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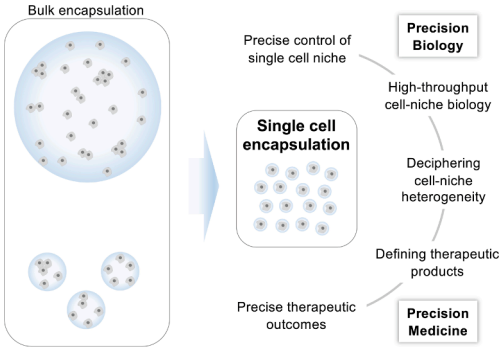
Towards single cell encapsulation for precision biology and medicine

Prerak Gupta [a,b], Omar Alheib [c,d], Jae-Won Shin [a,b,*]

[a] Department of Pharmacology and Regenerative Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA
[b] Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL 60607, USA
[c] 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal
[d] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal

GRAPHICAL ABSTRACT



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ABSTRACT

The primary impetus of therapeutic cell encapsulation in the past several decades has been to broaden the options for donor cell sources by countering against immune-mediated rejection. However, another significant advantage of encapsulation is to provide donor cells with physiologically relevant cues that become compromised in disease. The advances in biomaterial design have led to the fundamental insight that cells sense and respond to various signals encoded in materials, ranging from biochemical to mechanical cues. The biomaterial design for cell encapsulation is becoming more sophisticated in controlling specific aspects of cellular phenotypes and more precise down to the single cell level. This recent progress offers a paradigm shift by designing single cell-encapsulating materials with predefined cues to precisely control donor cells after transplantation.

1. Introduction

Cell encapsulation in biomaterials has a long history in the field of cell therapy; it was initially designed to broaden the availability of donor cell sources by protecting them from immune rejection while providing a semipermeable barrier [1,2]. As a consequence, cell encapsulation enables sustained, long-term release of therapeutic molecules from donor cells, such as insulin. More recently, biomaterials have been

* Corresponding author.
E-mail address: shinjw@uic.edu (J.-W. Shin).

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designed to enable a gradual exchange between donor cells and host cells in order to achieve regenerative outcomes; for instance, donor bone marrow mononuclear cells in biodegradable scaffolds are cleared over time but host stromal cells are recruited to form tissue-engineered vascular grafts [3]. Recent advances in understanding mechanisms of cell-material interactions highlight more active roles of biomaterial properties in directing cellular behavior [4–6]. Moreover, the extracellular matrix (ECM) in host tissue undergoes substantial changes in its properties during disease progression, as exemplified by increased rigidity during fibrosis [7] and cancer [8], which in turn can adversely impact donor cell function [9]. Thus, encapsulation materials need to be precisely designed not only to achieve desired donor cell function, but also to prevent any undesired interactions between donor cells and host tissue during therapeutic intervention. Technological advances in advanced biomanufacturing enable more sophisticated biomaterial design to control specific aspects of cell biology down to the single cell level.

Here, we present a case that single cell encapsulation enables precision biology and medicine. We start by highlighting some of the major advances in cell encapsulation therapy. We then elaborate on rationales behind miniaturizing encapsulation materials down to the single cell level and technical progresses to achieve this goal. We examine biophysical determinants of cell-material interactions and how single cell encapsulation helps reveal new insights behind this process. We put cell therapy into the context of quantitative pharmacology and discuss design criteria for encapsulation materials to improve therapeutic outcomes. We posit that enhancing the resolution of cell encapsulation down to the single cell level will enable a new conceptual advance where encapsulating materials can be considered as a 'bit' to precisely control donor cell function. We conclude by discussing the implication of single cell encapsulation in deciphering functional heterogeneity of cells and facilitating bottom-up tissue assembly.

