Shiver me timbers: Pulsatile contractility in model tissues

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Cells self-assemble and differentiate to form functional structures. This theme is ubiquitous throughout multicellular life and happens all along an organism's lifespan, from embryogenesis to tissue maintenance in a mature adult. Understanding how cells interact to form functional structures is a fundamental challenge in biology and biophysics; characterizing and targeting interactions such that they can be used for regenerative or therapeutic purposes is a major goal in bioengineering and medicine. In PNAS, Guevorkian et al. (1) study a multicellular aggregate that serves as a model tissue. Through experiments and analytical and numerical modeling, they find that small clusters of cells can respond to an externally imposed stretching force by pulsed contractions, which they dub "shivering" (1). The pulsatile nature of this response results from a threshold value of deformation required to trigger contractility; if the applied deformation is appropriately sized, contraction can reduce the deformation such that it then falls below the threshold value, causing the contraction to stop and then restart if the deformation continues. This active thresholded response constitutes an important potential class of interaction for sensitively shaping tissues.

Liquid-Like Tissues

On sufficiently long timescales, many tissues are shaped like liquid droplets and behave like liquids, in which individual cells can move and have measurable surface tensions. Probably the most well-known biophysical description of intercellular interactions that shape liquid-like tissues is the differential adhesion hypothesis (DAH) (2, 3). The DAH describes the surface tension in a tissue as a consequence of the adhesion between neighboring cells. The strength of this adhesion determines the energy cost of having nonadhering area and thus, acts as a surface tension (4). The DAH is particularly appealing, because it also describes how cells can be segregated by type as a result of the expression of different types and/or amounts of surface proteins (cadherins) that control adhesion; minimizing the system's total interfacial energy segregates cells (5, 6). Another description of intercellular interactions focuses on the mechanical interaction arising from cortical tension, which is generated in the actin cytoskeleton underlying the cell membrane. This differential interfacial tension hypothesis (DITH) describes cell positioning as a result of mechanical equilibration of the forces arising at points where two or more cells are joined (3, 7, 8). Recent work has shown that both adhesion and cortical mechanics work together to shape model tissues (ref. 9 and references therein).

Both the DAH and the DITH take a quasiequilibrium approach. That is, although the tissues in question are living systems that consume energy and thus are inherently far from equilibrium, the configuration of the system can be described by minimizing energy in the form of surface tension or mechanical tension. As a result, it is often easy to view the cells in these models as passive respondents instead of active participants in the system. Of course, this view is not strictly true; active motility allows cells to rearrange their position and shape, and cortical tension must be actively generated. Nevertheless, cellular activity is often a secondary consideration in work based on these hypotheses. A striking feature of the paper under consideration here (1) is that it explicitly probes active cellular response in tissues, using an experimental technique that is often used for equilibrium measurements. Understanding systems far from equilibrium is a major challenge to biological physics, and one often fruitful approach to doing so is to approximate one aspect of the system as being near some local equilibrium. Here, the authors began with experiments that might have seemed likely to lend themselves to such a quasi-equilibrium interpretation. Instead, they saw an active response clearly indicating that equilibrium concepts are insufficient to understand this system.

Small clusters of cells can respond to an externally imposed stretching force by pulsed contractions.

Active Tissues

As Guevorkian et al. (1) point out, the active mechanical response of cells to mechanical cues from the environment dictates a number of processes essential to building multicellular, differentiated, functional structures (10, 11). A spectacular example from embryogenesis is found in morphogenesis, when tissues are rearranged into new forms and even new topologies, largely as a result of cell-generated forces that drive local changes in cell shape (3). Examples of morphogenetic processes are gastrulation, when an embryo infolds to transform from a single-layer blastula to a trilayered gastrula (12), creating an opening, and fusion and closure processes that seal openings. Indeed, dorsal closure in late *Drosophila* embryogenesis is driven by pulsed contractile forces that helped inspire the model developed in the work of Guevorkian et al. (1, 13). There are a number of parallels between fusion and closure in embryonic morphogenesis and wound healing in mature adults (14, 15), suggesting that the work here is likely to connect with a variety of essential multicellular processes.

Guevorkian et al. (1) begin with experiments that use a small glass micropipette to aspirate a multicellular aggregate that acts as a model tissue. Micropipette aspiration applies controlled, negative pressure to draw material into the hollow micropipette, while imaging under a microscope; this technique is elegant and powerful, and it has been well-developed for sensitive measurements of isolated membranes and single cells (16, 17). Using this approach, a constant stress is applied, and the strain (which may vary with time) is measured to infer viscoelastic material properties. This technique was previously applied to multicellular aggregates by Guevorkian et al. (18). In contrast, measurements of the mechanical response of model tissues are most commonly done using parallel plate tensiometry (19), in which the tissue is held at a constant strain and the stress is measured while the system relaxes to equilibrium. Using micropipette aspiration instead of tensiometry or another bulk measurement method also means that stress and strain are directly applied to a much smaller number of cells (20).

Seeing Shivering

The differences between micropipette aspiration and parallel plate tensiometry allow Guevorkian et al. (1) to observe

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the shivering phenomenon that they study. According to the model developed by Guevorkian et al. (1), this shivering occurs because the experimenters apply constant stress but allow the strain to vary. Cells in the aggregate contract in response to the imposed strain as long as the imposed strain is over a threshold value. If the cellular contraction is sufficient to relax the strain to a below-threshold value, the contraction stops. With time, cells relax so that they are stretched beyond the threshold strain, and active contraction begins again; this results in shivering. A constant-strain measurement would not activate this phenomenon.

Furthermore, in micropipette aspiration, the cells stressed in the measurement need not be the entire volume of the aggregate. Rather, Guevorkian et al. (1) estimate that the cells involved are those cells in the micropipette itself and those cells in a subvolume of the aggregate that depends on the size of the micropipette ($\sim R_p^3$). Guevorkian et al. (1) also observe that large-amplitude shivers, which they attribute to contractions that are synchronized between multiple cells, are only seen when the aggregate radius is less than three times the micropipette radius. This finding corresponds to synchronization occurring over much of the aggregate only when a large volume of the aggregate is under strain. In contrast, Guevorkian et al. (1) see small contractions, attributed to the unsynchronized contractions of individual cells, across a much wider range of aggregate to micropipette size ratios.

The most immediate implication of this work is another instance of the old truism that the type of measurement done can qualitatively as well as quantitatively alter the phenomena studied. Specifically, studies of cellular and multicellular mechanical response may give very different results depending on the rheological approach (constant strain, constant stress, or other). Furthermore, the number of cells probed by a measurement can change the phenomenon observed, which is shown by the introduction of synchronized contractions by appropriate aggregate to micropipette size ratios. This finding highlights the importance of cooperative effects in multicellular systems and cautions against too aggressive scaling of models between systems of different sizes. Finally, Guevorkian et al. (1) show both that the strain required to activate contractions is thresholded and that cooperative, synchronized contractions primarily occur only when a sufficient fraction of the aggregate is strained. Thus, this work presents two possible mechanisms for fine-tuning and localizing active cellular response to specific stimuli.

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