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Investigating the effect of cell substrate on cancer cell stiffness by optical tweezers

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ABSTRACT

The mechanical properties of cells are influenced by their microenvironment. Here we report cell stiffness alteration by changing the cell substrate stiffness for isolated cells and cells in contact with other cells. Polydimethylsiloxane (PDMS) is used to prepare soft substrates with three different stiffness values (173, 88 and 17 kPa respectively). Breast cancer cells lines, namely HBL-100, MCF-7 and MDA-MB-231 with different level of aggressiveness are cultured on these substrates and their local elasticity is investigated by vertical indentation of the cell membrane. Our preliminary results show an unforeseen behavior of the MDA-MB-231 cells. When cultured on glass substrate as isolated cells, they are less stiff than the other two types of cells, in agreement with the general statement that more aggressive and metastatic cells are softer. However, when connected to other cells the stiffness of MDA-MB-231 cells becomes similar to the other two cell lines. Moreover, the stiffness of MDA-MB-231 cells cultured on soft PDMS substrates is significantly higher than the stiffness of the other cell types, demonstrating thus the strong influence of the environmental conditions on the mechanical properties of the cells.

1. Introduction

Tumor microenvironment plays a decisive role in cancer progression, invasion and metastasis (Schedin and Elias, 2004). One salient feature of the tumor microenvironment is its mechanical property, because it can affect cell survival, proliferation, adhesion and differentiation (Bissell and Hines, 2011; Hoffman and Crocker, 2009). The extracellular matrix (ECM) and neighboring cells are the main players of the mechanical interaction with the cell. The cell has the ability to change its mechanical properties in response to physical stimuli from the surrounding microenvironment (Discher et al., 2005). Cell stiffness is one of the mechanical properties which changes in the context of disease and has been identified as a possible marker for cell health (Hanahan and Weinberg, 2011). Cancer cells, cultured as isolated cells, have been found to be softer than the non-neoplastic cells (Coceano et al., 2015) and since this behavior has been verified for many types of cells (Suresh, 2007) it tends to be generalized also for cells in contact. However, we have recently shown that the neighboring cells differently affect the cell stiffness for different type of breast cancer cells (Yousafzai et al., 2016a). On the other hand, it has been shown that a stiffer extracellular microenvironment supports better the tumors with high invasive potential (Suresh, 2007).

Breast epithelial cells are not viable in suspension and are therefore reported to be anchorage dependent (Frisch and Francis, 1994). Usually, *in vivo* based experiments, cells are cultured on stiff substrates, like glass (E = 60–64 GPa) or polystyrene (E = 2.28–3.28 GPa) (Callister, 2001). However, cells *in vivo* are surrounded by tissues, such as neural, muscle and collagenous bone, that have a stiffness in the range of 1–100 kPa (Engler et al., 2006). Since cells try to adapt to the surrounding environment, different attempts have been made to tailor the stiffness of the underlying substrate to that of tissues and ECM (Chen et al., 2013; Pelham and Wang, 1997).

Polydimethylsiloxane (PDMS) is a biocompatible material which has been used to create substrates with tissue-like stiffness in diverse research fields to study neurite growth, stem cell

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differentiation, cell adhesion and growth, and cancerous cells proliferation and migration (Chen et al., 2013).

In this study, we fabricated PDMS cell substrates with three different stiffness values: 173 kPa, 88 kPa and 17 kPa, close to that of physiological tissues (Chen et al., 2013). We investigated the stiffness of breast cancer cells cultured on these substrates by vertical indentation of the cell membrane, using Optical Tweezers (OT). We present and discuss the results obtained from three human breast cell lines with different level of aggressiveness: HBL-100, MCF-7 and MDA-MB-231, showing that substrate stiffness and the cell-cell contact greatly influence the cell stiffness.

2. Materials and methods

2.1. Cells culture preparation

We used three human breast cell lines: normal myoepithelial (HBL-100), luminal breast cancer (MCF-7) and basal breast cancer (MDA-MB-231) cells (ATCC numbers HTB-124, HTB-26, and HTB-22, respectively). Cells were seeded overnight on 18 mm glass-cover slips coated with PDMS at a density of 10×10^4 cells/ml and 20×10^4 cells/ml in 2 ml of medium. Before starting the experiment, cells were washed three times with PBS and rinsed with medium before every measurement session.

2.2. Polydimethylsiloxane (PDMS) coating

Silicone base and curing agent (Sylgard® 184, Dow corning) were mixed in (m/m) ratio of 15:1, 35:1 and 50:1 and spin coated on glass coverslips to obtain the stiffness values of 173 kPa, 88 kPa and 17 kPa respectively, following the protocol given by Chen et al. (2013).

