An engraved surface induces weak adherence and high proliferation of nonadherent cells and microorganisms during culture

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BioTechniques 69: 113–125 (August 2020) 10.2144/btn-2020-0022

First draft submitted: 4 March 2020; Accepted for publication: 21 May 2020; Published online: 12 June 2020

ABSTRACT

When cells are cultured in a Petri dish, the adherent cells attach to the bottom of the dish; whereas, the nonadherent cells float in the culture medium. It was observed that nonadherent cells could be induced to adherent-like cells when cultured in an engraved plastic dish (biosimulator). The adherence of these cells to the engraved surface could be prevented with inhibitors specific for adhesion. It was also observed that culturing microorganisms of the environment in a biosimulator induced weak adhesion and high proliferation. Analysis of the microbiome using 16S rRNA profiling demonstrated that the biosimulator was more efficient in inducing proliferation of several phyla of microorganisms compared with culture by conventional techniques.

METHOD SUMMARY

Nonadherent cells could be induced to adherent-like cells when cultured in an engraved plastic dish (biosimulator). The adhesion induced proliferation of eukaryotic and prokaryotic cells.

KEYWORDS:

adherence • biosimulator • cell culture • diagnostics • engraving • environment • microbiome • microorganisms • nonadherent cells • Petri dish

Cell culture is routinely employed in laboratories for biomedical research and has multiple applications, including diagnosis, drug development, therapy and production of biological resources. The traditional process of culturing adherent cells in a Petri dish still serves as an important tool for cell culture applications [1]. During cell culture, the adherent cells attach to the bottom of a plastic Petri dish, whereas nonadherent cells float in the culture medium. There are no specialized dishes for transforming nonadherent cells to adherent cells; this is an impediment in several drug development studies, especially in those involving autoreactive T cells or cells involved in atherosclerosis.

The majority of microorganisms of the environment are nonculturable [2]. In addition, some microorganisms exist in a viable but nonculturable state and are difficult to culture in laboratory conditions. Such cells are characterized by a lack of culturability using conventional culture techniques that impairs their detection by routine analytical techniques. This leads to an underestimation of total viable cells in environmental or clinical samples, and thereby poses a risk to public health [3]. Hence, there is a need to develop a strategy to increase the proliferation of such microorganisms.

In this paper, it is demonstrated that nonadherent cells exhibit adherence when cultured in an engraved polystyrene Petri dish. Though the cells cultured were from the same clone, when cultured on an engraved dish, they exhibited polarity. It was also demonstrated that specific inhibitors could be used to prevent adhesion of nonadherent cells on an engraved surface. Further, we demonstrate that the biosimulator enhanced the proliferation of microorganisms. The phenomenon of cell adhesion on an engraved dish has wide applications in cell biology and microbiology.

Materials & methods

Development of a biosimulator to induce adhesion & proliferation of cells

For culturing cells, nonpyrogenic, noncytotoxic and polystyrene BioLite cell culture dishes (Thermo Scientific, NJ, USA) were used. To induce adhesion of cells, the plastic surface was engraved with parallel lines using a sterile sharp blade under aseptic conditions. The engravings had a width of 30–50 µm and depth of 5 µm.

Cell culture in the biosimulator

The nonadherent hybridoma cell lines 4B7, 10D9, 1A10, 99D, Sp2/0, B56T (1×10^6 cells/dish) were cultured in 10 ml Dulbecco's modified Eagle medium, and incubated at 37°C, 5% CO₂. The cells were monitored daily. All the experiments were repeated at least three times.

For cell proliferation assays, hybridoma cells were grown in a conventional dish or a biosimulator. Cells were collected every other day, depending on the proliferation. The absorption of cells was measured at 600 nm using a spectrophotometer; a hemocytometer was used for cell count.

For inhibition of cell adhesion, 0.1% salicylic acid (Fisher Scientific, NJ, USA) and 0.01% pectasol (gift of ecoNugenics, CA, USA) were added to the culture medium during cell culture.

