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Supporting Information

Bilayer Networks within a Hydrogel Shell: A Robust Chassis for Artificial Cells and a Platform for Membrane Studies

*Divesh K. Baxani, Alex J. L. Morgan, William D. Jamieson, Christopher J. Allender, David A. Barrow, and Oliver K. Castell**

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Supporting Information

Materials and Methods

Materials

Polyether ether ketone (PEEK) fingertight fittings, capillary sleeves and fluidic T-junctions as well as fluorinated ethylene propylene (FEP) tubing were all purchased from Kinesis (UK). Glass capillaries were purchased from CM Scientific (USA). Poly lactic acid (PLA) filament for 3D-printing was purchased from Faberdashery (UK). DI Water was used from a Milli-Q filtration system (Merck Millipore, USA). Hexadecane, mineral oil, sodium dihydrogen phosphate, 2-propanol, chloroform, sulphorhodamine B, lissamine green, low viscosity sodium alginate, glacial acetic acid, sodium chloride, potassium chloride, low melting point agarose and $CaCO_3$ were all purchased from Sigma-Aldrich (UK). N-(Triethoxysilylpropyl)-O-poly(ethylene oxide) urethane was purchased from ABCR (Germany). 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) was purchased from Avanti polar Lipids (USA).

Surface modification of glass capillaries

Glass capillaries (ID: 2.15 mm OD: 2.8 mm or ID: 2 mm OD: 2.4 mm) to be incorporated into the microfluidic device were sonicated (FB15055, Fisherbrand UK) for 10 minutes in acetone and then methanol and thoroughly dried prior to plasma activation (Diener FEMTO, Germany) using oxygen plasma (0.35 mbar, 15 sccm, 10 minutes). Following plasma activation, activated glass capillaries were soaked overnight in a 2 % (v/v) solution of N-(Triethoxysilylpropyl)-O-poly (ethylene oxide) urethane in 2-propanol to render them permanently hydrophilic. Silanised glass capillaries were then rinsed in 2-propanol, dried and cured overnight at 120ilic. ight in a 2 %ace modification was confirmed by contact angle measurement.

Preparation of fluids

Aqueous inner cores consisted of deionised water with 50 mM sodium dihydrogen phosphate and sulphorhodamine B or lissamine green for colour (50μM) (pink or blue, respectively) The conditions for the lipid in oil phase was based on previously reported methods[1,2] and was produced via the evaporation of chloroform containing dissolved DPhPC using a nitrogen gas stream, and by re-dissolving the resultant dry lipid film in a 1:1 mixture of hexadecane and silicone oil AR20 to a final concentration of 5 mg/mL. Alginate solution was prepared by dissolving sodium alginate (2% w/v) and suspending $CaCO_3$ particles (75 mg/mL) in DI water. The alginate solution was stirred at 60ing sodium alginate (2% w/v) and sonicated for 1 minute to remove air bubbles. The carrier oil phase was composed of mineral oil with 0.5% glacial acetic acid.

Device design, fabrication and operation

A 3D printed microfluidic device comprising integrated channels interfacing with FEP tubing and glass capillaries was engineered in order to facilitate the production of triple emulsions (water-in-oil-in-water-in-oil) eDIBs as described in the main text and illustrated in Figure 2. The 3D printed fluidic device and manifold was designed with the aid of CAD software (Solidworks, Dassault Systemes, France) and fabricated by 3D-printing

(Ultimaker 2 desktop 3D-printer, Ultimaker, Netherlands) parameterised with CAM slicing software (Cura, Ultimaker, Netherlands). Devices were printed in layers of 50ers of 5050f 50were printed in layers of 50 laypolylactic acid (PLA) filament. FEP tubing (ID: 750 t. FEP tubing in) and glass capillaries were secured in place with epoxy resin to create a leak-free seal. All fluids were delivered by syringe drivers (KD Scientific, World Precision Instruments).