2. Major advances in cell encapsulation therapy

Delivering drugs and small molecules to tissue by various administration routes is a prevalent approach for the treatment of many diseases. However, long-term sustained delivery of therapeutic agents would be more desirable to fundamentally treat chronic conditions, such as diabetes, cancer, and fibrosis. Cell therapy has potential to achieve this goal due to its ability to deliver therapeutic molecules in a sustainable manner and to integrate with the host tissue. With advances in synthetic biology, cells can be rewired or programmed to perform a desired function *in vivo* [10]. The most common examples of cell therapy are blood transfusion [11] and bone marrow transplantation [12]. Building upon advances in immunology and genetic engineering, engineered immune cell therapy with chimeric antigen receptor (CAR) has recently emerged as an effective treatment modality for previously incurable blood cancer conditions [13]. While blood cells can readily traffic in and out of tissue [14–16], the majority of tissue-resident cells interface with the ECM comprising of various signaling domains for an extended period of time [17]. In the absence of ECM presentation, some cells produce more reactive oxygen species and decrease nutrient uptake, leading to anoikis [18]. In addition, the ECM limits cellular overgrowth, which is important to reduce the risk of tumor development after cell transplantation [19,20]. In disease, host tissue undergoes significant changes in the ECM [21], which can adversely influence the function of donor cells. The importance of cell-ECM interactions provides a biological rationale behind developing strategies to deliver donor cells in well-defined biomaterials.

The main motivation of cell encapsulation for clinical applications has been to enable allogenic transplantation, which can help address donor shortage. The initial concept was reported in the 1960's where encapsulation provides a semipermeable barrier for donor cells to evade the host immune system while simultaneously allowing selective release of bioactive molecules from cells in a sustained manner [22]. It took several years to show preclinical demonstration. In the 1980's, microencapsulated islet cells xenografted in rats resulted in correction of diabetic phenotypes for 2–3 weeks [23]; alginate was used along with polylysine to encapsulate cells due to the facile nature of electrostatic crosslinking. This advance was translated to the first clinical trial where transplantation of microencapsulated islets in alginate with increased mechanical stability led to insulin independence in a type 1 diabetic patient for 9 months [24]. Since then, extensive efforts have been made to improve cell encapsulation technologies. Cell encapsulation therapy has been extended to treat other diseases by using genetically engineered xenogeneic or allogeneic cells to sustainably deliver therapeutic factors. For instance, in a phase 1 clinical trial, six amyotrophic lateral sclerosis patients were surgically implanted with encapsulated, genetically modified xenogeneic cells in the lumbar intrathecal space to sustainably deliver ciliary neurotrophic factor (CNTF), which otherwise showed adverse effects upon systemic delivery; CNTF was present in cerebrospinal fluid 17 weeks after implantation [25]. Another clinical trial aimed to use the similar approach to deliver nerve growth factor (NGF) to the cholinergic basal forebrain in order to minimize the degeneration of cholinergic neurons for Alzheimer disease patients; there was a persistent NGF release in half of the patients over 12 months [26]. Encapsulation technologies have also been implemented to improve mesenchymal stromal cell (MSC)-based therapeutics. For example, there is an ongoing clinical trial to treat diabetic foot ulcers by adipose-derived MSCs encapsulated in a hydrogel sheet [27].

The first generation of cell encapsulation materials was originally considered to be bioinert [28]. However, it has become clear that most biomaterials trigger a certain degree of foreign body response [29]. Fibrous capsules can limit the viability of encapsulated cells by inhibiting the diffusion of nutrients and can also impair the release of cell-secreted therapeutic molecules [30]. Several efforts have been made to reduce the foreign body reaction of cell encapsulating materials, especially in the context of encapsulated islet transplantation. Polyethylene glycol (PEG) is considered to be a 'gold standard' as it is hydrophilic and hence minimizes rapid protein adsorption. However, PEG is prone to degradation in a oxidative *in vivo* environment, limiting its application for long-term cell encapsulation [31]. Zwitterionic functional groups were shown to be superior to PEG in terms of hydrophilicity and anti-fouling properties [32,33]; they prolong the residence time of microencapsulated islets *in vivo* [33]. In addition, a combinatorial hydrogel library approach was used to identify triazole as a functional group that increases the residence of microencapsulated islets in primates albeit without impacting protein adsorption [34]. Simply tuning spherical dimensions of microencapsulation can also minimize the foreign body response [35] by modulating colony stimulating factor-1 pathway [36]. Functionalizing encapsulation materials with naturally-occurring immunomodulatory ligands, such as the immune checkpoint protein programmed death-ligand 1 [37] represents a biologically-inspired approach to evade immune recognition, and was recently implemented to promote the residence of microencapsulated islets [38].

