Transworld Research Network
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

The Bioartificial Pancreas and Other Biohybrid Therapies, 2009: 37-50 ISBN: 978-81-7895-415-8
Editors: Jean-Pierre Hallé, Paul de Vos and Lawrence Rosenberg

3 Microencapsulation technologies for a bioartificial endocrine pancreas

Denis Poncelet[1] and Susan K. Tam[2]
[1]École Nationale d'ingénieurs des techniques des industries agricoles et alimentaires (ENITIAA), UMR CNRS 6144 GEPEA, rue de la Géraudière BP82225, 44322 Nantes cedex, France; [2]Université de Montréal, École Polytechnique de Montréal, Biomedical Engineering (LIAB), C.P. 6079, Stn. Centre-Ville, Montreal, Qc, H3C 3A7, Canada

Abstract

This contribution offers an overview of the technologies suitable for pancreatic islet encapsulation. It proposes a comprehensive outline of the technologies from the point of view of the physico-chemist and engineer. After presenting methods for producing droplets of appropriate size, it classifies techniques for converting droplets into microcapsules.

Introduction

The concept of encapsulating islets of Langerhans for solving problems of immune rejection was published as early as 30 years ago. Starting with the work of Lim and Sun [1] on the entrapment of islets within alginate beads, the principle has remained relatively

Correspondence/Reprint request: Dr. Denis Poncelet, ENITIAA, UMR CNRS 6144 GEPEA, rue de la Géraudière BP82225 44322 Nantes cedex, France. E-mail: poncelet@enitiaa-nantes.fr

the same over the years. However, many groups around the world have worked intensively to understand the limitations of this system, not only from the point of view of the encapsulation technique itself, but also of the biocompatibility of the system and the biology surrounding and inside the microcapsules. The object of this contribution is to concentrate on the description of the encapsulation technologies from the point of view of the engineer and/or the physico-chemist. The biomedical aspects will not be treated directly in this chapter.

We provide a few (simple) equations to build basic theory, but we combine them as much as possible with drawings and graphs for easier understanding.

Microcapsule size

What may be one of the most important parameters of a capsule for cell immuno-protection is its diameter, or its size. If the capsules are to be injected, the diameter must be, at maximum, a third of the internal needle diameter. Larger capsules will sediment more in the syringe, creating risk of blockage. However, larger capsules are easier to recover for post-transplantation analysis, such as in the case of a foreign body reaction.

Clinical islet transplantation usually involves about 600 000 islets, i.e. a volume of 5 to 10 ml [2]. If an equivalent number of islets are to be immobilized in capsules of 250 to 500 µm diameter, this represents a volume of 20 to 160 ml to be transplanted. Because of this increased volume, the transplantation of large capsules is practical only in the peritoneal cavity [3]. In addition, it has been demonstrated that the use of larger capsules created a delay in the systemic response to glucose level [4] as well as in the transfer of oxygen into the capsules. These mass transfer problems are attributed to the increased distance between the capsule membrane and the encapsulated islet, which may be one to several hundred micrometers. As a consequence, authors [5] report that oxygen (as an example) decreases radially in the capsules, leading to a lowering of the islet activity.

For capsules smaller than 320 µm diameter, transplantation in the portal vein is possible without observing an important and permanent increase of the portal pressure [6]. Small capsules also offer improved oxygen and nutrient supply (including glucose) and good release properties of the insulin. One problem that may be presented with using smaller capsules is an increased probability of islet protrusion, which can induce a foreign body reaction [7], and a higher rate of empty capsule production.

Concerning the size of the capsules for islet encapsulation, one must also consider:

- Size distribution. Capsules that are too large or too small may lead to problems as described above. A wide size distribution also requires more work when handpicking capsules that are suitable for implantation.
- Homogenous distribution of the islets within the capsules. Most other authors refer to a single islet per capsules. However, in practice, islet distribution may follow Poisson's law with the effect that all capsules are assumed to fill a mean of 2 or 3 islets [8]. Some artifacts (such as sedimentation in the extrusion system) may improve this situation.

Conventional capsule diameters typically range between 250 and 800 micrometers. Thus, there is a tendency to develop technologies that target this capsule size [3, 6]. Yet, some authors propose to coat or graft polymers directly to the islets [9, 10] by putting the

islets into contact with a limited volume solution containing reagents for interfacial precipitation or polymerization. Wilson and Chaikof propose a very good review that discusses the selection of the microcapsule size to optimize the cell encapsulation and transplantation process, from the point of view of both the production and the performance of the system for diabetes treatment [11].

Droplet production (making the capsule core)

Many books classify methods for producing capsules as chemical, physicochemical or mechanical methods. However, many technologies combine such aspects and thus similar methods can be placed in more than one of these categories.

