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MICROCAPSULES AND COATING TECHNOLOGIES BASED ON OLIGOCHITOSAN – FORMATION, PROPERTIES AND POTENTIAL APPLICATIONS

The encapsulation and coating of biological materials in polymeric hydrogels having permeable or semipermeable membranes have recently attracted considerable attention due to their potential application in various industries including medicine, agriculture and biotechnology. A wide variety of different approaches, based on different polymer chemistries, different processes for membrane formation and different encapsulation technologies have been evaluated. Recently the new system based on polylelectrolyte complexes of the ionic polysaccharides with low molar mass cationic chitosan has been evaluated Š1Ć. Specifically, effects of reaction parameters on the formation, physical properties and release characteristics have been previously emphasized [2]. This presentation involves the elucidation of the properties of such system in respect to their specific applications for immobilization of living cells, such as islets of Langerhans and CHO cells.

MATERIAL AND METHODS

Polysaccharides

Sodium Alginate – Keltone HV (lot. 54650A) (Kelco/NutraSweet, San Diego, CA, USA) with intrinsic viscosity, [η] of 880 mL/g in 0.1 M NaCl at 20°C was used for capsule preparations. This corresponds to a molar mass (MM) of 440,000 [2]. Iota–carrageenan (lot. 9062–07–1) was purchased from Fluka Chemie AG (Buchs, Switzerland). Samples of oligochitosan with varying molar masses 1–4 kiloDaltons (kDa) were prepared by controlled radical degradation via continuous addition of hydrogen peroxide to 2.5% chitosan solution of pH 3.5–4.0 at 80°C. Chitosan with a MM of 50 kDa and a degree of deacetylation >97% was used as the starting material (Hutchinson/McNeil Int., Philadelphia, USA, product E–055). All samples, after degradation as chloride salts had similar polydispersities in MM (1.5–1.8) and high degrees of deacetylation (>95%). The detailed procedure of degradation and purification of chitosan, characterization of polysaccharides, and preparation of solutions have comprehensively been described elsewhere [2].

Cytotoxicity tests

Cytotoxicity of the prepared oligochitosan samples were estimated by measuring the viability of two types of mammalian cells:

– rat islets of Langerhans,

– CHO SSF3 cell line (suspension serum free Chinese hamster ovary cells).

Both types of cells were cultivated in the particular culture medium with addition of oligochitosan at 0.1% concentration. In the case of islets of Langerhans their viability was evaluated after 24 hours contact with oligocation solution by their ability to produce insulin in response to the glucose challenge during the static stimulation test. The CHO cell line of inoculation concentration $5 \cdot 10^4$ cell/cm^3 was culturing in the 0.1% oligochitosan solution, where the cell growth and their viability were used as the cytotoxicity indexes. Additionally, the ratio of dead to alive cells was estimated during the experiment.

Encapsulation of islets of Langerhans

The sterile 1.2% sodium alginate (Keltone HV) in 0.9% NaCl solution was mixed with freshly isolated rat islets (4000 islets/cm^3 of polyanion solution). The islets/polyanion suspension was extruded at 5 cm^3/h using an air–stripping apparatus (22 G needle –Becton Dickinson, Ireland) for 5 min into the receiving bath (plastic Petri plate with 30 cm3 of 1% oligochitosan solution in 0.9% NaCl at pH 7.0). The capsules (400–600 μm in diameter) were allowed to react in receiving bath for additional 5 min, afterwards the reaction solution was discard over plastic tube with nylon net, washed several times with PBS (phosphate buffered saline) solution and finally they were transferred with 25 cm^3 plastic pipette into the culture media. All preparations were performed under the sterile laminar fumehood to avoid a bacterial contamination. The viability and activity of encapsulated rat islets were evaluated after five days by insulin

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secretion using a standard perifusion test with a flow rate 0.1 cm^3/min.

Encapsulation of CHO cells

In this study the CHO SSF3 cell line (suspension serum free Chinese hamster ovary cells – Novartis, Basel, Switzerland) was used. The specially prepared mixture (5 cm^3 in total) of 1.2% anionic polysaccharide solution with 10^6 cell/cm^3 was extruded using a vibrating nozzle encapsulator (InnoTech, Oberntfelden, Switzerland) into 200 cm^3 of 1% oligochitosan/0.9% NaCl at pH 6.8 solution. After 20 min reaction under sterile conditions the obtained capsules were separated, washed twice in saline and stored in culture media Chomaster HP–1 at pHdic pipette into the culture media. All preparations were performed under the sterile laminar fumehood to avoid a bacterial contamination. The viability and activity of encapsulated rat islets were evaluated after five days by insulin secretion using a standard perifusion test with a flow rate 0.1 cm^3/min.

