution, streptomycin and penicillin were purchased from Gibco BRL (USA). DMEM (Dulbecco's Modified Eagle's Medium) and FBS (fetal bovine serum) were purchased from HyClone (USA). Cell adhesive peptide RGD capped with polymerizable acryloyl group (AryRGD) was purchesed from ChinaPeptides Company Limited, China. FITC-phalloidin (Enzo) and 4'-6-diamidino-2-phenylindole (DAPI) (Invitrogen) were used as received. All other reagents were of analytical grade and were purchased from Shanghai Reagent General Factory and used as received. Phosphate buffer solution (PBS, 0.02mmol/L, pH=7.2) was prepared use ultrapure water (purified with a Thermo Scientific Barnstead NANOpure Diamond Water Purification Systems to give a minimum resistivity of 18.2 MΩ·cm) and a purchased phosphate buffer salt (Beyotime Biotechnology, China, AR).

Preparation of PNIPAAm-co-PAAPBA hydrogel layer

The hydrogel layer was prepared according to our previous method.[2] Typically, NIPAAm (225 mg, 2.0 mmol), AAm (42 mg,0.6 mmol), MBAAm (16 mg,0.10 mmol) and cell adhesion peptide monomer AryRGD (2.46 mg, 0.005 mmol) were dissolved in 4mL of ultrapure water. APBA (19 mg, 0.1 mmol)was dissolved in 0.875 mL NaOH solution(0.2 M). Polymerization was carried out by mixture of the two solutions together and addition of of 5 mg of APS and 5 μL of TEMED at 37℃ for 30min under the argon flow. Subsequently, the solutions were placed between two glass plates (Biorad,Miniprotein-3) with a space of 0.75 mm. The reaction was lasted for 2 hours at 37℃. After the reaction, the resultant AcryRGD-hydrogels were punctured into disks with a diameter of 14 mm. Each piece of the hydrogel disks were alternately washed with PBS solution at 37℃ and 20℃ alternately for 2 days to remove the unreacted monomers, residual initiators and NaOH. Immersed the hydrogel disks in pure PBS solution at room temperature prior to use. Two kinds of control hydrogels without AcryRGD or without APBA were both prepared.

Fourier Transform Infrared(FTIR) analysis

Attenuated Total Reflectance FTIR (ATR FTIR) spectra of dual responsive hydrogels were obtained in the wave number range of 650 ~ 4000 cm^{-1} at 2 cm^{-1} resolution using a Nicolet

6700 FTIR Spectrometer. Thirty-six scans were performed for each sample and OMNIC software was used to process the data.

Measurement of the swelling ratio of the hydrogels

The swelling of hydrogels was determined by immersing the hydrogel disks in PBS solution with different concentration of sugar at different temperature. All the hydrogel disks sample were purified before use. Measurement of the size of hydrogel disk was firstly taken after immersing it in pure PBS for 2 h at 20℃, and then increased the temperature to 37℃ for another 2h to record the size change. Replaced the pure PBS for in which there's a certain amount of sugar (1 g/L, 5 g/L, 10 g/L and 20 g/L glucose or fructose PBS solution were implemented in this research, respectively) but at the same time decreased the temperature to 20℃ again. Repeated the prior steps between 37℃ and 20℃ for 5 times to get the average size of the hydrogel disks in PBS with different concentration of sugar at different temperature.The following equation was used to calculate the eauilibrium swelling ratio(S_{eq}):

$$S_{eq}(\%) = \frac{D - Do}{Do} \times 100$$

where D_o is the diameter of the hydrogel disk at 37℃ in pure PBS and D is the diameter of the swelled sample.

Measurement of air bubble contact angle of the hydrogels

A KRÜSS DSA25 contact angle equipment (Germany) was utilized to determine the air bubble contact angles of the hydrogel layers. The hydrogel disks was washed with PBS solution for 2 days before testing. 4 μL air bubble was blowed below the hydrogel disk for the measurement, and the average was used for the analysis.

Cell culture and cell sheet detachment

MC3T3-E1 cells (an osteoblastic cell line from normal mouse calvaria) were grown under a humidified atmosphere in low-glucose DMEM supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin in 5 % CO_2 at 37 ℃. The medium was changed twice weekly, and the cells were harvested using 0.25 % trypsin and 0.26 mM EDTA in PBS after reaching subconfluency.

