Alginate-chitosan microbeads/microcapsules with entrapped synthetic antigen for specific antibody removal

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Introduction

Removal of anti-carbohydrate antibodies from patient blood is needed in transplantation as well as for treatment of autoimmune diseases. The gap between the number of patients waiting for organ transplantation and the availability of suitable donor organs is significant (US OPTN, 2006). Removal of blood group anti-A and anti-B antibodies can prevent hyperacute organ rejection in ABO-incompatible transplantation. Antibody-removing treatments, including plasma exchange and column immunoadsorption with antibody specific antigen covalently linked to solid beads techniques are used in pre- and post-transplant periods to avoid hyperacute organ rejection. Rydberg (2004) showed specific removal of anti-A/B antibodies using ABO immunosorbent with synthetic carbohydrates linked to Sepharose matrix. Nevertheless similar to other techniques, complicated procedure of plasma separation is required before removal of the specific antibody by the adsorption column. Alginate-chitosan microspheres are potentially hemocompatible, and therefore could be proposed as immunoadsorbents contacting with whole blood. We are developing a new type of immunosorbent based on alginate-chitosan microbeads/microcapsules with semipermeable membrane loaded with synthetic A and B antigens (glycoconjugates) for the direct removal of ABO blood group antibodies from whole blood, without the need for plasma separation and plasma exchange. The membrane of microbeads/microcapsules can prevent glycoconjugate release while allowing antibody to penetrate inside microspheres/microcapsules and to specifically bind antigen. The sorption capacity of supports obtained was compared to the capacity of Sepharose modified by the same oligosaccharides.

Materials and Methods

Chemicals. In this research sodium alginate (Alg) (medium viscosity, Sigma), chitosan (30 kDa, DD 90%) and oligochitosan 3.5 kDa, DD 89 %, were used. Synthetic glyococonjugates B_{di}–PAA A_{tri}–PAA, A_{tri}–PAA–FITC (all samples with MM ~ 2000 кДа) were prepared as described earlier (Shilova 2005)

Encapsulation of glycoconjugates in alginate-chitosan microbeads/microcapsules was carried out using a two-step procedure. Sodium alginate solution (4% (w/v)) was mixed with glycoconjugate solution (1.0-2.5 mg/ml) in the ratio 1:1 (v/v), and the mixture was added to a 2% (w/v) $CaCl_2$ solution (25 ml) dropwise using a peristaltic pump. To provide formation of small calcium alginate (CaAlg) microbeads, an electrostatic bead generator (7.8 kV) or a special sprayer with air flow were used. The bead size could be varied within the range of 200-900 μm. The obtained CaAlg microbeads were washed 3 times with 0,9% NaCl, and then incubated in 0.2 -0.4 % (w/v) oligochitosan solution for 8-10 min at room temperature. To prepare oligochitosan solution, oligochitosan sample was easily dissolved in 0.9 % NaCl giving pH 3.8. The pH value of the polymer solution was adjusted to pH 4.5 by adding 2M NaOH. Then the beads were washed twice with physiological solution. To get hollow

microcapsules, the alginate core was dissolved by treatment of the microbeads with 50 mM EDTA at room temperature for 10-30 min. The obtained alginate-chitosan hollow microcapsules were again washed with 0,9% NaCl solution and stored at 4° C.

Characterization of microbeads/microcapsules was carried out using an optical microscopy. The membrane thickness was determined by using polymer latex nanoparticles.

Study of antibody sorption Aliquots of microbeads, microcapsules or Sepharose (each 50 µl) were incubated with antibody solution (1:10 v/v) for 1 hour and 24 hours, and sorption was carried out at 24 и 37^{o} C. Two types of antibodies, namely anti–Bdi (IgG + IgM, 254 µg/ml) which have been isolated from human serum (Khraltsova L., 2000), and monoclonal antibodies anti-Atri (IgM) mouse antibody got from ascitic liquid were provided by company Hematolog (Russia). The sorption efficacy of IgG and IgM antibodies was determined by ELISA as decribed earlier (Selina 2008)