Electrophysiology experiments

eDIBS were placed in a Petri dish within a custom built Faraday cage. Custom Ag/AgCl electrodes were prepared from 100 µm diameter silver wire embedded within a pulled glass capillary (initial internal diameter = 1 mm) to provide mechanical stability. The electrode designated for insertion into the internal aqueous cores of the eDIB was sealed with insulatory PDMS except for ~ 500 µm at the electrode tip. The PDMS was used to insulate from electrical leakage as the electrode was required to pass through the common alginate shell. The second electrode was inserted into the alginate shell. Electrodes were mounted on micromanipulators (Narishige International, USA) and connected to an Axopatch 200B with a 203BU headstage (Molecular Devices, USA). Electrophysiology recordings of bilayer capacitance and ion flux were made respectively under applied potentials of a +/-23 mV triangular wave at 10 Hz or a fixed potential of 10-50 mV. Data was recorded with WinEDR (University of Strathclyde) analogue filtered at 5 kHz. Electrophysiology traces were digitally filtered post-acquisition with either a 1 kHz or 100 Hz low-pass filter.

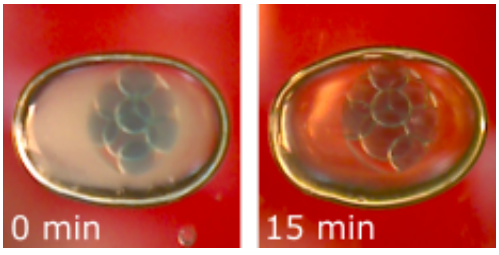
Supplementary Data and Discussion

eDIB alginate shell transparency

The alginate solution that forms the outer shell initially appears partially opaque due to suspended CaCO3 particles. During formation of individual eDIB constructs the alginate phase is brought into contact with the oil containing acetic acid. Partitioning of acetic acid from the organic to the aqueous phase initiates liberation of free calcium ions and dissolution of the CaCO3 particles in accordance with reaction 1.

$$CaCO_3 + 2H^+(aq) \rightarrow Ca^{2+}(aq) + CO_2 + H_2O \text{ (reaction 1)}$$

This gives rise to shell gelation as alginate monomers are cross-linked by the free divalent calcium ions, whilst dissolution of the calcium carbonate renders the outer shell transparent. Here, an excess of calcium carbonate is used, giving rise to rapid gelation whilst still in the microfluidic device, with complete transparency achieved subsequently upon reaction and dissolution of the remaining calcium carbonate.



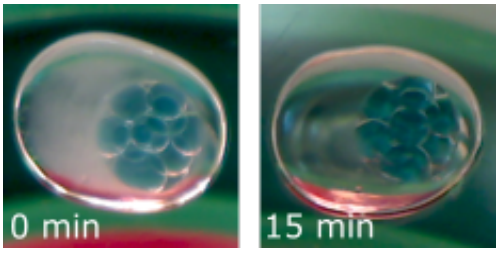


Figure S1. The outer shell of a single eDIB becomes transparent over a period of approximately 15 minutes. The alginate shell of eDIBs becomes transparent as partitioning of acetic acid from the carrier oil phase causes a reduction in pH reacting with the particulate $CaCO_3$ producing soluble calcium and carbon dioxide. The removal of suspended calcium carbonate results in transparency of the eDIB shell. Here, alginate phase contains 2% (w/v) low viscosity alginate and 75 mg/mL $CaCO_3$. In accordance with the gelling mechanism transparency is realised more rapidly at the core perimeter as acetic acid-partitions into the shell from the external oil phase.

The rate of this reaction can be modified to provide control over shell morphology. For example, early gelation was found to produce asymmetric, elongated, ovoid shaped constructs with internal cores often residing at one end, as external rigidity was achieved within the microfluidic channel. Whereas it was found slower gelation gave rise to spherical constructs as greater shell flexibility was maintained under flow conditions until full rigidification was achieved on exit from the device.

Whilst not the subject of detailed study here, we note that control of this gelation may be achieved by modulation of rate and extent of calcium liberation from calcium carbonate. This means that physical parameters such as particle size, concentration, acetic acid concentration, capsule surface area:volume ratio, capsule size and flow conditions (e.g. flow rate dependent advective mixing and oil:alginate volume ratio), can be used to control gelation. A reduction in $CaCO_3$ particle size from be upa to ticle size from be used to control in particle surface area : volume ratio, resulting in more rapid dissolution and progression to transparency. Similarly, buffering conditions of the alginate phase can be used to control the rate and extent of pH change on partitioning of acetic acid to modulate the conditions under which gelation proceeds. In the protein insertion and electrophysiology experiments reported here we employ 0.5x phosphaste buffered saline (PBS) in the alginate phase to produce a rugged, yet flexible, outer shell suitable for manual handling and protein diffusion through the hydrogel. After gelation, constructs may be washed in aqueous media to perfuse the gel and enable control of the outershell environment.

