Title

Protein Adsorption Alters Hydrophobic Surfaces Used for Suspension Culture of Pluripotent Stem Cells

Journal

The Journal of Physical Chemistry Letters, 6(3)

ISSN

1948-7185

Authors

Jonas, Steven J
Stieg, Adam Z
Richardson, Wade
et al.

Publication Date

2015-02-05

DOI

10.1021/jz502520r

Peer reviewed

HHS Public Access

Author manuscript

J Phys Chem Lett. Author manuscript; available in PMC 2016 February 05.

Published in final edited form as:

J Phys Chem Lett. 2015 February 5; 6(3): 388–393. doi:10.1021/jz502520r.

Protein Adsorption Alters Hydrophobic Surfaces Used for Suspension Culture of Pluripotent Stem Cells

Steven J. Jonas[†], **Adam Z. Stieg**[§,⊥], **Wade Richardson**[†], **Shuling Guo**[#], **David N. Powers**[‡], **James Wohlschlegel**[‡], and **Bruce Dunn**[†,§,||,*]

[†]Department of Materials Science and Engineering, University of California, Los Angeles, 420 Westwood Plaza, Los Angeles, California 90095, United States

[‡]Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, 615 Charles East Young Drive South, Los Angeles, California 90095 United States

[§]California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, United States

[||]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA, University of California, Los Angeles, 615 Charles East Young Drive South, Los Angeles, California 90095, United States

[⊥]WPI Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

Abstract

This Letter examines the physical and chemical changes that occur at the interface of methyl-terminated alkanethiol self-assembled monolayers (SAMs) after exposure to cell culture media used to derive embryoid bodies (EBs) from pluripotent stem cells. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy analysis of the SAMs indicates that protein components within the EB cell culture medium preferentially adsorb at the hydrophobic interface. In addition, we examined the adsorption process using surface plasmon resonance and atomic force microscopy. These studies identify the formation of a porous, mat-like adsorbed protein film with an approximate thickness of 2.5 nm. Captive bubble contact angle analysis reveals a shift toward superhydrophilic wetting behavior at the cell culture interface due to adsorption of these proteins. These results show how EBs are able to remain in suspension when derived on

[*]Corresponding Author: bdunn@ucla.edu.

[#]Isis Pharmaceuticals, Inc., 2855 Gazelle Court, Carlsbad, California 92010, United States

Author Contributions

S.J.J. conceived the experiments and prepared samples for all associated studies. S.J.J. and A.Z.S. performed AFM imaging and measurements. J.W. and D.N.P. carried out the MudPit analysis. B.D. conceptualized and directed the research project. All authors discussed the results and contributed to the manuscript.

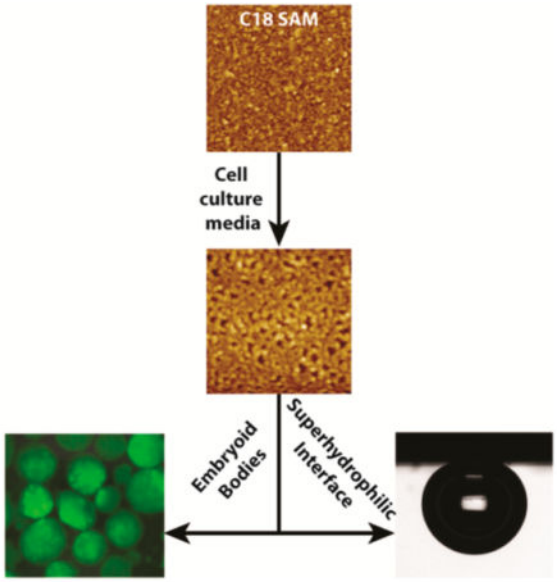
Notes

The authors declare no competing financial interest.

[*]Supporting Information

Materials and experimental section details as well as results from MudPIT analysis of adsorbed media components, ellipsometry, additional SPR studies, and movies illustrating the superhydrophilic behavior observed during captive bubble analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

hydrophobic materials, which carries implications for the rational design of suspension culture interfaces for lineage specific stem-cell differentiation.