RESULTS AND DISCUSSION

The immobilization of biological active materials is a multidisciplinary problem where many questions related to chemistry, biology and technology remain. Moreover, there are many technical obstacles which have to be correctly identified. Therefore, during the formation and the optimization of microcapsules containing living cells one should consider additional variables related to the new components of the culture medium, changes of the hydrodynamic behavior of the polyelectrolyte solution, polymer cytotoxicity, membrane biocompatibility, technological problems related to the membrane size, maximizing the rate of packing and centering of the cells within the capsules. In addition to the maintenance of viability, the encapsulated cells obviously have to retain their functional or differentiated state, i.e. synthesize and secrete specific biomolecules.

In every bioencapsulation system, the polymers that contact the cells must be non–cytotoxic, while the PEC membrane contacting the physiological fluid should be biocompatible. It is well known that high molar mass natural polysaccharides such as sodium alginate and carrageenans are non–cytotoxic. Although, it is clear that in the case of synthetic polymers or chemically modified natural biopolymers cytotoxic response can be a result of reagent quality and toxicity of residual solvents especially in case of chemically degraded chitosan. Consequently two separate tests were performed to evaluate the cytotoxicity of prepared oligochitosans toward following types of cells:

– rat islets of Langerhans,

– genetically modified CHO cells.

In both tests two sorts of degraded chitosan were used, either non–purified or purified by precipitation in polar non–solvent. In the first experiment the influence of the oligochitosan on rat islet function in vitro was tested.

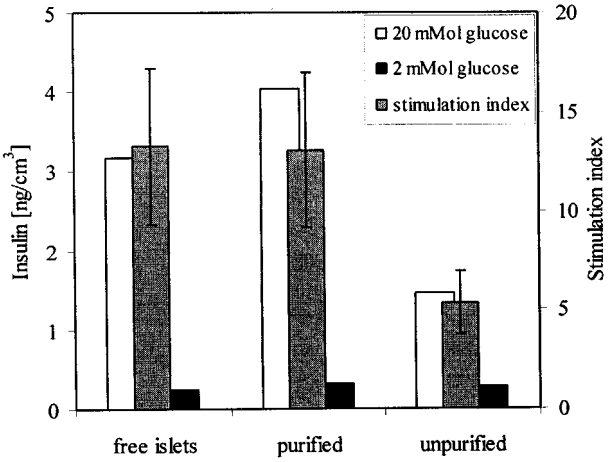


Figure 1. Insulin stimulation tests for rat islets cultured for 24 h in three different solutions: "0" (standard culture medium), purified (0.1% oligochitosan 3 kDa, purified by precipitation in polar non–solvent in culture medium) and unpurified (0.1% oligochitosan 3 kDa, non–purified in culture medium)

The functionality of the islets were measured after 24 hours culturing in 0.1% chitosan (3 kDa) solution. For the non–purified oligochitosan one could observe the significant reduction of the insulin stimulation index in comparison to the untreated islets (Fig. 1). Although, when the same oligocation sample was purified by precipitation in polar non–solvent the viability of islets was not affected what is validated by almost identical values of their stimulation indexes.

In the case of the CHO cells the significant differences in metabolic activity were observed in function of type of oligochitosan dissolved in the culture medium. The cell activity was measured using two types of indicators, either the cell growth or their viability (Fig. 2). Four types of oligochitosan samples, both purified and non–purified either at low or neutral pH, have been selected. The presence of both non–purified samples

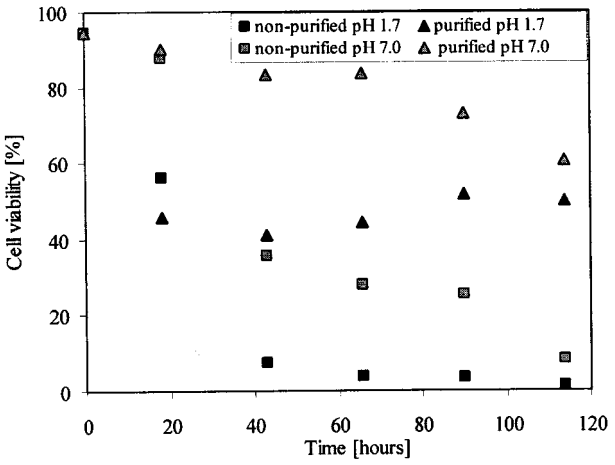
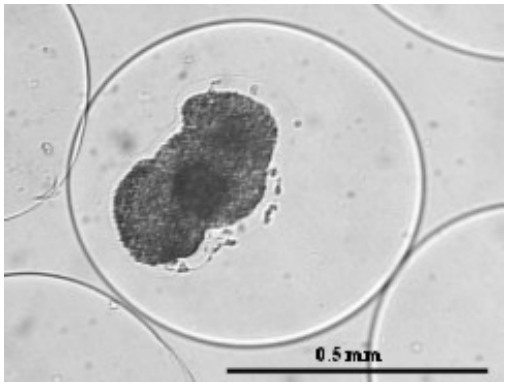


