

In cell encapsulation, transplanted cells are protected from immune rejection by an artificial, semipermeable membrane, potentially allowing transplantation (allo- or xenotransplantation) without the need for immunosuppression. Yet, despite some promising results in animal studies, the field has not lived up to expectations, and clinical products based on encapsulated cell technology continue to elude the scientific community. This commentary discusses the reasons for this, summarizes recent progress in the field and outlines what is needed to bring this technology closer to clinical application.

## Cell encapsulation: Promise and progress

In 1964, T.M.S. Chang<sup>1</sup> proposed the idea of using ultrathin polymer membrane microcapsules for the immunoprotection of transplanted cells and introduced the term 'artificial cells' to define the concept of bioencapsulation, which was successfully implemented 20 years later to immobilize xenograft islet cells. When implanted into rats, the microencapsulated islets corrected the diabetic state for several weeks<sup>2</sup>. Since then, there has been considerable progress toward understanding the biological and technological requirements for successful transplantation of encapsulated cells in experimental animal models, including rodents and non-human primates. Bioencapsulation has provided a range of promising therapeutic treatments for diabetes<sup>3</sup>, hemophilia<sup>4</sup>, cancer<sup>5</sup> and renal failure<sup>6</sup>. Additionally, the functional applicability of cell encapsulation in humans has also been reported in several clinical trials<sup>7,8</sup>.

Despite considerable interest, however, the field has not lived up to expectations<sup>9</sup>. A lack of reproducibility, or uncertainty surrounding the reproducibility of prior studies has been a major problem. For example, in the treatment of diabetes, researchers have been plagued by the inability to reproduce one excellent trial in dogs undertaken by Metabolex of Hayward, California (<http://www.metabolex.com>), one important primate study on rhesus monkeys<sup>3</sup> and a single patient study<sup>7</sup>. To put it simply, despite the Edmonton protocol<sup>10</sup>, in which islet transplantation in patients with type I diabetes resulted in insulin independence for a year, very few groups have isolated sufficiently good islets and developed a suitable biocompatible encapsulation material.

Indeed, the consensus is that microencapsulation still represents a sort of 'in-house' procedure, administered by a small number of laboratory groups who are reluctant, or who are unable because the technology is proprietary, to share complete information and protocols. Also retarding progress of this field is a lack of standardized technology, including optimized tissue and clinically proven materials for membrane manufacturing produced in reproducible batches. These limitations have bedeviled universities and startup companies alike. University-based laboratories have had the added difficulty of scaling up technologies to produce materials in sufficient quantities to permit duplicate studies. As a consequence, many essential research questions, such as the exact selection of membrane materials, their final properties, site of transplantation, cell source and choice of purification methods, remain unanswered.

### Current challenges

Technological and biological limitations, as well as ethical, political and regulatory obstacles, must be overcome if the promise of cell encapsulation technology is to be realized.

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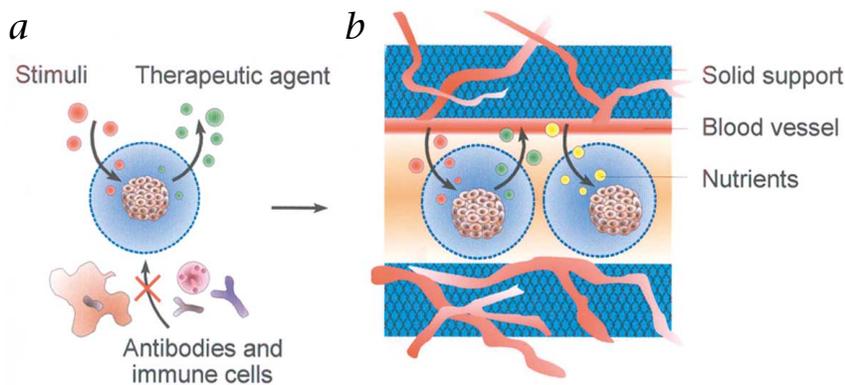
Some of the important considerations for consistent clinical success of cell encapsulation include a source of functional cells; a biocompatible, as well as mechanically and chemically stable, membrane of a suitable permeability cut-off value that provides immune protection to the implant; functional performance; biosafety; and long-term survival of the graft.

