

Cell encapsulation: technical and clinical advances

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Treating many chronic diseases will require a tight, minute-to-minute regulation of therapeutic molecules that is currently not achievable with most pharmaceutical therapies. For these diseases, implantable living cellular systems may be able to provide unlimited drug delivery, enabling seamless matching of treatment duration with disease longevity. Cell encapsulation is an advanced technology that achieves this goal and represents a viable therapeutic option. The advanced state of the field has allowed researchers to inch forward into therapeutic domains previously untouchable because of the myriad disparate fields that intersect biomaterials and cells. Here, we discuss the next generation of clinical trials and potential approaches, ‘smart’ and responsive encapsulation systems, sophisticated and multifunctional devices, and novel imaging tools, together with the future challenges in the field.

Controlled drug delivery

Improvements in controlled drug delivery have silently crept into our everyday lives. Nasal sprays, rapidly degradable oral strips, transdermal patches, various coatings on pills, and injectable drug-loaded microspheres are all in routine medical and over-the-counter use [1]. However, many diseases are chronic, progressive, and characterized by a life-long struggle and poor symptomatic control using conventional daily systemic drug administration. These treatments cannot provide the minute-to-minute regulation offered by endogenous physiological cellular systems. While advances in mechanical- and material-based drug delivery systems have elongated the duration of drug effectiveness by substituting hourly dosing with daily to weekly dosing, long-term drug delivery is more than a simple engineering problem [2]. Effective, controllable, and tunable long-term drug delivery will likely require the use of long-living cellular systems for essentially

unlimited drug delivery and the ability to match treatment duration with disease longevity [3].

Cell encapsulation is one of the current leading methodologies aimed at the immobilization of allogeneic or xenogeneic cells in a semipermeable but immunoprotective membrane to deliver biological products to patients without the need of immunosuppression. The overarching principle of this technology is to provide a long-lasting, perhaps life-long, solution for treating secretory cell dysfunction [4–6]. This is accomplished with the added benefits of reducing the burden of cell sourcing, obviating the need for complex repeated surgical procedures, and providing targeted drug delivery with more beneficial safety profiles. While other approaches that are currently under investigation (including direct tissue infusions [7], various gene therapy approaches [8], cell therapies [9,10], and biomaterial-based drug-delivery systems [11,12]), cell encapsulation is the only approach that, in principle, meets all of the essential prerequisites for a truly transformative medicine. This technology overcomes many of the fundamental obstacles encountered by other approaches by providing a targeted, continuous, *de novo* synthesized source of molecules that can be distributed over significant portions of the body or within tightly regulated compartments, such as the brain [13]. *A priori*, encapsulated cell therapy combines the potency of *de novo in situ* synthesis of cell-derived molecules with the safety of an implantable and retrievable medical device. Cells are enclosed in a semipermeable capsule that is implanted into the desired region. The capsule is constructed with a pore structure that allows oxygen and other nutrients to nourish the encapsulated cells while providing diffusive control of proteins and other molecules as they exit the capsule into the surrounding vasculature or tissue. Rejection of the cells is prevented by the construction of the immuno-isolating membrane, eliminating the entry of the most damaging elements of the host immune system into the cell-containing lumen. This is accomplished by the control of nominal pore size, distribution, tortuosity, and surface chemistry. An additional advantage of the technology is that the capsule can be configured so that it is easily removed and/or replaced if necessary or desired. The continued refinement in imaging

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and robotic surgical procedures inherently provides a means of selectively targeting those areas of the body where the secreted factor will be optimally therapeutic. Given that multiple implants can be used within the same target region, it is possible to achieve greater spread of the molecule throughout the targeted region than can be achieved with crude infusion of protein [14]. Fifty years after the initial bench-top studies, we describe here the state-of-the-art and the new directions in the basic research and translational medicine aspects of field of cell encapsulation (Figure 1). We highlight the next generation of clinical trials and potential approaches, 'smart' and responsive encapsulation systems, sophisticated and multifunctional devices, and imaging tools, together with future challenges in the field.

From choosing biomaterials to developing 'smart' delivery systems

Criteria for selecting biomaterials

Over the past 20 years, numerous polymers have been proposed and evaluated for encapsulation purposes, including alginate, agarose, chitosan, poly(ethylene glycol) (PEG), polyvinyl alcohol, among others. Many never made it to the application phase because they did not meet one or more basic requirements for their use. Polymers for immuno-isolating capsules should never reduce the functionality or the viability of encapsulated cells. Also, the polymers should form scaffolds that are flexible, soft yet mechanically stable, and allow diffusion of the therapeutic molecules into the surrounding host. Of particular importance is that the polymers need to be compatible with host

immune responses and should not provoke responses that interfere with viability of the enveloped cells.

The most commonly used polymers have been recently reviewed in view of their potential clinical application [15]. These are PEG, polyvinyl alcohol, polyurethane, polyethersulfone (PES), polypropylene, sodium polystyrene sulfate, polyacrylate, agarose, chitosan, cellulose, collagen, xanthan, and alginate [15]. Although many of these polymers have promising properties for cell encapsulation, a greater understanding is needed of the optimal structural conformation and how they can best be used to avoid inflammatory responses in humans. Currently, only one source, the naturally occurring polymer alginate, has passed most of the scientific and regulatory issues to qualify as safe for human application. Alginate is a heterogenic family of natural polymers obtained from algae that vary greatly in mannuronic (M) and glucuronic (G) acid content. It basically comprises homopolymers of M–M, G–G, and G–M. Variations in the ratios of these polymers determine the flexibility, pliability, and even the biological responses of final particles *in vivo*. Understanding how the basic chemical and physical properties of alginate improves the ability to manufacture implantable capsules could lead to the identification of synthetic molecules that can be reproducibly manufactured to have characteristics comparable or superior to those of alginate.

