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Interactions between Cells and Nanoscale Surfaces of Oxidized Silicon Substrates

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***Abstract*—**The importance for manipulating an incorporated scaffold and directing cell behaviors is well appreciated for tissue engineering. Here, we developed newly nano-topographic oxidized silicon nanosponges capable of being various chemical modifications to provide much insight into the fundamental biology of how cells interact with their surrounding environment in vitro. A wet etching technique is exerted to allow us fabricated the silicon nanosponges in a high-throughput manner. Furthermore, various organo-silane chemicals enabled self-assembled on the surfaces by vapor deposition. We have found that Chinese hamster ovary (CHO) cells displayed certain distinguishable morphogenesis, adherent responses, and biochemical properties while cultured on these chemical modified nano-topographic structures in compared with the planar oxidized silicon counterparts, indicating that cell behaviors can be influenced by certain physical characteristic derived from nano-topography in addition to the hydrophobicity of contact surfaces crucial for cell adhesion and spreading. Of particular, there were predominant nano-actin punches and slender protrusions formed while cells were cultured on the nano-topographic structures. This study shed potential applications of these nano-topographic biomaterials for controlling cell development in tissue engineering or basic cell biology research.

***Keywords*—**Nanosponge, Cell adhesion, Cell morphology

I. Introduction

THE necessity of cell-cell connection and cell-extracellular matrix (ECM) contact in vivo is known for formation of tissues and organs. A number of molecular mechanisms by which cells perceive and respond to the surrounding environment have been progressively unraveled include integrin-ligand interactions, influences of surface chemistry, the topographical effects of the matrix, etc. Of particular, cell adhesion to ECMs through integrin receptors plays a central role in numerous physiological and pathological processes. In this regard, utility of protein adsorbed material surfaces to provide and support cell attachment and architecture is important to many biomedical and biotechnological applications, such as tissue engineering [1,2]. Tissue engineering is a potential approach for restoring organ functionality and overcoming the shortage of transplantable organs. To achieve this, in fact, the inter-disciplinary principles of engineering and life sciences continuously aim on developing biological substitutes, typically composed of biological and synthetic components that restore, maintain, or improve tissue function in vitro.

An in vitro control of cellular environment at micro and nanoscale resolution is a challenge for tissue engineering. Traditional macroscale techniques have been limited in the ability to form spatially regulated patterns of molecules [3,4]. Most of in vitro studies have utilized plastic or glass surfaces for cell culturing, that the topography is not found in nature. The results may explain why differences are often found while comparing results from in vivo and in vitro studies. To resolve the aforementioned difficulties, micro and nano-engineering approaches have been employed to generate in vitro gradients of molecules on substrates to mimic natural environments. Since the importance of adhesion in directing cell properties and functions, engineering of surfaces that prevent or enhance cell adhesion represents an important issue for biomaterials research [5-7].

Numerous micro and nanoengineering approaches have been used to manipulate cell-substrate interactions in vitro through presenting specific molecules to cells. In principle, these studies are divided into the responses of cells interaction with varied substrate topographies or in the presence of a chemical contrast along a substrate. For example, self assembled monolayers (SAMs) of alkanethiols have been shown to affect the capacity of action potential of neurons [8]. Coating chemicals or proteins on defined areas allow us to study how cells adhere to specific substrates or/and topography [9], such as engineered fibronectin (FN) adsorption/conformation accompanying with different functional chemistries enables regulating cell adhesion and gene expression [10,11]. Moreover, nanoscale topography with engineered surfaces can affect protein adsorption, cell adhesion and spreading [12-15]. To be noted, in fact, the topographic scale of adhesive protein molecules such as fibronectin, laminin, and collagen fibers is on the order of roughness [12,13]. Other nanoengineered surface, such as porous silicon, has already been used to investigate the biocompatibility in the tissues of rat eyes [16], providing another biomaterial suitable for investigating the interactions between cells and

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biomimicking surfaces. Herein, we generated silicon nanosponge structures coated with different functional groups using the vapor deposition technique in order to investigate cell behaviors on bio-mimetic substrates in an in vitro condition [17,18].