3. Towards single living cell encapsulation

Encapsulation of a few cells in miniaturized hydrogels (<1 mm) offers a number of advantages over bulk hydrogels in the context of cell therapy. Practically, microencapsulation facilitates the delivery of donor cells in gels to tissue via injection. Another important advantage of microencapsulation is oxygen and nutrient exchange, since it was shown that beta cell clusters larger than ~ 150 μm in size form a necrotic core in the absence of vasculature *in vivo* [39]. To facilitate this process, microencapsulation is typically done in a spherical shape as it provides the maximum surface-to-volume ratio for diffusion. In addition, small microcapsules offer superior mechanical stability and may provide better protection to the encapsulated cells from external mechanical stresses (e.g. shear stress) [40]. Bulk encapsulation systems of large size with sharp edges are prone to foreign body response; smooth, spherical

microcapsules are relatively more immune compatible [41]. For instance, agarose microcapsules of ~ 100 μm show lower fibrotic response that their larger counterparts of ~ 300–1000 μm upon implantation in mice and rats [42]. Microencapsulation technology also allows high throughput imaging analysis as it minimizes the limitations associated with visualization of encapsulated cells due to thickness and light diffraction [43].

While encapsulating cell clusters in miniaturized microgels resolves several issues associated with conventional bulk encapsulation, single cell encapsulation represents another paradigm shift in the field of cell therapy. Miniaturization of microgels to the single cell level facilitates niche modeling and *in vivo* delivery [44]. Moreover, it allows precise control over volume of encapsulation material and spatial presentation of ligands around each cell [45,46]. From a tissue-engineering perspective, single cell-encapsulating microgels of 50 μm in size enable a minimum physiological cell density of ~ 10^6 cells per cm^3 construct after assembly [47]. Successful single cell encapsulation depends on advances in emulsification technology to generate and maintain monodisperse, microscale droplets of crosslinkable polymer solutions containing single cells. This is difficult to achieve with conventional approaches to generate microscale droplets, such as aerosolization and vortexing. Droplet-based microfluidics has served as a promising approach to obtain live single cells in monodisperse crosslinked microgels [45,46,48–52]. This approach uses two immiscible phases along with a surfactant to form and compartmentalize aqueous droplets in oil. With smaller droplets, the choice of surfactant becomes more critical to maintain cell viability since cells collide with surfactant molecules to a greater extent. This issue can be resolved by implementing biocompatible surfactants [53] or rapid gelation right after droplet formation [46]. Various polymers have been used to acquire gel-coated single cells via droplet microfluidics, including alginate [44–46], collagen, gelatin [54], fibrinogen-containing hyaluronic acid [55], and PEG [56]. While droplet-based microfluidics generally results in spherical gel coatings, microwell-based approaches can be used to vary the geometry of single cell-encapsulating microgels [57,58]. Single cell encapsulation in nanoscale (<1 μm) gel coatings can be done via direct hydrogel polymerization on the cell surface [59].

