Process Biochemistry 48 (2013) 1429–1435

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

A simple process for the isolation of epithelial cells from bacteria-contaminated samples using anchoring molecules

Ji Yeong Won [a], Jeong-Woo Choi [a,**], Junhong Min [b,*]

[a] Department of Chemical and Biomolecular Engineering, Sogang University, #1 Shinsu-dong, Mapo-gu, Seoul 121-742, Republic of Korea
[b] School of Integrative Engineering, Chung-Ang University, Heukseok-dong, Dongjak-gu, Seoul 156-756, Republic of Korea

ARTICLE INFO

Article history:
Received 8 February 2013
Received in revised form 3 May 2013
Accepted 25 June 2013
Available online 4 July 2013

Keywords:
Epithelial cell isolation
Bacteria removal
Anchoring molecule
BAM
Physical adsorption

ABSTRACT

A simple and efficient tool to isolate epithelial cells from bacteria-contaminated samples has been developed using two different microparticles functionalized with chemical molecules. The epithelial cells could be captured simply by biocompatible anchors for membranes (BAM), consisting of poly(ethylene glycol) functionalized with oleyl-chain-conjugated NHS (*N*-hydroxysuccinimide) on glass microparticles, whereas bacteria were adsorbed on 3-aminopropyltrimethoxysilane (ATPS)-functionalized magnetic microparticles. In the case of samples highly contaminated with bacteria, epithelial cells were not isolated successfully by both of the single BAM- and antibody-functionalized microparticles. Therefore, serial isolation steps of these two different chemical functionalized microparticles were introduced. The concentration of bacteria was decreased dramatically by using APTS-functionalized magnetic particles prior to the isolation of epithelial cells by BAM microparticles. With these serial processes, successful isolation of epithelial cells was achieved from bacteria-contaminated epithelial samples. The applicability of this method was verified with bacteria-contaminated intestinal samples biopsied from a BALB/C mouse for primary cell cultivation.

1. Introduction

Cells or tissues have been widely researched in the clinical and biomolecular fields to define cancer cell characteristics and behaviors, and to develop anti-cancer drug screenings [1,2]. The cultivation of primary cells from biopsy samples has been performed frequently in the drug-screening field [3], because it may not be guaranteed that the characteristics of commercially available stabilized cell lines accurately represent *in vivo* cells or tissues.

Various processes to release and culture cells that primarily come from a biopsy sample have been developed [4]. Normally, biopsy samples are chopped prior to collagenase treatment. This representative method has been widely utilized, but primary cultivation has often failed because of sample contamination by bacteria, inherently short passages, and low proliferation time [5,6]. As such, the initial concern for primary cell cultivation is the purification of cells against bacteria. Even a single bacteria cell can have a tremendous negative effect on primary cell cultivation, because bacteria grow at least 15 times faster than general epithelial cells [7,8]. Bacteria contamination continually occurs when a biopsy is performed from digestive organs, such as the stomach or intestine [9].

Penicillin streptomycin and gentimicin are powerful antibiotics, and have been widely used to prevent bacterial contaminations [10]. However, high doses of antibiotics might be required to eliminate highly concentrated bacteria in biopsy samples from digestive organs, which may induce antibiotic resistance [11]. Therefore, fast and effective isolation of epithelial cells from bacterially contaminated samples, or at least a dramatic concentration reduction in the production of bacteria in cultivated samples, is needed to minimize the usage of antibiotics, which may induce variation in the cell characteristics.

Physical methods such as percoll gradients and centrifugation due to density differences have been widely used in primary cell cultivation procedures. Biological methods involving enzymatic reactions and antibody affinities have also been employed [12–14]. These tools have not provided perfect environments (for long-term primary cell cultivation free of bacteria, because processes that include antibacterial drugs or severe washing steps may reduce cell concentration, and induce ambiguous culturing conditions with chemical complexes.

Microfluidic and microbead-assisted isolation methods of epithelial cells from various sample matrices have also been reported. Magnetic bead-assisted antibody affinity [15,16], electronic field flow [17], and hydrophobic interaction [18] were employed to isolate epithelial cells from samples. However, the

* Corresponding author. Tel.: +82 02 820 5348; fax: +82 02 814 2651.
** Corresponding author. Tel.: +82 2 705 8480; fax: +82 2 3273 0331.
E-mail addresses: jwchoi@sogang.ac.kr (J.-W. Choi), junmin@cau.ac.kr, jmin.email@gmail.com (J. Min).

1359-5113/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.procbio.2013.06.030

effective isolation of epithelial cells from bacteria-contaminated biopsied samples has not yet been published. This may be due to the fact that biopsied samples are normally to highly concentrated with large volume to be applied to microfluidic systems. Magnetic beads combined an antibody specific to epithelial cells have been applied to Magnetic-assisted cell sorting systems (MACS) [19], which are an alternative to FACS (Fluorescence assisted cell sorters)

Magnetic microparticles (MMP) are one of the effective tools used to separate certain targets from a biomatrix. Various biomolecules such as DNA, RNA, and proteins have been isolated from a biomatrix using MMPs [20]. Bacteria could be also separated from samples by magnetic nano structures [21]. Epithelial cells were separated from a sample using antibody [GAPDH]-conjugated magnetic microparticles. The antibody-assisted magnetic microparticles provide high separation efficiency for epithelial cells from a sample, and have been commercialized by Bioclone, Invitrogen, and others. However, antibody-conjugated magnetic particles have demerits regarding cost, shelf life, and separation time, because they utilize the affinity of antibodies to a specific protein to capture epithelial cells. Moreover, their ability to completely remove bacteria has not been verified.

