Decreased cell stiffness facilitates cell detachment and cell migration from breast cancer spheroids in 3D collagen matrices of different rigidity

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Abstract

Purpose: Tumour-cell detachment is a critical early event in the metastatic cascade. Although several mechanisms have been reported, the role of cell mechanical properties in facilitating cell detachment and migration is not well understood. We, therefore, investigated how cells alter intracellular stiffness during these processes.

Methods: MDA-MB-231 cells were embedded as 10,000-cell spheroids in 2 and 4 mg/ml collagen matrices. Using mitochondrial-based particle tracking microrheology (PTM), the intracellular stiffness of cells that have migrated different distances from the spheroid were assessed. Here, $0d_C$, $4d_C$ and $6d_C$ represented no, medium and high migration, respectively.

Results: For 2 and 4 mg/ml collagen matrices, the MSD and cell stiffness of $0d_C$ cells were larger than for migrated $4d_C$ and $6d_C$ cells. The MSD of $4d_C$ and $6d_C$ cells were similar; however, the cell stiffness of $4d_C$ cells was smaller than that of $6d_C$ cells. The stiffness of $0d_C$ cells was lower for higher matrix concentration and rigidity compared to lower matrix rigidity, whereas matrix rigidity did not affect the stiffness of $4d_C$ and $6d_C$ cells.

Conclusions: PTM was capable of quantifying intracellular mechanics during tumour detachment and migration in 3D environments. Based on our findings, it is proposed that decreased cell stiffness drives cellular detachment and migration. Increased matrix rigidity physically hinders migration and cells need to either soften or remodel the environment to migrate. The finding that matrix rigidity did not affect the stiffness of migrated cells suggests that cells facilitate migration by remodelling their environment through cleavage of matrix proteins.

Keywords: Breast cancer spheroids, Cell detachment, Cell migration, Cell stiffness; Mitochondrial-based particle tracking microrheology, 3D matrix rigidity

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1. Introduction

Metastatic cancer cells alternate between various labour-intensive tasks associated with disease progression, including detachment from the tumour, invasion into the tumour environment and formation of new tumours (Gal and Weihs 2012). Considerable attention has focussed on understanding the role that the mechanical properties of cancer cells play in these processes. It has been shown that the mechanical properties have crucial implications in regulating chemical and mechanical cues and in maintaining cell or tissue architecture in several cell types, including metastatic cancerous cells (Cui *et al.* 2017, Mak *et al.* 2014).

Many of these studies have probed cells in two-dimensional environments. However, cells in two-dimensional (2D) and three-dimensional (3D) environments exhibit substantial differences in morphology, gene expression, and mechanical and structural properties. Several studies reported a marked decrease in intracellular fluctuations from 2D to 3D environments (Baker *et al.* 2009, Mak *et al.* 2014). Additionally, 3D *in vitro* systems can recapitulate *in vivo* conditions more closely than 2D systems (Baker *et al.* 2009).

Studies of solid tumours should, therefore, incorporate 3D models that mimic complex tumour architecture, including cell-cell and cell-matrix interactions (Close *et al.* 2018). Cell spheroids are powerful in mimicking many aspects of solid tumours, including architecture (i.e. an outer proliferative layer of cells, a middle layer of senescent cells and a core of necrotic cells), enhanced cell-cell and cell-matrix connections, and gene expression (Costa *et al.* 2016, Fennema *et al.* 2013).

Tumour spheroid assays have addressed essential questions relating to spheroid morphology, growth, viability, drug development (Close *et al.* 2018), signalling pathways associated with cell detachment and invasion (Naber *et al.* 2012), cancer stem cell content (Reynolds *et al.* 2017). However, only few studies have investigated cell mechanics in the detachment and invasion process – owing to technical challenges of quantifying the mechanics of live cells in 3D environments during detachment and invasion. Microrheology can offer non-invasive techniques that allow passively probing intra-cellular mechanics to characterise single cell and collective cell behaviour without introducing cellular and matrix deformation (Mak *et al.* 2014).