**Lack of clinical-grade polymers.** In the quest for a better microencapsulation design, many types of natural and synthetic polymers are being explored. A substantial challenge related to the biomaterials used in cell encapsulation has been the lack of clinical-grade polymers. Although its intrinsic properties make alginate the current encapsulation material of choice, batches of alginate need to be standardized to minimize endotoxin and protein content, both of which can affect biocompatibility. This requires standardized protocols to eliminate such impurities<sup>11</sup>. In this regard, an international task force should be instituted to set up a 'central alginate factory' that would prepare standardized prototypes for use by participating laboratories in their transplant studies. The European Community and Norway now support the formation of such dedicated centers.

**Production of uniform capsules.** Another challenge involves the production of uniform capsules with excellent repeatability and reproducibility both within and between batches. The adoption of automated machines for microencapsulation could result in improved reproducibility in terms of shape, size and morphology. As such, the recent development of an automated chemical reactor<sup>12</sup> may help to address this problem.

**Use of polycations.** The discovery of suitable immune-compatible polycations represents another principal area of study. After 20 years of research, the clinical application of polycations for microcapsule formulation remains controversial. Although some believe that poly-L-lysine (PLL) polycation has a low probability of success as a result of its poor biocompatibility<sup>13</sup>, others have obtained promising *in vivo* results replacing PLL with poly-L-ornithine<sup>14</sup> or poly(methylene-co-guanidine) hydrochloride<sup>15</sup>. It may be that ultimately the simplest system of them all, the alginate bead with its non-uniform density, will be sufficient to provide immune protection in the case of allogeneic models (intraspecific human), whereas the development of microcapsule materials for xenogeneic models (interspecific





**Fig. 1** Cell microencapsulation. *a*, Nutrients, oxygen and stimuli diffuse across the membrane, whereas antibodies and immune cells are excluded. *b*, Pre-vascularized solid support system to facilitate optimal nutrition of the enclosed cells.

nonhuman) will remain a challenge. This is because alginates are too porous to offer suitable immune protection to the implant.

**Considerations before transplantation.** Still other challenges involve the assessment of the exact dosage<sup>10</sup> and molecular-weight cutoff value, as well as the overall biocompatibility of the system. X-ray photoelectron spectroscopy and Fourier-transform infrared spectroscopy techniques could shed light on the latter, helping to identify the chemical groups causing bioincompatibility on the surface of a capsule and predicting the biosafety of the devices before implantation<sup>16</sup>.

**Selecting suitable cell types for immobilization.** A number of issues should be carefully evaluated when selecting suitable cell types for immobilization. Indeed, encapsulation requires an appropriate source of functional cells. In this regard, the potential use of allogeneic versus xenogeneic cells has provoked important social and ethical debates<sup>17</sup>. The principal controversy surrounds the potential risk of inadvertent transfer to humans of animal viruses present in the xenotransplant, particularly the porcine endogenous retrovirus<sup>18</sup>, and many forums have concluded that research should proceed with allotransplantation over xenotransplantation<sup>19</sup>. Regulatory issues and selective moratoriums<sup>20–22</sup> aside, xenogeneic graft pilot trials could benefit from the use of special multicompartmental microcapsules. These may embody, for example, anti-oxidizing, anti-apoptotic and  $\beta$ -cell pro-mitogenic factors that could prolong primary cell survival and provide functional competence<sup>23</sup>.

**Transplantation site.** The choice of transplantation site is another important consideration. Here, it is necessary to weigh issues such as the safety and possibility of re-transplantation (peritoneal cavity, subcutaneous transplantation) against proximity to the circulation<sup>24</sup> (intrahepatic transplantation or membranes supporting vascularization). Still another pertinent issue is that permanent graft survival of encapsulated cells has never been reported. Some groups attribute these graft failures to the lack of direct vascularization of the enclosed cells, with consequent gradual tissue necrosis and death. To address this problem, pre-vascular-

ized solid supports are being studied to improve the nutrition of the encapsulated cells<sup>25</sup> (Fig. 1). However, the question of vascularization remains open, because other groups have obtained promising results by transplanting capsules into the peritoneal cavity of large animals without direct contact with blood vessels<sup>14</sup>.

#### Regulatory and ethical issues

With advances in the science of encapsulated cell therapies, regulatory authorities have been gradually adjusting their policies to accommodate these new therapeutic approaches. In the United States, for example, all islet transplant studies (and presumably all future encapsulation-type clinical

studies) will be regulated by the US Food and Drug Administration (FDA) under an investigational new-device submission. For now, Europe will likely rely on FDA guidelines, because specific regulations in this field are presently lacking. Recently, the US Pharmacopeia and National Formulary included a new therapeutic category for cell-based products<sup>26</sup>, which constitutes a significant step toward accepting this technology and encouraging clinical trials.