Biocompatible anchors for membranes (BAM) are one of the representative anchoring molecules, and consist of poly(ethylene glycol) functionalized with oleyl-chain-conjugated NHS (*N*-hydroxysuccinimide) [22]. The oleyl chain is an unsaturated fatty acid, and can be inserted into epithelial cell membranes since it has a similar cell membrane composition. This molecule has been widely utilized to selectively immobilize epithelial cells, although it is unavailable as an anchoring molecule in serum conditions [23].

The purpose of this study is to develop a simple and fast epithelial cell isolation method/KIT from biopsy samples highly contaminated with bacteria, using non-biological BAM molecules. To achieve easy concentration and purification to isolate epithelial cells from the sample, two different chemically functionalized microbeads were utilized as a separation platform.

A two-step procedure was employed, the first of which was for bacteria removal using surface-modified magnetic microbeads, and the second involved epithelial cell isolation steps with BAM-functionalized silica glass beads, because of the size difference between bacteria and epithelial cells, as shown in Fig. 1. Bacteria are adsorbed and captured on the positively charged magnetic particles by electrostatic interaction [24], whereas epithelial cells are concentrated on the BAM-functionalized glass beads [25]. The epithelial cell isolation efficiency of BAM molecules over time was compared with that of GAPDH antibody. The epithelial cell isolation process developed was enhanced by the employment of a pretreatment step for the removal of bacteria. This method was confirmed and verified by introduction to a primary cell cultivation process from an intestinal biopsy sample from a mouse.

2. Materials and methods

2.1. Cultivation of epithelial cell and bacteria

Human mammary cell lines (AGS, WI-38, PC3, HeLa) were purchased from ATCC (US), and cultivated using RPMI-1640 (PAA, Austria) containing 10% FBS (GIBCO, NY, US), 1% penicillin-streptomycin (GIBCO, NY, US), and 1% GutaMAX (GIBCO, NY, US). A commercial culture dish used as a positive sample was purchased from Falcon (NJ, US). Mammalian cells were cultivated under normal conditions of 37 °C and 5% CO_2, with an initial seeding amount of $5 \times 10^5/3.8\ cm^2$. *E. coli* (ATCC 43895) was cultivated using 3% TSB (Becton, Dickinson and Company, France) under normal conditions of 37 °C at 150 rpm.

2.2. Preparation of the functionalized micro beads

Glass (silica) microparticles (SP, $D = 100\ \mu m$) and magnetic microparticles (MP, 2 μm, MoBiTec, Germany) were silanized with 100 mM 3-aminopropyltrimethoxysilane (APTS, Gelest Inc., PA, US) in 95% ethanol for 1 h at room temperature. After washing with 100% ethanol, microbeads were dried

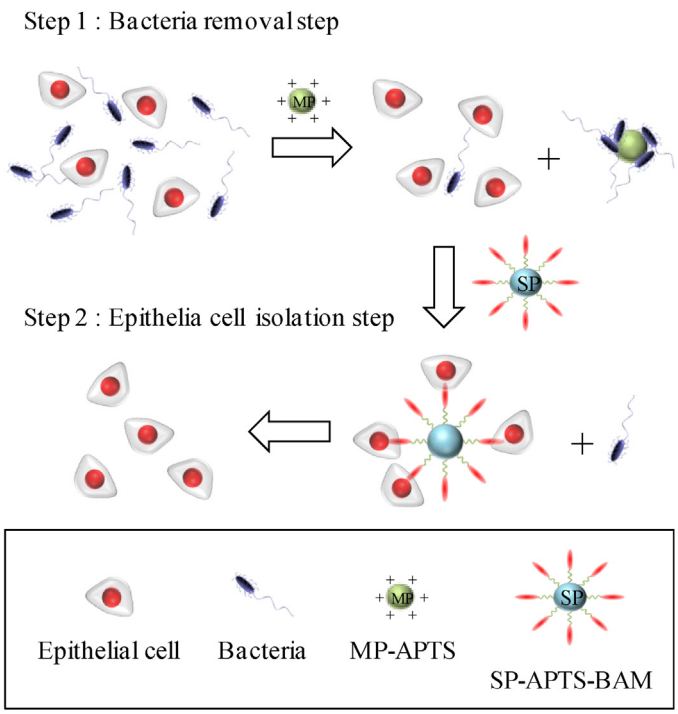


Fig. 1. Schematic illustration of 2-step isolation of epithelial cells from bacteria-contaminated samples.

for 1 h at 85 °C. The MP had a positively charged surface (MP-APTS). To achieve SP-APTS-BAM, SP-APTS was functionalized with 100 μM oleyl-O-poly(ethylene glycol)-succinyl-*N*-hydroxy-succinimidyl esters (BAM, NOF Corporation, Japan) in PBS buffer for 10 min. Antibody-functionalized SP (SP-APTS-antibody) was fabricate by functionalizing with 1 μg/1 mL of GAPDH antibody (Santa Cruz Biotechnology Inc., CA, US) in PBS buffer (10 mM, pH 7.4) for 30 min at room temperature. The prepared beads are shown in Fig. 2.

