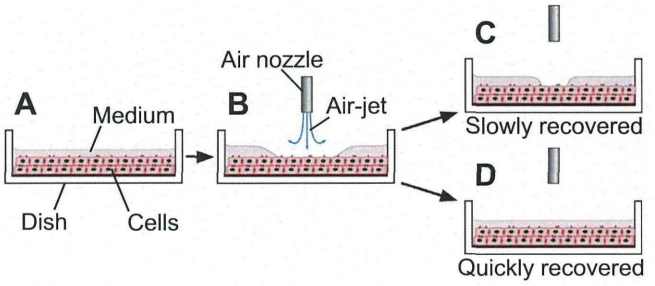

Fig. 1. Procedure of wettability assessment of cultured-cell surface. (A) Confluent cells on a cell culture dish were covered with a constant volume of culture medium. (B) Air-jet was given from an air-nozzle to the surface of culture medium, and the culture medium was squeezed by the air-jet. (C) In the case of hydrophobic surface, the squeezed area of culture medium was still remained. (D) On the other hand, in the case of hydrophilic surface, the culture medium recovers quickly.

surface of culture medium covering over cultured cells, the squeezed area of culture medium by air-jet was measured (Fig. 1B). After the cease of air-jet application within 1 s, medium on a low-wettability cell-surface was still squeezed or slowly recovered (Fig. 1C). On the other hand, culture medium recovered quickly over the cultured cells having a high wettability (Fig. 1D). Furthermore, the relationships between the expressions of mucous glycoprotein of cells, which were cultured in either fatal-bovine-serum-contained or eliminated cell-culture media, and the squeezed area were investigated.

2. Materials and methods

2.1. Development of measurement setup

The measurement setup was mainly composed of both an air-jet application unit (Fig. 2A) and observation unit (right side in Fig. 2B). In the observation unit, a handy digital movie camera (HDR-SR1) (SONY, Tokyo, Japan) (item No. 5 in Fig. 2B) was installed for observing and recording experiments, and an LED lamp (GH-LED08CLK) (GREEN HOUSE, Tokyo, Japan) (item No. 6 in Fig. 2B) illuminated the air-jet application unit area for obtaining clear images. The air-jet application-unit consisted of an air-nozzle (0.5 mm i.d., 20 mm long) (Air Blow Nozzle, ABNZNL5-1.0-20) (MISUMI, Tokyo, Japan) (item No. 2 in Fig. 2A) and a high speed solenoid valve (VA01PSP23-1P) (KURODA Pneumatics, Chiba, Japan) (item No. 3 in Fig. 2A) with a maximum switching frequency of 333 Hz. The air pressure of air source was controlled by an electro-pneumatic regulator (ITV2050-312CSQ) (SMC, Tokyo, Japan) before passing through the solenoid valve. Both solenoid valve and regulator were controlled by a laptop computer (ThinkPad X61) (Lenovo Japan, Tokyo, Japan) via an analog input/output interface module (CSI-360112) (Interface, Hiroshima, Japan). Compressed air as an air source was supplied from an oil-free type air-compressor (DPP-AYAD) (Koganei, Tokyo, Japan) through a membrane filter (Millex-GV, 0.22 μm, PVDF, 33 mm) (EMD Millipore, Billerica, MA). The air-jet application unit was attached on an *x*-*y*-*z*-axes linear slider device (custom-made item) (Sigma-koki, Tokyo, Japan) (item No. 4 in Fig. 2B) for obtaining fine alignment within a position accuracy of 0.1 mm. The movie camera and the linear slider with the air-jet application unit were rigidly fixed to a home-made base-plate made of aluminum frame (item No. 7 in Fig. 2B). The lamp was mounted on an appropriate position for observing around the air-jet application unit and a measurement object. To keeping temperature around the object, hot plate was installed on the base plate directly under the object.

2.2. Characterization of air-jet application

The pressure distribution of air-jet application was measured by a pressure sensor (PA-400) (Nidec Copal Electronics, Tokyo, Japan). By using the position adjuster, the horizontal distance between the air-nozzle and the probe hole of pressure sensor was able to be changed from 0 to 3 mm with a step of 0.1 mm (the inset in Fig. 3A), and the vertical distance between the air-nozzle and the probe hole was able to be changed from 1 to 3 mm with a step of 1 mm (the inset in Fig. 3B). Then, one air-jet was given from the air-nozzle with a specific horizontal and vertical distance. The pressure of air source was set to be from 0 to 0.4 MPa with a pressure step of 0.05 MPa. After the measurement of pressure distribution, the pressure was converted into fluid force by calculating the following equation:

$$f = \sum_{i=1}^{N} \left\{ p_i (2i-1)\pi \left(\Delta r^2 \right) \right\} \quad (1)$$

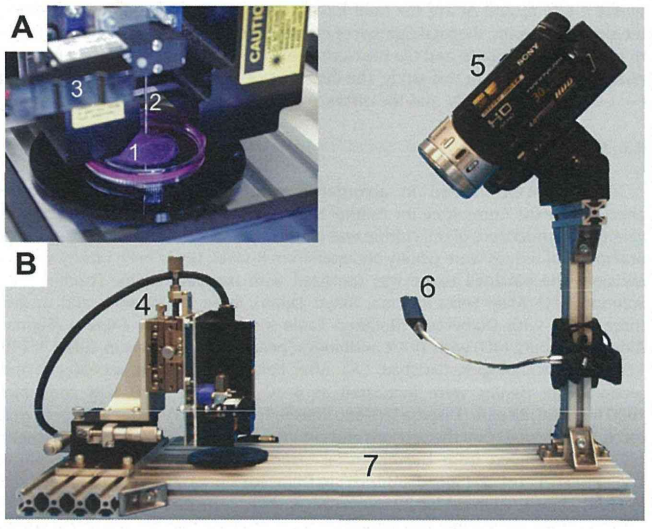

Fig. 2. Experimental setup for assessing the wettability of cultured cells. (A) Close-up view of air-jet application unit. (1) Cultured cells on a cell-culture substrate, (2) 0.5-mm-inner-diameter Air-nozzle, and (3) A electrical solenoid valve. (B) General view of experimental setup. (4) A position adjuster for *x*-*y*-*z*-axes directions, (5) A full-hivision movie camera, (6) An LED illumination, and (7) A home-made base-plate made of aluminum frames.

