Microencapsulation for cell therapy of type 1 diabetes mellitus: The interplay between common beliefs, prejudices and real progress

When Lim and Sun\(^1\) showed, for the first time in 1980, that intraperitoneal grafts of allogeneic rat pancreatic islets containing alginate-poly-L-lysine microcapsules reversed hyperglycemia in non-immunosuppressed diabetic rats, a new era for cell therapy of diabetes had started. As a unique and unmatched result, years later, Sun reported success in diabetic primates, that on intraperitoneal transplant of microencapsulated porcine islets, showed long-term remission of hyperglycemia.

However, at that time, the international academic and scientific community greeted with skepticism such a revolutionary approach. Our laboratory, at the University of Perugia (Perugia, Italy), had the privilege, since the middle/late 1980s, to enjoy Sun’s mentorship for learning the basics on the microencapsulation procedure. This fundamental step permitted us to further expand and critically refine the original technology, over the following three decades. Microcapsules efficiently prevented islet graft-directed immune destruction, with no recipients requiring immunosuppression, while allowing for passive diffusion of oxygen, nutrients, metabolites and hormones, all indispensable for the embodied islet cell survival and function. Nevertheless, microencapsulation hid a number of not immediately apparent technical skills, including, but not limited to, chemical materials selection and biocompatibility, long-term polymer stability, and appropriate membrane’s molecular weight cut-off selectivity. All these items became increasingly stringent, along with progress of microencapsulation into higher mammalian graft trials.

Work on microencapsulation has always been associated with struggle and progress. Variability of the communicated results, over the past few years, has favored recent, critical review analyses on still controversial issues of this technology. In particular, a recent article\(^2\) raised a number of reflections and concerns. However, while some of these are sharable, others look questionable, in that some observations on microencapsulation technical aspects look possibly biased, and based more on theoretical assumptions/common beliefs than on careful scrutiny of the actually achieved experimental and clinical data.

After 30 years of intense work, we believe we have learned the following lessons from the microencapsulation technology:

MICROCAPSULES BASIC CHEMISTRY AND BIOPOLYMER SELECTION

Not all potentially employable, and so far used polymers, are suitable for fulfilling the many stringent fabrication requirements, which an efficient microencapsulation procedure should comply with. At this juncture, except for alginates (AG), all alternative polymers, tentatively used to fabricate microcapsules, have never entered significant transplant studies, and many of them are today of mere historical memory. As far as AG are concerned, highly performing purification protocols of the raw product, including selection of polymeric molecular patterns, have enabled success in few dedicated centers. On the contrary, use of poorly purified/unpurified AG resulted in failure of many microencapsulation programs. We have shown that microcapsules formulated with ultrapurified, ‘clinical grade’ AG, comprising prevalent mannnarurate dimeric blocks, covalently bound to poly-L-ornithine (PLO), uniquely used by us, are extremely proficient (Table 1), also enabling per se, inhibition of \textit{in vitro} cellular immunity. In fact, these AG/PLO empty microcapsules, on \textit{in vitro} co-incubation with peripheral blood mononuclear cells of patients with recent-onset type 1 diabetes, blunted peripheral blood mononuclear cells proliferation\(^3\). This observation showed that highly purified AG might render microcapsules an immunomodulatory tool per se, regardless of their immunobarrier competence. This observation confirms that selection of qualified AG for cell microencapsulation purposes is essential.

