Droplet-Based Membranous Soft Materials

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KEYWORDS biologically-inspired materials, stabilized adhesive emulsions, droplet microfluidics, ferrofluids, lipid membranes, self-assembly, mechanotransduction, light-sensitive materials, cellular-inspired tissue reconfiguration, synthetic membranes.

ABSTRACT

Inspired by the structure and functionality of natural cellular tissues, DIB (droplet interface bilayer)-based materials strategically combine model membrane assembly techniques and droplet microfluidics. These structures have shown promising results in applications ranging from biological computing to chemical microrobots. This review briefly explores recent advances in the areas of construction, manipulation and functionalization of DIB networks, discusses their unique mechanics, and focuses on the contributions of our lab in the advancement of this platform. We also reflect on some of the limitations facing DIB-based materials and how they might be addressed, highlighting promising applications made possible through the refinement of the material concept.

INTRODUCTION

Biologically inspired engineering is an interdisciplinary effort (including organic chemistry, materials science, mechanical engineering, biological engineering, and biology to name a few

contributing fields) with the goal of mimicking naturally-evolved systems to produce new technologies. One approach in bioinspired engineering involves the bottom-up approach to synthetic biology, which replicates biological functionality in simplified structures by assembling the system from individual components. With many potential applications including chemical robots[1-4], artificial tissues capable of responding to external environmental stimuli[5], targeted drug delivery and diagnosis[6], and molecular computing[7], bottom-up synthetic biology offers many new opportunities in material design.

A subset of bottom-up synthetic biology is protocell creation, defined by its goal of constructing minimal cellular systems and cell-inspired materials. While fully replicating cellular tissues *in vitro* is not a foreseeably attainable goal, synthetic biology can still seek inspiration from particular cellular behaviors[8], using self-assembly mechanics[9] to replicate desired cellular features (**Figure 1-g** and **Figure 1-h**). Ideally these biologically-inspired structures would retain several characteristics of living cells, including their modularity, compartmentalization, and communication[6], widely considered part of the foundation for basic living systems per Ganti's chemoton model[10]. One particular subfield of bioinspired engineering and cell-inspired materials involves the use of stabilized adhesive emulsions[11]. The droplet interface bilayer (DIB) technique assembles lipid membranes at the interfaces of lipid-coated aqueous microdroplets dispersed in an oil phase[12-13]. The lipid membranes reproduced in this technique mimic an essential cellular structure that is highly impermeable to hydrophilic molecules and provides cellular boundaries and architectures[14], and the DIB technique produces an interconnected web of these membranes.

Formed at the interface of two connected lipid-coated water droplets (as compared to traditional liposomes- **Figure 1-a**), DIB membranes possess properties that depend on the composition of their bordering compartments (**Figure 1-b** and **Figure 1-c**). Using varying sets of spatially

arranged aqueous droplets with varying compositions, 2D and 3D droplet-based tissues (**Figure 1-d** and **Figure 1-e**) with different functionalities can be formed by altering the relative placement of the droplets within the tissue. In this article we discuss the basic mechanics of DIBs, followed by an overview of new platforms developed for constructing and functionalizing (**Figure 1-c**) larger synthetic droplet-based tissues as well as their inherent structural properties.

Aqueous microdroplets are introduced into an oil medium with lipids either dispersed in the oil phase (the lipid-out technique[15]: **Figure 2-e**), in the water phase (the lipid-in technique[16-17]: **Figure 2-d**), or in some instances in both phases for enhanced stability[18] (**Figure 2-f**). The permeability, structure, and stability of the generated membranes may be modulated by varying each one of these compositions (most notably the lipid compositions[6, 19-22]). In some cases, amphiphilic block copolymers are used for additional stability[21]. After sufficient time for the assembly of lipid monolayers at the water-oil interface[23], lipid-coated water droplets can be brought into contact with their neighboring droplets. The interfacial lipid bilayer spontaneously forms at their points of contact as the hydrophobic phospholipid tails arranged at the droplet surfaces self-assemble in a zipping motion, expelling the oil initially separating the droplets. By repeating this connection process multiple times within a collection of microdroplets, 2D[15, 18, 24-25](**Figure 2-g**) and even 3D[26-28] (**Figure 1-e** and **Figure 1-f**) tissue-inspired structures may be formed. In addition to the convenient modularity and scalability of DIBs, the DIB technique enables sequential assembly of membranes through separation and reassembly of the lipid bilayers between the droplets without jeopardizing the integrity of the rest of the structure or causing the droplets to coalesce. The DIB platform also allows for the simple creation of asymmetric membranes[17-18] (**Figure 1** and **Figure 2**) by varying the lipid composition between adjacent droplets.

DIB-based materials can be functionalized using bioinspired methods for initiating exchange between the adhered compartments, temporarily adjusting the characteristics of the lipid barriers between the droplets. Often dispersed among the aqueous phases, transmembrane channels self-assemble within membranes bordering the droplet in which they reside, altering the permeability of the interfacial bilayers as shown in **Figure 2-g**. Many of these embedded channels respond to varying stimuli including electrical, chemical, and mechanical inputs[14, 29], producing controlled droplet-droplet exchange on demand. The collective behavior of these membranes working in parallel determines the emergent functionality of the tissue and enables their responses to varying external conditions. These droplet-based materials have been used for soft chemical robots[1-2], energy storage and conversion[13, 30-31], light-sensing[18, 32-33], and the creation of engineered tissues[28, 34-36].

DIB-based membranous materials inherently satisfy many of the chemoton model criteria essential for sustainable life. DIB tissues are highly compartmentalized and modular yet can initiate droplet-droplet communication on-demand through governed membrane permeability. However, DIB-based tissues still face multiple challenges that need to be addressed, including difficulty in their manufacturing, associated limitations in their complexity, and issues with membrane instability. Since the functionality governed by droplet-droplet exchanges is dependent on the relative arrangement of the droplets within the material, novel droplet deposition schemes are often required for creating larger interconnected networks droplet by droplet[28, 35, 37-38]. Additionally, the responses of the droplet-based tissues are limited by available membrane activation mechanisms – original DIB materials relied on embedded electrodes but recent effort have expanded to include more biologically-relevant schemes[18, 39] to enable their use outside of the laboratory, or suggested activation through mechanical forces[40-41]. Finally the droplets are often prone to coalescence given

their fluid-in-fluid construction and metastable nature of the adhered droplet pairs. Substantial efforts have focused on either reducing their coalescence through encapsulation[28, 42] or harnessing the phenomena for enabling droplet-droplet mixing[43-44] and restructuring[24].

Formulating solutions to these obstacles requires familiarity with the mechanics of the droplet-based material. We begin by examining the influence of the solvent and surfactant selection on the macroscopic material properties, and how these selections govern membrane assembly and stability. Next, we discuss recent advances in the construction, manipulation, characterization and functionalization of these membrane-based soft materials with an emphasis on dynamic structures. Final notes will include the current challenges facing the proposed material and how these challenges may be potentially addressed.

MECHANICS OF DROPLET INTERFACE BILAYERS

DIBs provide a liquid-in-liquid platform for producing webs of lipid membranes. Their mode of formation results in a collection of unique mechanics, combining aspects of lipid membrane physics and droplet mechanics. We will begin with an overview of the relations between DIB composition and membrane properties, then we will move to how these relations lead to unique, emergent behaviors of DIB tissues.

Membrane Properties and Influences

The adhered structure of a DIB-based synthetic tissue is determined by the properties of its individual interfaces. The properties of each lipid membrane are primarily determined by the monolayer compositions of the bordering microcompartments as well as the selected solvent that provides the external phase. The functionality of the membranous tissue as a whole is shaped by the properties of the individual membranes within the tissue (**Figure 1** and **Figure 2**).

Consequently, any change in the properties or relative arrangements of the droplets comprising the membranes will induce changes in the overall characteristics of the tissue. These changes may include either macroscopic changes in the arrangement of the droplets or local changes within the individual membranes themselves.

The emulsive nature of DIBs offers parallels to several discussions of the mechanics of living tissues. Emulsive phenomena observed in natural tissues are commonly explained through the DAH (differential adhesion hypothesis[45] **Figure 3-f**), which suggests that both living tissues and emulsive systems exhibit similar packing behavior based on the minimization of interfacial energy[46]. This aggregation driven packing with droplets is enabled through the ability of microdroplets to deform from their original spherical shape. On an elementary DIB level, upon the formation of an interfacial bilayer, the droplets transition from a spherical shape to a spherical cap delimited by the dimensions of the membrane. The area of the membrane is governed through a balance of monolayer and bilayer tensions reflected through an external angle of contact (**Figure 2-d**) and gradually expands until the tensions at the annulus are balanced[20, 47-48]as given by Young's equation:

$$\gamma_b = 2\gamma_m \cos\theta \qquad (1)$$

where γ_m , γ_b, and θ are the monolayer, bilayer tension and the contact angle, respectively. Upon assembling multiple droplets into 2D and then 3D tissues, the droplet compartments adopt more complex polyhedral shapes governed by the number of neighboring droplets and their position relative to open and solid surfaces. Previous studies in oil-in-water[49] and water-in-oil[11, 47] stabilized microemulsions have shown that the external angle of contact between microdroplets is a crucial factor in the structural stability of compact systems. The importance of this angle of contact is mostly noted in emulsions and systems where the interfacial film thickness is significantly

reduced; this decrease in thickness is generally accompanied with an increase in the volume fraction φ, or the volume of the aqueous phase divided by the volume of the oil phase in the synthetic tissue. As the film thickness further decreases, an angle approaching 35.3º yields a volume fraction approximating unity[49]. The same study by Princen *et al.* found that the type of packing assumed does not significantly matter when evaluating the film thickness and volume fraction and their dependence on certain parameters[49]. Similarly, in DIB structures, Alcinesio *et al.* have reported the equilibrium angle of contact to be the key factor governing the structural stability of 3D DIB tissues (defined as resistance of the adhesive droplets to coalescence) and a key geometrical constraint in the positioning of water microdroplets relatively to one another[37]. When θ approaches the theoretically derived angle from the geometry of the system, valued at 35.3º, the DIB networks exhibit regular hexagonal close-packing lattices distribution with minimal geometric defects with monodisperse droplets. The optimal droplet packing here was described to the closest possible packing that significantly reduces unoccupied space between microdroplets[46] (**Figure 1-e** and **Figure 2-d**). Tightly packed membranous tissues significantly reduce the possibility of droplets sliding between different tissue levels and allow for the precise patterning of materials with conductive pathways one-droplet thick[6, 18, 26, 33, 35, 39]. From **Equation 1**, the equilibrium θ depends on interfacial tensions (monolayer and bilayer), both of which are affected by the oil[20, 22, 47], and surfactant compositions[22]. Consequently, the structural stability of DIB tissues can be regulated by varying the contents of the polar and apolar phases.