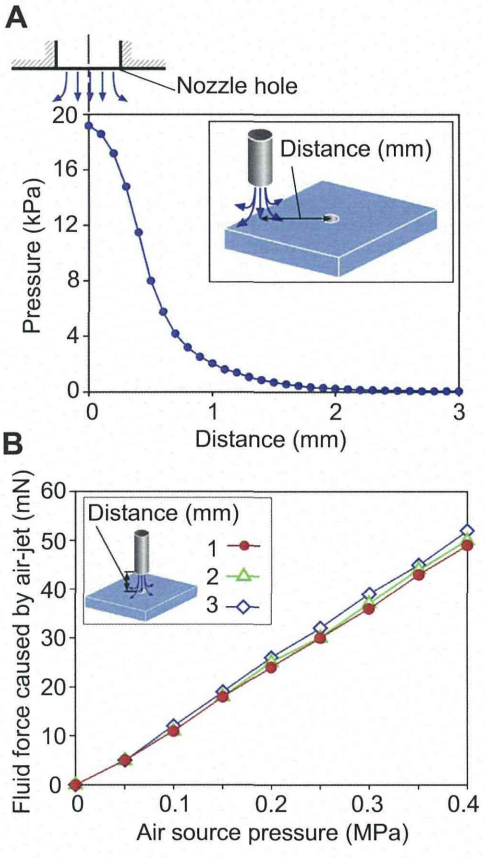

Fig. 3. Air-jet characterization of the wettability assessment device. Graph (A) The pressure distribution of air-jet was measured by a pressure sensor, which was able to move to the horizontal direction with an interval of 0.1 mm. The vertical axis is the pressure measured by the pressure sensor. The horizontal axis is the distance between a specific point under the air-nozzle and the probe hole of pressure sensor. Graph (B) shows the relationship between fluid force caused by air-jet and air source pressure at the inside of air-nozzle. Red, green, and blue lines show distances between air-nozzle and an object of 1, 2, and 3 mm, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

where f, i, N, p_i, and Δr are the fluid force caused by air-jet, the index number of measurement point, the total number of measurement points, the pressure data in i-th measurement points, and the measurement step to the radial direction from air-nozzle, respectively. In this study, the distances of 0 and 3.0 mm corresponded to $i = 1$ and 31, respectively, and the distance of measurement step Δr was 0.1 mm.

2.3. Cell culture

Animals were treated in accordance with the experimental procedures approved by the Committee for Animal Research of Tokyo Women's Medical University. The procedure of cell culture was based on the previous studies [26–29]. Rat oral mucosal tissues were wholly obtained from 8-week Lewis male rats by surgical excision. The obtained tissue was sterilized with povidone-iodine (Isodine Field Solution 10%) (Meiji Seika Pharma, Tokyo, Japan), dried for 30–60 s, and washed three times with Dulbecco's Modified Eagle Medium (DMEM, D6429) (Sigma–Aldrich, St. Louis, MO) with 1v/v% antibiotics (penicillin-streptomycin, Gibco 15140-122) (Life Technologies, Carlsbad, CA). After being cut into approximately 5 mm sections, the tissues were incubated at 4 °C overnight in DMEM containing 1000 units/mL dispase (Dispase I) (Godo Shusei, Tokyo, Japan). Epithelial layers were carefully removed from the sections and cut into a size of less than 1 mm. Epithelial cells were isolated from the epithelial layers by treating with 5-time-diluted Trypsin-EDTA (T4174) (Sigma–Aldrich) in Dulbecco's phosphate buffer saline (PBS) (D1408) (Sigma–Aldrich) for 20 min at 37 °C. After trypsin-EDTA was inhibited by adding DMEM containing 10% fetal bovine serum (FBS) (Lot No. 83300124) (Moregate BioTech, Queensland, Australia) and 1% antibiotics, a single-cell suspension was obtained by filtering with a 40-μm cell strainer (Falcon 352340) (Becton Dickinson, Franklin Lakes, NJ), and the medium of suspension was changed with DMEM containing only 1% antibiotics. Two types of culture media were prepared. Both media were mainly composed of a mixture of DMEM and Ham's F-12 (N6658) (Sigma–Aldrich) in a ratio of 1–3 with 0.4 μg/mL hydrocortisone (080-05581) (Wako Pure Chemical, Osaka, Japan), 2 nmol/L triiodothyronine (592-12121) (Wako Pure Chemical), 1 nmol/L cholera toxin (030-16331) (Wako Pure Chemical), 5 μg/mL insulin (Gibco 41400-045) (Life Technologies), 5 μg/mL transferrin (Gibco 11107-018) (Life Technologies), 10 ng/mL epidermal growth factor (EGF) (Invitrogen 13247-051) (Life Technologies), and 1% antibiotics. The base media were further supplemented with 5% FBS for the normal medium, which was referred to as keratinocyte culture medium (KCM), and 2.5 mg/mL bovine serum albumin (BSA) (A8806) (Sigma–Aldrich) as FBS-free medium. Primary rat oral epithelial cells were seeded in temperature-responsive culture inserts (23 mm i.d., UpCell Insert) (CellSeed, Tokyo, Japan) on a 6-well cell culture plate (353046) (Becton Dickinson, Franklin Lakes, NJ) at an initial densities of 4×10^4 cells/cm^2 for KCM and 8×10^4 cells/cm^2 for FBS-free medium. These cells were cultured for 10 days with KCM and 14 days with FBS-free medium in a humidified condition with 5% CO_2. Cell morphology was monitored with a phase-contrast microscope (ECLIPSE TE2000-U) (Nikon, Tokyo, Japan).