Figure 2. Monitoring of CHO cell viability cultured for 120 hours in 0.1% of oligochitosan 3 kDa (non–purified and purified by precipitation at pH 1.7 and/or 7.0)



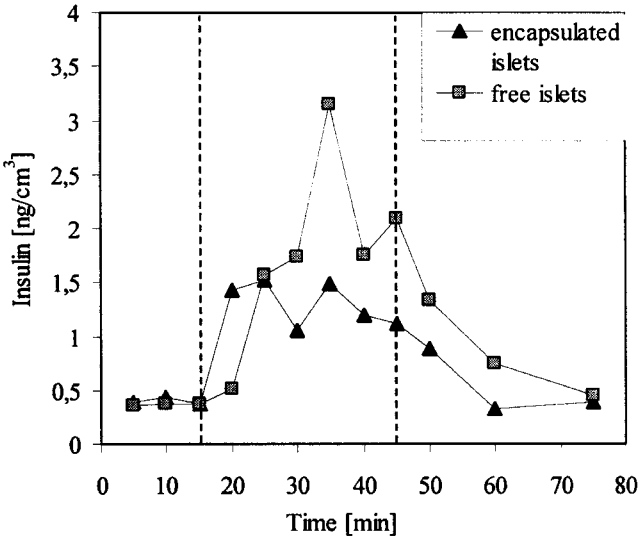


Figure 3. Insulin secretion by encapsulated and non–encapsulated islets of Langerhans using a perifusion test with a glucose/culture medium flow rate 0.1 cm^3/min (0–15 min/2 mMol, 15–45 min/20 mMol, 45–75 min/2 mMol of glucose)

leads to moderate lost of cell living functions expressed by gradual decrease in their total number. Although, despite the fact of similar growth the viability results indicate that pH of oligochitosan solution (low or neutral) during the precipitation process does significantly influence the biological quality of the final products. Already in the first 20 hours of culturing in the medium with addition of both oligochitosans prepared at low pH 1.7 the viability has decreased down to around 50%, while after the same time for the cell contacting with samples prepared at neutral pH their viability remains at high level (>90%). Therefore, it is important to adjust the pH of radically degraded oligochitosan solution before the precipitation process close to neutral values.

The efficiency of the new binary encapsulation system was evaluated by observation of immobilized rat islets of Langerhans *in vitro* activity via glucose/insulin perifusion test. The freshly isolated rat islets were encapsulated in sodium alginate/oligochitosan system according to the procedure described in the experimental section. The encapsulated islets of Langerhans were viable after 5 days in culture, where capsules of 600 μm in diameter remain mechanical stable without any change of their size or geometry. The encapsulated islets respond very fast to the higher glucose concentration (20 mMol) similar to the free islets (Fig. 3). Therefore, using such binary microcapsules one can achieve glucose homeostasis, where for safe control in diabetics the rapid insulin delivery (<15 min) in response to a glucose challenge is required.

In the second part of the investigation simmilar immobilization systems based on i–carrageenan and alginate have been evaluated as micro–reactors for CHO cells. All prepared capsules were perfectly spherical and remained intact and mechanical stable, with significant grow of cells during the culturing especially in case of i–carrageenan system.

CONCLUSIONS

The binary encapsulation system based on ionic polysaccharides and oligochitosan can be applied in various immobilization technologies. Low molar mass chitosan, obtained in radical degradation and purified by precipitation, is noncytotoxic against islets of Langerhans and genetically modified CHO cells. This new capsule chemistry indicates high versatility with potential application as bioartificial organs in medicine or immobilization carriers in biotechnology.

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REFERENCES

[1] Bartkowiak, A. (2001) Optimal conditions of binary polyelectrolyte microcapsule formation: Ann. N.Y. Acad. Sci. vol. 944: 120–134.

[2] Bartkowiak, A., Hunkeler, D. (2000) Alginate–Oligochitosan microcapsules: II. Control of mechanical resistance and permeability of the membrane. Chem. of Materials 12 (1): 206–212.