Another way to describe the contact angle is through droplet-droplet adhesion. A strong droplet-droplet adhesion results in more tightly packed and hence more stable DIB structures (**Figure 3-a**). This phenomena can be quantified using adhesion energy, defined as the energetic cost for

formation of the interfacial bilayer starting from two distinct amphiphilic monolayers as a reference point (also referred to as membrane's free energy of adhesion ε or $-\Delta F$[47]):

$$-\Delta F \ = \ 2\gamma_m \ - \gamma_b = 2\gamma_m(1 - cos\theta) \qquad (2)$$

The energy of adhesion is directly associated with the interactions of the two surfactant monolayers[50], and provides a point of comparison for membranes with varying lipid/oil/aqueous compositions. The higher the amount of energy saved by the formation of a bilayer, the more favorable and consequently more stable the produced membranes (**Figure 3-e**).

Figure 3-b shows the normalized capacitance of different membranes scanned using a hydrogel microelectrode and formed using different continuous phases (data produced by our group from Challita *et al.* was extracted and processed for the creation of this figure[51]). Since all membranes formed with this technique have a similar area dictated by the dimensions of the hydrogel microelectrode, such measurements isolate the effect of the oil phase on the membrane formation. The influence is quantified by observing the time it takes these bilayers to reach 2/3 of their equilibrium area referred to here as the initial rapid rise time (τ). By fitting the capacitance into a function similar to the one depicted by Freeman *et al.*[40], fast rise times for different membranes can be obtained and analyzed in light of calculated Laplace numbers (*La*). The Laplace number also known as the Suratman number (*Su*) can be found using the following equation:

$$La = \frac{D\rho\Delta F}{\mu^2} \sim \frac{inertia\ x\ surface\ forces}{viscous\ forces^2} \qquad (3)$$

where μ is the dynamic viscosity of the oil, ρ is the relative density of the aqueous/oil phases, and D is the characteristic length scale (here defined as the droplet diameter D). Plotting the characteristic rise time for membrane formation in different oils vs. *La*, an empirical equation

linking the two and reflecting the dynamics of the membrane formation (**Figure 3-c**) is found. Smaller Laplace numbers indicate a greater influence of the viscosity while larger ones show a behavior dominated by the inertia and surface forces. Smaller *La* have shown larger rise times (τ) indicating that these bilayers typically require more time to form. While this time difference might seem insignificant for single membrane applications, larger DIB tissues involving collections of microdroplets require rapid adhesion as depositing the droplets faster that the membrane stabilization time may result in structural instabilities and ultimately failure. Consequently the 1:1 hexadecane:silicone oil AR20 mixture is frequently used for rapid assembly of DIB networks[26, 28].

This particular mixture of hexadecane and silicone oil AR20 with suspended zwitterionic phospholipids (DPhPC 1,2-diphytanoyl-sn-glycero-3-phosphocholine) provides increased membrane stability[20], rapid membrane assembly time[23] and a closer density balance between the oil and aqueous phases to reduce gravitational influences. This lower relative density allows sufficient time for monolayer formation before the droplet settles and limits gravitational forces when constructing 3D tissues[15, 24, 26, 35] . The enhanced properties can be attributed to a reduction in trapped oil within DIBs formed in silicone oil AR20:hexadecane oil mixtures compared to membranes formed with hexadecane or decane or other alkane oils[20] (**Figure 3-b** , **Figure 3-c** and **Figure 3-e**), and the addition of silicone oil AR20 was found to increase the stability of the adhesive water-in-oil emulsions[47]. A 1/1 volume mixture of hexadecane and silicone oil AR20 produces interfacial phospholipid membranes displaying a θ of 30.06°[15] .

The lipid mixtures also influence the properties of the interfacial membranes[22-23, 52]. Mixtures of cholesterol and DPhPC have been shown to restrict the movement of DPhPC phospholipids within the monolayers, leading to a more rigid, well-packed membranes as demonstrated recently by our measurements of the membrane response to electrocompression[22]. Cholesterol molecules integrate

themselves between the acyl chains of DPhPC molecules, increasing the monolayer tension (**Figure 3-d**). This influence varies with the chain length of the lipid with which the cholesterol is mixed, and the effect can be predicted by comparing the hydrophobic tail chain length of both surfactants. Reports have shown that bilayers formed between phospholipids with up to 16-carbon acyl chains are thickened by the introduction of cholesterol, in contrast to bilayers formed between lipids with 18-carbon chains upon the introduction of cholesterol[20, 22]. Alcinesio *et al.* have analyzed the effects of oil phase and lipid composition on the stability of DIB-tissues by introducing both silicone oil and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) lipids into a undecane DPhPC solution used to form 2D and 3D DIB networks[37]. Their results show that θ can be related back to the selected volume fraction of silicone oil AR20 in a mixture of undecane and silicone oil as well as the selected lipid composition (the mole fraction of POPC in a mixture of DPhPC and POPC).

Electrical Properties and Modeling

The lipid membrane provides a low-permittivity hydrophobic core that is near impermeable to dissolved species within the droplets. Consequently, a DIB is often electrically approximated as a capacitor in parallel with a high amplitude resistor[13, 30](**Figure 2-e**). When perfectly sealed, the membrane's resistance is often in the order of GΩ-TΩ[13] hence the ohmic current is often negligible, and the overall current can be approximated as capacitive. Furthermore, the DIB platform brings an aspect of modularity. The droplets are an unsupported liquid-in-liquid structure, and it is possible to connect multiple droplets together forming networks of interconnected lipid membranes. The electrical properties of networks of lipid membranes are typically modeled using a network stencil through nodal voltage analysis, producing a system of differential equations describing the voltage distribution across each of the connected membranes

as modeled extensively by our group[16, 53]. These stencils have been combined with equations describing the underlying exchange of charged species associated with the currents to describe gradual shifts in droplet contents[29], and linked to the behavior of osmotically-driven actuators[35]. Furthermore, lipid membranes can be functionalized with protein channels and pore forming toxins strategically dispersed within aqueous compartments yielding conductive communication pathways spanning the tissue (**Figure 6**). This produces variable resistances between the droplets, in addition to triggering active transport using chemical fuel such as ATP[54].

Mechanical Properties and Behavior

Another unique aspect of DIB-based materials is their response to mechanical perturbation. This may be attributed to the weak elasticity of microfluidic droplets and capillary forces[55-57]. The energetic cost of formation for DIB networks is obtained by multiplying the tension of each interface (monolayer/bilayer) by their respective areas and can be combined with gravitational influences and other contributions as demonstrated for modeling DIBs[24]. The shape of the adhered droplets at equilibrium represent the minimization of this value with given volume constraints on the droplets[57]. Perturbing the droplets leads to a distortion away from their desired minimal shape[55], providing a form of weak elasticity where the droplets continually seek their minimum energy configuration in response to changing environmental constraints or boundaries. This has been used to regulate the membrane area with great success, either pulling the droplets apart[22, 48, 58] or varying the dimensions of an aperture between the droplets[59]. Furthermore, is possible to shift adhered droplets between metastable positions through the careful application of mechanical forces and constraints.

The stiffness and stability of the adhered droplet configuration is linked to their interfacial tensions (**Figure 2-a**). If the membrane formation is energetically favorable, then the energy well

representing the metastable state becomes deeper and requires greater forces for separation as demonstrated by our group (**Figure 2-e**). Consequently, if reconfiguration or separation of the droplets (defined as adaptation of the structure) is desired then a balance between ease of membrane formation and ease of separation must be achieved. The overall stiffness of the material is related to the monolayer tension, and the difficulty in separating the droplets is related to the energy of adhesion. Selecting the proper solvent and lipid combination is crucial when tailoring a network for a desired stiffness (**Figure 2-a**).

DIB based tissues are inherently different from their natural inspiration in the very description of the interfaces between their constituent modules. In natural living tissues, cell-cell interfaces are comprised of two adjacent cellular barriers. Consequently, separating two cells often does not significantly alter the activity of each individual unit comprising the pair. Meanwhile, in the case of DIB systems, the lipid barriers are comprised of the lipid-leaflets coating bordering compartments (**Figure 1-a** and **Figure 1-c**). Hence, the properties of DIB membranes reflect the compositions of the adhered droplet pair[17-18, 32] and rearranging droplets within DIB-structures fundamentally changes the properties of the membranes within the tissue by breaking these pairs apart and reforming them between different droplets. The functionality of the overall material is hence linked to its own internal structure.

FORMATION OF DROPLET INTERFACE BILAYERS

DIB materials are best described as a combination of stabilized adhesive emulsions and model membrane assembly techniques. As discussed previously, several DIB properties such as membrane thickness, dimensions, and droplet stiffness are largely determined by the selected solvents and lipids, allowing for flexibility in their manufacturing. It is possible to scale the DIB platform from a single membrane up to an entire membrane-based tissue by continuing to add

adhered droplets in the desired pattern. Techniques for generating DIB tissues can be characterized according to the droplet manipulation mechanism, the size/complexity of the resulting material, and the speed of assembly. Here we provide an overview of these techniques based on the number of membranes present in the individual functional units, referred to as the membrane density.