2.3. Isolation of epithelial cell and bacteria by using the functionalized micro beads

The 2-step isolation of epithelial cells from bacteria-contaminated samples is illustrated in Fig. 1. MP-APTS was first applied to a mixture of AGS cells (5×10^5 cells) and *E. coli* (10^5 CFU) in 1 ml of PBS buffer (10 mM, pH 7.4), and incubated for 10 min at room temperature. Then, bacteria-bound MP-APTS was separated from the solution using a magnet.

The supernatant containing epithelial cells was transferred into a tube containing SP-ATPS-BAM and incubated for 10 min at room temperature. Finally, the second supernatant was gently removed, and SP-ATPS-BAM was rinsed 3 times with PBS buffer solution. The remaining SP-ATPS-BAM (epithelial-bound) was transferred onto a cell culture dish for primary cell culture. The epithelial cells immobilized on the micro beads were cultured with RPMI 1640 containing 10% FBS for 42 h at 37 °C with 5% CO_2.

2.4. Counting of epithelial cells and bacteria

For the quantification of *E. coli* and AGS cells isolated by MP-APTS and SP-APTS-BAM, real-time PCR was utilized with a Total RNA Isolation NucleoSpin® RNA II kit (Qiagen, Germany) and commercialized PCR master mixture (SyBr Premix Ex Taq™ (Takara, Japan)). Real-time PCR results were verified with agar plating for bacteria, and a hemocytometer for epithelial cells. All experiments were conducted in quintuplicate.

2.5. Preparation of primary cell sample from the intestinal tissue of mouse

Intestinal tissue sample was biopsied from a 4-week-old BALB/C mouse, and washed with PBS buffer (10 mM, pH 7.4). The sample was finely chopped and incubated in 200 μl of collagenase (Sigma–Aldrich, MO, US) for 1 h at 37 °C. This tissue sample was re-washed by PBS buffer and re-suspended with DMEM (Gibco, NY, US). The cells from the mushy tissue samples (by collagenase) were isolated using a cell strainer (BD Falcon, PA, US) and incubated for 48 h at 37 °C with 5% CO_2. After all procedures, the cells and all impurities including bacteria were treated by trypsin EDTA prior to finally preparing a primary cell solution (bacteria contaminated) in PBS buffer.