It is generally accepted that elevated tissue rigidity is a characteristic of solid tumours (Wullkopf *et al.* 2018) and that cells adapt their stiffness to the environmental stiffness (Mitrossilis *et al.* 2010). It is of interest to investigate whether and how cells adapt their

stiffness during tumour detachment and invasion as the disease progresses. It has been shown that reduced cell-cell adhesion decreases cell stiffness (Omidvar *et al.* 2014) and increases migration (Geiger *et al.* 2019) and that increased migration and decreased cell stiffness are directly correlated (Guck *et al.* 2005, Remmerbach *et al.* 2009). However, it is not yet known whether these observations of cellular mechanics translate directly to physiologically relevant environments that mimic the tumour-cell detachment process. Considering that the mechanical properties of cells depend on the properties of the extracellular matrix (ECM), it is essential to clarify how cells regulate their mechanical properties when the properties of the ECM change.

This study aimed to determine the intracellular stiffness of metastatic breast cancer cells during tumour detachment and invasion by combining a 3D spheroid model and mitochondrial particle tracking microrheology (PTM). Different levels of migratory capacity were established by considering cells increasingly migratory with increasing migration distance from the spheroid.

2. Materials and methods

2.1. Cell culture

Metastatic breast adenocarcinoma cells, MDA-MB-231, were cultured in DMEM (Dulbecco's Modified Eagle Medium, Life Technologies), supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained in 25 cm^2 tissue flasks (Costar, Corning Life Science, Acton, MA) at 37°C and 5% CO_2 until near-confluency. Media was replaced every 3-4 days. Cells were used in passages 6-16.

2.2. Spheroid formation

To form spheroids, cell suspensions were grown in agarose-coated well plates according to the liquid overlay method (Metzger *et al.* 2011). An agarose solution was prepared by diluting 0.18 g agarose (Sigma-Aldrich, St. Louis, MO) in 12 ml 1XPBS and heated in a microwave oven for 36 s or until the agarose dissolved. The solution was immediately transferred to sterile conditions and kept on a hot plate to prevent premature gelation. A 96-well plate was prepared by pipetting 70 µl of the hot liquid agarose solution onto the flat bottoms of the wells, ensuring the solution covered the entire surface and contained no air bubbles. The agarose-coated 96-well plate was exposed to ultraviolet light for 30 minutes for sterilisation. Separately, cell monolayers were lifted from the culture flask using standard laboratory trypsinisation procedures. Spheroids of 10,000 cells were created by placing 100 µl trypsinised cells (100,000

cells/ml containing 2.5% Matrigel) into the agarose-coated wells, centrifuged at 1,000 rpm for 5 mins, and incubated at 37°C and 5% CO_2 for 72 hrs.

2.3. Spheroid collagen embedding

MDA-MB-231 spheroids were embedded in Type I Rat Tail Collagen Solutions (BD Biosciences) at final collagen concentrations of 2 and 4 mg/ml. Type I Rat Tail Collagen at a stock solution of 9.61 mg/ml was combined with equal amounts of neutralising solution (100 mM HEPES in 2X PBS with pH 7.3), and further diluted with 1XPBS on ice to create 2 and 4 mg/ml collagen solutions. Spheroids were added to the unpolymerised collagen solutions on 35mm glass-bottom dishes, with each 50 µl collagen solution containing one spheroid to avoid spheroid-spheroid interaction, and incubated at 37°C and 5% CO_2 for 1 hr. After 1 hr, the collagen solutions polymerised and 500 µl media were added.

2.4. Mitochondrial particle tracking microrheology

Embedded MDA-MB-231 tumour spheroids were prepared for mitochondrial tracking by adding 500 nM Mitotracker Red solution (Life Technologies, Carlsbad, CA) to the supplemented DMEM media 24 hrs before experimentation to allow the Mitotracker solution to sufficiently diffuse through the spheroid. Before imaging, the media was removed from the wells and the wells placed in an environmental chamber at 37°C and 5% CO_2.

