From protocells to prototissues: a materials chemistry approach

Prototissues comprise free-standing 3D networks of interconnected protocell consortia that communicate and display synergistic functions. Significantly, they can be constructed from functional molecules and materials, providing unprecedented opportunities to design tissue-like architectures that can do more than simply mimic living tissues. They could function under extreme conditions and exhibit a wide range of mechanical properties and bio-inspired metabolic functions. In this perspective, I will start by describing recent advancements in the design and synthetic construction of prototissues. I will then discuss the next challenges and the future impact of this emerging research field, which is destined to find applications in the most diverse areas of science and technology, from biomedical science to environmental science, and soft robotics.

Introduction

The past decade has seen the field of bottom-up synthetic biology try to fill the gap between chemistry and biology to better understand the boundary between non-living and living matter. To do this, major efforts have been devoted to construct so-called *protocells*. From a general perspective, protocells are cell-like entities designed to mimic basic aspects of living cells. Strictly speaking, protocells have many different definitions due to the rapid evolution of this research field. Xu *et al.* [1] suggested the possibility of classifying them into two main categories: *typical* and *non-typical protocells*. Typical protocells are by all means artificial cells. They exhibit a cell-like structure and at least one or more of the key characteristics of living biological cells such as self-sustainability, self-reproduction, or the ability to evolve or metabolise. Non-typical protocells are instead materials engineered to mimic one or more features of biological cells such as shape, morphology, function, or endogenous reactivity [2–11]. Most importantly, non-typical protocells do not need to strictly mimic living cells and have no restrictions on the type of materials, methodologies and chemical reactions that can be used to build them [12,13]. This perspective article focuses on this latter category and the term *protocell* here refers to *non-typical protocells*.

While the focus is currently kept on increasing the levels of biofunctionality and autonomy by advancing the biochemical complexity of individual protocells, less progress has been made towards the fabrication of *prototissues* (or *synthetic tissues*) from protocell building blocks. Prototissues comprise free-standing three-dimensional (3D) networks of interconnected protocell consortia that communicate and display synergistic functions, providing a new paradigm for the field of bottom-up synthetic biology. Important technological advancements in this research direction were pioneered by Prof. Hagan Bayley and co-workers, who developed synthetic tissues based on micro-droplets connected by interface bilayers (DIBs). These are lipid-coated picolitre aqueous droplets in oil that adhere at their interface to form stable bilayers, in which membrane proteins can be inserted (Figure 1a,b) [14].

Prototissues do not need to strictly mimic living tissues in the same way that protocells do not need to strictly mimic living cells. In fact, DIB-based prototissues might be close mimics of living tissues allowing us to study biology in a simplified and highly controlled environment, but present limited

technological applications as they are highly impermeable to molecules and suffer from low stability towards variations in pH, ionic strength, and temperature [15]. To advance the development and impact of prototissue-based technologies, a new *materials chemistry approach* is required. This approach combines advanced concepts of materials chemistry and synthetic biology to enable the generation of prototissues from protocell models constructed from synthetic functional molecules and materials. The resulting *protocellular materials* would benefit from greater chemical stability, mechanical robustness, and tuneable permeability, providing new opportunities in application areas beyond mimicking living tissues such as in the emerging field of soft robotics.

As a first step towards this objective, our group at the University of Bristol developed a synthetic approach to the programmed assembly of a binary community of functional protein–polymer protocell membranes termed *proteinosomes*, into prototissue spheroids, which were capable of thermally induced muscle-like contractions [16]. Instead of patterning by 3D printing, we pre-programmed two populations of proteinosomes to self-assemble by generating covalent bio-orthogonal protocell–protocell adhesions via an interfacial strain-promoted alkyne-azide cycloaddition (I-SPAAC) reaction. To achieve this, we synthesised a bovine serum albumin/poly (N-isopropylacrylamide)-co-methacrylic acid (BSA/PNIPAM-co-MAA) nanoconjugate and functionalised it either with azide (N_3–) or bicyclononyne (BCN) moieties (Figure 1c). The prototissue spheroids were then produced from water-in-oil-in-water (w/o/w) Pickering emulsion droplets, comprising of an outer non-reactive BSA/PNIPAM-co-MAA membrane caging a binary community of N_3- and BCN-functionalised proteinosomes. The w/o/w Pickering emulsions were then stabilised by a cross-linker and transferred to the aqueous phase. The removal of the encapsulated oil phase triggered the bio-orthogonal adhesion of the caged reactive proteinosomes via the I-SPAAC reaction (Figure 1d). This produced membrane-bound spheroids with a spatially integrated tissue-like structure as shown by fluorescence microscopy images (Figure 1e). Significantly, the prototissue spheroids displayed high stability in water media (>6 months) and were permeable to small molecules, thereby opening the possibility of building the first enzymatically active protocellular materials capable of triggering a programmed response to chemical stimuli received from the surrounding environment. Stuhr-Hansen et al. [17] applied a similar synthetic approach to generate adhesion points between binary populations of functional giant unilamellar vesicles (GUVs) by using both covalent (I-SPAAC reaction, disulfide bond formation, phenyl ester-hydrazine ligation), and non-covalent (coordination chemistry, streptavidin-biotin) interactions (Figure 1f). These works exemplify how functional molecules and materials combined with bottom-up synthetic biology concepts can provide an increased scope for advancing the chemical assembly of prototissue structures and the design of collective functions.

