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Mini review

Methods to mechanically perturb and characterize GUV-based minimal cell models

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ABSTRACT

Cells shield organelles and the cytosol via an active boundary predominantly made of phospholipids and membrane proteins, yet allowing communication between the intracellular and extracellular environment. Micron-sized liposome compartments commonly known as giant unilamellar vesicles (GUVs) are used to model the cell membrane and encapsulate biological materials and processes in a cell-like confinement. In the field of bottom-up synthetic biology, many have utilized GUVs as substrates to study various biological processes such as protein-lipid interactions, cytoskeletal assembly, and dynamics of protein synthesis. Like cells, it is ideal that GUVs are also mechanically durable and able to stay intact when the inner and outer environment changes. As a result, studies have demonstrated approaches to tune the mechanical properties of GUVs by modulating membrane composition and lumenal material property. In this context, there have been many different methods developed to test the mechanical properties of GUVs. In this review, we will survey various perturbation techniques employed to mechanically characterize GUVs.

Contents

1. Introduction

Theoretical physicists date the formation of elementary particles back to 13.8 billion years ago after the Big Bang, following which

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these particles formed atoms and then molecules of various complexity. Somewhere between 13.8 billion years ago and now, various inanimate molecules, complex and simple, organic and inorganic, self-assembled to form a condition we call life. Biologists have studied numerous cellular components and pathways to understand life and how it came to be, however, due to intrinsic complexity of even the modest of life forms with few hundred genes, how molecules synergistically self-assemble and give rise to a complex self-replicating, metabolizing and evolving matter remains elusive to all. Nature has mastered creation and sustenance of life by the way of making it robust enough to thrive, and to be efficient, dynamic and durable to endure abrupt environmental changes; in cells, this requires machineries that can interact in extremely entangled web of networks, with auxiliary processes having complex and redundant cellular components to ensure that life thrives. This makes the dissection of cellular processes to understand how they self-organize to produce life an impractical feat.

Bottom-up reconstitution of a minimal cellular process inside a cell-mimicking environment has recently become a popular approach to understand the making of life from its non-living parts. The bottom-up approach seeks to assemble biology one part at a time and evolve the hierarchy of complexity to understand emergent behavior of cellular processes. A fair number of reviews over the years have illustrated the advantages, the aims, and novel findings from bottom-up assembly of cell models [1–9]. Of the major challenges in reverse engineering a cell is creating a 3-dimensional, few micrometer-sized container that segregates the cytosolic lumen from the external environment. Thus, one of the initial tasks in bottom-up reconstitution was to identify a confining substrate that closely mimics the function and chemical composition of the cell membrane. Among other protocell compartments such as droplets, coacervates enriched with charged molecules [10,11], capsules made of polymeric amphiphiles (including block copolymers and peptides) [12–15] and proteinosomes [16,17], GUVs made of lipid bilayers have been largely used to create a cell-sized (1–100 μm) confinement to encapsulate numerous cellular components. Like natural cell membranes, GUVs can be made from various compositions of lipids, mainly phospholipids and cholesterol, whose amphiphilic nature allow them to self-assemble into spherical compartments in an aqueous solution. Various methods have been developed to efficiently generate GUVs [18–20] and numerous review articles have described these methods [21].

Many in the field of synthetic biology resorted to using GUVs for encapsulation of minimal set of biomolecules, thereby disentangling a specific cellular phenomenon from the cytosol that is present in cells. Encapsulation of purified proteins in GUVs and synthesis of proteins in GUVs using transcription-translation (TX-TL) have been used to study cellular processes in a minimal artificial setting. Protocells reconstituting eukaryotic and prokaryotic cytoskeletal dynamics [22,23], communication through membrane pores of various kinds [24,25], and protein-induced membrane remodeling [26,27] are among notable GUV-based cell model studies over the years. Among these, many groups have made significant advances in the study of cytoskeletal dynamics and self-assembly [28]. Characterization of actin architecture in confinement and mechanical characterization [23,29–32], microtubule-assisted GUV deformation [33] and FtsZ remodeling in response to loading [22] are examples among a large cohort of studies reconstituting cytoskeletal proteins. Mechanosensitive channels [24], toxin pores such as alpha hemolysin [34], and other membrane proteins have also been inserted into GUV bilayers to recapitulate numerous cellular processes including mechanotransduction [24], inducible chemical reactions and proximity sensors [35]. Furthermore, others have also taken on ambitious aims to realize cellular processes such as motility [36], division [37], and metabolism [38].

Cells are able to dynamically change their biophysical properties, particularly their mechanical characteristics, in response to environmental cues, in order to endure and thrive in different environments. Cells do this by changing their membrane composition, cytoskeletal organization, and cell shape, of which none are mutually independent. As simplified cell models, there is a large body of work characterizing biophysical properties of GUVs including GUV domain formation as a result of liquid-liquid lipid phase separation [39], GUV mechanics [40], permeability and electrical property [41]. However, an overly simplified cell model like the GUV is not nearly as durable. Thus, many have taken on the challenge to modulate the mechanical property of GUVs. In this review, we will explore approaches used to alter the mechanical properties of GUVs and on techniques used to mechanically characterize GUVs.

2. Modulating GUV mechanics

Mechanical robustness and durability of cells is one of the key characteristics for cell survival in changing and hostile physical and chemical environments. Mechanical make-up of a cell is endowed by complex cytoplasmic content comprised of numerous proteins and a heavily reinforced membrane with diverse lipid and membrane protein composition. Unlike cells, inherently and by design, GUV-based minimal cell models are not complex enough to possess a robust mechanical profile for them to endure and survive harsh physical environments. However, recent advances have focused on enhancing the mechanical property of GUV-based minimal cell models by changing luminal and membrane make-up of GUVs. The definitions of commonly characterized mechanical properties are summarized in Table 1. In this section, we will highlight and describe advances in improving mechanical durability of GUV-based minimal cell models.

2.1. Membrane modulation

Lipid bilayers are 2-dimensional fluids held by compressive forces from surrounding water molecules due to their attractive hydrogen bonding forces [42]. Since lipid bilayers alone do not construct a versatile and durable boundary, cells mitigate this by utilizing different lipid compositions and membrane proteins. About half the surface area of the cell membrane is covered by proteins which reinforces membrane mechanics and shape by lowering membrane fluidity via restricting lateral diffusion in response to protein crowding [43,44]. Unlike cells, bare GUV-based cell models are not naturally enriched with a diverse set of lipids and membrane proteins. Thus, GUVs are too fragile to endure perturbations in changing environments, consequently requiring delicate handling. However, studies have utilized and demonstrated different successful means to enhance the mechanical property of GUV bilayer membranes.

One of the commonly used methods to modulate mechanical property of GUV lipid bilayer is by tuning the lipid composition. For example, Kato et al. revealed, using optical tweezers, that membrane rigidity can be enhanced by increasing acidic phospholipid content in GUVs [45]. Furthermore, it was also shown that, using micropipette aspiration, different membrane sterols differentially regulate the compressibility modulus and lysis tension of membranes in GUVs [46]. Others, using electrodeformation of GUVs, have demonstrated that cholesterol plays a lipid-specific differential role in regulating membrane bending rigidity [47]. In this work, they showed that addition of cholesterol to DOPC vesicles results in little to no difference in bending rigidity whereas addition of cholesterol to sphingomyelin vesicles reduces bending rigidity [47]. Additionally, it was also found that bilayer asymmetry plays a role in GUV membrane rigidity due to changes in bending energetics

Table 1
List of GUV mechanical properties and their definition.