Let us therefore use a more appropriate method of classification based on the three steps for producing the capsules [12]:

- **Incorporation**: The active component (the islets of Langerhans in this case) has to be incorporated into a solution that will serve as the future capsule core (for example an alginate solution).
- **Dispersion**: The capsule core is dispersed. In the case of a liquid, this involves forming droplets. In the case of a solid core, the dispersion consists in mixing the particles with a coating solution.
- **Stabilization**: The droplets (or the liquid coating) have to be solidified.

Methods could be classified based on the dispersion steps (dripping, spraying, emulsification, fluidization…). Most of the works devoted to the encapsulation of fragile

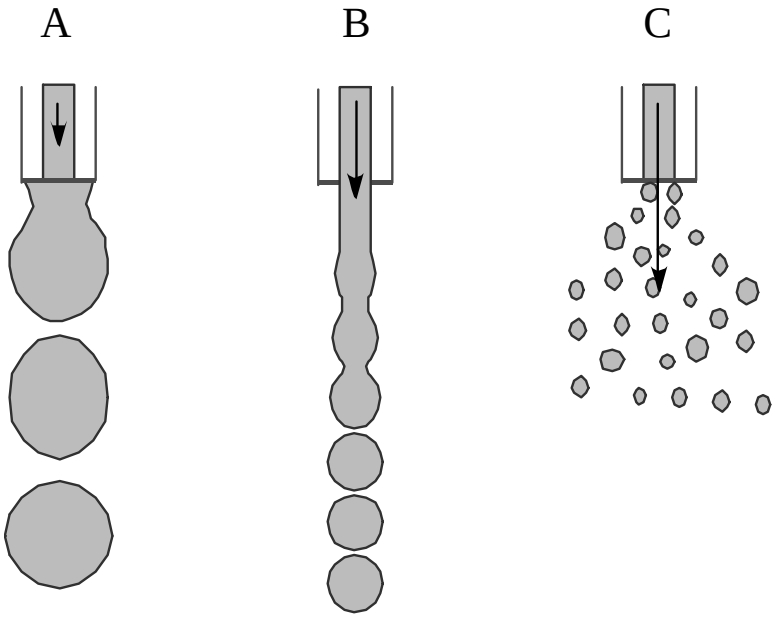

Figure 1. From dropping to spraying methods (Poncelet ©).

islets refers to dripping technologies. While a liquid is extruded from a simple needle or nozzle, one could observe that:

- For a very low flow rate, droplets are formed and detached one by one from the needle. We are in **dropping** conditions (Fig 1a).
- Above a certain flow rate, the liquid is ejected from the needle as a jet, which could break into droplets naturally (Raleigh's theory) or be broken up using a moderate external force. The latter may be a mechanical force to physically “cut” the jet, or a vibrational force to “shake off” the droplets. We are in **jet breakage** conditions (Fig 1b).
- For a high flow rate, or if a high external force is applied (such as high speed coaxial airflow), the liquid is exploded into number of small droplets. We are in **spray** conditions (Fig 1c).

Spraying involves high energy, shear forces, cavity effects and high pressure. Obviously, such conditions would be detrimental for the survival of the cells. We then have to mainly consider dropping and jet breakage for the encapsulation of islets of Langerhans.

Simple dropping

In simple dropping conditions, the detachment of the droplet occurs when the gravity force (linked to the mass of the droplet) becomes higher than the surface tension force maintaining the droplet on the needle [13]. The rupture point could then be defined by:

Gravity forces = surface tension forces

$$m\ g = \pi\ d_e\ \gamma \tag{1}$$

where m is the mass of the droplet, g the gravity force, d_e the external needle diameter, and γ the surface tension. From the mass of the droplet, we could deduce the diameter:

$$m = \frac{\pi}{6} d^3 \rho \tag{2}$$

where d is the droplet diameter, and ρ is the density of the droplet.

To reduce the size of the droplet, one would propose to reduce the diameter of the needle. However, the droplet diameter is only affected by the cubic root of the needle diameter. Moreover, the detachment of the droplet does not happen directly on the needle and the diameter of the liquid at the breakage point is only quasi constant. More importantly, Figure 2 shows that in practical simple dropping conditions, the droplet size will be too large for cell transplantation purposes, i.e. above ~1.8 mm in diameter.