In the reported experiments, in situ acetic acid partitioning from oil to water was employed to trigger gelation at the point of droplet formation. However, other methods of gelation, such as calcium addition, or the release of photo-caged calcium, could be used, and the kinetics of calcium delivery could be harnessed to control alginate morphology.[3] It should also be possible to use other hydrogels or alternative polymers for encapsulation, providing the ability to tailor external surface chemistry and permeability.

Variability in the number of internal aqueous cores per eDIB.

Microfluidic production of eDIBs was found to be reliable and reproducible. However, it was noted that under constant operating conditions some instability in number of internal aqueous cores encapsulated was observed, usually resulting in encapsulation of one greater or one fewer than the anticipated number of internal droplets. This variability was attributed to the ability of aqueous droplets to come into contact and form a bilayer during the encapsulation process, providing an adhesive force between contacting droplets

reducing the likelihood of separation at this point. Despite the non-linear pressure profiles in droplet formation and breakup mechanisms,[4,5] the microfluidic device was able to tolerate these fluctuations with minimal observable impact on subsequent construct formation (Figure 4f).

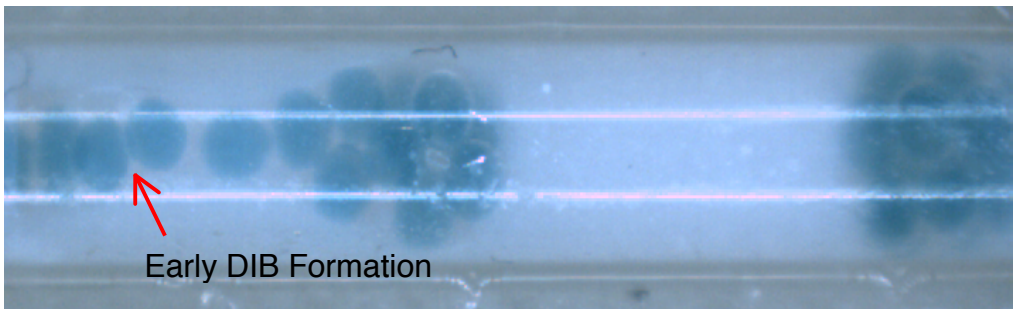


Figure S2. Droplet Interface Bilayer formation between neighbouring droplets during the encapsulation process. Occasionally it was observed that aqueous droplets could contact and form a bilayer during the encapsulation process providing an adhesion between the droplets. If this occurred close to oil droplet breakup, as pictured, it could result in the inclusion or exclusion of additional droplet(s), giving rise to the slight variability in the number of aqueous cores encapsulated as characterised in figure 4f.

Stability

We have found that eDIBs are stable for periods of weeks, with samples surviving over a month freestanding in an aqueous environment. We have been able to store eDIBs in aqueous solution in Eppendorf sample tubes (Figure S3a) where they are able to withstand mechanical agitation, allowing for transport from one University department to another (1.2 km, walking). eDIBs may be pipetted (Figure S3c) or manipulated by tweezing to make subsequent electrophysiology or fluorescent measurements (Figure S3b).