Pluripotent stem cells (PSCs) possess extraordinary potential for revolutionizing medicine based on their unique ability to proliferate indefinitely in culture and give rise to cells from each embryonic germ layer.[1–5] Research directed toward understanding how to control stem cell fate decisions has driven the development of defined protocols for manipulating differentiating PSCs and their derivatives toward generating new therapeutic tools for regenerative medicine.[6] Of these approaches, the formation of 3-D stem cell aggregates known as embryoid bodies (EBs) is the most common intermediate used to prime PSC populations prior to the induction of lineage specifoc differentiation.[7,8] EBs can be generated through several methodologies including suspension culture on commercially available low attachment tissue culture plates, hanging drop, and methylcellulose-based platforms.[9] However, these traditional strategies tend to produce heterogeneous populations of EBs that vary in size and morphology as well as being prone to agglomeration, which both limits homogeneous differentiation and leads to low production yields.[10] While technology-based approaches that make use of stirred bioreactors,[11,12] rotating culture platforms,[13–15] microfluidic devices,[16,17] and microfabricated cell culture substrates[18–20] have been proposed for generating homogeneous EB populations, the primary drawback to the widespread adoption of these methods stems from the need for specialized tools and equipment that are either not commonly found in most stem-cell biology laboratories or are simply cost-prohibitive.

We have previously investigated the efficacy of various materials toward the formation of more uniformly sized and functionally enhanced EB populations in suspension. This work revealed EB size to represent a critical parameter for lineage specific differentiation, where EBs with diameters between 100 and 300 μm displayed higher cellular viability, a lower degree of cell death, and enhanced differentiation potential across all three embryonic germ

layers.[21] In particular, materials with methyl-terminated hydrophobic surfaces such as polydimethylsiloxane (PDMS) and various alkanethiolate self-assembled monolayers (SAMs) with specific chain lengths were found to promote the formation of EBs within this optimal size range as compared with traditionally derived EBs under both serum-containing and serum-free cell culture conditions.[21] This observation is illustrated in the Supporting Information Figure S1, where suspension cultures of EBs prepared on octadecanethiol (C18) SAMs appear less prone to aggregation and possess a more consistent spherical morphology compared with those formed using a standard commercially available low attachment tissue culture plate (LAC, Corning). Controlling EB size in this manner ultimately translated to functional improvements that included higher expression of lineage-specific differentiation markers and improved yields of differentiated cells that were directed toward endodermal, ectodermal, and mesodermal lineages.

An interesting question that arose in this prior research was the means by which hydrophobic cell culture surfaces were able to support EBs in suspension. We address this question by tracing the evolution of an initially hydrophobic surface into one that is both hydrophilic and an effective material for use in suspension culture of EBs. Specifically, in the present work, we find that the chemical, compositional, and structural changes elicited by the adsorbate layer lead to a superhydrophilic surface that prevents EBs from binding to the substrate.

In general, biomolecules such as proteins are prone to adsorb onto the surfaces of methyl-terminated hydrophobic materials.[22,23] Given that cell culture media are complex solutions composed of proteins, carbohydrates, and other biomolecules, adsorption was monitored at the surface of a C18 SAM using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy before and after exposure to a standard EB formation culture medium. This serum-containing formulation is outlined in the experimental section and is used for all subsequent surface characterization studies reported here unless otherwise stated. It is significant to note that the presence of absorption peaks associated with amide I and II vibrations at 1644 and 1547 cm^{-1} in the spectra for the C18 SAM (Figure 1A) is consistent with the protein adsorption behavior observed previously on PDMS.[21]

The surface coverage and time scale for adsorption of the protein layer was monitored in real time via surface plasmon resonance (SPR) analysis.[24] Characterization of a C18 SAM, as provided in Figure 1B, indicates an increase in the baseline of the response curve of 1965 RU after a single injection of EB medium into the analysis chamber. For most proteins a change of 1000 RU corresponds to a concentration of 1 ng/mm^2 of adsorbed protein on the sensor surface, which for our materials equates to 1.97 ng/mm^2 of protein adsorbing at the SAM interface. The SPR response curve plotted in Figure 1B also indicates that the adsorption of proteins from the EB medium proceeds quickly at the SAM interface and is essentially complete after a single injection. Injecting a dilute detergent into the analysis chamber results in a drop in the baseline of the SPR response curve, suggesting that the proteins are loosely bound and physically adsorbed at the C18 SAM interface (Supporting Information, Figure S2).