In this study, we used silicon nanosponge structures with various functional group chemicals for the study of cell interaction with substrate. Cell adhesion, organization of cytoskeleton, and biochemical changes are examined and compared among cells responding to varied nanosponge structures and functional chemicals. Our results suggest that the effect of nanometer-size topographical features is similar to ECMs, which may provide a biomimic in vitro environment to facilitate a better manipulation and understand of cell behaviors resemblance with those in in vivo environment.

II. Materials and Methods

A. Surface Treatment of Oxidized Silicon Substrates

Nanosponges are fabricated on monocrystalline silicon wafers using Ag-nanoparticles (AgNPs) assisted etching as shown in Fig. 1 [19-21]. After the etching process, the nanosponges and silicon wafers were oxidized with 20 nm thick SiOx to enrich the biocompatibility and cut into pieces with 1 cm square in size suitable for using in the tissue culture wells. The organo-chloro-silane chemicals used for surface treatment are either hydrophilic or hydrophobic. For hydrophilic surface treatment, highly pure (> 97%) 3-aminopropyltrimethoxysilane (APTMS) with amino group (NH_2) was obtained from Alfa Aesar (Ward Hill, MA). On the other hand, highly pure (> 96%) perfluorodecyltrichlorosilane (FDTS) with fluorine (F) was used for hydrophobic surface treatment (Alfa Aesar, Ward Hill, MA). Surface treatment began with oxygen plasma for surface hydroxylation, followed by vapor deposition of silanol groups from the chemical precursors. A molecular layer is self-assembled on the surface via silanol-hydroxyl reaction. The reaction times required are 15 minutes and 1 hour for FDTS and APTMS, respectively. Overall, six different substrates in combination with nano-topography and chemical modifications include pristine SiOx substrate, pristine oxidized nanosponge, SiOx substrate with FDTS, oxidized nanosponge with FDTS, SiOx substrate with APTMS, and oxidized nanosponge with APTMS.

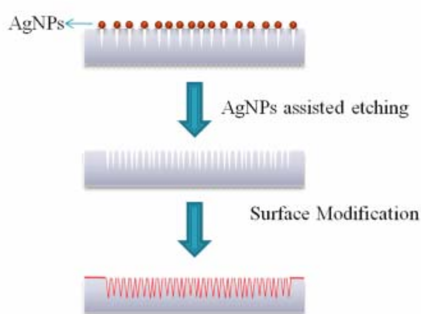

Fig. 1 Fabrication process of the substrates.

B. Surface Hydrophobicity

The surface hydrophobicity of substrates was characterized using contact angle measurement. The contact angle meter MD 100SB (Sindatek Instruments Co., Taiwan) analyzed droplets of deionized water placed on the substrates and the software MagicDroplet V1.0d calculates the contact angle of the droplets (i.e. the hydrophobicity.)

C. Cell Culture

Chinese hamster ovary (CHO) cells were cultured in normal tissue culture T25 flasks or 100-mm plates (BD) with Ham's F-12 medium (Invitrogen molecular probes, CA) containing 10% fetal bovine serum (FBS; Sigma) and supplementing with 1% glutamine as well as 1% penicillin and 1% streptomycin. Cells were maintained at 37 ℃ in a humidified 5% CO_2 incubator. Cells were trypsinized with trypsin-ethylenediaminetetraacetic acid (EDTA) and replated on the aforementioned six different substrates resided in 6-well or 12-well cultured plates for 2 hours to perform cell adhesion and fluorescent staining experiments as indicated. Prior steam sterilization of these substrates is undertaken while they are subjected to cell culture.

