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Current Opinion in Cell Biology

The dynamic mechanical properties of cellularised aggregates

Nargess Khalilgharibi[1,2,6], Jonathan Fouchard[1,6], Pierre Recho[3,6], Guillaume Charras[1,4,5] and Alexandre Kabla[3]

Cellularised materials are composed of cells interfaced through specialised intercellular junctions that link the cytoskeleton of one cell to that of its neighbours allowing for transmission of forces. Cellularised materials are common in early development and adult tissues where they can be found in the form of cell sheets, cysts, or amorphous aggregates and in pathophysiological conditions such as cancerous tumours. Given the growing realisation that forces can regulate cell physiology and developmental processes, understanding how cellularised materials deform under mechanical stress or dissipate stress appear as key biological questions. In this review, we will discuss the dynamic mechanical properties of cellularised materials devoid of extracellular matrix.

Addresses
[1] London Centre for Nanotechnology, University College London, UK
[2] CoMPLEX PhD Program, University College London, UK
[3] Department of Mechanical Engineering, Cambridge University, UK
[4] Department of Cell and Developmental Biology, University College London, UK
[5] Institute for the Physics of Living Systems, University College London, UK

Corresponding authors: Charras, Guillaume (g.charras@ucl.ac.uk) and Kabla, Alexandre (ajk61@cam.ac.uk)
[6] These authors contributed equally.

Current Opinion in Cell Biology 2016, **42**:113–120

This review comes from a themed issue on **Cell dynamics**

Edited by **Kenneth M Yamada** and **Roberto Mayor**

For a complete overview see the Issue and the Editorial

Available online 29th June 2016

http://dx.doi.org/10.1016/j.ceb.2016.06.003

Introduction

Cellularised materials are composed of cells interfaced through specialised intercellular junctions. These link the cytoskeleton of one cell to that of its neighbours allowing for transmission of forces over large length-scales. Cellularised materials are common in early development and adult tissues where they can be found in the form of cell sheets, cysts, or amorphous aggregates and in pathophysiological conditions such as cancerous tumours. In this review, we will discuss the mechanical properties of cellularised materials devoid of extracellular matrix (ECM). Early in development, the ECM is either absent or present in such scant quantities that it may play a polarisation role rather than a mechanical one [1–4]. In contrast, in adult tissues, the ECM tends to dominate mechanical responses and its remodelling by cells can conceptually be thought of as a very slow process.

The mechanics of cellularised materials are influenced by organisation and dynamics at both the molecular-scale and the cellular-scale. The relative importance of these two length-scales remains an open question and likely depends on what time-scale is being considered. For example, at time-scales shorter than a second, the contributions of genetic and biological regulatory pathways can safely be ignored whereas, for longer time-scales, biochemistry and signalling must to be taken into account.

In this review, we will first summarise experiments examining the rheology of cellularised materials, then we will turn to the respective roles of molecular-scale and cellular-scale phenomena in tissue mechanics, and finally, we will discuss the various theoretical approaches used to investigate tissue mechanics.

The rheology of cellularised materials

Cellularised aggregates possess complex mechanical properties. Indeed, dependent on the duration over which stress is applied, they behave either as liquids or solids. On time-scales of the order of 10s, cellular aggregates behave elastically, recovering their original shape after a transient application of force [5–8]. Similar relaxation times have also been measured in epithelia *in vivo* by monitoring the shape relaxation of a large tissue domain after its separation from the *Drosophila* pupa dorsal thorax epithelium by laser ablation [9]. Such consistency in mechanical relaxation time-scales may reflect the large degree of conservation of proteic constituents and molecular-scale organisation of intercellular junctions across species. An interesting feature of cellularised aggregates is that they often display non-linear behaviours. For example, monolayers devoid of a substrate display two clear regimes with different stiffnesses when subjected to increasing extension [10], suggesting that some mechanical elements may only be solicited above a certain threshold. In contrast with this short time-scale elastic response, on long time-scales (tens of minutes to hours), cell aggregates exhibit a liquid-like behaviour [5,8]. This tissue

fluidity, often associated with cellular-scale rearrangements, plays a central role during morphogenetic movement in *embryogenesis* [11]. At intermediate time-scales (typically tens of minutes), living materials, alongside most soft materials such as polymer melts, gels or elastomers, are neither purely elastic nor purely liquid. Indeed, experimental work by many groups has shown that the stress imposed on the cellularised material cannot be entirely dissipated implying that some cellular components must bear part of the tension applied [10,12,13]. Another manifestation of this dual nature is the slow flow (or creep) observed in some cellularised tissues when subjected to constant tension over minute time-scales [10]. Despite physiological relevance for the respiratory and cardiovascular systems, epithelial tissue rheology on sub-second time-scales has so far been insufficiently probed.