Mechanical Property	Definition
Young's modulus	A physical quantity that measures the general stiffness of elastic solids. Generally not a quantity used to characterize GUVs.
Bending rigidity	Physical quantity that measures resistance of an elastic material to changing its curvature. Membrane bending rigidity is one of the commonly measured GUV physical quantities.
Area expansion modulus/stretching modulus	Under tensile stress, an elastic material can expand to failure. Area expansion modulus measures the resistance of an object to expand/stretch under load. In GUVs, area expansion modulus measures how GUV bilayers withstand area expansion.
Membrane tension	Membrane tension is a state property measuring the force per area acting on a membrane cross-section. For GUVs, different approaches can be used to either measure membrane tension of GUV bilayer at an unperturbed state or to modulate membrane tension.
Viscoelasticity	Like most biological materials, GUVs exhibit both elastic and viscous properties. Viscoelasticity measures time dependent behavior of a material by measuring loss modulus from the relaxation curve during loading and unloading phases of the material.
Area compressibility modulus	This is the same as area expansion modulus when the load is compressive
Deformability	In GUVs, deformation modes are often elliptical. Deformability measures how much elliptically deformed GUVs deviate from a unit circle by calculating the ratio of major axis to minor axis.

resulted from how lipids with different spontaneous curvatures are distributed in a bilayer [48].

Additional to modulating membrane mechanics via tuning lipid content, another approach is to introduce external molecules such as proteins and polymers. For example, mechanical tests using micropipette aspiration of GUVs show that addition of membrane proteins Ca^{2+} ATPase into lipid bilayer of GUVs regulate membrane fluctuation and increase bending rigidity [49]. Similarly, a recent investigation revealed the insertion of lactose permease into the GUV bilayer nonlinearly regulate membrane rigidity [50]. Besides proteins, addition of other polymers can also alter GUV mechanics. For example, addition of block copolymers to lipid bilayer in GUVs greatly increases the stretching modulus [51].

2.2. Lumenal modulation

Cytoskeletal proteins self-assemble into complex and dynamic structures that endow the mechanical property of a cell [52]. This equips cells with the ability to endure and survive mechanically and chemically changing environments, thus making them capable of migrating in extremely constricted spaces or bearing heavy loads. Cells achieve this not by simply being physically tough but also by being adaptive enough to change their physical property in response to the changing environment mainly via cytoskeletal reorganization triggered by mechanotransductive signaling. Unfortunately, bare GUVs are helpless against the slightest physical disturbance. Similar to tuning bilayer content, this encourages researchers to study how lumenal content regulate the mechanical property of the whole GUV.

There are numerous studies encapsulating different synthetic and biological molecules to regulate the mechanical property of GUVs. Among these, one work demonstrated that the presence of agarose in GUVs increases the viscoelastic property of GUVs by increasing relaxation time during electric field perturbation as compared to agarose-free GUVs [53]. Others have also illustrated the role of other biopolymers in regulating deformation modes of GUVs subjected to osmotic shock [54]. Towards understanding cytoskeletal networks in an isolated environment and equipping GUVs with cell-like mechanical characteristics, many have encapsulated cytoskeletal proteins inside GUVs. For example actin cortex has been shown to increase the compressibility modulus of GUVs compared with cortex-free GUVs [55]. Similarly, GUVs encapsulating actin cortex subjected to electric field showed increased resistance to electroporation [56]. Furthermore, we recently demonstrated that different actin architectures, particularly filaments, crosslink networks and cortex shells, differentially regulate GUV mechanics [30].

While identifying methods towards making robust GUVs will propel the field of bottom-up biology to create versatile cell models, it is equally important to understand and utilize methods and techniques that allow us to manipulate and perturb GUVs to better understand their physical properties.

3. Methods to perturb GUV-based cell models

In order to extract quantities that will inform us about mechanical properties, GUV-based cell models must be subjected to a perturbation that results in deformation. The resulting deformation profile with respect to the perturbing load (stress) allows us to obtain various mechanical characteristics. Several methods have been developed to perturb GUVs and here, we will describe some of the principal methods that have been applied to mechanically characterize GUVs. Almost all of these methods were initially designed for mechanical characterization of cells. The mechanical property of a cell is intimately linked to a myriad of its pathological and developmental states, and thus can be used as indicators of hematologic diseases, cancer progression and metastasis, and cardiovascular health. This incentivized the development of various force application techniques to measure Young's modulus, viscous response, and membrane bending rigidity of the whole cell and cell components such as the cytoskeleton and the membrane. These techniques have been repurposed and applied to mechanically characterize GUV-based protocells.

3.1. Micropipette aspiration

The predecessor of what we now call micropipette aspiration, then referred to as "sucker" or "cell elastimeter", was developed in the early 30s as described by Vles [57] and further modified in the early 50s by Mitchison and Swann in its application to investigate membrane properties of sea-urchin eggs [58]. Over the following years, the set up morphed to its most familiar and current version by notable efforts from Rand and Burton [59] and Evans and Hochmuth [60]. Micropipette's ability to seamlessly apply well controlled and defined stresses onto cell-sized samples has made it among one of the most popular mechanical perturbation methods towards material characterization of a cell and extracted nuclei. Young's modulus of cells (considered as homogenous solids), surface tension, and viscous properties for creep profiles are among the mechanical properties measured by using micropipette aspiration.

Principally, micropipette aspiration applies negative pressure (aspirating) to single cells/GUVs suctioning them into a parallel-walled capillary of diameter smaller than the sample (Fig. 1 A). Although there are various designs of micropipette aspiration setup