Low Membrane Density Methods

First we discuss techniques for forming DIB networks with a smaller number of connected droplets, or low-membrane density methods. These methods are often compatible with parallel or rapid formation. The simplest structure formed through the DIB technique involves two aqueous droplets pipetted into an oil dish and manually driven into contact using either the tip of a micropipette (or the tip of a finely pulled glass rod) or guided by the electrodes[13]. The rate of formation of the lipid monolayers at the water-oil interface depends on the adsorption kinetics of phospholipids and by extension on the phase in which the amphiphiles are present[20, 23]. After a sufficient time for the acquisition of a lipid monolayer at the droplet surfaces, the droplets are brought into contact resulting in an initial thinning of the lipid-lipid interface. Lipid tail-groups spontaneously draw together in a zipping motion and exclude a significant quantity of the trapped oil between the droplets triggering the formation of an interfacial lipid bilayer (**Figure 2**). The inclusion of microelectrodes allows for the application of an electrical potential to confirm the formation of the membrane as well for the assessment of the ionic current flowing through embedded transmembrane channels using electrophysiological techniques. Simple 2D and even 3D (pyramids containing 14 droplets)[26] membrane structures can be formed using this technique as well. 2D membrane structures can likewise be patterned within pre-designed microfluidic devices[36, 60] and channels[25] (**Figure 4-a**).

Microfluidic channels provide manipulation through hydrodynamics for high throughput generation of DIB structures in rapid succession[61-63], connecting several droplets together in microfluidic traps. These approaches are best applied towards screening applications wherein the smaller DIB clusters provide a model environment for studying an interaction in parallel[62, 64]. Superhydrophobic oil-coated surface have been used to connect colliding water droplets otherwise suspended in air into interfacial bilayers[65] with potential applications for screening compounds in the air (**Figure 4-b**).

Optical strategies for droplet manipulation have also been developed exploring light-induced capillary effects and displaying many advantages including contact-free minimally invasive droplet manipulation. One of these strategies used the thermocapillary effect and convective fluid motion to optically manipulated lipid-coated microdroplets into contact using lasers[66] (**Figure 4-c**). Optical-tweezers approaches have been adopted for the manipulation of DIB-structures[66] resulting in the formation and effective manipulation (and even droplet fusion) of 2D membranous structures. This strategy was also explored for sculpting and fusing biomimetic vesicle networks[67] where vesicles are interconnected by axon-like tethers. The further use of transmembrane channels facilitated molecular transport and initiating protein expression across these structures post laser-induced vesicle fusion.

Standard droplet manipulation techniques are compatible with DIB formation as well. Using electrowetting on dielectric (EWOD), two or more aqueous microdroplets can be manipulated together into contact forming DIB-based structures[68-69]. Highly conductive electrolyte solutions can be directed using relatively low currents with minimal joule heating resulting in droplets' motion and increased wettability[68, 70].

The ability to magnetically influence aqueous microdroplets was first explored by Wauer *et al.* by embedding select compartments with magnetic beads[26, 44]. Magnetic droplets were used to drag and position the remaining aqueous droplets to a submillimeter precision using a permanent magnet. Another alternative was developed by our group through the incorporation of biocompatible ferrofluids into the DIB platform[15, 24, 71-72]. First we demonstrated that the ferrofluid was compatible with DIBs, exhibiting minimal changes in functionality and able to accommodate transmembrane channels[71]. Two types of magnetically infused DIB networks were formed: ferrofluid-based structures and mixed water-ferrofluid based structures. Homogeneous ferrofluid based DIB networks were formed by injecting magnetically infused droplets into a lipid-oil mixture and then gradually bringing a permanent magnet closer to the dish so that droplets adhered together and spontaneously formed bilayers[15, 24, 71-72] (**Figure 4-d**). Mixed networks with both ferrofluid and water droplets were formed by manually injecting both magnetic and aqueous droplets into a lipid-oil medium and then using ferrofluid droplets manipulated using permanent magnets to progressively recruit water drops into a connected structure[15, 24, 71-72].

High Membrane Density Methods

When forming larger functional networks of DIBs containing many interconnected droplets, several considerations are necessary since the precise relative arrangements of the droplets is essential. Larger structures approaching the tissue-scale often require a droplet-on-demand approach where the droplets are deposited sequentially with varying components. The concept of using 3D printer-based systems for the generation of DIB tissues enabled the production of compartmentalized tissues with preset functionalities determined by the droplet patterning. A key advantage of combining 3D printing with the DIB-platform is the ability to link multitudes of microcompartments with different chemical profiles together with high spatial/temporal control to

produce more complex membranous structures in a relatively short time[28, 35, 73]. However, such approaches are faced with possible structural instabilities arising from droplet coalescence or sliding prior to membrane formation. Hence, the properties of the oil phase and surfactant mixture as well as membrane formation time ought to be optimized for further development of the printing algorithm.

Villar *et al.* developed a 3D micro/nanodroplets printer comprising two printing nozzles connected to piezoelectric transducers[35]. Micro and nanodroplets can be injected in an oil bath and their respective x-y positions regulated through a computer-controlled micromanipulator. Droplet ejection and dimensions are a function of the pulse width and voltage supplied, with several droplet characteristics that provide a repeatable and reliable droplet ejection in the range ∼30–60 μm[35]. Since positioning of the printing nozzle in the *z* direction was unnecessary for droplet release, droplet deposition, incubation time (∼1 s) and bilayer formation (∼1-3 s) could be achieved in under 5 s[35]**.**

A second approach was later adopted by our lab by developing a pressure-based 3D droplet printer for the formation of DIB-based tissues[28, 73](**Figure 5-a**, **b**, **c** and **f**). A glass capillary is mounted on a micromanipulator and connected to a microinjector, both synchronized and controlled via computer. A multi-capillary holder was design to allow for the use of multiple capillary tubes containing different lipid mixtures simultaneously. Microdroplets are first formed at the tip of the glass capillary by controlling the pressure provided for the capillary tubes connected to a pneumatic system (computer-controlled). Following formation, droplets are released by vertically removing the tip of the capillary tube from the oil medium into air in a snap-off motion using capillary forces. The process is then repeated as needed to produce networks of aqueous droplets with varying inflated dimensions (on average a single droplet every few seconds). The snap-off

motion results in longer deposition time (in comparison to the previously described approach) as the printing nozzle is introduced and removed from the oil medium for each droplet. However this approach allows for variable dimensions of the printed droplets based on inflation time and pressure[28].

Microfluidic channels have also been adapted for the controlled and high-throughput generation of larger 2D and 3D DIB structures[74-75]. Branched microfluidic devices generate DIB structures according to the contours of the canals while a linear channel is used to construct 2D and 3D networks. Although the above experiments focus on droplets of differing lipid composition, the methodologies could equally be extended to incorporate droplets with varying encapsulated components.

Encapsulation of Synthetic Tissues

Although these structures remained stable for several days when printed in oil, one of the key challenges facing further development of these materials is their fragility, causing possible degradation when outside of controlled laboratory conditions. One approach to ensuring stability involves using hydrogels within the aqueous phase to provide a solid droplet core[44, 76-77]. Hydrogels are water-swollen networks of crosslinked hydrophilic polymers often used in cell culture applications as an artificial extracellular matrix. The hydrogels are ionically conductive and permit electrical measurements when hydrated with a buffer solution. Hydrogels can be used as building blocks for DIB materials with applications ranging from electrical circuits to mechanical devices[77].

Alternatively, DIBs may be fully encapsulated and shielded from the environment in a solid substrate, producing a liquid-in-solid configuration. The first approach for this employed a flexible substrate containing two enclosures for the droplets to further control membranes properties[78] introducing the regulated attachment method. Similar approaches suggested encapsulating bilayer

membrane structures in hydrogels[79-80] forming multiple interconnected membranous zones inspired by the concept of proto-organs.

Recent research by our group and others has focused on using thermoreversible organogels within the oil phase to provide a robust and flexible encapsulation strategy that may be reset with heat[28, 42] (**Figure 5-d** and **Figure 5-e**). This involves the dissolution of a thermoreversible triblock copolymer within the oil phase (SEBS, Poly(styrene-b-ethylene-co-butylene-b-styrene)). SEBS forms a polymeric matrix within the gel at room temperature, but shifts to a fluid phase when the temperature is elevated to a higher level dependent on the polymer concentration[28] (**Figure 5-c**). The result is a liquid-in-gel material that may be temporarily softened to enable droplet deposition and assembly, then cooled to provide a soft encapsulating matrix.

The oil phase of DIB-based systems poses challenges to their use in physiological aqueous environments and in applications such as biosensors or compartmentalized drug delivery systems. Multisomes have been explored as an alternative encapsulation technique. Aqueous interconnected lipid-coated microdroplets can be embedded in an oil-in-water droplet[81-82], producing a multiphase emulsion. The functionalization of multisomes was studied through their response to pH and temperature, demonstrating transmembrane channel activity and communication to the external water phase[82]. Further steps in fully functionalizing multisomes towards medicinal applications established their use as compartments for "genomes" in an *in-situ* protein synthesis in defined regions[83]. The ability to establish communication with the exterior water phase makes multisomes a promising encapsulation strategy for drug-delivery and detection applications and paves the way for future works where higher-order cellular characteristics can be integrated into the synthetic tissues.

FUNCTIONALIZED DROPLET INTERFACE BILAYER MATERIALS

Efforts have been directed at enabling DIB-tissues with the ability to sense and react to external triggers. These membranous tissues can be designed to either change their internal chemical composition or their overall droplet architecture (more ambitiously a combination of both) to adapt to external events. DIB-based materials traditionally have been designed to respond to external stimuli by changing their internal chemical compositions, accomplished via internal diffusion pathways between droplets that allow for molecular flow akin to chemical computing[6, 39]. More recent attempts have explored adaptive architectures within DIB tissues, a strategy also inspired by nature where function follows form[84-86]. In the following sections, we will discuss these various methods for enabling DIB functionality.