While such a materials chemistry approach presents many advantages compared with the use of lipid-based systems, such as lower costs of production, easier scalability, and production of a wider range of protocellular materials with interesting physical, chemical, mechanical, and permeability properties, there are also some important drawbacks that need to be considered. For example, synthetic materials may be toxic or non-biodegradable and methodologies to generate protocells or prototissues may require harsh conditions or toxic reagents which could remain present in the system. This would hinder potential applications of protocellular materials in biomedical sciences and environmental science. Moreover, such a *synthetic* bottom-up approach that aims to build protocells and protocellular materials from the molecular level is limited in the biomimetic complexity that it can achieve compared with methodologies that directly exploit living cells' components or machineries.

Current challenges of prototissue engineering

To achieve future impact, several challenges on prototissue design and synthetic construction still need to be addressed. Here I highlight those that can be overcome primarily with the support of materials chemistry.

Upscaling and 3D architecture

To date, prototissues have been assembled in the millimetre and submillimetre size range using a variety of techniques. Bio-orthogonal chemistry coupled with a double Pickering emulsion technique provided an effective way to assemble robust spheroids of protocells 75–200 μm in diameter that could be easily transferred into water media (Figure 1e) [16]. Magnetic fields could be used to manipulate diamagnetic GUVs into various spatially coded configurations a few hundred micrometres in size (Figure 1g) [18]. 3D printing proved to be a powerful technology for the patterning of protocells into synthetic tissues up to a millimetre scale with rather elaborate protocell architectures (Figure 1b) [19–22]. However, these approaches present several pitfalls. The Pickering emulsion method does not provide spatial control over the protocell organisation and is currently