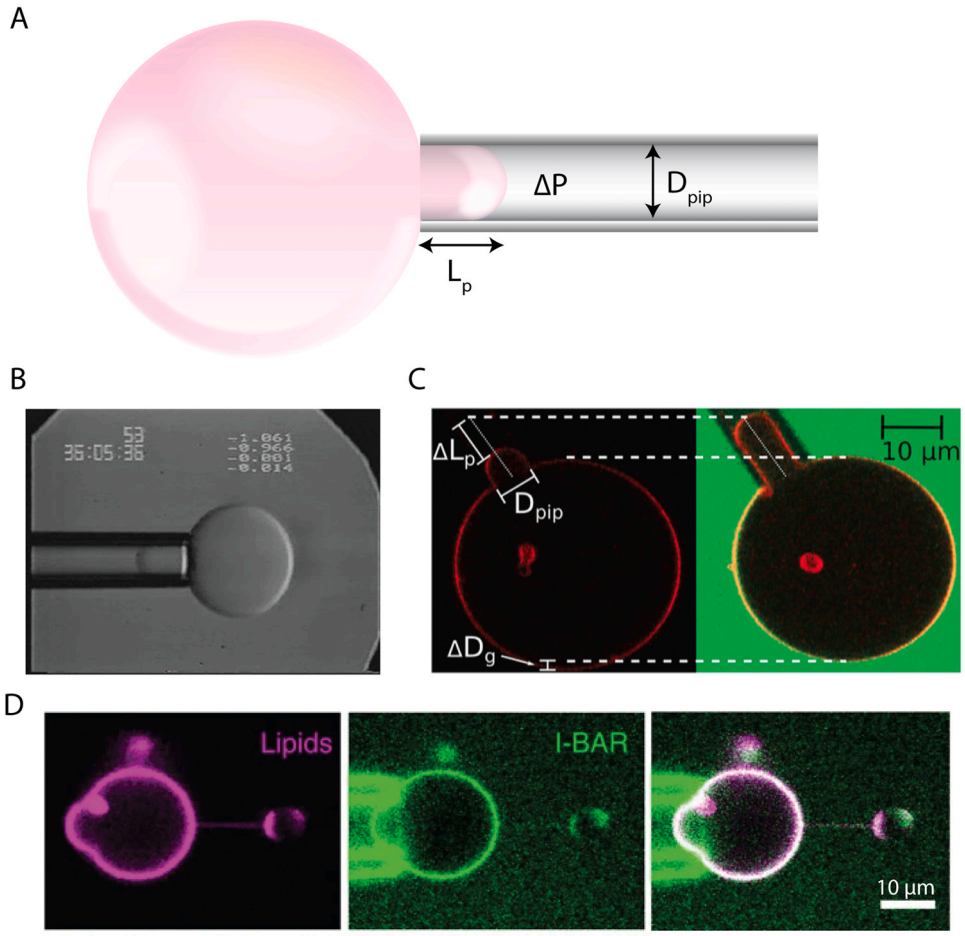

Fig. 1. Micropipette aspiration of GUVs. (A) Schematic representation of a micropipette capillary aspirating on a GUV. ΔP is the pressure difference between ambient pressure outside of the pipette and pressure inside the pipette. D_{pip} is the diameter of the pipette and L_p is the protrusion length of GUV at ΔP. (B) Representative brightfield image of an aspirated GUV to measure elastic area expansivity modulus. (C) Aspirated GUV with fluorescently labeled lipid bilayer (red) under iso-osmotic condition (left) and GUV immersed in a hyperosmotic buffer containing carboxyfluorescein (green) with a larger aspirated protrusion length (right). (D) Relationship between GUV and membrane curvature sensing protein (I-BAR) by modulating membrane tension using micropipette aspiration and optical tweezers for measure aspiration force and pulling membrane nanotubes, respectively. Panels B, C, D are adapted from [65], [70], and [71], respectively.

over the years depending on researchers' preference, the setup generally comprises of a custom-prepared glass capillary pipette, a pipette holder, a reservoir for pressure control and a pressure controller of some sort. Briefly, pipettes are pulled commonly using a pipette puller and further cut using a microforge to the desired diameter at the pipette mouth. For GUV aspiration, pipette inner diameters in the range of 2–10 μm are typically used. Pipettes are surface-coated to minimize sample adherence and friction using reagents such as BSA. Manipulation of pipette to selectively aspirate on an isolated sample is done via a micromanipulator onto which a micropipette holder is mounted. In earlier days of micropipette aspiration development, negative aspiration pressure was attained by adjusting height of a reservoir inducing a change in hydrostatic pressure. However in recent years, high precision pressure controllers can replace water columns to apply high resolution and stable pressure points [61]. The micropipette aspiration setup is typically installed on an inverted microscope mounted on a vibration isolation table.

Since the 1980s, numerous micropipette aspiration studies have been conducted to measure material properties of model membrane. Elastic properties such as bending rigidity, area expansion and tensile strength of GUVs with different bilayer compositions (Fig. 1B) [4,5,62–65], role of increasing membrane tension and membrane curvature in lipid domain formation [66,67], thermomechanical properties [68] and viscous properties of GUVs with gel phase bilayers [69] are among the notable works. In addition to characterization of bare GUVs, other studies have also used micropipette aspiration to study various aspects of GUV cell models. These include studies of characterizing the hydraulic conductivity of membrane water channel permeability (Fig. 1 C) [70] and identifying membrane curvature sensing proteins (Fig. 1D) [71]. Later developments have combined micropipette aspiration with other perturbation setups. For example, micropipette aspiration combined with optical tweezers provides the ability to induce high membrane curvatures while controlling of GUV membrane tension [72] and has been used to acquire precise measurements of membrane tension [73] and protein clustering [71]. Although a powerful tool to manipulate and apply load to GUVs, limitations such as low measurement yield, setup complexity requiring expertise and specialized equipment to prepare capillaries remain a challenge.

3.2. Atomic force microscopy

Successor to the scanning tunneling microscope (STM), a Nobel Prize winning invention for profiling surface topology at the atomic scale, atomic force microscopy (AFM) replaces the current tunneling tip of STM with a force sensing cantilever [74]. The original invention of the AFM was to correct critical surface imaging limitations of the STM, which was restricted to electrically conductive metals and semi-conductors. However, its ability to apply and sense forces as

