A bio-synthetic hybrid hydrogel formed under physiological conditions consisting of mucin and a synthetic polymer carrying boronic acid

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Abstract: Mucin-containing bio-synthetic hybrid hydrogel is successfully formed under physiological conditions upon mixing aqueous solutions of native mucin and synthetic polymers carrying boronic acids. The mechanical properties and stability of the hydrogel in physiological solutions, e.g., cell culture media, are tunable depending on the boronic acid content of polymers. The hydrogel dissolved in the physiological solutions releases native mucin and boronic acid-containing polymer, which can control the adhesion of mammalian cells to the surface.

1. Introduction

Biomacromolecules exert sophisticated functions such as the formation of higher-order structures, biochemical reactions, and intra- and intercellular communications. Alternatively, synthetic polymers have high robustness and functional properties arising from tailor-made

molecular design. Hence, bio-synthetic hybrid polymeric materials have attracted much attention due to the sophisticated functions of biopolymers tunable by design freedom of synthetic polymers.[1-4] Most biopolymers, e.g., proteins, polysaccharides, and nucleic acids are sensitive to the external environment and easily be denatured under harsh conditions.[5] Thus, bio-synthetic hybrid materials should be used under mild conditions throughout the lifecycle of materials including preparation, use, and degradation.

Mucin is a family of glycoproteins consisting of a hydrophobic polypeptide main chain densely modified with hydrophilic polysaccharide side chains. Mucins are categorized into various types including membrane-bound mucin, secreted mucin, and others. Mucin and other proteins are cross-linked through noncovalent bonds, e.g., disulfide bonds, ionic bonds, and hydrogen bonds[6,7] to form mucus. The main roles of animal mucins are (1) physical barrier preventing tissues and organs from drying, harmful substances, and viruses,[8,9] (2) intercellular communication through molecular recognition,[10] and (3) living environment for symbiotic bacteria.[11] Early studies on mucins have focused on their biological activity mainly from a physiological point of view.[12] Recently, mucin as a naturally-derived macromolecule having such a unique structure and properties has been regarded as a building block of biomaterials,[10] as represented by coating for cell-repellent[13] or cell-selective[14] surfaces, and a component of therapeutic agent for wound healing.[15] Animal mucus forms through the interaction of mucin and non-mucin proteins via dynamic bonds, forming hydrogel having shear moduli in the order of 1–100 Pa.[6,7,16] Similarly, interactions between mucin and various macromolecules including cationic or mucoadhesive proteins,[17,18] polysaccharides,[19-22] and lectins,[23,24] have been extensively studied. Generally, chemical modification is needed for mucins to be used as building blocks of bio-synthetic hybrid materials. For example, mucin modified with methacryloyl groups was cross-linked to form hydrogels for controlled release of drugs.[25] Trimming sugar chains of mucin by chemical treatment gave polymers forming nanoparticles capable of loading and releasing lysozyme.[26] However, to the best of our knowledge, there have been only a few reports on the interaction or formation of hybrid materials using native, unmodified mucin in combination with synthetic polymers; the interaction of mucin with poly(acrylic acid),[27] disulfide bond formation with poly(ethylene glycol) derivatives,[28] the interaction with polymer-coated liposomes,[29] and the formation of coacervate particles at basic pH.[30] Specifically, hybrid materials of mucin and synthetic polymer which can be formed, used, and degraded under physiological conditions using mild stimuli[23,24] are promising to be a candidate for wide

applications in biomedical field, such as injectable hydrogel for artificial mucus, antiadhesion agent, and drug delivery system.

Boronic acid is a well-known synthetic molecular recognition tool[31] forming reversible covalent bonds in aqueous media with diol or polyol compounds, e.g., saccharides.[32] Molecular recognition properties of boronic acids have been utilized for construction of functional materials,[33-36] especially for biomedical applications.[37-39] Boronic acids have also been used for functional "bridge"[40] for cross-linking biopolymers and synthetic polymers.[41,42] Generally, the molecular recognition of boronic acid in aqueous media efficiently works at pH above pK_a. Typical phenylboronic acid derivatives have pK_a at basic pH > 8, which limits the potential for applications at neutral pH. Structural modification of phenylboronic acid moieties, of which pK_a is decreased to a neutral or acidic pH, allows ones to use boronic acid residues in combination with biomacromolecules under physiological conditions.[43]

This work focuses on a novel hybrid hydrogel using the interaction of type III gastric mucin glycoprotein with synthetic polymers possessing boronic acid moieties, which is formed under physiological pH and degraded under mild conditions. Mechanical properties, stimuli-responsiveness, and degradability of the obtained hydrogels are investigated. Furthermore, in anticipation of its application to artificial mucus, interaction of the hydrogel with mammalian cells is discussed.

2. Results and discussion

2.1. Material design and preparation of polymers

Figure 1 shows the molecular design for mucin-incorporated hydrogel in the present study. In this work, crude, commercially available porcine stomach mucin (Sigma, M1778) was used without further purification. A synthetic polymer designed to interact with mucin at neutral pH consists of poly(*N*,*N*-dimethyl acrylamide) (pDMA) as a hydrophilic and nonionic main chain which is widely used in biomedical fields,[45] having 2-acrylamidophenylboronic acid (2APBA) as pendant groups (pDMA-2APBA). 2APBA has a unique molecular structure in which the intramolecular B-O bond facilitates boronate ester formation at pH above 5.[33] This enables interaction with diol-containing polymers at neutral pH in cross-linking to form a hydrogel.[43] Since the formation of boronate ester is dynamic and reversible, the crosslinks are cleavable on-demand using sugars as a mild chemical stimulus. In other words, native mucin molecules can be released upon competitive molecule-induced gel-to-sol transition of

the hydrogel. pDMA-2APBA was synthesized by conventional free-radical copolymerization of *N*,*N*-dimethylacrylamide (DMA) and 2APBA in ethanol using 2,2'-azobisisobutyronitrile (AIBN) as a radical initiator. Figure S1 shows ^{1}H NMR spectra of pDMA-2APBA (*x*) in D_2O (*x* = 10, 15, 20, 25, and 30%). Calculation from the area intensities of the corresponding peaks in the ^{1}H NMR spectra showed successful preparation of polymers with boronic acid contents slightly larger than the feed ratio (Table S1). Figure S2 shows the GPC chart of pDMA-2APBA(*x*) (*x* = 10, 15, 20, 25, and 30%) measured with PBS as the eluent. Table S2 summarizes the molecular weight and molecular weight distribution estimated using pullulan as a standard. These data show pDMA-2APBA (*x*) have average molecular weights of ca. 10^3–10^4 Da with a relatively wide molecular weight distribution due to non-controlled free-radical polymerization.