2.4. Wettability assessment

A cell culture insert with confluent cultured cells was moved from 6-well culture plate onto a cell culture dish (353001) (Becton Dickinson). After old culture medium covered on cultured cells was carefully removed, 1.2 mL fresh KCM was poured into the cell culture insert, which was put on the hot plate under the air-nozzle. An air-jet was applied to the surface of culture medium covering the confluent cells. Three different applied pressures at 6, 10, and 16 kPa that had been calibrated in advance were used. The air-jet application time controlled by a laptop computer was 1.0 s. A dynamic phenomenon appearing on culture medium under the air-nozzle was recorded by the handy digital movie camera from 0.5 s before the air-jet application to 3.5 s after the cease of application. Upon the recording, one pixel of each frame of movie images was calibrated to a real length. The width of squeezed area on culture medium in every frame of every recorded movie was measured by using homemade pixel counter software. The width data at 3.5 s after the cease of air-jet application on confluent cells cultured in FBS-free medium were compared to those of KCM. For evaluating significant difference between two groups, Welch's t-test was used.

2.5. Alcian blue staining

After being cultured for 10 days (KCM) or 14 days (FBS-free medium), rat oral mucosal epithelial cells were rinsed with PBS three times and fixed with 4% paraformaldehyde phosphate buffer solution (163-20145) (Wako Pure Chemical). After being rinsed with 3% acetic acid (017-00256) (Wako Pure Chemical), the fixed cells were stained with Alcian blue solution at pH 2.5 (015-13805) (Wako Pure Chemical) for 24 h at room temperature. The fixed and stained cells were then rinsed with PBS three times again and photographed by a digital camera. The stained status of cells in KCM was qualitatively compared with that in FBS-free medium.

2.6. Histology

To harvest a rat oral mucosal epithelial cell sheet in the normal or FBS-free KCM, the temperature of a thermo-responsive culture insert was reduced to 20 °C after confirming the confluence of cells. Harvested cell sheets were fixed with 4% paraformaldehyde phosphate buffer solution and processed into 4-μm thick paraffin-embedded sections on slid glasses. Hematoxylin and eosin (HE) staining was performed by conventional methods. Sections were deparaffinized and rinsed with 3% acetic acid for 3 min. Then, sections were stained with Alcian blue solution at pH 2.5 for 1 h. After being washed with 3% acetic acid for 3 min and tap water for 5 min, the sections were counter-stained with kern echtrot stain solution (40872) (Muto Pure Chemicals, Tokyo, Japan) for 5 min. The sections were washed with tap water for 1 min, covered with cover glasses, and observed with a microscope (ECLIPSE E800) (Nikon, Tokyo, Japan). The maximum differences between the thinnest and the thickest parts of section, the thickness of section, and the thickness of Alcian blue stained parts were measured in the microscope images of sections.

2.7. Immunohistochemistry

The deparaffinized sections on slid glasses were heated at 96 °C in 10-time-diluted citrate buffer solution (Target Retrieval Solution S1699) (Dako, Glostrup, Denmark) in PBS for 20 min for retrieving antigens. After being washed with PBS with 0.1% Tween 20 (161-0781) (Bio-Rad, Hercules, CA), (PBS-T) for 5 min, the sections were reacted with a few drops of peroxidase blocking solution (S2023) (Dako) for 5 min at room temperature. After being washed with PBS-T for 5 min, the sections were reacted with 5% donkey serum (317-000-121) (Jackson ImmunoResearch Laboratories, West Grove, PA) in PBS-T at room temperature for 1 h mouse monoclonal anti-mucin 4 antibody (6A134) (ab85717) (Abcom, Cambridge, UK) diluted (1:100) with 1% Donkey serum in PBS-T was reacted with the sections at 4 °C overnight. After the sections were washed with PBS-T for 5 min three times at room temperature, anti-mouse IgG antibody conjugate with horseradish peroxidase (715-036-150) (Jackson ImmunoResearch Laboratories) diluted (1:500) with 1% donkey serum in PBS-T was reacted to the section at room temperature for 1 h. After being washed with PBS-T for 5 min three times at room temperature, the sections were reacted with 3,3′-diaminobenzidine solution (K3466) (Dako) under microscopic observation for 2 min at room temperature. Immediately, the sections were washed with PBS-T. The counterstaining was performed with hematoxylin for 5 min. The stained sections were observed with an upright microscope (ECLIPSE E800) (Nikon). The thicknesses of anti-MUC4 antibody reacted parts in the sections were measured in the microscope images of sections.

2.8. Gene expression assay

The expression of messenger RNA (mRNA) was quantified by a quantitative reverse transcription polymerase chain reaction (RT-PCR) method. Cells cultured in KCM and FBS-free medium were lysed with an RNA extract kit (RNeasy Mini Kit, 74105) (Qiagen, Hilden, Germany), and total RNA in the lysate of cells was isolated with the kit. The single stranded complementary DNA (cDNA) was synthesized from the isolated RNA with a single stranded cDNA synthesis kit (First-strand cDNA Synthesis System for Quantitative RT-PCR, 11801) (Marligen Biosciences, Ijamsville, MD) by using a thermal cycler (iCycler) (Bio-Rad Laboratories, Hercules, CA). Primer pairs and TaqMan MGB probes (TaqMan Gene Expression Assays) (Life Technologies) were designed for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Rn01775763_g1), mucin 1 (MUC1; Rn01462585_m1), mucin 4 (MUC4; Rn01475265_m1), mucin 13 (MUC13; Rn00709919_m1), mucin 15 (MUC15; Rn01747580_m1), and mucin 16 (MUC16; Rn01749838_g1). The quantitative RT-PCR was performed by using a real-time PCR system (StepOnePlus) (Life Technologies) with PCR reagents (TaqMan Fast Advanced Master Mix 4444556) (Life Technologies). The mRNA expression levels of mucin genes were normalized with the expression level of GAPDH. For evaluating significant difference between two groups, Welch's t-test was used.