After 24 hrs, cells started detaching from the spheroid and migrating into the collagen gel predominantly as linear branches with a leader cell and follower cells.

To avoid boundary effects of the rigid 2D glass-bottom surface and to ensure full engulfment, cell branches near the middle of the collagen gel were located and imaged. Mitochondrial fluctuations of branching cells at the spheroid surface and varying distances from the spheroid were captured using time-lapse fluorescent imaging for 120 s (exposure time: 50 ms/frame; spinning disk confocal microscope, 63x 1.4 oil immersion objective and CCD camera, Hamamatsu Photonics, Hamamatsu, Japan). Fluorescent images were acquired of surface and migrated cells (branched). The distance migrated (d_m) from the spheroid was reported in terms of cell diameter (d_C), where $d_m = 0d_C$, $4d_C$ and $6d_C$ correspond to the cell at the spheroid surface, intermediately migrated, and furthest migrated, respectively. These experiments were repeated on two independent days (n = 2), with data taken from at least two spheroids, each with at least five branches comprising at least six migrated cells.

Post-processing involved constructing mitochondrial trajectories using TrackMate (Tinevez *et al.* 2017) in Fiji Image J (Schindelin *et al.* 2012). The tracking assumed particles with a

diameter of 1 µm and allowed up to 4 missed frames between subsequent spots to account for missing detections. Mitochondrial trajectories were imported into MATLAB (The MathWorks, Natick, MA), and the ensemble-averaged, time-dependent mean square displacement (MSD) was calculated using @msdanalyzer (Tarantino *et al.* 2014) according to:

$$\langle \Delta r^2(\tau)_{xy} \rangle = \langle [x(t+\tau) - x(t)] + [y(t+\tau) - y(t)] \rangle^2 \quad (1)$$

Here, $\langle \Delta r^2 (\tau)_{xy} \rangle$ is the ensemble-averaged MSD, τ is the time interval or delay time between the first and last image frame used for the analysis, and *x(t)* and *y(t)* refer to the spatial coordinates of particle positions at time *t*.

The MSD increases with delay time according to a power-law relationship, $\langle \Delta r^2 (\tau) \rangle \sim \tau^{\alpha}$ (Baker *et al.* 2010). The MSD-dependent power-law coefficient or diffusivity coefficient $\alpha = \partial \ln \langle \Delta r^2 (\tau) \rangle / \partial \ln (\tau)$ represents the intracellular fluidity (deformability).

2.5. Statistical analysis

Data are reported as mean ± standard deviation in the text. Error bars in graphs represent the standard error of the mean (SEM). Two-way ANOVA was employed to detect differences in MSD and fluidity of cells at different delay times. ANOVA assessed two main effects, i.e. distance from the spheroid and changes in collagen concentration, and one interaction between main effects. The migrated distance contained three levels, i.e. no migration, medium migration, and large migration, with which posthoc analysis was performed using Tukey's HSD test. The collagen concentration contained two levels, i.e. 2 mg/ml and 4 mg/ml collagen, and ANOVA was followed up with linearly independent pairwise comparisons among estimated marginal means.

A criterion for statistical significance of $p < .05$ formed the basis of all evaluations. SPSS Statistics for Windows (Version 25.0, IBM Corp., Armonk, NY) was used for all statistical analyses.

3. Results

To provide a platform for cell detachment from tumours and migration in physiologically relevant environments, metastatic breast cancer cells (MDA-MB-231) were embedded as tumour spheroids in 3D collagen matrices. Mitochondrial PTM experiments (n = 2) were conducted after 24 hrs of incubation when cells started detaching from spheroids.

The use of mitochondria as tracer particles to determine the viscoelastic response of cells has been validated; Mak *et al.* (2014) showed that the MSD of mitochondria and ballistically injected nanoparticles was similar when used in metastatic breast cancer MDA-MB-231 cells (Mak *et al.* 2014). For short delay times, the mitochondrial MSD primarily represents the viscoelastic properties of a cell, whereas active motor-driven cellular processes dominate the MSD for long delay times (Kim *et al.* 2018, Mak *et al.* 2014).