Atomic force microscopy (AFM) was used to characterize changes in surface topography at the C18 SAM interface following exposure to the EB growth medium. Representative tapping-mode AFM images of a C18 SAM on gold acquired under ambient conditions before and after immersion in EB medium for up to 1 day are shown in Figure 2. These topographic images provide insight into the morphology of the protein layer during its formation. After only 10 min of exposure to EB medium, a substantial degree of surface coverage was observed. Following 1 day of exposure, the protein layer appears as a porous, mat-like film that completely covers the alkanethiol monolayer. AFM was also used to assess the thickness of the adsorbed layer and the possibility of multilayer formation over extended exposure periods. These AFM-based measurements were performed under a phosphate-buffered saline (PBS) solution to maintain complete hydration of the adsorbed protein layer. By applying sufficient force to the surface so as to penetrate and displace the soft layer of adsorbed cell culture media components, square trenches were fabricated using the tip of the AFM probe, as illustrated in Figure 3. These AFM tip-induced perturbations appear to behave similarly to those reported by Dupont-Gillain et al. in assessing strength and time dependent changes of albumin adsorption on SAMs.[25] Displacement of the adsorbed layer in this manner enabled the direct measurement of its thickness in a fully hydrated form. A summary of the protocol used for this analysis is provided in the Supporting Information. Cross-sectional height analyses of these 1 μm × 1 μm areas indicated a film step height of ~2.5 nm. This direct height measurement agrees closely with an optically obtained value of ~3 nm measured by ellipsometry (Supporting Information, Table S1). The observed layer thickness was the same for samples that were immersed in EB medium for periods of 2 and 24 h, suggesting that the adsorbate layer forms completely within 2 h and does not exhibit continued, multilayer adsorption. In addition, no tip-induced damage to the underlying C18 SAM was observed in images acquired directly after manipulation of the adsorbed protein layer.

The ATR-FTIR, SPR, and AFM studies illustrate how the methyl-terminated hydrophobic interfaces of C18 SAMs are altered chemically and morphologically upon exposure to the cell culture medium used to form EBs. To complement these experiments, we used contact-angle analysis to establish how cells and ultimately EBs respond to these changes. The wettability of a C18 SAM was assessed before and after exposure to EB formation medium for up to 1 day using the sessile drop method. This analysis indicates that the substrate-cell culture media interactions lead to a shift from hydrophobic to hydrophilic behavior, where the water contact angle is reduced from 108 to 5° (Figure 4). It is important to recognize, however, that the traditional sessile droplet technique might not provide the most accurate representation of what actually occurs during EB formation because the samples must be dried prior to analysis. During cell culture, the adsorbed protein layer remains hydrated and is covered by culture media throughout the duration of a typical experiment. Thus, to better simulate the interactions between the hydrophobic surfaces and the EB formation medium, the wetting behavior was reexamined using captive bubble contact-angle analysis. This technique is analogous to an inverse contact-angle measurement, where the surface of interest is immersed in an analysis solution (e.g., water or cell culture media). Surface wettability can subsequently be assessed based on the contact angle made by an air bubble placed on the sample surface. The C18 SAMs examined with the captive bubble method

exhibit a hydrophobic to hydrophilic transition, analogous to what we observed for the sessile drop experiment (Figure 4). However, in this case, the immersed C18 SAM surface appears to display superhydrophilic behavior, whereby the air bubble rolls off the sample. A real-time movie of this phenomenon is provided as Supporting Information.

Components of the culture medium used in EB formation are the building blocks that dictate this transition to superhydrophilicity. Cell culture media are often supplemented with plasma proteins in the form of fetal bovine serum, combinations of growth factors, small molecules, or other relevant biomolecules that may be required to properly sustain the cell line being cultured. In particular, plasma proteins rapidly adsorb onto synthetic materials in a biological environment, where the amount of adsorption, conformation, and composition of the adsorbed proteins is in part governed by the surface chemistry of the synthetic cell culture substrate.[22,23] The resultant adsorbed protein layer subsequently establishes a new cell culture interface with surface properties that may be distinct from the underlying substrate. This behavior is somewhat reminiscent of so-called “protein corona” that forms around nanostructures in physiologic environments, which has been demonstrated to alter nanoparticle–cell interactions.[26,27]

To assess the composition of the adsorbed protein layer on the C18 SAMs, we utilized multidimensional protein identification technology (MudPIT) mass spectroscopy.[28] Not surprisingly, a majority of the peptide fragments identified at the C18 SAM-cell culture medium interface were consistent with serum albumin. This component was observed regularly throughout the analysis of four distinct EB medium formulations that were either supplemented with fetal bovine serum or meant for serum-free cell culture (Supporting Information, Tables S2–S6). Because serum albumin, which is commonly employed to passivate substrates against cellular adhesion, is a primary component of fetal bovine serum used in cell culture, surfaces that readily adsorb this protein will resist cell attachment. In addition, the MudPIT study did not identify peptides corresponding to common extracellular matrix (ECM) proteins such as laminin, collagen, fibronectin, or fibrinogen among the top 10 sequences collected from the adsorbed layer (Tables S3–S6 in the Supporting Information). This trend held true for all EB formation media studied. The presence of these ECM components within the adsorbed layer, even at relatively low concentrations, could act as undesirable anchorage points for EBs through the so-called “albumin rescuing effects.”[29]