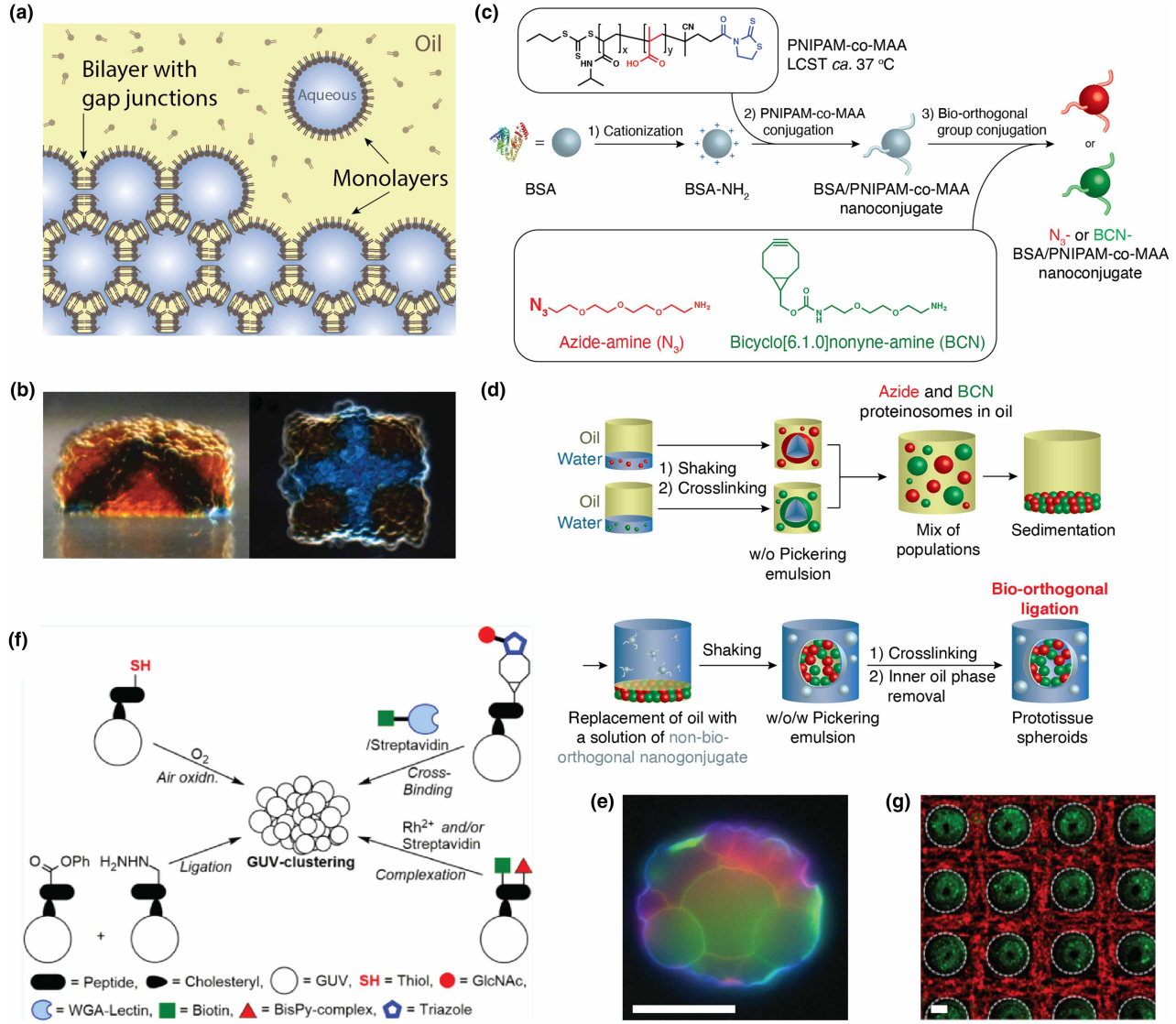

Figure 1. Synthetic strategies for the assembly of prototissues.

(**a**) Aqueous droplets submerged in a lipid-containing oil become encapsulated in lipid monolayers. When they are brought in contact, a kinetically stable lipid bilayer is formed at the interface. Membrane proteins can then be installed in the bilayers to promote communication between the different aqueous micro-compartments. (**b**) Bright-field microscopy images of two different DIBs patterned with two droplet types (blue and orange), imaged from the side (left) or the top (right). Image from reference [14]. (**c**) Synthetic pathway for the preparation of amphiphilic thermoresponsive protein–polymer nanoconjugates with bio-orthogonal functionalities for I-SPAAC reaction. (**d**) Scheme showing an experimental procedure for the preparation of proteinosome-based prototissue spheroids, starting from the N_3- and BCN-functionalised BSA/PNIPAM-co-MAA nanoconjugates described in (**c**). (**e**) Fluorescence microscopy image of a prototissue spheroid comprising a DyLight405-labelled non-reactive BSA/PNIPAM-co-MAA outer membrane (blue fluorescence) caging a binary community of interconnected rhodamine B-labelled N_3- and fluorescein-labelled BCN-functionalised proteinosomes (red and green fluorescence, respectively). Scale bar, 50 μm. Image from reference [16]. (**f**) Scheme showing different chemical strategies to generate covalent connections between GUVs. Image from reference [17]. (**g**) Fluorescence microscopy image of spatially organised FITC- and RITC-labelled GUV colonies (green and red fluorescence, respectively) formed via the subsequent application of magnetic fields with different orientations. Scale bar, 100 μm. Image from reference [18].

limited to the generation of spheroids of protocells. The diamagnetic GUVs require an aqueous media containing $MnCl_2$ and a constant magnetic field to maintain the patterns. The 3D-printed DIBs require the presence of an oil phase and present a shelf life of only a few hours [15]. A materials chemistry approach would allow the advancement of current prototissue construction technologies and enable the precise assembly of protocells

into large-scale materials (>1 cm in size) that are robust, free-standing, and stable in water media. This is critical for future technological applications of prototissues and would represent a major achievement in the field. This new approach will open up a route to the design and manufacture of protocellular materials with higher-order functions, where the prototissue structural properties could be coupled to carefully engineered collective mechanical and communication capabilities.

Mechanical properties

The mechanical properties of living tissues such as hardness, toughness, ductility, strength, and stiffness determine their physiological function and depend on the cells' and extracellular fibres' 3D arrangement. For example, bones have a structural role, facilitate vertebrates' movements, and protect their vital organs. Nature designed them to be hard and with an ultrastructure that enables them to withstand high compression forces applied longitudinally. In contrast, cartilage is flexible and elastic. It bends, resists stretching, and is specifically structured to absorb loads applied both transversally and longitudinally.