Changing Internal Compositions

Communication between the droplets is what enables changes in the chemical composition within the tissue and determines the functionality of the network, whether it be signal rectification[31], osmotic actuation[35], detection and sensing[82, 87], or mechanosensing[40, 88-89]. Communication defined here as molecular transit between compartments is an important condition for modular multi-cellular design of engineered materials, stemming directly from the chemoton requirements of life[10]. In DIB systems, communication is achieved by governing the permeability of the interfacial bilayers in response to external stimuli, functionalizing the tissues and individual membranes. Here, targeted permeability can be achieved either using integral transmembrane channels, lipid packing allowing for the diffusion of different species across the lipid membranes, or some combination of the two methods.

Due to their bioinspired structure, model lipid membranes can be functionalized with integral protein channels and pore forming toxins strategically dispersed within the aqueous compartments

for the production of communication pathways spanning DIB tissues (**Figure 6** and **Figure 2-g**). Most intrinsic protein/peptide channels whether single-pass, multi-pass or multi-subunit include residues with hydrophobic side chains that interact with fatty acyl groups of the membrane phospholipids and α helices or multiple β strands spanning the membrane[90]. Standard approaches to characterizing DIB-based materials have involved the use of electrophysiological equipment, which allows the user to prescribe voltages within the droplets which may then be used to detect and measure the activity of transmembrane channels or activate electrically sensitive ones. Typically, the presence of an ohmic current is an indicator of a transmembrane porous activity. Extensively reported in the literature[13, 15-18, 26-28, 30-31, 34-35, 39, 73], alpha-hemolysin (*α*HL) has been the main channel of choice when establishing communication in membranous DIB networks (**Figure 6-2-a**). One-droplet thick pathways can be created by dispersing *α*HL in aqueous droplets and used to transport molecules across the structure. This allows for printed conductive pathways within the otherwise high-impedance material. 7R-*α*HL, a modified form of the pore, provides a diode-like behavior where steady-state conductivity is only enhanced for positive voltages. This was used to successfully demonstrate emergent properties in a four droplet cluster through signal rectification[31]. Similarly, DIBs can be functionalized with water-soluble peptides such as alamethicin[18-19, 25, 36, 91] producing a voltage-dependent pore activity. Alamethicin is particularly noteworthy as it provides a voltage-dependent conductivity similar to 7R-*α*HL; however, it requires an input voltage above a threshold for activation as shown in **Figure 6-1-a** and **Figure 6-2-c**. Gramicidin channels across DIBs[18, 70, 92] are formed from the dimerization of monomers from each monolayer leaflet, these pores exhibit a selectivity for small monovalent cations[93-94] and provide a way of establishing directional communication only between compartments containing the antimicrobial dimer.

More complex membrane proteins can also be used functionalize DIB structures. These are reconstituted into proteoliposomes and added within the aqueous droplet dispersion. The activity of potassium channels (KcsA[70]) and different mechanosensitive channels (Piezo-1[95], MscS[95]and MscL[96-100]) (**Figure 6-1-c** and **Figure 6-2-b**) has been investigated in lipid membranes. Cyclically producing changes in the membrane tensions[97, 101] at lower frequencies[97-100] through mechanical distortions to the droplets bordering the membrane has been successfully used to activate V23T-MscL (**Figure 6-1-c**).

In many cases these embedded electrodes are impractical either due to difficulties in manufacturing, challenges in portability, or difficulty in simultaneously interrogating multiple membranes. Alternative means of establishing internal communication relying on more biologically relevant activation mechanisms have been developed. Active transport applications may be defined as cases which facilitate "uphill" transport against an energetic gradient within these soft materials to establish and maintain out-of-equilibrium conditions rather than relying on preset gradients[6, 102]. The most common approach employed in DIB-based materials involves the transduction of light energy into chemical energy through light-sensitive bacteriorhodopsin[30, 87, 103] (**Figure 6-2-d**). This has been used for the creation of artificial eyes and light sensors by patterning the droplets in an appropriate fashion.

Alternatively, the lipid composition of the membrane itself may be tailored to enhance permeability. This is the approach typically taken for liposomal drug delivery, and there are a variety of options to ensure transport across a lipid membrane[104]. Droplet-droplet communication can be achieved by utilizing lipid self-assembly (raft formation) and polymerization principles to alter membrane permeability without channels or pore-forming toxins. This alternative route for membrane permeabilization occurs through two mechanisms: graded or all-or-none[105-106]. The all-

or-none mechanism typically results from a complete bilayer failure as in the case of membrane fusion during vesicle trafficking or cellular apoptosis[105]. Meanwhile, a graded-mechanism permeabilization process typically results either from pore formation/insertion in bilayers[106] or transient alterations associated with lipid rearrangement and phase boundary defects[104]. The methods selected in DIBs focus more on graded release rather than all-or-none to limit droplet coalescence. Similar methods would be appropriate in polymer-based DIBs, taking advantage of the wealth of research available on stimuli-responsive liposomal/polymersomal drug delivery[107]. Multi-component lipids bilayer mixtures show a potential for triggered disruption of lipid packing leading to a graded permeability mechanism that operates in a similar way to pore forming proteins (PFPs) or toxins (PFTs). The incorporation of photopolymerizable lipids in the DIB platform is relatively recent[32] despite their presence in liposome-based drug-delivery applications[104, 108-109] for decades[108, 110]. Our approach to this idea demonstrated that cross-membrane transmission with these light-sensitive lipids is shown to occur only in bilayers that incorporate polymerizable lipids within both leaflets, necessitating compatible lipid profiles combined with exposure to appropriate wavelength UV-light[18](**Figure 6-1-b** and **Figure 6-2-e**). This produces exchange within the tissue dependent on the properties of the adhered droplet pairs[18, 91].

A rapidly developing area is the integration of living cells and artificial cells by encapsulating cellular machinery or whole living cells within the droplets. The use of cell-free machinery for the production of αHL enables the initiation of communication between droplets through biological mechanisms and gene circuitry[39]. Encapsulating living cells within the membranous architecture and observing their collective response was just recently demonstrated as well [38]. The integration of living and artificial cellular systems is an emerging field[6, 111] that provides exciting new alternatives for these membrane-based materials.

Shape Shifting

DIB tissues are often employed as static structures and the structural layout of these tissues is confined to the initial selected pattern. Thus, each printed tissue provides a singular formulation and functionality. While such membranous tissues offer opportunities for the study of tissue mechanics and fundamental scientific processes in biological interfaces and self-assembly, their static nature poses limitation when exploring potential applications where adaptability is required. More robust functionalities such as chemical microrobots for example would require more dynamic structures where the material functionality may be altered as needed.

Inspired by natural instances such as the capability for bone tissues response to chronic and occasional mechanical stresses for example[84-86] through changes in the cell shape (**Figure 7-1-a**), DIB-materials can be functionalized to respond to external stimuli through structural adaptations either at the single droplet level or at the entire tissue level. Again, a unique property of DIB materials relative to living tissues is that the membranes determining droplet-droplet exchanges are formed between droplet pairs. Therefore, the properties of the membranes are in part determined by the qualities of the individual droplets, including the membrane structure[18, 64] and gradients across the membrane generated by droplet compositions[35, 112]. Consequently, changes in the overall shape of the droplets/entire structure will also affect transport properties within the synthetic tissue. This is particularly relevant for cases where transport is only enabled with either matching or asymmetric lipid compositions[18, 52].

Transmembrane voltages provided through external electrodes may be used to drive electrowetting (**Figure 7-2-d**), altering the membrane areas through electrical signals and providing a form of single-interface shape change. As noted previously, the relative dimensions of the monolayer and bilayers are a function of their minimized interfacial energies, with membrane dimensions

dependent on the energy of adhesion. If transmembrane voltages are supplied, this produces an apparent reduction in the bilayer tensions through electrowetting[20-22, 113] which in turn produces a temporary increase in the membrane area between the droplets. Recent research has proposed using the inherent asymmetry in wetting/dewetting rates[40, 114] in DIBs to produce a form of neuromorphic materials as well, utilizing the DIB membranes as memcapacitors[115]. Membrane-based memory is obtained by applying a variable transmembrane potential and observing the transient wetting behaviors of the droplets. These phenomena allow for the membrane dimensions to gradually adapt to repeated pulses of voltages, approximating short-term synaptic activity.

Another instance mostly unique to DIBs is the ability to trigger the material structural response by mechanically perturbing certain constitutive droplets, exploiting the fact that DIB tissues are deformable given the innate weak elasticity of the adhered droplets[55-56]. Distorting the droplets produces transient changes in the membrane tensions[97, 101] as well as changes in the membrane dimensions[59]; consequently mechanical force may be used to either modulate membrane activities or produce changes in the structure of the adhered droplets. Traditionally, such functionalization at the individual membranes level typically involves direct contact with the droplets comprising the DIB, either electrodes attached to micromanipulators[48, 58, 78] or deformable flexible substrates[78] or even magnetic fields[15] (**Figure 7-2-c**). These approaches offer dynamic control over the DIB membrane dimensions. Our recent research highlights the ability of interfacial bilayers to generate capacitive currents (akin to a Kelvin probe) when subjected to periodic deformations[40-41, 89, 116-117]. Periodic droplet distortion in a high-frequency regime generates flexoelectric currents through membrane distortion[40-41]. This flexoelectricity has been used as a basis for enabling DIB structures with sensing abilities particularly vibration detection and detection of nearby air flow using single membrane DIBs[88-89, 117] as well as larger membrane networks[40, 116].

While direct manipulation of two adhered droplets to generate a desired response within the membrane is readily achievable using micromanipulators, direct mechanical contact with each droplet is impractical in high membrane-density cases. To rectify this, microfluidic methods for droplet manipulation may be used as an alternative. Many of these techniques have been applied towards the creation of droplet structures as discussed previously; here we discuss their use in promoting changes in the membranous structure.