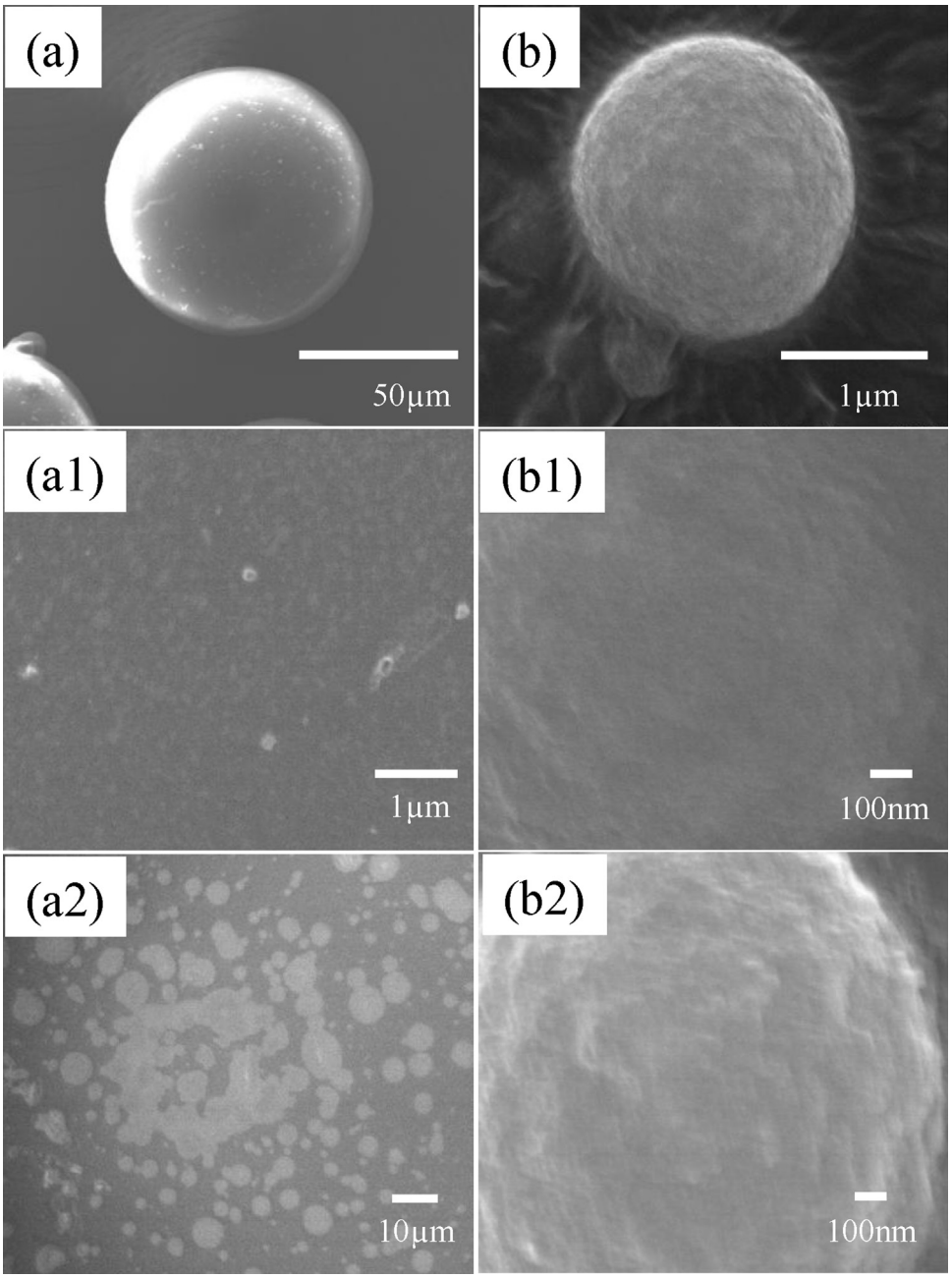


Fig. 2. SEM images of (a) SP (silica micro particles) and its surface (a1) before and (a2) after the modification with BAM molecules, and (b) silica coated magnet particle (MP) and its surface (b1) before and (b2) after the modification with APTS.

3. Results and discussion

3.1. The capturing efficiency of ATPS and BAM modified surface

Both the gram-negative and positive bacteria could be adsorbed on the positively charged surface, because it is likely that most bacteria have a net-negatively charged cell wall containing peptidoglycan in neutral pH [24]. We tested the bacteria adsorption on the positively charged surface with respect to the APTS concentration loaded on the surface, as shown in Fig. 3. The adsorption % of *E. coli* on the surface was proportional to the amount of amine residue (APTS molecules). This implied that the *E. coli* (net-negatively charged particles) could be adsorbed on the APTS surface by electrostatic interaction, and bacteria adsorption could be accelerated by increasing the positive charge and surface area. This result corresponds well with previous results, which showed *E. coli* or salmonella had net-negative charge, and were well adhered to a positively charged surface [21,24].

We chose BAM molecule as a capturing molecule specific to epithelial cells from the sample, because oleyl group in BAM could penetrate into cell membrane. We confirmed this capability of BAM with 4 different mammalian cells (AGS (human gastric cancer cell), WI-38 (human lung fibroblast cell), PC3 (human prostate cancer cell), and HeLa (human cervical cancer cells)), and red blood cells. These cells were spread out onto a BAM-coated glass surface and washed out 10 min later. 5 different cells (3 epithelial cells, 1 fibroblast, and 1 endothelial cell) were captured by BAM in a short period of time, as shown in Fig. 4. This result showed good agreement with other published results, which indicates BAM molecules can penetrate mammalian cell membranes in the absence of serum [23].

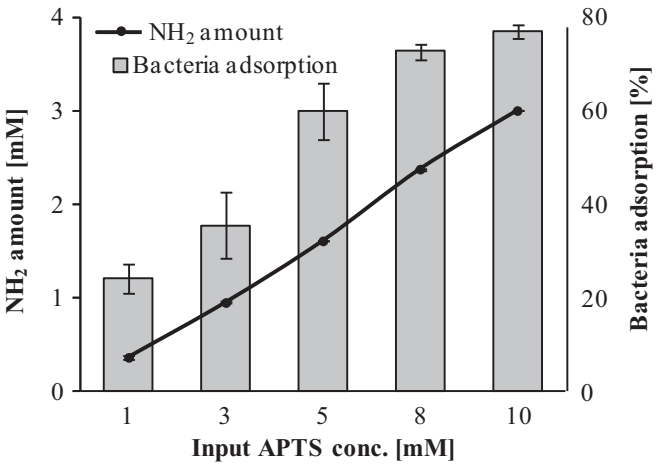


Fig. 3. The effect of positive charge (APTS amount) on bacteria adsorption [based on Kaiser Test].

More ever, this result implies that BAM molecules might be one of the best materials compared with antibodies for the isolation of mammalian cells from bacteria-contaminated biopsied samples, with regard to processing cost, material price, process time, and the shelf life of materials.

We also investigated the selectivity of BAM and APTS-functionalized surfaces on the epithelial cells (AGS cells), gram-negative bacteria (*E. coli*), and gram-positive bacteria (*B. cereus*), as shown in Fig. 5(a). The BAM-functionalized surface had great selectivity for AGS cells compared with bacteria. Bacteria were not adhered to or captured by the BAM-functionalized surface, whereas a high adsorption of bacteria was found on the