Following detachment from the tumour surface, cells invaded the tumour environment predominantly in linear branches. In these branches, several cells trailed a leader cell and migrated away from the tumour (Figure 1).

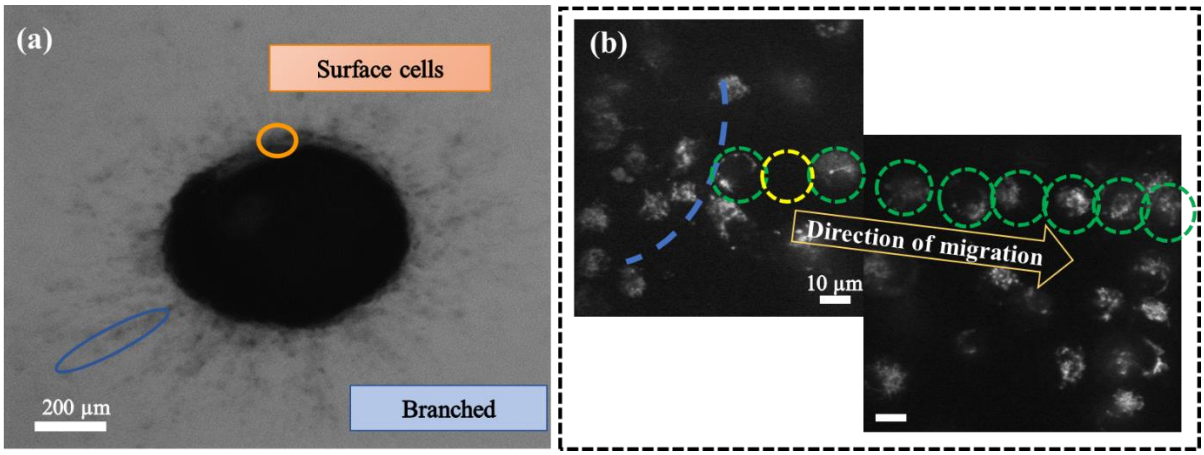

Figure 1: Illustration of detaching (surface) and migrated (branched) cells as (a) bright-field image (5x magnification) and (b) mitochondrial fluorescent image (63x magnification). The blue dashed line indicates the spheroid border, green dashed circles indicate the approximate diameter of cells migrating away from the spheroid in a branch, and the yellow dashed circle indicates a cell out of focus.

3.1. Effect of detachment and migration: Migrated cells are more deformable than detaching cells

The effect of detachment and migration of cells on mitochondrial fluctuations and cell deformability was assessed in 2 and 4 mg/ml collagen (Figure 2). The MSD of $0d_C$, $4d_C$, and $6d_C$ cells in 2 and 4 mg/ml collagen increased with increasing delay time. To determine the viscoelastic properties of cells as the distance from the spheroid increased, MSD and α data were evaluated and reported below for short delay times of $\tau = 0.05$ s and 2 s, respectively.

In 2 mg/ml collagen matrices, mitochondrial fluctuation was larger and intracellular fluidity was smaller for cells at the spheroid surface, i.e. $d_m = 0d_C$, than for cells detached and invaded the environment (Figure 2a and b). The MSD in $0d_C$ cells was 0.016 ± 0.004 µm^2 compared to

4d$_C$ cells with 0.012 ± 0.002 μm^2 (p = .012) and 6d$_C$ cells with 0.012 ± 0.003 μm^2 (p = .010). The MSD of 4dc and 6dc were similar (p = .946). The intracellular fluidity α of 0d$_C$ cells (0.311 ± 0.298) was lower than that of 4d$_C$ (0.815 ± 0.243, p < .001) and 6d$_C$ cells (0.664 ± 0.159, p = .003), and α was larger for 4d$_C$ cells than for 6d$_C$ cells, although non-significant (p = .173). The mitochondrial motion was strongly sub-diffusive in 0d$_C$ cells, closely diffusive in 4d$_C$ cells, and weakly sub-diffusive in 6d$_C$ cells.