Results and Discussion

Preparation and characterization of alginate-chitosan microbeads/microcapsules
As well known, Ca-alginate-based beads can be prepared from sodium alginate by using Ca^{2+} ions which provide a hydrogel network..Chitosan as a polycation is widely used for coating Ca-alginate beads with entrapped biomolecules, in order to prevent their release from microparticles. A-trisaccharide (Atri) and B-disaccharide (Bdi) previously covalently attached to PAA macromolecules were entrapped in alginate-chitosan microbeads or microcapsules. The microcapsules were prepared by treatment of the microbeads with EDTA solution. Ca-alginate microbeads were got using a special sprayer (with air flow) or electrostatic bead generator, voltage 7.8 kV (Table 1). Microbead size could be varied within 200-900 µm. by varying concentration of a sodium alginate solution and the needle diameter (Table 1). The stability of the microcapsules depends upon the inner structure and the thickness of the polyelectrolyte membrane.

Concentration of sodium alginate solution, %	The needle diameter, mm	The mean microcapsule size in physiological solution, µm
2.0	0.60	791.8
2.0	0.33	425.4
1.5	0.45	512.0
1.5	0.33	367.7
1.5	0.25	289.5

Table 1. The microcapsules size as function of alginate solution concentration and needle diameter.

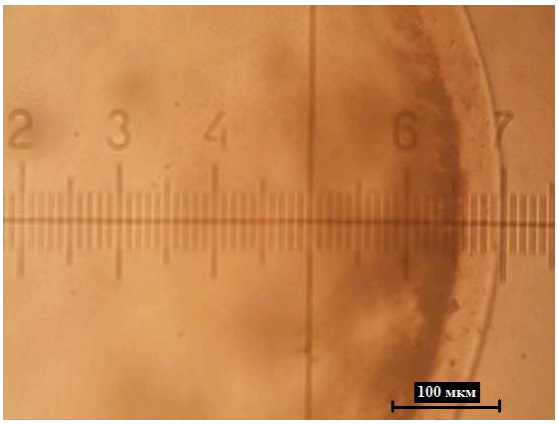

Fig.1. The micrograph of microcapsule with membrane.

One of the key parameters affecting the membrane formation process, the membrane thickness and its stability was chitosan molecular weight. The use of oligochitosan (3.5 kDa) allowed us to prepare stable microcapsules with memrane thickness 40-50 µm. These membranes were 10-20

μm thicker compared to those ones made of chitosan with MM 30 kDa. In its turn, for every polysaccharide sample (chitosan or oligochitosan), the membrane thickness depended upon 2 parameters : 1) pH value of chitosan solution; 2) the incubation time of CaAlg beads in the chitosan solution. The incubation time of CaAlg beads in chitosan solution had the profound influence on the process of microcapsule formation.. The obtained microcapsules were stable after centrifugation for 10 min at 1000 rpm.

Study of glycoconjugate efficacy entrapment into alginate-chitosan microbeads and microcapsules

The efficacy of glycoconjugate entrapment was studied using FITC- labeled Atri-PAA and Bdi-PAA glycoconjugates. About 40 % of glycoconjugate initial quantity was found in microbeads while 45 % of FITC- labeled glycoconjugates was found in $CaCl_2$ solution to which sodium alginate was added to get Ca-alginate microbeads. The rest 15 % of FITC- labeled A-tri-PAA was found in washings. Since, in order to get A-tri-PAA glycoconjugate, we used PAA (2000 kDa) without any previous fractionation, the release of low molecular weight conjugate fractions during encapsulation procedure was expected. It should be mentioned that there was no further glycocongugate release from the microbeads after encapsulation procedure was over, and only 3.5% of the released conjugate was found after its storage at 4 C for 16 days. That means that we found a desirable microbead design which provided keeping A-tri-PAA inside microbeads without its release through polyelectrolyte alginate-chitosan membrane. After treatment of alginate-chitosan microbeads with EDTA, the glycoconjugate content inside the formed hollow microcapsules 2-fold decreased. This fact can be explained by the additional release of low molecular weight glycoconjugate fractions due to the increase of the membrane permeability after the treatment with EDTA.