For cell culture, the glass slides were placed in cell culture plate. MC3T3-E1 cells were seeded onto the plate at 1×10^4 cells/cm^2, and cultured at 37 ^{o}C under a humidified atmosphere of 5 % CO_2 in low-glucose Dulbecco's modified Eagle medium (DMEM-L, 1 g/L of glucose). For saccharide-induced cell sheet release experiments, the medium was carefully changed with a 37^{o}C DMEM (supplemented with 10 g/L of glucose or fructose) and the cells were incubated at 37^{o}C under a humidified atmosphere of 5 % CO_2. Cell sheet morphology was

recorded under a microscope equipped with a digital camera at definite time intervals. The area of adhered cell sheet in the photos was counted and averaged from three separate experiments.[3]

Fluorescence staining

For the staining of cells, the culture medium was removed after each experiment and the slides were washed with PBS and then fixed using a 4% paraformaldehyde and 1 mM $CaCl_2$ solution in PBS. After 30 minutes the slides were washed 3 times with PBS and incubated for 5 minutes with 0.4% triton-X and 1 mM $CaCl_2$ in PBS to punch the cell membrane at room temperature. Again the slides were washed three times with PBS. Subsequently, the cells were stained with FITC-phalloidin (for staining F-actin stress fibers) and 4'-6-diamidino-2-phenylindole (DAPI, for staining nuclei) for 15 min. After staining, the slides were washed three times with PBS and and then examined under a fluorescence microscope.

References

[1] D. Roy, J. N. Cambre and B. S. Sumerlin, *Chem. Comm.*, **21**, 2008, 2477-2479.

[2] G. Pan, Q. Guo, Y. Ma, H. Yang and B. Li, *Angew. Chem. Int. Ed.*, 2013, **52**, 6907-6911.

[3] G. Pan, B. Guo, Y. Ma, W. Cui, F. He, B. Li, H. Yang and K. J. Shea, *J. Am. Chem.* Soc., 2014, **136**, 6203–6206.

Figures

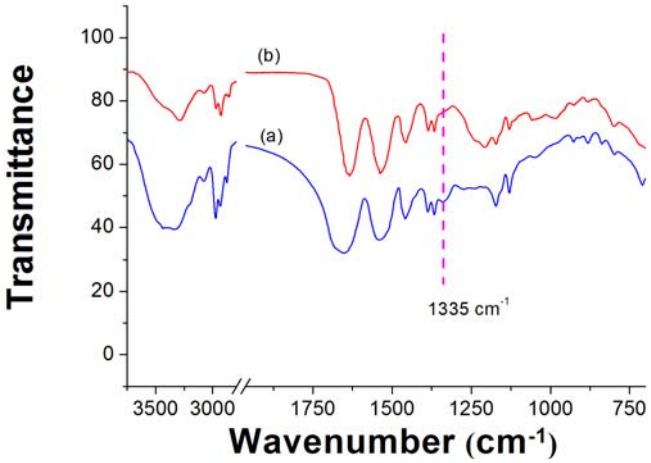

Figure S1. FTIR spectra of (a) the dual-responsive hydrogel and (b) the control hydrogel without PBA groups in the network.

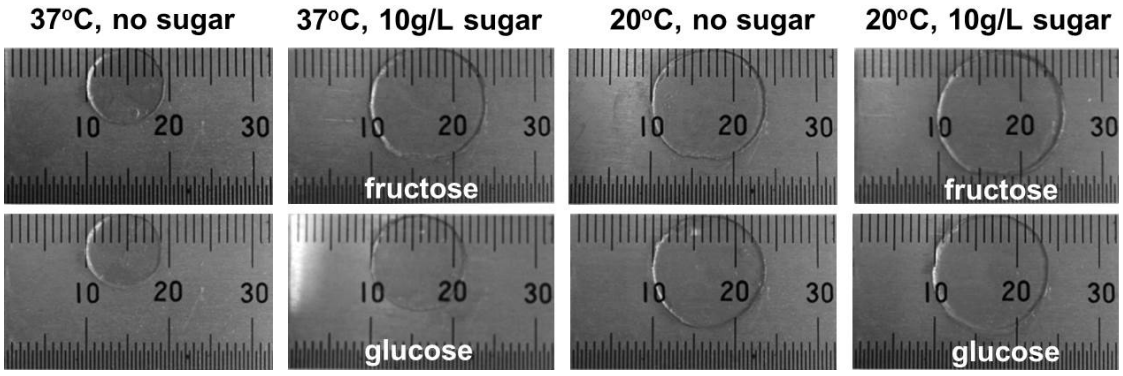

Figure S2. Photographs of the dual-responsive hydrogel layer in PBS with different sugar concentration and at different temperature after immersion for 30 min. The diameter of the hydrogel is 14 mm at 20°C in PBS without sugar.