To obtain a capsule diameter lower than 1 mm, one has to add some moderate external force. Three options that are commonly applied include the use of the coaxial airflow, an electrostatic potential, and a vibrating nozzle:

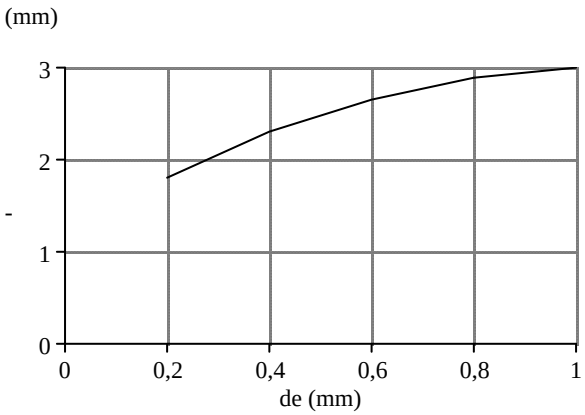

Figure 2. Size of falling droplet versus external nozzle diameter.

Dropping using a coaxial airflow

A coaxial airflow may be applied around the needle to create a drag force and reduce size of the droplet. In this case, the droplet falls from the needle when:

Gravity forces = surface tension forces – drag forces

$$m\ g = \pi\ d_e\ \gamma - F_g \tag{3}$$

wherein Fg is the drag force [8].

If the coaxial airflow is not well controlled or is increased too much, it will create turbulence, droplet size dispersion and finally spraying. Such a system is therefore suitable to reduce the size of the droplet to a minimum of 500 micrometers.

There now exists some commercial devices to produce microcapsules using a coaxial airflow (as an example consult www.nisco.ch). A very promising device supporting multi-60µm-nozzles surrounded by micro-channels with a controlled airflow has been used to produce 150µm capsules [14]. Building such system is based on microfluidic engineering, a relatively complex technology. However, because the nozzle diameters are so small, pancreatic islets would have to be carefully screened for their size in order to avoid blockage of the nozzles.

Dropping using an electrostatic potential (electro-dripping)

An alternative method to reduce the size of droplets is to apply an electrostatic potential between the nozzle and the receiving solution [6]. The existence of this electrostatic potential leads to charge accumulation on the surface of the droplet, creating repulsion and consequently reducing the surface tension [15]. In this case, the force balance is described by :

$$m\ g = \pi\ d_e\ \gamma_o\left(1 - \frac{U^2}{U_{cr}^2}\right) \tag{4}$$

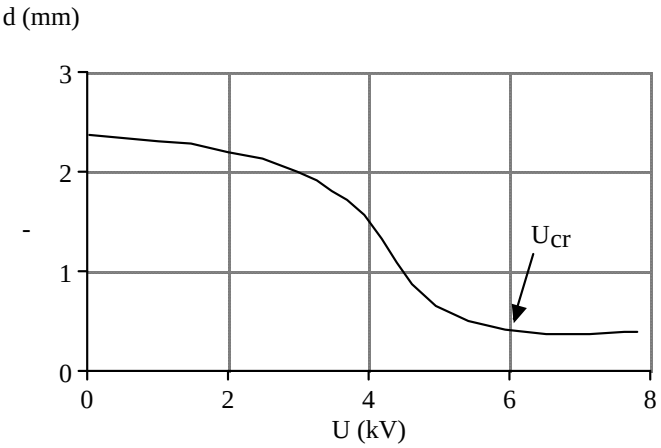

Figure 3. Droplet size versus electrostatic potential (adapted from [16]).

where γ_o is the surface tension without electrostatic potential, U is the electrostatic potential and U_{cr} the critical electrostatic potential. Figure 3 shows the evolution of the size in function of the electrostatic potential. Equation 4 and Figure 3 suggest that:

- For very low electrostatic potentials, the size of the droplet is similar to when no electrostatic potential is applied.
- For medium electrostatic potentials, the droplet size decreases quickly with increasing potential.
- As the electrostatic potential approaches its critical value, the droplet size stabilizes towards a minimum value. In fact, at the critical electrostatic potential, the surface tension is null and the liquid leaves the needle as a jet. Thus, we are no longer in the dropping mode, and the droplet size is defined by Raleigh's theory, which is presented in the next section.
- Note that others have used electrostatic pulses instead of a continuous electrostatic potential. This approach is described in chapter 5.

With the electrostatic droplet generator, depending on the design of the system, the manipulator could produce droplets (and then capsules) as small as 50 micrometers in diameter. One may be concerned by the high electrostatic potential applied to droplet. However, it has been shown that even high electrostatic potential does not significantly affect cell survival [17]. Improvements of the reliability of the electrostatic droplet generator and the control of the electrodripping process (size, size distribution of the capsules, productivity …) are still in development, but commercial equipments already exist (as an example, consult www.nisco.ch).