Study of antibody removal using alginate-chitosan immunosorbents

Sorption of anti-B antibodies by alginate-chitosan microbeads/microcapsules at 24 and 37^0 C was studied (Table 2). Percentage of bounded antibodies was calculated as a difference of optical densities before and after incubation of the microbeads/microcapsules with antibody solution.

Type of antigen carrier	Conc of B_{di}-PAA entrapped in carriers mg/μL	Quantity of bounded anti-B_{di} (IgG+IgM) antibodies , %*			
		in 1h		in 24 h	
		24^0C	37^0C	24^0 C	37^0C
microbeads	$1 \cdot 10^{-3}$	60	62	100	100
microcapsules	$0.6 \cdot 10^{-3}$	38	46	66	68

Table 1 Table 2. Sorption of anti-B (IgG+IgM) antibodies by alginate-chitosan

From Table 2 one can see that 62 and 100 % of antibodies were bounded to glycoconjugate (antigen) entrapped in microbeads after 1 h and 24 h of incubation, respectively. In the case of microcapsules total sorption was lower, but both results were similar when being calculated for one unit of entrapped glycoconjugate mass. As can be also seen, the temperature did not affect sorption efficacy. Blank microcapsules (without glycoconjugate) were used as a control. Non-specific sorption was about 5 %. The developed microbeads were compared to modified Sepharose which was chosen as the most effective immunoabsorbent commonly used today. Modified Sepharose contained covalently attached the equal quantity of the same glycoconjugate (0.6 μM/ml) which has been prepared as previously described (Rieben R., 2000) . Results are shown in Fig.2. (a, b).

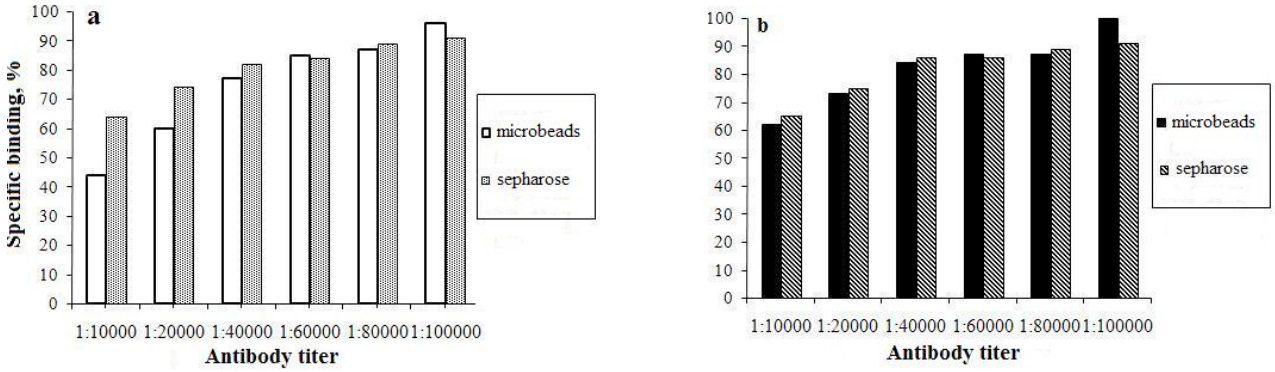

Fig. 2. Sorption of antibodies by alginate-chitosan microbeads and Sepharose after 1 hour (a) and after 24 hours (b).

As can be seen from Fig.2, the efficacy of antibody sorption for both immunosorbents (alginate-chitosan microbeads and Sepharose) was similar.

Conclusion

Thus, the obtained alginate-chitosan microbeads were demonstrated to be able to eliminate both IgG and IgM antibodies. It should be noted that the quantity of glycoconjugate (antigen) entrapped in microbeads can be increased at least 10-fold (unlike in the case of Sepharose for which the quantity of covalently attached antigen is limited). We suggest that this possibility can be used for increasing sorption capacity of microbeads compared to Sepharose. At the same time microbead size (which is at least 10-fold bigger compared to Sepharose bead size) could allow us to prepare a novel hemocompatible immunosorbent for removal of antibodies directly from whole blood without the need for plasma separation and plasma exchange.

References

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