Supporting Information for

Fabrication of pocket-like hydrogel microstructures through photolithography

Kimio Sumaru,* Toshiyuki Takagi, Kana Morishita, Taku Satoh, Toshiyuki Kanamori

Matererials

Hydroxypropyl cellulose (HPC, M.W. 100,000, #19188-4, Sigma-Aldrich Co.) was used as a water-soluble hydroxyl-rich polymer composing a hydrogel sheet. Fluorescein isothiocyanate (FITC-I, Dojindo Molecular Technologies, Inc.) was used to prepare a fluorescently-functionalized HPC (fHPC). 1,3,4,6-tetrakis(methoxymethyl)glycoluril (TMMGU, #T2058, Tokyo Chemical Industry Co., Ltd.) was used as an acid catalyzed crosslinker. Polystyrene petri dish (#351008, Becton, Dickinson & Co.) with 35 mm diameter was used as a basal substrate. Human induced pluripotent stem (iPS) cell line (#201B7) was purchased from RIKEN Bioresource Center (Tsukuba, Ibaraki, Japan) and repeatedly subcultured under feeder-free condition on a substrate coated with Matrigel solution (hESC-qualified, #354277, Becton, Dickinson & Co.). ReproFF2 (ReproCELL Inc.), supplemented with 5 ng/mL human recombinant FGF-2 (bFGF, ReproCELL Inc.), was used as a culture medium for human iPSCs.

Apparatuses

A spin coater (ASS-301, Able Co., Ltd.) was used for polymer solution coating. Micro-patterned light irradiation was carried out by using a PC-controlled micro-projection system (DESM-01, Engineering System Co.) installed in an inverted research microscope (IX70, Olympus Co.) through a 2X objective lens (PlanApo 2X, Olympus Co.).[1,2] Bright field images were taken with a cooled CCD camera system (VB-7000, Keyence Co.) installed on the same microscope. 3-dimensional observation of the microstructures was carried out by using a confocal laser scanning microscope (FluoView 300, Olympus Co.) installed in an inverted research microscope (IX71, Olympus Co.).

1 K. Sumaru, J.-I. Edahiro, Y. Ooshima, T. Kanamori and T. Shinbo, *Biosens. Bioelectron.,* 2007, **22,** 2356-2359.
2 K. Sumaru and T. Kanamori, *Methods Cell Biol.,* 2014, **120,** 185-197.

Polymer Synthesis

We synthesized a photo-acid-generating poly(methyl methacrylate) (pPAGMMA) functionalized with near UV–visible light responsive PAG moiety[3] at 2 mol% through the radical copolymerization of methyl methacrylate and styrene derivative having PAG group as we have already reported.[4,5] fHPC was synthesized by heating to dry a THF solution containing 1.0 wt% HPC and 0.10 wt% FITC-I at 110 ˚C.

3 M. Shirai and H. Okamura, *Prog. Org.c Coat.,* 2009, **64,** 175-181.
4 K. Sumaru, K. Kikuchi, T. Takagi, M. Yamaguchi, T. Satoh, K. Morishita and T. Kanamori, *Biotechnol. Bioeng.,* 2013, **110,** 348-352.
5 K. Sumaru, K. Morishita, T. Takagi, T. Satoh and T. Kanamori, *Eur. Polym. J.,* 2017, **93,** 733-742.

Preparation of photoresponsive bilayer

A photoresponsive bilayer (PRBL) composed of HPC pregel layer and pPAGMMA layer was prepared as follows. Firstly, a pPAGMMA thin layer was formed by spincoating a 1.0 wt% pPAGMMA solution in 2,2,2-trifluoroethanol containing 10 wt% n-buthanol on the surface of a polystyrene petri dish under N_2 atmosphere. Then a methanol solution containing 5.0 wt% HPC, 0.072 wt% fHPC, 0.40 wt% TMMGU and 0.78 mM/kg H_2SO_4 was spincoated on the pPAGMMA layer under N_2 atmosphere, and baked for 5 minutes at 85 ˚C.

Photoresponsive lifting-off of cHPC layer from pPAGMMA layer

We examined the photoresponsive lifting-off of the HPC layer, which had been crosslinked uniformly by sulfuric acid, from a pPAGMMA layer. Fig. S1 shows the time required for the cHPC hydrogel sheet to lift off of a substrate under the light irradiation (wavelength: 436 nm, intensity: 300 mW/cm^2) dependent on the composition of the ethanol-water mixed solvent. After the lifting-off, the cHPC layer reached the fully swelled state in a minute.

Adhesion of cells captured in pocket-like structures

We examined to introduce Madin-Darby canine kidney cells (RIKEN Bioresource Center, Tsukuba, Ibaraki, Japan) into pocket-like hydrogel structures and cultured. Fig. S2 shows the microscopic image of the system after “kangaroo culture” for 1 day. The cells adhered spreading on the surface of the substrate appearing after the lifting-off of cHPC hydrogel sheet.

