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Development and optimisation of alginate-PMCG-alginate microcapsules for cell immobilisation

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Abstract

Mechanical stability, uniformity of size, complete encapsulation of cells and optimal microenvironment are major challenges in the design and development of microcapsules for cell immobilisation purposes. In this work, a novel microcapsule chemistry based on polyelectrolyte complexation between alginate and poly(methylene-co-guanidine) (PMCG) is presented. We have characterised the effect of PMCG concentration and time of exposure on microcapsule diameter and membrane thickness, selecting a PMCG concentration of 0.5% (v/v) and an exposure time of 1 min as optimal parameters for a correct coating. Afterwards, the mechanically most resistant alginate-PMCG-alginate (A-PMCG-A) microcapsule type was chosen according to two different stability studies. Beads with a solid core and an inhomogeneous internal configuration resulted in stronger microcapsules. Further, the selected A-PMCG-A beads presented both an increased stability compared to classical Ca^{2+}/alginate and alginate-poly-L-lysine-alginate (APA) microcapsules, and had an adequate microenvironment for cell viability. This new chemistry allows the controlled adjustment of microcapsule size and wall thickness, offering new alternatives for cell transplantation.

Keywords: Microcapsule; Alginate; Mechanical stability; Cell encapsulation

1. Introduction

Cell encapsulation aims to entrap viable cells within immobilisation devices surrounded by semipermeable membranes. The membranes are expected to be permeable to molecules essential for cell survival such as nutrients, oxygen and growth factors, but not to allow inward diffusion of molecules larger than a desired critical size. In fact, the access of host immunomodulators should be physically prevented. In the last two decades, the alginate-poly-L-lysine (PLL)-alginate (APA) microcapsules, pioneered by Lim and Sun (1980), have been the most frequently employed devices to transplant biomaterials to the host in the absence of immunosuppression. These microcapsules have been employed for various applications including the development of a bioartificial pancreas (Soon-Shiong et al., 1993, 1994) and the delivery of therapeutic gene products such as factor IX for haemophilia (Hortelano et al., 1999), nerve growth factor for central nervous system injuries (Maysinger et al., 1993), the inducible nitric oxide synthase for tumor suppression (Xu et al., 2002) or vascular endothelial growth factor for vascular regeneration (Springer et al., 2000). However, the long-term in vivo success of APA microcapsules has been limited

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principally due to the mechanical fragility of the membrane complex and to the difficult handling, high cost, and immunogenicity and cytotoxicity of PLL (Strand et al., 2001).

The principal factors directly affecting the future development of successful cell microencapsulation devices in terms of stability are: (i) careful selection of polymers and polycations and stringent control on the whole capsule making procedure and (ii) precise and reproducible assays to evaluate the membrane strength of the microcapsules. Considering these two main points, we have developed and optimised new microcapsules employing a guluronic acid rich alginate and poly(methylene-co-guanidine) (PMCG), a cationic methylol amid as polycation, with the aim of improving the uniformity of size of beads, wall thickness, and mechanical stability of the devices for cell immobilisation.

In the design of these novel capsules, we have chosen purified guluronic acid rich alginates, as they posses a lower impurity content and avoid an antibody response (Orive et al., 2002a). Further, these alginates form rigid gels, as divalent ions such as Ca^{2+} bind preferentially to G blocks in a highly preferential manner (Smidsrød and Skjåk-Bræk, 1990; Draget and Skjåk-Bræk, 1997). Regarding the polycation, we have selected PMCG which binds to alginate polymer leading to the formation of a semipermeable membrane on the surface of the microcapsule. The polyelectrolyte complexation between alginate and PMCG has multiple advantages such as short reaction and processing time, thereby providing a high microcapsule production rate, a well controlled simple production method, and good mechanical properties (Canaple et al., 2001).

The present paper reports on new alginate-PMCG-alginate (A-PMCG-A) microcapsules processed by an electrostatic droplet generator to control the uniformity of size and smoothness of the beads. Initially, the effect of PMCG concentration and time of exposure on microcapsule diameter and membrane thickness was studied. Once elaboration parameters were selected, eight different microcapsules (inhomogeneous, homogeneous, solid, liquefied, empty and cell-loaded with C2C12 myoblasts) were produced and their mechanical stability properties were evaluated. Two different mechanical stability studies were optimised to analyse the swelling pressure resistance and compression resistance of the devices. Moreover, stability properties of the selected A-PMCG-A beads were compared to Ca^{2+}/alginate, Ba^{2+}/alginate, APA and barium alginate-PLL-alginate (BPA) microcapsules. Finally, another cell line (RINm5F cells) was immobilised in the selected A-PMCG-A beads with the aim of studying its metabolic behaviour and thereby evaluating the microenvironment conditions of the novel A-PMCG-A beads.

2. Materials and methods

2.1. Cells and materials

RINm5F cells were obtained from the Animal Cell Culture Department (CIB, CSIC, Madrid, Spain) and were cultured in a growth medium consisting of Dulbecco's modified essential medium (DMEM) containing 10% calf serum and 1% antibiotic/antimycotic solution. C2C12 myoblasts were kindly provided by the Department of Biochemistry and Molecular biology of UAB (Spain) and cultured under standard conditions as described elsewhere (Hortelano et al., 1996). Cultures were maintained at 37 °C in a humidified 5% CO_2/95% air atmosphere and passaged every 2–3 days. All reagents were bought from Gibco BRL (Invitrogen S.A., Spain).

Purified low molecular weight, high guluronic acid content alginate extracted from stipes of *Laminaria hyperborea* was purchased from Pronova Biomedical (Oslo, Norway). PMCG was obtained from Scientific polymer products, Inc (Ontario, NY). PLL (Mw: 29,300) as well as the rest of chemicals were obtained from Sigma (St Louis, MO, USA).