D. Scanning Electron Microscopy

The morphology of CHO cells adhered onto the different substrates was visualized by the scanning electron microscope S-4300 (Hitachi, Japan). Briefly, the adhered cells were at first fixed in a fixative containing 2.5% glutaraldehyde / 0.1M PBS at 4 ℃ for 30 minutes. After washing twice with 0.1M PBS, the samples were postfixed with 1% osmium tetroxide (Sigma) at room temperature for 30 minutes. Then, the samples were washed twice with PBS, dehydrated through a serial gradients of ethanol at ten minutes for each gradient, and finally dried out by the critical point dryer HCP-2 (Hitachi, Japan). At last, the substrates were sputtered with gold and examined under the SEM.

E. Laser Scanning Confocal Microscope

Immunofluorescent staining was performed according to previous description with few modifications [22]. Briefly, CHO cells were allowed for adhering onto the different substrates for two hours and following washed twice with PBS. Then, cells were fixed with fresh 4% paraformaldehyde for 15 minutes at room temperature. After washing with PBS, the substrates were immersed with 0.1% Triton X-100 in PBS for 10 minutes to permeabilize cell membranes. Non-specific binding sites were blocked by incubating with 1% bovine serum albumin (BSA; Sigma) for 30 minutes at room temperature. Rhodamine conjugated phalloidin (Invitrogen molecular probes, CA) was used for staining actin filaments, and 4',6-diamidino-2-phenylindole (DAPI; Invitrogen molecular probes, CA) was used for staining DNA in nuclei. After mounted on coverglass slides, all fluorescence images were acquired with a laser scanning confocal microscope (Zeiss LSM

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510 META, Germany), equipped with 10X eyepiece and 100X oil (NA 1.4) objective lens.

F. Cell Adhesion Assay

CHO cells of density of fifty thousand per milliliter were seeded on the aforementioned substrates for various adhesion time intervals as indicated (i.e. 30, 60, 120, and 240 minutes). The substrates were washed twice with PBS after a specific duration of cell culture. The adhered cells were harvested with trypsin-ethylenediaminetetraacetic acid (EDTA) and the cell number was counted using a hemocytometer.

G. Western Blotting

Briefly, cell lysates were collected after CHO cells were replated for two and half hours on the different substrates with or without FDTS or APTMS, and then equal amounts of whole cell lysates were resolved on SDS-PAGE followed by Western analyses using anti-FAK, anti-phospho-Tyr397, anti-phospho-925, and anti-actin antibodies, respectively.

III. RESULTS

A. Surface Properties of Substrates

The surface properties of six different substrates, those are pristine SiO_x wafer, pristine oxidized nanosponge, SiO_x wafer with FDTS, oxidized nanosponge with FDTS, SiO_x wafer with APTMS, and oxidized nanosponge with APTMS, were characterized by contact angle measurement. The contact angles are 29 ± 2 degree, 1 ± 1 degree, 110 ± 3 degree, 148 ± 4 degree, 41 ± 2 degree and 2 ± 1 degree, respectively. Accordingly, FDTS gives rise to a high contact angle on both SiO_x wafer and oxidized nanosponge, suggesting a hydrophobic property on these modified surfaces in contrast to the APTMS remaining a low contact angle on both surfaces. Interestingly, we found that both of the pristine and APTMS coated nanosponges confer to an extreme low contact angle. These observations are consistent with the Gibbs surface energy theory, in which the surface physical properties could be enhanced by nanostructures [23-25]. For instance, an increased hydrophobicity or hydrophilicity of a surface can be achieved by roughening its surface in related to the corresponding characteristic. In accordance with the above theory, the results showed the increase of hydrophilicity on the pristine oxidized and APTMS coated nanosponges in compared with the SiO_x wafer counterparts. Likewise, the hydrophobicity of FDTS is augmented on the oxidized nanosponge.

