G. ORIVE[1]
A. BARTKOWIAK[2]
S. LISIECKI[2]
M. DE CASTRO[1]
R.M. HERNÁNDEZ[1]
A.R. GASCÓN[1]
J.L. PEDRAZ[1]
[1]Laboratory of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of the Basque Country, Vitoria–Gasteiz, Spain
[2]Chair of Food Packaging and Biopolymers, Faculty of Food Sciences and Fisheries. University of Agriculture in Szczecin, Poland

BIOCOMPATIBLE MICROCAPSULES BASED ON LOW MW OLIGOCHITOSAN/ ALGINATE/Ca^{+2} SYSTEM

In the last few years, many different alginate–chitosan microcapsules have been described in the scientific literature and several of the preparation methods have been patented. However, such procedures usually require the previous alkalinization of the chitosan samples because the acidic properties of the polymer could cause damage over cell cultures. To address this problem, many efforts have been directed to obtain low molar mass chitosans (<7,000 g/mol) which would permit the formation of capsules at physological pH, representing a strong advantage over other chitosan based microcapsules. Furthermore, it has been observed that these low molar mass chitosans, obtained in radical degradation and purified by precipitation, are non–cytotoxic against islets of Langerhans and genetically modified CHO cells (Bartkowiak, A. 2002).

Assuming this knowledge, we have evaluated the use of low molar mass chitosans as coating agents in the elaboration of rigid, semipermeable and biocompatible microcapsules for cell immobilization. As polycations might diffuse during the coating process within the alginate gel matrix and penetrate through the cell membranes interfering with cell internal structures, we have carried out a complete *in vitro* biocompatibility study of the oligochitosan samples. In fact, the morphology, viability (MTT assay) and lactate dehydrogenase release (LDH assay) from C_2C_{12} myoblast cells exposed to two oligochitosans with varying molar masses (Mn in range 3.3–4.6 kDa) has been performed. In addition, alginate–oligochitosan–alginate microcapsules prepared with a two–stage procedure and using both oligochitosans were elaborated for this work (Gåserød, O. et al. 1998). Finally, the osmotic swelling and permeability properties of the microcapsules were studied.

MATERIALS AND METHODS

Materials: Chitosan of MM >1,200 kDa and a degree of deacetylation 82–87% was exposed to a radical degradation as explained elsewhere (Bartkowiak, A. 2002). Low viscosity LVG alginates were obtained from FMC Biopolymers (Norway). All tissue culture media and serum were purchased from Gibco BRL (Invitrogen S.A., Spain).

MTT and LDH assays: 10000 C_2C_{12} myoblast cells/well were cultured in 96 well cell culture clusters. Complete DMEM medium was employed to set the 100% viability and 0.1% (w/v) Triton–100 to set the 0% viability. Serial dilutions of both oligochitosans (0.5 and 0.05 mg/mL) were added to the cells and incubated for 24 hours. Cell viability was determined by MTT assay as described by Fischer et al. (2003). Similarly, the LDH concentration in the supernatants was quantified using a commercial kit (DG 1340, Sigma). Results are expressed as [test viability]/[control viability] x 100 and [LDH release test]/[total LDH release] x 100.

Microcapsule elaboration: A 2% (w/v) solution of alginate was extruded into 0.05 M solution of $CaCl_2$. Once polimerized, alginate beads were coated with a 1% (w/v) solution of the oligochitosans for 20 min and 0.1% (w/v) alginate solution for 5 min.

Osmotic swelling test: 50–80 microcapsules of each type were cultured in 900 µL of PBS. Capsules were put a shaker at 500 rpm and 37 °C during 1 hour. After the supernatants were retired, 800 µl of purified water was added and the size of the capsules was analyzed. This process was repeated during the following 7 days. Results are expressed as $[(D_f \times D_i)/D_i] \times 100$, where D_i is the diameter of the capsule before the treatment and D_f is after treatment.

Permeability study: 2 mL of a 0.2% dextran mixture (200 kDa, 110 kDa, 70 kDa and 15 kDa (Polfa Works,

Author address: G. Orive, Laboratory of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of the Basque Country, Vitoria–Gasteiz, Spain

Paper presented as a lecture.

Kutno, Poland) was added under agitation to 1 mL of microcapsules. Further details are explained elsewhere (Bartkowiak, A. 2002).

RESULTS AND DISCUSSION

Oligochitosan samples were characterized and the main details are exposed in Table 1.