2.2. Microcapsule elaboration

2.2.1. Alginate-PMCG-alginate microcapsules

Eight types of A-PMCG-A microcapsules were prepared for this work modifying the structural properties of the immobilisation devices: inhomogeneous-empty microcapsules both solid (IES) and liquefied (IEL) and homogeneous-empty beads solid (HES) and liquefied (HEL). The surface of microcapsules with an inhomogeneous core has a higher alginate concentration than that of homogeneous microcapsules. On the other hand, solid capsules, in contrast to the de-gelled liquefied system have the most solid part bordering the polyanion–polycation membrane (Thu et al.,

1996b). Finally, another set of microcapsules was prepared enclosing C2C12 cells (C) within the beads (ICS), (ICL), (HCS) and (HCL).

Microcapsules were prepared at room temperature employing an electrostatic droplet generator. By applying an electrostatic potential between the droplet generator device and the collecting solution, it is possible to obtain controlled size droplets in the range of micrometers (Poncelet et al., 1999). Briefly, cell-loaded solid beads were prepared suspending C2C12 myoblasts in 1.7% sodium alginate solution at a density of 2.5×10^6 cells/ml. The cell–gel suspension (only gel solution in the case of empty beads) was connected to a 0.4 mm needle using a 22 cm length plastic tube from a peristaltic pump and extruded into 0.05 M solution of $CaCl_2$ (for inhomogeneous beads) or 0.05 M $CaCl_2$ + 0.9 wt.% NaCl solution (for homogeneous beads). Once recovered, alginate droplets were coated with PMCG and added to a 0.1 wt.% solution of alginate for 5 min. The elaboration of liquefied beads was performed by suspending the solid microcapsules with a calcium sequestering agent such as sodium citrate (0.055 M) for 5 min. Finally, microcapsules were washed several times with Dulbecco's PBS (Gibco BRL, Invitrogen S.A., Spain) and cultured in complete medium.

Once all the stability studies were performed, RINm5F cells were enclosed by the same protocol described above in the selected A-PMCG-A beads at a density of 5×10^6 cells/ml and their metabolic behaviour was analysed over the next 2 months.

2.2.2. *Alginate-poly-L-lysine-alginate microcapsules*

A suspension of C2C12 myoblasts (2.5×10^6 cells/ml) in 1.7% sodium alginate solution was extruded into 0.05 M $CaCl_2$ solution. Droplets formed were initially coated with 0.05% PLL for 4 min and later with another layer of 0.1% alginate for 5 min. Solubilisation of the internal core was made by suspending the solid beads in sodium citrate (0.055 M) for 5 min. After two rinses in Dulbecco's PBS, beads were transferred to complete cell culture medium and kept under normal culture conditions.

2.2.3. *Barium alginate-poly-L-lysine-alginate microcapsules*

Microcapsules were prepared according to the procedure of Peirone et al. (1998) with slight modifications. The cell–gel suspension of C2C12 myoblasts (2.5×10^6 cells/ml) in 1.7% sodium alginate solution was extruded into 0.05 M $BaCl_2$ solution and the created droplets were coated with PLL and alginate. Solubilisation of the beads was performed as described above.

2.3. *Microcapsule morphological characterisation*

The diameter and wall thickness of the different microcapsules were checked under an inverted optical microscope. We focused special attention on the smoothness and uniformity of the beads as these factors play an essential role in the biocompatibility of the devices. Experiments were repeated for 20 capsules of each type in order to obtain statistically relevant data.

2.4. *Mechanical stability studies*

2.4.1. *Osmotic and mechanical resistance test*

This stability assay is a brief modification of the one described by our group for testing alginate-agarose microcapsules (Orive et al., 2001). In short, 100 μl of microcapsule suspension (with approximately 50 beads) were mixed with another 400 μl of sodium citrate (0.055 M) solution and placed in 24 well cell culture cluster, which was then put in a shaker at 800 rpm and 25 °C. In this way microcapsules are exposed to a combination of destabilising forces comprised of the osmotic swelling on the core induced by the citrate solution, the slow dissociation of the polyelectrolyte complexation between alginate and PMCG, and the shear forces produced by the shaker. At 1, 10, 30, 60, 180 and 360 min, ruptured capsules from each well were counted with an inverted microscope for statistical evaluation. Results are presented as the percentage of ruptured capsules as a function of time.

2.4.2. *Compression resistance study*

The compression resistance of all type of microcapsules was determined as the main force (g) required to generate a 70% compression of a sample of beads using a Texture Analyser (Model TA-XT2i, Stable MicroSystems, Surrey, GB). The apparatus consisted of a mobile probe moving vertically, up and down, at constant and pre-defined velocity. For this assay, larger capsules of approximately 1.7 mm diameter were prepared. The force exerted by the probe on

the beads was recorded as a function of the displacement leading to a force versus strain curve. Twenty capsules per batch were analysed in order to obtain statistically relevant data.

2.5. Metabolic cell activity

Cellular activity of the immobilised cells was determined by the tetrazolium assay (MTT assay) as described by Uludag and Sefton (1990). The level of viability detected is an indicator of the mitochondrial cell activity and therefore the physiological state of the enclosed cells. Results were expressed as mean ± standard deviation for three replicates.

3. Results

3.1. Effect of PMCG concentration and time of exposure

Our first goal was to study the effect of PMCG concentration and time of exposure on bead diameter and microcapsule membrane thickness in order to determine if this system allows the controlled adjustment of capsule size and wall thickness as well to select the suitable parameters for an optimal coating.

Initially, we prepared four solid and inhomogeneous A-PMCG-A microcapsule batches with different PMCG concentrations (0.35, 0.5, 0.7 and 1.1 % (v/v), respectively) and with a constant coating time of 1 min because the polyelectrolyte complexation between alginate and PMCG requires only a short reaction time. As it is shown in Fig. 1A and B, the membrane of the microcapsules was more defined and increased in thickness with the concentration of the polycation, reaching a maximum thickness of 14 μm, which represents 4.25% of the total bead diameter. On the other hand, the diameter of the beads decreased with the PMCG concentration ranging from 456 μm for the lowest concentration to 337 μm for the highest one.