2.3. Optical tweezers indentation and cell stiffness calculation

We employed an Optical Tweezers (OT) vertical indentation technique for cell elasticity measurements, previously described in Yousafzai et al. (2016b) and schematically shown in Fig. 1. Laser power of 25 mW was used to produce a stable trap with a stiffness of 0.015 pN/nm with a bead diameter of 3 μm. Cells were indented vertically by moving the cell holder, coupled with Piezo stage, against the trapped bead by a sinusoid signal (amplitude A = 1 μm, one period T = 5 s, frequency = 0.2 Hz) and the displacement of the bead in the trap was acquired at a sampling frequency of 10 kHz. All measurements were performed at 37 °C. The apparent elastic modulus, E, was calculated using the Hertz-model.

3. Results

Breast cancer cells are anchorage-dependent and adapt their elasticity to the stiffness of the substrate where they are cultured. We studied cells elastic moduli on PDMS coated glass coverslips to mimic the ECM stiffness similar to that of physiological conditions. Twenty cells from each substrate were analyzed from different sets of cultures. Measurements were performed for all cells in their central region, above the nucleus (Coceano et al., 2015; Yousafzai et al., 2016a) in isolated and connected conditions. The results obtained from a cell indentation approach can be influenced by the cell thickness (Darling et al., 2007; Gavara and Chadwick, 2012). For instance, if the cell is too thin, the probe begins to feel not only the cell but also the substrate, mainly when AFM is used. We used the region above the nucleus because here the cell is usually the highest and the measurement do not require corrections. Moreover, this allows a practical and accurate way to measure the cells under similar conditions. All the three cell lines show viability and biocompatibility with PDMS of varying stiffnesses.

3.1. Cell stiffness alteration on PDMS: Isolated cells

MDA-MB-231 cells exhibit elasticity considerably lower than HBL-100 and MCF-7 on bare glass and is clearly distinguishable from the other two cell lines on the basis of their elasticity (Coceano et al., 2015) as summarized in the histogram shown in Fig. 2(a). As the stiffness of the substrate decreases the elasticity of HBL-100 and MCF-7 cells decreases as well, while MDA-MB-231 increases its elastic modulus with PDMS stiffness. On PDMS, all the three cell lines have comparable elasticity and become indistinguishable (Fig. 2(a)). Fig. 3 shows bright field images of the typical morphology of the three cell lines on glass and PDMS substrates. The softness of the substrate turns HBL-100 and MCF-7 to adopt compact morphologies, while, MDA-MB-231 shows polarization on soft substrates (Fig. 3d–f). MDA-MB-231 cells have higher aggressiveness and highly motile nature; therefore, their response is different to compliant substrates. MDA-MB-231 increases its stiffness with the softness of the substrate as show in Fig. 2(a). Fig. 4(a) shows that on glass the cells' average spread area is $552 \pm 37\ \mu m^2$ but on PDMS the spread area is of

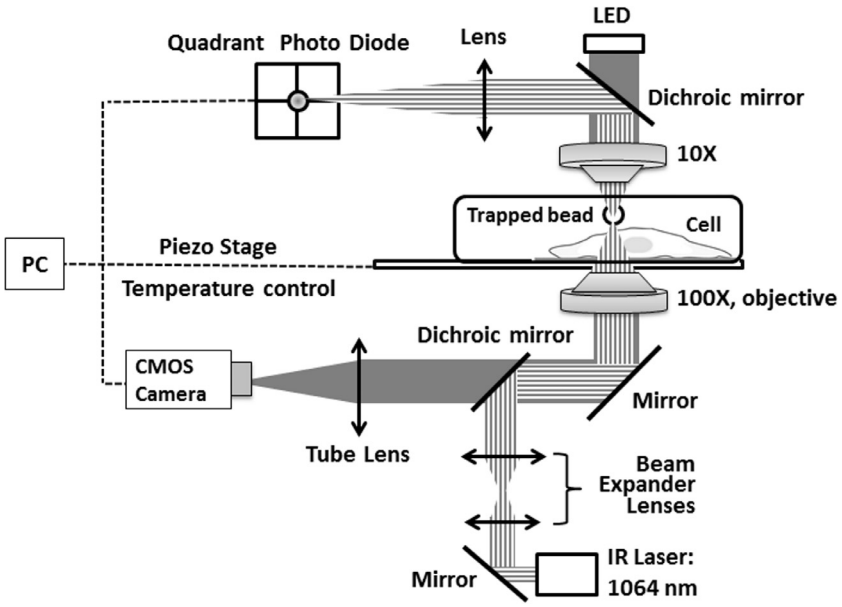

Fig. 1. Optical tweezers indentation and force measurement setup. IR trapping Laser path (gray solid lines) and bright-field imaging path (gray dashed).