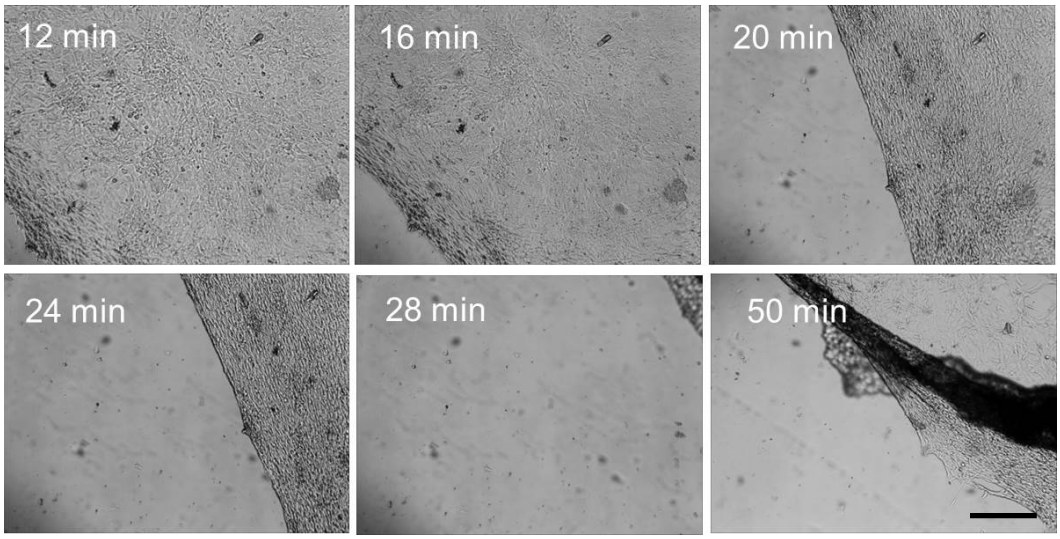

Figure S3. Detachment of a cell sheet from the dual-responsive hydrogel after incubated in 10 g/L of glucose-containing DMEM at 12, 16, 20, 24, 28 and 50 min at 37^{o}C, respectively. Note that the cell sheet migrated out of the view of the microscope after 28 min. However, after 50 min, the cell can not completely detached from the hydrogel layer. Scale bar 400 μm.

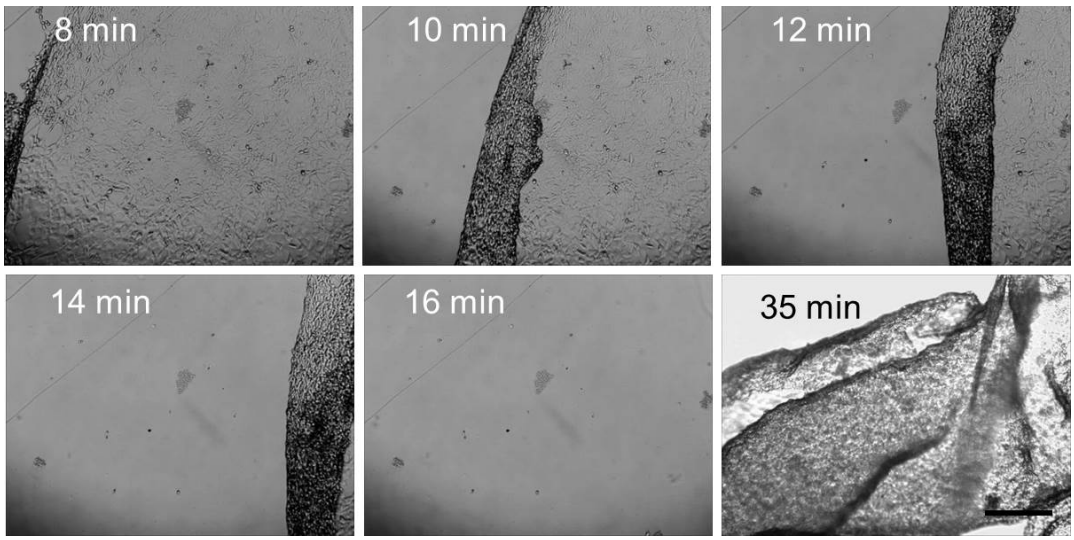

Figure S4. Detachment of a cell sheet from the dual-responsive hydrogel after incubated in 1g/L of glucose DMEM at 8, 10, 12, 14, 16 and 35 min at 20^{o}C, respectively. Note that the cell sheet migrated out of the view of the microscope after 16 min. However, a harvested cell sheet was found in the medium after 35 min. Scale bar 400 μm.