Proliferation & analyses of microorganisms in a biosimulator

Samples from soil, spring water, air (office) and host oral microbiome were cultured in a biosimulator in Luria-Bertani medium for 48 h. After 48 h, the media were pelleted in a centrifuge at 5000×g and the samples subjected to 16S rRNA sequencing (Arizona State University Microbiome Core) for taxonomic identification. Microbial DNA was extracted from samples using the DNeasy PowerSoil Kit (Qiagen, Germany) following the manufacturer's directions.

Microbiome library preparation methodology

Bacterial community analysis was performed via next generation sequencing on the Illumina MiSeq platform. Amplicon sequencing of the V4 region of the 16S rRNA gene was performed with the barcoded primer set 515f/806r designed by Caporaso *et al.* [4] and following the protocol by the Earth Microbiome Project (<http://www.earthmicrobiome.org/emp-standard-protocols/>) for library preparation. PCR amplifications for each sample were done in triplicate, then pooled and quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, CA, USA). A no-template control sample was included during library preparation as a control for extraneous nucleic acid contamination. About 240 ng of DNA per sample were pooled and then cleaned using the QIAquick PCR purification kit (Qiagen). The pool was quantified using the Illumina library Quantification Kit ABI Prism® (Kapa Biosystems, MA, USA), then diluted to a final concentration of 4 nM, denatured, and diluted to a final concentration of 4 pM with 15% of PhiX control library (Illumina, CA, USA). Finally, the DNA library was loaded in the MiSeq and run using the version 2 module, 2 × 250 paired end, following the directions of the manufacturer.

Analysis of sequencing results was performed by the University of North Carolina Microbiome Core. The sequencing output from the MiSeq platform was converted to FASTQ format and demultiplexed using Bcl2Fastq 2.18.0.12 (Illumina). The resulting paired-end reads were processed using QIIME 2 2018.11. Index and linker primer sequences were trimmed using the QIIME 2 invocation of cutadapt. The resulting paired-end reads were processed with DADA2 through QIIME 2, including merging paired ends, quality filtering, error correction and chimera detection.

Amplicon sequencing units from DADA2 were assigned taxonomic identifiers according to Green Genes release 13.08, the Human Oral Microbiome Database release 15.1 and Silva database release 132.

Alpha diversity with respect to Faith PD whole tree, Evenness (Shannon) index and observed species number metrics was estimated using QIIME 2 at a rarefaction depth of 5000 sequences per subsample. Beta diversity estimates were calculated within QIIME 2 using weighted and unweighted Unifrac distances as well as Bray–Curtis dissimilarity between samples at a subsampling depth of 5000. Results were summarized, visualized through principal coordinate analysis and significance was estimated as implemented in QIIME 2. Significance of differential abundance was estimated using AnCom as implemented in QIIME 2.

Statistics

Unpaired two-tailed Student *t*-tests were used to compare sets of data obtained from independent groups. Statistical significance was considered at the $p < 0.05$ level.

Results & discussion

An engraved surface could induce adhesion of nonadherent cells

Based on the observation that a cell culture plate with a compromised surface has high affinity for cells, parallel lines were engraved on a tissue culture Petri dish and used the dish for cell culture. The cells were nonadherent and were found floating on the first and second day of culture. After 3 days of culture, all the cell lines tested (4B7, 10D9, 1A10, 99D, Sp2/0, 99D) formed distinct patterns on the engraved plastic surface; pattern formation corresponded to the engraving. When the Petri dish was engraved with parallel lines, the nonadherent cells were seen on top of the engraved line, whereas on the lower half of the dish the nonadherent cells were below the engraved line (Figure 1). The orientation of the cells is shown in Figure 1C & D. The cells were closely packed on the engraved line. The alignment of nonadherent cells was found to be stable on the engraved line; minor disturbances to the plastic dish did not destroy the patterns exhibited. This failure to destroy the patterns demonstrated that the cells were adhered near the engraved surface. The experiment demonstrated that an engraved surface could induce nonadherent cells to become adherent cells.