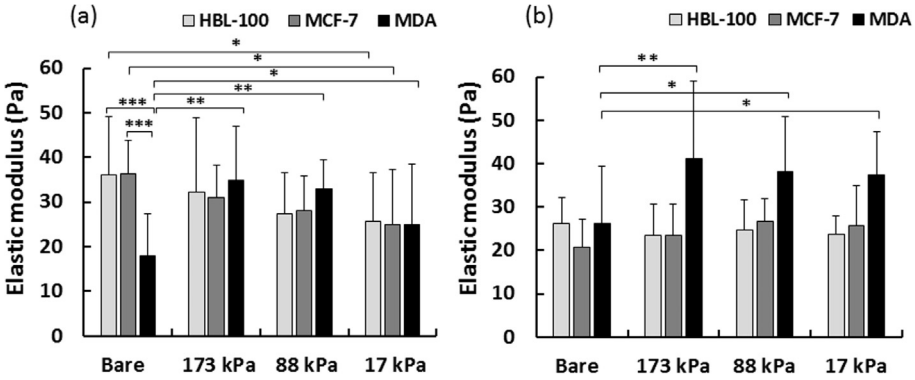

Fig. 2. Elasticity alterations of HBL-100, MCF-7 and MDA-MB-231 cells on bare and PDMS coated glass substrates in (a) isolated and (b) connected conditions. Error bars represent Standard deviation. (*t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, N = 20).

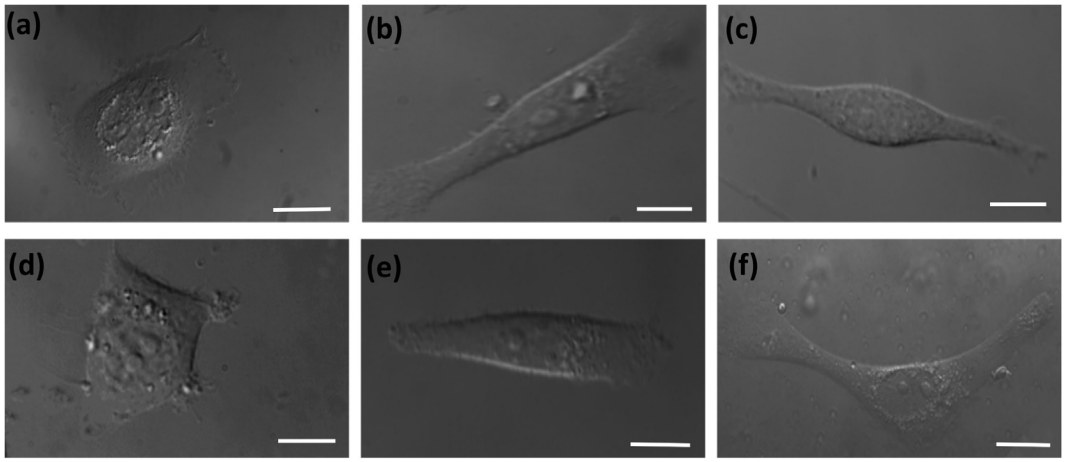

Fig. 3. Bright field images show morphology of MCF-7 cells on glass (a) and PDMS (d); HBL-100 on glass (b) and PDMS (e); and MDA-MB-231on glass (d) and PDMS (f) substrates. HBL-100 and MCF-7 show compact structures on PDMS while MDA-MB-231 shows polarization at two ends. (Scale bar 5 μm).

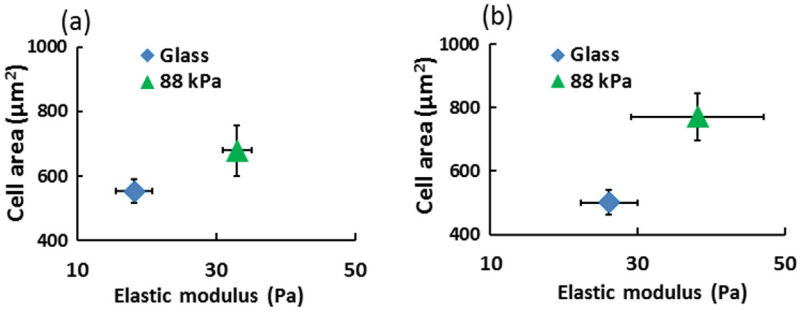

Fig. 4. MDA-MB-231 cells spread area on glass and PDMS (88 kPa) substrates in (a) isolated and (b) connected conditions. The cells show increased polarization and spread on soft substrates.