To describe the dynamic mechanical behaviours of tissues, linear rheology is often adopted as a conceptual framework (for an introduction see [14,15•]). This relies on describing a material's dynamic mechanical properties based on arrangements of standard mechanical components: springs that are elastic, dashpots that are viscous, and more complex active elements. Springs are parametrized by a stiffness that sets the force necessary to extend them and, when the force applied is removed, they return to their initial shape (Figure 1a). Dashpots are parametrized by a viscosity that sets the rate at which they can be deformed; in contrast to springs, they do not recover their initial length if the applied force returns to zero. Combinations of springs and dashpots arranged in series or in parallel can be used to mimic the dynamic mechanical properties of just about any passive material in a linear regime. For instance, a spring in parallel with a dashpot behaves on short time-scales like a fluid, but on long time-scales like a solid, with a transition between the two regimes controlled by the ratio between the viscosity and the stiffness (Figure 1a). A faithful description of the dynamic mechanical properties of cellularised materials often necessitates the inclusion of multiple rheological elements to take into account aspects of the macroscale behaviour that emanate from the molecular-scales and cellular-scales at different time-scales and different strain regimes (Figure 1b). The larger the number of components, the richer and more complex the behaviour can be. An extreme example is power law behaviour, which has been observed in single cells as well as cellularised materials [10,16,17]. It implies either the existence of a large number of relaxation timescales due to the multitude of distinct biochemical and physical phenomena taking place within the material or a hierarchical spatial organisation of the same units within the tissue (Figure 1b). Another fundamental challenge comes from the fact that constant metabolic energy generation allows for the generation of active stresses and strains inside the material due for instance to cell growth or contractility which must to be added to the passive rheological description. Though it is usually possible to design a rheological model that accurately fits the observed behaviour of tissues, the challenge resides in identifying the molecular-scale or cellular-scale origin of the observed mechanical response.

Mechanical behaviours of tissues arising directly from cellular mechanics

As cellularised materials are constituted of cells, cellular rheological properties naturally influence those of the aggregate. We now discuss what is currently known about the links between rheological properties of single cells and those of cellularised aggregates.

The actomyosin cytoskeleton is widely thought to be the most important determinant of cellular mechanical properties while microtubules are mostly involved in intracellular transport and cell division. Consequently, actomyosin rheology has been the subject of intense study (for a review [18,19]). Filaments within the actin cytoskeleton continuously turnover on time-scales of minutes. They are connected to one another through specialised crosslinkers whose attachment-detachment endows crosslinked actin networks with viscous liquid behaviours, dictates their relaxation time, and allows them to rearrange to adapt to new configurations [20–24]. Myosin motors play a dual role as crosslinkers and force dipoles generating tension within filaments. At time-scales long compared to F-actin turnover (>1 min), crosslinked actomyosin networks generate a constant tension. Overall, the exact rheological behaviour of cellular actin networks depends on their turnover, connectivity and contractility, all of which can be modulated by signalling [25•]. In a continuum limit and close to thermodynamic equilibrium, the cytoskeleton is well understood within the theoretical framework of active gels (reviewed in [26•]). Many studies on monolayers in embryos and *in vitro* suggest that as in isolated cells, myosin contractility plays a central role in tissue mechanics and dynamics [9,10,27•]. In particular, the actin cortex, a submembranous layer of actin, myosin, and actin-binding proteins present in most isolated animal cells [28] has been shown to reorganise embryonic epithelia *via* pulses of contractility arising in the medial apical cortex of individual cells [29,30]. To add further complexity, the mechanical properties of cells can be altered by forces through mechanotransduction [21].

Though much work has concentrated on F-actin, there is mounting evidence that intermediate filaments play an important role in the mechanics of isolated cells [31–34]. Intermediate filaments are strain-stiffening [35] and can withstand far higher strain than F-actin [36] (reviewed in [36]). In isolated cells, keratin or vimentin depletion both lead to a decrease in cytoplasmic elasticity