B. Morphologies of CHO cells on different substrates

To characterize how cells interact with various SiO_x surfaces at a nanoscale level, CHO cells were cultured on 1 cm square SiO_x wafers and oxidized nanosponges with or without surface modifications manufactured as described in Materials and Methods (Fig. 1). After 4 hours re-plating, the morphological appearance of adhered CHO cells on these different substrates was obtained by scanning electronic microscopy (SEM) as shown in Fig. 2. In comparison to normal culture dishes or glasses (data not shown), the morphology of CHO cells on pristine SiO_x wafers shows the similarity with 20~30 μm in size (Fig. 2a). Intriguingly, there are long and radial nanospikes stretched out from the cell edges prominently visualized as well as obvious rounded-up appearance when CHO cells were cultured on the pristine nanosponges as shown in Fig. 2b. In contrast, in Fig. 2c and 2d, while CHO cells were cultured on the SiO_x wafers with FDTS or the oxidized nanosponges with FDTS, nanospikes were no longer visualized despite these cells remained a rounded-up shape with relatively smaller sizes. As for the SiO_x wafers with APTMS and the oxidized nanosponges with APTMS, CHO cells appeared extended spreading on these surfaces with no or shorter nanospikes, respectively, as shown in Fig. 2e and 2f. Eventually, cells enabled spreading to 100 μm and even larger on the SiO_x wafers with APTMS (see Fig. 2e) and this effect is attributed by the chemical property of APTMS. The reason is derived from the observation of that CHO cell on the oxidized nanosponges with APTMS are spreading as well as on the pristine SiO_x wafers, obviously differing from those on the oxidized nanosponges with or without FDTS (see in Fig. 2b and 2d).

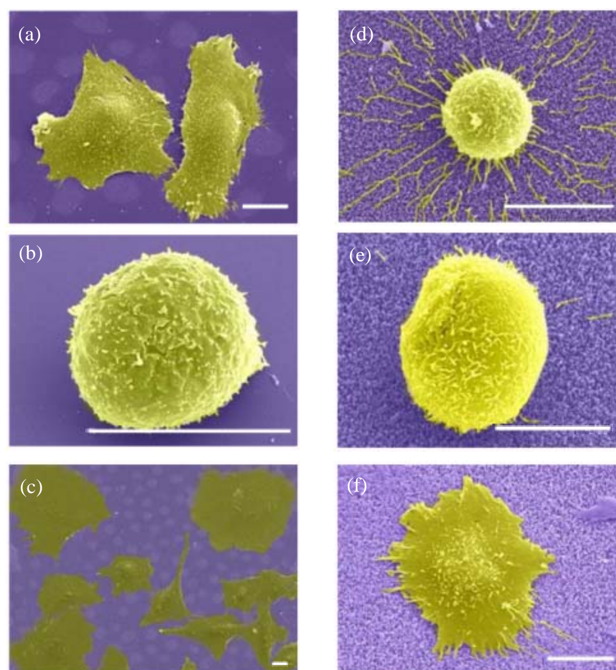

Fig. 2 SEM morphologies of CHO cells cultured on functionalized substrates after re-plating cells for 2 hours on the below substrates: (A) Pristine oxidized silicon substrate; (B) FDTS-coated oxidized silicon substrate; (C) APTMS-coated oxidized silicon substrate; (D) Pristine oxidized silicon nanosponge; (E) FDTS-coated oxidized silicon nanosponges; (F) APTMS-coated oxidized silicon nanosponges. All scale bars are 10 μm