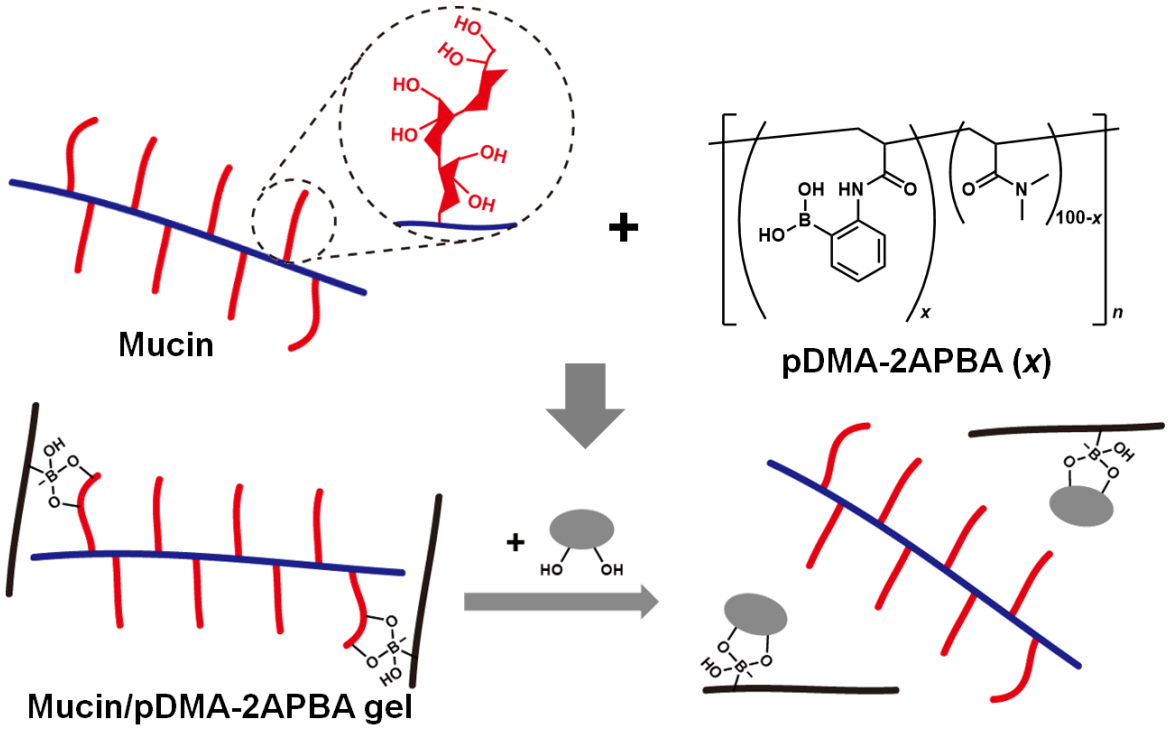

Figure 1. Design principle and chemical structure of stimuli-degradable Mucin/pDMA-2APBA gel used in this study.

2.2. Preparation and characterization of hydrogel

The hydrogel samples obtained were characterized. Figure 2a shows photographs of aqueous solutions of mucin (ca. 3.5 w/v% in PBS), pDMA-2APBA (*x*) (15 w/v% in PBS and 1/1 mixture (v/v) of them (*x* = 10, 15, 20, 25 and 30%). Samples with *x* = 10, 15, and 20% remained sol state after mixing, whereas samples with *x* = 25 and 30% turned to gel within 1 min. Figure S3 summarizes the gelability of mucin and pDMA-2APBA (25) and (30) at

varying concentrations in PBS solution. These observations indicate that mucin interacts with pDMA-2APBA and the higher boronic acid content leads to the lower polymer concentration required to form a hydrogel. All the samples, especially those in the sol state, had a light-yellow color. It is likely that the boronic acid residues were somehow oxidized in air. A sample of x = 30 formed a cloudy hydrogel, indicating that larger aggregates (~ μm) formed through intermolecular interactions. A previous study has reported the formation of coacervate particles between mucin and boronic acid polymers at basic pH.[30] It can be assumed that macroscopic gelation occurred in this case because the concentration of coacervate particles became high enough to fill up the system. The rheological properties of the hydrogel samples at 37 °C were investigated using a rheometer with parallel plate geometry. Figure 2b shows a photograph of Mucin/pDMA-2APBA gel (x = 25%) dyed with green-color dye injected from syringe through a 27G needle into PBS. The hydrogel was soft enough to be injected into PBS, but did not diffuse or dissolve instantly and formed into fiber-like shape. Figure 2c shows the dependence of storage elastic modulus (G'), loss elastic modulus (G''), and tan δ (= G''/G') on the frequency of the mixtures of solutions of mucin and pDMA-2APBA (x) containing final concentrations of the polymers at ca. 1.8 and 7.5 w/v%, respectively. G', G'', and tan δ at f = 1 Hz are summarized in Figure 2d. Samples with x = 10, 15, and 20% showed sol or critical gel-like properties with G' and G'' being almost the same. The sample with x = 25 and 30% showed viscoelastic gel-like, G'-dominant rheological properties. As can be seen in Figure 2c, the samples with higher x had larger G', larger G'', and smaller tan δ values. In comparison with mucus hydrogel, the sample with x = 25 has G' of ca. 1–100 Pa, which is close to that of native mucus.[6,7,16]

To elucidate the mechanism of hydrogelation, mucin and pDMA-2APBA polymer solutions were mixed in the presence of competitive sugars. Mucin (ca. 3.5 w/v%) and pDMA-2APBA (x = 25%) (15 w/v%) were dissolved respectively in PBS non-containing or containing 100 mM sugar (glucose, galactose, or fructose) before mixing. Figure S4a shows photographs of 1/1 mixture (v/v) of the solutions. Figures S4b and S4c show rheological properties of the solution mixtures in the absence and presence of a competitive sugar. G' and G'' of the samples were in the order of PBS > PBS with glucose ~ PBS with galactose > PBS with fructose. The binding constants (K) of phenylboronic acid with glucose, galactose, and fructose are reported to be $K = 1.1 \times 10^2$, 2.8×10^2, and 4.4×10^3 M^{-1}, respectively.[46] These observations indicate that these sugars partially inhibited cross-linking in accordance with binding strength to boronic acid moieties. It is thus concluded that the hydrogelation is based on the formation of boronate ester bonds (Figure 1). It is noteworthy that the mechanical

properties of the hydrogel can be controlled by tuning of the composition of polymers and sugar.