Smart delivery systems

Over the past few years, several important studies have examined the possibility of developing advanced 3D microcapsules capable of responding to external stimuli to provide

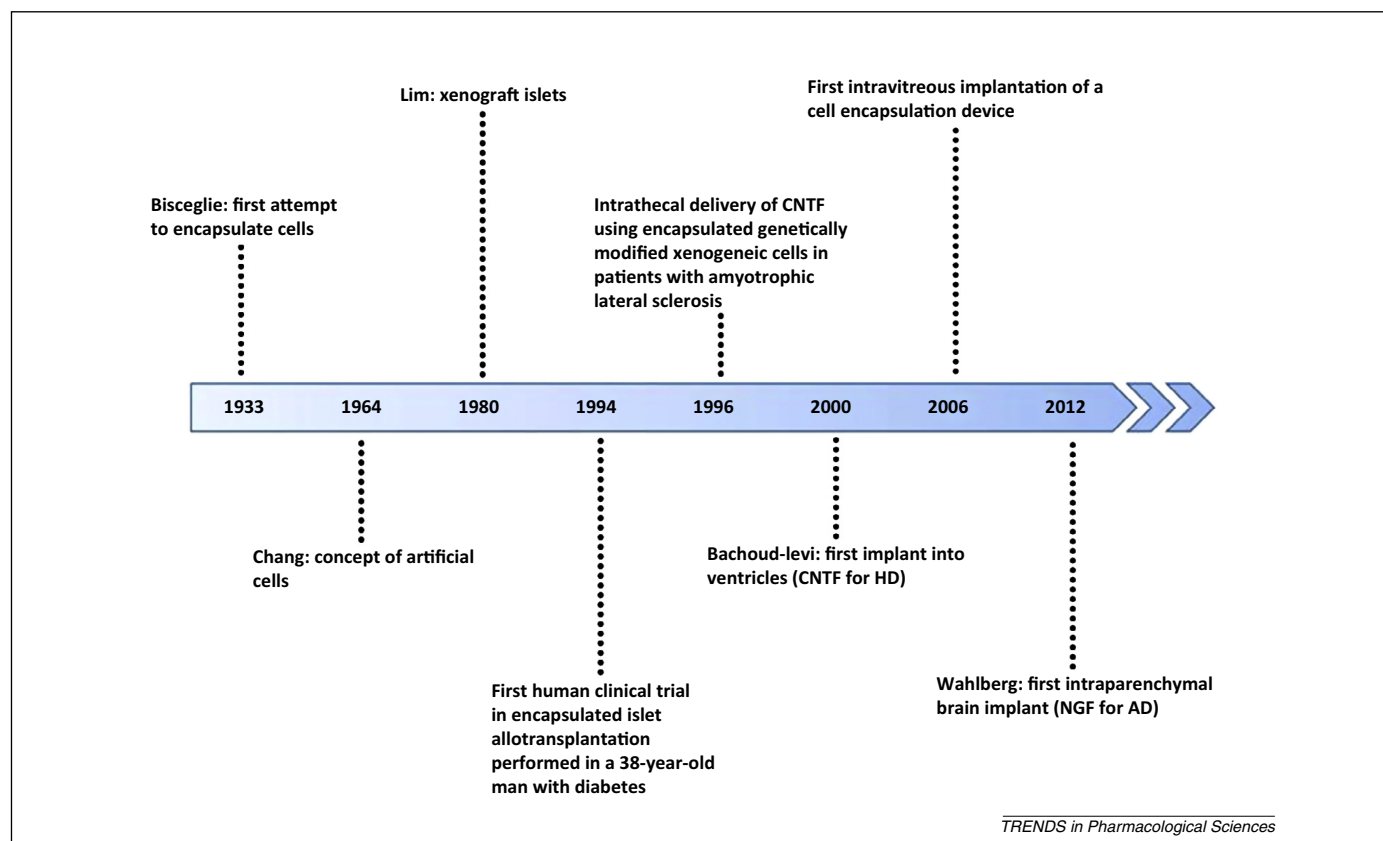


Figure 1. Timeline describing the milestones of cell microencapsulation since its first conception.

dynamic control over the presentation of molecules and cells [16,17]. For example, Huebsch *et al.* achieved a nearly digital release of chemotherapeutic mitoxantrone from injectable alginate scaffolds by ultrasound pulses. This resulted in more effective drug delivery manifested by enhanced suppression of tumor growth *in vivo* [18]. Pulsatile release profiles have also been developed by loading agarose and/or alginate capsules with polypyrrole (PPy) nanoparticles, which react to near-infrared light by acting as photothermal nanotransducers that melt the agarose to release the reagents [19]. Similarly, alginate ferrogels can be triggered by magnetic fields to deliver bioactive agents, including mitoxantrone, plasmid DNA, chemokines, and mesenchymal stem cells (MSCs) [20]. These examples highlight a burgeoning trend of designing microcapsules that extend beyond the traditional immunobarrier to act as dynamic vehicles that are responsive to external, non-invasive stimuli to bring out superior pharmacokinetic features for both drug and cell delivery purposes.