C. Cytoskeleton of cells on different substrates

The effect of substrate topography on cytoskeleton orientation and focal adhesion formation has been correlated to the shape and adhering nature of cells in response to specific extracellular environments [10]. In light of distinguishable morphologies as described above, promptly, we then examined

the actin cytoskeleton organization of CHO cells on the aforementioned different surfaces by immunofluorescent staining using Rhodamine-conjugated phalloidin as described in Materials and Methods. As shown in Fig. 3, the distribution of actin filaments (stress fibers) were profoundly across the centers of cell bodies while cultured on the pristine SiO_x wafers, which resembles those on ECM coated glass coverslips (data not shown). This result is consistent with the similar spreading morphology of CHO cells on the pristine SiO_x wafer and ECM coated plates/coverslips. On the other hand, we found that the F-actin was mainly clustered on the small punches which might represent the contact sites of cell-extracellular nanoscale surfaces while CHO cells were cultured on the pristine oxidized nanosponges (Fig. 3b). In addition, we observed that cortical actin bundles predominantly appeared on the peripheries of cells, which consistently representing a less-spreading and rounded-up morphology. Collectively, these results implicate a potential restriction of actin polymerization in an opposite direction while cells encounter with nanoscale contact sites on the oxidized nanosponges rather than a flatten actin fiber network connected by a 70° angle at the leading edge of adhered cell membrane on the planar surface of the SiO_x wafer [26,27].

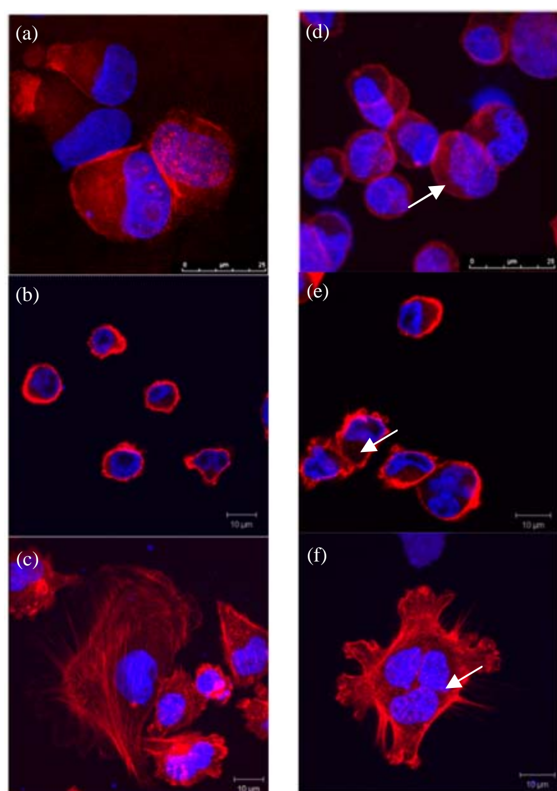

Fig. 3 Laser scanning confocal microscope images of the cytoskeleton and nuclei of cells cultured on functionalized substrates: (A) Pristine oxidized silicon substrate; (B) FDTS-coated oxidized silicon substrate; (C) APTMS-coated oxidized silicon substrate; (D) Pristine oxidized silicon nanosponge; (E) FDTS-coated oxidized silicon nanosponges; (F) APTMS-coated oxidized silicon nanosponges. The arrows in D-F indicate F-actin clustering into small punches

To be noted, this restriction of actin polymerization in an opposite direction against the nanoscale contact sites is not influenced by the surface chemistry since it remained markedly displayed in the cells adhered on the oxidized nanosponges coated with either APTMS or FDTS (Fig. 3f) in comparison with the SiO_x wafers (Fig. 3c-e). Taken together, the observations resulted from cells interaction with the nanoscale contact sites of our oxidized nanosponges provide novel insights on biomimicking *in vivo* cell-ECM interaction, which might differ from general cells-planar surface interactions using at present in *in vitro* cell culture conditions.