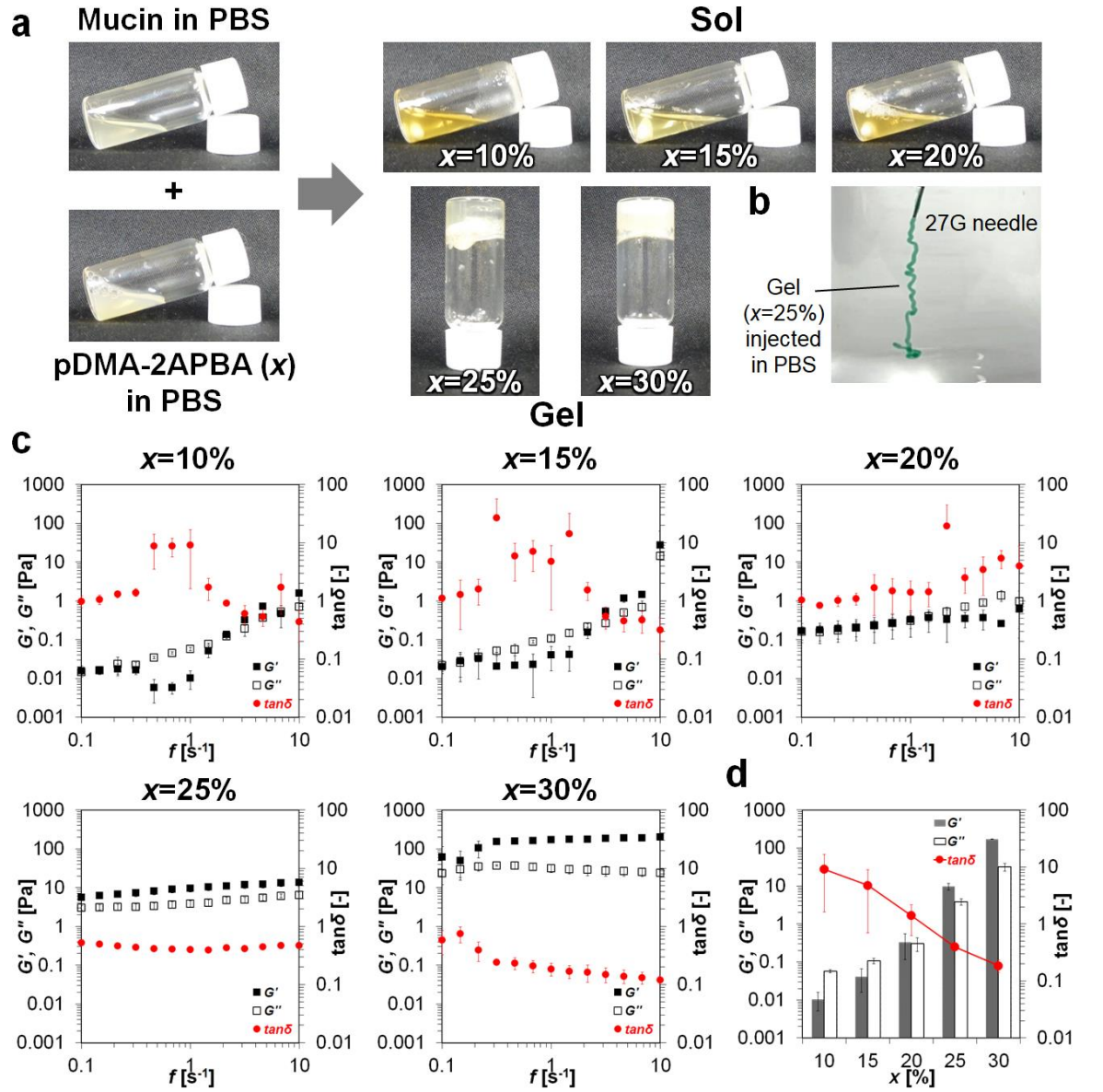

Figure 2. (a) Formation of the hydrogel by mixing aqueous solutions of mucin and pDMA-2APBA (*x*). (b) Mucin/pDMA-2APBA gel (*x* = 25%) dyed with green-color dye injected from 27G needle into PBS. (c) Dynamic viscoelastic measurements of the mixtures of solutions of mucin and pDMA-2APBA (*x*) (*x* =10, 15, 20, 25, and 30%) at 37 °C. (d) Dependence of *G'*, *G''*, and tan δ (at *f* = 1 Hz) on *x*.

2.3. Stability of hydrogels in aqueous media

Stability of the hydrogel under physiological conditions was investigated. Figure 3a shows a schematic illustration of the experimental procedure. Mucin/pDMA-2APBA gel (*x* = 25 or 30%) (0.1 mL) was formed in a 12-well plate. The gel was immersed in 1 mL of PBS, 0.1 M

glucose in PBS, Dulbecco's modified Eagle medium (DMEM), or DMEM containing 10 v/v% fetal bovine serum (FBS) in the well at room temperature in air. Then, the gels were incubated in 5% CO_2 at 37 °C. Photographs of the hydrogels after immersion for 1 and 24 h are shown in Figure 3b. Most of the Mucin/pDMA-2APBA gel samples (x = 25%) dissolved soon after the immersion, and the hydrogel samples almost disappeared within 1 h under all the conditions examined. On the other hand, Mucin/pDMA-2APBA gel (x = 30%) did not dissolve after 24 h under all the conditions examined. These observations indicate that the difference in the boronic acid content is critical for the stability of the hydrogel samples. Figure 3c shows ratio of diameter ($D/D_{initial}$; D indicates diameter at each time, $D_{initial}$ indicates diameter soon after immersion) of the Mucin/pDMA-2APBA gel (x = 30%) over time under each condition. It should be noted here that the hydrogel (x = 30%) in PBS with glucose swelled after 1 h and recovered to almost the original size after 24 h, whereas the hydrogel sample (x = 30%) in PBS shrank, indicating that competitive sugar dissociates the cross-linking through the boronic acid–sugar interaction. The hydrogel sample (x = 30%) in DMEM without FBS also swelled (ca. 20% increase in diameter after 24 h). This is likely that not only glucose (ca. 25 mM) but also some amino acids in the medium interact with boronic acid residue in the hydrogel.[47] Interestingly, the hydrogel sample (x = 30%) shrank upon immersion into DMEM with 10% (v/v) FBS (ca. 30% decrease in diameter after 24 h). The exact reason is unclear, but it is likely that biomacromolecules in FBS may have an intermolecular interaction with either mucin[48] or boronic acid residues[47] in the hydrogel. To elucidate the interaction of the hydrogel and cell culture medium, the dissolved hydrogel was analyzed using membrane electrophoresis. Figure 3d shows the membrane electrophoresis experiment after dissolution of hydrogel samples. A 0.1 mL of Mucin/pDMA-2APBA gel (x = 25%) in 1 mL of PBS, 0.1 M glucose in PBS, DMEM, or DMEM with 10 v/v% FBS dissolved. After 24 h, the resultant solution (1 µL) was then applied onto a polyvinylidene difluoride (PVDF) membrane (8 cm × 8 cm) for electrophoresis. The control sample contains mucin (0.4 w/v%) in PBS. After the electrophoresis with constant current (8 mA) for 10 min, the membrane was stained with an alcian blue solution, which is an anionic polysaccharide-specific dye. Figure 3e shows a photograph of the membrane after washing. As shown in Figure 3e, the samples degraded in PBS, PBS with glucose, whereas the sample in DMEM without FBS showed almost the same bands of mucin (shown in white arrows) as those shown in the control sample. The sample in DMEM with FBS also gave bands from mucin, but the band with higher mobility were almost missing. Instead, a band with lower mobility (shown in a red arrow) appeared. This result is consistent with the tendency in Figure

3b. Previous studies have reported interactions between mucin and serum albumin,[49,50] in which hydrophobic interactions between macromolecules play an important role. In the hydrogels in this study, it is likely that the hydrophobic interactions between mucin, serum proteins, and pDMA-2APBA are rather strong, resulting in gel shrinkage. The potential interaction with such biomacromolecules should be considered when using this hydrogel for biomedical applications.