Afterwards, we produced six new batches of solid and inhomogeneous A-PMCG-A beads maintaining a constant PMCG concentration of 0.5% (v/v) but modifying the coating time from 0.5 to 6 min. Once again, the increase in exposure time reduced significantly the diameter of the beads while it increased membrane thickness to 15 μm for 6 min of coating time (Fig. 2A and B).

It was clear that both PMCG concentration and coating time induced a similar effect, increasing wall thickness while reducing bead diameter. This behaviour is analogous to that observed for PLL (Thu et al., 1996a). In fact, the polyelectrolyte complexation generated by the PMCG coating produced a shrinkage effect on beads, reducing the size of the microcapsules due to the pressure exerted by the polycation. Further, we noticed that when PMCG concentration was higher than 0.5% (v/v) or time of coating superior to 1 min, some of the microcapsules became irregular, showing multiple striations and craters and some of them broke down. On the contrary, if both variables were small enough the capsule membrane was quite thin.

3.2. A-PMCG-A beads prepared with the selected conditions: morphological characterisation

According to the results obtained and the considerations explained above, we selected a PMCG concentration of 0.5% (v/v) and a final coating time of 1 min. Beads created with these conditions had a critical diameter of 410 μm, which has been considered the preferred device geometry by various experts in the field (Hunkeler, 2001a). In addition, images obtained with an inverted optical microscope showed that microcapsules presented a visible membrane, and were totally spherical and uniform.

However, though the semipermeable membrane parameters were characterised, we still had to define the most suitable microcapsule configuration in order to obtain rigid and mechanically stable beads. In fact, we needed to select between solid or liquefied beads and between homogeneous or inhomogeneous core beads. Moreover, we also intended to evaluate the effect of cell immobilisation on the mechanical properties of the devices. In the quest for the best immobilisation system, eight different types of A-PMCG-A beads with the selected parameters were prepared. Half of them were inhomogeneous, with the highest alginate concentration in the periphery of the bead. Among them, two were empty (one solid: IES and the other liquefied: IEL), and the other two were cell-loaded, solid: ICS and liquefied: ICL, respectively. The other half were homogeneous with a regular distribution of the alginate throughout the bead, and were divided with the

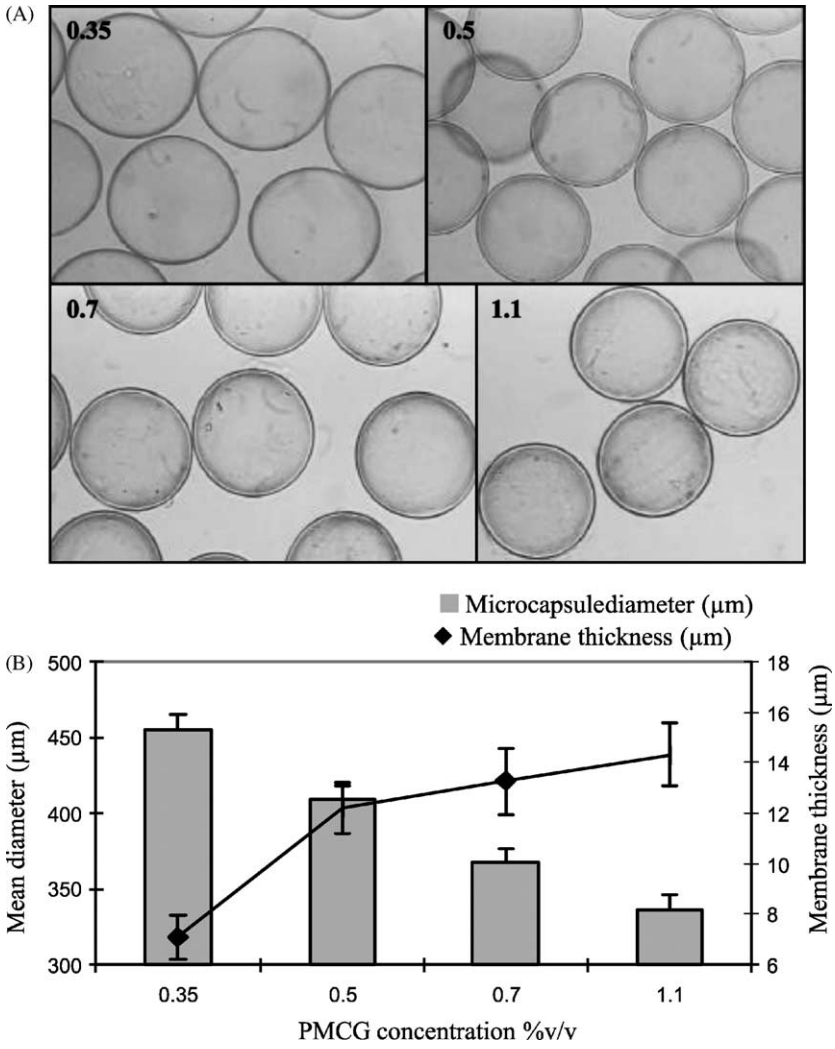

Fig. 1. (A) Images of A-PMCG-A microcapsules prepared with different PMCG concentrations (0.35, 0.5, 0.7 and 1.1% (v/v)). (B) Effect of PMCG concentration on membrane thickness and diameter of the beads.

same protocol as above: HES, HEL, HCS and HCL. A microcapsule of each type is presented in Fig. 3.

When the morphological characterisation of all types of A-PMCG-A beads was performed, the most significant difference was found between solid and liquefied microcapsules. In fact, the former had a main diameter of 409 μm while the latter had an increased size to 550 μm. This is a consequence of the treatment of solid beads with citrate or another sequestering agent, which assumes that the strongly bound calcium ions are exchanged with non-gelling sodium or potassium ions, increasing the swelling pressure inside the capsule and the diameter of the bead from its original volume (Thu et al., 1996b).