D. Cell adhesion on different substrates

To investigate the characteristic of the different substrates on cell adhesion, we performed cell adhesion assays by replating CHO cells on these surfaces for a serial point of time, such as 30, 60, 120, and 240 minutes, and then washing with PBS. The measurement of adhered cells was proceeded by harvesting the retained cells on the wafers and counting them under a microscope. As shown in Fig. 4, cell adhesion is increased in association with a prolonged time period after replating as expected. And, the least cell adhesion displayed on the SiO_x wafers with FDTS in comparison with other substrate surfaces. Interestingly, as the replating time lasted to four hours, the cell adhesion on SiO_x wafers with FDTS suddenly augmented, which might be due to that cells enable secreting ECM proteins to their surrounding extracellular fluid and surface, of which provide adherent substances and thereby enhance adherent strength. On the contrary, APTMS coated surfaces exhibited enhanced cell adhesion on the SiO_x wafer but moderate or not on the oxidized nanosponges in comparison with their pristine counterparts, suggesting the physical property resulted from the oxidized nanosponges effectively affecting cell adhesion than the chemical effect. As a matter of fact, we found that the oxidized nanosponges give rise to a lesser adhesion for CHO cells regardless the chemical property of the adhering surface, which empowers our above hypothesis.

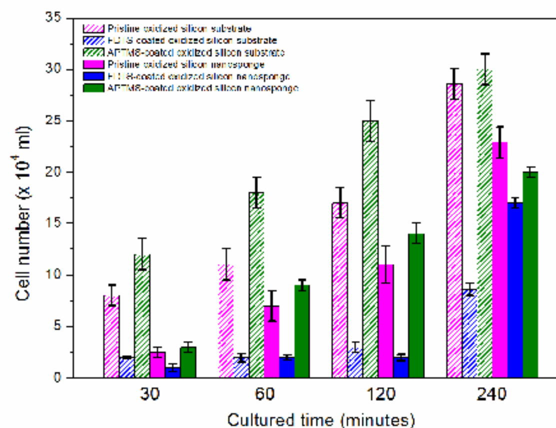

Fig. 4 The relationship between the detached cell number and the culture time on arious functionalized substrates. Cell adhesion increased with respect to a prolonged re-plating period, as expected

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E. Biochemical insights

Focal adhesion kinase (FAK) is a 125KDa cytoplasmic tyrosine kinase that plays a key role in integrin-mediated signaling transduction pathways in response to cell adhesion, migration, proliferation and so on [28-30]. Upon integrins engagement with ECM molecules, FAK is translocated to focal contacts and autophosphorylates on Tyr-397 [29,30]. The activation of FAK is followed by its association with a number of SH2 (Src homology 2) domain-containing signaling proteins including Src family kinases [31-34], p85 regulatory subunit of PI3K [35], phospholipase C-γ [36] and Grb7 [37]. The formation of the FAK/Src complex has been demonstrated to allow phosphorylation of Y925 of FAK by Src, which binds to the SH2 domain of Grb2 and relays FAK signaling to MAP kinase cascade by which FAK enables regulating cell cycle progression [33].

To further explore the interaction between CHO cells and the different chemical modification substrates, we attempted to employ biochemical analyses to explain the cellular phenotypes we have observed above. As shown in Fig. 5, focal adhesion kinase (FAK), a key signaling protein tyrosine kinase downstream of cell-ECM adhesion receptor family called integrins, was subjected to examining its phopshorylation status on Tyr-397 and Tyr-925, respectively. Our results revealed that the phospho-Tyr-397 of FAK significantly elevated on the pristine SiO_x wafer, nanosponge, and SiO_x wafer with APTMS. Unexpectedly, the phoshpo-Tyr-397 was not seen on the oxidized nanosponges with APTMS. As mentioned previously, it is well-known that the increase of phospho-Tyr-397 of FAK is associated with activation of integrins in response to cell adhesion with ECM molecules [29,30]. However, its levels were moderate or absent on SiO_x wafer with APTMS or oxidized nanosponges with APTMS although cells were extensively spreading on these APTMS surfaces as described above. Therefore, we hypothesize that cell adhesion mediated by APTMS might be independent of integrins. On the other hand, the elevated phospho-Tyr-925 was only seen on the pristine SiO_x wafer but not on other surfaces, suggesting that nanosponges might be unable to promote cell proliferation although the ability of cell survival on nanosponges showed similar to that on the pristine SiO_x wafers (data not shown).