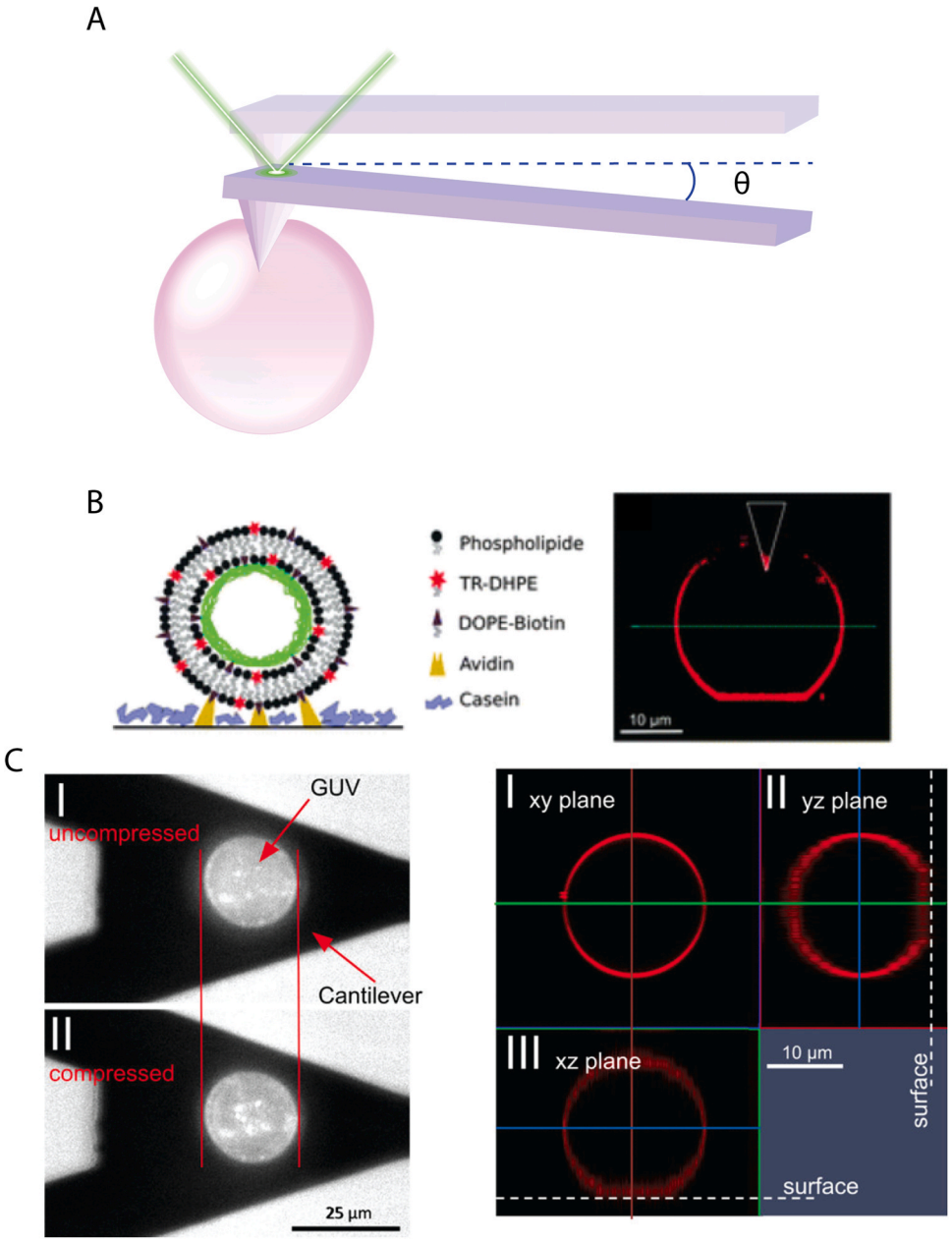

Fig. 2. AFM indentation of GUVs. (A) Schematic representation of AFM indentation of a GUV. Laser light reflecting from tip of the cantilever is used to measure deflection, θ, of cantilever in response to GUV indentation. (B) Actin cortex encapsulating GUVs immobilized via biotin-avidin linkage subjected to AFM indentation using a conical tip to measure compressibility modulus and pre-stress membrane tension. (C) Compression of actin cortex encapsulating GUVs using tip-less cantilever. Similar to (B), parallel plate compression using AFM is used to extract physical properties of actin cortex GUVs including compressibility modulus and membrane tension. Panels B and C are adapted from [88] and [55], respectively.

low as a few piconewtons allowed scientists to repurpose AFM for measuring physical properties of biological samples.

In principle, AFM is an elastography instrument that uses a cantilever of specific spring constant with a microscale indentation probe at the tip (Fig. 2 A). Typically, conical silicon indentation tips are commonly used for indentation experiments, however, spherical geometries using glass tips and no tip (used for compression as opposed to indentation) have been frequently used. AFM is equipped with a laser reflecting off the back of the cantilever tip and a photodiode detects shifts of the reflected laser. These shifts enable the precise measurement of cantilever deflection as it comes to contact with a sample. A piezoelectric driver moves the cantilever towards immobile biological samples (adherent cells or substrate-immobilized suspension samples), thereby applying stress resulting in the indentation of the sample and deflection of the cantilever. Given that the cantilever has a known and calibrated spring constant, the detected cantilever deflection is then used to determine the precise loading force by the indenter. This results in a force-indentation curve that can be converted to a stress-strain curve.

Numerous studies have used AFM to characterize mechanical variability among cell types from bacteriophage [75] to eukaryotic cells [76–79], detect disease progression such as cancer metastasis [2, 80, 81], identify the mechanical topology of cytoskeletal networks [82,83], study viscoelasticity of the cell membrane [84,85], and to investigate dynamic mechanical properties of protein complexes [86,87]. Given its ubiquitous use in biophysics, AFM can easily

be adopted to study GUV mechanics. However, as of yet, mechanical studies of GUV-based and other cell models using AFM remain largely underutilized. Studies investigating GUV membrane tension and area compressibility modulus using conical indenters (Fig. 2B) [88], and measurement of the bending rigidity of DPPC liposomes [6] are among the few works using AFM to mechanically characterize GUV cell models. Additionally, using a modified AFM with tip-less cantilever, Schaefer et al. investigated area compressibility modulus of GUVs with and without reconstituted actin shells and found that actin shells significantly stiffen GUV membrane with up to 10-fold increase in compressibility modulus from 0.12 N/m (actin-free DOPC GUV) to 1.25 N/m (actin-shell GUVs) (Fig. 2 C) [55]. Although AFM can be implemented for GUV mechanical studies, limitation such as complexity of setup, prone to noise, requirement of substrate adhered samples and extended measurement time are worth noting.

3.3. Acoustic manipulation

Acoustic perturbation is a field gradient perturbation approach used in numerous cell manipulation experiments as the preferred non-invasive perturbation technique with high precision. One of the early works implementing acoustic waves to cells was conducted in the early 70s by Dyson et al. on blood circulation where they observed “arranged” red blood cell aggregates [89]. Alignment and separation of red blood cells was further investigated and attributed to standing waves induced by ultrasound by Baker the following year [90]. Following these seminal works, commonly integrated with microfluidic devices, acoustic perturbation has been used for cell manipulation studies including patterning for tissue engineering [91,92], trapping for single cell analysis [93–95], sorting specific cell types from a heterogeneous population [96–98], and single cell mechanical testing [99–102].

A sound source induces acoustic radiation force creating a time-averaged pressure field deviating from the local ambient static pressure due to velocity change in the medium. An acoustic device for cell or GUV manipulation leverages this characteristic to generate standing waves by using two opposing and identical acoustic waves (Fig. 3 A). At a given frequency and acoustic intensity, a stable and steady pressure field is created with high pressure nodes (antinodes) and zero-pressure nodes simply referred to as nodes (Fig. 3 A). Particles like cells, with a positive acoustic contrast factor, are driven by the acoustic radiation to localize at the nodes of the pressure field away from the antinodes. Theoretically, the node is a single point and thus a 3-dimensional particle suspended in the pressure field will be subjected to some level of radiation force and this can result in deformation of cells and GUVs. There mainly exist two different types of acoustic manipulation platforms utilized in perturbation of biological samples and these are surface acoustic waves (SAWs) and bulk acoustic waves (BAWs). SAWs are used to induce acoustic waves that are spatially specific to perturb a thin layer of liquid at the surface of the substrate. Interdigital transducers (IDTs) are used to drive a piezoelectric substrate, commonly lithium niobate, to transmit acoustic waves resulting in radiation capable of creating a pressure field to a thin layer of fluid dispensed on top of the piezo substrate [102–104]. BAWs on the other hand are transmitted through the sample medium. Commonly, for standing BAW, two piezo transducers or one piezo transducer and a reflector, are arranged with a space in between where the sample solution will be dispensed. Driven by a function generator, acoustic waves will be transmitted through the solution resulting in a pressure field with stable nodes and antinodes.

Although there is not a large body of work implementing acoustic radiation to perturb GUV-based minimal cell models, some studies have used this platform to perturb and manipulate GUVs in order to study chemical signaling between GUVs and red blood cells [105], characterize membrane elasticity (Fig. 3B) [106], and spatially align GUV colonies (Fig. 3 C) [107]. Unlike cells, some minimal cell models, either GUV-based, condensates or otherwise, can be delicate and easily perturbed by contact-based and invasive perturbation methods. Thus, remote perturbation techniques such as acoustic manipulation can be of great benefit for characterization and manipulation of cell models. Furthermore, with the fast-growing field of synthetic biology where structurally and biochemically complex cell models are developed, the use of acoustic perturbation for characterization and perturbation of minimal cell models will be of great utility. For GUV-based cell models, which are not as robust as cells, remote manipulation and perturbation render a useful platform for mechanical studies. However, lack of spatial resolution for high precision control remains a limitation of acoustic manipulation devices.