In conventional cell culture, adherent cells grow to confluence, and must be dissociated from the dish using trypsin. In our experiment, the nonadherent cells (unlike the adherent cells) were not strongly adhered to the biosimulator. Liquid media were pipetted to disrupt

Figure 1. Engraved surface-induced adhesion of nonadherent cells. (A) Photomicrograph of nonadherent cells (4B7) exhibiting polarity on an engraved dish (biosimulator). (B) Ponceau S staining of cells cultured in a biosimulator. (C) The orientation of cells in a biosimulator. Cells are aligned on top of the engraving in the upper half of the biosimulator, whereas they are aligned below the engraving in the bottom half of the biosimulator. (D) Drawing of an engraved Petri dish showing the orientation of nonadherent cells. Experiments were repeated five times.

the cells from a distance of 1 cm. Seventy percent of the cells were removed when the media was pipetted at a speed of 5 ml per 10 s (repeated five times). The experiment demonstrated that the nonadherent cells are weakly adhered to the engraving of the biosimulator.

An engraved surface induced proliferation of nonadherent cells

We determined whether the engraved surface induced proliferation of nonadherent cells. B cell hybridoma cell lines 99D (Figure 2) and 4B7 (Figure 3) were cultured in an engraved biosimulator, using conventional Petri dishes as a control. We counted the cells and also determined the absorbance (optical density at 600 nm) at regular intervals. The biosimulator induced proliferation of the nonadherent cells, as determined by cell counting and absorbance measurements (Figures 2 & 3). Culture of nonadherent cells in a biosimulator led to a statistical increase in the number of cells compared with culturing in conventional Petri dishes. The study demonstrated that engraving patterns in a biosimulator could induce adhesion and proliferation of nonadherent cells.

Adhesion of nonadherent cells in a biosimulator could be inhibited by specific inhibitors

Salicylic acid is known to prevent cell–cell interaction and is used in animal models of diabetes [5], but its mechanism of action is not clearly known. When nonadherent cells (4B7) were treated with 0.1% salicylic acid, cell adhesion was inhibited (Figure 4). We also used the adhesion inhibitor pectasol (which prevents cancer metastasis) in our studies [6]. Treatment of nonadherent cells with 0.01% pectasol did not prevent cell proliferation; however, it prevented cells adhering to the plastic surface (Figure 4C). The nonadherent cells lost the orientation property; the cells were found floating in the medium and did not have any affinity for the engraved surface. These *in vitro* experiments demonstrate that the phenomenon of pattern formation could be used in drug discovery studies.

The engraved surface-induced proliferation of microorganisms

As the engraved surface in the biosimulator could induce cell proliferation, we were interested to determine whether it could also induce proliferation of microorganisms. One of the impediments in microbial studies or diagnosis is the nonculturable property of most of the microorganisms. Only a very limited number of microorganisms are culturable by conventional culture techniques; there is therefore a

Figure 2. Engraved surface-induced proliferation of 99D nonadherent cells. The hybridoma cell 99D was induced to proliferate by the engraved surface. (A) Photomicrograph showing 99D cells adhering to the engraving of a biosimulator compared with (B) growth in a conventional dish. (C) Absorbance at 600 nm of 99D cells growing in a biosimulator or conventional dish. (D) Cell count of 99D cells growing in a biosimulator or conventional dish using a hemocytometer. Experiments were repeated five times.

* $p < 0.05$, ** $p < 0.01$ as determined by t-test.

need to develop new strategies to induce proliferation of microorganisms. The microbiome is involved in health and diseases [7] and there is a need to determine dysbiotic microorganisms to facilitate diagnosis and treatment.