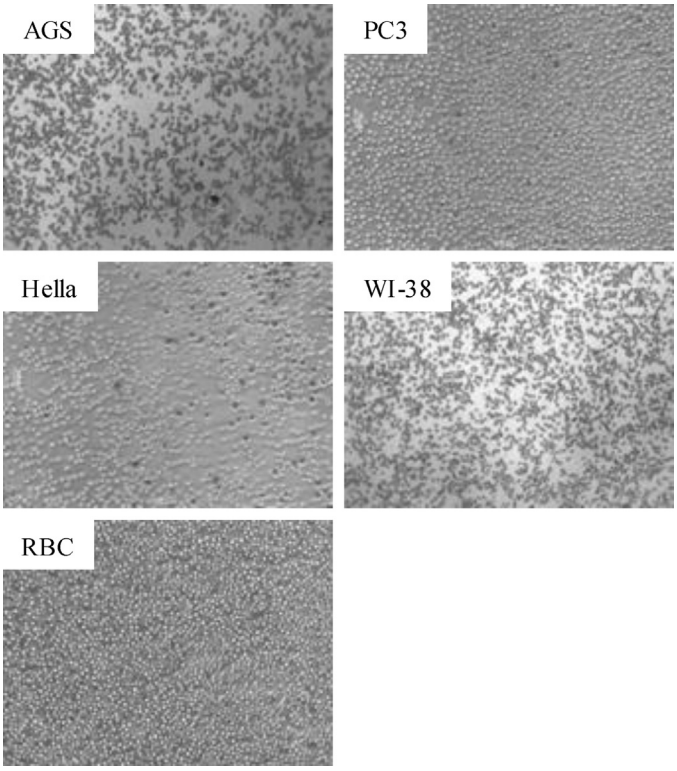
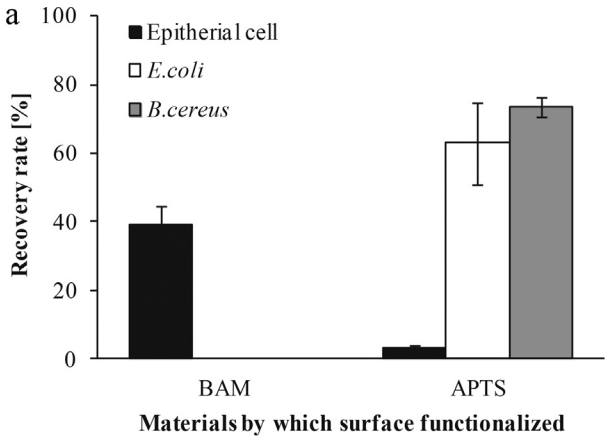


Fig. 4. The capability of BAM modified surface to capture 5 different mammalian cells (epithelial cells: AGS, PC3, HeLa, Fibroblast: WI-38, Endothelial cell: Red Blood Cell).



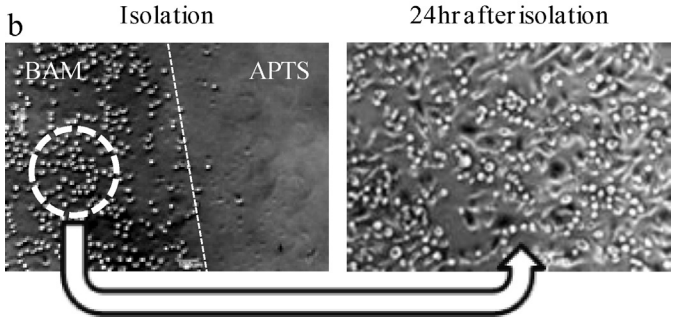


Fig. 5. The selective binding of epithelial cells and bacteria by using BAM and APTS surfaces: (a) the recovery rates and (b) microscopic images of epithelial cells and *E. coli* and *B. cereus* on BAM surface and APTS surface.

APTS-functionalized surface. This result was confirmed by a visual experiment, as shown in Fig. 5(b). The AGS epithelial cells were well adhered on the BAM functionalized surface, whereas no cells were found on the APTS surface.

3.2. Isolation and re-cultivation test of epithelial cell (AGS cells) using SP-APTS-BAM

When silica microbeads (100 μm in diameter) were utilized instead of a flat surface, the simple adhesion of epithelial cells was observed with SEM and microscopic images. As shown in Fig. 6(a), the epithelial cells could bind to the BAM immobilized silica microparticles (SP-APTS-BAM). It was also confirmed that AGS cells captured by SP-APTS-BAM could spread out well and proliferate on the culture dish, because cell transfer from the beads to the surface (re-cultivation or re-proliferation) is important in primary cell cultivation from tissue.

To separate and re-collect epithelial cells from the samples, an antibody specific to housekeeping protein such as GAPDH and β-actin can be utilized [26]. Therefore, the epithelial cell-capturing efficiency using BAM was confirmed and compared with that by GAPDH-specific antibody over time, as shown in Fig. 6(b). The adsorption rate of epithelial cells (AGS cells) by SP-APTS-BAM was much faster than that by SP-antibody. The recovery rate was almost saturated at 10 min, whereas that by SP-antibody was less than 10% when the reaction time was 10 min. The saturation of the recovery rate seems to depend on the amount of BAM. According to this result, BAM materials could be a great alternative to antibody to isolate epithelial cells from a sample, because its rate of binding epithelial cells is faster than the antibody's rate. Moreover, this material is an organic chemical that can be handled and stored easily.

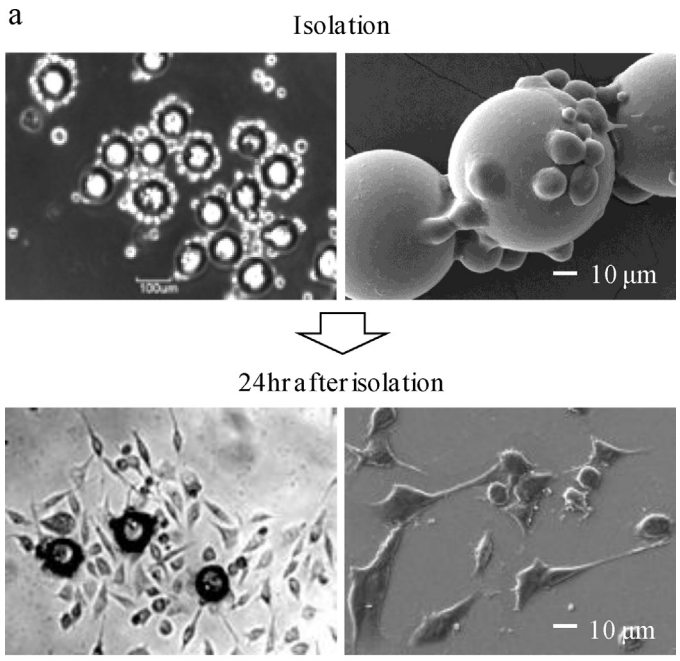


Fig. 6. The capabilities of BAM molecules to capture and release epithelial cells: (a) the demonstration of cell capturing and releasing procedures by BAM-modified silica microparticles (SP-APTS-BAM) and (b) the comparison of BAM and anti-GAPDH antibody for the epithelial cell isolation.