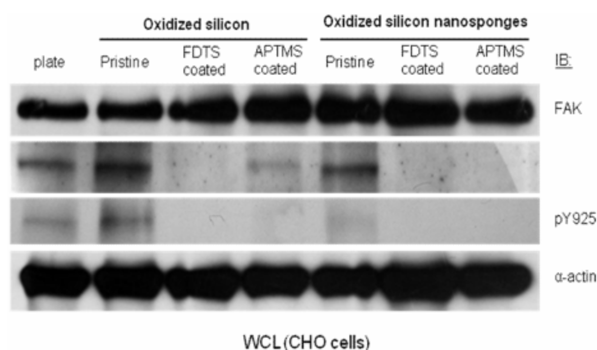

Fig. 5 Biochemical analyses of focal adhesion kinase (FAK) on the various functionalized substrates. CHO cells were re-plated on the various functionalized substrates for 2.5 hours, and lysates were collected for western blotting using antibodies as indicated

The phospho-Tyr397 and phospho-Tyr925 were detected to determine the potentially activated signaling events related to cell adhesion and cell proliferation. Total FAK and actin blots were used as controls.

IV. Discussions

Microenvironmental cues direct a number of functionalities for cells, such as adhesion, growth, survival, proliferation, migration, differentiation, cell death and so on. Through physical interactions between cells and these surrounding cues/surfaces, some changes in morphology, biochemistry, and physiology are occurring to adapt themselves into an appropriate situation and behavior in the presence of specific extracellular conditions. Here, we have generated and demonstrated a bio-mimic 3D oxidized nanosponge surface to allow cells adhered and maintained on it. In addition, we are able to modify the chemical property of nanostructure in terms of the vapor deposition technique to lead to a denser and durable outcome as well as remain *per se* surface nanostructure. By analyzing cell morphological appearance, biochemical changes, and functional characteristic among the SiO_x wafers and the oxidized nanosponges in combination with FDTS or APTMS chemical modification, we depict the merits of the oxidized nanosponges for understanding the responses of cell in encountering with nanoscale surfaces and bio-mimicking the *in vivo* nanoscale environment for bioengineering applications.

Filopodia are slender, fingerlike membrane projections that function as antennae for cells to probe their environment. Therefore, the filopodia were stretched out from the edge to finally form adherent structures. These filopodia-like structures are markedly visualized when cells cultured on pristine oxidized nanosponges as shown in Fig. 2d, it is consistent to the aforementioned contact guidance hypothesis. However, they were no longer seen on substrates coated with FDTS with a relatively rounded cell shape. Numerous studies investigate how the surface wettability and charge may influence cell adhesion, shape and spreading [38-40]. For example, enhanced cell spreading on hydrophilic surfaces relative to hydrophobic surfaces has been observed by these studies. In Fig. 2, the cellular morphology indeed is varied with surface wettability but seems no direct relationship with the topography of the substrates, that is planar- versus nano-surface. When substrates are coated with same functional group, the cell shape on different topography is quite similar. However, as there is no chemistry on the substrates, the surface topography will influence the cell behavior. (Fig. 2a and 2d). These data implicate the chemical properties of contact surfaces will also influence cellular behaviors, at least cell adhesion.

Previous reports demonstrated while cells adhere on anisotropic structures, most of cells display elongated and preferentially follow along the structures [41-43]. This phenomenon is called "contact guidance" in different cell types, such as macrophages, epithelia, fibroblasts and neuronal cells