Jet-breaking by a vibrating nozzle (nozzle resonance dripping)

By increasing the liquid flowrate leaving a nozzle, it forms a liquid jet [18] whose velocity can be described by

$$u_j \geq 2\sqrt{\frac{\gamma}{\rho\ d_i}} \tag{5}$$

where u_j is the linear velocity of the liquid leaving the nozzle as a jet, γ is the surface tension of the liquid, ρ is the liquid density and d_i is the internal nozzle diameter. Generally, for the encapsulation of islets, the liquid is an aqueous solution with a fixed surface tension and density. The minimum jet velocity is therefore mainly a function of the nozzle internal diameter.

Equation 5 also suggests that there is a maximum flowrate limit for dropping methods. To scale-up droplet production, one has to use jet-breaking methods.

A capillary liquid jet has a tendency to absorb certain vibrations and break into small droplets [19]. The transition from liquid jet to droplets can be described by the Rayleigh break-up theory [20]. According to this theory, the optimum conditions for droplet formation occur when the jet enters in resonance with an applied vibration (Figure 4), which is defined by [20]:

$$f = \frac{u_j}{\lambda} \quad \text{and} \qquad \lambda = 4.058\ \ d_j \tag{6}$$

where f is frequency of vibration applied to the liquid jet, u_j is the jet linear velocity at the exit of the nozzle, λ is the natural resonant wavelength of the fluid jet and d_j is the jet

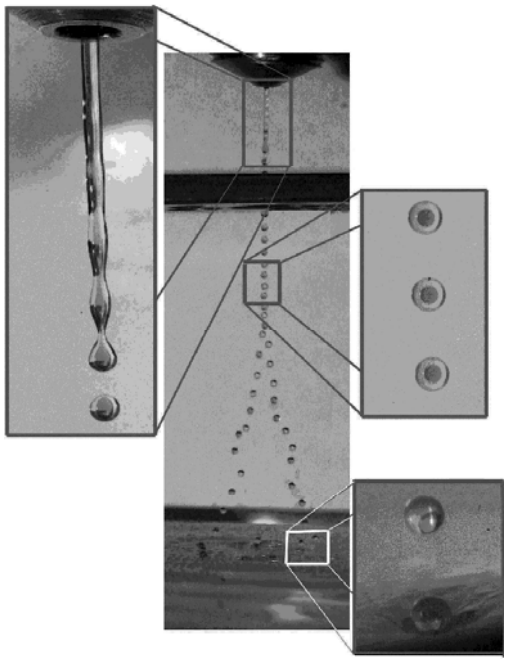

Figure 4. Jet breakage by resonance (Inotech ©).

diameter. The jet breaks in cylinders with a length equal to λ and diameter d_j. The jet linear velocity may be 2 to 5 times the minimum jet velocity (and should be, to get a good flow regime). The flowrate, Q, is then given by the product of the jet cross-sectional area times the jet linear velocity :

$$Q = \left(\frac{\pi d_j^2}{4} \right) u_j \tag{7}$$

The volume of the liquid cylinder formed by breakage of the jet is equal to the volume of resulting droplet. Mathematically, this is represented by :

$$\frac{\pi}{6} d^3 = \frac{\pi}{4} d_j^2 \lambda \tag{8}$$

Combining Equations 6 and 8, and subsequent simplifying, leads to a very simple rule relating the droplet and jet diameters:

$$d = 1.89 \ \ d_j \tag{9}$$

Equation 9 provides a mean droplet diameter because the jet is submitted to many vibrations that, in practice, can cause the formation of smaller or larger droplets. That is, even if the applied vibrational frequency is constant, one is expected to observe a droplet size distribution due to interfering vibrations. However, by isolating the jet from external vibrations and applying a specific vibration, one can obtain quasi mono-dispersed droplets (standard deviation between 5 and 10 % of the mean diameter) [21].

The factor 4.058 in equation 6 is defined for ideal liquids. Viscosity variations (especially for non-Newtonian fluids such as alginate solutions) or the presence of particles (islets) may slightly affect this value. In practice, Schneider and Hendrick [22] observed that uniform droplets could be formed in the range of wavelength defined by:

$$3.5 \ \ d_j < \lambda < 7 \ \ d_j \tag{10}$$

To simplify the production of mono-dispersed capsules, most commercial equipments (www.nisco.ch, www.inotech.ch, www.brace.de) are equipped with diodes that flash at the same frequency as the jet. Setting the system is then easy.

- Select a nozzle to obtain the correct droplet size (in practice the internal diameter of the nozzle should be half of the desired droplet diameter).
- From the data provided by the supplier (or from Equations 6 and 7) define an appropriate flowrate and frequency of vibration.
- Fine-tune the frequency by observing the jet. When the frequency is correct, the droplets appear to be immobile in the diode flashing light.

