Mechanopathology

https://doi.org/10.1038/s41551-023-01121-2

A window into solid stresses within tumours

Bashar Emon & M. Taher A. Saif

Intravital microscopy reveals that the transmission of solid stresses within tumours depends on tumour size and on the tumour microenvironment.

The heterogenous compressive and tensile stresses resulting from dynamic cellular forces within solid tumours affect tumorigenesis and treatment outcomes[1–3]. Such solid stresses can compress blood and lymphatic vessels, and inhibit the delivery of drugs into the tumour[4,5]. They can also influence the movement of immune cells, and promote tumour cell invasiveness[6,7]. There have been considerable advancements in the development of techniques for the measurement of solid stresses in tumours ex vivo and in vitro. Yet, measuring solid stresses in tumours in vivo has remained difficult[3,8]. Writing in *Nature Biomedical Engineering*, Hadi Nia and colleagues now describe a technique that combines computational modelling and the multimodal intravital imaging of fluorescently labelled polyacrylamide beads as sensors to enable high-resolution three-dimensional measurements of local solid stresses within tumours in vivo, from the cellular scale to the tissue scale[9].

When incorporated into tumours, the hydrogel beads undergo deformation, owing to local solid stresses. These stresses can be computed from the measurements of the degree of deformation of the beads and their known elastic modulus. Specifically, Nia and co-authors used a computational model to determine the stresses on the basis of three-dimensional visualizations of the deformations measured by confocal microscopy, two-photon microscopy and optical coherence tomography (Fig. 1a). The beads were modified with fibronectin

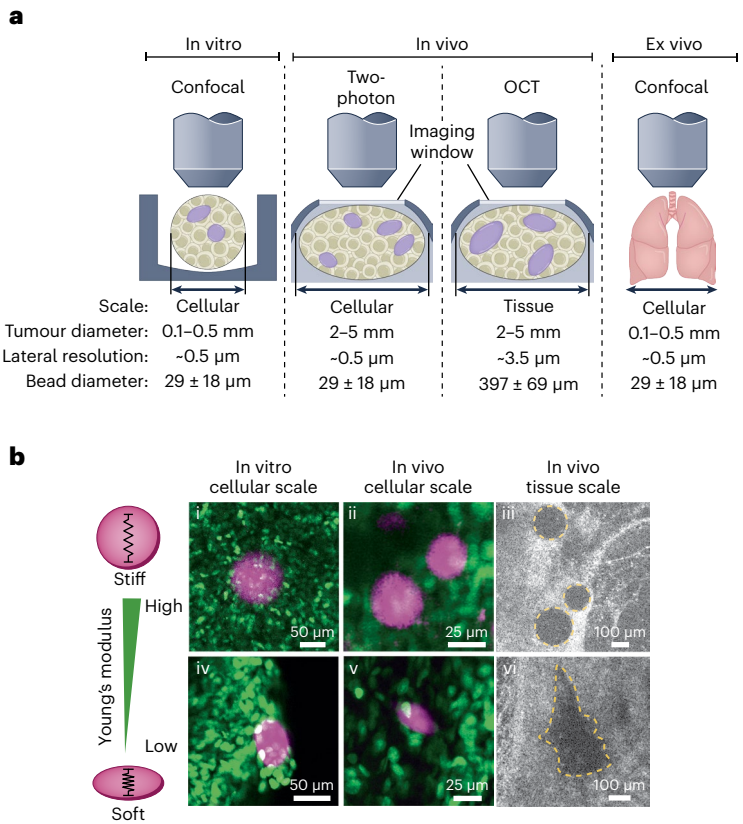

Fig. 1 | Measurement of solid stresses from the cellular scale to the tissue scale, in vitro, ex vivo and in vivo. a, By using fluorescently labelled polyacrylamide beads (shown in purple) as sensors, solid stresses in vitro, in vivo and ex vivo can be measured intravitally at the cellular and tissue scales via confocal microscopy (using cellular-scale beads in tumour spheroids in vitro and in lung metastases ex vivo), two-photon microscopy (using cellular-scale beads in tumours in vivo) and optical coherence tomography (OCT; using tissue-scale beads in tumours in vivo). **b**, The size and stiffness of the beads can be adjusted to alter the sensitivity and scale of the solid-stress measurements. The stiff beads (top panels) are circular, indicating low deformation, whereas the soft beads (bottom panels) undergo detectable levels of deformation. The yellow dashed lines in the images on the right indicate the outlines of the beads. Figure adapted with permission from ref. 9, Springer Nature.