To date, research has been focusing on the development of methodologies to assemble protocells together in a controlled and reproducible manner. However, if we want to develop prototissue-based technologies, it is of paramount importance that we start advancing the prototissue's mechanical properties. In this regard, current studies have been limited to the characterisation of the prototissue's tensile strength and elastic modulus. For example, the tensile strength of DIBs was measured to be *ca.* 25 Pa and their Young's modulus was found to be in the order of ∼100–200 Pa, which is comparable to the elastic moduli of brain, fat, and other soft tissues [19]. Prototissue spheroids based on proteinosomes and capable of thermally induced reversible contractions were instead found to be more robust with a Young's modulus of *ca.* 5 kPa, and when expanding back to their original shape were capable of exerting a force of 62.2 ± 6.5 nN, which was commensurate with typical forces determined for biological systems such as beating cardiomyocyte clusters, migrating endothelial cells and motile bacteria colonies [16]. It is now critical to construct protocellular materials with a range of mechanical properties that mimic those of living tissues and perhaps even outperform them. To achieve this ambitious goal, experimental methodologies to reinforce the protocell building blocks must be devised. This can be implemented by engineering protocell membranes from new materials with tuneable mechanical properties. In this regard, good candidates are, for example, block copolymers and inorganic or polymeric nanomaterials. Other possibilities are to endow the protocells with a hydrogel cytoskeleton [23,24], or to generate protocells of different shapes, which could be assembled together with specific orientations to generate protocellular materials capable of withstanding loads applied from different directions. Finally, standardised experimental methodologies to characterise the protocellular material's mechanical properties and investigate their relationship with the protocell spatial arrangements need to be devised. It is clear that materials chemistry and engineering will provide invaluable opportunities to advance this important aspect of prototissue engineering.

Chemical communication

Communication is an essential characteristic of living tissues. Within a tissue, living cells communicate by secreting diffusible signalling molecules and molecular effectors. This triggers specific processes in neighbouring cells such as cell growth, division, exocytosis, and apoptosis. Communication also enables higher-order tissue functions that cannot be achieved by single and isolated cells, such as homeostasis, the regulation of differentiation processes, the secretion of oscillating gradients of signalling molecules, and collective mechanical responses [25–27]. Therefore, the engineering of communication pathways between interconnected and spatially organised consortia of protocells represents a fundamental step towards the generation of prototissues capable of higher-order functions.

Communication in a prototissue must happen at two levels: internally (i.e. among the protocells that compose the material), to trigger specific collective and higher-order responses; and with the external environment, to receive nutrients, and sense and release chemical signals. To date, communication within a synthetic tissue has been promoted by gap junctions [19–21,28–30] (Figure 2a) and passive diffusion (Figure 2b,c) [16]. The next challenge that needs to be addressed is the implementation of internalised, sophisticated, and extended communication pathways to access prototissue's higher-order functions. To achieve this, prototissues need to be assembled from protocells endowed with a receiver, a transductor, and an emitter of various outputs (e.g. light, pH, temperature, mechanical movement, etc.). Synthetic gene circuits and DNA nanotechnology present invaluable opportunities for developing this type of system. A plethora of synthetic gene circuits have been implemented and tested in cell-free transcription–translation extracts and in living cells [31], even though