Although the adsorbed protein layer is weakly bound, it effectively leads to superhydrophilic behavior that prevents cells from binding to the underlying substrate. As illustrated in the captive bubble experiments, when the air bubble is forcibly pressed onto the sample it rolls off the analysis surface and falls back into solution. An EB floating in suspension can be thought to behave analogously to the air bubble, where under typical cell culture conditions an EB approaching the cell culture interface would be pushed back into suspension by the thin layer of water that continuously wets the superhydrophilic surface. The manner in which water molecules surrounding the adsorbed protein film prevent cell attachment during EB formation thus appears to resemble mechanisms based on prior theoretical and experimental works that describe how PEG-terminated SAMs remain biologically inert.[30–33] The presence of similar hydrated layers might also explain the poor performance of $–CH_3$-terminated materials in the cell binding assays of Tidwell, Lopez, and Faucheux.[23,34,35]

The superhydrophilic behavior exhibited by the adsorbed protein layer was observed during the course of monitoring C18 SAMs under simulated suspension culture conditions. While the height of the adsorbed layer does not change, it appears that the density of the film may increase over time, as noted by the increase in material displaced to the trench edges by the AFM tip in the 24 h sample (Figure 3). This apparent increase in density most likely results from reorganization of the proteins that compose the film. It is generally accepted that proteins undergo structural rearrangements at hydrophobic interfaces such as at the C18 SAMs used in this study.[36] Structural modifications to proteins adsorbed on to hydrophobic SAMs tend to be more dramatic and promote more conformational changes than those on hydrophilic SAMs, as noted by Ostuni et al.[37] These changes result in thinner adsorbed protein layers at hydrophobic interfaces due to the greater degree of denaturation and spreading. We suspect that these rearrangements are responsible for establishing the superhydrophilic interface. What is clear from the captive bubble studies is that we do not see samples display superhydrophilic characteristics until they have been immersed in EB formation media for at least 4 h. This observation would suggest that the adsorbed protein layer requires additional time to reorganize itself into a configuration that can achieve superhydrophilicity.

In summary, we have established that proteins present in standard stem-cell culture media formulations used for EB formation readily modify the surface properties of hydrophobic materials. This interaction produces a superhydrophilic cell culture interface that, in turn, contributes to the enhancements in EB-mediated stem-cell differentiation seen in our previous research. The results presented here clearly illustrate how the interplay between a synthetic substrate and the cell culture medium that bathes it can alter the physical and chemical properties of the environment in which stem cells are cultivated. Because PSCs are notoriously sensitive to environmental cues, stem-cell biologists are increasingly interested in utilizing chemically defined and serum-free media formulations in their studies to more rigorously control cellular behavior in vitro.[38] Our results indicate that culture media–substrate interactions are an important consideration when designing stem-cell culturing tools to be compatible with these defined conditions. Moreover, this study provides a set of design rules for developing synthetic materials that can be used for creating improved stem-cell culturing technologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work has been partially supported by the National Institutes of Health through Grant P01 GM081621 (B.D., W.R., and S.J.J.) and the Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA. S.J.J. also acknowledges the NIH Medical Scientist Training Program and the National Science Foundation NSF-IGERT program. Cell culture media was generously provided by Prof. Owen Witte and Prof. Hong Wu. We thank Dr. Bahram Valamehr, Dr. Julien Polleux, Eric Gschweng, and Nancy Lin for their helpful comments and technical support. We would also like to thank Prof. Yoram Cohen for access to the contact angle measurement system as well as the Nano and Pico Characterization Laboratory for AFM instrumentation.