3. Results and discussion

Measurement setup was successfully assembled, confirmed to work normally, and found to satisfy the expected functional requirements. Pressure distribution of air-jet was measured by a pressure sensor, which moved to the horizontal direction to the center axis of air-nozzle (Fig. 3A). The profile of pressure distribution exhibited a bell-like shape where the peak of shape was located directly under the nozzle hole, and the skirt of shape spread out at a point from the probe hole of pressure sensor, confirming that the radius of spread skirt was 3 mm. Equation (1) was applied to the pressure distribution for every measurement condition: the vertical distance between the air-nozzle and the pressure sensor, and the pressure of air source, individually. The relationship between the fluid force and air-source pressure was obtained (Fig. 3B). The fluid force increased linearly with increasing air-source pressure in the measured range (Fig. 3B). When the vertical nozzle-sensor distance was changed from 1 to 3 mm, the fluid

force varied within only 2% at 0.30 MPa at maximum, and the variation of fluid force was negligible at 0.05 MPa, which corresponded to the pressure of air source for this study. This result suggested that the alignment for the vertical distance between the air-nozzle and an object was almost insensitive at the condition of this study.

Primary rat oral mucosal epithelial cells were cultured for 10 days in KCM ($n = 3$) and for 14 days in FBS-free medium ($n = 3$), and then the wettability assessment of cultured cells in both conditions was performed (Fig. 4A and Supplementary Movie 1). Before air-jet application, the surface of cells was confirmed to be fully covered KCM (Fig. 4A at 0 s). At 0.5 s after the start of video recording, one air-jet was applied to the surface for 1 s. Both the normal and FBS-free media were squeezed out outer direction from the air-nozzle (Fig. 4A from 0.5 s to 1.5 s). After the cease of air-jet application, KCM (Fig. 4A at 5 s) recovered the squeezed region. On the other hand, the squeezed area of FBS-free culture medium still remained at 5.0 s (Fig. 4A at 5 s). Generally, when liquid surface is unlevel

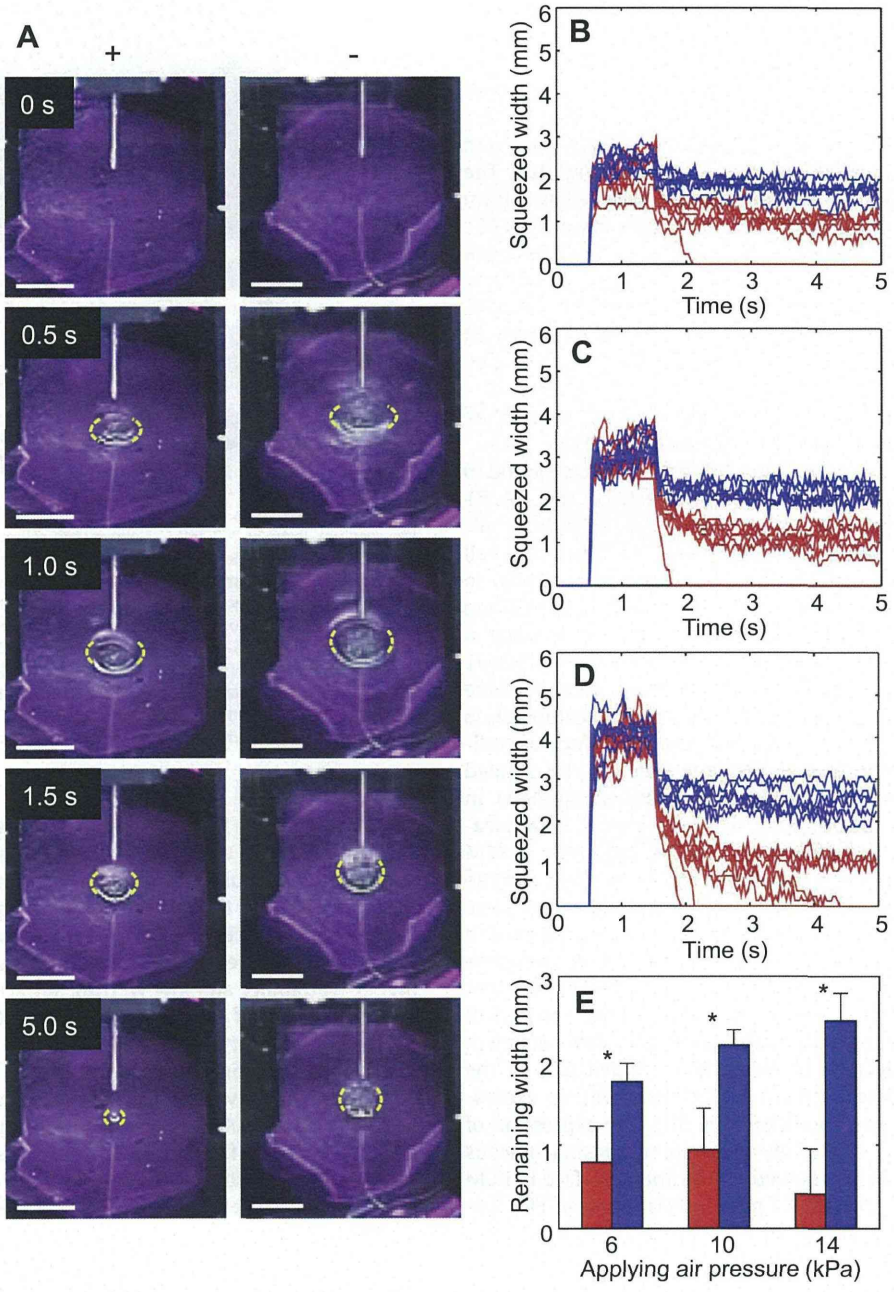

Fig. 4. Culture medium removal by air-jet application. Photographs in columns (A) show the time-series of video images captured by a video camera at 0, 0.5, 1.0, 1.5, and 5.0 s. Air-jet was applied from 0.5 to 1.5 s. Left and right columns (A) show the surfaces of culture-medium-covered cells cultured in KCM and FBS-free medium, respectively. Symbols "+" and "−" above both columns indicate the conditions of FBS-addition (KCM) and FBS-free, respectively. Scale bars represent 10 mm. Yellow dashed lines show the outline of squeezed area of culture medium. Graphs (B), (C), and (D) show the time-courses of squeezed width of culture medium at pressures of 6, 10, and 14 kPa, respectively. Red and blue lines are the data of KCM and FBS-free medium, respectively. Bars in graph (E) show the remaining widths of culture-medium-squeezed area at pressures of 6, 10, and 14 kPa. Red and blue bars are data areas in KCM and FBS-free medium, respectively. Error bars represent the standard deviations of six-time measurement. Asterisks indicate a significant difference ($p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