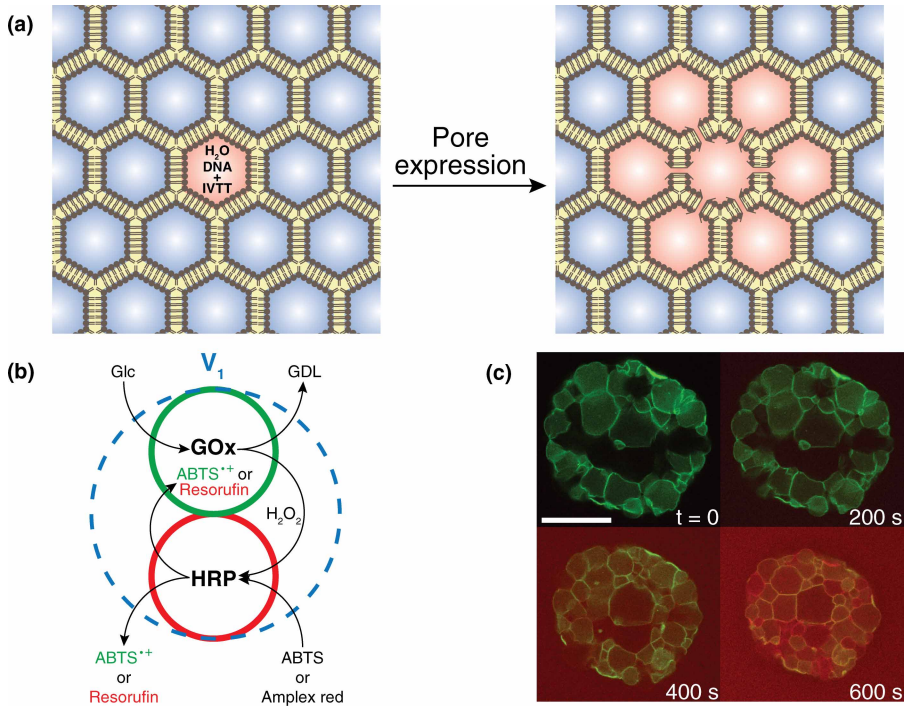

Figure 2. Communication within prototissues.

(**a**) Scheme showing gap junction-promoted communication within a 3D-printed droplet network. A central protocell containing DNA and an *in vitro* transcription/translation (IVTT) system expresses α-hemolysin (αHL) and forms gap junctions with the adjacent protocells. (**b**) Scheme showing the functioning principles of chemical communication based on passive diffusion. A GOx/HRP enzyme cascade reaction is hosted within a prototissue spheroid (dashed blue circle) comprising GOx-containing BCN-functionalized proteinosomes (green circle) and HRP-containing N_3-functionalized proteinosomes (red circle). Due to their small molecular size glucose (Glc), ABTS or Amplex red can passively diffuse through the protocell membranes. As a consequence, the prototissue spheroid is capable of sensing the presence of Glc in the environment and trigger the synthesis and release of D-glucono-1,5-lactone (GDL) and $ABTS^{\bullet+}$ or resorufin. Image from reference [16]. (**c**) Time-dependent fluorescence confocal microscopy images of a single prototissue spheroid structured as described in (**b**) and in the presence of glucose and Amplex Red (14 mM and 0.5 mM, respectively). Green fluorescence: FITC-labelled GOx-containing BCN-functionalized proteinosomes; red fluorescence: time-dependent resorufin production. Scale bar, 50 μm. Image from reference [16].

their implementation in protocells so far has been limited [32,33]. DNA-based communication pathways in distributed proteinosome consortia has also been implemented. To achieve this, the modularity and scalability of enzyme-free DNA strand-displacement circuits was exploited, and signalling cascades, negative feedback loops, and Boolean logic circuits within proteinosomes trapped in a microfluidic device were implemented [34]. It should be now possible to employ these available technologies to engineer protocellular materials capable of collective communication functions such as signal amplification, bidirectional communication, distributed computational operations, oscillations and pattern generation, and homeostasis maintenance upon exposure of the prototissue to specific physical–chemical perturbations.

Finally, in order to generate more complex forms of communication within prototissues the design, construction, and use of more structured types of protocell building blocks is required. Living cells owe their complexity to membrane-bounded internalised organelles. These, by sub-segregating specific bio-components, provide a framework for internalised specialised functions such as signals and energy transduction, removal of toxic elements, generation of local concentration gradients of substrates, information regulation and trafficking, and metabolic processing [35]. Models of multi-compartmentalized protocells endowed with functional proto-organelles have already been implemented [36–39]. These systems provide excellent examples of the different chemistries and experimental methodologies that could be exploited to generate multi-compartmentalised protocell building blocks for the assembly of protocellular materials capable of proto-organelles-mediated communication and more complex forms of internalised metabolism.

Accessing higher-order functions in synthetic tissues through the implementation of synthetic gene and DNA circuits and the design of multi-compartmentalised materials-based protocell models will advance prototissues research and could lead to important applications in diagnostics and personalised therapy.

Towards forms of chemo-mechanical and mechano-chemical transduction

Thus far, I have discussed the mechanical and chemical signalling properties of prototissues separately. However, signalling molecules and molecular effectors can do far more than simply diffuse through or reside inside a passive prototissue architecture. They can be part of the architecture itself. This would open up a route to non-equilibrium systems where the mechanical and chemical components continuously adjust to each other resulting in forms of mechano-chemical and chemo-mechanical signal transduction.