With such equipment, production rates may be as high as liters per hour for 1 mm diameter beads. However, the production rate decreases proportionally with the square of the droplet diameter (Equation 7). For beads around 250 micrometers in diameter,

productivity is thus limited to a few hundred milliliters per hour, which is nonetheless sufficient for creating a bioartificial pancreas.

The main limitation of the jet-breaking approach to droplet formation is that the liquid viscosity must be lower than 200 mPa·s. For higher viscosity fluids, the breakage could be provoked by a mechanical force (as an example, using a wire to cut through the jet, see www.geniaLab.de) in place of a vibration. This approach is generally not used for medical applications, however, because the mechanical forces tend to injure the living cells to be encapsulated.

Converting droplets to microcapsules

In the previous paragraphs, we described methods that are suitable for producing droplets for the encapsulation of islets of Langerhans. The conversion of these droplets into microcapsules requires a "stabilization" process. This may be based on different processes such as solidification by cooling, jellification, polymerization, coacervation (see below), drying or solvent evaporation. However, for islet encapsulation, ionic jellification (or gelation) and interfacial coacervation are most suitable, since the other methods generally require strong temperature fluctuations, the use of harsh chemicals, or pH ranges that are not compatible with living cells.

Gelation

The most common method of pancreatic islet encapsulation consists of mixing the islets in an alginate solution and dropping this suspension into a calcium chloride solution. An example of a protocol to produce calcium alginate (gel) beads is described as follows:

1. In a beaker, place 2 g of sodium alginate powder
2. Add a small quantity of water and mix with a glass rod to obtain a homogeneous paste
3. Slowly add the rest of water (100 ml) while mixing gently to avoid air incorporation
4. Let stand for overnight to allow complete re-hydration of the alginate
5. Add the islet suspension by gentle mixing
6. Introduce the mixture into the droplet generator
7. Allow droplets to fall into a 50 mM $CaCl_2$ solution agitated gently by a magnetic stirrer
8. Allow droplets to continue gelling in the $CaCl_2$ solution for 30 min
9. Filter the capsules on a 40 micrometer nylon mesh
10. Keep the capsules in adequate buffer solution (avoid phosphate, a chelating agent)

Calcium is sometimes replaced by barium to obtain stronger gel [23]. Other materials have also been proposed to encapsulate the pancreatic islets [24] such as agarose [25], poly(ethylene glycol) [26] or the more exotic chitosan-polyvinyl pyrrolidone [27]. The real future of such materials is yet to be evaluated. However, more than 90 percent of the literature on islet (and more generally cell) encapsulation is based on entrapment in alginate beads. The alginate system has strong advantages over most other methods:

- The surface of the alginate droplets jellify instantaneously upon contact with the calcium ion solution, allowing to obtain smooth and spherical beads. Most other materials would jellify at slower rates.
- Alginate jellification takes place at room temperature (or a little bit higher), at neutral pH, without toxic cross-linkers, without harsh chemical reactions, and at a physiological osmotic pressure and ionic strength. These conditions are compatible with living cells.
- It is one of the most well known systems, both in terms of the jellification process and of materials involved.
- In the case of islet encapsulation, a key point for success is not the encapsulation process itself, but the purity and quality of the materials. Alginate has been thoroughly analyzed and purified. Moreover, some groups have developed alginates with tailored properties and improved biocompatibility using enzymatic modification [28]

However, the domination of the calcium-alginate system over the literature has unfortunately limited the development of other alternatives. If, in the future, alginate beads are discovered not to be the ideal system for cell encapsulation, it is unlikely that some other system will be advanced enough to immediately replace it. Despite its apparent simplicity, producing alginate beads suitable for pancreatic islet transplantation requires a very careful selection of the materials and precise conditions of production [29]. As the purification and the modification of the alginate are probably the key to success, research groups have historically kept their alginate production process a secret [30]. Overall, drawing conclusions from the literature is difficult as there are many differences and discrepancies between published studies. These discrepancies are partially linked to the complexity of the biology involved, but are also the result of the different alginates that are being used for producing the microcapsules.

Coating of hydrogel beads

In general, alginate beads are not expected to offer adequate protection of the islets of Langerhans against the host immune system. That said, some groups do not agree and have reported successful immuno-protection using simple barium-alginate beads with a density gradient [31]. As a strategy to improve the immuno-protective capabilities of the system, alginate beads are more commonly coated with other polymers. This coating acts to provide desirable surface properties, a more selective permeability, and a greater mechanical and chemical stability of the capsule. The initial alginate beads generally serve as the inner core for the complete microcapsule system.