Scaling up the throughput of gel-coated single cells remains an important bottleneck for translation into clinical therapy. For droplet-based microfluidics, the probability of microgel droplets containing cells is governed by Poisson distribution; low cell concentration results in many empty microgels, whereas high cell concentration leads to polydisperse distribution of cell number per microgel [60]. A number of studies have been done to overcome this issue and obtain a pure population of gel-coated single cells. Earlier approaches relied on strategies to introduce single cells into droplets as they were formed. An optical tweezer method can bring cells to the aqueous-oil interface right before droplet production [61]; this method is precise but the throughput is limited. Another approach involves cell ordering in the aqueous phase to match with frequency of droplet production either by inertial microfluidics [62,63] or close cell packing [64]. While this approach is potentially high-throughput, consistent encapsulation requires synchronization of aqueous and oil flow rates, which can be difficult to achieve reproducibly. Because of these limitations, more recent approaches have implemented the sorting of single cell-containing microgels after formation. The system generally consists of a sensor connected to a microfluidic sorting device based on dielectrophoresis [65] or acoustic waves [66]. To date, electrical impedance [67], fluorescence [68–71] and label-free [72–74] imaging have been used as sensors. This system can be useful to isolate a specific subpopulation of gel-coated cells. However, most of these approaches involve complicated setups and can be difficult to scale. Most recently, chemical approaches have been developed where crosslinker precursors are adsorbed or grafted onto the cell surface, which are then released to induce selective gelation of droplets that contain cells. This concept was tested with $CaCO_3$ nanoparticles where Ca^{2+} is released from the cell surface in response to mild acid from the oil phase in order to crosslink alginate in a buffered solution [44]. This approach was also extended to covalent crosslinking by implementing polyethylene glycol (PEG) modified with a lysine donor-glutamine acceptor pair that undergoes conjugation in the presence of Ca^{2+}-dependent, transglutaminase factor XIIIa [75]. Chemical approaches are particularly useful to enable one-step purification of gel-coated single cells as they are formed while varying different types of parameters such as microgel volume [46] and compartmentalization [45]. However, one key consideration is to determine whether crosslinker precursors inadvertently impact cellular phenotypes. Designing click chemistry-based bioorthogonal crosslinkers will likely provide a promising path to mitigate this potential issue.

4. Encoding biophysical cues in cell microencapsulation materials for precision control of biology

Biological molecules, such as growth factors or cytokines, have been widely functionalized into biomaterials to direct the function of encapsulated cells [76]. However, it has become clear that biophysical properties of biomaterials also substantially influence cellular function through mechanotransduction [4,5,77]. This fundamental insight provides a wide range of opportunities to specifically design biomaterials for cell microencapsulation by tuning biophysical cues.

One of the most striking concepts in mechanotransduction is that biophysical cues control cell fate, which is a complex biological process that results in fundamental changes in gene expression. Seminal studies showed that varying elastic modulus (E) of 2D hydrogels functionalized with cell adhesion ligands [78] guides the differentiation of preosteoblasts [79] and multilineage differentiation of MSCs [80] independently of biochemical cues. The motivation behind this line of investigation was initially based on the intuition where organs exhibit a range of E (brain is soft and bone is stiff), which can differentially impact how the same cell type responds to its microenvironments [81]. Cells adhere to and pull on their substrate more stably when it is rigid; as a result, cells can generate greater contractile forces via actomyosin and increase cortical membrane tension [79,80]. The nucleus becomes squeezed, and nuclear pores are opened up, resulting in selective translocation of some transcription regulators [82]; one of them is Yes-associated protein (YAP), which is a co-factor of transcriptional enhanced associate domain [83]. The nuclear translocation of mechanosensitive transcription factors also becomes stabilized by increased polymerization of nuclear lamins due to force-dependent folding, which decreases phosphorylation [84]. A similar cellular behavior is also observed by seeding cells on micropatterned substrates with varied adhesion areas [85], geometry [86], and flexibility [87]. These studies have opened various avenues of investigation to control cellular function by physically tuning cell-material interactions.

To translate these insights to cell microencapsulation, it is important to understand the process of mechanotransduction in 3D environments. An early study showed that varying E in 3D alginate hydrogels with an adhesion ligand directs MSC differentiation but osteogenesis occurs at an optimum E (~20 kPa), rather than higher E [88]. This discovery was extended to preclinical demonstration where bone regeneration is enhanced by implanting MSCs in void-forming alginate hydrogels with higher E [89]. Unlike 2D substrates, cells are fully surrounded by polymer networks in a 3D space, and hence mechanically confined. Indeed, relieving mechanical confinement by cell-mediated polymer degradation [90] or accelerating substrate stress relaxation [91] increases cell spreading and osteogenic differentiation of MSCs. Stress relaxation is the key consequence of viscoelasticity, which is exhibited by most biological tissues regardless of their E [92]. In fast stress relaxing hydrogels, MSCs exhibit volume expansion during spreading via the mechanosensitive ion channel transient receptor potential vanilloid 4 (TRPV4), which activates runt-related transcription factor 2 (RUNX2), rather than YAP to drive osteogenesis [93]. Fast stress relaxing hydrogels also facilitate cell division [94] and filopodia-mediated