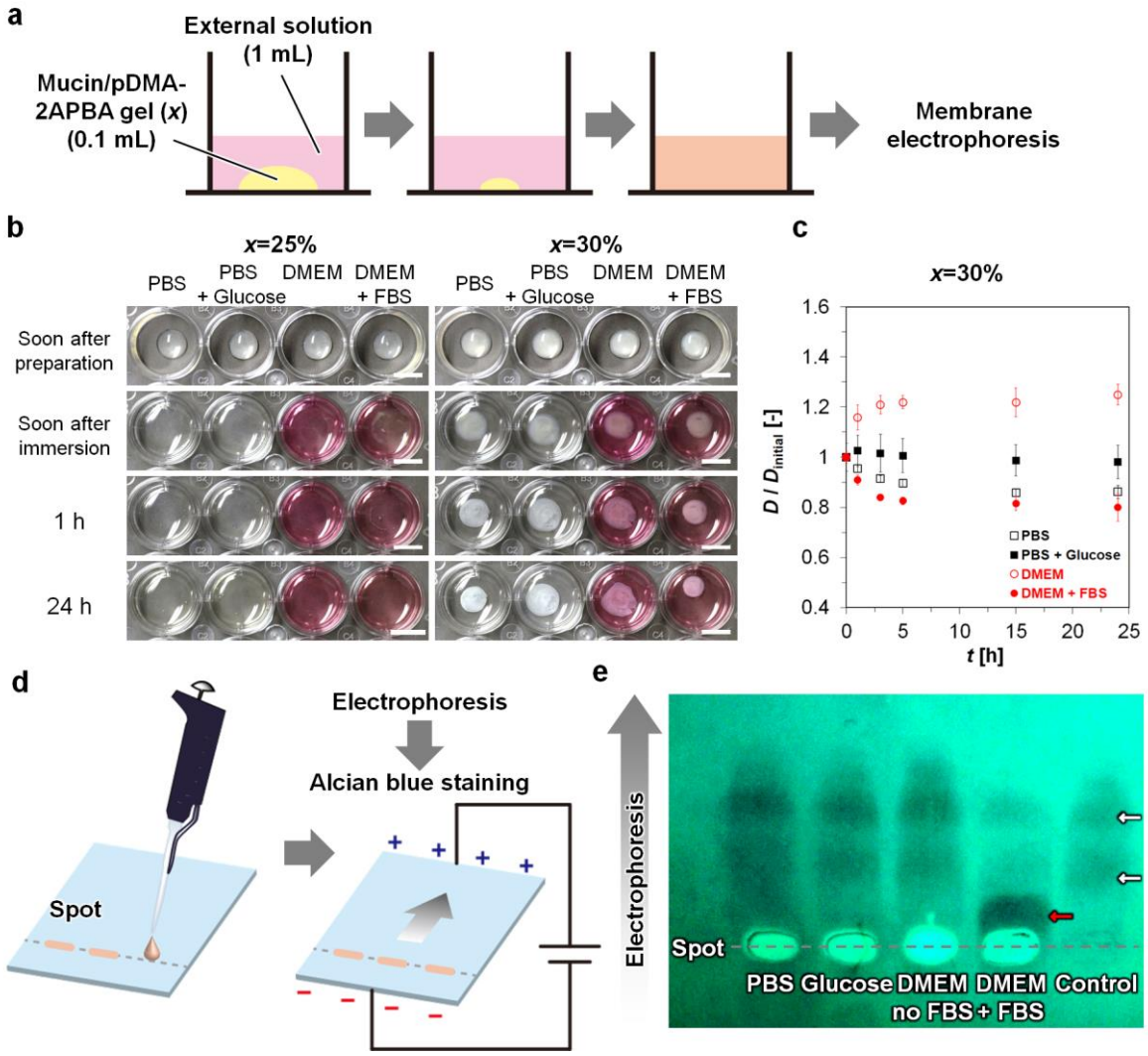

Figure 3. (a) Schematic illustration showing experiments for estimating the stability of the gel. (b) Photographs and (c) change in diameter of the hydrogels over time showing the difference in the degradability of Mucin/pDMA-2APBA gel (x = 25 and 30%) under each condition. (d) Schematic illustration of membrane electrophoresis experiment. (e) Photograph showing membrane electrophoresis of degradant of Mucin/pDMA-2APBA gel (x =25%).

2.4. Cell studies

To demonstrate the potential for biomedical applications of this injectable Mucin/pDMA-2APBA gel (x =25%), mammalian cell culture in the presence of the hydrogel was investigated. Figure 4a shows a schematic illustration of the experimental procedure under the following conditions. (1) 0.1 mL of PBS, (2) 0.05 mL of PBS and 0.05 mL of pDMA-2APBA (x = 25%) (15 w/v%) in PBS, (3) 0.05 mL of PBS and 0.05 mL of mucin (ca. 3.5 w/v%) in PBS, and (4) 0.1 mL of Mucin/pDMA-2APBA gel (x =25%) were placed in each well of 12-well cell culture plate. Then, a suspension containing trypsinized cells in DMEM with 10 v/v% FBS (1 mL) was added to each well so that the surface density of the cells be 1×10^4 cells cm^{-2} and cells were incubated for 24 h in a 5% CO_2 incubator at 37 °C. Fibroblast (10T1/2), myoblast (C2C12), and cancer epithelioid (HeLa) cells were chosen here to see the difference depending on cell type, according to a previous study by Crouzier et al.[13] Figures 4b, S5b, and S6b show micrographs of 10T1/2, C2C12, and HeLa cells under each condition soon after seeding, after incubation for 4 and 24 h. Most of the 10T1/2 cells attached and proliferated in the presence of pDMA-2APBA (x = 25%) and mucin, whereas no adhesion to the surface was observed after 24 h in the presence of hydrogel (Figure 4b). The C2C12 cells showed almost the same tendency as that of the 10T1/2 cells. Cells formed a confluent layer in the absence and presence of pDMA-2APBA (x = 25%) and mucin, whereas no adhesion was observed in the presence of a mixture of both the polymers (Figure S5b). HeLa cells also attached and proliferated in the absence and presence of pDMA-2APBA (x = 25%) and mucin. It should be noted that HeLa cells attached to the surface and proliferated even in the presence of hydrogel (Figure S6b). As shown in Figure 4c, cell adhesion and proliferation strongly depended on cell type. Although cells attached and proliferated in the presence of pDMA-2APBA (x = 25%) or mucin in solution, using polymer concentrations used in these experiments may harmful to the cells depending on cell type. Previous studies suggested that some types of purified mucin prevent cell-adhesion due to their hydrophilic sugar chains,[10,13,51] whereas crude mucin has less effect on such cell-repellent properties.[13] Also, previous studies and Figure 3e suggest that mucin forms intermolecular complexes with non-mucin biomacromolecules.[6,47,52] As for the difference between polymer and hydrogel, polymers had dissolved in PBS instantly diffuse and dissolve when the medium containing cells was added. Thus, the local concentration in the medium should be low throughout the experiments. In contrast, hydrogel does not immediately dissolve into the medium. This means that the local concentration near the bottom of the well should be higher in the early stage of cell culture. This may be the reason why the adhesion of each cell was relatively inhibited in the cases of using hydrogels. Previous studies have shown that cancer cells have

relatively higher expression of sialic acid, one of the sugars that can interact with boronic acid, on the cell surface.[53] Thus, boronic acid-containing polymers have been used to selectively interact with cancer cells.[54] Based on the observations in Figures 3 and 4, it is likely that (1) crude mucin and boronic acid-containing polymer were released from the hydrogel sample to coat the surface, making the local concentration of polymers higher close to the surface. (2) Balance between cell-repellent properties of mucin and cell-adhesive properties of boronic acid-containing polymer and biomacromolecules in FBS determines the cell behavior on the surface.

