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The cytoskeletal organization of breast carcinoma and fibroblast cells inside three dimensional (3-D) isotropic silicon microstructures

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ABSTRACT

Studying the cytoskeletal organization as cells interact in their local microenvironment is interest of biological science, tissue engineering and cancer diagnosis applications. Herein, we describe the behavior of cell lines obtained from metastatic breast tumor pleural effusions (MDA-MB-231), normal fibrocystic mammary epithelium (MCF10A), and HS68 normal fibroblasts inside three dimensional (3-D) isotropic silicon microstructures fabricated by a single-mask, single-isotropic-etch process. We report differences in adhesion, mechanism of force balance within the cytoskeleton, and deformability among these cell types inside the 3-D microenvironment. HS68 fibroblasts typically stretched and formed vinculin-rich focal adhesions at anchor sites inside the etched cavities. In contrast, MCF10A and MDA-MB-231 cells adopted the curved surfaces of isotropic microstructures and exhibited more diffuse vinculin cytoplasmic staining in addition to vinculin localized in focal adhesions. The measurement of cells elasticity using atomic force microscopy (AFM) indentation revealed that HS68 cells are significantly stiffer ($p < 0.0001$) than MCF10A and MDA-MB-231 cells. Upon microtubule disruption with nocodazole, fibroblasts no longer stretched, but adhesion of MCF10A and MDA-MB-231 within the etched features remained unaltered. Our findings are consistent with tensegrity theory. The 3-D microstructures have the potential to probe cytoskeletal-based differences between healthy and diseased cells that can provide biomarkers for diagnostics purposes.

1. Introduction

Cell cytoskeleton is a highly dynamic polymeric network which defines the cell shape and its mechanical rigidity [1]. Any change in the cytoskeletal structure can affect the interaction of cells with their surrounding microenvironments [2]. Biological events in normal cells such as embryonic development, tissue growth and repair, and immune responses as well as cancer cell motility and invasiveness are dependent upon or regulated by cytoskeletal reorganization and the biomechanical properties of the cytoskeleton [1,3–5]. Understanding how the cell cytoskeleton reorganizes during its interaction with the surrounding environment is a fundamental biological question with applications to tissue engineering [6] and cancer diagnosis and therapy [7].

The extracellular matrix (ECM) proteins *in vivo* form a complex and textured interconnecting network and a (3-D) surface topography [8,9]. Cells are exposed to several mechanical, chemical and three dimensional topographical stimuli, which modulate their behaviors such as migration, growth, and adhesion. With advances in micro- and nano-fabrication technology, researchers have been able to create substrates comprised of precise micro- and nano-topographical and chemical patterns in order to mimic more *in vivo* microenvironments for biological and medical applications. These studies have provided valuable information on several cellular processes such as migration [10–12], cytoskeletal organization [12,13], contact guidance [14–16] and differentiation [17] on the proposed micro- and nano-environments. However, many of the previous approaches have relied on using either microstructures comprising anisotropic geometries (grooves) [18–21], or polymeric fibrous networks [22]. It is well known that 3-D microenvironments influence cell functions to a great extent and are different from standard two-dimensional (2-D) culture environments [23,24].

Our group recently reported the development of 3-D silicon microstructures which comprised of curved isotropic surfaces to characterize and compare the growth and adhesion behavior of normal fibroblast and metastatic human breast cancer cells [25,26].

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The isotropy of the curved surfaces determined by an approximately constant curvature is an excellent characteristic of our microstructures since it can be used for understanding the mechanism of deformation, adhesion and force balance within the cytoskeleton of different cells. This eliminates variability in the cell behavior introduced by the geometrical anisotropy of the microstructures. In this paper, by building upon our previous work, we are interested in studying the biological behavior of the cell lines obtained from metastatic breast tumor pleural effusions, normal fibrocystic mammary epithelium, and normal fibroblasts inside 3-D isotropic microstructures. Breast carcinoma frequently originate with cells that normally line the milk ducts within the mammary gland [27]. It is also well known that inside the body fibroblast cells are intimately embedded within the breast microenvironment [28]. In invasive breast carcinoma, the tumor cells fill the duct, and the basement membrane, which normally separates the ductal epithelial cells from the stromal elements, primarily the fibroblasts, is disrupted resulting in close juxtaposition of carcinoma cells and the fibroblast cells of the breast stroma. This structural reorganization constitutes a critical pathobiological transition leading to disease progression [29].