3.3. Bacteria effect on the epithelial cell isolation by BAM

Prior to applying SP-APTS-BAM to real samples, with various artificial sample mixtures (1 mL) of AGS cells and *E. coli* (0, 10^1, 10^2, 10^3, 10^4, and 10^5 CFU), the bacteria effects on the isolation capability of SP-APTS-BAM (10,000 ea) on the epithelial cells were investigated and compared with the antibody-assisted method. As shown in Fig. 7, in cases of both SP-APTS-BAM and SP-APTS-antibody, AGS cells isolated were contaminated with bacteria when the concentrations of bacteria were in the sample were relatively high. However, the number of bacteria separated from epithelial cells by BAM or antibody was very low (less than 200 CFU), which could induce severe problems, since bacteria grow quickly compared with epithelial cells. This result means that these BAM and antibody-assisted methods to isolate epithelial cells are not perfect for application to real research, and antibiotics are still required after physiochemical isolation. This result also provides a clue to isolate epithelial cells from samples if the bacteria

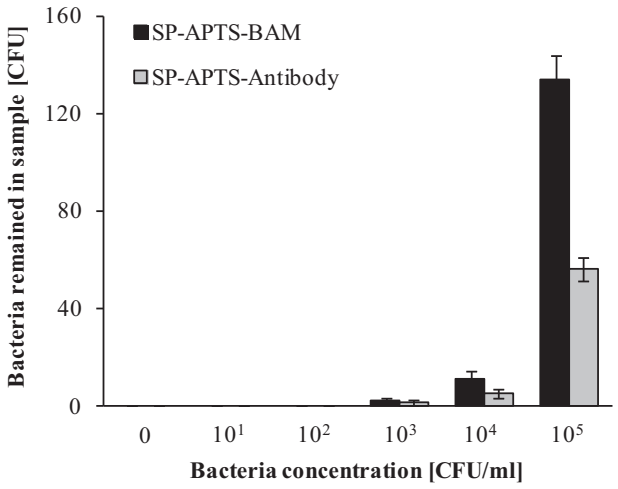


Fig. 7. The comparison of BAM and anti-GAPDH antibody for bacterial removal efficiency.

concentration is relatively low (~10^2 CFU/mL). Therefore, we added bacteria removal steps to this process.

3.4. Two-step optimization on cells separation

Two sequential steps, a bacteria removal step using MP-APTS and an epithelial isolation step by SP-APTS-BAM, were utilized to isolate epithelial cells completely from the bacteria-contaminated samples (over 10^5 CFU/mL). MP-APTS was utilized as a first step for the easy removal of bacteria from the sample. Fig. 8 shows the % concentration (defined as % concentration/input concentration = C/C_o %) of bacteria and AGS cells in the samples in each step. The % concentration of bacteria was dramatically decreased after the removal process (first step, 10 min) by MP-APTS, whereas that of AGS cells remained in the tube, because bacteria have a

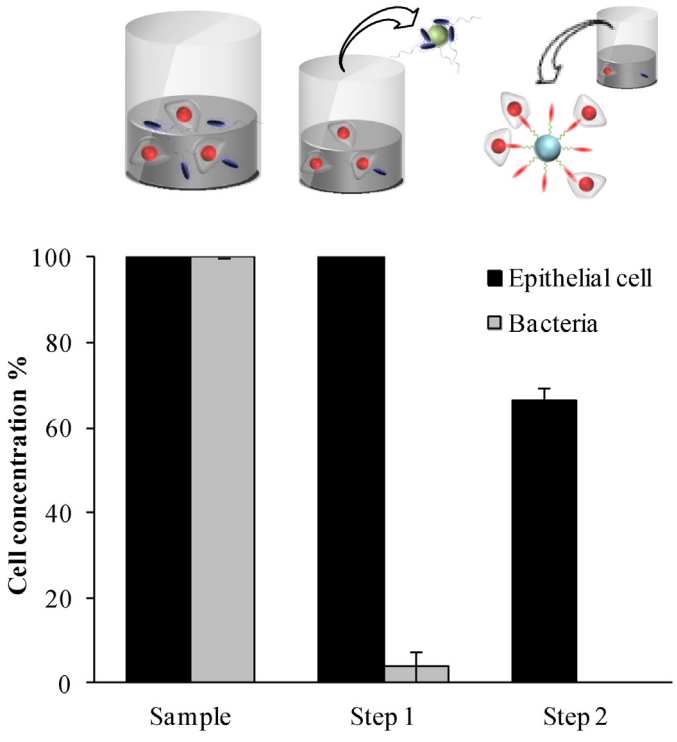


Fig. 8. The step by step the epithelial cell isolation efficiencies of 2-step process.