Materials can also be designed in ways that enable real-time imaging to confirm implant location and function of cells encapsulated into those materials. The precise location of the implanted cell-loaded capsules may be determined by traditional imaging modalities, including magnetic resonance imaging (MRI) [21–24] or ultrasound [25]. For example, microcapsules can be coated with self-assembled gold nanoparticles, which enable them to be monitored by X-ray micro-CT scanners [26,27]. In addition to confirming the implant location of encapsulated cells, imaging can be used to simultaneously confirm the viability and function of the entrapped cells. Adding reporter genes to enable bioluminescence and/or fluorescence imaging allows accurate non-invasive follow-up of the encapsulated cells, providing quantitative, real-time, live-imaging determination of those cells [28–32]. The biosafety of the implant may also be reinforced by including suicide genes that enable external inactivation of entrapped cells once the therapy is complete, if dosing changes are desired, or in the case of deleterious effects. For instance, both suicide and reporter genes (e.g., TGL triple-fusion reporter gene–GFP, firefly luciferase, and Herpes Simplex virus type 1 thymidine kinase) have been incorporated into cell encapsulation systems to provide concurrent monitoring and inactivation of encapsulated cells *in vivo* [29,33].

Current state of microencapsulation

Recent advances in microtechnology-based cell encapsulation methods have enabled the production of microcapsules with precise control over the enclosed cell number, particle size, or polymeric structure [34]. For instance, taking advantage of flow-focusing technologies, small particles ($\approx 100 \mu\text{m}$) have been fabricated to reach difficult targets such as the intravitreal space [35]. Either with flow-focusing systems (a technology whose aim is the production of drops or bubbles by straightforward hydrodynamic means [36]) or with a microfluidic-based electrospray [37], structured capsules with individually incorporated components can be constructed that give investigators the option of encapsulating different molecules and/or cells into spatially separated compartments within the same particle. Regardless of the encapsulation technology used,

coaxial flows have been harnessed to simultaneously produce bilayers in ‘core-shell’ microcapsules [38–40]. Such a strategy is being used, for example, to form cell spheroids or to avoid the exposition of enclosed cells in the particle surface. Similarly, using a re-encapsulation process, Bhujbal *et al.* produced a multilayer system that included a non-cell survival-supporting external alginate ‘shell’ to impede the protrusion of cells from the inner cell permissible alginate core [41]. Finally, the use of superhydrophobic surfaces is being investigated as a means of obtaining multicompartamental particles with diverse hierarchical structures and without the need for any precipitation bath [42–44].

A significant amount of effort has been devoted to reducing the size of the encapsulating particle, especially with regard to pancreatic islet transplantation (Figure 2A), with the aim of minimizing the engraftment volume:surface area ratio [45]. Thus, conformal coating has arisen as a new and potentially more effective encapsulation model for the immunoprotection of islets of Langerhans. Fabrication procedures for conformal coating are diverse and are evolving rapidly, ranging from the first alginate emulsification methods to the more modern layer-by-layer assembly, which enables the bio-orthogonal conjugation of bioactive compounds, such as immunosuppressants or labeling motifs [46,47]. Recently, a flow-focusing approach provided a thin, complete, and uniform coating adapted to the shape and size of individual islets, resulting in the successful protection and maintenance of islet function (Figure 2B) [48].

Encapsulation of allografts versus xenografts

Therapeutic cells are being used in immuno-isolating devices such as allografts or xenografts. Allografts are cells that are being transferred from a host to a recipient of the same species (e.g., human to human), while xenografting involves transplanting cells over a species barrier (e.g., from animals to humans). In most applications of encapsulated primary cells, such as pancreatic islets, it is almost impossible to obtain adequate tissue. Given that the number of diseased recipients simply outnumbers the available pancreas donors, xenografting is a logical alternative. Up to now, almost the same capsule systems have been used for allografts and xenografts. This might have to change because allografts and xenografts provoke different immune responses (outlined below) likely requiring capsule configurations capable of protecting encapsulated cells against variable cellular and humoral immunological environments.

For protection of allogeneic tissue, it is probably sufficient to avoid contact between immune cells of the host and the donor cells [49]. Therefore, simple cation-alginate capsules without any diffusion limitation for immuno-active molecules, such as immunoglobulins can prevent allograft rejection [50]. For xenografts, this is different and simple systems are probably not effective. Higher mammals, including humans, respond strongly to xenogenic epitopes and have different mechanisms by which they can eliminate encapsulated xenogeneic cells. Xenogeneic cells contain highly immune reactive epitopes, such as galactosyl (Gal) residues. These Gal are ligands for naturally occurring (anti-Gal) and