In the 4 mg/ml collagen environment, the MSD of the 0d$_C$ cells of 0.014 ± 0.005 μm^2 was slightly larger than for 4d$_C$ (0.012 ± 0.004 μm^2) and 6d$_C$ cells (0.011 ± 0.004 μm^2) (Figure 2c). These differences were, however, not significant (p = .436). The observations for intracellular fluidity α were similar to those in the 2 mg/ml collagen, although differences were not significant (p = .151). The 0d$_C$ cells exhibited lower fluidity (0.487 ± 0.284) than the 4d$_C$ (0.734 ± 0.220) and 6d$_C$ cells (0.659 ± 0.349). The fluidity values indicated that the migrated 4d$_C$ and 6d$_C$ cells displayed weaker sub-diffusive motion than the 0d$_C$ cells at the spheroid surface (Figure 2d).

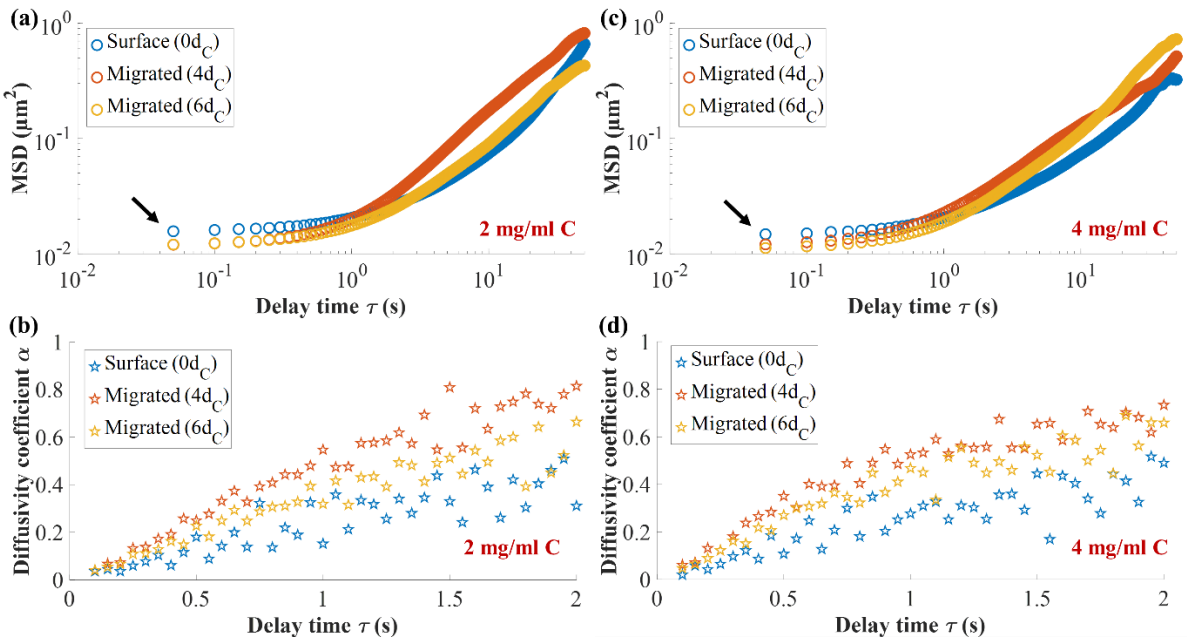

Figure 2: Intracellular fluctuations from spheroids (n = 2) in 2 and 4 mg/ml collagen gels. MSD and diffusivity coefficient α versus delay time of cells in 2 mg/ml collagen (a, b) and in 4 mg/ml collagen (c, d). Error bars omitted for clarity. Arrows indicate MSD at short delay times of τ = 0.05 s. Trends of MSD and α of cells were similar in 2 and 4 mg/ml collagen. Mitochondrial fluctuations of 0d$_C$ were larger than migrated 4d$_C$ and 6d$_C$ cells. 0d$_C$ cells exhibited the largest cell stiffness, followed by 6d$_C$ and then 4d$_C$ cells.