Samples were cultured from different environments – air, water, soil and host oral cavity – in the biosimulator. The microorganisms were found to adhere to the engravings of the biosimulator within 24 h (Figure 5). After 48 h, they proliferated and covered the entire biosimulator. Analysis of the soil microbiome by spectrometry demonstrated that the biosimulator induced more proliferation of the microorganisms compared with conventional techniques (Figure 5).

The biosimulator induced proliferation of a large number of Firmicutes in samples from air, soil, water and oral cavity compared with culture using conventional techniques (Figure 6). The phylum Firmicutes includes the class Clostridia and genus *Clostridium* and consists of a large group of anaerobic to aerotolerant spore-forming bacilli found in soil and in the gut flora of humans and animals. Clostridia include both gram-positive and gram-negative species, although the majority are gram-positive. Interestingly, many gram-positive species lose the Gram reaction, resulting in gram-negative cultures [8]. Some species of *Clostridium* can become aerotolerant on subculture; however, only a few (*C. carnis*, *C. histolyticum*, and *C. tertium*) can grow under aerobic conditions. The vast majority of human oral Clostridia comprises the families Lachnospiraceae, Peptostreptococcaceae and Veillonellaceae [9]. Microbial sequencing demonstrated that the biosimulator induced proliferation of bacterial phyla of air (Figure 7, Supplementary Table 1), water (Figure 8, Supplementary Table 2), soil (Figure 9, Supplementary Table 3) and oral samples (Figure 10, Supplementary Table 4).

Figure 3. Engraved surface-induced proliferation of the 4B7 nonadherent cells. The hybridoma cell 4B7 was induced to proliferate by the engraved surface. (A) Photomicrograph showing 4B7 cells adhering to the engraving of a biosimulator compared with (B) growth in a conventional dish. (C) Absorbance at 600 nm of 4B7 cells growing in a biosimulator or conventional dish. (D) Cell count of 4B7 cells growing in a biosimulator or conventional dish using a hemocytometer.

* $p < 0.05$, ** $p < 0.01$ as determined by *t*-test.

Figure 4. Treatment of nonadherent 4B7 cells with specific inhibitors prevented adhesion of cells to the engraved plastic surface. (A) Nonadherent cells adhering to the engraved plastic surface. (B) Salicylic acid treatment prevented adhesion of the nonadherent cells in an engraved plastic dish. (C) Treatment with pectasol prevented cell adhesion in an engraved plastic dish. Experiments were repeated three times.

Culturing the microbiome of air from the built environment (office) using a biosimulator led to the proliferation of the genera *Comamonas*, *Aeromonas*, *Bacillus*, *Clostridium* (*sensu stricto*), *Shigella*, *Escherichia*, *Klebsiella*, *Providencia*, *Serratia*, *Exiguobacterium*, *Acinetobacter*, *Neisseria*, *Lysinibacillus*, *Pseudomonas*, *Shewanella*, *Lactococcus* and *Streptococcus*. However, these microorganisms were not observed when cultured by conventional techniques.

Culturing the microbiome of spring water using a biosimulator led to the proliferation of the genus *Proteus*. Culturing the microbiome of soil using a biosimulator led to the proliferation of the genus *Comamonas*.

The principle of contact guidance or topographic guidance was introduced by Ross Harrison in 1914 [10] and refined by Paul Weiss in 1945, when he demonstrated that cells elongate along the direction of the groove and that migration is influenced by the grooves [11]. The cultured cells exhibit thigmotropism, sensing the surface topography of their environment and reacting to these surface cues.

Figure 5. The biosimulator induced proliferation of bacteria from the oral cavity, air, water and soil samples. The engraving in the biosimulator induced proliferation of the bacteria from the (A) oral cavity, (B) air, (C) water and (D) soil samples. Experiments were repeated three times.

Cellular behavior, including cell shape, adhesion, orientation, migration and proliferation, is influenced by surface chemistry and surface topography [12,13].