The coating process is relatively simple. Alginate beads (charged negatively due to the carboxyl group of alginate) are suspended in a low concentration (e.g. 0.2 %) solution of a positively charged polymer. Initially, poly-L-lysine was selected as the polycation [1]. Alternatives have since been proposed, including chitosan [32] or poly-L-ornithine [33], but polylysine remains the most commonly applied of them all.

It must be pointed out that the term "coating" is not appropriate as it has been demonstrated that the cationic polymer in fact interpenetrates the alginate gel [34]. The thickness of the polymeric membrane increases with the time of incubation in the polycation solution, and some of the polycation may potentially even reach the center of

the beads [34]. Physico-chemical analyses of the microcapsule surface have also supported the notion that the polycation does not form a distinct layer [35].

Poly-L-lysine (PLL)-coated alginate beads are expected to have a positively charged surface that is suspected to provoke a foreign body reaction. PLL-coated beads are thus re-suspended in a low concentration alginate solution [36]. The process is sometimes repeated to reach optimum membrane properties.

Interfacial coacervation

Probably the main alternative to the alginate bead system is based on interfacial coacervation, also called "polymer complex membrane formation". Developed originally by Dautzenberg in Germany [37], it consists of dropping a polymer solution (cellulose sulfate) into a polymeric solution of opposite charge (poly(dimethyldiallylammonium chloride)). If well selected, the two polymers interact at the droplet interface to form insoluble complexes that are still hydrated, called "coacervates". The coacervates coalesce to form a membrane surrounding the droplet and thusly form the capsules.

Hunkeler has tested more than 1 500 combination of polymers, defining rules to obtain strong and biocompatible capsules [38]. However, most of the research is focused on the coupling of alginate with chitosan [39].

In comparison to alginate beads (whether coated by a polycation or not), microcapsules formed by interfacial coacervation have a more specific molecular cut-off (down to 3000 Daltons) [40]. Authors reported that even though some polymers may independently have some cytotoxicity, the formed membrane shows high biocompatibility [41]. Interfacial coacervation allows the formation of a capsule in one step, while coated beads may require many steps (particularly in consideration of the washing steps between the coating steps). However, it is a more delicate technology to drive.

Co-extrusion

The co-extrusion technology consists of concentrically extruding two liquids as droplets then solidifying the external layer (Figure 6). This approach for encapsulation has been tested for mammalian cells and is of interest for islet encapsulation [42]. This technology allows a lot of freedom in selecting the membrane material (even if the polymer or its solvent could have some toxicity) [43].

Even if the principle is simple, optimizing such a system is complex. To obtain nice, spherical, core-centered capsules as presented in Figure 6, one has to consider the viscosity of each phase, their surface tension, their flow rates, the size of the concentric nozzles, the breaking process, and the properties of the collecting solution. The most advanced system of this kind is currently based on the nozzle resonance method [44]. The main limitation in applying this method to the encapsulation of islets is the size of the capsules (which actually cannot be smaller than 500 µm diameter).

Coated islets

To minimize the transplant volume, a coating may be deposited or grafted directly onto the islets. The simplest solution is to suspend the islets in a reactive solution. Polyethylene glycol (PEG) derivatives are generally used as the grafting material as

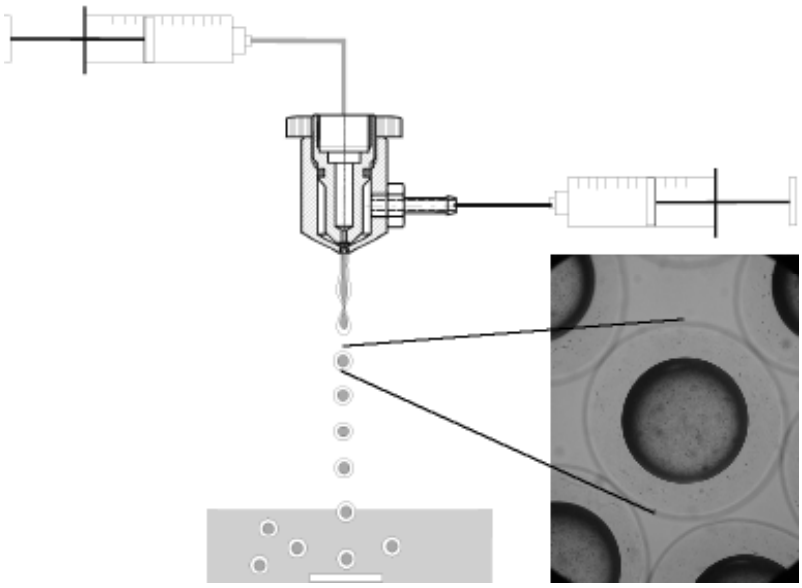

Figure 6. Coextrusion process (Capsulae © adapted from Inotech ©).