IDEAL SIZE OF MICROCAPSULES

Still controversial remains the issue of whether small-sized, possibly ‘conformal,’ microcapsules would perform better than conventional-sized microcapsules (these are commonly defined by an equatorial diameter of 600 \(\mu\)m). If a major concern with the use of larger-sized microcapsules was the unfavorable molecular diffusion kinetics, this has not been technically proven. On the contrary, using standard 600-\(\mu\)m microcapsules, fabricated according to our technology, including the use of ultrapurified AG, Pipeleers’ group\(^4\) reported that microcapsules containing human \(\beta\)-cells, retrieved from the peritoneal cavity of nude mice at 5 weeks of graft, on \textit{ex vivo} perifusion with glucose, showed excellent patterns of physiological insulin secretory kinetics. Hence, 600-\(\mu\)m AG microcapsules, if properly formulated, do not impair insulin and other metabolites diffusion. Consequently, older studies cited from the late 1980s by Chicheportiche et al.\(^5\) speaking against the use of large-size microcapsules today look at least unreliable, simply because decades ago, microcapsules were comprised of impure AG, which today is known to heavily interfere with insulin secretion.
Furthermore, the issue that large-sized microcapsules would impair oxygen diffusion, postulated by a number of published reports, prevalently came from \textit{in vitro} studies, but it was never clearly confirmed \textit{in vivo}. It is likely that more than the individual capsules’ size, the selected graft site might adversely affect oxygen/nutrient supply to the microencapsulated islets/cells.

**IMMUNOPROTECTIVE PROPERTIES OF AG-BASED MICROCAPSULES**

If microcapsules have been proven, in selected centers, to retain clear immunobarrier competence, this goal has been missed by many others. In general, variability of data between laboratories can be ascribed, at least partially, to the lack of a standardized method for microencapsulation, including, but not limited to, the basic polymer composition/purity grade. In some instances, this relates to the fact that many standard operatory procedures for microencapsulation are proprietary, with limited if not inexistent, interlaboratory collaborative exchanges.

To comply with requirements of the regulatory agencies, in order to apply microencapsulated islet/cell grafts to patients, the use of ultrapurified, ‘clinical grade’ AG that optimizes per se microcapsules physical-chemical properties, is mandatory. This has indeed been the case for the first early pilot human clinical trial of AG/PLO microencapsulated islet allografts in non-immunosuppressed patients with type 1 diabetes, carried out by our group. These microcapsules uniquely prevented both graft rejection and sensitization of the recipients to the encapsulated islet cell allogeneic antigens, virtually making the embodied islets ‘bioinvisible’ to the host’s immune system. On the contrary, ‘biovisibility’ is not achievable by unencapsulated allogeneic islet grafts in pharmacologically immunosuppressed patients with type 1 diabetes, where antigen sensitization cannot be obviated. For this purpose, the issue of human leukocyte antigen class I and II donor/recipient mismatch, viewed as a potential, still unsurmounted problem with currently fabricated microcapsules, does not apply to microcapsules that are formulated appropriately. In fact, we have provided clear evidence in our pilot clinical study, that our microencapsulated human islets were associated with a complete lack of sensitization towards human leukocyte antigen class I and II, islet cell antibodies, glutamic acid decarboxylase 65 and insulin islet donor antigens, throughout 5 years of post-transplant follow up. Finally, correctly fabricated AG/PLO microcapsules containing islet/cells are associated with long-term robustness and durability over long periods of time. As a matter of fact, we retrieved physically intact microcapsules from one of our patients at 5 years post-transplant.

Minimal-sized microcapsules look very attractive, and they would certainly be advantageous in terms of occupied final graft volume, permitting access to wider graft site selection. Nevertheless, these coatings are still burdened with a number of technical pitfalls, mainly dependent on the selected basic polymers, spanning from questionable immunoprotection capacity, to uncertain biocompatibility, to unknown chemical endurance. The latter especially applies to layer-by-layer constructs that are comprised of very thin films surrounding the individual islet cells/cell clusters. These artificial membranes might be prone to consumption, degradation and loss of selective permeability. Development of new polymeric molecules might attenuate these problems in the next years to come. Furthermore, quite recently, Veiseh \textit{et al.}\(^7\) postulated that bigger might be more proficient than smaller microcapsules, in terms of immunobarrier competence. On a final note, our 600-µm microcapsules performed very well in both experimental non-obese diabetic mice and patients with type 1 diabetes, where neither regeneration of native islet cells, nor downregulation of plasma glucose levels could ever occur.