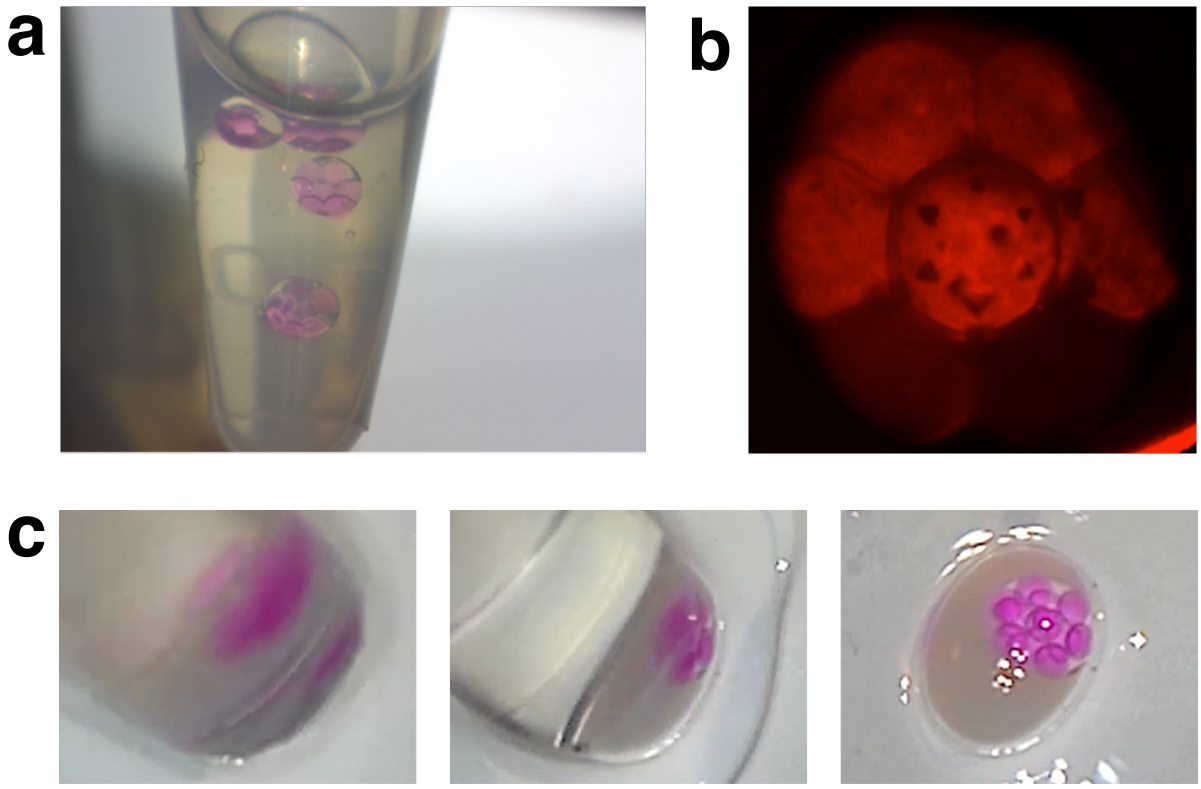


Figure S3. a) eDIBs stored in aqueous solution in a sample tube 11 days after preparation. The alginate shell of the constructs is not readily visible owing to complete dissolution of calcium carbonate and equilibration with the external environment. b) Fluorescent microcopy image of eDIB construct with internal cores containing 50 11 sulphorhodamine B, eDIBs were pipetted onto a microscope slides for imaging. Image acquired with a custom built epi-fluorescence microscope with a 4x objective and using a 532 nm laser illumination. c) Sequence of images demonstrating the pipetting of eDIBs from an oil solution onto the surface of a polymer Petri dish.

References

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[4] P. Garstecki, M. J. Fuerstman, H. A. Stone, G. M. Whitesides, *Lab on a Chip* **2006**, 6, 437-446.

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Supplementary Movies

SI Video 1. Coaxial flow producing water-in-oil droplets in a continuous stream of un-gelled alginate solution. Aqueous droplets contain lissamine green for visualisation. Video taken using a high-speed camera (Megaspeed, USA) recording at 50 frames per second, mounted on a stereomicroscope (AZ100 Multizoom, Nikon, Japan). The video is reproduced at 20% speed.

SI Video 2. Coaxial flow producing water-in-oil-in-alginate droplets in a continuous stream of mineral oil containing acetic acid. Video taken using a high-speed camera (Megaspeed, USA) recording at 50 frames per second, mounted on a stereomicroscope (AZ100 Multizoom, Nikon, Japan). The video is reproduced at 20% speed.

SI Video 3. Handling of eDIBs: a) An eDIB in oil being deposited on a Petri dish surface with use of a pipette. b) An eDIB with two internal droplet cores of different composition being transferred from oil to water via tweezers. c) An eDIB with 10 internal cores being transferred into an oil environment via tweezers.