Table 1. Comparison of the two oligochitosan samples studied

Oligochitosan	Mn	Mw	Polid
Oligochitosan–1	4640	6680	1,44
Oligochitosan–2	3380	4680	1,38

Cell viability evaluation after exposition to both oligochitosans revealed that cells produce blue formazan depots due to their physiological activity (Fig. 1a) and maintain at least the 80–90% of their activity at the end of the study (Fig. 1b). This clearly reflects that both oligochitosans presented a very reduce citotoxicity for C_2C_{12} myoblasts. However, little cell damaged was detected after incubation with oligochitosan–2, reducing the viability in a 36% compared to the control cells when a concentration of 0.5% (w/v) was assayed. Assuming that the relative change in MM of both oligochitosans is more than 30% (oligo–2 and oligo–1; 3400 and 4600 g/mol respectively), we could expect that the shorter chains of chitosans could diffuse faster through the cell membranes and interfere with cell internal structures resulting in decrease of cell viability.

Interestingly, a similar profile was observed when the LDH release was quantified (Fig. 2). Oligo–1 induced a lower LDH secretion comparing with oligo–2. These are perfectly tolerable values assuming that other polycations employed in cell encapsulation such as poly–L–lysine (PLL) and poly(ethylenimine) (PEI) provoke a release of 70–80% after only 1 hour of incubation (Fischer, D. et al. (2003).

Microcapsules coated with both oligochitosans were fabricated and exposed to a purified water treatment which provoked a higher swelling effect in capsules elaborated with oligo–2 (Fig. 3a). Moreover, permeability studies demonstrated that oligochitosan coating had a significant effect on reduction of capsule porosity (Fig. 3b).

CONCLUSIONS

Depolymerized and purified low molar mass oligochitosans appeared to be non–cytotoxic for C_2C_{12} myoblasts and they were able to reduce the membrane cut–off of the alginate capsules. However, the extend of such effects is clearly related to the MM of the polymer.

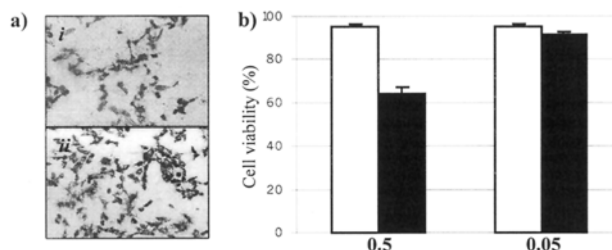

*Figure 1. **a)** Formation of blue formazan depots due the metabolic activity: i) oligo–1 and ii) oligo–2. **b)** Viability of the cells exposed to oligo1(□) and oligo–2 (■).*

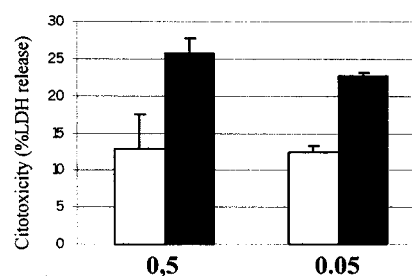

Figure 2. LDH release after exposition to oligochitosan–1(□) and oligochitosan–2 (■).

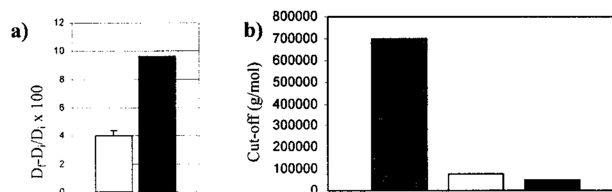

Figure 3. a) Swelling of capsules elaborated with oligo–1 (□) and oligo–2 (■). b) Membrana cut–off values for non–coated (■) and coated microcapsules with oligo–1(□) and oligo–2 (■).

ACKNOWLEDGEMENTS

We thank the Ministry of Education Culture and Sports of Spain (CYCYT SAF 2002–02268). We also would like to thank prof. Edward Kolakowski from Department of Food Technology (University of Agriculture in Szczecin) for enable to perform the mechanical characterization of microcapsules using SMS Texture Analyzer.

REFERENCES

[1] Bartkowiak, A. 2002. Binary polyelectrolyte microcapsules based on natural polysaccharides. Habilitation thesis, Technical University of Szczecin.

[2] Gåserød, O. et al. 1998. Microcapsules of alginate–chitosan–I. A quantitative study of the interaction between alginate and chitosan. Biomaterials (19): 1815–25.

[3] Fischer, D. et al. 2003. In vitro cytotoxicity testing of the polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials (24): 1121–31.