Hydrogel microspheres for cell immobilization: Approaches to improve stability and biocompatibility

R. Mahou[1] and C. Wandrey[*]
Ecole Polytechnique Fédérale de Lausanne
Laboratoire de Médecine Régénérative et de Pharmacobiologie
CH-1015 Lausanne, Suisse
redouan.mahou@epfl.ch

Introduction

Considerable progress in the field of cell immobilization/encapsulation biotechnology has been made during recent years as a result of extensive research. Immobilization of living cells has advanced to a multidisciplinary topic with an impact on many fields, especially in pharmacology and biomedicine. In fact, there are several biomedical applications where the transplantation of immobilized cells is being employed to restore, maintain or improve tissue functions. Nowadays it is indisputable that many other biotechnological processes could benefit from this technology. Cell encapsulation techniques are based on the principle that a semi-permeable membrane protects entrapped cells from the host immune system. The membrane allows nutrients, waste, and therapeutic products to pass freely, but serves as a barrier to the diffusion of antibodies and cytokines. Despite the simplicity of the principle, progress in the fields of immunoisolation has been hampering for several reasons, in particular due to difficulties concerning the maintenance of cell viability, insufficient stability of the capsules, and biocompatibility issues.

Microencapsulation in alginate-based hydrogels, as originally described by Lim and Sun (Lim et al, 1980), is the most commonly applied procedure for cell immunoisolation. During recent years, important advances have been made with this technology. Alginate (Alg) is a linear biopolymer formed by guluronic (G) and mannuronic (M) acid units. In nature, the monomers are arranged in blocks of MM, GG, and MG along the chain. Alginate with specific structures can also be produced by enzymatic modification using mannuronan C-5 epimerases. Alg provides many advantages over other materials. It is one of the few materials that allow processing under physiological conditions. It has been shown that alginate did not interfere with the functions of the entrapped cells. Another observation was that Alg-based hydrogels provide an adequate microenvironment for novel cell regeneration (Finegood et al, 1995). Nevertheless, Alg-based microspheres and capsules frequently suffer from mechanical stability deficiency, bioincompatibility problems, and permeability drawbacks, and some studies shown that microcapsules do not completely prevent diffusion of antibodies and cytokines. To overcome these problems, the most utilized approach is the subsequent coating of the initially formed Alg beads.

During the last decade, Alg/poly(L-lysine) capsules were the most commonly and extensively studied system. The capsules comprise three main components: a core material of ionically gelled Alg, surrounded by a polyanion-polycation complex semi-permeable membrane, and sometimes an additional outer coating of polyanions. The functionality of the additional coating is to neutralize non-reacted polycation, and thus mask undesirable immune responses to the polycation. It has been shown that long-term stability can be reached either by controlling the swelling behavior of the capsules, or by increasing the membrane strength. Further was shown that the mechanical stability is directly correlated with the type of the applied material, since Alg-based capsules formed from high-G Alg are more durable (Thu et al, 1996). Some other authors reported that the use of high-G Alg is associated with more extensive inflammatory responses than intermediate-G. This was

explained by different affinity and binding properties of poly(L-lysine) (PLL) to Alg having different G/M ratio. Other drawback of this coating system is the use of PLL, which can cause fibrosis as it was shown when comparing the biocompatibility of high-G Alg beads and high-G Alg capsule prepared with PLL (Strand et al, 2001).

To overcome the biocompatibility and mechanical stability drawbacks, we envision two strategies. The first one is to use Alg beads as a template for the formation of a covalently bond polyethylene glycol (PEG) hydrogel. Good biocompatibility was demonstrated for such PEG hydrogels. Numerous papers reported PEG gels for drug delivery devices (West et al, 1995; Scott et al, 1999), for islet encapsulation (Sawhney et al, 1993), to prevent adhesions and inhibit thrombosis after surgery, and as biocompatible surface treatment for cell adhesion-resistant surfaces (Drumheller et al, 1995). The second strategy addresses functionalizing of the raw material (Alg) prior to capsule formation or coating.

Material and Methods

Starting material: Alg beads were prepared by the extrusion method, using the coaxial air-flow droplets generator. PEG-vinylsulfone was synthesized adapting a previous protocol (Lutolf et al, 2003). Briefly, multiarm PEG (Shearwater Polymers, Huntsville, AL) was dried by azeotropic distillation in toluene (VWR, Nyon, Switzerland), divinylsulfone (Fluka, Buchs, Switzerland) was added in excess in presence of sodium hydride (Sigma–Aldrich, Buchs). The degree of PEG functionalization with vinylsulfone was determined by proton NMR spectroscopy (in $CDCl_3$) using a Bruker 400 spectrometer (Bruker BioSpin, Faellanden, Switzerland). Characteristic peaks were observed at 6.1, 6.4, and 6.8 ppm. The degree of end group conversion was found to be ≈ 97 %. Gel permeation chromatography was used to confirm that the PEG-OH and PEG-VS have identical molar mass distribution. The polymer was stored under argon at -20 °C until use.