3.3. Mechanical stability study

To address the problem of A-PMCG-A microcapsule type selection, two different quantitative mechanical stability studies were performed: the osmotic-mechanical test and the compression resistance study.

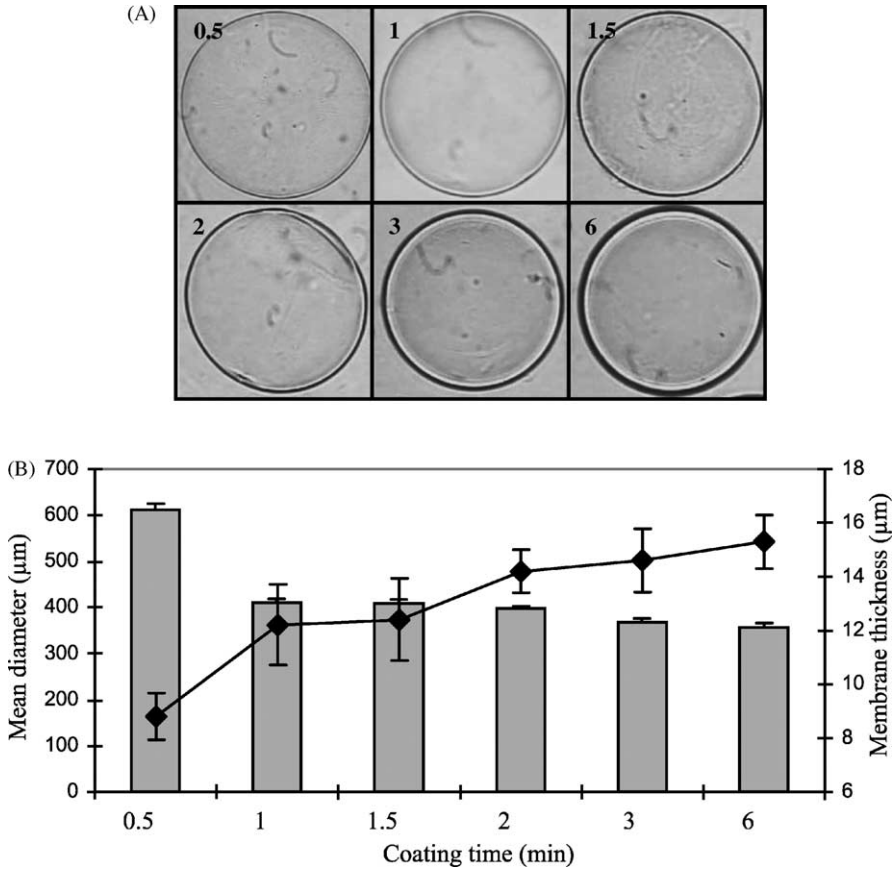

Fig. 2. (A) Microphotographs of A-PMCG-A microcapsules produced with different PMCG coating times (0.5, 1, 1.5, 2, 3 and 6 min). (B) Effect of PMCG exposure time on wall thickness and size of the devices.

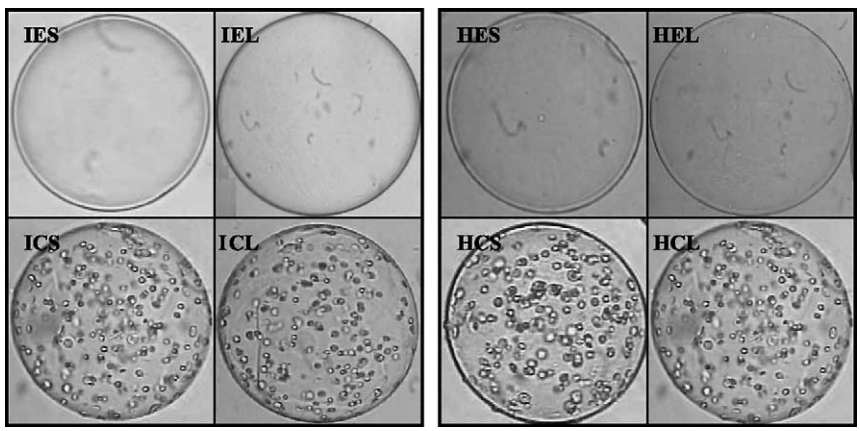

Fig. 3. Images of the eight different A-PMCG-A beads prepared with the selected conditions of PMCG concentration of 0.5% (v/v) and coating time of 1 min. Identification: I, inhomogeneous; H, homogeneous; E, empty; C, cell loaded; S, solid and L, liquefied.

3.3.1. Osmotic and mechanical resistance test

In this assay, beads were exposed to an osmotic swelling pressure which mimics the main cause of microcapsule breakage under physiologic conditions (Hu, 1996), and to mechanical forces that will progressively weaken the beads. Results obtained showed that solid microcapsules were mechanically more resistant than liquefied ones, both in the case of empty and cell loaded beads, presumably due to the swelling effect induced by the citrate solution (Fig. 4A and B). Similar data have been reported by our group for alginate-agarose beads (Orive et al., 2001) and by others for APA microcapsules (Van Raamsdonk and Chang, 2000). However, only 10% of the solid microcapsules broke during the assay, thus we were not able to find significant differences between inhomogeneous and homogeneous beads, nor between empty and cell loaded beads.

3.3.2. Compression resistance study

The previous study showed that solid beads had improved mechanical properties when compared to the solubilised microcapsules. Nevertheless, we still needed to differentiate among solid capsules in order to choose the most resistant internal configuration for our A-PMCG-A beads. With this purpose, resistance against compression of the devices was evaluated.