One proposed contact-free microfluidic method for accomplishing changes/deformations in the shape of the material without moving individual cells is through osmotic flux. Naturally, tissues have the ability to deviate from their initial shape through swelling/deswelling without having to shift/move individual cells. Preset concentration gradients drive water exchange between the droplets, causing swelling and shrinking phenomena. This was used to great effect when printing self-folding droplet patterns[35], converting preset ionic concentration gradients between the droplets into changes in shape.

However, living tissues also exhibit the ability to rearrange the relative positions of their individual cells to adapt to external environments. Reproducing these events within DIBs is being explored by our group, taking cues from reconfiguration observed in living tissues. These events most notably occur during early stages of embryonic development (including cell shape change in **Figure 7-1-a**, cell fusion in **Figure 7-2-b**, rosette formation in **Figure 7-1-c**, tissue elongation through either elongation in **Figure 7-1-e** or intercalation in **Figure 7-1-f**, and non-apoptotic extrusion[45] in **Figure 7-1-d**).

Cell fusion (**Figure 7-1-b**) has been emulated in DIBs previously through targeted droplet fusion (**Figure 7-2-a**), allowing for the mixing of their contents and adjustment of the membranous structure[24, 44]. Reconfiguration without requiring coalescence was recently investigated by our

laboratory, applying forces on the adhered droplets to shift then between metastable states and change their connected neighbors and organization[71-72]. Hybrid membranous tissues containing both aqueous and ferrofluid (magnetic sensitive) droplets were generated and adhered together. Strategically applying external magnetic force fields yielded rearrangement events in the membranes neighboring magnetic micro-droplets marked by the separation of interfacial bilayers followed by the formation of new ones (a simple preliminary example of this was demonstrated in **Figure 7-2-b** through magnetically induced droplet translocation). Magnetic compartments shift and reach new equilibrium situations, adjusting the underlying internal structure of the overall tissue (**Figure 7-2-b**). This may be used to adjust the composition of the membranes within the DIB, altering the exchange of information[18, 52]. DIB tissues with the ability to adapt to external environment (an essential component of Ganti's postulated chemoton and of the hallmarks of life) can be used and built on in the further development of smart tissues where internal structure dictates functionality. The function of such tissues can be directly controlled by triggering changes in their structures.

CONCLUSION AND FUTURE PROSPECTS

Droplet-based materials offer a robust model platform for recreating simple cellular phenomena associated with exchange across the membranous barriers. In this review, we have explored recent advances in the construction, manipulation, and functionalization of these materials and how the selected components shape the overall properties and stability of the resulting droplet-based tissue. Next, we summarized how these materials may be used and improved, highlighting how their unique physics allow for new applications that combine synthetic and living systems and exploit their unique emulsive elasticity for adaptability.

There are still many challenges facing these materials. They are delicate and relatively simplistic when compared to living cellular systems. Ongoing research attempts to address each of these shortcomings, including new encapsulation techniques for prolonging durability, new surfactants that provide more stable membranes, and incorporation of living and artificial elements within the same DIB tissue. Each of these fields has rapidly expanded in recent years, prompted by a more thorough understanding of the underlying emulsive mechanics responsible for DIB characteristics.

While admirable research has been performed shifting these droplet-based materials towards more biomimetic designs, we must still recognize that the best path forward should blend the natural and synthetic aspects, opting to take advantage of the non-biological aspects of the platform to augment cellular functionalities. Based on the summarized material presented here, we propose that future directions in the development of DIB-tissues should combine droplet rearrangement with changes in membrane permeability, implementing both functionalization strategies for the material. Ideally, DIB-materials should be designed to adapt their droplet structure in response to supplied external forces, leading to evolutions in droplet composition through diffusion across newly formed pathways. In these applications, the form of DIB-based materials would dictate their function and the emulsive foundation of DIBs provide a metastable, dynamic form. Linking membrane permeability to relative droplet positioning and reliably generating reconfiguration events within the tissue will provide new functionalities for the material concept (**Figure 7-3**).

Our research group has been investigating strategies that enable DIB materials with the ability to respond to external force fields, self-support in an organogel matrix and establish communication between neighboring droplet compartments is governed by matching droplet compositions. It is believed that this platform could have additional applications in compartmentalized chemistry, membrane particle interactions studies, chemical microrobots specializing in biocompatible

precise actuation and high-throughput membrane screening once these envisioned advances are completed.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

The authors graciously acknowledge support from the National Science Foundation (NSF) under grants #1537410 and #1903965.

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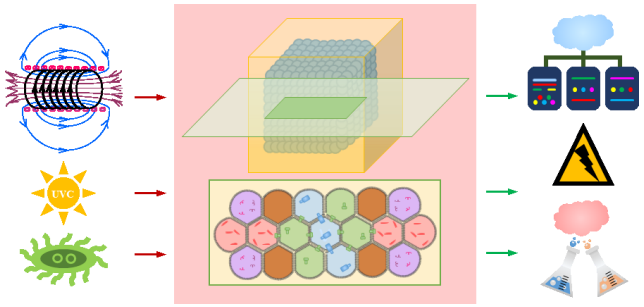
Table of Content Figure



Author Biographies

Michelle M. Makhoul-Mansour joined the Biomembranes Engineering Laboratory in the spring of 2016. Her work focuses on artificial materials that are inspired by the structure, modularity, and functionality of natural tissues. She is particularly interested in materials employing self-assembly and interfacial chemistry principles and constructed from basic molecular components. She is also exploring how systems of membrane mimics may be activated through mechanical, optical, and magnetic forces and is examining how the rearrangement and reconfiguration of membranous interfaces can be inspired by tissue cohesion and natural cellular rearrangement. Michelle graduated with a BE degree in Mechanical Engineering from the Lebanese American University in the spring of 2015 (Byblos, Lebanon).

Eric C. Freeman received his Ph.D. in 2012 from the University of Pittsburgh in Mechanical Engineering and Material Science then studied as a postdoctoral associate at Virginia Tech from 2012 to 2014. He joined the faculty at the University of Georgia as an assistant professor within the College of Engineering in 2014, founding the Biomembranes Engineering Laboratory with the goal of developing, characterizing, and simulating new biologically inspired materials based on cellular mechanics. He is now an associate professor in the School of Environmental, Civil, Agricultural and Mechanical Engineering at the University of Georgia.

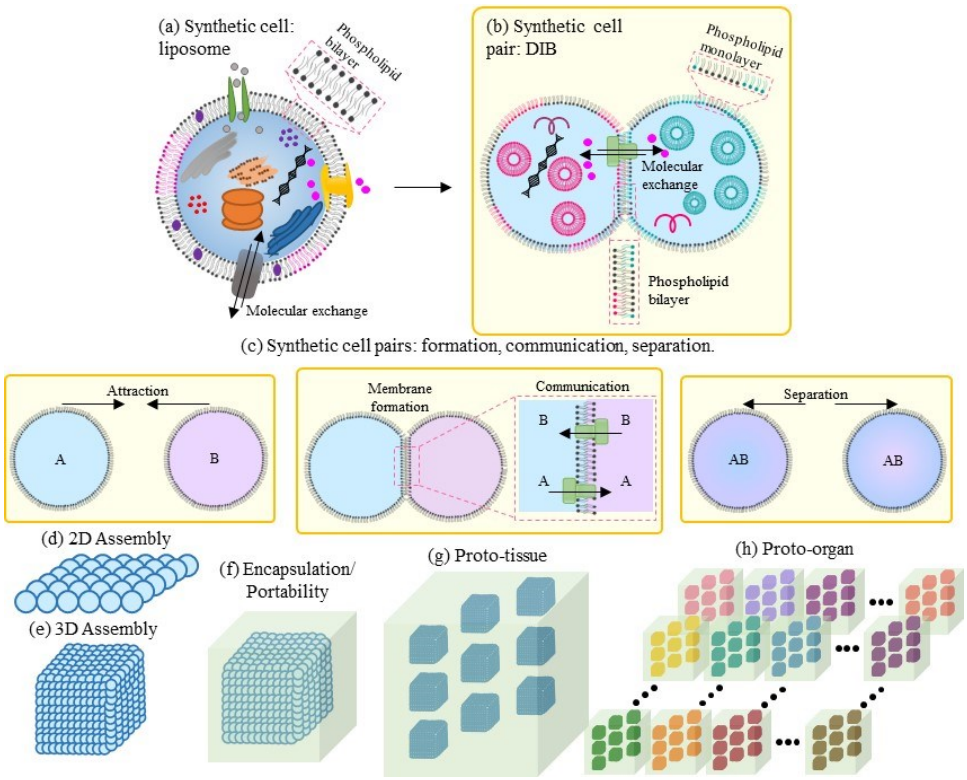


Figure 1 - (a) It has been proposed that basic molecular components can be used to construct synthetic cellular systems. One example of this are empty cells or liposomes, which are bordered with semi-permeable lipid bilayers and mediate exchange through transmembrane channels. Each liposome represents a standalone functional unit. (b) In contrast droplet pairs are connected with an interfacial lipid bilayer in the DIB technique to produce a single functional unit. Unlike liposomal systems, the properties of lipid membranes are in this case dictated by the bordering droplet pair and hence any rearrangement in the placement of these droplets will result in changes in the properties and composition of the interfacial bilayers. (c) These droplet pairs can be brought together to initiate droplet-droplet exchange. Afterwards, droplet pairs can be separated resulting in two compartments with updated molecular composition. DIBs can be used to construct (d) 2D and (e) 3D membranous tissues in oil and water mediums. (f) Further efforts are focused on enhancing the portability of these materials by developing various encapsulation strategies. It has been suggested that the multicompartmentalization of DIB-structures within an organogel could approximate a proto-tissue as shown in (g), and a collection of connected proto-tissue units would ambitiously approximate a proto-organ as shown in (h).