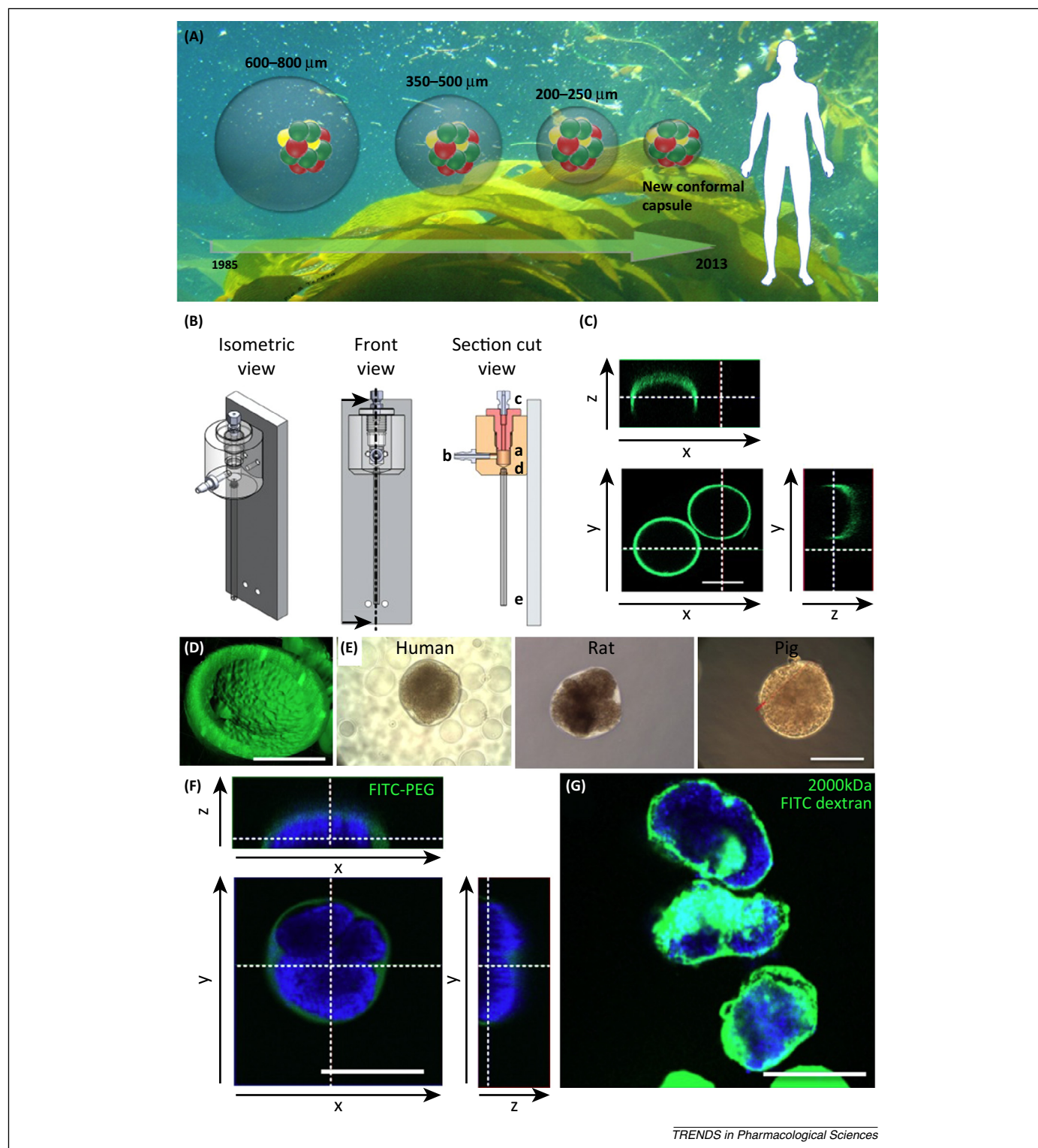


Figure 2. Conformal coating in pancreatic islet transplantation. (A) Evolution of pancreatic islet encapsulation from large microcapsules to current conformal coatings. (B–G) Design of the flow chamber and optimization of conformal coating encapsulation of model beads and pancreatic islets with polyethylene glycol (PEG)–vinyl sulfone (VS) crosslinked with dithiothreitol (DTT). (B) Computer-aided design (CAD) drawings of the encapsulation chamber in isometric, front, and section cut views. Orthogonal projections (C) and a 3D surface plot (D) of islet model beads conformally coated with fluorescein isothiocyanate (FITC)-labeled PEG. Scale bar = 200 μm. (E) Phase-contrast images of conformally coated human, rat, and pig islets. Scale bar = 100 μm. Orthogonal projections (F) and maximum projection on the z axis (G) of rat islets conformally coated with FITC-labeled PEG (F) or with a solution of PEG containing 1 mg/mL 2000 kDa FITC dextran (G). Scale bars = 200 μm. Reproduced from [45] (A; with permission) and [48] (B–G).

non-Gal immunoglobulin (IgM) antibodies. When ligated, these Gal and natural antibodies complexes are strong activators of the classical complement pathway. Upon diffusion of Gal residues out of the capsules, complexes of antibodies and complement may accumulate on the surface of capsules. These complexes, in turn, lead to chemotaxis of

undesired cell types, such as neutrophils [51], with strong inflammatory responses elicited near or on the capsules. During these responses, many cytokines that are small enough to pass through the membrane of the capsule are produced, further contributing to the failure of the encapsulated cells.

After the initial innate response, a second response occurs: the so-called ‘delayed-type hypersensitivity response’. This is an IgM-mediated reaction against xenogeneic epitopes [51] that induces a secondary influx of inflammatory cells in the vicinity or on the capsules themselves. During this response, chemokines and cytokines are also produced that are deleterious to the cells. The final result of this cascade is often envelopment of the capsules by inflammatory cells and fibroblasts that scavenge almost all local nutrients and lead to ischemic compromise of the surviving cells in the capsules. A further loss of cell functionality can occur because of epithelial-to-mesenchymal (EMT) transition. This EMT may be induced under the influence of hypoxia-inducible factor-1 α (HIF-1 α), which is produced by cells during ischemia [52]. HIF-1 α induces Twist expression, which subsequently leads to progressive fibrosis.

As a consequence of the differences in immunological responses to allo- versus xenogeneic cells, stricter encapsulation requirements exist for xenografts. The membranes should be less permeable and not allow entry of the large molecular structures of the complement system and, preferably, the cutoff should be low enough to retain hyperinflammatory xenogeneic epitopes, such as Gal residues. In a recent rat-to-pig xenograft experiment, pancreatic islets were encapsulated in a membrane system that included an additional barrier between the immunoprotective membrane and the interface with the recipients. This system prevented rejection of the islets for the 3-month period of the experiment [53]. Of note, a multifunctional hydrogel-based scaffold was recently proposed that, in a single injectable system, incorporates xenogenic cell-entrapping alginate-poly-L-lysine-alginate (APA) microcapsules together with dexamethasone (DMX)-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres [54]. However, it should be mentioned that therapeutics such as DMX can not be applied in fields such as the encapsulation of islet cells, because DMX is diabetogenic by itself.