678 ± 78 μm^2. This increment in area is a marker for cell motility and invasive behavior of MDA-MB-231 cells.

3.2. Cell stiffness alteration on PDMS: Connected cells

We studied the three cell lines in connected conditions on glass and PDMS substrates. On bare glass, in connected condition, MDA-MB-231 has stiffness similar to HBL = 100 (as previously reported in Yousafzai et al. (2016a)), but on soft substrates, MDA-MB-231 is clearly distinguishable from the other two cell lines (Fig. 2(b)). HBL-100 and MCF-7 show similar elasticity values, which are much lower than MDA-MB-231. The spread area of MDA-MB-231 in connected conditions for PDMS (771 ± 75 μm^2) is also larger than for glass (503 ± 38 μm^2), as shown in Fig. 4(b). The increased stiffness and spread area of MDA-MB-231 cultured on softer substrates can be used together with the measurements performed on bare glass to confirm cell elasticity as a possible marker to characterize cell aggressiveness.

4. Discussion and conclusion

Cells constantly interact mechanically with their underlying substrate through integrin based focal adhesions and neighboring cells through E- cadherins based tight junctions (Mierke, 2014).

Studies show that by tailoring matrix stiffness, cancer cell fate may change to normal ones (Bissell and Hines, 2011; Ingber, 2008). Invasive cells show increased elasticity alterations and acto-myosin cortex contractility, which give them the ability to apply normal forces on soft gels (Kristal-Muscal et al., 2013) as well as lateral forces on neighboring cells for increased migration (Lee et al., 2012). In contrast, benign cells remain rounded on soft gel and apply small normal and traction forces. Our results (Fig. 2) show that elasticity of the cell is influenced by substrate stiffness and cell aggressiveness. In isolated conditions, more aggressive cells are softer on bare glass, but acquire similar elasticity, comparable to normal and less aggressive tumor cells, on soft substrates. The three cell lines are, indeed, mechanically indistinguishable on PDMS substrates. HBL-100 and MCF-7 cells are getting softer with the softness of the substrate, while MDA-MB-231 becomes stiffer. Moreover, we observed MDA-MB-231 cells on softer substrates become even stiffer when in contact each other, while the stiffness does not change for the other cells (Fig.2b). This confirms the result obtained previously on hard substrates (Yousafzai et al. 2016a), suggesting that soft substrate and cell neighboring have a cumulative effect on the cell stiffness. This indicates also that MDA-MB-231 cells are very adaptable to the mechanical properties of the environment and hence complex measurements in different conditions should be performed when using cell stiffness as a disease marker. This increment in stiffness may be attributed to the increment in acto-myosin activity during cell motility and force generation abilities (Kristal-Muscal et al., 2013; Suresh, 2007). These cell lines adhere to PDMS substrates and adapt their morphology according to their physical state and aggressiveness.

Tissue cells not only feel the mechanical changes in their ECM but they are also influenced by their neighboring cells, and transmit mechanical signals to their neighboring cells in a coordinated way. Our data indicate that for HBL-100 and MCF-7, as the softness of the substrate increases, cell-cell interaction becomes irrelevant. Though on Glass, cell-cell interaction is the prominent contributor for the cell softening. However for MDA-MB-231 cells, even if they are on soft substrate, their interplay with the neighboring cells is strong and this behavior is likely the explanation of their invasive potential.

In conclusion, cell-microenvironment mechanical interaction has a strong impact on the mechanical behavior of cells. Cell elasticity alters with the compliance of the substrate as well as with the neighboring cells. These alterations vary with the aggressiveness of the cells. For HBL-100 and MCF-7 the cell-cell interaction becomes irrelevant when they experience soft substrate, however MDA-MB-231 cells respond to both neighboring cells and ECM actively. More aggressive and invasive cells show increased rate of spreading and polarization. Alteration of the cell stiffness and cell spreading area can be considered as a possible marker to characterize cell aggressiveness and invasive potential. In summary, mechanical study on these cell lines in relation to their microenvironments can give a further insight into the peculiar mechanisms of tumor growth and invasion.

Conflict of interest

The authors declare no conflict of interest associated with this work.

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