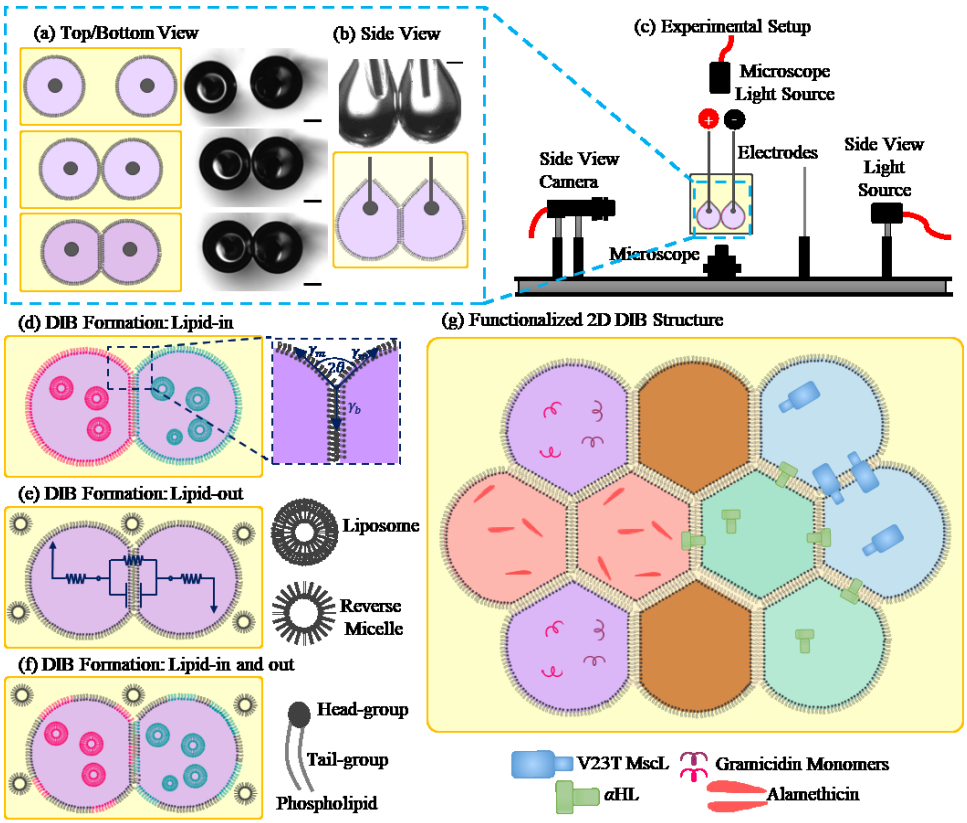


Figure 2 - The droplet interface bilayer (DIB) technique. A single lipid bilayer is formed at the interface of two lipid-coated aqueous microdroplets brought into contact. Schematic and experimental images of the formation of a DIB viewed (a) from the top/bottom and (b) from the side. (c) Schematic representation of the experimental setup used for DIB formation/characterization. Aqueous droplets are deposited on agarose-coated silver/silver chloride electrodes submerged in oil. Side view and top view cameras connected to a microscope are used to acquire images of the formed bilayer. (b) and (c) are adapted from ref (Makhoul-Mansour, M. M.; El-Beyrouthy, J. B.; Mumme, H. L.; Freeman, E. C., Photopolymerized microdomains in both lipid leaflets establish diffusive transport pathways across biomimetic membranes. Soft Matter 2019.) by permission of the Royal Society of Chemistry 2019. Copyright 2019 Royal Society of Chemistry. Lipids can be dispersed in the (d) water phase (lipid-in), (e) oil phase (lipid-out) or (f) both phases. The area of the DIB is governed through a balance of monolayer and bilayer tensions reflected through an external angle of contact. (g) Multiple droplets can be connected to form a 2D or even 3D membranous structure. Upon the formation of a single membrane, the spherical droplets deform into spherical caps, delimited by the dimensions of the interfacial bilayer. On a larger scale, similarly to tightly packed emulsive systems, DIB networks can exhibit regular hexagonal close-packing lattices distribution when optimal oil phases and lipid mixtures are used. Various integral

transmembrane channels can be embedded in droplets and used to establish communication pathways within the material. Scale bar 250 μm.

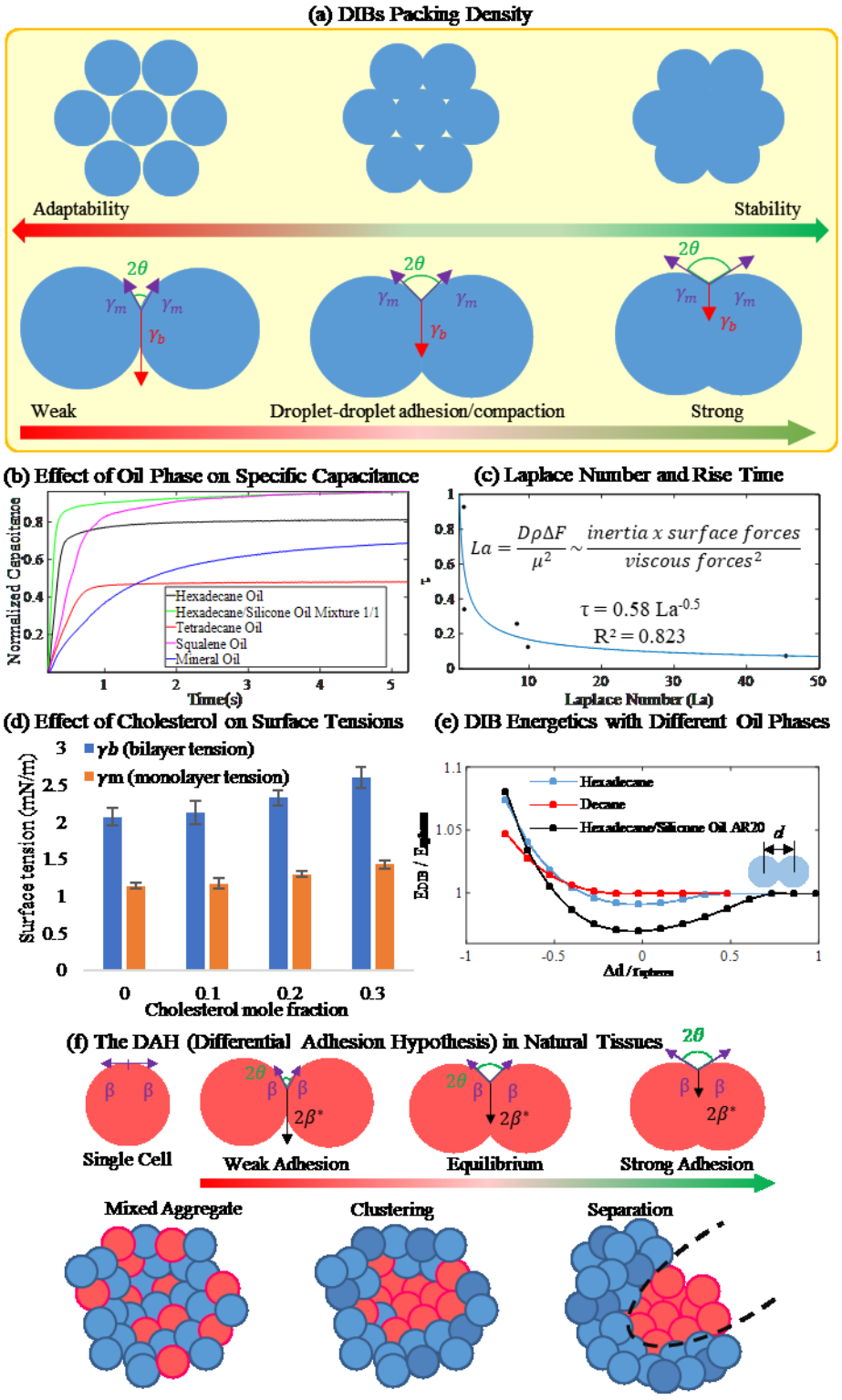


Figure 3 – Summary of DIB mechanics and dependencies. (a) Schematic representation of the effect of packing density on the adaptability and the stability of DIB tissue. As adhesion between droplets is increased, the contact angle, membrane area and adhesion energy are increased, leading to denser networks. (b) Properties of DIBs were assessed using custom fabricated hydrogel microelectrodes, investigating the effect of the oil used on the specific capacitance of lipid membranes and their rate of formation. (c) The time for the membrane to reach 2/3 of its normalized size (defined here as the rise time) can be plotted against the Laplace number (mirroring the effect of inertia, surface forces and viscous forces). (b) and (c) are adapted (data adapted) with permission from ref (Challita, E. J.; Freeman, E. C., Hydrogel Microelectrodes for the Rapid, Reliable, and Repeatable Characterization of Lipid Membranes. Langmuir 2018, 34 (50), 15166-15173.). Copyright 2018 American Chemical Society Langmuir. (d) Cholesterol molecules insert themselves between phospholipid molecules resulting in more rigid membranes which is reflected through an increase in both the monolayer and bilayer tension. Data adapted with permission from ref (El-Beyrouthy, J.; Makhoul-Mansour, M. M.; Taylor, G.; Sarles, S. A.; Freeman, E. C., A new approach for investigating the response of lipid membranes to electrocompression by coupling droplet mechanics and membrane biophysics. Journal of the Royal Society Interface 2019, 16 (161), 20190652.). Copyright 2019 Royal Society Interface. (e) Further investigation into the effect of the oil phase showed that the interfacial energy for a pair of adhered droplets as a function of the normalized distance between their centers (offset by the equilibrium distance) is also varying for different oils. Membranes that are more favorable to form show a greater range of deformation prior to separation. Adapted with permission from ref (Challita, E. J.; Makhoul-Mansour, M. M.; Freeman, E. C., Reconfiguring droplet interface bilayer networks through sacrificial membranes. Biomicrofluidics 2018, 12 (3), 034112.). Copyright 2018 AIP Biomicrofluidics. (f) Intended to provide an explanation for the spontaneous liquid-like tissue behavior, the DAH models tissues as emulsive systems: cells behave similarly to microdroplets whose varying degrees of surface adhesion (illustrated by using different colors blue and pink) causes them to spontaneously reorganize and minimize their interfacial free energy (β: cortical tension at free cell surface and β*: residual tension in a cell at contact).