Still, these and other studies demonstrate that xenografting is possible with adapted membranes and can even aid the temporal release of anti-inflammatory drugs. However, they also illustrate that many features remain to be studied before a successful efficacious immuno-isolating system for xenografts can be universally applied in humans.

Clinical evaluation of encapsulated cells

Encapsulated islets for diabetes

Numerous clinical trials have been conducted or are underway evaluating the use of encapsulated cells in human diseases (Table 1). A significant proportion of these efforts have focused on type 1 diabetes mellitus (T1DM), where encapsulation offers protection of alternative cell sources for pancreatic islets of rare donors, including xenogeneic islets, genetically engineered insulin-producing cells, and stem cell-derived islets. Soon-Shiong *et al.* reported the successful transplant of human islets encapsulated in APA microcapsules [55]. The intraperitoneal graft of 15 000 islet equivalents (IEQ)/kg resulted in insulin independence and glycemic control for 9 months. At approximately the same time, Scharp and colleagues [56] demonstrated that human islets encapsulated in poly(acrylonitrile-co-vinyl chloride) (PAN-PVC) macrodevices could survive in a subcutaneous implantation without eliciting an immune response in patients. Calafiore *et al.* [57] transplanted alginate-poly-L-ornithine (PLO) microencapsulated human islets into the peritoneum of four patients. The first two patients showed increased C-peptide serum levels together with an ephemeral decline in exogenous insulin consumption. A subsequent report confirmed that no adverse effects or immune sensitization occurred and that all patients had lowered (approximately 50%) insulin requirements [58]. Tuch and colleagues [59] transplanted allogeneic islets encapsulated in Ba²⁺-alginate microbeads into four patients with diabetes. While a single treatment resulted in a transient increase in C-peptide, three separate islet infusions resulted in C-peptide that was detectable

Table 1. Examples of clinical trials with encapsulated cells

Biomaterial	Cell source	Graft site	Refs
Diabetes			
APA microcapsule	Allogeneic islets	Peritoneal cavity	[55]
PAN-PVC macrocapsule	Allogeneic islets	Subcutaneous	[56]
Alginate-PLO-alginate microcapsule	Allogeneic islets	Peritoneal cavity	[57,58]
Ba ²⁺ alginate microbeads	Allogeneic islets	Peritoneal cavity	[59]
Ca ²⁺ /Ba ²⁺ alginate microbeads	Allogeneic islets	Peritoneal cavity	[60]
Alginate-PLO-alginate microcapsule	Xenogeneic islets (porcine)	Peritoneal cavity	[67]
Encaptra macrodevice	Allogeneic beta cell precursors	Subcutaneous	http://ViaCyte.com/
AD			
PES macrocapsule	NGF-producing ARPE-19 cells	Intraparenchymal (Ch2 and Ch4)	[14,72]
ALS			
PAN-PVC macrocapsule	Ciliary neurotrophic factor (CNTF)-producing hamster fibroblasts	Intrathecal	[68,69]
Chronic pain			
PAN-PVC macrocapsule	Bovine chromaffin	Intrathecal	[69]
HD			
PAN-PVC macrocapsule	CNTF-producing hamster fibroblasts	Intrathecal	[70]
Pancreatic cancer			
Cellulose sulfate microcapsules	Allogeneic 293 cells secreting CYP2B1	Tumor blood vessels	[94]
Retinitis pigmentosa			
	CNTF-producing ARPE-19 cells	Eye	[71]

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for 30 months in a single patient. Changes in insulin requirements or glycemic control were not observed. Most recently, Jacobs-Tulleneers-Thevissen *et al.* transplanted Ca²⁺/Ba²⁺-alginate microbeads containing allogeneic islets into a single patient [60]. The alginate microbeads were harvested 3 months after transplantation. The microbeads were clustered and conglomerated in the peritoneal cavity, demonstrating insufficient biocompatibility, although the cells remained glucose responsive.

These studies have all shown partial function of the encapsulated islet grafts but at the same time limitations to the survival time. A first critical issue that has to be overcome is tissue responses against the capsules. Tissue response can be caused by numerous factors. For example, it can be provoked by contaminations in alginate [61]. It has been shown that commercially available alginates, such as those used in the studies mentioned above, contain so-called 'pathogen associated molecular patterns' (PAMPs) [62]. PAMPs are strong initiators of inflammatory responses. Almost all systems have been associated with protrusion of cells [63]. Above a certain threshold, this leads to a strong inflammatory response resulting in complete fibrosis of the capsules, with necrosis of the cells [64]. Mechanical stability might also be an unresolved issue because many encapsulation systems have variable elasticity and mechanical resistance. These forces are not only recipient, but also site dependent and the optimal parameters for cell survival are still unknown in humans [65]. These factors, in addition to broadly recognized issues, such as the surface roughness, protein adsorption, and the presence or absence of negative charges, are all critical in the success and failure of the grafts [66]. Unfortunately, none of the clinical trials so far have addressed these parameters

and questions will remain until side-by-side comparisons are made.

One of the few clinical studies addressing the efficacy of xenogenic islets has been initiated by Living Cell Technologies (LCT). LTC initiated a clinical trial with porcine islets that were encapsulated in alginate-PLG microcapsules and implanted into the peritoneal cavity of patients without immunosuppression. While few published data are available, it was reported that live porcine islets could be retrieved from one of the patients 9.5 years post implantation [67] (<http://www.lctglobal.com/Products-and-Services/Diabecell>). Although most cells appeared to be necrotic, the long-term demonstration of viability is impressive and shows the potential applicability of the approach once we understand why some capsules failed while others survived.