3.4. Optical stretching

In the mid-80s, we learned from Ashkin et al. that the momentum of a gradient laser light with a point focus is capable of trapping particles with sizes ranging from micrometers to atomic scales in 3 dimensions [108,109]. Using this method, for the first time, visible argon-laser light was used to trap viruses and bacteria [108]. Through the years, optical tweezers have been deemed one of the crucial methods for manipulations of cells and sub-cellular biological materials. In 2000, Guck et al. demonstrated that optical tweezers can be transformed into optical stretchers using identical opposing laser gradients that are capable of stretching/deforming biological samples [110]. Over the following years, optical stretchers were extensively used for mechanical characterization of cells. Mechanical properties of eukaryotic cells [111,112], differential mechanical properties of cells in response to drug treatment [113], differential mechanics of healthy and metastatic cancer cells [114,115] have been studied using optical stretchers.

Stretching principles of optical stretchers are similar to the trapping mechanism of optical tweezers. When an unfocused gradient laser light passes through a transparent material with a different refractive index compared to the native external environment, take a cell for example in a solution, light will change its path and gain momentum which will be transferred to the material as a scattering force that propels the cells in the direction of light path. Having two identical and opposing gradient laser lights, opposing propulsion of a cell will result in stretching of cell/GUV (Fig. 4 A) [110]. Unlike single beam optical tweezers which require focusing for 3-dimensional trapping of samples, double beam optical stretchers do not require focusing thereby minimizing damage of biological samples from high intensity lasers [110,116]. For mechanical characterization of biological materials, optical stretchers are often integrated with microfluidic devices in order to easily focus and deliver samples to the trapping/stretching region [117–119].

In recent years, optical stretchers have been utilized for mechanical perturbation of GUV cell models. Among these, dual beam optical stretchers integrated into a microfluidic device have been used to study elastic constants [120] and bending modulus of GUVs (Fig. 4B) [121]. Viscoelastic properties of GUVs have also been investigated using optical stretchers (Fig. 4 C) [122]. Others also have investigated lipid oxidation in GUVs in response to optical stretcher-induced change in membrane tension [123]. Beyond direct mechanical characterization of optical tweezers to perturb GUVs, they have also been used together with micropipette aspiration approach to modulate membrane tension [72,73]. Over the years, many of the limitations of optical stretchers, such as laser-induced damage, have been improved, yet the effect of heat and laser-induced damages on

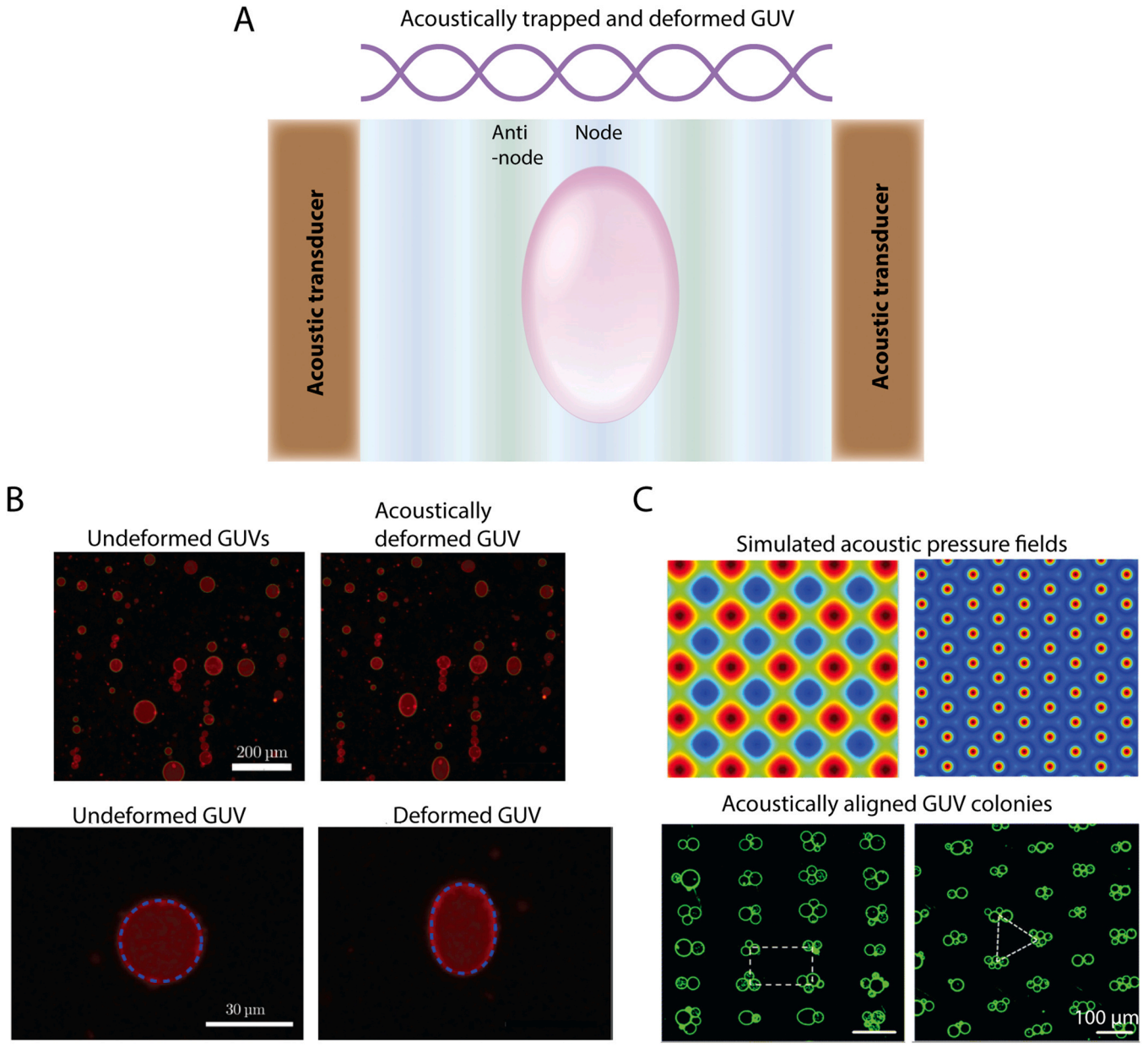

Fig. 3. GUV manipulation via acoustic radiation. (A) Schematic representation of an acoustically deformed GUV. A pair of piezo transducers operated at identical frequency are used to create standing waves generating pressure fields of nodes and anti-nodes in the GUV-containing solution. (B) Acoustic radiation used to mechanically deform GUVs. Membrane elasticity and other physical properties are obtained using acoustic radiation-induced GUV deformation. (C) Precise control of pressure field in GUV-containing solution allow the formation of aligned GUV colony formation. Chemical interaction between GUVs and GUV-cells are studied using acoustic manipulation/alignment of GUVs and cells. Panels B and C are adapted from [106] and [107], respectively.

GUVs and GUV-encapsulated proteins, especially during extended perturbation periods, remain an unresolved issue.