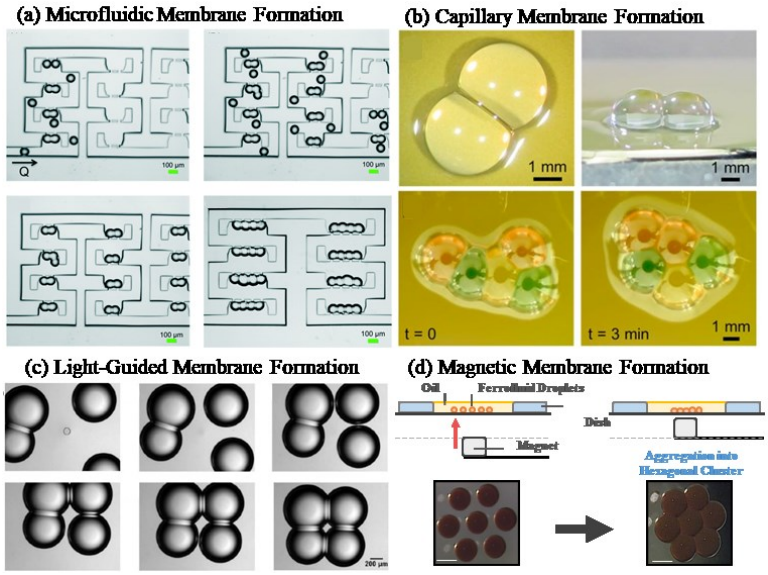


Figure 4 – Various methods for assembly smaller DIB networks in a repeatable fashion (a) Experimental images of DIBs formed using microfluidic channels. Each hydrodynamic trap can hold several lipid bilayer formed between two aqueous droplets. Reprinted with permission from ref (Nguyen, M.-A.; Srijanto, B.; Collier, C. P.; Retterer, S. T.; Sarles, S. A., Hydrodynamic trapping for rapid assembly and in situ electrical characterization of droplet interface bilayer arrays. Lab on a Chip 2016, 16 (18), 3576-3588.). Copyright 2016 Lab on a Chip RSC. (b) Lipid-coated water droplets can be deposited into oil infused hydrophobic surfaces and brought into contact forming 2D DIB structures. Reprinted with permission from ref (Boreyko, J. B.; Polizos, G.; Datskos, P. G.; Sarles, S. A.; Collier, C. P., Air-stable droplet interface bilayers on oil-infused surfaces. Proc Natl Acad Sci U S A 2014, 111 (21), 7588-93.). Copyright 2014 PNAS. (c) Laser-induced heating may be used to drive aqueous droplets into close proximity to each other resulting in the formation of lipid bilayers. Reprinted with permission from ref (Dixit, S. S.; Kim, H.; Vasilyev, A.; Eid, A.; Faris, G. W., Light-driven formation and rupture of droplet bilayers. Langmuir 2010, 26 (9), 6193-6200.). Copyright 2010 American Chemical Society Langmuir. (d) Magnetic fields may be used to assemble DIB networks by selectively infusing droplets with ferrofluids. The scale bar is 800 μm. Reprinted with permission from ref (Challita, E. J.; Makhoul-Mansour, M. M.; Freeman, E. C., Reconfiguring droplet interface bilayer networks through sacrificial membranes. Biomicrofluidics 2018, 12 (3), 034112.). Copyright 2018 AIP Biomicrofluidics.

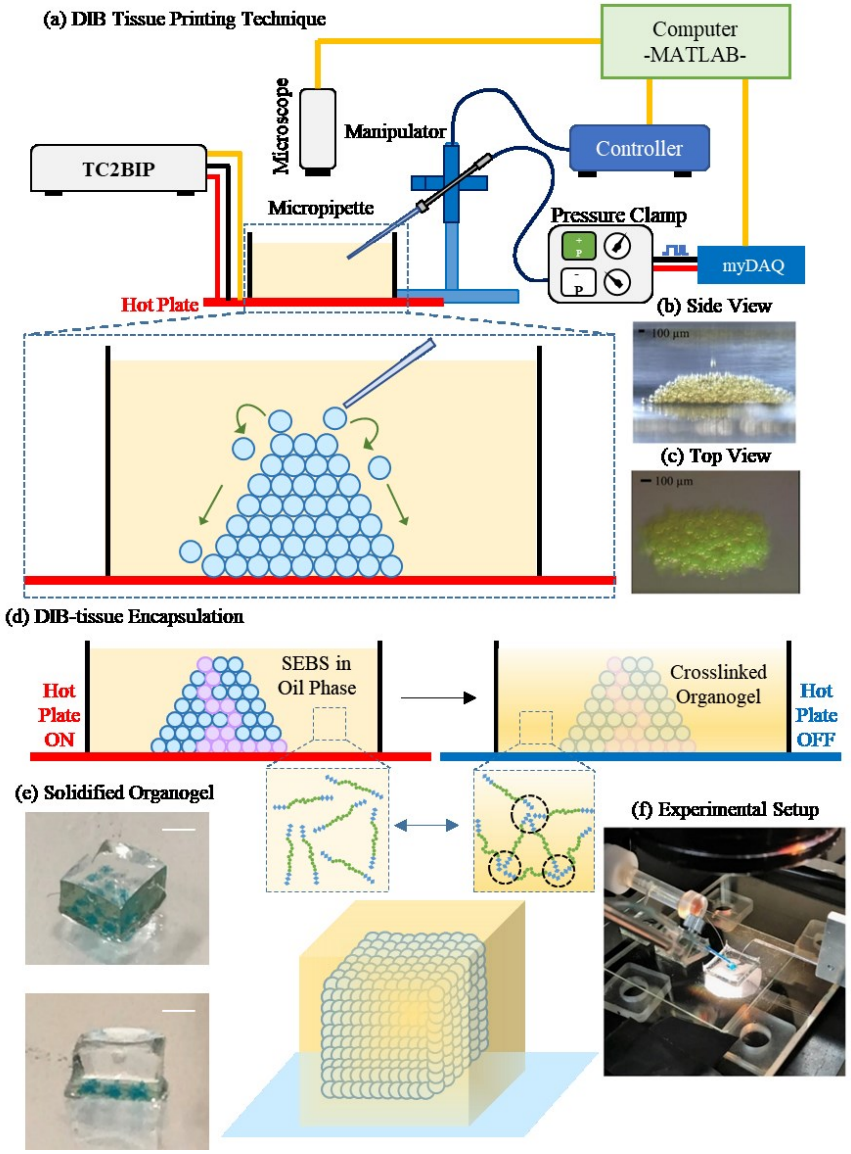


Figure 5 - High membrane-throughput printing of encapsulated DIB tissues. (a) A glass micropipette is connected to a pressure clamp for pneumatic droplet deposition. A 3-axis manipulator is used to manipulate the micropipette to specific predefined coordinates. Adapted with permission from ref (Challita, E. J.; Najem, J. S.; Monroe, R.; Leo, D. J.; Freeman, E. C., Encapsulating Networks of Droplet Interface Bilayers in a Thermoreversible Organogel. Scientific reports 2018, 8.). Copyright 2018 Scientific Reports, Nature. (b) Side and (c) top view experimental images of a printed 3D DIB network. (b) and (c) are adapted with permission from ref (Challita, E. J.; Najem, J. S.; Freeman, E. C.; Leo, D. J. In A 3D printing method for droplet-based biomolecular materials, Nanosensors, Biosensors, Info-Tech Sensors and 3D Systems 2017, International Society for Optics and Photonics: 2017; p 1016712.). Copyright 2017 International Society for Optics and Photonics SPIE. (d) Using the previously developed pneumatic droplet printing

system, the process is repeated to generate webs of aqueous droplets. The printing process is achieved on a heating plate using the experimental setup shown in (f) ensuring that the encapsulating organogel is molten. (e) Once the final structure is produced, the heat is removed allowing the organogel to solidify. The organogel preserves the structural integrity of the material and can be removed from the substrate. (e) and (f) are reprinted with permission from ref (Challita, E. J.; Najem, J. S.; Monroe, R.; Leo, D. J.; Freeman, E. C., Encapsulating Networks of Droplet Interface Bilayers in a Thermoreversible Organogel. Scientific reports 2018, 8.) Copyright 2018 Scientific Reports, Nature.