Important steps in this direction have already been undertaken. A self-folding DIB-based synthetic tissue was assembled by 3D printing a network bilayer of two types of communicating water-in-oil droplets with different osmolarity. The flow of water from one layer to the other caused the swelling of the layer with higher osmolarity and the shrinkage of the layer with lower osmolarity, respectively (Figure 3a). This was exploited to design and print synthetic tissues that could self-fold into different shapes [19]. Similar droplet networks were also exploited as templates to manually assemble hydrogel structures capable of undergoing a variety of shape changes in response to thermal and light stimuli [40]. To achieve this, nanolitre aqueous pre-gel droplets were connected through lipid bilayers and photopolymerized to yield continuous thermally responsive hydrogel structures. By photo-generating domains of thermoresponsive polymers with different lower critical solution temperatures, the overall hydrogel structure could undergo thermally induced reversible curling and could be exploited to construct a simple hydrogel gripper. It was also possible to entrap gold and magnetic nanoparticles within specific areas of the network. This allowed for the construction of a multi-responsive hydrogel that could curl when irradiated with green light and could be magnetically driven through a maze (Figure 3b).

Our research group at the University of Bristol synthesised prototissue spheroids capable of undergoing thermally induced reversible contractions, which could be enzymatically modulated and exploited for mechano-chemical transduction. This was accomplished by coupling the reversible collective contractility of the prototissue spheroids to the down/up regulation of a co-ordinated glucose oxidase/horseradish peroxidase (GOx/HRP) enzyme cascade hosted within the interlinked protocell network. When the prototissue spheroid underwent a contraction the protocell membranes became more hydrophobic, hindering the access of substrate molecules to the prototissue and consequently causing the down-regulation of the hosted enzyme cascade (Figure 3c,d) [16].

These examples highlight that materials science and bottom-up synthetic biology need to work synergistically to achieve protocellular materials capable of complex forms of emergent behaviours. Therefore, it is critical to continue to explore the boundary between chemistry and mechanics and create more complex forms of signalling pathways that interweave with the reconfiguration of polymers, fibres, and protocell membranes that constitute the prototissue. This will eventually lead to the development of protocellular materials capable of self-sustained collective beating, muscle-like contractions, motility, chemotaxis, haptic sensing, self-repairing, and much more.

Conjugation to living tissues

Currently, a significant challenge in bottom-up synthetic biology is the generation of chemical synergy between living matter and non-living matter [41–44]. An important achievement in this research direction would be the engineering of prototissues capable of expanding the sensing capabilities of living tissues, influencing their growth and differentiation, or supplementing specific molecules or drugs to failing living tissues. However, this research area remains essentially unexplored due to the low stability and bio-compatibility of the current prototissue models. The possibility of generating robust protocellular materials from functional molecules, polymers, and nanomaterials would represent a feasible way to address this multidisciplinary scientific challenge. By exploiting the broad library of biocompatible functional materials that is now available, it would be feasible to select appropriate starting molecular systems to self-assemble synthetic protocell membranes that can be used as building blocks for the generation of biocompatible protocellular materials endowed with interfacial functional groups for adhesion to living cells and tissues.

Impact of prototissues on science and technology

Prototissue research, as discussed so far, is still at an embryonic stage and has a low technological maturity. However, in the upcoming years, the combined research efforts of materials chemistry and synthetic biology are destined to lead to unprecedented forms of non-equilibrium materials capable of higher-order functions.