References

1. Martin GR. Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells. Proc Natl Acad Sci U S A. 1981; 78:7634–7638. [PubMed: 6950406]
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic Stem Cell Lines Derived from Human Blastocysts. Science. 1998; 282:1145–1147. [PubMed: 9804556]
3. Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R, Clark AT, Plath K. Generation of Human Induced Pluripotent Stem Cells from Dermal Fibroblasts. Proc Natl Acad Sci U S A. 2008; 105:2883–2888. [PubMed: 18287077]
4. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell. 2007; 131:861–872. [PubMed: 18035408]
5. Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006; 126:663–676. [PubMed: 16904174]
6. Keller G. Embryonic Stem Cell Differentiation: Emergence of a New Era in Biology and Medicine. Genes Dev. 2005; 19:1129–1155. [PubMed: 15905405]
7. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in Vitro Development of Blastocyst-Derived Embryonic Stem Cell Lines: Formation of Visceral Yolk Sac, Blood Islands and Myocardium. J Embryol Exp Morphol. 1985; 87:27–45. [PubMed: 3897439]
8. Robertson, EJ. Teratocarcinomas and Embryonic Stem Cells: A Practical Approach. IRL Press; Washington, DC: 1987. p. xiip. 254
9. Kurosawa H. Methods for Inducing Embryoid Body Formation: In Vitro Differentiation System of Embryonic Stem Cells. J Biosci Bioeng. 2007; 103:389–398. [PubMed: 17609152]
10. Ng ES, Davis RP, Azzola L, Stanley EG, Elefanty AG. Forced Aggregation of Defined Numbers of Human Embryonic Stem Cells into Embryoid Bodies Fosters Robust, Reproducible Hematopoietic Differentiation. Blood. 2005; 106:1601–1603. [PubMed: 15914555]
11. Gerecht-Nir S, Cohen S, Itskovitz-Eldor J. Bioreactor Cultivation Enhances the Efficiency of Human Embryoid Body (Heb) Formation and Differentiation. Biotechnol Bioeng. 2004; 86:493–502. [PubMed: 15129432]
12. Dang SM, Gerecht-Nir S, Chen J, Itskovitz-Eldor J, Zandstra PW. Controlled, Scalable Embryonic Stem Cell Differentiation Culture. Stem Cells. 2004; 22:275–282. [PubMed: 15153605]
13. Carpenedo RL, Sargent CY, McDevitt TC. Rotary Suspension Culture Enhances the Efficiency, Yield, and Homogeneity of Embryoid Body Differentiation. Stem Cells. 2007; 25:2224–2234. [PubMed: 17585171]
14. Chapman RG, Ostuni E, Takayama S, Holmlin RE, Yan L, Whitesides GM. Surveying for Surfaces That Resist the Adsorption of Proteins. J Am Chem Soc. 2000; 122:8303–8304.
15. Ungrin MD, Joshi C, Nica A, Bauwens Cl, Zandstra PW. Reproducible, Ultra High-Throughput Formation of Multicellular Organization from Single Cell Suspension-Derived Human Embryonic Stem Cell Aggregates. PLoS One. 2008; 3:e1565. [PubMed: 18270562]
16. Lillehoj, P.; Nan, L.; Tsutsui, H.; Ho, CM. A Compact Microfluidic Continuous Flow Separator for Particle and Cell Sorting. Proceedings of the IEEE 21st International Conference on Micro Electro Mechanical Systems (MEMS); Tucson, AZ. Jan 13–17, 2008; Piscataway, NJ: IEEE Operations Center; 2008. p. 292-295.
17. Torisawa Y, Chueh B-h, Huh D, Ramamurthy P, Roth TM, Barald KF, Takayama S. Efficient Formation of Uniform-Sized Embryoid Bodies Using a Compartmentalized Microchannel Device. Lab Chip. 2007; 7:770–776. [PubMed: 17538720]
18. Bauwens CL, Peerani R, Niebruegge S, Woodhouse KA, Kumacheva E, Husain M, Zandstra PW. Control of Human Embryonic Stem Cell Colony and Aggregate Size Heterogeneity Influences Differentiation Trajectories. Stem Cells. 2008; 26:2300–2310. [PubMed: 18583540]
19. Hwang YS, Chung BG, Ortmann D, Hattori N, Moeller HC, Khademhosseini A. Microwell-Mediated Control of Embryoid Body Size Regulates Embryonic Stem Cell Fate Via Differential

Expression of Wnt5a and Wnt11. Proc Natl Acad Sci U S A. 2009; 106:16978–16983. [PubMed: 19805103]