similar to the surface of culture medium squeezed by air-jet, liquid can be moved by hydrostatic pressure, which makes the surface level. Therefore, the reason why the squeezed area remained after the cease of air-jet application was speculated to be a surface tension on a hydrophobic surface, and the surface tension produces the opposite direction pressure against hydrostatic pressure. The result suggested that the wettability of cells in KCM was higher than that in FBS-free medium. The time-courses of the squeezed width of culture medium were obtained from the analysis of recorded movie images (Fig. 4B–D). With increasing the applied pressure, 6, 10, and 14 kPa, the squeezed width during air-jet application also increased. However, there was no difference in squeezed width between the normal and FBS-free KCM during air-jet application. On the other hand, after air-jet application, the squeezed width in KCM was narrower than that in FBS-free medium. In some cases of KCM, culture medium fully recovered over cell surface within 3.5 s after the cease of air-jet application. This trend notably expressed especially in a larger applied pressure of 14 kPa (Fig. 4D). The remaining squeezed width of culture medium was obtained from the width at 3.5 s after the cease of air-jet application (Fig. 4E). There were significant differences in the remaining width between the normal and FBS-free KCM at the applied pressures of 6, 10, and 14 kPa. The remaining width in KCM was one sixth of that in FBS-free medium at 14 kPa. Therefore, the wettability of cells in KCM condition was speculated to be higher than that in FBS-free medium.

Supplementary video related to this article can be found online at http://dx.doi.org/10.1016/j.biomaterials.2013.08.029.

The cultured cells in the normal and FBS-free KCM were found to be confluent after 10- and 14-day culture, respectively (Fig. 5A, B). Although primary rat oral mucosal epithelial cells in KCM proliferated at a higher rate than those in FBS-free medium, the cell morphology in KCM, especially its size, was found to be similar to that of FBS-free medium. The region of keratinization in KCM group was larger than that in FBS-free group. Primary rat oral mucosal epithelial cultured cells were stained with Alcian blue (Fig. 5C, D). The whole surface of culture cells in KCM was clearly stained in blue (Fig. 5C). On the other hand, that in FBS-free medium exhibited no significant stained area (Fig. 5D). Therefore, mucous layer was well-formed in KCM, but unformed in FBS-free medium. HE-stained sections clearly showed the structure of stratified epithelia in both KCM and FBS-free medium (Fig. 5E, F). Moreover, keratinization and cell exfoliation were found to be in the apical side in KCM (Fig. 5E). The maximum differences between the thinnest and the thickest part of cultured cells in KCM and FBS-free medium were 13.7 and 2.9 μm, respectively. The thicknesses of cultured cells in KCM and FBS-free medium were 20 and 9.4 μm, respectively. Cultured cells in KCM were rougher and two times thicker than that in FBS-free medium. Alcian-blue-stained area on the sections of cultured cells was expressed only on the apical side of the section in KCM (Fig. 5G). The thickness of Alcian blue stained part in the section of cultured cells was 3.6 μm in KCM. However, no expression was found in FBS-free condition (Fig. 5H). The expression of MUC4 protein, which is one of glycoprotein composing mucous layer, on the section strongly showed on the apical surface in KCM (Fig. 5I), and in contrast, no MUC4 protein was found in FBS-free medium (Fig. 5J). The thickness of anti-MUC4 antibody reacted part in the sections was 2.3 μm in KCM.

The expression levels of messenger RNAs (mRNAs) of mucin family (MUC1, 4, 13, 15, and 16) were detected. The expression level of only MUC4 in KCM was 30 times higher than that in FBS-free medium (Fig. 6). And the only expression level of MUC4 in KCM was more than 10% that of GAPDH. There were significant differences between cells cultured in KCM and FBS-free medium with a significant level of 5% in MUC1, 4, 13, and 15 (Fig. 6).