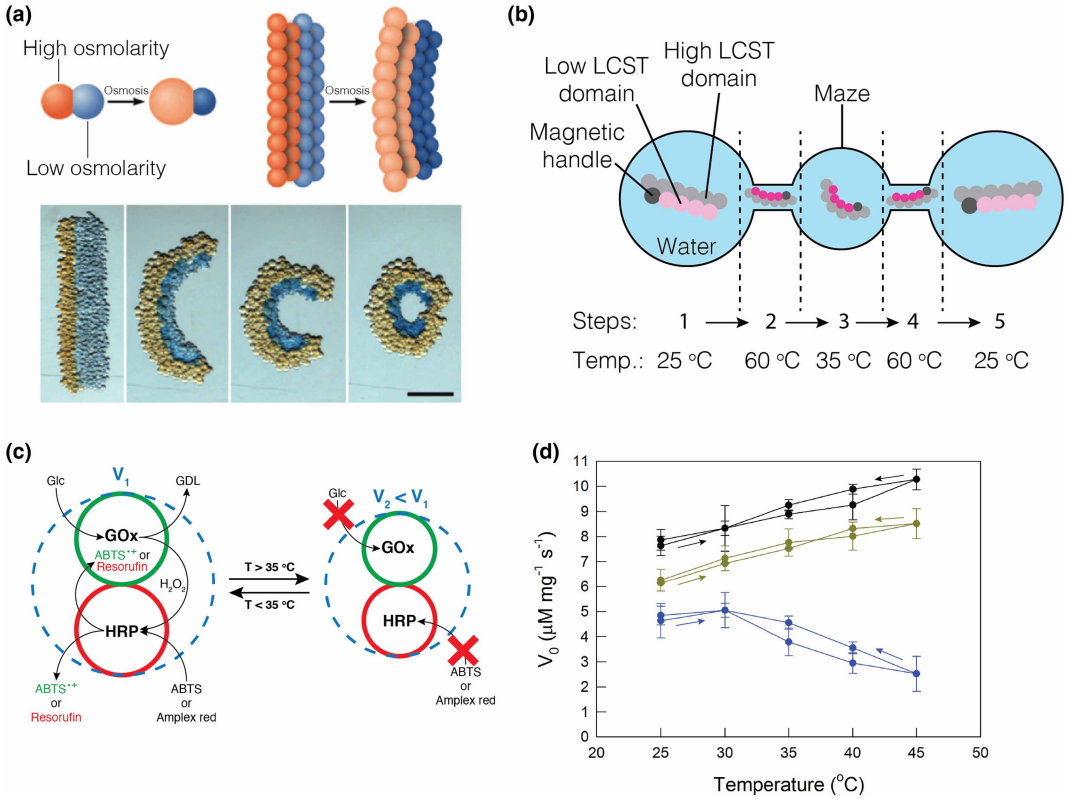

Figure 3. Forms of chemo-mechanical and mechano-chemical transduction within prototissues.
(**a**) A self-folding DIB network. Top: Scheme showing the functioning principle of the self-folding droplet network. Bottom: Photographs of a rectangular network folding into a circle over ~3 h. Scale bar, 250 mm. Image from reference [19]. (**b**) A multi-responsive hydrogel droplet network. The scheme shows the design principles of a multi-responsive hydrogel droplet network that is magnetically driven through a maze. The vertical dashed lines indicate the different steps and the corresponding temperature changes required to contract the material [40]. (**c**) Scheme representing coupling of the thermally induced contractile behaviour to a GOx/HRP enzyme cascade reaction hosted inside a prototissue spheroid (dashed blue circle). The prototissue spheroid comprises a 1:1 binary population of thermoresponsive GOx-containing BCN-functionalized proteinosomes (green circles) and HRP-containing N_3-functionalized proteinosomes (red circles). In the relaxed prototissue (V1), glucose (Glc) and ABTS or Amplex red freely diffuse through the system, and are transformed into D-glucono-1,5-lactone (GDL) and green $ABTS^{\bullet+}$ or red fluorescent resorufin, respectively. In the contracted form (V2), diffusion of the substrates towards the enzymes is hindered. Image from reference [16]. (**d**) Plots of reversible temperature-dependent changes in initial reaction rates for the GOx/HRP-mediated cascade reactions undertaken in the presence of glucose and ABTS Red (5 mM and 0.1 mM, respectively) for free enzymes in solution (control, black plot), a dispersed 1 : 1 population of non-coupled GOx-containing and HRP-containing proteinosomes (control, dark yellow plot) and GOx/HRP-containing prototissue spheroids (blue plot). Image from reference [16].

But what is the potential impact that these materials will have on science and technology? In this section, four research fields that are likely to be influenced once protocellular materials reach technological maturity are discussed. The goal of this section is to provide the reader with some food for thought on the next research directions to undertake in order to increase the prototissues technology readiness level, rather than to describe specific applications of prototissues.

Fundamental science

In the same way, as the bottom-up synthesis of protocells might be a way to understand the origin of cellular life [45,46], the bottom-up assembly of protocellular materials might provide insights into the molecular basis of multicellularity, which is one of the major transitions that occurred during the course of early evolution. Why did living cells, at some point in the evolutionary pathway, decide to join forces and aggregate together?

Clearly, this happened to gain a greater advantage. This could have been a biological advantage, for example building up defences against phagotrophic organisms or providing a more effective environment for the storage of nutrients; or a physico-chemical advantage, for example, increased stability towards shear forces or chemical gradients. However, so far this remains at the centre of a scientific debate due to the unavailability of appropriate experimental data [47,48]. Protocellular materials might provide a framework to experimentally evaluate these hypotheses and especially those involving fundamental physical–chemical interactions between matter. Moreover, in living tissues different cells are spatially arranged in specific 3D architectures to collectively perform specific biological functions, but can their performances be enhanced by modifying the cellular arrangement? If we can develop methodologies to spatially arrange protocells into structurally complex materials that are resilient and capable of sensing and communicating with the external environment, we will be able to shine some light on the molecular origin of multicellularity and open up a route to engineer synthetic tissues with emergent functions that outperform their natural counterparts.