Results showed that A-PMCG-A beads presented a global high impact resistance while maintaining their elasticity (Fig. 5A and B). Once again, solid beads were mechanically more resistant than liquefied ones, but in this case it was also possible to discern among all type of microcapsules. In fact, it was observed that those beads prepared with a solid core and an inhomogeneous internal structure (both IES and ICS) were mechanically most resistant. This could be explained by the higher network density on

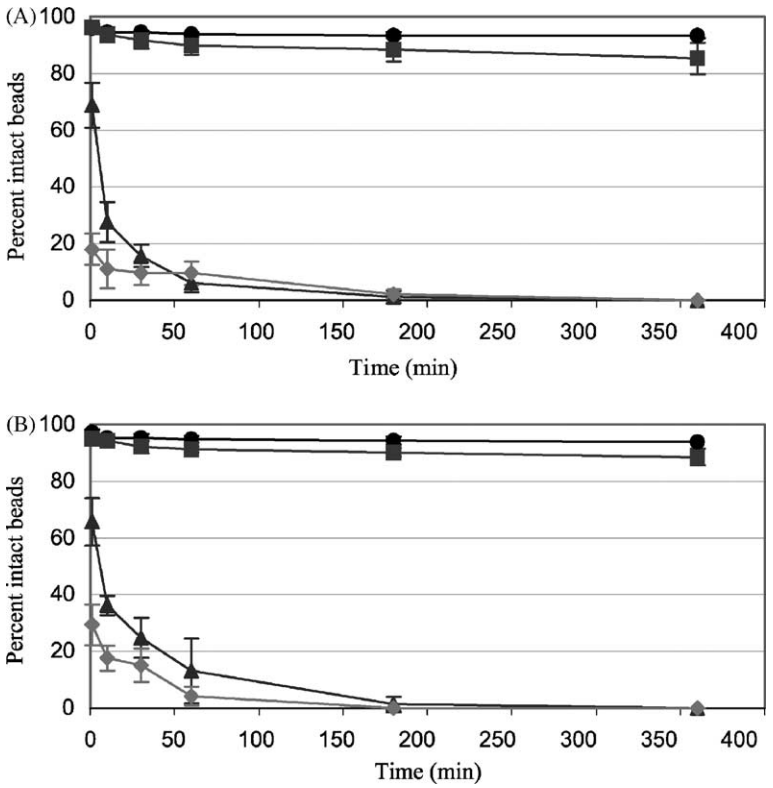

Fig. 4. Mechanical and osmotic pressure resistance of empty (A) and cell loaded (B) A-PMCG-A analysed by the osmotic-mechanical test. (●): inhomogeneous and solid beads; (■): homogeneous and solid beads; (▲): inhomogeneous and liquefied beads and (◆): homogeneous and liquefied beads. Results were expressed as mean ± standard deviation for three different microcapsule batches.

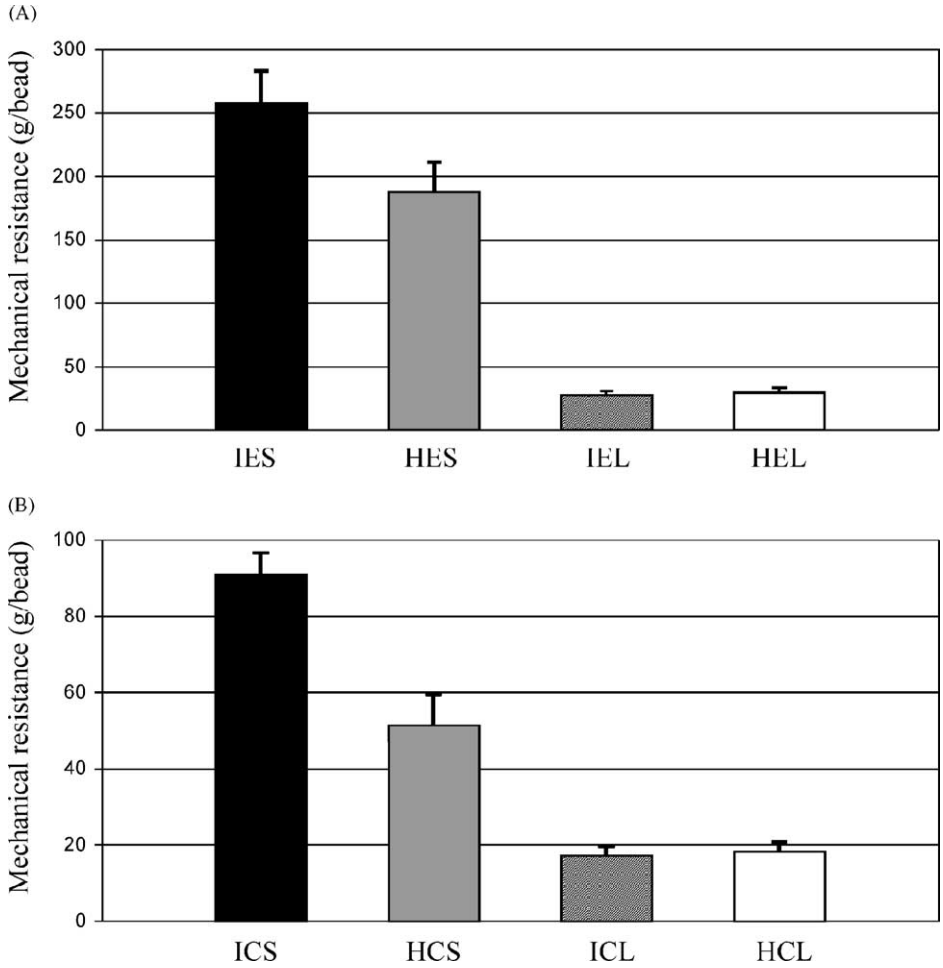

Fig. 5. Compression against resistance of empty (A) and cell loaded (B) A-PMCG-A studied by the compression resistance assay. Identification: I, inhomogeneous; H, homogeneous; E, empty; C, cell loaded; S, solid and L, liquefied. Results were expressed as mean ± standard deviation for three different microcapsule batches.

the bead surface induced by an inhomogeneous core. Further, as was expected, empty beads were shown to be stronger than microcapsules containing cells, which could be a consequence of the alginate network disruption provoked by the enclosed cells.