During cell culture, cells attach to the surface via focal adhesions that connect the surface to the cytoskeleton. The surface chemistry, electrostatic charge, wettability and elastic modulus influence cell growth. The topographical surface provides clues to cell alignment, migration and outgrowth of neurites along a specific orientation. In cell biology studies, to understand the ability of cells to respond to surface topography, grooved substrates are often used. The cells are physically guided along the direction of the grooves, reorganized by the cytoskeleton. However, the mechanism by which cells recognize surface geometry is not clearly understood. The literature suggests it may be a passive process, whereby adhesion molecules best fitting the local topography simply draw cells or cellular components into the observed shape. In several cell lines, surface topography has been shown to be important for the early events of attachment and formation of focal adhesions, activating mechanotransduction events which may eventually determine cell fate and consequent

Figure 6. Relative abundance of bacteria at the phylum level cultured in the biosimulator or conventional dishes. Bacteria from air, soil, water and oral cavity samples were cultured in a biosimulator or conventional dish and the taxonomy determined by 16S rRNA sequencing. Each bar represents the mean of three samples.

tissue formation. Among different topographies, micrometer-sized grooved surfaces have been extensively studied for their effects on cell alignment because they can be easily fabricated. As an example, culture of osteogenic cells on grooved surfaces results in strong orientation in the direction of the grooves – unlike on flat surfaces, where a random orientation is generally observed. It has also been demonstrated that microgrooves with widths similar to the cell size induce better cell guidance, whereas the guidance is weaker when cells are cultured in grooves with widths larger than the cells [14–16]. Focal adhesions and actin microfilament bundles and microtubules are found to align along micrometer-sized grooves and ridges, whereas, on smooth substrates, no preferred orientation is exhibited by cells and cytoskeletal elements [17]. In the culture of osteoblastic cells on microgrooves with widths ranging from 4 to 38 μm , it was shown that the narrower grooves (4–16 μm ; $0.5\text{--}2 \times$ cell size) are more effective in guiding the cell orientation. Similarly, it was shown that microgrooves with widths ranging from 2 to 12 μm exhibit great contact guidance effects on the shape and orientation of rat bone marrow cells and fibroblasts [16].

Knowledge of the phenomenon that cells have affinity for grooves on the substrate has led to the development of the field of cellular micropatterning. Cellular micropatterning is a tool to accurately design cell–substrate attachment, tissue engineering, biological assays and biosensors, and is used for drug development studies [18]. A broad range of techniques and materials have been employed to fabricate well-defined topographical and chemical cues to assess cell micropatterning. Some of these fabrication techniques are based on photolithography and reactive ion etching that may be followed by anisotropic etching [19]. In recent years, microfluidics encompassing sophisticated micropatterning designs has led to the development of powerful tools for single cell analysis. The localized microenvironment is precisely controlled by microfluidic techniques, thereby increasing accuracy. The technique is important for disease diagnosis and personalized medicine [20].

Micropatterning can be fabricated into microwell arrays to achieve three different dimensions: single cell (1D), cell monolayer (2D) and cell spheroid (3D). As cells in the *in vivo* environment are surrounded by other cells in three dimensions, 2D cell culture does not adequately take into account the natural environment of cells. As a result, 2D cell culture tests sometimes provide misleading and non-predictive data for *in vivo* responses [21]. 3D *in vitro* cell models provide a more realistic cellular environment and permit the reproduction of *in vivo* cellular phenotypes [22]. Spheroids realistically reflect *in vivo* cell behaviors and provide results more closely aligned to those of *in vivo* tests, thereby making them useful in drug developmental studies [21].