Alginate dissolution by enzymatic degradation: To check the degradation of gelled Alg, beads were submerged in triethanolamine (TEA) solution (0.05 M, pH= 8) in a ratio of 10 mL/g of beads. Alginate Lyase (Sigma–Aldrich, Buchs, Switzerland) was added to the suspension to a concentration 1mg/mL. The degradation was followed by microscopy

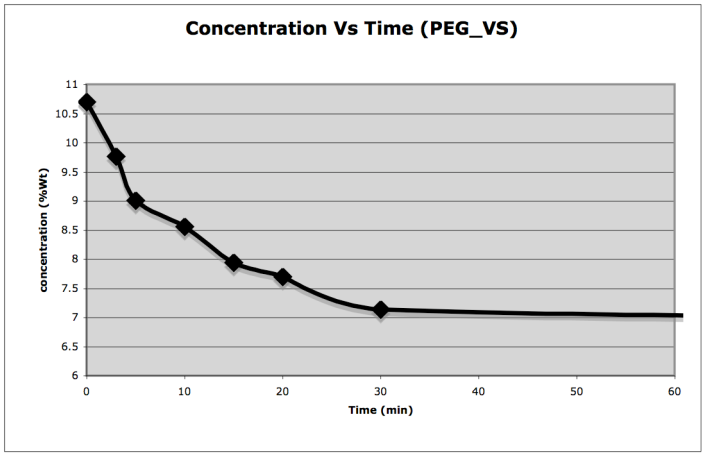
Alginate degradation by repeating washing: Ionic gelling of Alg and divalent cations is an equilibrium process. In presence of sufficient monovalent salt, the hydrogel is expected to swell until total dissolution. To study this process, Ba-Alg beads were submerged in a solution of 0.9% w/v NaCl in TEA (0.05 M pH= 8). The suspension was stored at 5 °C over night. Subsequently, the bead diameters were controlled, and the supernatant was replaced by a freshly prepared solution. The diameters were measured and solution replaced daily until total dissolution of the beads.

PEG-Alg bead formation: Two different methods were used to form PEG-Alg hydrogels. All reactions were performed using an aqueous solution of TEA (0.05 M, pH= 8).
Method 1: Beads were prepared by atomizing the solution of 1.5 wt % of high viscosity high-M Alg (Keltone HV, Chemical Company, San Diego, USA). Droplets were gelled during 5 minutes at room temperature in a gelation bath containing 2.2 wt % $CaCl_2$ (or 1.5 wt % $BaCl_2$). Size distributions were determined using an optical microscope connected to a digital camera. Samples of thirty beads were withdrawn and measured. Average diameters (D) and standard deviations (SD) were evaluated for a given size distribution. Satisfactory results were obtained ($SD \leq 4\%$). Beads were washed with 0.9 wt % NaCl solution, and submerged in a solution of PEG-VS. The concentration change was followed by refractive index measurement, and the exact amount of the

cross-linker was then calculated before its addition to beads. PEG-Alg beads were collected by filtration, washed by 0.9% wt NaCl solution, and stored in their washing solution.
Method 2: PEG-VS was directly mixed with a solution of Alg, and extruded in a gelation bath as described for method 1. The bath also contained the cross-linker for PEG hydrogel formation during 30 min at room temperature.