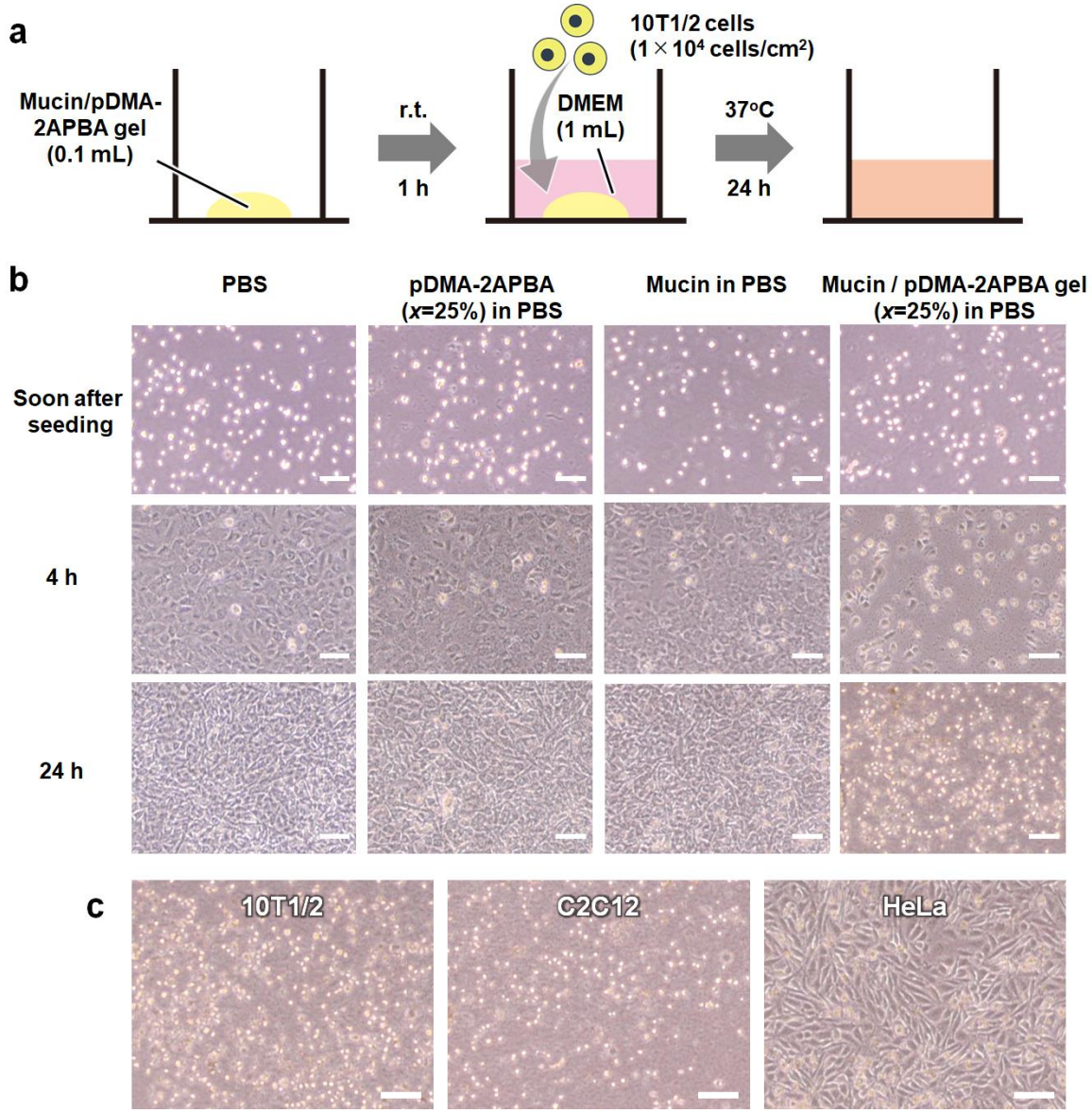

Figure 4. (a) Schematic illustration of cell seeding experiment. (b) Micrographs of 10T1/2 cells soon after seeding, after 4 h and 24 h of culture. (c) Micrographs of 10T1/2, C2C12, and HeLa cells on the well with Mucin/pDMA-2APBA gel after 24 h of culture. Scale bar: 200 μm.

3. Conclusion

We successfully prepared a bio-synthetic hybrid hydrogel through molecular interaction between native mucin as a functional biopolymer and pDMA-2APBA as a synthetic polymer. The mechanical properties and stability of the resultant hydrogel was tunable by changing the boronic acid content as a parameter for material design. The hydrogel affects the adhesion of mammalian cells on the surface depending on the cell type. The mucin-containing hydrogel formed under physiological conditions can be a candidate for various biomedical applications, e.g., coating of medical devices, functional sacrificial scaffolds for tissue engineering, and artificial mucus in which symbiotic bacteria form microbiota. Using the hydrogel system reported in this study, specific applications will be reported in our subsequent paper, in which the hydrogel properties will be optimized for the applications.

4. Experimental Section/Methods

Materials: Acryloyl chloride, D-(+)-glucose, D-(+)-galactose, D-(−)-fructose, diethyl ether, NaCl, KCl, $Na_2HPO_4{\cdot}12H_2O$, KH_2PO_4, $NaHCO_3$, molecular sieves 4A, certified Australian fetal bovine serum (FBS), penicillin G potassium, and streptomycin sulfate were purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). L-Glutamine was obtained from Peptide Institute (Osaka, Japan). 2,2′-Azobisisobutyronitrile (AIBN) was obtained from Kishida Chemical Co. Ltd (Osaka, Japan). Ethanol was obtained from Japan Alcohol Trading Co. Ltd. (Tokyo, Japan). Mucin from porcine stomach (type III, bound sialic acid 0.5–1.5 %, partially purified), *N*,*N*-dimethylacrylamide (DMA), and trypan blue were obtained from Sigma-Aldrich (St. Louis, MO, USA). D_2O was obtained from Cambrige Isotope Laboratories, Inc. (Tewksbury, MA, USA). 2-Aminophenylboronic acid was obtained from Combi-Blocks (San Diego, CA, USA). Water used for the preparation of the aqueous solutions (except for NMR measurements) was purified with a Milli-Q Advantage A10 system (Merck Millipore, Burlington, MA, USA). All reagents were used without further purification.

Measurements: ^{1}H NMR spectra were recorded at 400 MHz with a JNM–ECS400 NMR spectrometer (JEOL, Tokyo, Japan). In all NMR measurements, chemical shifts were referenced to the solvent values (δ = 4.79 ppm for D_2O). Rheological measurements were performed using a HAAKE MARS III (Thermo Fisher Scientific, Waltham, MA, USA) at a constant shear strain of 1% at 37 °C. The gap between the parallel plate (φ = 40 mm) and the stage was set to 0.5 mm. A sample (1.0 mL) was placed between the parallel plate and stage.

The sample was covered with a lid, and the inside was filled with humidified air throughout the measurements. Optical micrographs were taken by an inverted light microscope (CKX-41 and IX-71, Olympus, Tokyo, Japan). Gel permeation chromatography (GPC) analysis was carried out using a GL-7400 HPLC system (GL Science, Tokyo, Japan) equipped with Inertsil WP300 Diol column (GL Science, Tokyo, Japan) and refractive index (RI) detector (RID-20A, Shimadzu, Kyoto, Japan), using PBS as an eluent at the flow rate of 0.3 mL min^{-1}. at 25 ºC. Standard samples of ReadyCal-Kit Pullulan (purchased from PSS Polymer Standards Service GmbH, Mainz, Germany) were used for calibration.

Preparation of polymers: 2-Acrylamidophenylboronic acid (2APBA) was prepared according to the procedure of Yang et al.[44] with a slight modification. ^{1}H NMR spectrum is shown in Figure S1a. The polymer pDMA-2APBA was prepared by conventional radical copolymerization of 2APBA and DMA initiated with AIBN.

Briefly, 2APBA (x/100 M), DMA ((100 – x)/100 M) and AIBN (0.01 M) were dissolved in ethanol dried with molecular sieves 4A. Here x represents the feed ratio of 2APBA monomer per all monomers on a molar basis. The solution was purged with nitrogen gas for 1 h, sealed, and heated with an oil bath thermostated at 65 °C for 12 h. The solution was poured into a 10-fold volume of diethyl ether. The precipitated polymer was collected by filtration, washed with diethyl ether thrice, and dried under reduced pressure at room temperature.

Preparation of hydrogel: Mucin/pDMA-2APBA gel was prepared by mixing aqueous solutions of mucin and pDMA-2APBA. Typically, mucin was mixed with PBS so that the final concentration be 4 w/v%. After stirring overnight at room temperature, the milky solution was centrifuged (12,000 rpm, 4 °C) to remove the insoluble part. The clear solution containing ca. 3.5 w/v% of mucin was used. pDMA-2APBA was mixed with PBS so that the final concentration be 15 w/v%. After stirring overnight at room temperature, a clear solution was obtained and used as it was. Aqueous solutions of mucin and pDMA-2APBA were mixed at 1/1 (v/v) to form a hydrogel.

Sugar-responsiveness of hydrogel: Mucin/pDMA-2APBA gel (100 μL) was formed in a 12-well plate. After standing for 1 h at room temperature, 1 mL of an external solution was added to each well. Then, photographs of the gels were taken at a specific time during incubation in a humidified 5% CO_2 incubator at 37 °C.

Membrane electrophoresis: Supported molecular matrix electrophoresis (SMME) was used to separate and characterize mucin contained in aqueous solution according to electrophoretic mobility based on net charge. Mucin membrane electrophoresis kit (SMME-01) and

electrophoresis unit (SMME-02) (Cosmo Bio, Tokyo, Japan) were purchased and used according to the manufacture's protocol.

Cell culture: Human cervix epithelioid carcinoma HeLa cells, mouse embryo fibroblast cell line 10T1/2 cells, and mouse striated muscle myoblast C2C12 cells (the Riken Cell Bank, Ibaraki, Japan) were cultured in Dulbecco's modified Eagle's medium (DMEM, Nissui Pharmaceutical, Tokyo, Japan) supplemented with FBS (10 v/v%), penicillin G potassium (100 U mL^{-1}), streptomycin sulfate (100 mg L^{-1}), L-glutamine (0.59 g L^{-1}), and D-(+)-glucose (4.5 g L^{-1}) at 37 °C in humidified air containing 5% CO_2. The viability of cells was estimated using a trypan blue exclusion method.