3.2. Matrix rigidity affects cell stiffness during detachment but not during subsequent migration

The effect of changes in matrix rigidity on mitochondrial fluctuations and cell deformability was assessed for varying migration distances from the tumour spheroids.

The increased concentration and rigidity of the collagen matrix did not affect the mitochondrial fluctuations of the cells. The MSD of $0d_C$, $4d_C$ and $6d_C$ cells in 4 mg/ml was similar to that in 2 mg/ml collagen (p = .290, p = .773 and p = .426, respectively) (Figure 3a).

An increase, although not significant, in intracellular fluidity α with increased collagen concentration was observed for the $0d_C$ cells at the spheroid surface (p = .182) but not for the migrated $4d_C$ (p = .444) and $6d_C$ cells (p = .964) (Figure 3b).

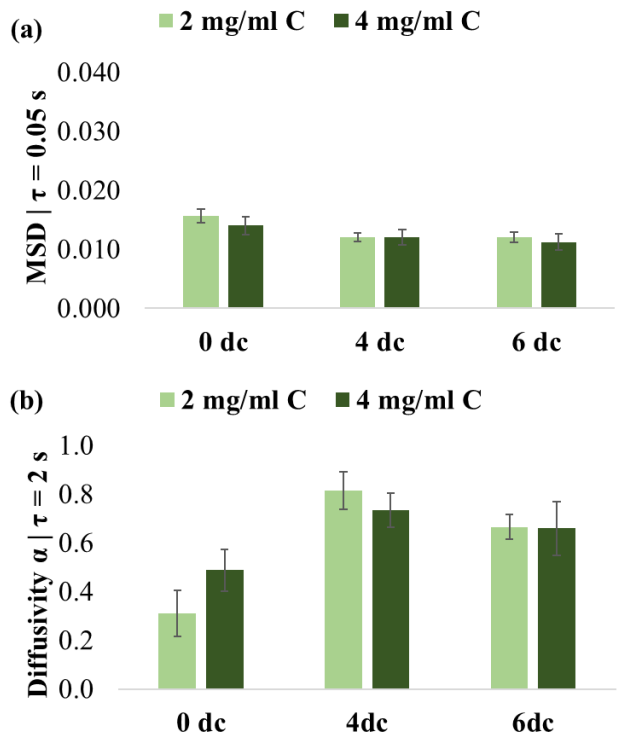

Figure 3: Comparison of the MSD and fluidity of cells at the spheroid surface ($0d_C$) and after migration of a distance of $4d_C$ and $6d_C$ from the spheroid surface in 2 and 4 mg/ml collagen matrices (C) (d_C indicates cell diameter). Increased matrix rigidity increased the fluidity of $0d_C$ cells but did not affect the fluidity of $4d_C$ and $6d_C$ cells. a) The MSD of $0d_C$ cells was 0.016 ± 0.001 μm^2 (in 2 mg/ml C) and 0.014 ± 0.002 μm^2 (in 4 mg/ml C), of $4d_C$ cells was 0.012 ± 0.001 μm^2 (in 2 mg/ml C) and 0.012 ± 0.001 μm^2 (in 4 mg/ml C), and of $6d_C$ cells was 0.012 ± 0.001 μm^2 (in 2 mg/ml C) and 0.011 ± 0.001 μm^2 (in 4 mg/ml C). b) The diffusivity coefficient α of $0d_C$ cells was 0.311 ± 0.094 (in 2 mg/ml C) and 0.487 ± 0.086 (in 4 mg/ml C), of 4dc cells was 0.815 ± 0.077 (in 2 mg/ml C) and 0.734 ± 0.069 (in 4 mg/ml C), and of 6dc cells was 0.664 ± 0.050 (in 2 mg/ml C) and 0.659 ± 0.110 (in 4 mg/ml C). All differences were non-significant. Values are mean ± SEM, error bars are SEM.