cell migration [95]. Since cell microencapsulation has mostly been done with ionically crosslinked alginate, which is relatively viscoelastic compared to covalently crosslinked materials, these insights will be useful to understand how microencapsulation material properties control cellular behavior.

In many native tissues, cells interface with the ECM with a low ECM-to-cell volume ratio (Fig. 1**A**). However, most investigations on 3D cell-material interactions were done by encapsulating cells in bulk hydrogels, which exhibit a high material-to-cell volume ratio and contribute to mechanical confinement. The material-to-cell volume ratio can be reduced by implementing granular hydrogels [96] or forming cell spheroids prior to or after encapsulation [97]. However, single cell encapsulation technologies enable precisely controlled deposition of hydrogels around cells with smaller material volume per cell (Fig. 1**B**). Thus, microencapsulation offers tremendous opportunities to recapitulate and control properties of pericellular microenvironments around single cells and to discover new biological insights that were not previously revealed by using bulk hydrogels. These new insights can potentially be leveraged to improve cell encapsulation therapy.

A number of cell instructive cues can be tuned in a single cell encapsulation format, including spatial presentation of cell binding ligands, biophysical cues, substrate volume, and presence of cell-secreted factors (Fig. 2). New insights are beginning to be discovered in this context. As the amount of alginate gel coating with the adhesion ligand Arg-Gly-Asp (RGD) around single cells decreases, MSCs undergo rapid isotropic volume expansion and increased membrane tension, thereby driving osteogenesis [46]. Alternatively, asymmetric distribution of RGD in alginate coating around single cells is sufficient to elongate them and recruit the cell polarization factor cell division cycle 42 (Cdc42), resulting in the polarization of membrane tension and enhanced osteogenesis [45]. MSCs singly coated with soft alginate-RGD gel coatings were also shown to exhibit higher expression of interstitial collagenases in response to tumor necrosis factor-α (TNFα) than MSCs in the bulk gel counterpart [52]. Thus, a picoliter volume of biomaterials can be used to profoundly impact cellular phenotypes for therapeutic applications.

5. Cell microencapsulation for precision control of therapeutic outcomes

With the convergence of multidisciplinary knowledge, it is now evident that cell-based engineering has remarkable scope in precision medicine, wherein desirable therapeutic outcomes can be achieved by manipulating the cell decision-making process. Cell microencapsulation technology can rationally be placed at the interface of precision biology and precision medicine which may potentially solve critical limitations associated with basic and applied sciences, respectively. In the previous section, we discussed how microencapsulation can pave a way to understand the cell decision-making process as a function of microenvironmental cues, providing critical biological insights. A precise control over cell decision making can be further leveraged to guide cells in a way that they confer desirable therapeutic outcomes. To achieve this goal, it will be important to put cell therapy into a quantitative context and to understand the role of microencapsulation in this context. Pharmacokinetics (PK)/pharmacodynamics (PD) modeling serves as an important quantitative framework for the development of therapeutic small molecules and biologics [100]. The feasibility of developing PK/PD models for a given cell therapy product depends on whether its mechanism of action (MOA) is well-defined. Although there are multiple mechanisms by which donor cells may result in therapeutic effects, they can be attributed to a finite number of well-defined biological processes [10].