Another issue to consider was a comparison of the mechanical properties of the selected cell-loaded A-PMCG-A microcapsules, both solid (ICS) and liquefied (ICL), to other frequently employed cell loaded immobilisation devices for biotherapeutic purposes. We determined the resistance against compression of solid non-coated beads such as Ca^{2+}/alginate and Ba^{2+}/alginate capsules and also of solid and liquefied APA and barium alginate-PLL-alginate (BPA) capsules. It was not possible to liquefy non-coated beads because the internal swelling pressure provoked the breakage of the devices. Fig. 6 reflects the different resistance forces achieved during the assay. As shown, capsules made with calcium were weaker than those prepared with Ba^{2+} ions, which can be explained by the higher affinity of the latter to alginates, leading to more resistant and rigid gels (Haug, 1964; Smidsrød and Haug, 1972). However, Ba^{2+} gels are less physiologically compatible than Ca^{2+} ones and could induce toxic effects when implanted in the host. On the other hand, the mechanical stability

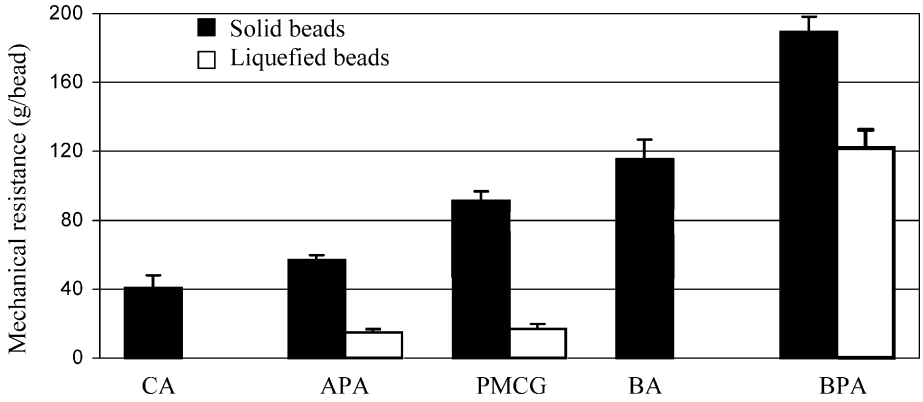

Fig. 6. Compression resistance comparison among the selected A-PMCG-A beads (PMCG) and other frequently employed devices such as Ca^{2+}/alginate (CA), Ba^{2+}/alginate (BA), alginate-PLL-alginate (APA) and barium alginate-PLL-alginate (BPA) capsules. Results were expressed as mean ± standard deviation for three different microcapsule batches.

of coated capsules was significantly improved in relation to non-coated ones. The semipermeable membrane surrounding the beads not only allowed the bi-directional diffusion of molecules, but also enhanced the mechanical properties of the devices.

However, the most interesting issue that the novel A-PMCG-A capsules were stronger than the classical APA ones, presumably because the polyelectrolyte complexation between Ca^{2+}/alginate and PMCG improves the mechanical stability of the devices.

3.4. Metabolic activity of the enclosed cells

ICS beads were shown to be the most resistant to breakage among all cell loaded A-PMCG-A capsules and mechanically more stable than classical APA beads. Nevertheless, we still needed to demonstrate that the semipermeable membrane produced by the PMCG coating allowed the inward diffusion of oxygen and nutrients to the entrapped cells. For this aim, we enclosed RINm5F cells within the selected ICS microcapsules and then cultured them for the next 2 months. Fig. 7 reflects that the metabolic activity of the immobilized cells increased in the first week and then decreased, reaching an equilibrium state during the rest of the assay. This meant that cellular viability of the enclosed cells at day 60 was similar to that obtained in the beginning of the assay. Therefore, it can be concluded that once cells were adapted to their new microenvironment, they were able

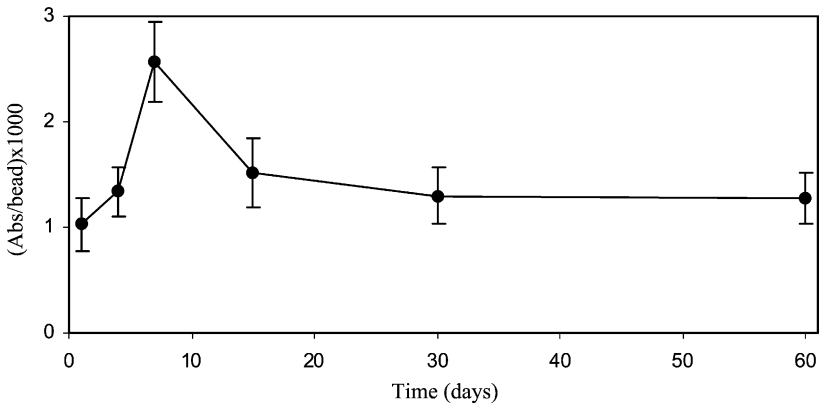

Fig. 7. Metabolic activity of RINm5F cells enclosed in inhomogeneous and solid A-PMCG-A microcapsules. Results were expressed as mean ± standard deviation for three separate sets of experiments.

to maintain their mitochondrial activity for at least 2 months.

4. Discussion

Encapsulated cell technology has the potential to treat a wide range of diseases by the controlled and continuous delivery of biological products to the host. In the last decade, a large number of attempts have been carried out and some exciting pre-clinical and clinical results have been achieved (Orive et al., 2002b, 2003). The principal procedure for producing these microcapsules involve coating cell loaded alginate droplets with PLL and alginate through ionic interactions, creating the APA beads. Though APA microcapsules have succeeded in small animal assays, a number of issues needed to be addressed before their future implementation in long-term as well as in large animal studies. These include the elevated cost, complex manipulation required to coat with PLL, and the mechanical fragility observed in these devices.