A major ethical concern surrounding the use of microencapsulated cells is to ensure that patients are treated with a technology that demonstrates a clearly proven biosafety based on standardized protocols and procedures. In this regard, it is important to avoid poorly conducted studies that put individuals at unnecessary risk and unfairly raise hopes and expectations. For example, the recent trial of pig islet xenotransplantation in children by Valdes' group at the National University of Mexico has sparked a fresh round of debate<sup>27</sup>, because it is in direct contravention of the Helsinki Agreement on the ethical performance of clinical trials. Moreover, reports of this kind run the risk of hampering future progress of the entire field.

#### Recent progress

In recent years there have been some interesting research developments. For example, considerable effort has focused on the identification of alternative natural and synthetic materials for use in cell encapsulation. These efforts have resulted in the polyanionic material recently patented under the name Biodritin<sup>28</sup>, the photopolymerizable poly(ethylene glycol) polymer to immobilize cell clusters (<http://www.novocell.com>, Irvine, California), and the genetically modified alginates with a highly controlled chemical structure<sup>29</sup>. Recently, microfabricated silicon membranes have been reported to shed light on the issue of permeability control<sup>30</sup>. This thin membrane possesses an extremely uniform pore size of only a few nanometers wide, providing strict control over the inward diffusion of immunoglobulins and, hence, greater protection against immunorejection of the transplanted cells.

In addition, genetic engineering has contributed to the development of modified cells that have superior cell viability and are therefore capable of providing an improved supply of therapeutic products. In one study, recombinant



mouse myoblasts enclosed in alginate-polylysine-alginate microcapsules, showed continuous expression of human Factor IX for at least 7 months *in vivo*<sup>4</sup>. In another study, hybridoma cells enclosed in cellulose sulfate capsules showed detectable levels of antibody in mouse serum for as long as 4 months after transplantation<sup>31</sup>, indicating that this approach might be useful for antibody-based gene or cell therapy. In regard to cancer therapy, researchers used encapsulated genetically modified allogeneic cells expressing pro-drug-activating enzymes such as cytochrome P450 (ref. 32) as a possible treatment for inoperable pancreatic carcinoma. Moreover, promising anti-angiogenic results have been obtained with endostatin-transfected cells encapsulated in alginate in the treatment of malignant brain tumors<sup>33,34</sup>. Finally, another group has developed an approach to tumor suppression that involves genetically modified myoblast cells secreting interleukin-2 linked to the Fv region of a humanized antibody with affinity to tumors over-expressing the oncogene *ERBB2* (also known as *HER-2* or *NEU*)<sup>35</sup>.

The gross insufficiency of suitable cadaveric and fetal cells could likely be circumvented through the use of stem cells. Once suitable sources of stem cells and appropriate means to control their differentiation become available, stem cells may constitute a universal cell line suitable for the large-scale manufacture of encapsulation devices. In any event, microencapsulation may be necessary for the immunoisolation of stem cells, in that recent studies have shown differentiated human embryonic stem cells to express high levels of major histocompatibility (MHC) class I proteins, which may cause them to be rejected on transplantation<sup>36</sup>.

**What the future holds**

Cell microencapsulation is a technology with enormous clinical potential for the treatment of a wide range of diseases<sup>37</sup>. Yet many difficulties remain, some of which will certainly challenge our scientific ingenuity. The stepwise analysis of the essential obstacles, coupled with increased international collaboration, should move the technology forward in a careful and controlled way and bring it much closer to clinical reality. Some of the most convincing arguments for bioencapsulation are that it could eliminate the need for immunomodulatory protocols or immunosuppressive drugs and permit the long-term *de novo* delivery of therapeutic products in either a local or systemic manner<sup>38</sup>.

Clinical trials of alginate microcapsules could begin soon. An application to initiate clinical trials of encapsulated human islets in non-immunosuppressed patients with type 1 diabetes, headed by Riccardo Calafiore and Paolo Brunetti of the University of Perugia, is currently pending at the Italian Ministry of Health. Clinical applications for other endocrine defects, such as pituitary dwarfism or thyroid and parathyroid disorders, are expected to follow.

Despite these promising developments, we believe that if cell encapsulation technology is to receive a full evaluation as a potential therapeutic alternative to overcome the limitations of whole-organ transplantation (such as, lack of suitable donors, need for immunosuppression, potential risks associated with major operation, cost), an international advisory committee should supervise the initiation of pilot clinical trials of microencapsulated cell allografts into carefully selected recipients. This paper represents a call for such a committee.

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