Cell encapsulation in inorganic polymers: recent achievements, future challenges

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INTRODUCTION

The sol-gel process relies on a polymerization reaction that starts from an inorganic precursor and leads to a metal oxide gel (Brinker C.J. 1990). Because this process can be performed at room temperature, in aqueous solvents and near neutral pH, it is now recognized as a useful alternative to polymer and biopolymer-based encapsulation strategy for proteins, enzymes and antibodies (Avnir D. 2006).

Although the encapsulation of living cells within sol-gel matrices has been discovered about 20 years ago, it has been much less explored compared to enzyme entrapment. This is because living organisms are usually much more sensitive to their environment, both during the synthesis of the matrix and upon ageing. Several strategies have been developed in order to improve the compatibility of the sol-gel reactions and materials with the long-term preservation of cellular activity (Livage J. 2006). They could be used for the encapsulation of yeasts, bacteria, algae and even animal cells (Böttcher H. 2006). Applications in bioreactor and biosensor design have been reported (Carturan G. 2006). The variety of inorganic materials that can be made compatible with living cells has also been recently extended (Amoura M. 2009b).

Despite these achievements, several challenges remain, both from a fundamental point of view and in term of material properties before the sol-gel process can be fully considered as a useful alternative to the current (bio)-polymer based technology.

SOL-GEL CHEMISTRY AND ITS APPLICATION TO CELL ENCAPSULATION

The conventional inorganic sol-gel reaction starts from a metal-organic precursor termed alkoxide of general formula $M(OR)_n$ where M is an alkaline, alkaline earth, semi-metal or metal atom and R is an alkyl chain, usually CH_3 or C_2H_5. When put in contact with water, this molecule undertakes a *hydrolysis* reaction converting the M-OR bond into a M-OH bond and releasing the parent ROH alcohol. Then a *condensation* reaction can occur, mainly between two hydrolyzed precursors, leading to the formation of a M-O-M metal oxide bond. Depending on the nature of M, the condensation reaction may continue until sub-micrometer size particles (sol) are formed. As a function of pH and ionic strength, growing particles can interact with each other leading to percolation (gel) or aggregation (precipitate). At the end of the process, hydrated metal oxide $M_nO_m.xH_2O$ materials are obtained that can be shaped as monoliths, fibers, coatings, thick films, micro- and nanospheres,..Overall, this technology is very similar to the organic polymer science. The main difference is that the material is inorganic, thus it usually present enhanced chemical, thermal and mechanical stability. Moreover, it is usually not biodegradable. Finally, it can exhibit specific properties (conductivity, magnetism) that can be used for building functional devices.

However, when trying to apply this technology to cell encapsulation, a major limit was soon discovered: during the hydrolysis step, ROH alcohol molecules are produced that may be harmful to living organisms. This problem can be overcome in several ways: evaporation/distillation of

released alcohol from the solution before cell addition and gel formation, deposition of films exhibiting a high contact surface with air to obtain rapid alcohol evaporation, pre-encapsulation of the living organisms in a biopolymer hosts or preparation of novel precursors $Si(OR)_4$ with R being a biocompatible alcohol (glycerol).

Another possible approach is to avoid metal organic precursors and to use only mineral (inorganic) precursors $M(OH)_n^{z+}$. In this case, the sol-gel process occurs only *via* condensation and can be performed in the absence of organic solvents or by-products. However, two new problems arise. First, the condensation reaction of inorganic precursors in water is often very difficult to control, strongly depending on pH and ionic strength. In other words, it is not always possible to form sol-gel materials from inorganic precursors near neutral pH and in suitable salinity conditions. Second, these precursors may show severe toxicity towards living cells. As an alternative, it is possible to set up a colloidal route to gel formation, *i.e*. to proceed in two steps consisting of i) synthesis of a sol of nanoparticles and ii) gel formation in the presence of the cell suspension. The advantage is that particles can be prepared in whatever conditions are required and direct contact between the cells and the inorganic ions is avoided. However, this approach is successful only if i) the cell culture medium allows sol gelation (and not aggregation or re-dispersion) and ii) if the colloids does not show detrimental interactions with cells, both aspects being related to the surface chemistry of particles. Until now, this approach was successful for the encapsulation of bacterial cells in silica, aluminium and iron oxide materials but failed with zirconium-based gels (Nassif N. 2004, Amoura M. 2007, Amoura M. 2009a, Amoura M. 2009b).