Cell seeding experiments: Mucin/pDMA-2APBA gel (100 μL) was formed in a 12-well plate. After standing for 1 h at room temperature, 1 mL of cell suspension was added to each well so that cell density in each well be 1×10^5 cells cm^{-2}. Then, micrographs of the cells were taken at a specific time during incubation in a humidified 5% CO_2 incubator at 37 °C.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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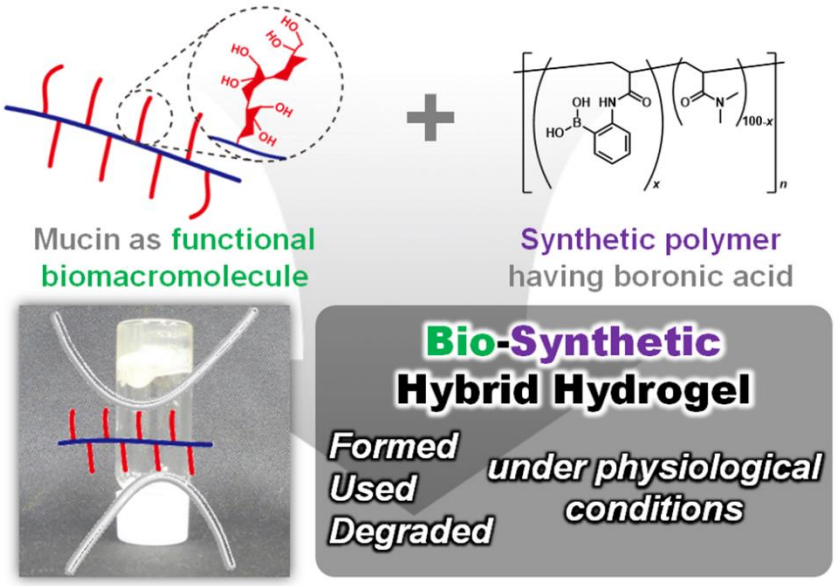
Mucin-containing bio-synthetic hybrid hydrogel is successfully formed under physiological conditions upon mixing aqueous solutions of mucin and synthetic polymer carrying boronic acids. Mechanical properties of the hydrogel are controllable depending on content of boronic acids. The hydrogel dissolved in the physiological solutions releases mucin and boronic acid-containing polymer, which can control the adhesion of mammalian cells to the surface.

M. Nakahata*, N. Tominaga, K. Saito, K. Nishiyama, Y. Tanino, K. Saiki, M. Kojima, S. Sakai

A bio-synthetic hybrid hydrogel formed under physiological conditions consisting of mucin and a synthetic polymer carrying boronic acid



Supporting Information

A bio-synthetic hybrid hydrogel formed under physiological conditions consisting of mucin and synthetic polymer carrying boronic acid

Masaki Nakahata, Naoki Tominaga, Keishi Saito, Keita Nishiyama, Yuya Tanino, Kiyoshiro Saiki, Masaru Kojima, and Shinji Sakai*

Characterization of monomer and polymers.

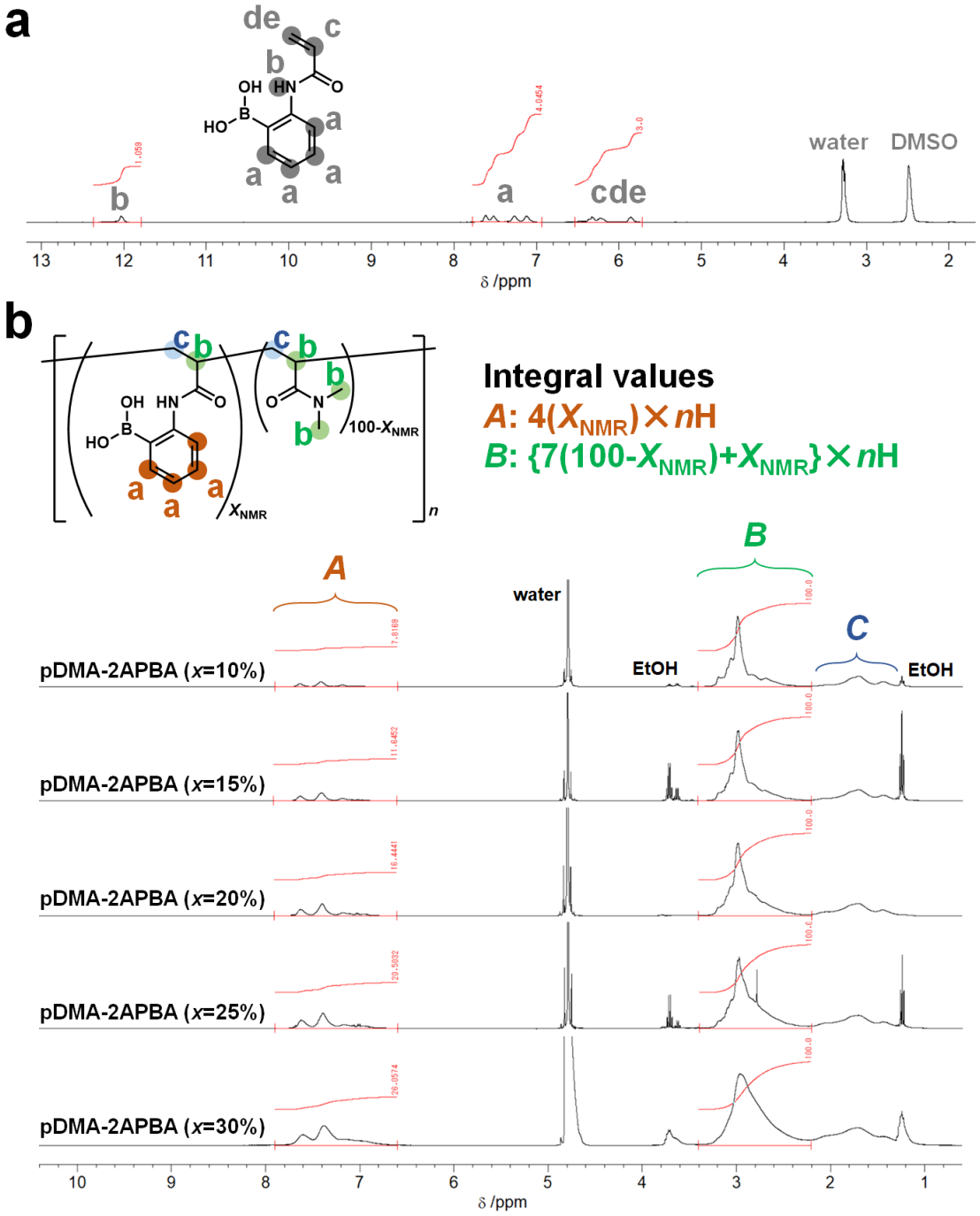


Figure S1. (a) ^{1}H NMR spectrum of 2APBA (400 MHz, DMSO-d_6, 30°C).

(b) ^{1}H NMR spectra of pDMA-2APBA (400 MHz, D_2O, 30°C).

The composition of pDMA-2APBA was determined from ^{1}H NMR spectra (Figure S1). Using integral values of peaks a (δ = 7.9-6.6 ppm, *A*) and b (δ = 3.4-2.2 ppm, *B*), the ratio of 2APBA unit (X_{NMR}) was estimated with the following equation:

$$A : B = 4X_{NMR} : 7(100\text{-}X_{NMR})+X_{NMR} \quad (1)$$

Table S1. Calculation of X_{NMR}.

Feed ratio (*x*) [%]	***A* [-]**	***B* [-]**	**X_{NMR} [%]**
10	7.82	100	12
15	11.6	100	17
20	16.4	100	23
25	20.5	100	27
30	26.1	100	33

In all samples, X_{NMR} was estimated to be slightly larger than *x*. This might be due to the evaporation of DMA monomer during nitrogen bubbling for 1 h.