Encapsulated cells for neurological diseases

Delivering therapeutic molecules across the blood-brain barrier (BBB) to the brain parenchyma is a formidable challenge. To be effective, delivery needs to occur in a long-term and stable manner at sufficient quantities directly to the target region in a manner that is selective but yet covers the desired target region. Cell encapsulation overcomes these issues by delivering therapeutic molecules directly to the brain area of interest (Box 1). Initial clinical trials for amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) [68–70] were based on solid preclinical data showing that encapsulated cells could be configured into implantable capsules that were biocompatible, maintained cell viability for extended periods of time, and were functional in animal models. However, during Phase I clinical trials, it became evident that obstacles remained. The cells that were used (e.g., modified fibroblasts) were

Box 1. The CNS as a unique challenge and opportunity for encapsulated cell therapy

Treating chronic, degenerative CNS diseases has traditionally been a challenge, in large part because of the inability to deliver potentially therapeutic molecules (trophic factors, antibodies, etc.) across the BBB directly to the target site in a stable, controlled, and continuous manner. Several strategies are under development to optimize the diffusion and spread of trophic factors into brain tissue. These include direct brain infusion, various gene therapy approaches, cell therapies, and biomaterial-based drug delivery systems. These approaches have advantages, but also inherent disadvantages, including cumbersome repeated surgical procedures, limited stability of the drug being delivered, and safety concerns related to immunogenicity and the inability to reverse the treatment once initiated. Encapsulated cell therapy overcomes all of these obstacles by providing a targeted, continuous, *de novo* synthesized source of molecules that can be distributed over significant portions of the brain in a manner that combines the potency of *de novo in situ* synthesis of cell derived proteins with the safety of an implantable and retrievable medical device. For CNS applications, the use of conventional imaging and stereotactic procedures provides a means of selectively targeting those areas of the brain where the secreted factor will be therapeutic. Given that multiple implants can be used within the same target region, it is possible to achieve greater spread of the protein throughout the targeted region than can be achieved with its crude infusion.

Cell encapsulation has been an intuitively appealing means of treating CNS diseases for 20 years with significant preclinical demonstrations of efficacy and safety. Despite establishing many of the essential prerequisites of this technology in animal models during the 1990s, the field had difficulty living up to expectations, and clinical

evaluation yielded promising but insufficient results. Implantable capsules were biocompatible, could maintain cell viability for extended periods of time, and were functional in animal models, but phase I clinical trials also revealed that these initial configurations produced results that were inconsistent enough to limit clinical investigation and that additional technical development was needed. Non-human, animal-derived cells, such as fibroblasts, with difficult-to-control cell division frequently overgrew the capsule environment, resulting in an accumulation of necrotic tissue that diminished the permeability characteristics of the membrane, further reducing cell viability and secretion. The techniques used for genetic modification resulted in low factor secretion and limited diffusion within brain tissue, making adequate distribution in the human brain difficult to achieve. The membranes used were generally biocompatible, but also permitted tissue ingrowth into the membrane walls, further limiting bidirectional diffusion while also raising concerns during device retrieval.

Despite these setbacks, relatively clear pathways emerged for transitioning this technology into a viable clinical product. Encapsulated cell technology now utilizes human cells with excellent long-term viability and function in animals and humans. Membranes and cell scaffolds have been developed under rigorous, well-controlled manufacturing processes and advances in molecular biology have increased the secretion of therapeutic molecules from encapsulated cells by several log orders. Finally, the development and continued refinement of clinical implantation systems compatible with conventional stereotactic techniques eases the transition from preclinical evaluation into widespread clinical implementation.

animal-derived cells with uncontrolled proliferation that frequently overgrew the capsule environment, producing necrotic tissue that diminished the permeability characteristics of the membrane, further reducing cell viability and secretion. The cells and the techniques used for inducing production of the therapeutic molecule were not effective, resulting in production that was too low. The therapeutic molecules were not adequately distributed because of their relative large size. Although the membranes used were generally biocompatible, they also permitted tissue ingrowth into the membrane walls that had the potential to further limit bi-directional diffusion while also raising possible concerns during device retrieval [13].

From these studies, relatively clear pathways emerged for transitioning this technology into a viable clinical product. Recently, a human cell line (ARPE-19) was used as a platform cell line for both preclinical and clinical evaluation. These cells can survive under stringent conditions, such as implantation into the eye, or even *in vitro* in extreme conditions of nutrient deprivation. Encapsulated ARPE-19 cells have survived for at least 2 years in the human vitreous [71] and for at least 1 year in the brain parenchyma of patients with Alzheimer's disease (AD) [14,72]. Membranes and cell scaffolds have also been developed under rigorous, well-controlled manufacturing processes, further augmenting the continued survival and function of encapsulated human cells. While most work within the central nervous system (CNS) has revolved around the use of PAN-PVC, the use of PES and polystyrene (PS) membranes appears to have addressed previous questions about modest biocompatibility. Thus, nerve growth factor (NGF)-secreting cells loaded within PES membranes were implanted into the brains of minipigs and were successfully explanted 12 months later [73]. All of the devices were removed and proved to be intact with no adherence of inflammatory cells. Viable cells were identified with continued secretion of NGF. These studies also illustrate the importance of the cell scaffolding within the capsule. It has long been known that the survival and differentiation of encapsulated cells can be influenced by matrix interactions. With ARPE-19 cells, the replacement of a polyvinylalcohol foam scaffolding with a polyethylene terephthalate (PET) yard significantly improved cell viability and function while also making the manufacture of the devices more reproducible. Moreover, advances in molecular biology have led to approaches to increase the secretion of therapeutic molecules from encapsulated cells by several log orders. This is a particularly important for CNS degenerative diseases because these diseases are not treatable by delivering drugs systemically or from the ventricular space, given that diffusion of compounds in brain tissue is severally limited when governed only by passive diffusion. Of note, ARPE-19 cell lines were generated using the Sleeping Beauty transposon system [73,74]. This process resulted in stable gene transfer with long-term expression and bioactivity both *in vitro* and *in vivo*. NGF levels were approximately 10 times higher than that achieved previously using other constructs. Notably, when implanted into minipig brain, the resulting diffusion of NGF in the target region (basal forebrain) was substantial [74], yielding diffusion