3D cell culture is used in stem cell and tumor cell research [23,24]. In 3D cell culture, human embryonic stem cells (hESCs) have the potential to differentiate into over 200 diversely functioning cell types. Microwell culture permits generation of hESC colonies with a defined size that can later form monodisperse embryoid bodies. When cultured in this system, hESCs retain pluripotency and self-renewal, and can be passaged to standard unconstrained culture conditions [23]. At the intersection between tissue engineering and oncology, 3D *in vitro* tumor models simulate the *in vivo* physiological microenvironment. Working in 3D involves the formation of spheroids – aggregates that can either be grown in suspension, encapsulated, or grown on top of a 3D matrix using different 3D methods [25]. Many cell lines show a reduced proliferation rate in 3D cultures compared with those cultured in 2D. Furthermore, many currently available 3D cell culture techniques are slow, time-consuming, expensive and lack reproducibility [21].

In the initial days of culture in the biosimulator, the pattern of the engraving influenced the nature of cell adherence: the cells were more adhered on the outer edge of the engraved line than the inner edge. It is not understood why the cells show polarity when cultured

Figure 7. Relative abundance of microorganisms of air (bacteria and fungi) at the genus level cultured in the biosimulator or conventional dishes. Bacteria from air (office) were cultured in a biosimulator or conventional dish for 48 h and the taxonomy determined by 16S rRNA sequencing (n = 4 per treatment).

on a dish with parallel engravings; however, the physical features influencing the cells to form patterns include the circular shape of the Petri dish and the engravings on the hydrophilic plastic surface.

The engraving on the plastic surface did not induce very strong affinity to the cells, which had only a weak adhesion to the engravings. Rapid shaking and extreme washing prevented adhesion of the nonadherent cells. Nevertheless, the adhesion induced proliferation of eukaryotic and prokaryotic cells.

The fundamental units of cell adhesion are protein complexes consisting of three classes of proteins: cell adhesion molecules/adhesion receptors, extracellular matrix proteins and cytoplasmic plaque/peripheral membrane proteins. The cell adhesion proteins include members of the integrin, cadherin, immunoglobulin, selectin and proteoglycan super families [26]. We have not deter-

- D_4__Aeromonadaceae;D_5__Aeromonas
- D_4__Bacillaceae;D_5__Bacillus
- D_4__Planococcaceae;D_5__Lysinibacillus
- D_4__Enterobacteriaceae;D_5__Providencia
- D_3__Bacillales;D_4__Planococcaceae
- D_4__Enterobacteriaceae;D_5__Klebsiella
- D_4__Burkholderiaceae;D_5__Comamonas
- D_3__Enterobacteriales;D_4__Enterobacteriaceae
- D_5__Myroides;D_6__Myroides marinus
- D_4__Moraxellaceae;D_5__Acinetobacter
- D_4__Flavobacteriaceae;D_5__Myroides
- D_3__Betaproteobacteriales;D_4__Burkholderiaceae
- D_4__Pseudomonadaceae;D_5__Pseudomonas
- D_4__Shewanellaceae;D_5__Shewanella
- D_4__Family XII;D_5__Exiguobacterium
- D_4__Enterococcaceae;D_5__Vagococcus
- D_4__Enterobacteriaceae;D_5__Pantoea
- D_5__Myroides;D_6__Myroides sp. OTU s6b
- D_4__Enterobacteriaceae;D_5__Morganella
- D_4__Enterobacteriaceae;D_5__Serratia
- D_4__Streptococcaceae;D_5__Lactococcus
- D_4__Enterobacteriaceae;D_5__Escherichia-Shigella
- D_4__Xanthomonadaceae;D_5__Stenotrophomonas
- D_4__Enterococcaceae;D_5__Enterococcus
- D_2__Bacilli;D_3__Bacillales
- D_4__enterobacteriaceae;D_5__Proteus
- D_4__Chromobacteriaceae;D_5__Chromobacterium
- D_4__Weeksellaceae;D_5__Chryseobacterium
- D_4__Vibrionaceae;D_5__Vibrio
- D_4__Hydrogenophilaceae;D_5__Thiobacillus
- D_4__Enterobacteriaceae;D_5__Hafnia-Obesumbacterium
- D_4__Weeksellaceae;D_5__Empedobacter
- D_4__Corynebacteriaceae;D_5__Lawsonella
- D_5__Joenoides;D_6__Joenoides intermedia
- D_5__Paenibacillus;D_6__Paenibacillus taiwanensis
- D_4__Clostridiaceae 1;D_5__Clostridium sensu stricto 1
- D_4__Neisseriaceae;D_5__Neisseria
- D_4__Sphingobacteriaceae;D_5__Sphingobacterium
- D_5__Paenibacillus;D_6__Paenibacillus sp. St-s
- D_5__Empedobacter;D_6__Empedobacter sp. PH7-1
- D_5__uncultured;D_6__bacterium DCE29
- D_3__Lactobacillales;D_4__Enterococcaceae
- DD_4__Nitrosomonadaceae;D_5__MND1
- D_4__Burkholderiaceae;D_5__Alcaligenes
- D_5__Microcoleus PCC-7113;D_6__Microcoleus sp. HTT-U-KK5
- D_4__Propionibacteriaceae;D_5__Cutibacterium
- D_4__Chromobacteriaceae;D_5__Vogesella
- D_0__Bacteria;__