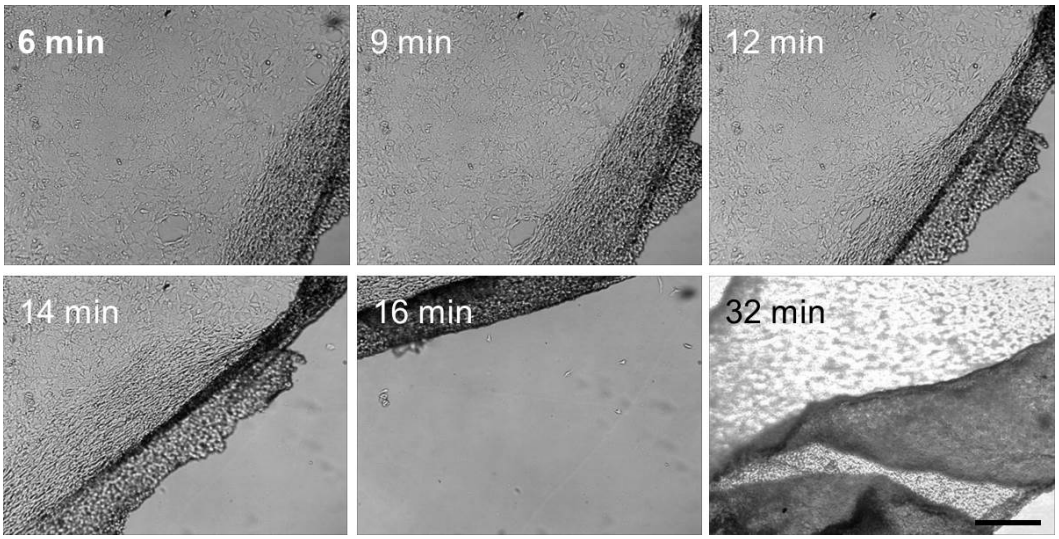

Figure S5. Detachment of a cell sheet from the dual-responsive hydrogel after incubated in 10 g/L of glucose-containing DMEM at 6, 9, 12, 14, 16 and 32 min at 20°C, respectively. Note that the cell sheet migrated out of the view of the microscope after 16 min. However, a harvested cell sheet was found in the medium after 32 min. Scale bar 400 μm.

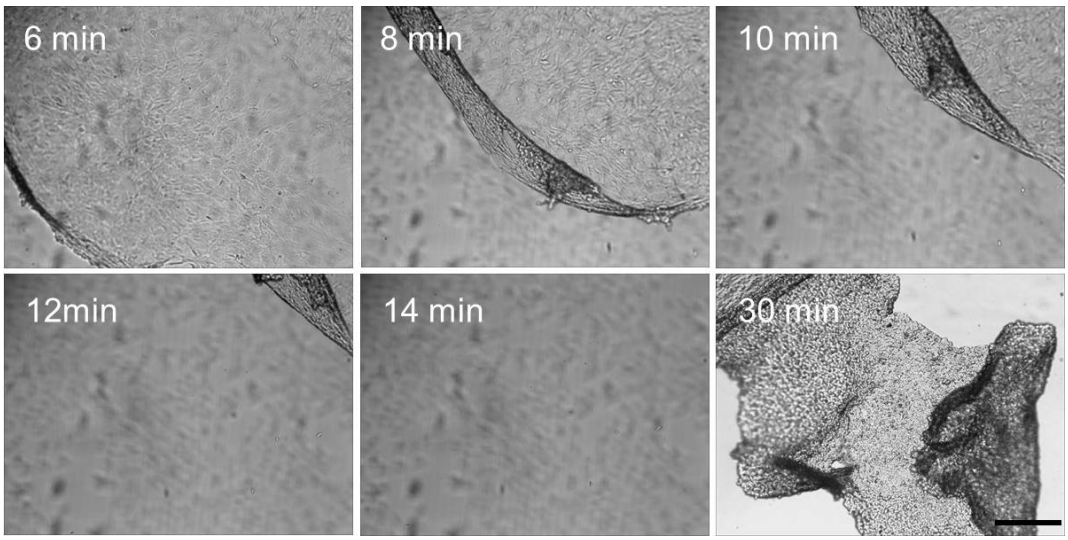

Figure S6. Detachment of a cell sheet from the dual-responsive hydrogel after incubated in 10 g/L of fructose-containing DMEM at 6, 8, 10, 12, 14 and 30 min at 20°C, respectively. Note that the cell sheet migrated out of the view of the microscope after 14 min. However, a harvested cell sheet was found in the medium after 30 min. Scale bar 400 μm.