20. Nguyen D, Sa S, Pegan JD, Rich B, Xiang G, McCloskey KE, Manilay JO, Khine M. Tunable Shrink-Induced Honeycomb Microwell Arrays for Uniform Embryoid Bodies. Lab Chip. 2009; 9:3338–3344. [PubMed: 19904398]
21. Valamehr B, et al. Hydrophobic Surfaces for Enhanced Differentiation of Embryonic Stem Cell-Derived Embryoid Bodies. Proc Natl Acad Sci U S A. 2008; 105:14459–14464. [PubMed: 18791068]
22. Roach P, Farrar D, Perry CC. Interpretation of Protein Adsorption: Surface-Induced Conformational Changes. J Am Chem Soc. 2005; 127:8168–8173. [PubMed: 15926845]
23. Tidwell CD, Ertel SI, Ratner BD, Tarasevich BJ, Atre S, Allara DL. Endothelial Cell Growth and Protein Adsorption on Terminally Functionalized, Self-Assembled Monolayers of Alkanethiolates on Gold. Langmuir. 1997; 13:3404–3413.
24. Lahiri J, Kalal P, Frutos AG, Jonas SJ, Schaeffler R. Method for Fabricating Supported Bilayer Lipid Membranes on Gold. Langmuir. 2000; 16:7805–7810.
25. Dupont-Gillain CC, Fauroux CMJ, Gardner DCJ, Leggett GJ. Use of AFM to Probe the Adsorption Strength and Time-Dependent Changes of Albumin on Self-Assembled Monolayers. J Biomed Mater Res, Part A. 2003; 67A:548–558.
26. Maiorano G, Sabella S, Sorce B, Brunetti V, Malvindi MA, Cingolani R, Pompa PP. Effects of Cell Culture Media on the Dynamic Formation of Protein–Nanoparticle Complexes and Influence on the Cellular Response. ACS Nano. 2010; 4:7481–7491. [PubMed: 21082814]
27. Walkey CD, Chan WCW. Understanding and Controlling the Interaction of Nanomaterials with Proteins in a Physiological Environment. Chem Soc Rev. 2012; 41:2780–2799. [PubMed: 22086677]
28. Yates JR, Ruse CI, Nakorchevsky A. Proteomics by Mass Spectrometry: Approaches, Advances, and Applications. Annu Rev Biomed Eng. 2009; 11:49–79. [PubMed: 19400705]
29. McClary KB, Ugarova T, Grainger DW. Modulating Fibroblast Adhesion, Spreading, and Proliferation Using Self-Assembled Monolayer Films of Alkylthiolates on Gold. J Biomed Mater Res. 2000; 50:428–439. [PubMed: 10737886]
30. Harder P, Grunze M, Dahint R, Whitesides GM, Laibinis PE. Molecular Conformation in Oligo(Ethylene Glycol)-Terminated Self-Assembled Monolayers on Gold and Silver Surfaces Determines Their Ability to Resist Protein Adsorption. J Phys Chem B. 1998; 102:426–436.
31. Ostuni E, Chapman RG, Holmlin RE, Takayama S, Whitesides GM. A Survey of Structure-Property Relationships of Surfaces That Resist the Adsorption of Protein. Langmuir. 2001; 17:5605–5620.
32. Wang RLC, Kreuzer HJ, Grunze M. Molecular Conformation and Solvation of Oligo(Ethylene Glycol)-Terminated Self-Assembled Monolayers and Their Resistance to Protein Adsorption. J Phys Chem B. 1997; 101:9767–9773.
33. Li L, Chen S, Zheng J, Ratner BD, Jiang S. Protein Adsorption on Oligo(Ethylene Glycol)-Terminated Alkanethiolate Self-Assembled Monolayers: The Molecular Basis for Nonfouling Behavior. J Phys Chem B. 2005; 109:2934–2941. [PubMed: 16851306]
34. Faucheux N, Schweiss R, Ļtzow K, Werner C, Groth T. Self-Assembled Monolayers with Different Terminating Groups as Model Substrates for Cell Adhesion Studies. Biomaterials. 2004; 25:2721–2730. [PubMed: 14962551]
35. Lopez GP, Albers MW, Schreiber SL, Carroll R, Peralta E, Whitesides GM. Convenient Methods for Patterning the Adhesion of Mammalian Cells to Surfaces Using Self-Assembled Monolayers of Alkanethiolates on Gold. J Am Chem Soc. 1993; 115:5877–5878.
36. Li L, Chen S, Jiang S. Protein Adsorption on Alkanethiolate Self-Assembled Monolayers: Nanoscale Surface Structural and Chemical Effects. Langmuir. 2003; 19:2974–2982.
37. Ostuni E, Grzybowski BA, Mrksich M, Roberts CS, Whitesides GM. Adsorption of Proteins to Hydrophobic Sites on Mixed Self-Assembled Monolayers. Langmuir. 2003; 19:1861–1872.
38. Nakagawa M, et al. A Novel Effcient Feeder-Free Culture System for the Derivation of Human Induced Pluripotent Stem Cells. Sci Rep. 2014; 4.