4. Discussion

In the present study, metastatic breast adenocarcinoma cells, MDA-MB-231, were embedded as tumour spheroids in collagen gels of two different concentrations (2 and 4 mg/ml collagen) to investigate the mechanical properties of the cancer cells associated with of tumour-cell detachment and migration in physiologically relevant 3D environments. Mitochondrial-based particle tracking microrheology was utilised to probe intracellular fluctuations. Cells were distinguished between cells attached to the spheroid, i.e. before tumour-cell detachment, and migrated cells that have employed the necessary processes to detach and gain enhanced migratory properties for cell dissemination away from the tumour spheroid. Cells were denoted according to the distance migrated from the spheroid in terms of cell diameter (d_C), where $0d_C$, $4d_C$ and $6d_C$ represented no, medium and high migration, respectively.

This study extends work on the microrheological characterisation of breast cancer cells in 3D environments. MDA-MB-231 cells were used since their mechanics has been extensively studied as single cells in both 2D and 3D environments (Baker *et al.* 2009, Mak *et al.* 2014), and with and without drug treatment (Kim *et al.* 2018, Wullkopf *et al.* 2018). However, the intracellular mechanics that aid cells in detaching from the tumour and invading the surrounding matrix is not well studied. Here, the microrheology of cells during physiological relevant processes such as tumour detachment and directed migration were characterised in 3D environments.

The findings of this study indicate that cells are more solid-like before tumour detachment than after detachment and migration into the surrounding matrix. The cells at the spheroid surface (i.e. $0d_C$) displayed a lower fluidity α, alluding to increased cell stiffness, than migrated $4d_C$ and $6d_C$ cells in both 2 and 4 mg/ml collagen concentrations. In the context of migration, studies have shown that increased migratory potentials were inversely related to increased cell stiffness, irrespective of whether different grades of cancers from the same cancer type or the same tumour specimen were used (Swaminathan *et al.* 2011, Xu *et al.* 2012).

(Swaminathan *et al.* 2011, Xu *et al.* 2012) used cell spheroids of a highly invasive breast cancer cell line 4T1 and pancreatic cancer cell line $KP^{R172}HC$ to measure the cell stiffness at the centre of the spheroid and in the stalk and the tip of branches of invading cells in compliant and rigid collagen matrices. Here, the cells in the spheroid centre, branch stalk, and branch tip represented no migration, medium migration, and large migration, respectively. Irrespective of

the rigidity of the collagen matrix, the stiffness of tip cells that had migrated furthest was lower than that of cells in the spheroid centre (Wullkopf *et al.* 2018). These results support our findings. However, it is important to mention that the cell stiffness of $4d_C$ cells that have migrated medium distances was lower than the stiffness of $6d_C$ cells that have migrated large distances in 3D matrices of different rigidity. In particularly, the diffusivity α of cells increased from $0d_C$ to $4d_C$ cells and decreased from $4d_C$ to $6d_C$ cells in 2 mg/ml and 4 mg/ml collagen matrices.

The $0d_C$ cells were connected to the spheroid through cell-cell adhesion, whereas $4d_C$ and $6d_C$ cells detached and migrated away from the spheroid. Migrated cells are, therefore, less mechanically influenced by the tumour and instead adapt to the rigidity of the matrix. Interestingly, the cell stiffness at $6d_C$ distance from the spheroid was higher than that of $4d_C$ cells. Reports on the migration of cells in 3D environments show that MDA-MB-231 cells require actomyosin-based contractility to invade the matrix despite their spherical morphology (Poincloux *et al.* 2011). It is therefore proposed that the increased migration of $6d_C$ cells compared to $4d_C$ cells requires increased actomyosin contractility, and hence increased cell stiffness. Furthermore, mitochondrial fluctuations in migrated $4d_C$ and $6d_C$ cells neared or entered the diffusive regime much earlier than in $0d_C$ cells, indicating heightened motor activity to contract the cytoskeleton in preparation for migration and invasion into the gel (Guo *et al.* 2014).