Herein, we present the detailed cytoskeletal organization and adhesion mechanism of normal breast epithelial cells, metastatic breast cancer cells and fibroblast cells, three key cellular components embedded in any breast tumor microenvironment [28], inside the 3-D silicon microstructures. The role of actin cytoskeleton in the cell adhesion behavior was established in our previous study [25]. In this work we explored the contribution of the cellular elasticity, focal adhesion complexes, and microtubules on the adhesion characteristics of the cells inside the isotropic (curved) 3-D microstructures. The role of microtubules in fibroblasts behavior on 2-D rigid surfaces and 3-D collagen matrices has been addressed before [30], but is relatively understudied in human breast cancer cells, where they might significantly impact pathological cell behaviors such as adhesion, migration and metastasis. We used atomic force microcopy (AFM) indentation to quantitatively measure cellular elasticity.

The results of the current research can provide important diagnostic and prognostic markers unique to the tumor, which could ultimately be used to develop new tools for the detection and treatment of breast cancer.

2. Materials and methods

2.1. Silicon device fabrication

Fig. 1(a, b) shows the photo image of the fabricated microdevice and the corresponding Scanning Electron Microscopy (SEM) images. The fabrication process of 3-D silicon microstructure is similar to our previous work. It relies on the application of reactive ion etching (RIE) lag and its dependence on geometrical patterns of the photomask layout to etch silicon to different depths [25]. Briefly, the fabrication process was started by depositing 8000 Å-thick plasma enhanced chemical vapor deposition (PECVD) oxide layer on a silicon wafer. After spinning and patterning photoresist, the oxide layer was etched for 3 min using deep reactive ion etching (DRIE) CH_4/C_4F_8 plasma. Next, silicon was etched using DRIE SF_6 plasma to form complex arrays of features composed of star- and circular-shaped microchambers. After removing photoresist, the oxide layer was subsequently removed using DRIE. As shown in the SEM images of the microchambers, the depth of the microchambers varies between 60 and 70 μm and the width ranges between 150 and 170 μm. It is notable that the described fabrication technique provides localized rough edges on the curved sidewalls and on the bottom surface of the microchambers. In the star

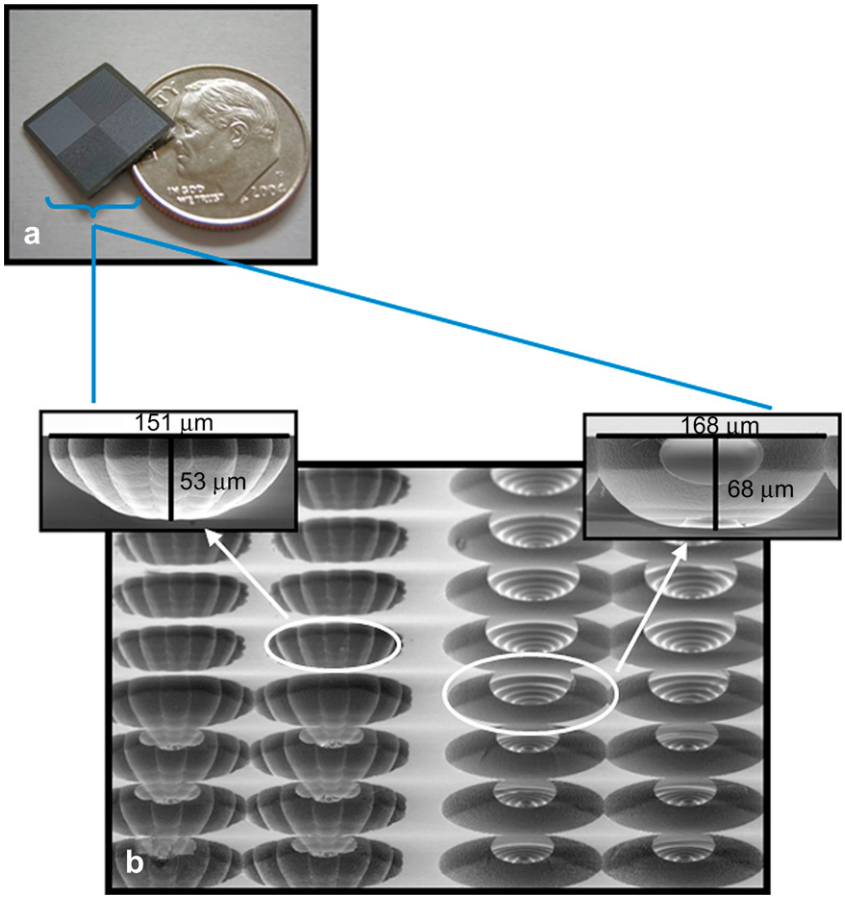

Fig. 1. (a) Photo image of the fabricated devices in silicon. (b) SEM images of the 3-D silicon microstructures comprising star- and circular-shape microchambers. With the fabrication technology utilized, scalloped edges can be formed on the curved sidewalls and the bottom surface of the microchambers.