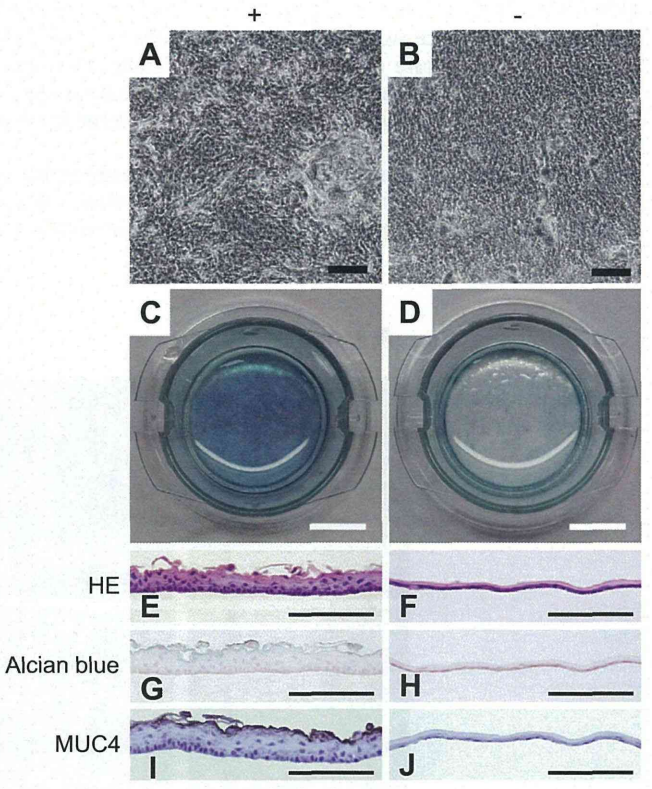

Fig. 5. The photographs of rat oral mucosal epithelial cells cultured in KCM and FBS-free medium. Symbols "+" and "−" above both columns of photographs indicate the conditions of FBS-addition (KCM) and FBS-free, respectively. Photographs (A) and (B) show the phase-contrast microscopic images of surface of cells cultured in KCM and FBS-free medium, respectively. Scale bars represent 100 μm. Photographs (C) and (D) are the results of mucous layer staining with Alcian blue on the surfaces of cells cultured in KCM and FBS-free medium, respectively. White scale bars represent 10 mm. Photographs (E, F), (G, H), and (I, J) are the images of section of the cells stained with hematoxylin-eosin, Alcian blue, and an anti-MUC4 antibody, respectively. Photographs (E, G, and I) and (F, H, and J) correspond the section of cells cultured in KCM and FBS-free medium, respectively. Black scale bars show 100 μm.

Generally, two factors affecting the wetting characteristic of surface are known to be the roughness of surface and the affinity of surface against liquid. However, the roughness of surface is hardly a factor to decide if the surface is hydrophobic or hydrophilic [30]. Roughness increases the hydrophobicity or the hydrophilicity of surface, and, in case of rough surface, high wetting surfaces become higher wettability and low wetting surfaces become lower wettability. The result of mucous-layer staining directly corresponded to those of wettability assessments of KCM and FBS-free medium. Alcian blue solution at pH 2.5 selectively stains acidic glycosaminoglycans (GAGs), which is one of the main components of mucous layer. Generally, GAGs have a high hydrophilic property, because they have a lot of hydroxyl group in their chemical structures. Therefore, in this study, the main cause of difference in wettability assessment between KCM and FBS-free medium was speculated to be difference in the expression of mucous layer.

The expression of MUC4 protein on the apical side of section in KCM suggested that some components derived from FBS except BSA would be expected to induce the expression of MUC4 protein, resulting in the appearance of the mucous layer and highly hydrophilicity on the cell surface in the wettability assessment. On the other hand, FBS-free medium was able to be important as the extreme case of the negative control for assessing mucous layer, when some essential components would be searched for the development of the

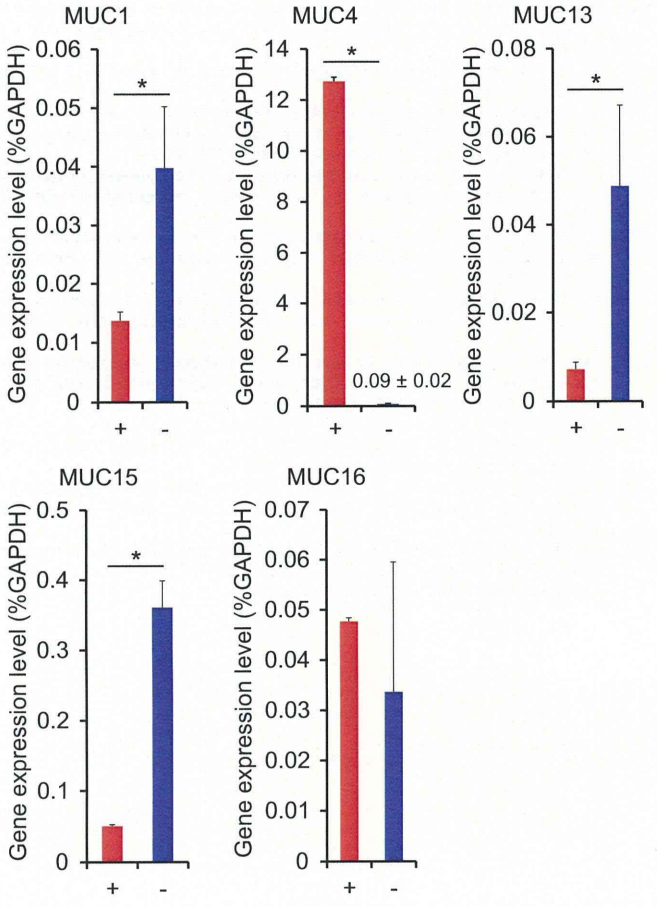

Fig. 6. Gene expression levels of mucin genes, MUC1, 4, 13, 15, and 16. Symbols "+" and "−" below graphs indicate the conditions of FBS-addition (KCM) and FBS-free, respectively. The asterisk indicates a significant difference ($p < 0.05$).

serum-free culture conditions [31,32] maintaining the appropriate production of mucous layer and MUC4 protein.

In some case of dry-eye treatment, several investigations using the topical application of autologous serum have been reported [33–35]. The previous investigation has found that mucin expression in cultured conjunctival epithelial cells is upregulated by human serum [33]. Furthermore, the mRNA expressions of membrane-associated mucins in cultivated human ocular surface epithelial cells have been reported to show MUC1, -4, and -16 dominantly [36]. In these mucins, the gene expression of MUC4 has been found to be the most sensitive for serum addition, and the addition of retinoic acid (RA) to serum-free culture medium has also strongly enhanced the mRNA expression of MUC4 [36]. These results were similar to the result of this study where the mRNA expression level of MUC4 in KCM was 30 times larger than that of FBS-free medium. Therefore, one of the reasons for the expression of MUC4 on the apical side of section in KCM was speculated to be the existence of RA in culture medium. Additionally, the noncontact wettability assessment method in this study would be useful for the quality control of cultured cells or drug screening for finding out drug candidates [37,38] in vitro for curing mucosal epithelial diseases such as dry eye syndrome.

4. Conclusion

By a noncontact culture-medium squeezing technique, the deficiency of fetal bovine serum was found to decrease the surface wettability of primary cultured rat oral mucosal epithelial cells. The results confirmed that the reduction of cell-surface wettability corresponded with the reductions of productions of mucous layer and MUC4 protein on the surface of cells. This method would be useful for the wettability assessment of mucous layer as a quality control during mucosal epithelial cell culture.