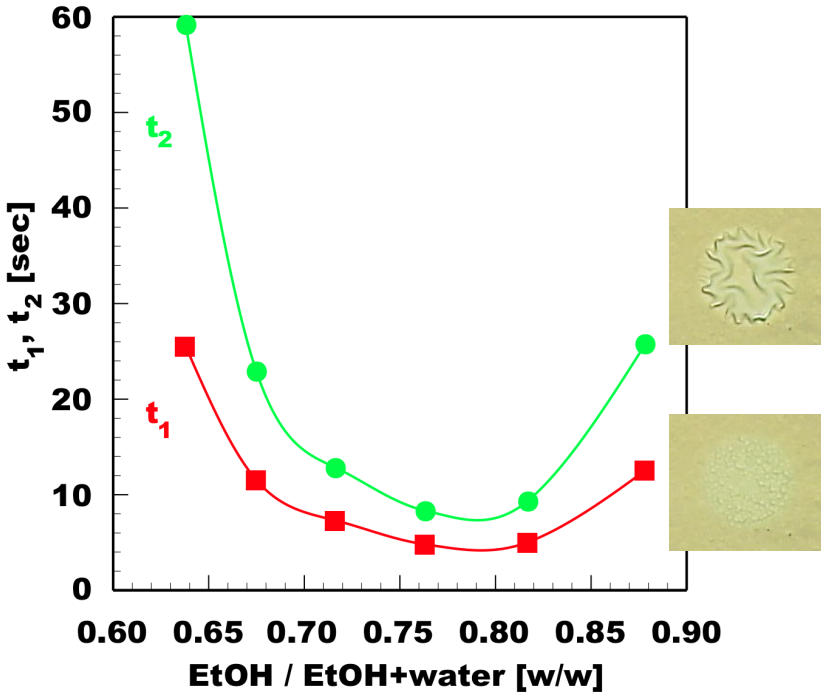

Fig. S1 Lifting-off rate of the cHPC hydrogel sheet under the light irradiation (wavelength: 436 nm, intensity: 300 mW/cm^2) dependent on the composition of the ethanol-water mixed solvent. t_1 and t_2 are the times when the cHPC hydrogel sheet to start to lift off and to accomplish lifting off, respectively.

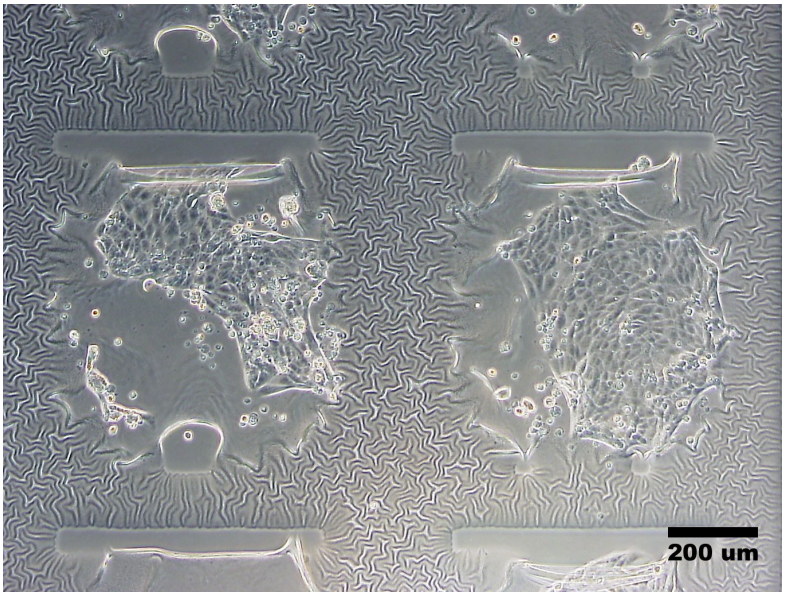

Fig. S2 Microscopic image of MDCK cells after "kangaroo culture" in pocket-like microstructures composed of hydrogel sheet.

Fabrication of balloon-like microstructures composed of partially hydrolyzed polyvinyl acetate

We examined to fabricate balloon-like microstructures using 40% hydrolyzed polyvinyl acetate (PVAcH, M.W. 72,000, #17561, Polysciences, Inc.) instead of HPC. A methanol solution containing 4.7 wt% PVAcH and 0.23 wt% TMMGU was used as a pregel solution. Fig. S3 shows the microscopic image of the microstructures in 80% ethanol.

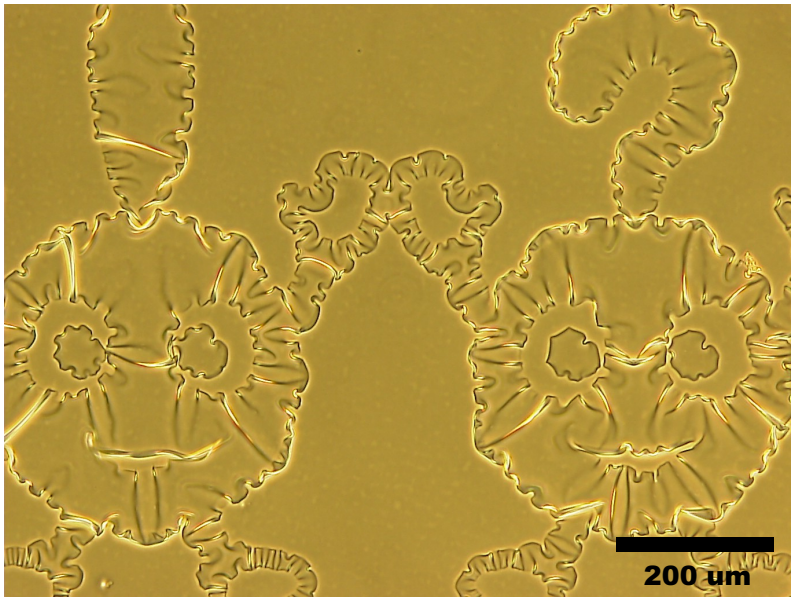

Fig. S3 Fabricated balloon-like microstructures composed of partially hydrolyzed polyvinyl acetate in 80% ethanol.

Supporting movie

An animation to help understanding of the 3-dimensional structure of a hollow trapezoidal circular cone structure composed of cHPC hydrogel sheet can be seen in the movie **SumaruAnim3D.avi**.