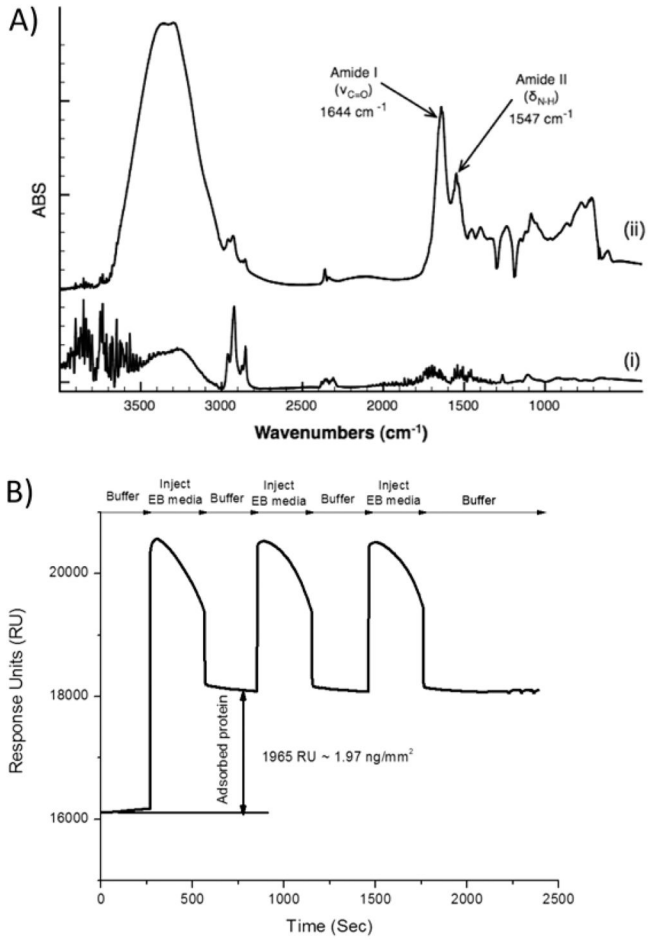


Figure 1.

ATR-FTIR spectra taken for: (i) a pristine C18 SAM substrate and (ii) after the C18 SAM was immersed for 3 days in a standard cell culture medium used to form EBs (A). The peaks noted at 1644 and 1547 cm^{-1} in the sample exposed to the EB formation medium are characteristic for amide I and amide II bonding, respectively. Surface plasmon resonance (SPR) sensogram showing the adsorption of cell culture media components onto a C18 SAM (B).

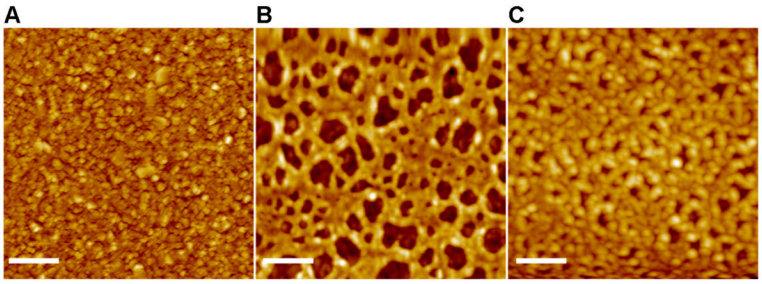


Figure 2.

Representative tapping-mode AFM height images of a freshly prepared C18 SAM (A) and after exposure to EB formation medium for 10 min (B) and 1 day (C). After exposure for 1 day, the adsorbed layer of cell culture media components completely covers the surface of the C18 SAM and possesses a porous mat-like appearance (scale bar = 200 μm).