LONG-TERM VIABILITY OF ENTRAPPED CELLS

The above-presented strategies were developed to improve the compatibility of sol-gel chemical reaction towards cell encapsulation at the time of gel formation or a few hours/days after. However, when considering long-term viability, then both the physics of the material and the biology of the cells have to be considered. From the physical point of view, two main factors are relevant: the interaction between the cell wall and the gel internal surface, the shrinkage of the gel porosity upon ageing. In the latter case, the evolution of the gel network upon drying should also be considered. Considering the biological aspect, it is usually considered that cells cannot divide inside the gel because the porosity is too small and also because, in contrast to biopolymers, the mineral host is, to one exception, not biodegradable. Thus, in a way or the other, cells have to adapt this situation and continue to survive without division.

Considering the problem of shrinkage and drying, it has been shown that sugars or polyols allow to limit porosity decrease and to maintain sufficient humidity (Fiedler D. 2007). Another very interesting approach use phospholipids. These molecules self-organize as lipid bilayers that surround the cells and thus provide a suitable interface between the cell surface and the inorganic material. Moreover, these bilayers appear to confine water inside the cells, allowing preservation of biological activity even under vacuum (Baca H.K. 2006). Alternatively, glycerol addition to inorganic gels allows the preservation of bacterial cells within sol-gel materials over month period (Nassif N. 2004). Glycerol was also used as an additive during a freeze-gelation process where the sol-gel transition is triggered by freeze-casting on a – 40°C metal plate (Böttcher H. 2006). In this case, the cell suspension is added to a sol and the resulting mixture freeze-casted on a – 40°C metal plate. In this case, the role of glycerol may be several: cryo-protective agent, interface between cell wall and mineral surface, porogen, moisture preservative, not mentioning any possible biological effect.

Looking at the biology, studies performed on silica-entrapped *E. coli* bacteria suggested that they enter a kind of dormant physiological state that allow them to adapt their metabolism to the impossibility of division. Indeed, such behavior of cells is usually related to inter-cell communication within a given population. However, within inorganic gels, bacteria are isolated one from another so that the exchange of chemical signals (quorum sensing) becomes difficult. In this case, it is possible to add quorum sensing molecules to the gel, leading to an enhanced survival of the entrapped bacteria over time (Nassif N. 2004).

However, in most cases, the viability of entrapped cells has not been monitored over a one-month period. Reports on islets of Langerhans encapsulated in silica-coated alginate beads showed preserved biological activity over several months but in this case, the inorganic material is not the main constituent of the cell environment (Carturan G. 2006). In the case of sporulating organisms, it was demonstrated that entrapped spores could be stored for several weeks after which they could be used for further germination (Böttcher H. 2006).

TOWARDS BIOTECHNOLOGICAL APPLICATIONS

The targeted applications of cell encapsulation in sol-gel materials are not different from the conventional (bio)-polymer based technology. Cell-based biosensors and bioreactors, bioremediation materials and even artificial organs have been developed that involve sol-gel chemistry (Böttcher H. 2006, Carturan G. 2006, Livage J. 2006)

Noticeably, in the recent years, many groups have studied the encapsulation of photosynthetic organisms, either plant cells, micro-algae or cyanobacteria (Dickson D.J. 2009, Fiedler D. 2007, Gautier C. 2006, Meunier C.F. 2009, Perullini M. 2005). The aim of these studies was related to the production of specific molecules or for the design of materials for alternative energy source (photobioreactors, biofuel cells). In the former case, it was shown that the sol-gel host could sustain the extraction process involving organic solvents that is usually required to recover the metabolite of interest. In the latter case, the main advantage of the inorganic material, especially silica, is that it is completely transparent and allows efficient access of light to the organisms while constituting an efficient mechanical protection.

Despite the increasing number and variety of living organisms that have been encapsulated in sol-gel inorganic materials, large-scale industrial applications still appear out of reach. Several challenges remain, including i) the absence of suitable process to form silica beads similar to polymer ones in conditions that are compatible with cell encapsulation, ii) the absence of cell division inside the inorganic networks which limit their use as bioreactors, iii) the poor knowledge of cell/mineral interfacial interactions. Another key limitation is that the sol-gel technology is still not well-known in the academic and industrial biotechnology community.

CONCLUSION

Indeed, further developments of sol-gel encapsulation will only become possible if this technology present some strong advantages over the current one. In this context, the set-up of novel processes that allow the entrapment of cells in functional inorganic materials that show magnetic or conductivity properties and the possibility to create some synergy between the biological activity

and the material properties to build “living” materials is one of the most promising approach in this field

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