Biomedical sciences

The use of protocellular materials as simplified biomimetic models to gain new insights into the structure-property relationship of living tissues has the potential to impact the fields of tissue engineering, regenerative medicine, and drug delivery. For example, if protocellular materials could closely mimic the 3D structure and the physical, chemical, and mechanical properties of specific living tissues, we would advance the biomechanical study of those tissues and investigate how signalling molecules or drugs permeate and distribute across living tissues and organs. This would lead to important insights into how to enhance the efficiency of current medical therapies. Moreover, one of the major issues in tissue engineering is the immune-rejection of living tissues grown from allogenic cells. This is because living cells are autonomous entities that can unpredictably trigger undesirable biological responses. However, protocellular materials have the potential to solve this issue as they can be constructed from scratch using biocompatible molecules and polymers [49], and could be chemically programmed to carry out specific biochemical functions. Of course, research on protocellular materials is just at the beginning and we are far away from these goals. However, the combination of materials chemistry and synthetic biology is destined to open new avenues for diverse biomedical applications.

Environmental science

Protocellular materials have the potential to impact the way industry deals with chemical waste. For example, they could be chemically programmed to sequester pollutants and toxins from wastewaters of industrial plants and transform them into non-harmful products. This would definitely require a massive scale up and low production costs, which could be achieved by synthesising protocell building blocks from low-cost functional and biocompatible polymers. Another important challenge in environmental science is the accumulation of plastics in the oceans. Synthetic biologists recently discovered a bacterium and enzymes that are capable of digesting plastics [50–52]. However, the consequences of further bioengineering such a bacterium to deliver more active enzymes and releasing it in the seas presents all kinds of unassessed hazards. Protocellular materials would represent a better option as they are non-living materials that could be chemically programmed to specifically hydrolyse plastics and then naturally degrade.

Soft robotics

Current challenges in robotics involve the engineering of robots of small scale (millimetre and submillimetre), that can work *in vivo*, are disposable and biodegradable, or constructed from hybrid living and non-living materials [53–57]. Soft robotics aims to address these challenges by developing robots or robotic components that are constructed from soft and smart materials. In this regard, protocellular materials can provide a modular and highly programmable material framework to construct soft-bots and robotic actuators and sensors that can respond to physical–chemical changes in the environment and trigger a mechanical response. In fact, as discussed above, the development of protocellular materials capable of chemo-mechanical transduction is very much achievable in the immediate future. This would open up the possibility of constructing, for example, grippers or muscle-like actuators capable of transducing physical (thermal, light, electric, or magnetic) or chemical (pH, or presence of specific biomolecules) stimuli into mechanical movements. The construction of the first autonomous and adaptive protocellular materials-based actuators would revolutionize the field of soft robotics, opening up new avenues to robotic parts capable of morphological computation [58,59]. However, the hardest challenge that will need to be addressed is the design of soft robots and robotic components capable of

forms of chemo-mechanical transduction that happen quickly (timescale of milliseconds to seconds) and generate forces at least in the mN range.

Perspectives

- Prototissues comprise free-standing 3D networks of interconnected protocell consortia that communicate and display synergistic functions.
- Current challenges in prototissue design and synthetic construction involve: (1) the assembly of prototissues in the centimetre to decimetre size range and with high spatio-temporal control; (2) the engineering of a wide range of mechanical properties; (3) the implementation of complex forms of communication; (4) the development of forms of chemo-mechanical and mechano-chemical signal transduction; and (5) the conjugation of synthetic tissues to living tissues.
- By bringing together in an original and synergistic manner the fields of synthetic biology and materials chemistry new routes to address these important multidisciplinary scientific challenges will be pioneered. Following this new approach, protocellular materials will be constructed from protocell models based on functional molecules and materials. This would lead to the generation of the first adaptive and autonomous forms of prototissues that compared with living tissues can be much more robust, exhibit a wider range of mechanical properties, function under extreme conditions, and undergo a wider range of bio-inspired metabolic functions.
- These protocellular materials will find applications in the most diverse areas of science and technology, from biomedical science to environmental science, and soft robotics.

Competing Interests

The author declares that there are no competing interests associated with the manuscript.

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Abbreviations

BSA/PNIPAM-co-MAA, bovine serum albumin/poly(N-isopropylacrylamide)-co-methacrylic acid; DIBs, water-in-oil droplets connected by interface bilayers;; GOx, glucose oxidase; GUVs, giant unilamellar lipid vesicles; HRP, horseradish peroxidase; I-SPAAC, interfacial strain-promoted alkyne-azide cycloaddition reaction; w/o/w, water-in-oil-in-water Pickering emulsion; αHL, α-hemolysin.

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