**OUTLOOK**

Undoubtedly, microcapsules need to face new and difficult challenges ahead. It is increasingly evident that, within microcapsules, pancreatic islets need to be replaced by other cell types, regardless of whether they are xenogeneic (i.e., pig) islets or human embryonic, or more likely, adult stem cells. Additionally, an implant site that couples easy and safe access with appropriate vascular supply, such as the omentum, could permit use of mini-invasive laparoscopy. This is a stringent issue, dictated by the notorious low-oxygen tension associated with the large peritoneal cavity. Here, sufficient oxygen/nutrient supply to the grafted encapsulated islet mass, hence physiological insulin secretory kinetics, could be hampered. Our pilot clinical trial, although mainly targeting safety and not efficacy, has partially confirmed this drawback. In fact, we showed that intraperitoneally grafted AG/PLO microcapsules afforded full immunoprotection to the grafted human islets, whereas metabolic proficiency of these grafts was only partial. The grafts affected glucose control parameters quite marginally, despite either well documented production of C-peptide, which was completely absent before transplant (all recipients bore long-standing type 1 diabetes), or improvements in glycated hemoglobin levels in all recipients. They indeed enjoyed long-term significant reduction, or in one instance transient withdrawal, of the daily exogenous insulin supplementation.

| Table 1 | Alginate polymer qualification for microencapsulation (University of Perugia) |
|---------------------------------|---------------------------------|
| **Alginites** | **Ultrapurification** | **Poly-L-ornithine** |
| Endotoxin | <1 EU/mL | |
| Pyrogens | Absent | |
| Proteins | <0.4% | |
| Heavy metals | ppm | |
| Arsenic | 0.04 | |
| Strontium | Undetectable | |
| Iron | 0.65 | |
| Manganese | 0.06 | |
| Mercury | 0.02 | |
| Magnesium | 0.72 | |
| Lead | 0.03 | |
| Copper | 0.15 | |
| Zinc | Undetectable | |
In terms of alternative cell sources, human post-partum umbilical cord Wharton Jelly-derived adult mesenchymal stem cells (hUCMS), retrieved in bulk in our laboratory, are extra-embryonic stem cells that do not incur the embryonic stem cells-associated safety and ethical issues. In particular, hUCMS hold powerful immunoregulatory properties. AG/PLO microencapsulation turned individual isolated hUCMS into viable and functionally active cellular spheroids, that were able to release immunomodulatory molecules, such as indoleamine 2,3 di-oxygenase-1 and human leukocyte antigen class G5. Preliminarily, we observed that grafts of microencapsulated hUCMS induced long-term reversal of hyperglycemia in non-obese diabetic mice with recent-onset, spontaneous diabetes, by reinstalling a condition of acquired immunotolerance.

In conclusion, pending issues still contend ground to already achieved milestones in microencapsulation. Nevertheless, the many accomplished results should not be neglected, but eventually regarded as the positive demonstration that microencapsulation might play a pivotal role in cell therapy of type 1 diabetes in the near future.

DISCLOSURE
The author declares no conflict of interest.

Riccardo Calafiore*
Section of Cardiovascular, Endocrine and Metabolic Clinical Physiology, Department of Medicine, School of Medicine, University of Perugia, Perugia, Italy
*E-mail: riccardo.calafiore@unipg.it

REFERENCES
1. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. Science 1980; 210: 908–910.
2. Korsgren O. Islet encapsulation: physiological possibilities and limitations. Diabetes 2017; 66: 1748–1754.
3. Montanucci P, Alunno A, Basta G, et al. Restoration of t cell subsets of patients with type 1 diabetes mellitus by microencapsulated human umbilical cord Wharton jelly-derived mesenchymal stem cells: An in vitro study. Clin Immunol 2016; 163: 34–41.
4. Jacobs-Tulleneers-Thevissen D, Chintinne M, Ling Z, Gillard P, et al. Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient; Beta Cell Therapy Consortium EU-FP7. Diabetologia 2013; 56: 1605–1614.
5. Chicheportiche D, Reach G In vitro kinetics of insulin release by microencapsulated rat islets: effect of the size of the microcapsules. Diabetologia 1988; 31: 54–57.
6. Basta G, Montanucci P, Luca G, et al. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. Diabetes Care 2011; 34: 2406–2409.
7. Veiseh O, Doloff JC, Ma M, Vegas AJ, et al. Size-and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates. Nat Mater 2015; 14: 643–651.

Doi: 10.1111/jdi.12788