As a result of these considerations, much effort has been devoted to the design and optimisation of new immobilisation devices which could reduce or eliminate the disadvantages described for the APA beads. Some of the attempts have been focused on the selection of a new polycation to replace the PLL. In this regard, dimethyldiallylammonium chloride has been employed to surround both alginate (Chia et al., 2002) and cellulose sulphate droplets (Pelegrin et al., 1998; Dautzenberg et al., 1999), a binary alginate-oligochitosan system has been reported for biotechnology applications (Bartkowiak et al., 1999) and finally, PMCG has been used as a polycation for cellulose sulphate-based microcapsules (Wang et al., 1997; Lacík et al., 1998).

In this study, we present a novel microcapsule type based on the polyelectrolyte complexation between alginate and PMCG with the objective of overcoming the principal limitations described for the previous devices. PMCG is a cationic methylol amid which is significantly cheaper than PLL and is liquid at room temperature, thereby facilitating its manipulation. The A-PMCG-A microcapsules shown here were made with an electrostatic droplet generator to produce microcapsules of a more uniform size. Initially, we evaluated the effect of PMCG concentration and time of exposure on bead diameter and wall thickness. Results confirmed that an increase in both variables exerted a similar effect, reducing the diameter of the beads and increasing wall thickness (Figs. 1 and 2). Therefore, this novel system allows the independent control of capsule size and wall thickness and improves batch repeatability. Further, the morphological characterisation of the beads helped us select a PMCG concentration of 0.5% (v/v) and an exposure time of 1 min as optimal parameters for a correct coating.

Once the membrane characteristics were defined, our next objective was to select the most suitable microcapsule core design according to its mechanical properties. With this aim, we prepared eight different types of microcapsules taking into account variables such as the solid or liquefied state of the core, the inhomogeneous or homogeneous distribution of the alginate over the beads, and the enclosure or absence of cells within the device (Fig. 3). To assess capsule stability, two different stability studies were performed in order to evaluate the mechanical and osmotic swelling resistance as well as the resistance against compression of the devices. This is important as results from two independent methods could provide more comprehensive information and more details of capsule stability (Hunkeler et al., 2001b). Both studies confirmed that solid beads were mechanically more resistant than liquefied ones (Figs. 4 and 5). Moreover, the compression resistance assay showed relevant differences among all type of beads evaluated, concluding that solid and inhomogeneous capsules, both empty and cell loaded, possessed improved mechanical properties.

Compression resistance of the selected cell loaded beads was also compared to other frequently employed devices such as Ca^{2+}/alginate, Ba^{2+}/alginate, APA and BPA capsules (Fig. 6). As other groups have reported (Van Raamsdonk and Chang, 2000), BPA beads were shown to be stronger that the rest of devices but are also less physiologically compatible. Solid and coated beads also had better stability than liquefied and non-coated ones respectively. More interestingly, capsules exposed to PMCG increased their stability when compared to those coated with PLL, which meant that the polyelectrolyte complexation between alginate and PMCG made stronger beads than the ionic interaction between the former and PLL. Finally, we also demonstrated that RINm5F cells enclosed

in the selected A-PMCG-A beads maintained their metabolic activity in vitro for up to 60 days (Fig. 7).

A new immobilisation device based on the complexation between alginate and PMCG has been designed. Coating parameters with PMCG have been optimised, morphological characterisation of the microcapsules has been carried out, and the mechanically most resistant microcapsule type has been selected according to two different stability studies. The A-PMCG-A beads were found to be stronger than classical APA beads, and also provided an adequate microenvironment for cell viability.

The optimised A-PMCG-A beads here presented, allow the controlled adjustment of microcapsule size and wall thickness, while they improve the mechanical properties of classical APA systems, offering new alternatives for cell transplantation.

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References

Bartkowiak, A., Canaple, L., Ceausoglu, I., Nurdin, N., Renken, A., Rindisbacher, L., Wandrey, C., Desvergne, B., Hunkeler, D., 1999. New multicomponent capsules for immunoisolation. Ann. N.Y. Acad. Sci. 18, 134–145.

Canaple, L., Nurdin, N., Angelova, N., Saugy, D., Hunkeler, D., Desvergne, B., 2001. Maintenance of primary murine hepatocyte functions in multicomponent polymer capsules, in vitro cryopreservation studies. J. Hepatol. 34, 11–18.

Chia, S.M., Wan, A.C.A., Quek, C.H., Mao, H.Q., Xu, X., Shen, L., Ng, M.L., Leong, K.W., Yu, H., 2002. Multi-layered microcapsules for cell encapsulation. Biomaterials 23, 849–856.

Dautzenberg, H., Schuldt, U., Grasnick, G., Karle, P., Müller, P., Lörh, M., Pelegrin, M., Piechaczyk, M., Rombs, K.V., Günzburg, W.H., Salmons, B., Saller, R.M., 1999. Development of cellulose sulfate-based polyelectrolyte complex microcapsules for medical applications. Ann. N.Y. Acad. Sci. 18, 46–63.

Draget, K.I., Skjåk-Bræk, G., 1997. Soon-Shiong P. Alginate based new materials. Int. J. Biol. Macrom. 121, 47–55.

Haug, A., 1964. Composition and properties of alginates. Rep. Norw. Inst. Seaweed Res. 30, 25–45.

Hortelano, G., Al-Hendy, A., Ofosu, F.A., Chang, P.L., 1996. Delivery of human factor IX in mice by encapsulated recombinant myoblasts: a novel approach towards allogeneic gene therapy of hemophilia B. Blood 87, 5095–5103.

Hortelano, G., Xu, N., Vandenberg, A., Solera, J., Chang, P.L., Ofosu, F.A., 1999. Persistent delivery of factor IX in mice: gene therapy for haemophilia using implantable microcapsules. Hum. Gene Ther. 10, 1281–1288.

Hu, B.J., 1996. Alginate Polycation Microcapsules: A Study of Some Molecular and Functional Properties Relevant to Their Use as a Bioartificial Pancreas. PhD Thesis. Norwegian University of Science and Technology, Trondheim, Norway.