Results and Discussion

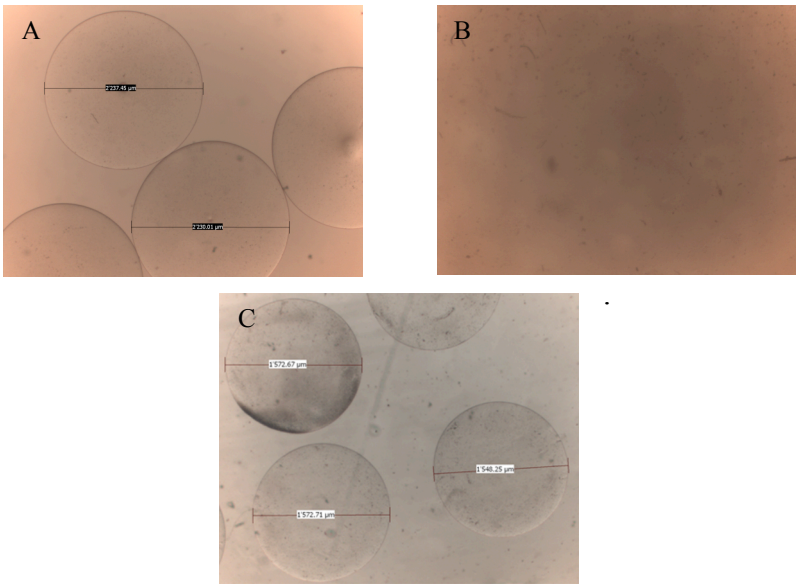
First strategy. Despite the complexity of method 1 (multi-steps, refractometer calibration), a good estimation of the concentration change of PEG-VS was possible using refractive index measurements (Figure 1). Method 2 was shown to be easier and less time consuming. Nevertheless, sperical PEG beads, with uniform dispersity were formed by both methods. The dissolution of Alg beads by enzymatic degradation was completed after 12 hours in alginate lyase solution. (Figure 2).



t

Figure 1:

Concentration change of PEG-VS**.**
The diffusion of PEG-VS into the Alg-beads was controlled by measuring the PEG-VS concentration in the supernatant.



.

Figure 2:

A): Ba-Alginate beads before degradation. B) Ba-Alginate after 12 hours in alginate lyase solution. C) PEG-Alginate capsules after 12 hours in alginate lyase solution**.**

Second strategy: Sodium Alginate could be modified first by grafting of side chains with desired functionality. After capsule formation, the side chains could be cross-liked in order to reinforce the capsules and to control the permeability (Figure 3).

.

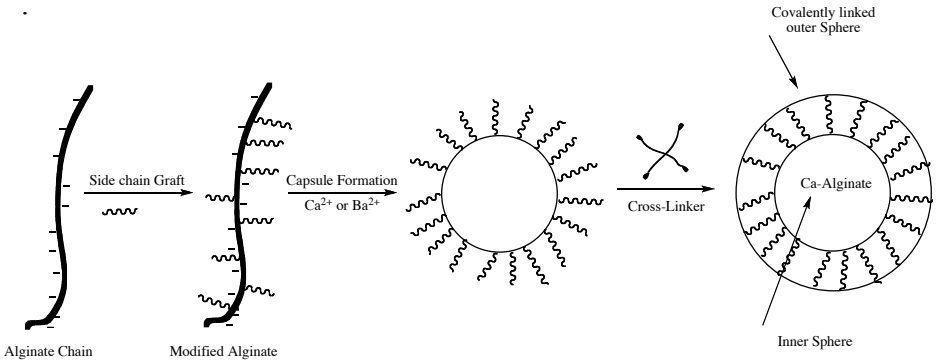


Figure 3:

Schematic representation of the second strategy

Conclusions

At this stage of research, the conclusions are tentative. Nevertheless, novel spherical microbeads, with well-controlled size could be formed by the methods presented herein, avoiding the use of polycations. Further studies are in progress.

References.

- Drumheller, P et al. (1995) *Densely crosslinked polymer networks of poly(ethylene glycol) in trimethylolpropane triacrylate for cell-adhesion-resistant surfaces* J Biomed Mater Res. 29(2) 207–215.

- Finegood, D.T et al. (1995) *Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model*. Diabetes 44(3) 249-256.

- Lim, F et al. (1980) *Microencapsulated islets as bioartificial endocrine pancreas*. Sciences. 210, 908-910.

- Sawhney, A et al. (1993) *Interfacial photopolymerization of poly(ethylene glycol)-based hydrogels upon alginate-poly(l-lysine) microcapsules for enhanced biocompatibility* Biomaterials 14(13) 1008–1016.

- Scott, R. A et al. (1999). *Highly crosslinked, PEG-containing copolymers for sustained solute delivery*. Biomaterials 20(15) 1371–1380.

- Strand, B. K et al. (2001) *Poly-L-Lysine Induces Fibrosis on Alginate Microcapsules via the Induction of Cytokines*. Cell transplantation. 10(3) 263-275.

- Thu, B et al. (1996). *Alginate polycation microcapsules: II. Some functional properties*. Biomaterials 17(11) 1069-1079.

- West, J et al. (1995). *Photopolymerized hydrogel materials for drug delivery applications*. Reactive Polymers 25(2): 139–147.