to promote cell–matrix interactions and to enhance their uptake by tumours. By adjusting the size and mechanical properties of the beads (Fig. 1b), the authors measured solid stresses at the tissue scale (with beads approximately 400 μm in diameter) and at the cellular scale (with beads approximately 30 μm in diameter). The authors quantified solid-stress distributions in primary mammary tumours or metastatic lung tumours in mice. However, it was challenging to measure tissue-scale stresses in tumours in the lung because the injection of large beads into the vasculature often resulted in vascular blockage and lethality.

Nia and colleagues also compared the mechanical microenvironments of metastases and primary tumours. They found that solid stresses in metastases of breast cancer in the lungs of mice were substantially higher than those in primary tumours. Also, spatial analyses of the stresses revealed that they were higher in the peritumoural region than in non-tumoural and intratumoural regions. These findings suggest that the core of a tumour experiences isotropic stresses, whereas tensional stresses at the periphery cause anisotropy, emphasizing the need to consider spatial heterogeneity when studying the biomechanics of tumours. Moreover, by monitoring cellular-scale stresses for 7 days, the authors observed that the stresses increased during the initial two days, reaching a plateau from the second day until day 6. This implies a rapid accumulation of solid stresses within the early stages of secondary tumour growth. Also, the stresses measured using the cell-scale beads were lower than those measured with the tissue-scale beads. This may result from two possible and non-exclusive scenarios: the nature of the interaction between the cells and the beads depends on the size of the beads, or the larger beads undergo higher deformation owing to the collective behaviour of a large number of cells.

There are a few limitations to adopting Nia and co-authors' imaging-based approach. First, it is currently feasible only for small tumours in animals with implanted intravital windows or for small in vitro and ex vivo tissues via confocal microscopy. Second, the attenuation of optical light by tissue limits the range of tissue depths that can be effectively examined, which also restricts the extent of longitudinal imaging to about two weeks (tumour growth and the development of fibrosis owing to the implantation of intravital windows can push beads beyond the maximum imaging depth). Although the authors observed that solid stresses did not vary considerably with depth within a range of 160 μm, the depth limitations of light microscopy prevented the observation of variations in solid stresses deeper into the tumour. In addition, fluorescent light can induce cell-traction relaxation, and hence reduce solid stresses in the region of measurement[10]. Choosing longer wavelengths and keeping the intensity of light below safe thresholds may prevent any artefacts from fluorescence imaging[9]. Third, the beads cannot be placed within the tumour at prescribed locations; they are randomly distributed within the tumour. Furthermore, manufacturing variabilities may affect the size distribution of the beads, and this may affect the precision of the solid-stress measurements (solid-stress calculations rely on the deformations of the beads with respect to their initial undeformed size). Further work will possibly address many of these limitations and refine the methodology.

The origins of the heterogeneity of the solid stresses and their downstream effects need further study. Perhaps the dynamic contractility of cancer cells and stromal cells contributes to the heterogeneity of the stresses, leading to the remodelling (either stiffening or softening) of tumour stroma. Nia and colleagues' intravital-imaging methodology is limited by the indirect nature of the measurements of bead deformation caused by solid stresses; hence, the measured stresses do not directly correspond to the forces generated by tumour cells nor to the mechanical changes in the surrounding extracellular matrix. However, integrating the technique with biomechanical sensors that can measure cellular forces and matrix remodelling may reveal whether there is a synergy between solid stresses, cell contractility and stromal stiffening[11,12]. Such insights would inform the study of the biophysical mechanisms of tumour growth and metastatic progression, and the development of therapeutics that target matrix stiffness, cell force or stress. One key advantage of Nia and colleagues' intravital-microscopy approach is the longitudinal characterization of the isotropic and anisotropic components of solid stress, and their quantification from the early stages of tumorigenesis in small metastatic tumours. The technique should thus be generally useful for the investigation of the complex interplay between biophysical and biochemical processes in cancer and in other pathophysiological processes.

Bashar Emon[1] & M. Taher A. Saif[1,2] ✉
[1]Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA. [2]Cancer Center at Illinois, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
✉e-mail: saif@illinois.edu

Published online: 9 November 2023

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Competing interests

The authors declare no competing interests.