Acknowledgments

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厚生労働科学研究費補助金

難病・がん等の疾患分野の医療の実用化研究事業
（再生医療関係研究分野）

ヒト幹細胞を用いた再生医療の臨床実用化のための基盤構築に関する研究

平成25年度　総括・分担研究報告書

研究代表者　中井　謙太

平成 26 (2014) 年　3 月

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V. 資料

Ⅴ．資料

１．ＥＬＳＩ委員会　活動報告

厚生労働科学研究費補助金
難病・がん等の疾患分野の医療の実用化研究事業（再生医療関係研究分野）

ＥＬＳＩ委員　委員名簿

（平成２５年８月～）

後藤　厚宏　　情報セキュリティ大学院大学
　　セキュアシステム研究所　所長

隅藏　康一　　科学技術政策研究所　総括主任研究官

（平成２５年９月～）

星野　利彦　　京都大学ｉＰＳ細胞研究所　所長補佐

○町野　朔　　上智大学生命倫理研究所　所長代行

三尾　美枝子　キューブＭ総合法律事務所　弁護士

宮田　満　　日経ＢＰ社　特命編集委員

山本　貴史　　株式会社東京大学ＴＬＯ　代表取締役社長

（平成２５年４月１日～平成２５年９月）

高須　直子　　京都大学ｉＰＳ細胞研究所　医療応用推進室　室長

○：委員長

平成２５年度厚生労働科学研究費補助金
難病・がん等の疾患分野の医療の実用化研究事業（再生医療関係研究分野）
「ヒト幹細胞を用いた再生医療の臨床実用化のための基盤構築に関する研究」

第一回ＥＬＳＩ委員会　議事要旨

日時/場所	２０１３年６月２８日(金)１６：００～１８：３０ 東京大学医科学研究所　総合研究棟８階　会議室
出席者(敬称略)	委員：　◎町野　朔（上智大学生命倫理研究所　所長代行） 隅藏　康一（科学技術政策研究所　総括主任研究官） 高須　直子（京都大学 iPS 細胞研究所　医療応用推進室　室長） 三尾美枝子（キューブM総合法律事務所　弁護士） 宮田　満　（日経 BP 社　特命編集委員） （◎は委員長） 事務局：中井研究代表、齊藤（東大医科研）
テーマ	（１）新委員の紹介 （２）議事録のとりかたについて （３）ELSI 委員会設置要綱（案）について （４）ELSI 委員会の活動について （５）ワーキンググループについて
資料	資料　１　　委員名簿 資料　２－１　ＥＬＳＩ委員会設置要綱（案） 資料　２－２　ＥＬＳＩ委員会の活動について（案） 資料　２－３　ＥＬＳＩ委員会ワーキング・グループ　メンバー（案）

１．議事内容　：

（１）　新委員の紹介

【事務局からの説明】

- 準備委員会での議論をふまえ、山本委員にご紹介いただき、三尾美枝子先生に御参加いただくこととなったが、ご承認いただきたい。

【委員から】

- 異議無し。

（２）議事録のとりかたについて

【事務局からの説明】

- 評価委員会等では速記業者による逐語記録を作成していたが、特に委員からの意見がなければ、事務局が作成した議事要旨にて対応したい。

【委員から】

- 厚労省に確認してみて、必要であれば逐語記録を、そうでなければ録音を残し、議事要旨で対応ということでよいと思われる。また、抄録は確認後ホームページへ掲載。

（3） ELSI委員会設置要綱（案）について

【事務局からの説明】

- 準備委員会での議論をもとに、加筆を行った。
- セキュリティの問題についても本ELSI委員会にて管理・審議したいので、前回までの準備委員会にて指摘があった加筆箇所に加えて、設置目的に「情報セキュリティに関する問題を含む」を加筆したい。
- そのため、セキュリティの専門家が委員として加わることをご検討いただきたい。

【委員から】

- 入れられたデータについてもデータ漏洩等のセキュリティの問題が関わると思うが、その前にどんな人がこのプロジェクトのデータを取り扱うのか、研究室のメンバーの定義とか、データ投稿時/後のこと、データを誰が閲覧できるのかという基本的なところについて、決めておかなければいけないと思われる。
- セキュリティ問題のポリシー作成等も ELSI 委員会で扱うのか。ルール作りと監督を両方行うのは、目的から外れていると思うが。
    -- ルール作りについては作業部会で練られたものについてご審議いただく。日々発生する問題等についてはこの委員会では取り扱わない。それよりも上位の問題、専門性が高い問題については、ここで審議していただくこともあると思う。
- セキュリティ問題というのは、情報セキュリティのことを指すのか、個人情報（データ）の保護という問題も入っているのか。
    -- データ漏洩、個人情報の保護ももちろん守備範囲だが、技術的な問題も含めている。
- “知財”という用語が何を指すのか。
    -- 法律で定められている意味での“知財”だけではなく、日々蓄積されている研究データや、そのデータの権利、等研究者からみたあらゆる情報・所有物についての権利問題等も含めて考えていただきたい。
    データの提供に関するルール（ガイドライン）を作成したほうがいいのか、ワーキンググループとも検討してみる。

2．決定事項

- 新委員として三尾美枝子先生に御参加いただくことになった。

- 議事録のとりかたについては、委員会としては要旨のみで構わない。研究代表が厚労省に確認して、指示に従う。
- ELSI委員会設置要綱については承認を得られた。
- セキュリティの専門家の選出は研究代表者が行う。

3．その他

委員会中に、本プロジェクトと ELSI 委員会の関わり及び事業運用方針について以下のような自由議論と提案がなされた。

（自由議論と提案については略）

4．次回開催予定　：

第二回ELSI委員会　平成25年7月25日（木）15時～17時

主な議題（案）

- 事業運用方針（ルール策定・契約書策定等）の検討
- ゲストスピーカーの講演

（以上）

平成２５年度厚生労働科学研究費補助金
難病・がん等の疾患分野の医療の実用化研究事業（再生医療関係研究分野）
「ヒト幹細胞を用いた再生医療の臨床実用化のための基盤構築に関する研究」