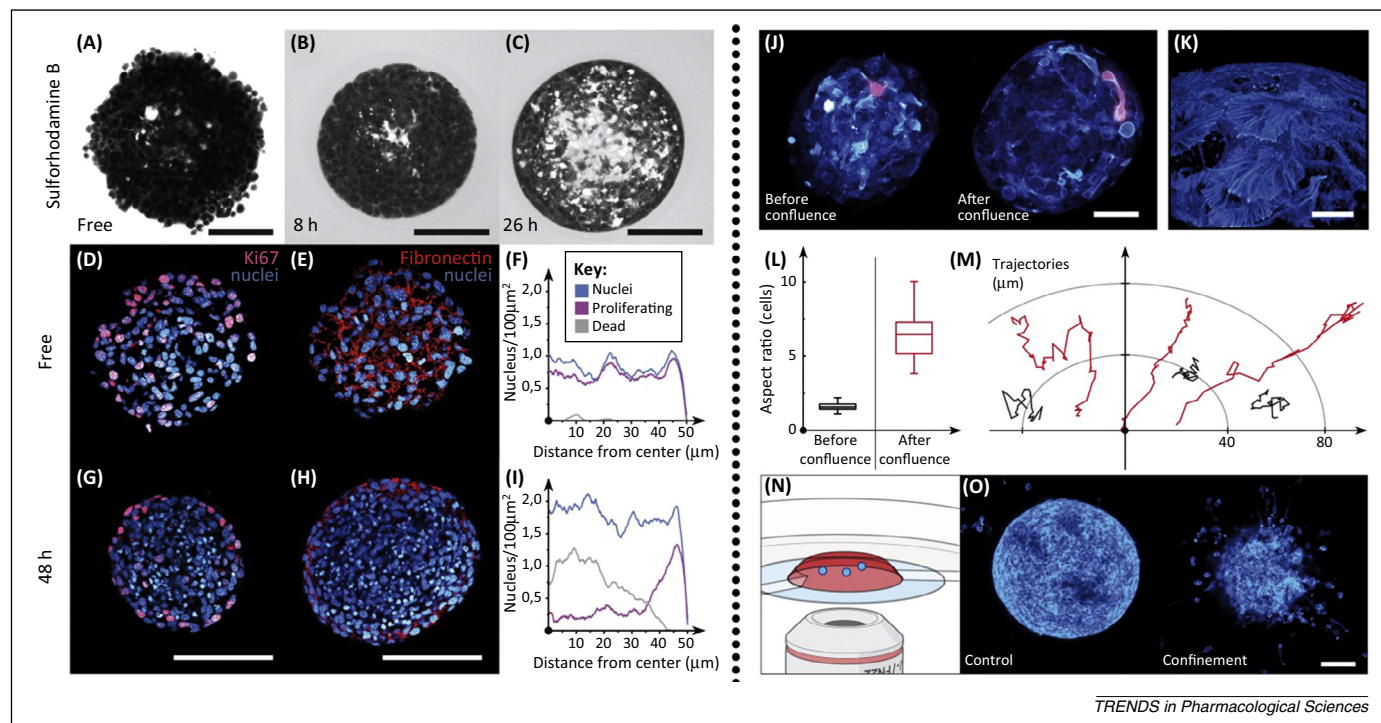
distances from the implant sites up to 2.5 mm, as illustrated by immunohistochemistry. Immunohistochemistry likely underestimates the distance that biologically active trophic factors, such as NGF, diffuse in the brain, making it probable that the measured 2.5 mm of diffusion was a low estimate in these studies. Cell lines have also recently been generated that secrete GDNF levels approaching microgram quantities per day making it conceivable for the first time that molecules such as glial cell-derived neurotrophic factor (GDNF) can be delivered in a sustained manner that covers the putamen of patients with Parkinson's disease. A recent clinical trial involved encapsulated ARPE-19 cells to deliver NGF to the brains of patients with AD [93]. This study confirmed the promise of recent advances. One centimeter-long capsules were attached to an inert polymer tether and placed bilaterally into the basal nucleus of Meynert (three patients) or both the basal nucleus of Meynert and the vertical limb of the diagonal band of Broca. A total of six AD patients were involved in the trial. Post-operative CT scans confirmed the appropriate placement of the capsules and MRI images at 3 and 12 months showed no evidence of inflammation or device displacement. At 12 months, implants were successfully retrieved. The devices were intact, and low but persistent NGF secretion was detected in 50% of the patients.

Opening new avenues in the use of cell encapsulation

Overcoming many of the basic obstacles of cell encapsulation has allowed researchers to legitimately set their sights on future developments, including delivery of enzymes, developing artificial organs through the combination of 3D material and/or cell complexes, the use of stem cells for drug delivery and tissue integration, the development of biodegradable capsules as a cell delivery system capable of parallel or sequential drug delivery, and even the use of cell-based tools as diagnostics.

It is accepted that cell-based therapies have potential for regenerative medicine, but the inefficient delivery and retention of therapeutic cells in the affected and/or implanted area has limited their clinical utility [75]. Cell encapsulation provides controllable delivery of therapeutic cells, growth factors, and/or a combination thereof. For example, in the field of cardiac tissue repair, cell microencapsulation represents a useful strategy to improve the modest engraftment and reduced long-term survival observed with the transplantation of unencapsulated cells [76,77]. Further optimization has been proposed for future studies by adding extracapsular proteins, such as proteoglycans or transglutaminase, to facilitate the attachment of the capsules to the host extracellular matrix [76].