Hunkeler, D., 2001a. Allo transplants xeno: as bioartificial organs move to the clinic. In: Hunkeler, D., Prokop, A., Cherrington, A., Rajotte, R. (Eds.), Bioartificial Organs III: Tissue Sourcing, Immunoisolation and Clinical Trials. Ann. N.Y. Acad. Sci. 944, 1–6.

Hunkeler, D., Rehor, A., Ceausoglu, I., Schuldt, U., Canaple, L., Bernard, P., Renken, A., Rindisbacher, L., Angelova, N., 2001b. Objectively assessing bioartificial organs. In: Hunkeler, D., Prokop, A., Cherrington, A., Rajotte, R. (Eds.), Bioartificial Organs III: Tissue Sourcing, Immunoisolation and Clinical Trials. Ann. N.Y. Acad. Sci. 944, 456–471.

Lacík, I., Briššová, M., Anilkumar, A.V., Powers, A.C., Wang, T., 1998. New capsule with tailored properties for the encapsulation of living cells. J. Biomed. Mater. Res. 39, 52–60.

Lim, F., Sun, A.M., 1980. Microencapsulated islets as bioartificial endocrine pancreas. Science 210, 908–910.

Maysinger, D., Piccardo, P., Filipovic-Grcic, J., Cuello, A.C., 1993. Microencapsulation of genetically engineered fibroblast secreting nerve growth factor. Neurochem. Int. 23, 123–129.

Orive, G., Hernández, R.M., Gascón, A.R., Igartua, M., Rojas, A., Pedraz, J.L., 2001. Microencapsulation of an anti VE-cadherin antibody secreting 1B5 hybridoma cells. Biotechnol. Bioeng. 76, 285–294.

Orive, G., Ponce, S., Hernández, R.M., Gascón, A.R., Igartua, M., Pedraz, J.L., 2002a. Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. Biomaterials 23, 3825–3831.

Orive, G., Hernández, R.M., Gascón, A.R., Igartua, M., Pedraz, J.L., 2002b. Encapsulated cell technology: from research to market. Trends Biotechnol. 20, 382–387.

Orive, G., Hernández, R.M., Gascón, A.R., Calafiore, R., Chang, T.M.S., De Vos, P., Hortelano, G., Hunkeler, D., Lacík, I., Shapiro, A.M.J., Pedraz, J.L., 2003. Cell encapsulation: promise and progress. Nat. Med. 9, 104–107.

Peirone, M., Ross, C.J.D., Hortelano, G., Brash, J.L., Chang, P.L., 1998. Encapsulation of various recombinant mammalian cell types in different microcapsules. J. Biomed. Mater. Res. 42, 587–596.

Pelegrin, M., Marin, M., Nöel, D., Del Rio, M., Saller, R., Stange, J., Mitzner, S., Günzburg, W.H., Piechaczyk, M., 1998. Systemic long-term delivery of antibodies in immunocompetent animals using cellulose sulphate capsules containing antibody-producing cells. Gene Ther. 5, 828–834.

Poncelet, D., Neufeld, R..J., Goosen, M.F.A., Bugarski, B., Babak, V., 1999. Formation of microgel beads by electric dispersion of polymer solutions. AlchE 45, 2018–2023.

Smidsrød, O., Haug, A., 1972. Properties of poly(1,4-hexuronates) in gel state. II. Comparison of gels of different chemical composition. Acta Chem. Scand. 26, 79–88.

Smidsrød, O., Skjåk-Bræk, G., 1990. Alginate as immobilization matrix for cells. Trends Biotechnol. 8, 71–78.

Soon-Shiong, P., Feldman, E., Nelson, R., 1993. Long-term reversal of diabetes by the injection of inmunoprotected islets. Proc. Natl. Acad. Sci. USA 90, 5843–5847.

Soon-Shiong, P., Heintz, R., Merideth, N., Yao Quiang, X., Yao, Z., Zheng, T., Murphy, M., Moloney, M., Schmehl, M., Harris, M., Mendez, R., Mendez, R., Saundford, P., 1994. Insulin independence in a type I diabetic patient after encapsulated islet transplantation. Lancet 343, 950–951.

Springer, M.L., Hortelano, G., Bouley, D.M., Wong, J., Kraft, P.E., Blau, H.M., 2000. Induction of angiogenesis by implantation of encapsulated primary myoblast expressing vascular endothelial growth factor. J. Gene Med. 2, 279–288.

Strand, B.T., Ryan, L., Veld, P.I., Kulseng, B., Rokstad, A.M., Skjåk-Bræk, G., Espevik, T., 2001. Poly-L-lysine induces fibrosis on alginate microcapsules via the induction of cytokines. Cell Transplant. 10, 263–275.

Thu, B., Bruheim, P., Espevik, T., Smidsrød, O., Soon-Shiong, P., Skjåk-Bræk, G., 1996a. Alginate polycation microcapsules. I. Interaction between alginate and polycation. Biomaterials 17, 1031–1040.

Thu, B., Bruheim, P., Espevik, T., Smidsrød, O., Soon-Shiong, P., Skjåk-Bræk, G., 1996b. Alginate polycation microcapsules. II. Some functional properties. Biomaterials 17, 1069–1079.

Uludag, H., Sefton, M.V., 1990. Colorimetric assay for cellular activity in microcapsules. Biomaterials 11, 708–712.

Van Raamsdonk, J.M., Chang, P.L., 2000. Osmotic pressure test: a simple quantitative method to assess the mechanical stability of alginate microcapsules. J. Biomed. Mater. Res. 54, 264–271.

Wang, T., Lacik, I., Brissová, M., Anilkumar, A.V., Prokop, A., Hunkeler, D., Green, R., Shahrokhi, K., Powers, A.C., 1997. An encapsulation system for the immunoisolation of pancreatic islets. Nat. Biotechnol. 15, 358–362.

Xu, W., Liu, L., Charles, I.G., 2002. Microencapsulated iNOS-expressing cells cause tumor suppression in mice. FASEB J. 16, 213–215.