Figure 8. Relative abundance of microorganisms of water at the genus level cultured in the biosimulator or conventional dishes. Bacteria from spring water were cultured in a biosimulator or conventional dish for 48 h and the taxonomy determined by 16S rRNA sequencing (n = 3 per treatment).

Figure 9. Relative abundance of microorganisms of soil at the genus level cultured in the biosimulator or conventional dishes. Bacteria from soil were cultured in a biosimulator or conventional dish for 48 h and the taxonomy determined by 16S rRNA sequencing (n = 3 per treatment).

mined which adhesion molecules were expressed on the nonadherent cells when cultured in a biosimulator. However, we determined whether inhibiting the expression of the adherent proteins would prevent the nonadherent cells from adhering to the engraving.

For these inhibition studies, we used salicylic acid and pectasol. It is known that salicylic acid prevents adhesion of eukaryotic and microbial cells [27–31]. The adhesion is mediated by ERK signaling and the inhibition of this signaling prevents adhesion [32,33]. For example, coating urinary catheters with salicylic acid reduces bacterial adherence and the risk of urinary tract infection [30].

Pectasol-C is a modified pectin compound used in the inhibition of adhesion and metastasis in cancer cells [6]. Galectin-3 (Gal-3) is a 29-kDa β -galactoside-binding adhesion protein and is expressed intracellularly and extracellularly by various cell types. Pectasol is known to inhibit Gal-3, thereby inhibiting adhesion [34]. In this study, we used both salicylic acid and pectasol for inhibition studies. The cells did not adhere to the engravings of the biosimulator in the presence of salicylic acid and pectasol, demonstrating that the adhesion was mediated by adhesion proteins.

Biofouling is a limiting factor in medical devices. When associated with the biological environment, biomedical devices are prone to surface biofouling due to adhesion of microbial or thrombotic agents [35]. We demonstrated that engravings could predict where

Figure 10. Relative abundance of microorganisms of the oral cavity at the genus level cultured in the biosimulator or conventional dishes. Bacteria from the oral cavity were cultured in a biosimulator or conventional dish for 48 h and the taxonomy determined by 16S rRNA sequencing (n = 3 per treatment).

nonadherent cells adhere. Based on this property, we could design better probes for biomedical applications that could resist biofouling. The phenomenon thus has wide applications in biomedical engineering.