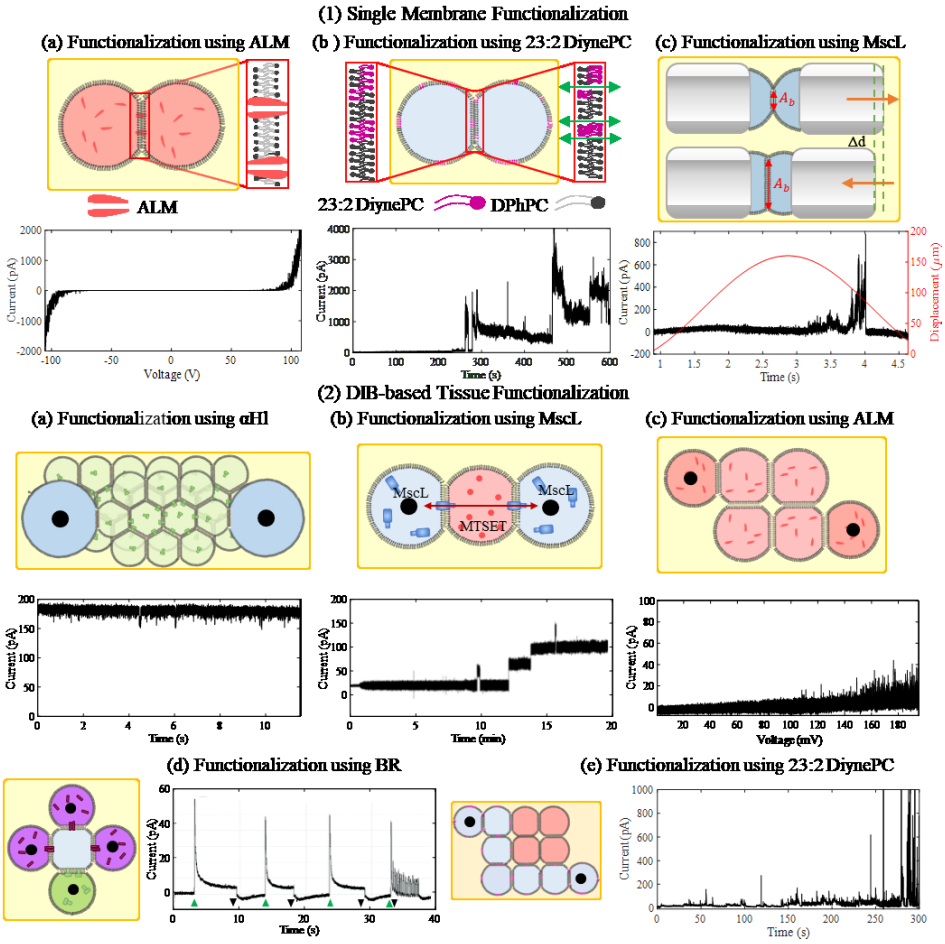


Figure 6- Various methods for functionalizing DIB materials presented as changes in membrane conductivity. (1) (a) Alamethicin peptides exhibit a characteristic nonlinear conductivity. Adapted with permission from ref (Makhoul-Mansour, M. M.; Freeman, E. C., Photo-Triggered Soft Materials with Differentiated Diffusive Pathways. In ASME 2019 Conference on Smart Materials, Adaptive Structures and Intelligent Systems, American Society of Mechanical Engineers Digital Collection: Louisville, Kentucky, USA, 2019; Vol. 1, pp 1-9.). Copyright 2019 ASME. (b) Photopolymerizable lipids can be crosslinked using UVC light forming transmembrane defects. Adapted from ref

(Makhoul-Mansour, M. M.; El-Beyrouthy, J. B.; Mumme, H. L.; Freeman, E. C., Photopolymerized microdomains in both lipid leaflets establish diffusive transport pathways across biomimetic membranes. Soft Matter 2019.) by permission of the Royal Society of Chemistry 2019. Copyright 2019 Royal Society of Chemistry. (c) V23T MscL can be activated in single DIBs by droplet compression. (2) (a) αHL can be used to establish exchange within a DIB tissue. Adapted with permission from ref (Challita, E. J.; Najem, J. S.; Monroe, R.; Leo, D. J.; Freeman, E. C., Encapsulating Networks of Droplet Interface Bilayers in a Thermoreversible Organogel. Scientific reports 2018, 8.) Copyright 2018 Scientific Reports, Nature. (b) MscL activity has been observed in networks of droplet interface bilayers (MTSET in the middle droplet). Adapted with permission from ref (Haylock, S.; Friddin, M. S.; Hindley, J. W.; Rodriguez, E.; Charalambous, K.; Booth, P. J.; Barter, L. M.; Ces, O. J. C. C., Membrane protein mediated bilayer communication in networks of droplet interface bilayers. 2020, 3 (1), 1-8). Copyright 2020 Nature. (c) In large DIB networks, alamethicin still requires transmembrane voltages of 70 mV. Adapted with permission from ref (Challita, E. J.; Najem, J. S.; Monroe, R.; Leo, D. J.; Freeman, E. C., Encapsulating Networks of Droplet Interface Bilayers in a Thermoreversible Organogel. Scientific reports 2018, 8.). Copyright 2018 Scientific Reports, Nature. (d) DIB networks can be rendered sensitive to green light through bacteriorhodopsin (BR). Adapted with permission from ref (Holden, M. A.; Needham, D.; Bayley, H., Functional bionetworks from nanoliter water droplets. Journal of the American Chemical Society 2007, 129 (27), 8650-8655.). Copyright 2007 American Chemical Society. (e) Droplets containing compatible polymerizable lipid profiles produce conductive pathways. Reprinted from ref (Makhoul-Mansour, M. M.; El-Beyrouthy, J. B.; Mumme, H. L.; Freeman, E. C., Photopolymerized microdomains in both lipid leaflets establish diffusive transport pathways across biomimetic membranes. Soft Matter 2019.) by permission of the Royal Society of Chemistry 2019. Copyright 2019 Royal Society of Chemistry.

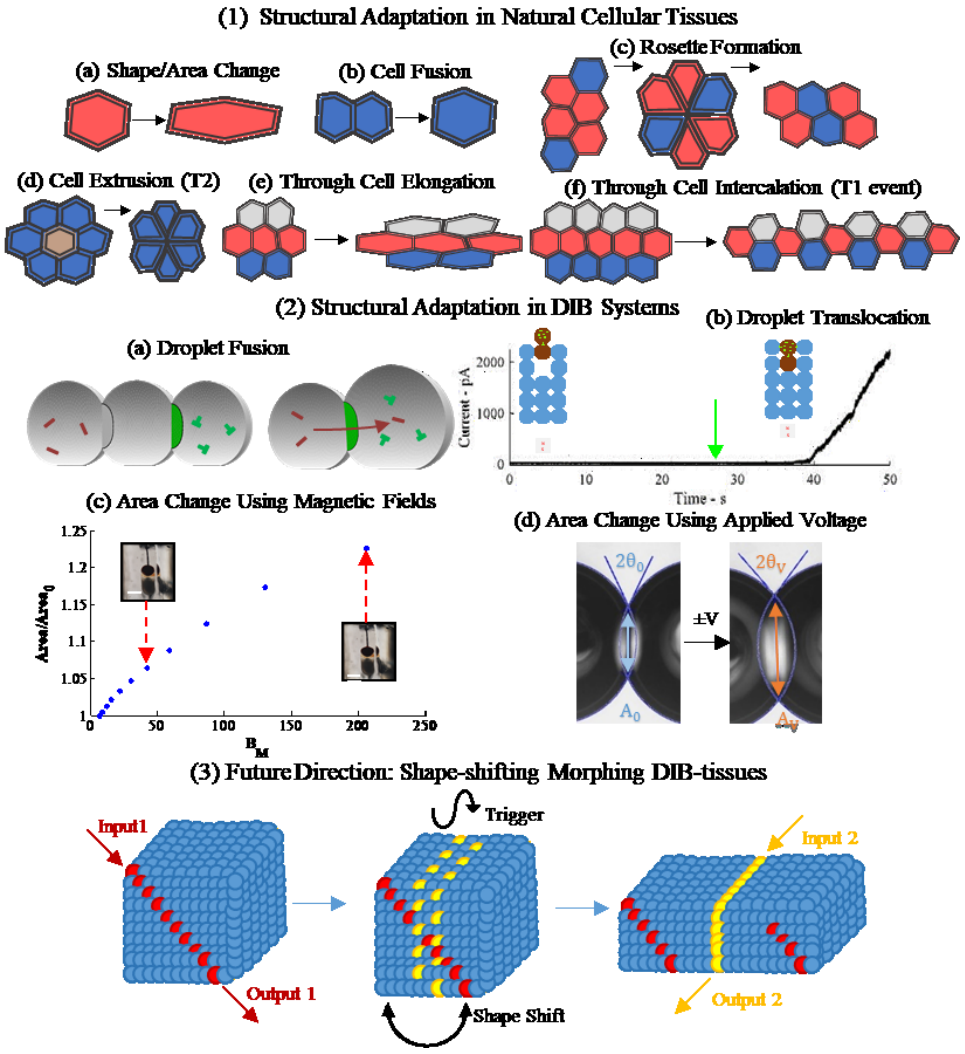


Figure 7- Structural adaptation in natural cellular tissues inspires future development of DIB-based tissues. (1) Living tissues possess the ability to rearrange and reconfigure their cells relative to one another as well as to alter the area/shape of individual cells. Various cellular processes drive adaptation such as (a) shape/area change, (b) cell fusion, (c) rosette formation, and (d) cell extrusion. (2) These processes are being explored within the DIB platform. (a) Droplet fusion has been triggered through the application of a high transmembrane voltage, enabling rapid mixing of droplet contents. Reprinted with permission from ref (Challita, E. J.; Makhoul-Mansour, M. M.; Freeman, E. C., Reconfiguring droplet interface bilayer networks through sacrificial membranes. Biomicrofluidics 2018, 12 (3), 034112.). Copyright 2018 AIP Biomicrofluidics. The incorporation of biocompatible ferrofluids within the platform has allowed for a contact free manipulation of stable DIB networks for the establishment of new communicative pathways shown in (b) or the control of the membrane and droplet shape/area. (b) is reprinted with permission from

ref (Makhoul-Mansour, M.; Challita, E. J.; Freeman, E. C. In Ferrofluid Droplet Based Micro-Magnetic Sensors and Actuators, ASME 2017 Conference on Smart Materials, Adaptive Structures and Intelligent Systems, American Society of Mechanical Engineers: 2017; pp V001T06A009-V001T06A009.) Copyright 2017 ASME. (c) is reprinted from ref (Makhoul-Mansour, M.; Zhao, W.; Gay, N.; O'Connor, C.; Najem, J.; Mao, L.; Freeman, E. C., Ferrofluid-Based Droplet Interface Bilayer Networks. Langmuir 2017.). Copyright 2018 American Chemical Society Langmuir. Alternatively, electrical currents can be also employed to influence the droplets' and membranes' size through wetting/dewetting. Adapted with permission from ref (El-Beyrouthy, J.; Makhoul-Mansour, M. M.; Taylor, G.; Sarles, S. A.; Freeman, E. C., A new approach for investigating the response of lipid membranes to electrocompression by coupling droplet mechanics and membrane biophysics. Journal of the Royal Society Interface 2019, 16 (161), 20190652.) Copyright 2019 Royal Society Interface. (3) Future work on adaptive DIB structures will emphasize coupling changes in membrane permeability with changes in relative droplet positioning, producing networks of droplets that swap between communication modes enabled by external forces.