The transplantation of encapsulated cells provides different therapeutic strategies for regenerative medicine. The most classical one is to use genetically engineered cells that overexpress one or several growth factors and biologically active agents. Following this traditional path, vascular endothelial growth factor (VEGF)-secreting human umbilical cord MSCs have been used in the vascularization of tissue-engineered dermis [78]. However, with recent advances in stem cell biology, tissue remodeling is increasingly being tackled by means of the paracrine



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Figure 3. Cell encapsulation as a platform for investigating the interplay between tumor growth and the mechanical stress imposed by surrounding tissues. (A–I) Imaging of the internal cellular organization of growing spheroids under elastic confinement. Snapshots taken by two-photon imaging of free (A) and encapsulated (B,C) spheroids stained with the polar dye SRB are shown. Time $t = 0$ corresponds to confluence. Confocal images of free (D,E) and confined (G,H) spheroids after cryosection and immunolabeling for DAPI (blue), Ki67 (magenta), and fibronectin (red). Quantification of cell nucleus (blue), proliferating cell (purple), and dead cell (gray) radial densities for free (F) and confined (I) CT26 spheroids. Scale bars = 50 μm (A–C); 100 μm (D,E,G,H). (J–O) Impact of elastic confinement on cell motility at the periphery of growing spheroids. (J) Confocal live imaging of an encapsulated spheroid grown from cells stably transfected with LifeAct-mCherry. Maximum intensity projections of the confocal stacks [a hot look-up table acquired using Fiji is shown (cyan)]. One representative cell is colored magenta before and after confluence to highlight the features quantified in (L). (K) Enlarged view of the surface of a fixed spheroid imaged by confocal microscopy after staining with phalloidin-Alexa 488 (Hot LUT, cyan). (L) Box plot shows the aspect ratio of cells before and after confluence ($n = 94$, five spheroids). (M) Representative projected trajectories of the center of mass of cells moving at the periphery of spheroids. The time interval is 15 min. (N) Cartoon describing cell migration assay. Spheroids (blue) are embedded in collagen matrix (red) in a Petri dish. (O) Confocal image taken 48 h after implantation in collagen for spheroids grown freely (control) and in a capsule past confluence after shell dissolution (confined). Scale bars = 50 μm (A); 10 μm (B); 100 μm (F). Reproduced from [83].

exchange of molecular signals [79] or by direct engraftment of transplanted cells [80]. Additionally, the use of injectable multifunctional microgels encapsulating outgrowth endothelial cells (OECs) and growth factors has produced notable results in the neovascularization of hindlimb ischemia, where VEGF and hepatocyte growth factor (HGF) release supported the paracrine effects of OECs and their possible migration and implication in the vasculogenesis process [81]. Interestingly, another innovative strategy relies on engineering the microenvironment of implanted cell capsules by harnessing the endogenous secretion of growth factors. Thus, bone marrow-derived MSCs have been delivered in microspheres preloaded with anti-bone morphogenetic protein 2 (BMP2) monoclonal antibodies (mAbs), so that the mAb captures the endogenous BMP2 and presents it to the MSCs, thereby directing their differentiation towards osteogenic lineage [82].

Beyond the multiple uses and versatility of this constantly evolving biotechnology, cell encapsulation also serves as a valuable and attractive platform for the study of many biological processes, either physiological or pathological. In a recent study, cellular capsules were used as a tool for investigating the interplay between tumor growth and the mechanical stress imposed by surrounding tissues [83]. This allowed researchers to elucidate the compressive forces that slow tumor progression but at the same time trigger cell invasion and metastasis (Figure 3). Similarly,

culturing mouse embryonic stem cells (mESC) in 3D alginate beads enhanced differentiation toward neural lineages when compared with 2D cultures [84]. Indeed, 3D models are gradually replacing many of the conventional 2D flat-surface culture systems [85,86].

Finally, immobilizing cells within polymeric matrices represents an advantageous 3D culture system for mass production in bioreactors [87,88] by maintaining the pluripotency of ESCs and induced pluripotent stem cells [89,90]. 3D spherical culture systems allow enrichment of cancer stem-like cells for cancer research and therapy development [91], and allow the production of a readily scalable liver cell biomass for the development of a bioartificial livers [92].

Concluding remarks

Cell encapsulation is an advanced technology that enables the implantation of living cellular systems to provide unlimited drug delivery, resulting in a seamless matching of treatment duration with disease longevity. Important advances have been made in cell encapsulation during recent years, with greater insight into the factors determining the success and failure of encapsulated cellular grafts in humans. Therapeutic cell encapsulation is expected to evolve significantly in the future. Dynamical control over the release of active compounds in response to external stimuli may dramatically improve the biosafety and efficacy of this biotechnology. In drug delivery

applications, managing the option to switch between sustained versus pulsatile releases or secreting multiple drugs in a sequential form would be advantageous. The field of regenerative medicine may also see some firsts. Cell delivery might be controlled by means of porous cell loaded scaffolds that degrade naturally or by external magnetic fields. The use of 3D microcapsules may also aid the study of the biology and spread of tumors and the evaluation of potential new treatments. Last but not least, cell encapsulation represents an excellent 3D culture system for mass production in bioreactors.

In summary, because the rate of advancement has increased significantly since the inception of encapsulation, it is envisioned that advances in engineering, biomaterials, and cell biology will only further accelerate the advancement of cell encapsulation into widespread clinical evaluation and, ultimately, medical practice.

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