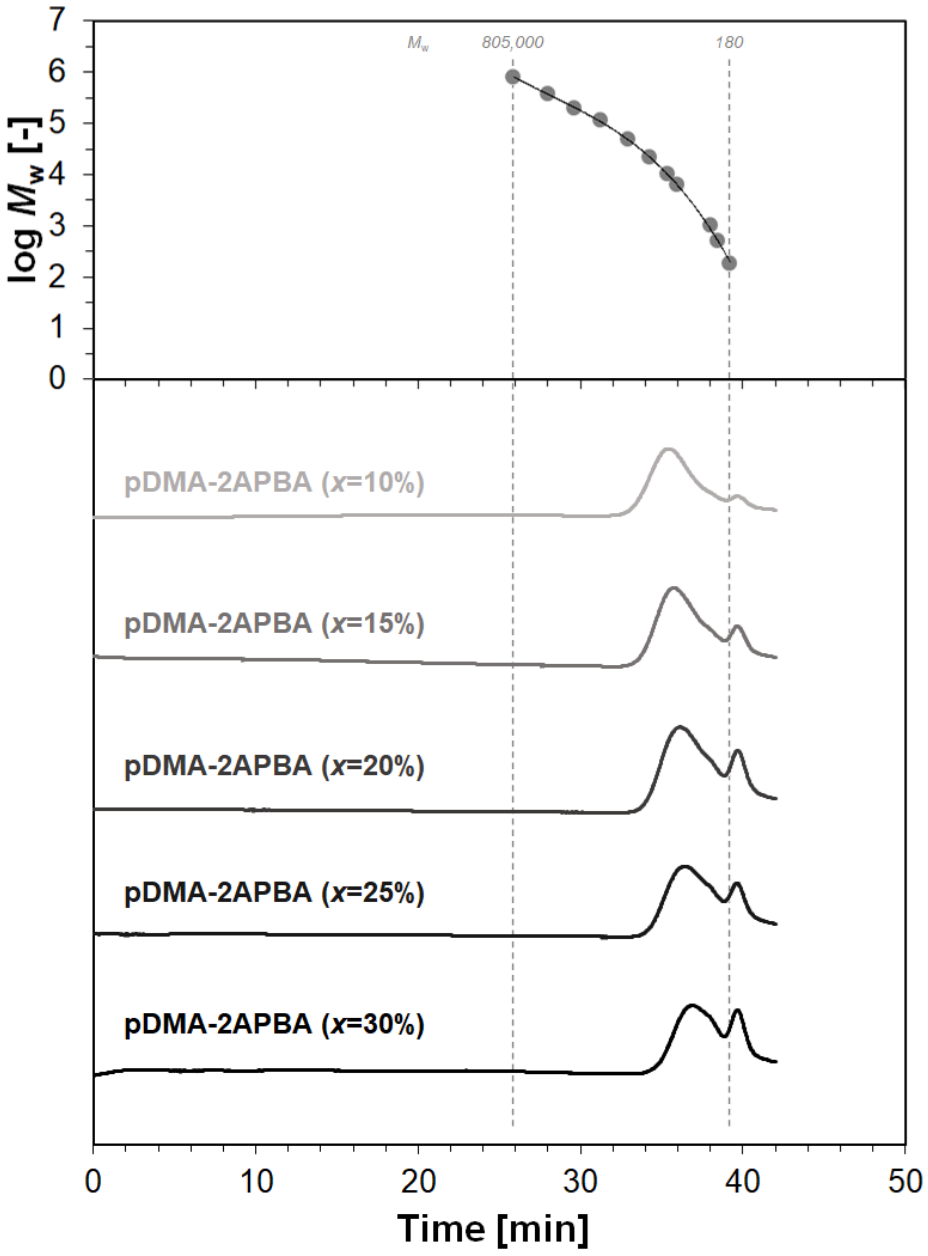
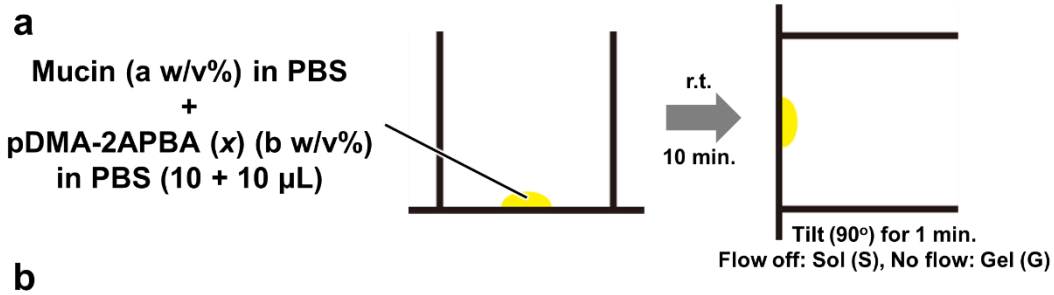


Figure S2. GPC charts of pDMA-2APBA shown with calibration curve (eluent: PBS, flow rate: 0.3 mL/min., 25 ºC. Pullulan was used as standard.).

Table S2. Weight-average molecular weight (M_w) and polydispersity index (PDI = M_w/M_n) of the polymers estimated using pullulan standard.

Feed ratio (*x*) [%]	**Peak top M_w [Da]**	**M_w/M_n [-]**
10	1.1×10^4	4.1
15	8.4×10^3	3.6
20	6.6×10^3	3.3
25	5.5×10^3	3.1
30	3.9×10^3	2.7

Dependence of polymer concentration on hydrogel formation.



b

pDMA-2APBA (25) [w/v%] \ Mucin [w/v%]	0	1	2	3	4
0	S	S	S	S	S
5	S	S	S	S	S
10	S	S	S	S	G
15	S	S	S	S	G

pDMA-2APBA (30) [w/v%] \ Mucin [w/v%]	0	1	2	3	4
0	S	S	S	S	S
5	S	S	S	S	S
10	S	S	G	G	G
15	S	G	G	G	G

Figure S3. (a) Schematic illustration of hydrogelation test. (b) Observation for hydrogelation test on Mucin/pDMA-2APBA (25) and (30). S: sol, G: gel.

Hydrogelation in the presence of competitive sugar molecules.