3.5. Electrical perturbation

Initial use of electrical pulses to perturb single cells dates as far as the mid 20th century where researchers observed motile responses of sperm cells [124] and aggregation of red blood cells [125]. However, the revolutionary use of electric fields to permeabilize the membrane, thus allowing access to the cytoplasm, was realized after seminal studies from Sale and Hamilton demonstrating cellular lysis when high energy electric pulses were applied [126,127]. With further control and modulation, non-lethal access to the cell cytosol via electropermeabilization enabled technologies such as delivery of molecules for therapeutic purposes [128–130] and transfection [131–133]. The use of electroperturbation to mechanically characterize cells began in the early 80s with studies showing deformability of red blood cells in response to electric fields [134] and studies measuring elastic modulus of blood cells [135]. Over the following decades, advanced and integrated variation of electroperturbation devices were used to mechanically characterize cells [136–140]. Although a facile approach to perturb single cells, complexity and variability of cells, which consequently result in variable electrical properties, such as membrane dielectric constant and

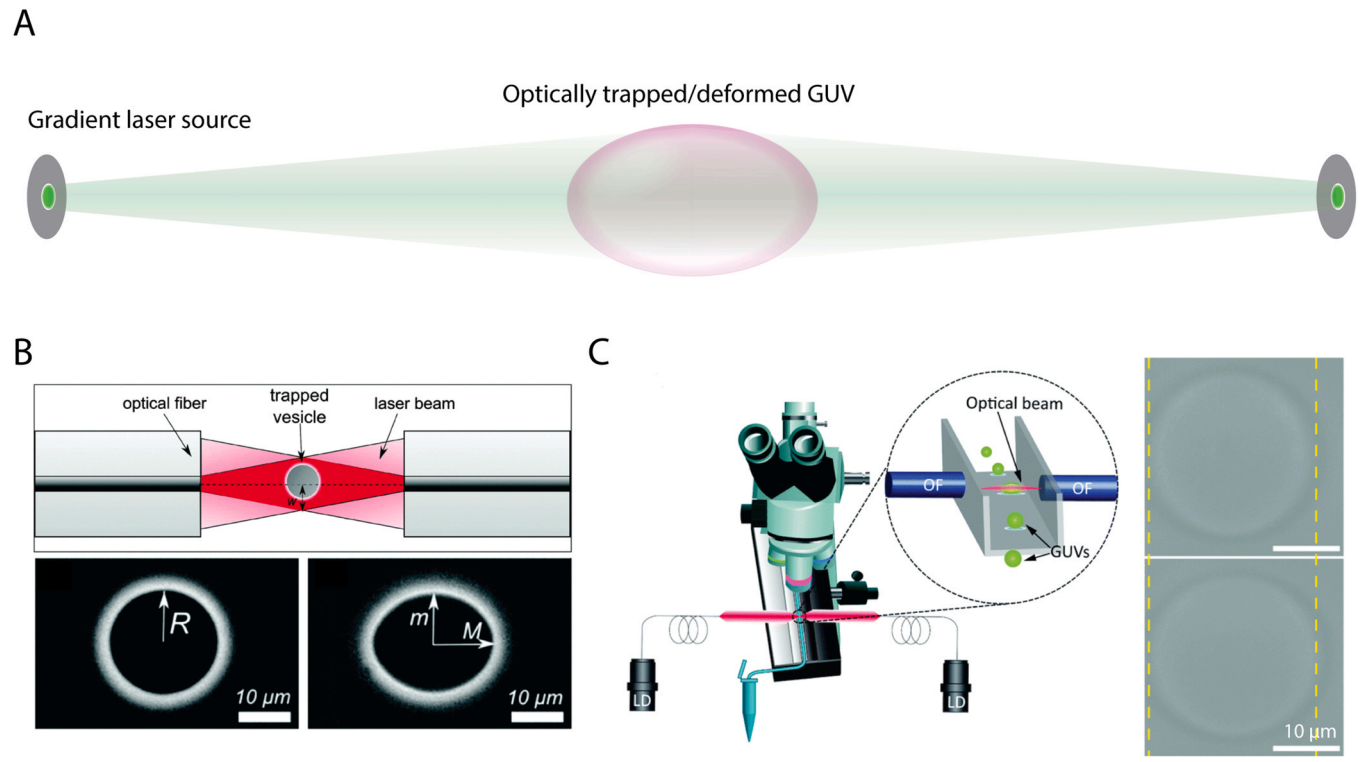

Fig. 4. Optical stretching of GUVs. (A) Schematic representation of an optically stretched GUV. Opposing light momentum results in the stretching of the GUV. Two opposing and identical gradient laser sources beam lasers on a GUV. (B) Optical trapping and stretching of GUVs to study elastic property of GUVs and the effect of generated heat from absorbed laser on GUV deformability. (C) Characterization of GUV bilayer viscoelastic properties. Stretching time constants in the slow stretching regime (elastic regime) are measured from optically stretched GUVs to characterize their viscoelastic properties. Panels B and C are adapted from [120] and [122], respectively

cytosolic conductivity, has made identifying a universally reliable mathematical model describing the influence of electric field to cell's mechanical property a difficult feat.

Easy to make, cheap and customizable aspects of the electroperturbation setup make it attractive as a facile method for mechanical characterization of biological samples. Essentially, the setup comprises a chamber with parallel electrode walls into which samples are dispensed in, a function generator, a microscope and an image acquisition system. Copper tapes or platinum wires can be used to make the chamber with a known distance between two electrodes for precise control of electric field [141]. Depending on the aim of the experiments, both AC and DC electric fields have been applied in numerous studies. AC fields induce steady, frequency dependent, elliptical deformation of GUVs and are largely used to measure properties including membrane bending rigidity, membrane capacitance [142], and area dilation [143]. DC fields, on the other hand, due to the high intensity pulses that can be induced, have been widely used to induce electroporation with aims to investigate membrane rheological dynamics and stability [144,145].

Unlike other perturbation methods that operate by applying stresses either through contact or propagation via a medium that does not depend on inherent property of the sample itself, electroperturbation leverages unique properties of lipid bilayers in biological samples. Lipid bilayers create a physical insulating barrier between the cytosol and the outer environment, thereby permitting separation of charges of molecules and ions (Fig. 5 A), which is the critical mechanism by which stresses are induced to result in electromechanical deformation of biological samples. Expectedly, this sparked researchers' interest to study electromechanical response of lipid bilayers in a cell-like environment, thus encouraging membrane biophysicists to use GUV-based cell models to study lipid bilayers of various compositions. Critical membrane voltage as a function of cholesterol in the lipid composition [146] to characterize membrane stability and permeabilization [147], characterization of membrane viscoelasticity [148], membrane bending rigidity [47] and capacitance (Fig. 5B) [142], and high resolution deformability dynamics of GUVs (Fig. 5 C) [144] are among notable works using GUV cell models for electromechanical characterization of lipid bilayers. Beyond mechanical characterization of GUVs, electroperturbation has been used for electromechanical characterization of polymer-based cell model microcapsules [149–151]. Although the majority of studies using electrodeformation of cell models are directed at towards understanding membrane properties, the setup can be of great utility for characterization of cellular mechanics by reconstituted cytoskeleton networks. Recent studies have utilized electroperturbation to investigate the relaxation time and electrically induced pore resealing time showing that actin-cortex GUV pores seal in minutes while actin-free GUV pores seal in ~1 ms post poration [56]. Additionally, differential regulation of GUV mechanics via different actin architectures was investigated by measuring deformability indicating ~25 % deformation dampening by actin network encapsulating GUVs versus actin-free GUVs [30].