PEGacylation has been shown to mask antigens from host antibodies [45]. PEG is fixed by direct covalent binding onto the cell surface amino groups or by insertion of a PEG-lipid or PEG-carbohydrate into the cell membrane [46].

An alternative is to absorb onto the islet surface a catalyst (i.e. eosin Y) and to suspend the islets in a monomer solution (i.e. PEG-diacrylate), then initiating photo polymerization of the monomer solution by light illumination [47]. Despite the need of a very careful optimization of the process, it is reported that cell viability can be greater than 90 % [48] and this technology is currently being evaluated for phase I/II clinical trial for human islet encapsulation (Novocell, Inc.).

Islets may alternatively be suspended in the top of a centrifugation tube filled with an alginate solution that is layered on top of a dextran calcium solution. During centrifugation, islets cross the tube while dragging a thin layer of alginate around them, which jellifies while entering in contact with the calcium solution [9]. By such technology, a coating layer as thin as 5 to 10 micrometers may be formed [10].

Conclusions

There are other alternatives to islet or cell encapsulation than the above-described methods. Many of them have been tested by a limited number of authors, while other will likely never be developed. However, it is important that researchers do not focus on too limited a number of methods. Considering the complexity of fulfilling all criteria necessary to succeed (biocompatibility, islet viability, vascularization of the capsule surrounding, mass transfer…), no one to this date could pretend to hold the optimum technology. Similarly, no one could pretend that a unique technology will be adequate for

all cases. Technologies may even need to be alternated over the period of therapy in order to avoid long-term body reaction.

The path has been long since 1980 when the first publication on islet encapsulation was published and, most probably, will continue to be long, in terms of the scale of the challenge before us, and the medical and social impact of diabetes. Treating this illness with a cell encapsulation system will open the door to many other medical applications. The main risk is that funding associations (public or private) will limit their effort to support this research in regard to the length of time required for its development.

References

1. Lim, F., and Sun, A.M. 1980. Science, 210, 908.
2. Canaple, L., Rehor, A., and Hunkeler, D. 2002, J. Biomater. Sci. Polym. Ed., 13, 783.
3. Chaikof, E.L. 1999, Annu. Rev. Biomed. Eng., 1, 103.
4. De Vos, P., Vegter, D., De Haan, B.J., Strubbe, J.H., Bruggink, J.E., and Van Schilfgaarde, R. 1996, Diabetes 45, 1102.
5. Dulong, J.L., and Legallais, C. 2007, Biotechnol. Bioeng., 96, 990.
6. Leblond, F.A., Simard, G., Henley, N., Rocheleau, B., Huet, P.M., and Hallé, J.P. 1999, Cell Transplant., 8, 327.
7. de Vos, P., De Haan, B., Pater, J., and van Schilfgaarde, R. 1996, Transplantation, 62, 893.
8. Poncelet, D., Poncelet, B., Beaulieu, C. and Neufeld, R.J. 1993, Fundamentals of Animal Cell Encapsulation, M.F.A. Goosen (Ed.) CRC Press, Boca Raton, 113.
9. Zekorn, T., Siebers, U., Horcher, A., Schnettler, R., Zimmermann U., Bretzel, R.G., and Federlin, K. 1992, Actia Diabetol., 29, 41.
10. Park, Y.G., Iwata, H., and Ikada, Y. 1999, Polym. Adv. Technol., 9, 734.
11. Wilson, J.T., and Chaikof, E.L. 2008, Adv. Drug. Deliv. Rev., 60, 124.
12. Poncelet, D. 2008, Science and Applications of Skin Delivery Systems, J.W. Wiechers (Ed.) Allured Publishing Corporation, Carol Stream, IL, USA, 251.
13. Harkins, W.D., and Brown, F.E. 1919, J. Am. Chem. Soc., 41, 499.
14. Sugiura, S., Oda, T. Aoyagi, Y., Matsuo, R., Enomoto, T., Matsumoto, K., Nakamura, T., Satake, M., Ochiai, A., Ohkohchi, N. and Nakajima, M. 2007, Biomed. Microdev., 9, 91.
15. Poncelet, D., Babak, V.G., Neufeld, R.J., Goosen, M.F.A., and Burgarski, B. 1999, Adv. Colloid Interf. Sci., 79(2-3), 213.
16. Bugarski, B., Qiangliang, L., Goosen, M.F.A., Poncelet, D., Neufeld, R.J. and Vunjak, G. 1994, AIChE Journal, 40(6), 1026.
17. Pjanovic, R., Goosen, M.F.A., Nedovic, V., and Bugarski, B. 2000, Minerva Biotecnol., 12, 24.
18. Lindblad, N.R., and Schneider, J.M. 1965, J. Sci. Instrum., 42, 635.
19. Savart, F. 1833, Ann. Chim., 53, 337.
20. Rayleigh, J.W.S. 1878, Proc. London Math. Soc., 10, 4.
21. Serp, D., Cantana, E., Heinzen, C., von Stockar, U., and Marison, I.W. 2000, Biotechnol. Bioeng., 70, 41.
22. Schneider, J.M., and Hendricks, C.D. 1964, Rev. Sci. Instrum., 35, 1349.
23. Tuch, B., Vaithilingam, V., Williams, L., Keogh, G., Lui, S., Foster, J., Williams, G., Chen, K., Jayadev V., and Phillips R. 2007, Xenotransplant., 14, 475.
24. Li, R.H. 1998, Adv. Drug. Deliv. Rev., 33, 87.
25. Kai-Chiang, Y., Ching-Yao, Y., Chang-Chin, W., Tzong-Fu, K., and Feng-Huei, L. 2007, Biotechnol. Bioeng., 98, 1288.
26. Kizilel, S., Wyman, J.L., Zhao, P., Nothias, J.M., Shah, M., Nagel, S., Mrksich, M., and Garfinkel, M.R. 2007, Xenotransplant., 14, 547.