For clinical bone marrow transplantation and chimeric antigen receptor (CAR) T-cell therapy, the main MOA is persistent reconstitution

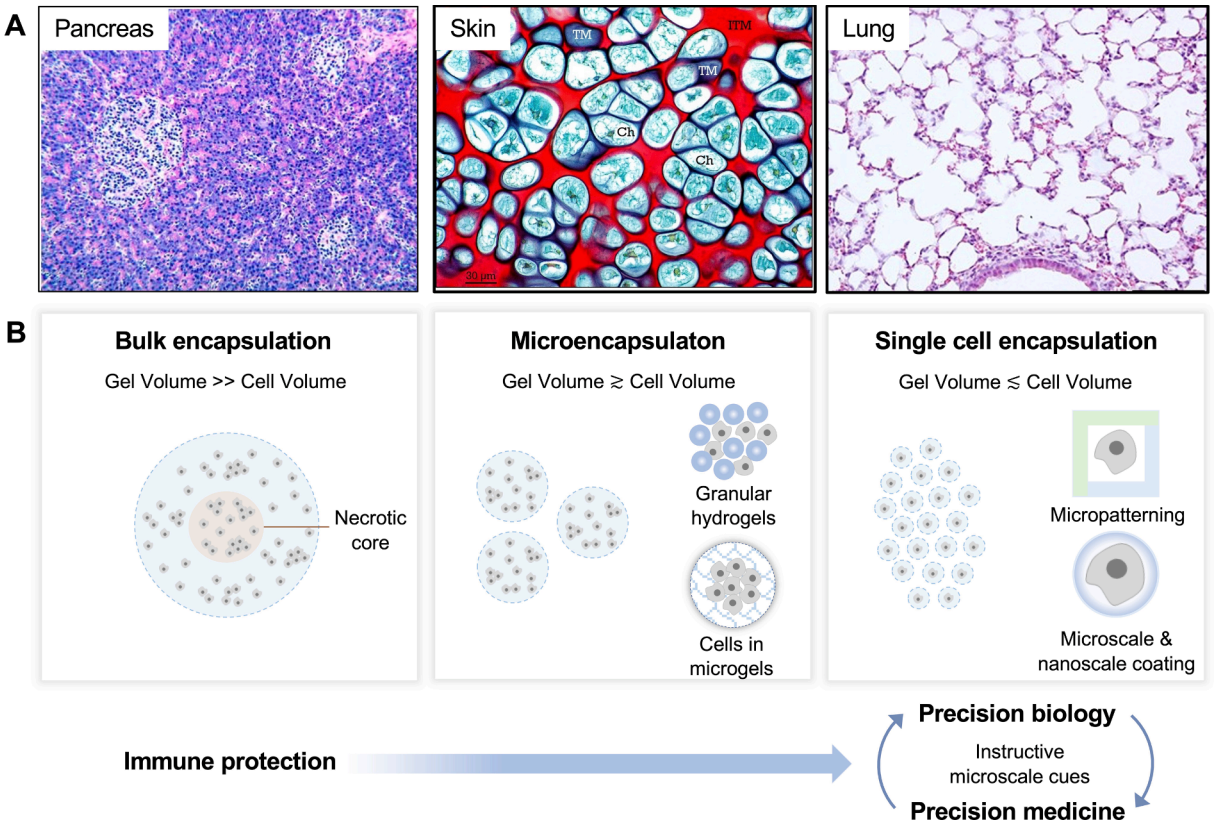

Fig. 1. Encapsulation of cells in hydrogels with decreasing material volume offers physiological relevance and methods to achieve precision biology and medicine. (A) Representative histology sections of pancreas, cartilage (Ch: chondrocytes, ITM: interterritorial matrix, TM: territorial matrix) and lung tissue showing low matrix-to-cell volume ratios. Adapted from references [98,99,144]. (B) Cell encapsulation approaches to achieve smaller material-to-cell volume ratios.