In the lower collagen concentration (2 mg/ml), the motion of the mitochondrial particles of $0d_C$, $4d_C$ and $6d_C$ cells was strongly sub-diffusive ($\alpha < 0.3$), diffusive ($\alpha = 1.0$) and weakly sub-diffusive ($0.7 < \alpha < 1.0$), respectively. In the higher collagen concentration (4 mg/ml), mitochondria of $0d_C$ were sub-diffusive, whereas mitochondria of $4d_C$ and $6d_C$ cells were weakly sub-diffusive. Importantly, these observations indicate that weakly sub-diffusive to diffusive behaviour is characteristic of cells that migrated away from their tumour spheroid.

The migrating cells exhibited amoeboid motility and moved in a straight path away from the spheroid. Amoeboid migration strongly is driven by increased myosin II-mediated contractility (Poincloux *et al.* 2011). Hence it is suggested that the diffusive mitochondrial motion we observed in migrating cells is based on increased myosin II-mediated contractility.

Increased matrix rigidity played a role in decreasing the cell stiffness of cells at the spheroid but had no impact on the stiffness of migrated cells. The intracellular fluidity α of $0d_C$ cells was lower in 2 mg/ml than in 4 mg/ml collagen, whereas α of $4d_C$ and $6d_C$ cells were similar

in 2 and 4 mg/ml collagen matrices. Increased matrix rigidity has been reported to increase intracellular activity and decrease cell stiffness of isolated cells in 3D environments (Baker *et al.* 2009, Butcher *et al.* 2009). The fluidity of $0d_C$ cells at the spheroid surface is consistent with these reports. For migrated cells, the similar fluidity of $4d_C$ and $6d_C$ cells in soft and stiff matrices are consistent with the results of Wullkopf *et al.* (2018). The authors showed that the stiffness of invasive breast cancer 4T1 and pancreatic cancer $KP^{R172}HC$ cells on the stalk and tip of invading branches of spheroids were similar in collagen matrices of high and low rigidity (Wullkopf *et al.* 2018). Combined with the effect of migrated distance, this finding suggests that cells acquire maximum deformability during migration irrespective of the rigidity of the matrix. It is known that cells can remodel their environment and that the secretion of matrix metalloproteases (MMPs) increase with cancer progression (Harjanto *et al.* 2011). It is therefore proposed that $4d_C$ and $6d_C$ cells degrade dense collagen matrices by secreting MMPs to facilitate migration.

The present study focused solely on cell detachment and migration from breast tumour spheroids. Future work should entail repeating the current study with a different cancer disease, such as melanoma, to provide a more comprehensive understanding of the adjustment of intracellular mechanics of cells during cell detachment and invasion.

Conventional spheroid invasion assays determine the signalling pathways involved in the invasion process (Naber *et al.* 2012, Wiercinska *et al.* 2011). Complementing these studies with PTM experiments would provide more powerful tools to understand how cancer cells regulate mechanical and biochemical cues to metastasise. For example, transforming growth factor β (TGFβ) signalling is commonly studied in spheroid invasion assays, and reports have shown that TGFβ plays a vital role in stimulating invasion and metastasis in advanced breast cancer (Wiercinska *et al.* 2011). Expansion of the present study could study the effects of TGFβ on the intracellular stiffness of metastatic breast cancer cells during tumour detachment and invasion.

5. Conclusion

It was shown that mitochondrial particle tracking microrheology could detect changes in cell stiffness of metastatic breast cancer cells as cells detached and migrated away from their spheroids in soft and stiff collagen matrices. Consistent with several reports (Guck *et al.* 2005, Remmerbach *et al.* 2009), this study suggests that decreased cell stiffness mediates the escape

of cells from the initial metastatic niche. Based on the difference in cell stiffness between $4d_C$ and $6d_C$ cells, it is proposed that upon escaping the initial metastatic niche, cells require increased actomyosin contractility to migrate. The findings of this study provide insights into the collective effects of enhanced migratory conditions and increased matrix rigidity on the mechanical properties of cells. These results are valuable and can be implemented in the development of therapeutics to target cells not only migrating in 3D but more importantly, cells at the initial tumour site, thereby preventing metastatic spread.

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