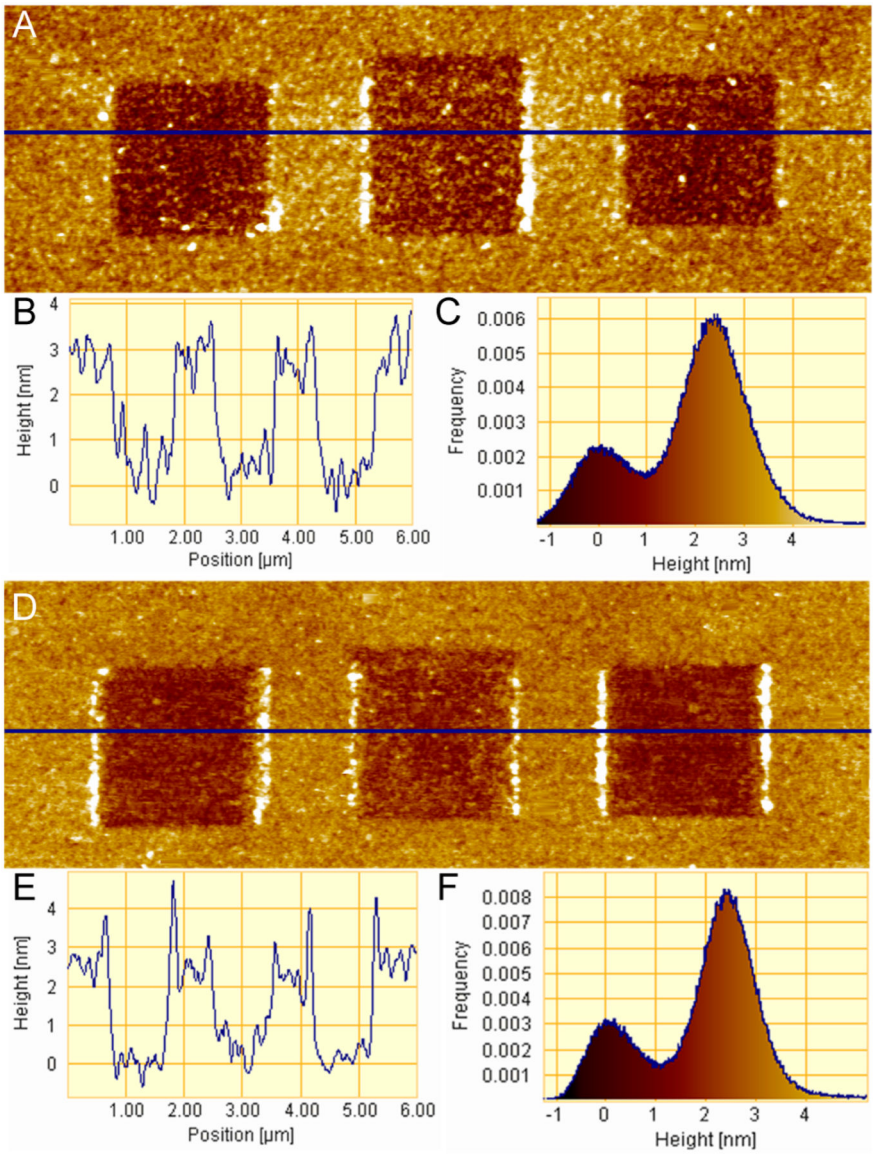


Figure 3.

Representative contact mode AFM height images, cross-sectional profiles, and histogram analyses used to characterize the thickness of the adsorbed protein layer. One μm × 1 μm square trenches were prepared by displacement of the adsorbed layer with the AFM probe under a loading force of ~3–5 nN to expose the underlying surface of C18 substrates that had been immersed in EB medium for 2 h (A) and 1 day (D). Normalized cross-sectional height profiles (B,E) and histogram analyses of pixel heights (C,F) indicate the layer of adsorbed proteins to be on the order of 2.5 nm, independent of exposure time.

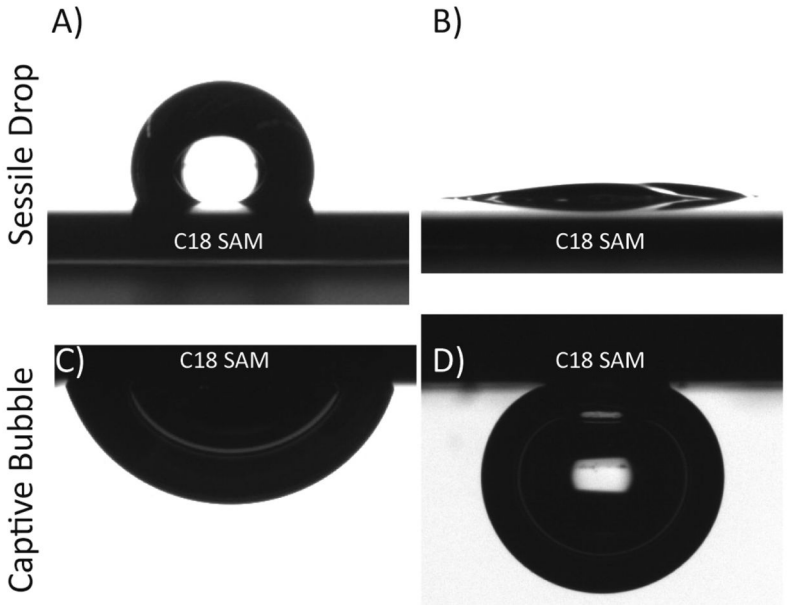


Figure 4.

Photographs of water droplets from sessile drop contact angle experiments on C18 SAM substrates before (A) and after (B) exposure to EB medium. Images from captive bubble contact angle analysis showing air bubbles on C18 surfaces completely immersed in either water (C) or EB medium (D) for 2 min.

Table 1

Contact-Angle Measurements Obtained for C18 SAMs Treated with EB Media

sessile drop θ_C (deg) [a]		captive bubble θ_C (deg)		
	EB media		EB media	
initial[b]	4 h[c]	initial[d]	2 min[e]	24 h[e]
108.3 ± 1.9°	5.1 ± 0.6°	103 ± 1.62°	34.1 ± 3.8°	<5°

[a] θ_C refers to the contact angle of water on the indicated C18 SAM surface.

[b] Contact angle of water droplet on a pristine C18 SAM surface.

[c] Contact angle C18 SAM that had been immersed in EB media for 4 h.

[d] Contact angle taken while a C18 SAM substrate was immersed in deionized water.

[e] Contact-angle measurement obtained while a C18 SAM was immersed in EB media for the indicated time.