3.6. Microfluidic devices

Unlike the above perturbation methods, there are numerous microfluidic designs manipulating biological samples in different ways. Some utilize solely fluidic flow inside microfluidic flow channels to manipulate cells, or solid features at the micron-scale and some, often the case with most microfluidic devices, are integrated with other perturbation techniques as discussed earlier. Among studies using flow-dependent microfluidic devices for mechanical characterization include pneumatically controlled single cell compression microfluidic device to measure Young's modulus [152] and study biochemical responses to compressive forces [153], microfluidic micropipette aspiration devices for measuring cellular

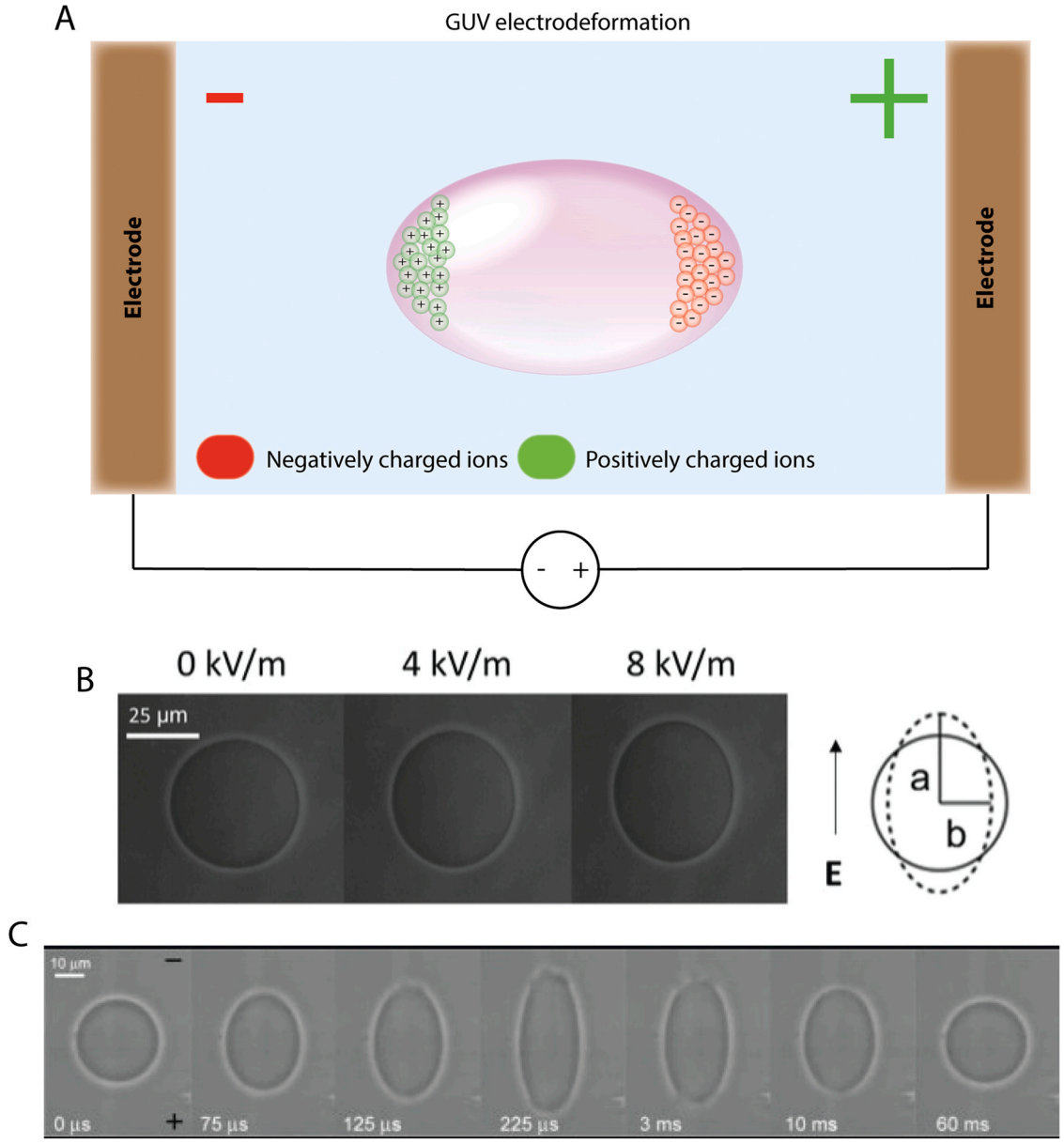

Fig. 5. Electrodeformation of GUVs. (A) Schematic representation of an electrodeformed GUV. Two parallel electrodes connected to a voltage source induce an electric field across a conductive GUV-containing solution. Charged ions inside GUV separate towards the opposing sides of the electrodes. This charge separation results in the deformation of GUVs. (B) Elliptical deformation of a GUV in response to an AC electric field. Deformation profiles of GUVs are used to measure membrane bending rigidity and membrane capacitance. (C) Electrodeformation of GUVs using DC pulses. Dynamic response of GUVs to electric field, including elliptical deformation and poration are captured at high temporal resolution. These dynamic responses are used to measure the critical transmembrane potential of GUV lipid bilayer. Panels B and C are adapted from [142] and [144], respectively

stiffness [154,155], microfluidic sorting devices for stiffness-based cell sorting [156,157], and constriction-based devices for single cell mechanical characterization [158,159]. Microfluidic platforms can be integrated with optical momentum [119], acoustic radiation [160], electric fields [161], or magnetic forces [162] to manipulate cells.

Microfluidic devices, both flow-based and integrated, have also been frequently applied to perturb GUV-based minimal cell models. Notably, Ganzinger et al. developed a microfluidic device capable of trapping and deforming FtsZ network encapsulating GUVs (Fig. 6 A) [22]. Using this device, they studied the remodeling modes of cytoskeletal network in response to constriction-induced deformation [22]. Others have utilized microfluidic devices for shaping GUVs using pH inducible microfluidic wells (Fig. 6B) [163], and for studying membrane biophysics using microfluidic micropipette aspiration device (Fig. 6 C) [164]. Integrating electric fields to microfluidics, Korlach et al. used dielectrophoretic field cages for deforming and reorienting GUVs (Fig. 6D) [165]. Others have creatively implemented microfluidic design principles for single GUV trapping [166], measuring GUV membrane permeability [167], and trapping and filtering devices for GUV-GUV communication studies and synthetic tissue formation by facilitating colony formation [168]. In Table 2, we have provided example studies that measured GUV mechanical properties by using various methods discussed in the sections above.

