Multimodal mechano-microscopy reveals mechanical phenotypes of breast cancer spheroids in three dimensions

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Background incl. aims

Cancer cell invasion relies on an equilibrium between cell deformability and the biophysical constraints imposed by the extracellular matrix (ECM), and multicellular spheroids are a powerful model to study biochemical and biophysical interactions between cancer cells during growth and progression. However, little is known about how the biomechanics of the three-dimensional (3D) tumour microenvironment (TME) control cancer cell behaviours. 3D TME models remain underutilised, because contemporary mechanical quantification tools are limited to surface measurements, and there is a lack of enabling technologies that can measure subcellular-scale elasticity and co-register it with the morphology and function of cells in a 3D microenvironment.

Here, we develop a multimodal mechano-microscopy system that integrates optical coherence microscopy-based elasticity imaging with confocal fluorescence microscopy. We use this multimodal microscope to quantify local mechanics of cancer cell spheroids in 3D TMEs.

Methods
Our multimodal mechano-microscopy setup integrates high-resolution interferometric detection of optical coherence microscopy (OCM), a high-resolution variant of optical coherence tomography (OCT), with compression elastography and confocal fluorescence microscopy.[1] The system utilises a supercontinuum laser, whose output was shaped to a spectral range of 650 nm to 950 nm. This spectrum corresponded to a full-width at half-maximum (FWHM) bandwidth of ~250 nm, providing a measured OCM axial resolution of 1.4 µm in air. The system was implemented as a Michelson interferometer in a dual-arm configuration with the same optics in the reference beam path to match optical dispersion. The sample arm beam was expanded to fill the entrance pupil of a 20X 0.75NA objective lens providing a measured lateral resolution of 0.5 µm. Scanning was achieved using a 2D galvanometer system. A spectrometer comprising a 2048-pixel line camera was used to detect the spectral interference at each xy location.

The integrated confocal fluorescence microscope comprised two laser lines at 405 nm and 488 nm for excitation and a photomultiplier tube as the detector. The confocal system used the same galvanometer scanning arm and optics as the OCM system to scan the focal point in the sample. The emitted fluorescence from the sample was focused into a multi-modal optical fibre, which also acted as the confocal pinhole. The measured lateral resolution of the confocal imaging system was 0.5 µm, and the axial resolution ~8 µm.

Sample preparation is described elsewhere in more detail [1]. Briefly, MCF-7 and MDA-MB-231 cells were suspended in gelatin methacryloyl (GelMA) solution. The solution was pipetted into moulds placed on glass slides, and exposed to UV light for 15 or 60 s to produce soft and stiff gels, respectively. The cells were then cultured for 6-16 days to allow spheroid formation and growth. Prior to imaging, the cells were labelled with nuclear dye Hoechst 33342 and membrane dye CellMask Green for fluorescence microscopy.

For imaging, the gel sample with a thickness of ~400–500 µm and a 1 mm thick compliant silicone layer were compressed between the coverslip and a rigid optical window attached to an annular piezoelectric actuator (Fig 1a). Two B-scans were acquired at each y-location, one at the unloaded and the other at the loaded state. Local displacement was calculated from the phase difference between the two B-scans and strain was estimated as the gradient of the axial displacement with depth.[2] Strain in the layer was related to stress through knowledge of the stress–strain response of the compliant layer in contact with the sample. Confocal images were acquired directly after the mechano-microscopy measurement.

Results

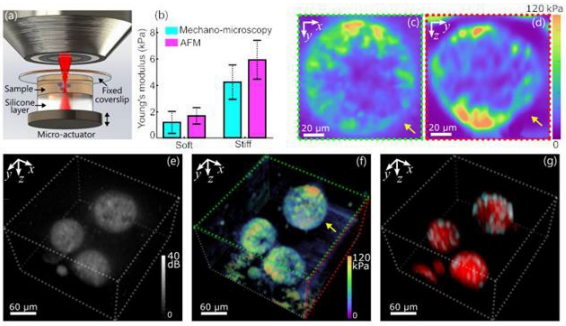
To test the setup, Young's modulus of blank, soft and stiff gelatin methacryloyl (GelMA) samples was first measured using mechano-microscopy and was found to be in good agreement with AFM surface measurement (Fig 1b). The multimodal setup was then used to obtain volumetric maps of living breast cancer cell spheroids cultured in the gels. Fig 1c,d shows example en face and B-scan Young's modulus of a non-metastatic spheroid, and Fig 1e-g show an example of a volumetric map where (e) OCM intensity, (f) Young's modulus and (g) confocal fluorescence image of the nuclear (cyan) and membrane (red) fluorescent dyes.

By imaging both metastatic (MCF-7) and non-metastatic (MDA-MB-231) breast cancer cell spheroids cultured in both soft and stiff gels (n≥4 for each condition), we observe that non-metastatic cancer spheroids show no invasion while showing increased peripheral cell elasticity in both stiff and soft environments. Metastatic cancer spheroids, however, show ECM-mediated softening in a stiff microenvironment and, in a soft environment, initiate cell invasion with peripheral softening associated with early metastatic dissemination. This exemplar of live-cell 3D mechanotyping supports that invasion increases cell deformability in a 3D context, illustrating the power of multimodal mechano-microscopy for quantitative mechanobiology in situ.

Conclusion

We demonstrate a multimodal imaging system that integrates an OCM-based subcellular mechano-microscopy system with a multi-channel confocal fluorescence microscopy system. This multimodal mechano-microscopy presents a new opportunity for quantifying local Young's modulus in live 3D samples with subcellular resolution, directly revealing mechanical phenotypes in situ.

Graphic:



Keywords:

Multimodal microscopy, elastography, OCM

Reference:

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