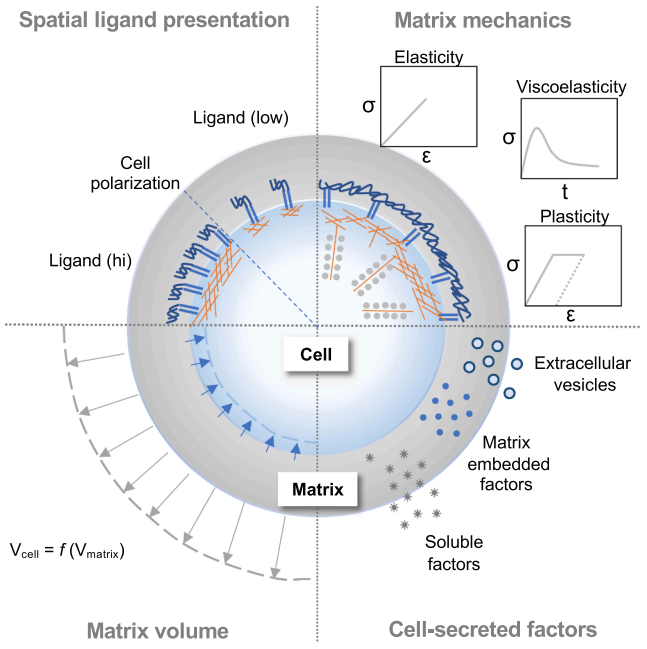

Fig. 2. Instructive cues capable of controlling the function of singly encapsulated cells.

of new cell populations in host tissue. In this case, both PK and PD profiles depend on donor or donor-derived cells. In an ideal scenario, the PK would show a one-phase association kinetics with different half-life values depending on the lineage of reconstituted cells, while the final outcome is the persistent presence of donor-derived cells [101]. The PK profile of different donor cells depends on their ability to migrate, engraft, differentiate and replicate (or self-renew for stem cells) in target tissue. A less persistent PK profile would indicate graft failure. The PD profile depends on whether donor-derived cells are competent in their known function, such as the role of CAR-T cells to target and kill tumor cells. In this context, microencapsulation materials will need to encode specific cues to initially enhance the survival of donor cells, but they will need to be degradable and promote subsequent engraftment of donor cells to host tissue. This can be achieved by using hydrogels that can sequester soluble factors known to promote cell survival and migration [102] or introducing a gradient of adhesion ligands or biophysical cues [103]. Microencapsulation will also need to train allogeneic donor cells to become resistant to clearance by the host immune system after material degradation; however, this challenge can potentially be overcome by implementing gene editing strategies prior to encapsulation to evade immune detection [104]. Another important consideration is that MSCs were shown to possess mechanical memory that retains information from past biophysical environments and influences biological phenotypes [105]. Understanding how mechanical memory can be controlled will enable the development of instructive microscale materials that can persistently program cells.

For islet transplantation and most disease indications of MSC-based therapeutics, the main MOA is persistent delivery of secreted therapeutic factors from cells to host tissue. For this MOA, the PK depends on the residence of donor cells and how they produce secreted factors *in vivo*, and the PD is determined by the properties of cell-secreted factors and their targets in host tissue. A successful PK profile does not require donor cells to become engrafted in host tissue via direct physical contact as long as there is a mechanism for the cells to remain viable and sustain their function at the vicinity of target tissue. As a consequence, it is likely not essential for donor cells to reach their known *in situ* locations as long as the secreted factors can reach their intended targets. Since the main goal here is to protect donor cells from clearance while providing necessary cues for cells to remain viable and secrete factors, microencapsulation materials will need to be non-degradable and minimize foreign body reaction. Both biochemical and biophysical cues of microencapsulation materials can be tuned to further optimize the quantity of therapeutic secretions per cell by modulating specific biological pathways [17,52,106–109]. Single cell encapsulation helps improve the accuracy of PK modeling, since the number of proliferating cells per encapsulation can be precisely tuned by material properties [44,46]. In this context, a deterministic model was developed to successfully predict and tune the therapeutic efficacy of gel-coated MSCs in a preclinical model of fibrotic lung injury [52]. For soluble factors from donor cells, they generally undergo rapid degradation after secretion. In this case, the PD closely follows the PK of transplanted cells [110]. However, for secreted proteins with a longer degradation time, such as ECM proteins, it is possible that therapeutic effects can be seen long after the clearance of donor cells. Metabolic labeling of donor cells along with mass spec analysis [111] can be used to delineate the bioavailability of donor-derived proteins in host tissue. Together, precision programming of biological phenotypes by single cell encapsulation will enable more predictable and accurate PK/PD models for cell-based therapeutics.