27. Risbud, M., Hardikar, A., and Bhonde, R. 2000, Cell Transplant., 9, 25.
28. Strand, B.L., Mørch, Y.A., Syvertsen, K.R., Espevik, T., and Skjåk-Braek, G. 2003, J. Biomed. Mater. Res. A., 64, 540.
29. King, A., Andersson, A., Strand, B.L., Lau, J., Skjak-Braek, G., and Sandler, S. 2003, Transplantation, 76, 275.
30. de Vos, P., Faas, M.M., Strand, B., and Calafiore, R. 2006, Biomaterials, 32, 5603.
31. Duvivier-Kali, V.F., Omer, A., Parent, R.J., O'Neil, J.J., and Weir, G.C. 2001, Diabetes, 50, 1698.
32. Gaserod, O., Sannes, A., and Skjak-Braek, G. 1999, Biomaterials, 20, 773.
33. Darrabie, M.D., Kendall, W.F., and Opara, E.C. 2005, Biomaterials, 26, 6846.
34. Gaserod, O., Smidsrod, O., and Skjak-Braek, G. 1998, Biomaterials, 19, 1815.
35. Tam, S.K., Dusseault, J., Polizu, S., Ménard, M., Hallé, J.P., and Yahia, L'H. 2005, Biomaterials, 26, 6950.
36. Schneider, S., Feilen, P.J., Slotty, V., Kampfner, D., Preuss, S., Berger S., Beyer J., and Pommersheim R. 2001, Biomaterials, 22, 1961.
37. Braun, K., Besch, W., Jahr, H., Loth, F., Dautzenberg, H., and Hahn, H.J. 1985, Biomed Biochim Acta, 44, 143.
38. Hunkeler, D. 1997, Trends Polym. Sci., 5, 286.
39. Bartkowiak, A., and Brylak, W., 2006, Polimery, 51, 547.
40. Mansfeld, J., Förster, M., Schellenberger, A., and Dautzenberg, H. 1991, Enz. Microb. Technol., 13, 240.
41. Angelova, N., and Hunkeler, D. 1999, Trends Biotechnol., 17, 409.
42. Crooks, C.A., Douglas, J.A., Broughton, R.L., and Sefton, M.V. 1990, J. Biomed. Mater. Res. A., 24, 1241.
43. Stevenson, W.T.K., Evangelista, R.A., Sugamori, M.E., Sefton, M.V. 1988, Biomat. Artif. Cells Artif. Organs, 16, 747.
44. Wyss, A., von Stockar, U., and Marison, I.W. 2004, Biotechnol. Bioeng., 86, 563.
45. Scott, M.D., Murad, K.L., Koumpouras, F., Talbot, M., and Eaton, J.W. 1997, Proc. Natl. Acad. Sci. USA, 94, 7566.
46. Kellam, B., De Bank P.A., and Shakesheff, K.M. 2003, Chem. Soc. Rev., 32, 327.
47. Cruise, G.M., Hegre, O.D., Lamberti, F.V., Hager, S.R., Hill, R., Scharp, D.S., and Hubbell, J.A. 1999, Cell Transplant., 8, 293.
48. Cruise, G.M., Hegre, O.D., Scharp, D.S., and Hubbell, J.A. 1998, Biotechnol. Bioeng., 57, 655.