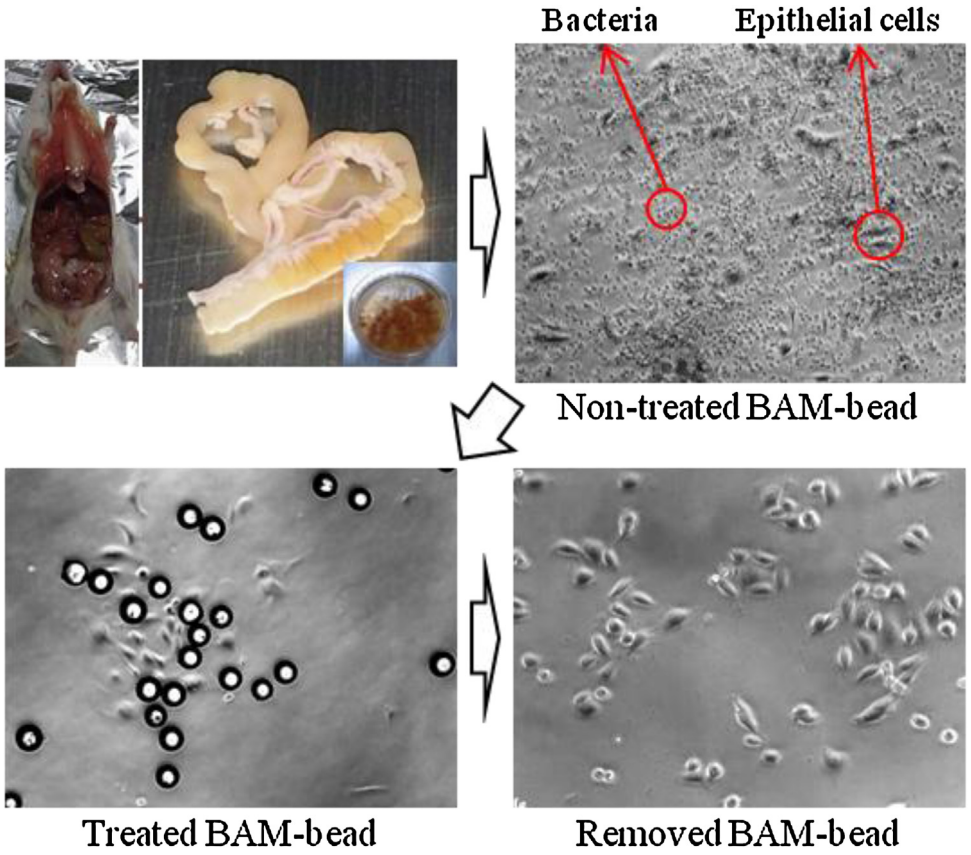


Fig. 9. The primary cell cultivation from the intestine biopsy of the mouse by using 2-step isolation process.

net-negatively charged surface at neutral pH [24]. It was clear that epithelial cells could adhere to the positively charged surface, but in this case, they could not bind to MP-APTS, because it could not adhere to the small area of the MPs [27]. In the first step, we could decrease the bacteria concentration to less than 10^3 CFU/mL, which means that epithelial cells would be perfectly isolated by SP-APTS-BAM, because the sample contains bacteria at a relatively low concentration (see Fig. 7). As a second step, the SP-APTS-BAM was added and incubated for 10 min in the remaining solution (from which most bacteria was removed). The solution was replaced with PBS buffer 3 times after 10 min of incubation. As a final result, a 60% recovery rate of epithelial cells from the sample without any bacteria could be achieved.

3.5. Primary cell cultivation from mouse intestinal sample

The successful isolation of AGS cells from samples contaminated with *E. coli* has been demonstrated using two different particles, MP-APTS and SP-APTS-BAM. To verify its applicability, this method was applied to real tissue sample treatment. An intestinal sample (upper-left in Fig. 9) from a mouse was selected, because digestive could achieve such as the stomach and intestine normally have various bacteria in high concentration. The tissue was biopsied from a BALB/C mouse and thoroughly washed to remove visible impurities. After normal treatment for the primary culture of tissue cells, cells were cultivated on a culture dish for 2 days. As expected, bacteria were cultivated more quickly than epithelial cells (upper-right in Fig. 9). The 2-step operation to remove bacteria (using MP-APTS) and isolate epithelial cells (using SP-SPTS-BAM) was applied to this bacteria-contaminated intestinal primary cultivation sample. We were able to isolate epithelial cells by SP-APTS-BAM (bottom-left), and verified that the epithelial cells were bacteria-free by 24 h of cultivation after removing SP-APTS-BAM (bottom-right). As shown in figure, bacteria could be removed from the sample in 20 min without the use of any antibiotics.

4. Conclusion

In cell-based research, it is mainly considered that the chemical, biological, and physical factors of the micro environments of cells may affect the cell characteristics, which could restrict the application of cell-based research to clinical research. Therefore, it is scientifically and empirically important to minimize the use of additives such as antibiotics to cultivate cells. We have developed a simple and effective tool to isolate epithelial cells from bacteria-contaminated tissue samples, by simply using two different chemically functionalized microparticles, MP-APTS and SP-APTS-BAM.

Bacteria-free primary cell cultivation could be achieved by only chemical methods. This methodology did not depend on antibiotics to remove bacteria and biological reactions (in antibodies). The method could reduce processing time and cost as well as increase the shelf life of a separation KIT. Also, this method could be easily automated by the introduction of microfluidics, because we used simple magnetic particles and silica particles. After adjusting the operating parameters for microfluidics, we anticipate the development of an automated cell treatment device for primary cell cultivation.