6. Outlook

Cell encapsulation initially began with the aim to provide donor cells with a protective barrier from host immune system while enabling therapeutic activity. Single cell encapsulation helps evolve the field towards precision biology and medicine. Advances in understanding cell-material interactions clearly show that cells sense and respond to various cues encoded in biomaterials. As control over material properties becomes more sophisticated and diverse at the single cell level, there is a greater need to develop methods that enable the tuning of multiple material parameters combined with high-throughput analyses of cellular behavior in a single experimental setting. Adopting various microfluidic control approaches, such as mixing [112,113] and droplet splitting [114] will help rapidly generate a library of singly coated cells with varied material properties. Machine learning has already been implemented in droplet microfluidics to optimize single cell encapsulation based on real-time image analysis of droplets and feedback control [115]. Technical advances in single cell encapsulation drive innovations in precisely developing therapeutic cells and engineered tissues.

Some cell therapies under clinical trials administer a heterogeneous population of donor cells. For instance, MSCs have been isolated by plastic adherence, followed by expansion in culture to obtain a sufficient quantity of cells for therapeutic administration [116]. However, lineage tracing and single cell RNA sequencing studies suggest that plastic adherent cells from the same tissue origin consist of different subpopulations [117–123]. These subpopulations also change substantially during culture expansion of MSCs [123,124]. This presents significant challenges in managing donor-to-donor variability and attributing mechanisms of action to specific MSC subpopulations [125]. Accordingly, individual cells are likely heterogeneous in how they interact with their environments. By interfacing MSCs with 2D bulk hydrogels and varying their stiffness, a recent study shows that MSCs consist of subpopulations with distinct mechanosensitivity, differentiation potential, and cell cycle status [126]. Another study shows that MSC heterogeneity from different donors and sources is related to differential expression of ECM-associated genes [127]. These studies highlight the importance of studying cellular subpopulations in physiologically relevant environments from the moment of isolation. Single cell encapsulation offers a promising solution to achieve this goal. Individual cells from a given tissue source can be encapsulated in microgels and cultured to capture desired biological phenotypes, which can then be linked to molecular profiles of the same cells. A recent report provides a feasible example of this approach where single B-cells are cultured in cavity-containing microgels that can record the quantity of IgG secretion by barcoded antibodies; this is followed by single cell RNA sequencing analysis to

identify cell surface markers that can be used to prospectively isolate high IgG-secreting B-cell subpopulations [128]. Thus, single cell encapsulation enables the isolation of precisely defined cell therapy products where mechanisms of action can be selected and tailored for different diseases.

Gel-coated cells can also serve as the basic units of bottom-up tissue assembly. By introducing specific ligand pairs to microgels, it is possible to self-assemble gel-coated cells into spheroid or organoid-like structures [129–131]. However, printing offers a means to precisely dispense each gel-coated cell into a desired location [132]. Success of printing gel-coated cells as voxels will depend on the resolution limit of a printing approach. Micropipette aspiration has long been used to capture and manipulate single cells [133–135]; it was adapted to pick individual tissue spheroids as small as 80 μm and position them into specific locations for assembly [136]. For a more rapid construction of 3D structures, embedded printing was recently developed to generate, deposit and assemble cell-encapsulating spherical microgels as voxels at a sub-millimeter resolution [137]. Achieving scalable voxelated printing at a higher resolution will likely require new approaches to perform on-demand single cell encapsulation from a cell-laden bioink, followed by rapid polymerization via photolithography, which can now be done at a single cell resolution [138–140]. While many cell types remain viable after single cell encapsulation in the presence of adhesion ligands [44–46,52,141], juxtacrine signaling plays important roles in directing necessary morphological changes for tissue development [142]. Using gel-coated cells as voxels enables precision control of physical contact between neighboring cells after assembly, since different gel coating materials can be designed to degrade via ester hydrolysis, enzymes or light [143]. Together, single cell encapsulation offers broad potential to advance precision biology and medicine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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