第二回ＥＬＳＩ委員会　議事要旨

日時/場所	２０１３年７月２５日(金)１５:００～１７：００ 東京大学医科学研究所　総合研究棟８階　会議室
出席者(敬称略)	委員：　◎町野　朔（上智大学生命倫理研究所　所長代行） 隅藏　康一（科学技術政策研究所　総括主任研究官） 高須　直子（京都大学 iPS 細胞研究所　医療応用推進室　室長） 三尾美枝子（キューブM総合法律事務所　弁護士） 宮田　満　（日経 BP 社　特命編集委員） 山本　貴史（株式会社東京大学ＴＬＯ　代表取締役） （◎は委員長） ゲストスピーカー： 田中　英彦（情報セキュリティ大学院大学　学長） 事務局：中井研究代表、齊藤（東大医科研）
テーマ	（１）ゲストスピーカーによるミニレクチャー （２）新委員のご紹介 （３）運用ルール（案）検討 （４）その他
資料	資料　１　　ミニレクチャー要旨・ゲストスピーカー略歴 資料　２　　委員名簿 資料　３　　運用ルール（案） 参考資料　　ＮＢＤＣヒトデータ共有ガイドライン

１．ゲストスピーカーによるミニレクチャー

「情報共有にむけた情報セキュリティのあり方」

情報セキュリティ大学院大学　学長　田中　英彦氏によるミニレクチャー

２．議事内容　：

（１）　新委員の紹介

【事務局からの説明】

- 当初、情報セキュリティ大学院大学　学長　田中英彦先生に就任していただく予定だったが、その後　同大学院大学　教授で、セキュアシステム研究所　所長の後藤厚宏氏に就任いただくことになった。

【委員から】

- 異議無し。

（2）運用ルール（案）検討

【事務局からの説明】

- 情報システムの運用ルールとして、知財のガイドライン（案）として作成したので検討していただきたい。

【委員から】

- まず事務局が作成した草案の各項目について
  - データや実験ノートの公開は、ある一定年数を経ても公開するのは無理（分担者に同意を得られない）のではないか。
  - 情報提供者が第三者の保有するデータを提供するときの義務が、（このルール上では）大きすぎて、データを出すことができなくなるのでは。
  - 「情報被提供者」の定義があいまい。二次利用をした人が「被提供者」なのか、二次利用しなくても提供者以外にそのデータを見たりした人も「被提供者」になりうる。
- 草案全体について
  - このプロジェクトでは「管理者」「情報提供者」「情報被提供者」という player がおり、それぞれの権利義務は、このDBの目的・利用方法・原則のもとにそれぞれを設定するべき。
  - このプロジェクトでは、他のプロジェクトと違い、個人情報を含むデータ、含まないデータの両方を取り扱うため、提供されたデータを研究のために使用し、成果とすることがあること、またその成果（またはデータ）を共有することがあること、をデータ提供者に許可を得る必要があるのではないか。
  - （このルール上で）プロジェクトの目的、趣旨、データをどこまで使用し、何を目指しているのかを明記したほうがいい。
  - 今回草案として作成されたものは、「知財ガイドライン」というより、「（主に）知財に関する運用ガイドライン」という内容になっている。まずは運用ルールを作成し、そこから知財、セキュリティ、等のルールをそれぞれ作成したほうがよい。
  - 最初から細部に渡ってルールを決めず、まずは大筋を決めて、その後に細則を決めるというやり方が今回は合理的であると思われる。

3. 決定事項

- 新委員として後藤　厚宏先生に参加していただくこととなった。
- 今回いただいた意見をもとに、運用ルール（案）の修正を行い、次回委員会前に一度メール等でご確認いただき、それを班員の皆様にも見ていただき、班員からのご意見・修正を加えた案を次回委員会にて皆様に再検討いただく。

4. 次回開催予定　：

第三回ELSI委員会　平成25年9月18日（水）15時～17時

主な議題（案）

- 運用ルール（案）ver2 の検討

（以上）

平成２５年度厚生労働科学研究費補助金
難病・がん等の疾患分野の医療の実用化研究事業（再生医療関係研究分野）
「ヒト幹細胞を用いた再生医療の臨床実用化のための基盤構築に関する研究」

第三回ＥＬＳＩ委員会　議事要旨

日時/場所	２０１３年９月１３日(水)１５：３０～１７：３０ 東京大学医科学研究所　総合研究棟８階　会議室
出席者(敬称略)	委員：　◎町野　朔（上智大学生命倫理研究所　所長代行） 隅藏　康一（科学技術政策研究所　総括主任研究官） 後藤　厚宏（情報セキュリティ大学院大学 セキュアシステム研究所　所長） 星野　利彦（京都大学 iPS 細胞研究所　所長補佐） 宮田　満　（日経 BP 社　特命編集委員） 山本　貴史（株式会社東京大学ＴＬＯ　代表取締役） （◎は委員長） 事務局：中井研究代表、齊藤（東大医科研）
テーマ	（１）新委員のご紹介 （２）運用ルール（案）再検討 （３）その他
資料	資料　１　　委員名簿 資料　２　　運用ルール（案）

１．議事内容　：

（１）新委員の紹介

【事務局からの説明】

- 高須委員より、委員交代依頼があったため、星野利彦氏をご推薦いただいた。
  所長補佐のため山中先生との距離も近く、また現在検討中の規約・ガイドライン検討の面からも適任であることから、星野委員をご推薦いただき、就任いただいた。

（２）運用ルール

- 前回の議論をもとに作成した運用ルール(案)について、再議論を行い、修正等を加え、ELSI 委員会でのルール作成作業を終了とした。

3. 決定事項

- 今回作成した運用ルール(案)について再度班員の皆様にも見ていただき、班員からのご意見があればELSI委員とのメール審議を行い、意見・修正がなければELSI委員会にて承認されたこととし、実際に運用する。

4. 次回開催予定 :

なし(必要に応じてメール審議)

(以上)