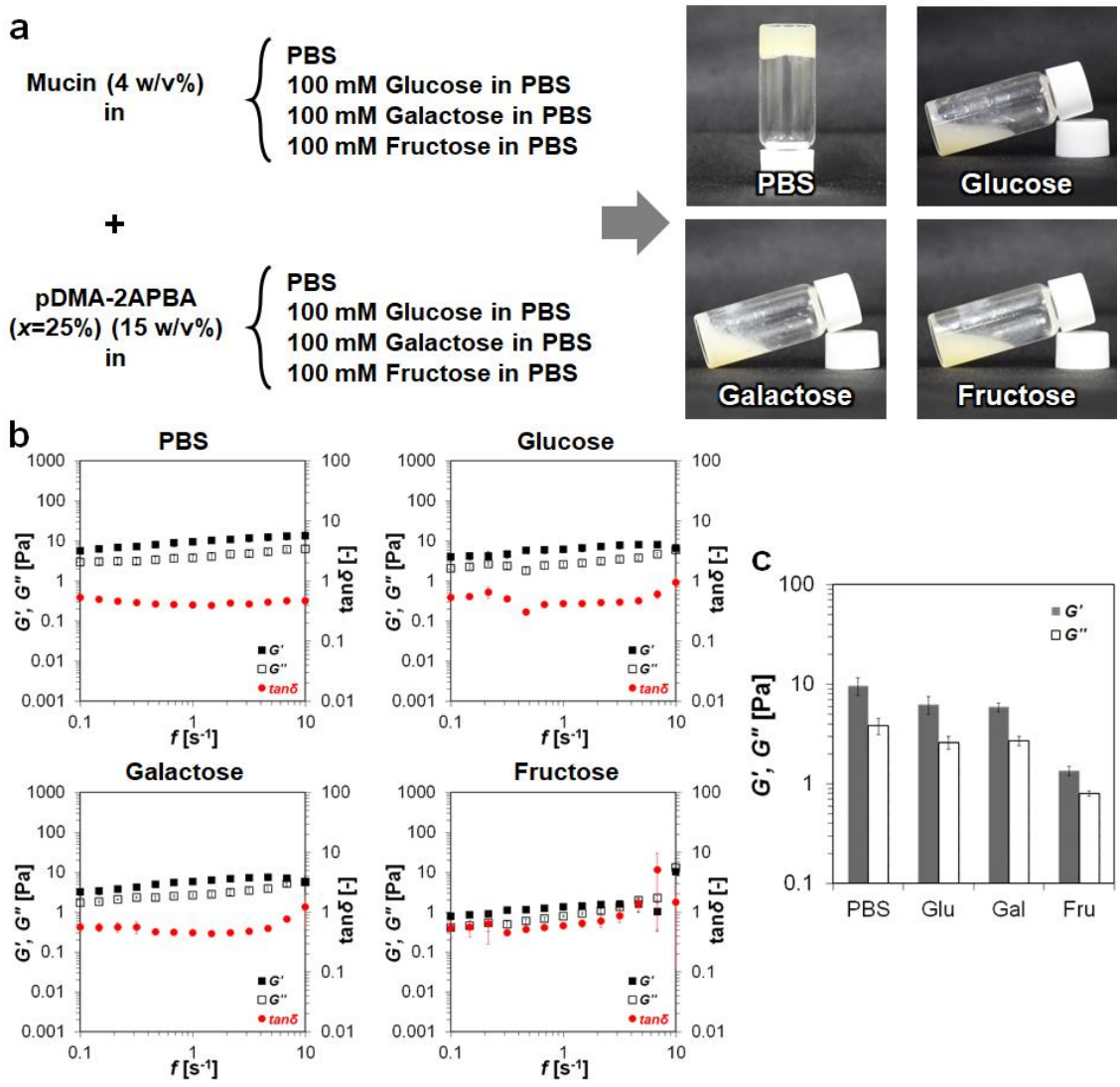


Figure S4. (a) Photographs of aqueous solution mixtures of mucin (4 w/v%) and pDMA-2APBA (*x*=25%) (15 w/v%) in the absence and presence of glucose, galactose, and fructose in PBS. (b) Dynamic viscoelastic measurements of the mixtures of solutions of mucin and pDMA-2APBA (*x*=25%) in the absence and presence of sugars at 37 °C. (c) Dependence of *G'* and *G''* (at *f* = 1 Hz) on sugar molecules.

Cell experiments using various types of cells.

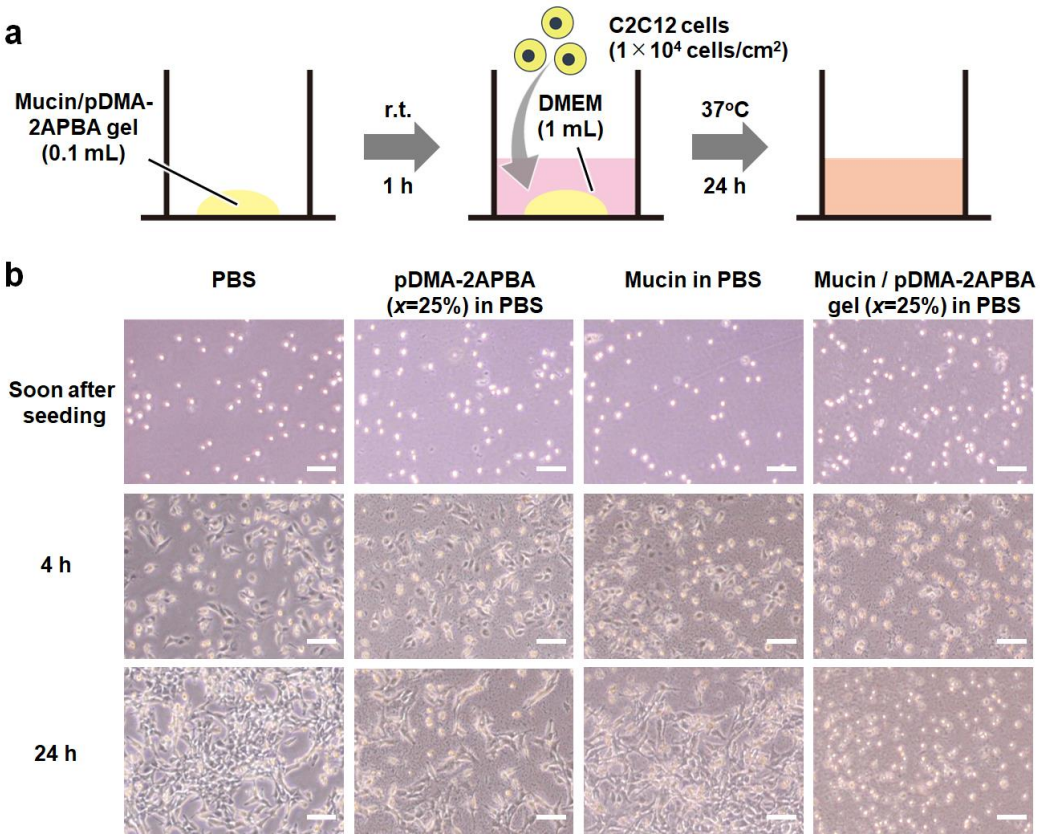


Figure S5. (a) Schematic illustration of cell seeding experiment using C2C12 cells. (b) Micrographs of C2C12 cells soon after seeding, after 4 h and 24 h of culture. Scale bar = 200 μm.

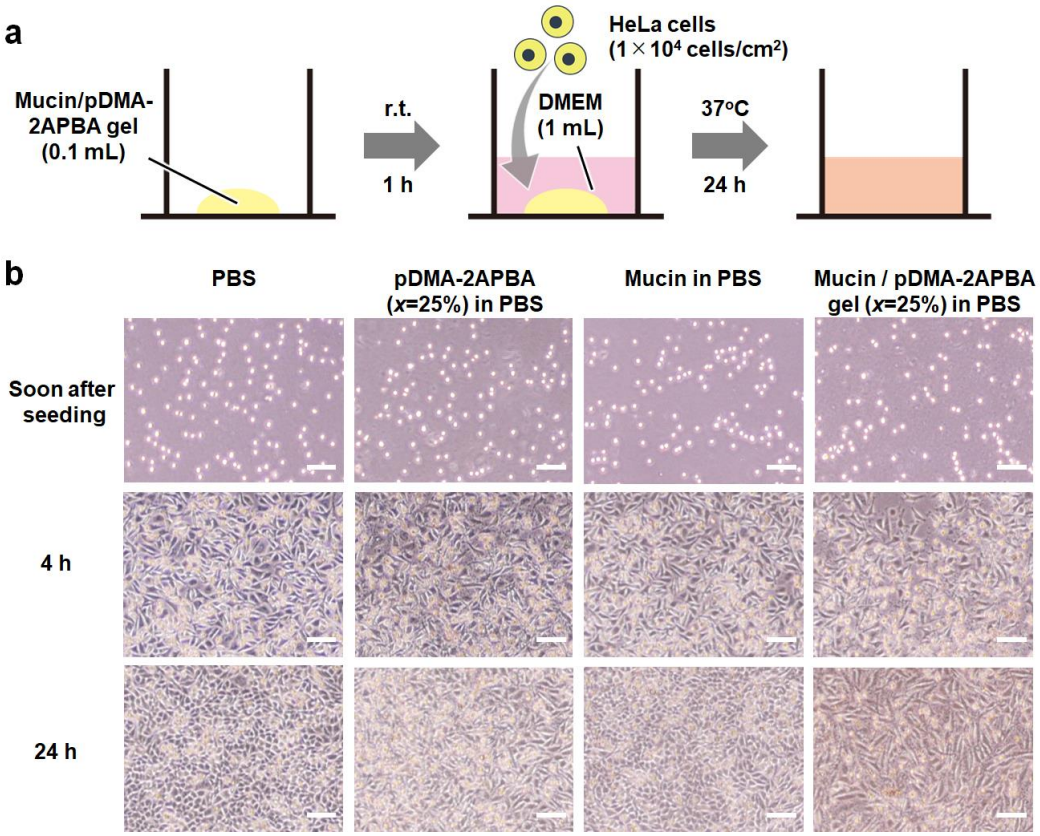


Figure S6. (a) Schematic illustration of cell seeding experiment using HeLa cells. (b) Micrographs of HeLa cells soon after seeding, after 4 h and 24 h of culture. Scale bar = 200 μm.