Use of animal models is the preferred method for drug development studies, and single cell analysis provides key information critical to understanding disease processes. However, animal models are expensive and cumbersome, and the researcher has to analyze and collect data of single cells from a pool of cells. Current strategies do not allow analysis of single cells without removing them from the context of interest, which not only destroys contextual information but also may impair the process under study [36]. The induction of adherence in nonadherent cells has wide application in cell biology. The phenomenon could be used to study the mechanism by which autoreactive T cells adhere to pancreatic β cells. Similarly, the mechanism by which monocytes or macrophages adhere to artery walls could be studied using a biosimulator. We have demonstrated that pattern formation can be prevented using drugs that prevent cell adhesion. Based on this principle, new drugs could be tested for their ability to prevent adhesion in different cells. Promising drug candidates that inhibit adhesion molecules in autoreactive T cells could help to combat diseases such as Type I diabetes. Use of the biosimulator to aid development of novel drugs by inhibiting adhesion of nonadherent cells is a more cost-effective strategy than using animal models.

The majority of microorganisms found in nature, including pathogens infecting humans, animals and plants, are nonculturable using conventional techniques [37]. A device that could be used to induce proliferation of these microorganisms will be beneficial in diagnostics as well as the development of new drugs and vaccines.

There have been few modifications in the design and construction of the Petri dish since its development in the 19th century by the German bacteriologist Julius Petri. Surface modified (hydrophilic) polystyrene plates are in vogue for cell culture studies. When adherent cells are cultured on a Petri dish, they spread rapidly and grow to confluence within a couple of days (the exact time required to form confluence depends on the nature of the cell line). However, when nonadherent cells are cultured, some cells attach to the bottom of

the Petri dish, but the majority remain suspended in the medium. Currently, there are no specialized plates for transforming nonadherent cells to adherent cells.

In this paper, it is demonstrated that the biosimulator is a 2D cell system. Most of the 2D systems in the literature are designed for adherent cells; we developed the biosimulator for nonadherent cells and the microbiome. The biosimulator does not have any special coating for inducing adherence. The only variable is the engraving that induces adhesion and proliferation of eukaryotic cells and the microbiome. The width of the engraving is larger than the nonadherent cells. Unlike the adherent cells that formed lamellopodia in the grooves, there was no lamellopodia formation in the nonadherent cells attached to the engravings, resulting in weak affinity of the cells to the engravings. The purpose of the biosimulator is to induce adhesion of nonadherent cells so that they could be used for drug developmental studies; however, further work is required to determine whether induction of adhesion by the biosimulator changes the properties of the nonadherent cells. In addition to induction of adhesion in nonadherent cells, the biosimulator also induced adhesion of microorganisms, and thus could also be used to monitor the microbiome of host and environmental samples.

We used the biosimulator to induce proliferation of microorganisms from different environments: air, water, soil and host. The microorganisms from the environmental samples proliferated within 24 h in a biosimulator. In the first 24 h, we observed that the bacteria had more affinity to the engraving; after 24 h the whole biosimulator was covered with bacteria. To determine if there were changes in the microbial species after culture in different conditions, we cultured the environmental samples in a biosimulator as well as a conventional Petri dish. Microbial sequencing demonstrated that the biosimulator could induce proliferation of microorganisms better than culturing in conventional dishes; we observed several species of bacteria in the environmental samples cultured in a biosimulator that were not seen in those cultured in conventional dishes. We hypothesize that the biosimulator may be useful in encouraging the proliferation of difficult-to-culture microorganisms.

To conclude, the induction of adhesion and proliferation of nonadherent cells and microorganisms on an engraved plastic surface will open new avenues of research in immunology, cell biology and microbiology.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2020-0022

Author contributions

S Thomas conceived, designed and performed the experiments, analyzed the data, and wrote the paper.

Acknowledgments

The author thanks MA Azcarate-Peril and J Roach of the University of North Carolina Microbiome Core for microbiome analysis.

Financial & competing interests disclosure

The author wishes to acknowledge receiving grants and support from the Wawa Foundation, Abraham Thomas Foundation and Women's Board of Lankenau. The UNC Microbiome Core is funded partially by NIH/NIDDK grant number P30 DK34987. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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