[41-43]. The chemical nature of the substrate is also important for eliciting contact guidance as a result of that cells cultured on substrate with different chemical compositions showed similar responses to topography [41]. In contrast, contact guidance was shown to be independent of surface chemistry but only affected by the architectures of the substrate in certain studies [44]. In agreement with this, these studies indicate that contact guidance are depending on the length scale of topographical features of the cell culture substrates [45,46]. In our studies, we found that cell adhesion on the oxidized nanosponges was enhanced in the presence of APTMS coated on the surface in compared with the pristine oxidized nanosponges. However, we have noted that the overall cell adhesion on the APTMS coated nanosponges was independent of integrins due to the phospho-Tyr-397 was not increased. In this regard, we also observed the actin stress fibers were less formed on the cells adhered onto the APTMS coated nanosponges. Instead, a number of contact punches between cell membrane and the nanoscale surfaces of the oxidized nanosponges were predominantly seen, suggesting that the physical topographical features of the oxidized nanosponges are mainly attributed for this "contact guidance". Currently, the physical characteristic of the surface of the oxidized nanosponges affecting the contact guidance is under investigating. Intriguingly, the nanoscale surface of the oxidized nanosponges may provide an *in vivo*-mimicking surface for understanding the interactions between cells and ECMs.

One of major challenges in developing tissue engineered devices is how to culture cells on synthetic materials *in vitro* along with maintaining their differentiated properties and functions. In our preliminary observations, cells often maintained in a quiescent state (BrdU lack) and easily exhibited as round-up aggregates while cultured on the pristine oxidized nanosponges (unpublished observations). Therefore, it is probably used for a replacement of complicated setting for bioengineering scaffolding and stem cell research in the future by employing the photolithography and vapor deposition techniques.

In summary, differential responses of CHO cells on the various SiO_x wafers are described as followings: First, cell adhesion is mainly determined by the surface hydrophobicity of surfaces although cells tend to adhere to hydrophilic surfaces rather than hydrophobic ones. Surface hydrophobicity primarily depends on the functional groups of the surfaces while the surface nano-topography pushes the surface hydrophobicity to the extremity. Second, a distinguishable morphology, flatten versus rounded, has been seen on either the planar SiO_x wafers or the oxidized nanosponges, respectively, which is corresponding to the central stress fibers occurred in the hydrophilic surfaces (i.e. pristine SiO_x, APTMS coated SiO_x, wafers, and APTMS coated oxidized nanosponge) rather than the hydrophobic ones with much cortical stress fibers (i.e. FDTS coated SiO_x wafers and the oxidized nanosponges). Interestingly, the pristine oxidized nanosponge surface remained some extent of rounded shape with significant cortical stress fibers and detectable central stress fibers, suggesting an extended regulation of cell morphology by a combination of physical and chemical characteristic. Additionally, the oxidized nanosponges give rise to some marked cytoskeletal organizations displayed as nano-actin punches and slender actin fiber protrusions from upper cell surfaces, whose dimension is similar to the nanorods of the oxidized nanosponges, onto the nano-contact sites between cells and extracellular surfaces (i.e. various types of ECMs). Third, both of the SiO_x wafers and nanosponges can provide an environment for cell survival. In agreement with this observation, the activation of FAK (i.e. phospho-Tyr397), a key regulator for promoting cell proliferation in response to cell adhesion, is elevated while CHO cells are cultured on both the SiO_x wafers and nanosponges. Moreover, the phospho-Tyr925 of FAK, which is an indicator for cell cycle progression upon FAK activation (i.e. phospho-Try397) by which FAK signaling enables relaying onto MAPK cascade (mitogen activated protein kinase) is obviously augmented on both the pristine SiO_x wafers and the oxidized nanosponges.

V. Conclusion

Our newly developed nano-topographic oxidized nanosponges provide a more flexibility to allow us in manipulating cell morphogenesis and understanding of biochemical, physiological and functional responses of a cell in a biomimicking environment *in vitro*. Of particular, we also unravel the close relationships for cell adhesion and the hydrophobicity as well as cell morphological regulation and nano-topography of contact surfaces. Accordingly, an anticipation on the potential utilization of this nano-topographic oxidized nanosponges on bioengineering could benefit for complex tissues manipulations and more biomimic.

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