3.7. Additional perturbation methods

Numerous other methods are used to perturb GUV-based minimal cell models. These approaches can be standalone methods or integrated with those discussed in previous sections. To list a few, for instance, magnetic beads have been used to pull membrane tubes with piconewton scale forces from micropipette-aspirated GUVs

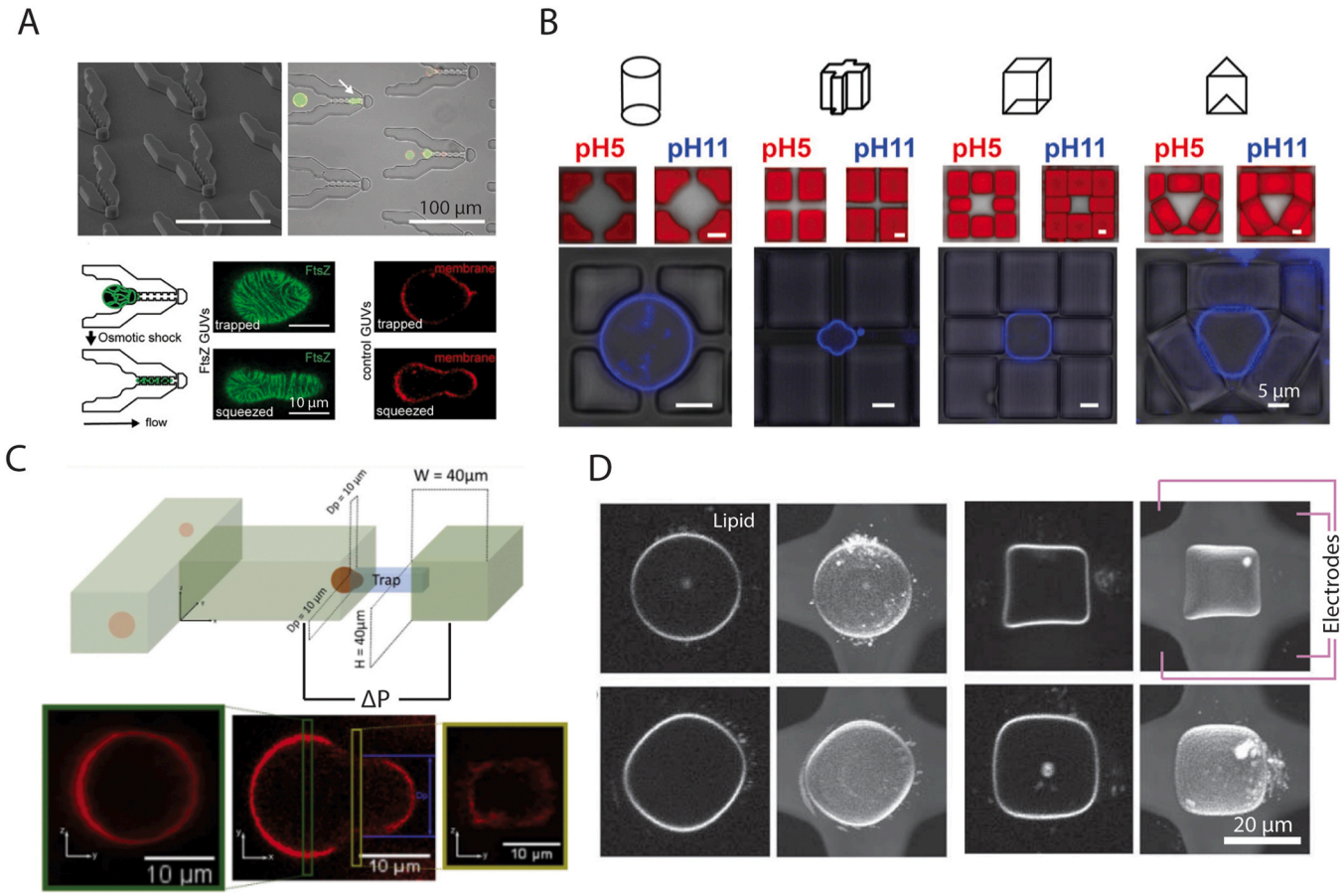

Fig. 6. Microfluidic manipulation of GUVs. (A) FtsZ-encapsulating GUVs trapped and deformed using a microfluidic device (top). Modulating the osmotic condition of the GUV solution changes GUV deformation and FtsZ organization (bottom). (B) A chemically tunable microfluidic device is used to trap and shape GUVs. Different trapping designs are used to tarp GUVs. Increasing solution pH results in swelling of trapping features, thus deforming the GUVs to specific shapes. (C) Implementation of micropipette aspiration in a microfluidic device. GUVs are trapped and aspirated in to a microchannel. Changing the flow rate inside the microfluidic channel results in change in aspiration pressure ΔP. (D) Integrated microfluidic device with electric field cages trap deform and reorient GUVs. Panels A, B, C, and D are adapted from [22], [163], [164], and [165], respectively.

Table 2
Methods of GUV perturbations and some of the measured mechanical properties.

Method	Measured mechanical property
Micropipette aspiration	Area expansion modulus (Ref. [65]) Bending rigidity (Ref. [4,62]) Membrane tension (Ref. [5])
Atomic force microscopy	Area compressibility modulus (Ref. [88]) Bending rigidity (Ref. [6])
Acoustic manipulation	Deformability (Ref. [106]) Membrane Young's modulus (Ref. [106])
Optical stretching	Viscoelasticity (Ref. [122]) Bending rigidity (Ref. [120,121]) Membrane tension (Ref. [120])
Electrical perturbation	Deformability (Ref. [144]) Viscoelasticity (Ref. [148]) Membrane bending rigidity (Ref. [47])
Microfluidic devices	Deformability (Ref. [22,163]) Stretching modulus (Ref. [164])

[169]. Furthermore, magnetic beads have been used to manipulate GUVs for trapping [170] and magnetic microrheometry was used to deform actin-encapsulating GUVs to measure viscoelastic relaxation modulus [171]. Similar to magnetic beads, encapsulated Janus particles have also been used to perturb GUVs to study membrane structural integrity [172]. Numerous other works have used various nanoparticles to generate GUV deformation and poration to study membrane integrity and fluidity [173–175].

Other GUV perturbation methods leverage controlling the native environment GUVs are dispersed in. These include changing the osmotic environment, regulating the surrounding temperature and the adhesive property of substrates onto which GUVs settle. For example, one study revealed the pulsatile property of bilayers by subjecting GUVs to hypotonic condition resulting in a swell-burst cycle [176]. Others have leveraged such a simple mechanism to study oscillatory lipid-lipid phase separation driven by differential membrane tension [177]. Furthermore, deformation modes of actin-reinforced GUVs were studied by changing osmotic gradient [178]. Additionally, thermal fluctuation of the GUV environment has also been widely used to characterize different biophysical properties of GUVs. Among the large cohort of studies, regulating GUV morphology [179] and temperature-regulated lipid phase separation and miscibility are notable [180].

4. Summary and outlook

Initially, GUVs were ubiquitously used as a model membrane system with control of membrane content. Recently, with the growth of bottom-up synthetic biology, GUVs were utilized as an ideal substrate for the creation of a synthetic cell-like system using biological parts. Perhaps, with the collective effort of scientists using GUVs to understand cellular mechanisms and creating synthetic systems, creating minimal life-form from non-living parts may be realized. Regardless, towards creating life or simply using them as model systems to understand cellular functions, GUV-based minimal cell models must be mechanically robust and their mechanical characteristics be extensively characterized. Without further

reinforcement, GUVs are just simply unfit to endure and survive the physiochemical environment native to cells and attempting to understand a cell outside of its biological environment will make the study incomplete. Thus, it is imperative to make all attempts to equip GUVs with the mechanical robustness and durability akin to that of cells. While we have yet to make a sturdy cell-like biological compartment with a boundary as versatile as the cell membrane, remarkable advances have been made to make GUV-based cell models more mechanically robust. Developing new techniques for effective mechanical characterization of GUV-based cell models is essential and innovative approaches for high precision, high resolution cell model manipulation will propel the field of synthetic biology and cell biology in general.

Conflict of interest

There are no conflicts to declare.

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