Acknowledgment

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology

(No. 2011-0022060) and by Fishery Commercialization Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (No. 112089031SB050) and by National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2013R1A2A2A03015620).

References

[1] Li D, Zang R, Yang S-T, Wang J, Wang X. Cell-based high-throughput proliferation and cytotoxicity assays for screening traditional Chinese herbal medicines. Process Biochem 2013;48:517–24.
[2] Butcher EC, Berg EL, Kunkel EJ. Systems biology in drug discovery. Nat Biotechnol 2004;22:1253–9.
[3] Quaroni A, Hochman J. Development of intestinal cell culture models for drug transport and metabolism studies. Adv Drug Deliv Rev 1996;22:3–52.
[4] Kasraeian S, Allison DC, Ahlmann ER, Fedenko AN, Menendez LR. A comparison of fine-needle aspiration, core biopsy, and surgical biopsy in the diagnosis of extremity soft tissue masses. Clin Orthop Relat Res 2010;468:2992–3002.
[5] Kinney TP, Kozarek RA, Raltz S, Attia F. Contamination of single-use biopsy forceps: a prospective in vitro analysis. Gastrointest Endosc 2002;56:209–12.
[6] Lee RM, Kozarek RA, Sumida SE, Raltz SL. Risk of contamination of sterile biopsy forceps in disinfected endoscopes. Gastrointest Endosc 1998;47:377–81.
[7] Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T. Interdependence of cell growth and gene expression: origins and consequences. Science 2010;330:1099–102.
[8] Wong J, Kelly K, Mittra A, Gonzalez SJ, Song KY, Simpson G, et al. A third-generation herpesvirus is effective against gastroesophageal cancer. J Surg Res 2010;163:214–20.
[9] Hartley CL, Neumann CS, Richmond MH. Adhesion of commensal bacteria to the large intestine wall in humans. Infect Immun 1979;23:128–32.
[10] Van der Valk J, Brunner D, De Smet K, Fex Svenningsen A, Honegger P, Knudesnm LE, et al. Optimization of chemically defined cell culture media – replacing fetal bovine serum in mammalian in vitro methods. Toxicol In Vitro 2010;24:1053–63.
[11] Defoirdt T, Sorgeloos P, Bossier P. Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr Opin Microbiol 2011;14:251–8.
[12] Pan D, Das A, Liu D, Veazey RS, Pahar B. Isolation and characterization of intestinal epithelial cells from normal and SIV-infected rhesus macaques. PloS ONE 2012;7:e30247.
[13] Booth C, O'Shea JA. Isolation and culture of intestinal epithelial cells. Culture of epithelial cells. 2nd ed. Glasgow, Scotland: Wiley-Liss Inc.; 2002. p. 303–35.
[14] Way AL. Isolation and culture of bovine oviductal epithelial cells for use in the anatomy and physiology laboratory and undergraduate research. Adv Physiol Educ 2006;30:237–41.
[15] Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 2007;450:20–7.
[16] Gudjonsson T, Villadsen R, Nielsen HL, Rønnov-Jessen L, Bissell MJ, Petersen OW. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. Genes Dev 2002;16:693–706.
[17] Yang J, Huang Y, Wang X-B, Becker FF, Gascoyne PRC. Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow fractionation. Anal Chem 1999;71:911–8.
[18] Chung SH, Min J. A microscopic investigation on the effect of hydrophobic properties on cell adhesion on a PDMS surface. Biochip J 2008;2:141–7.
[19] Choi S, Song S, Choi C, Park J-K. Continuous blood cell separation by hydrophoretic filtration. Lab Chip 2007;7:1532–8.
[20] Ly N, Foley K, Tao N. Integrated label-free protien detection and separation in real time using confined surface plasmon resonance imaging. Anal Chem 2007;79:2546–51.
[21] Won JY, Son SJ, Um SH, Choi J-W, Min J. The simple and fast isolation of Escherichia coli O157:H7 using magnet nanoparticle embedded silica nanotube for the nucleic acid based detection. J Biomed Nanotechnol 2013;9:886–90.
[22] Altankov G, Jankova K, Jonsson G, Thom V, Ulbricht M. Biocompatible materials. US Patent US20050053642 [05.03.10].
[23] Kate K, Umezawa K, Funeriu DP, Miyake M, Miyake J, Nagamune T. Immobilized culture of nonadherent cells on an oleyl poly(ethylene glycol) ether-modified surface. Biotechniques 2003;35:1014–21.
[24] Won JY, Min J, Park J-H. Bacteria adsorption on hydrophilic surface for the sensitive detection of pathogenic bacteria using a single tube chamber system. Biosens Bioelectron 2010;26:1763–7.
[25] Kate K, Umezawa K, Miyake M, Miyake J, Nagamune T. Transfection microarray of nonadherent cells on an oleyl poly(ethylene glycol) ether-modified glass slide. Biotechniques 2004;37:444–52.
[26] Ferguson RE, Carroll HP, Harris A, Maher ER, Selby PJ, Banks RE. Housekeeping proteins: a preliminary study illustrating some limitations as useful references in protein expression studies. Proteomics 2005;5(2):566–71.
[27] Kim JH, Seo S, Min J. Epithelial cell patterns on a PDMS polymer surface using a micro plasma structure. J Biotechnol 2011;155:308–11.