APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 8, 523-539 (1983)

REVIEW

Agarose–Polyaldehyde Microsphere Beads: Synthesis and Biomedical Applications

Cell Labeling, Cell Separation, Affinity Chromatography, and Hemoperfusion

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Received March 26, 1983; Accepted June 2, 1983

Abstract

Polyaldehyde microspheres, polyglutaraldehyde (PGL), and polyacrolein (PA) were synthesized by polymerizing glutaraldehyde and acrolein in the presence of an appropriate surfactant. The microspheres with average diameter of 0.2 μm were used for the specific labeling of human red blood cells (RBC) and mouse lymphocytes. The "naked" microspheres were encapsulated with agarose and formed agarose–polyaldehyde microsphere beads in sizes ranging from 50 μm up to 1 cm. The encapsulated beads, with diameters ranging from 50 to 150 μm were used as insoluble adsorbents for affinity purification of antibodies. Beads with diameters varied from 150 to 250 μm were used for cell fractionation purposes (mouse B splenocytes from T splenocytes). Uniform beads of 1 mm diameter were designed for hemoperfusion purposes. As a model, the removal in vitro of anti-BSA from immunized goat whole blood was studied.

Index Entries: Agarose–polyaldehyde microsphere beads; polyaldehyde–agarose microsphere beads; microspheres, agarose–polyaldehyde; synthesis, of agarose–polyaldehyde microspheres; cell labeling, with agarose–polyaldehyde microspheres; cell separation, with agarose–polyaldehyde microspheres; affinity chromatography, with agarose–polyaldehyde microspheres; hemoperfusion, with agarose–polyaldehyde microsphere; chromatography, affinity with agarose–polyaldehyde microspheres.

0273-2289/83/1200-0523$03.20

Introduction

The potential use of synthetic polymeric microspheres as markers for the visualization and identification of cell surface receptors has been investigated by several teams. Polystyrene latex particles have been utilized as immunological markers by the scanning electron microscopy (SEM) technique (*1*). However, the uses of polystyrene microspheres are limited by their hydrophobic properties, which cause the polystyrene particles to adhere non-specifically to the surface of many cells. Various kinds of hydrophilic polymeric microspheres based on acrylic monomers were prepared and used for mapping of cell surface receptors (*2, 3*). These microspheres carried on their surface a variety of functional groups, e.g., carboxylate, hydroxyl, amide, and/or pyridine groups, through which proteins were bound covalently by means of a series of reactions (*4*). In order to simplify the derivatization procedure, PGL microspheres (*5*) and, more recently, PA microspheres (*6*) were synthesized. These microspheres are provided with reactive aldehyde groups through which covalent binding of a variety of amino ligands, such as peptides, proteins, or drugs, can be achieved in a single step under physiological pH. The "naked" polyaldehyde microspheres were also encapsulated with agarose to form agarose–polyaldehyde microsphere beads in sizes ranging from 50 μm to 1 cm (*7*†). The synthesis of the "naked" and encapsulated polyaldehyde microspheres as well as some of their biological applications are described in this review article.

Synthesis and Characterization of the "Naked" Microspheres

PA microspheres were prepared by irradiating acrolein in the presence of the surfactant polyethylene oxide (*6*). PGL microspheres were synthesized by the aldol condensation polymerization of glutaraldehyde in presence of the surfactant polysurf 10-36B (*5*). Fluorescent microspheres were obtained by carrying out the polymerizations in presence of the appropriate fluorochromic compounds, e.g., tetramethyl rhodamine isothiocyanate. The polyaldehyde microspheres, after their formation, were washed by centrifugation and were then redispersed in distilled water or in phosphate buffer saline (PBS).

Both PGL and PA microspheres contain high concentration of aldehyde groups. The presence of these groups was ascertained by several methods. The infrared spectra indicate absorption bands at 1720 and 2740 cm^{-1} caused by the stretching of nonconjugate aldehyde and CH of aldehyde groups, respectively (*6, 8*). Additional evidence for the presence of aldehyde groups is the developed yellow color obtained by the interaction of the microspheres with 2,4-dinitrophenylhydrazine. Quantitavely, the aldehyde content of the microspheres was determined by the nitrogen percentage of the product that resulted from the reaction of the microspheres with hydroxylamine hydrochloride (*9*). Table 1 shows that both the aldehyde content and the yield for the formation of PA microspheres is higher than for PGL microspheres. The hydrophilic character of the microspheres, essential for higher specificity of the microspheres (*5*), is mainly caused by the hydration of the alde-

†Margel, S., patent pending.

TABLE 1
Aldehyde Content and Yield of Microspheres Formed by the Polymerization of Glutaraldehyde and Acrolein

Microspheres	Yield, %	N, %	No. of aldehyde groups/g $\times 10^{-21}$
PGL	5–8	7.5	3.4
PA	50	15.4	7.0

hyde groups (*10*) and by the quenching of these groups with hydrophilic compounds, e.g., ethanolamine or BSA. The hydrophilicity of the PGL microspheres is also a result of the presence of hydroxyl groups obtained by the Cannizzaro reaction that occurred during the polymerization of glutaraldehyde (*5*). Amino ligands, e.g., proteins, were covalently bound to the polyaldehyde microspheres to form the appropriate Schiff base products. Leakage of proteins bound to the microspheres was not detected because of the polyvalent Schiff base bonds formed. However, leakage of ligands containing a single amino group can be prevented by a further reduction of the Schiff base bond with sodium borohydride or sodium cyanoborohydride (*11*) to produce a stable single C—N bond.

$$RCHO + R'NH_2 \rightleftharpoons RCH = NR' \xrightarrow{NaBH_4} RCH_2NHR'$$

Cell Labeling with the "Naked" Microspheres (5, 6)

Different subpopulations of cells were resolved according to their different surface antigens by labeling the cells with immunomicrospheres, i.e., microspheres to which antibody is covalently bound. Figure 1 illustrates the procedure employed for the labeling. In the direct procedure, an immunomicrosphere selects out its target site and bind to it (Fig. 1a), and in the indirect method (Fig. 1b) a second antibody is employed. The high specificity of the polyaldehyde microspheres towards cells was demonstrated by the following systems:

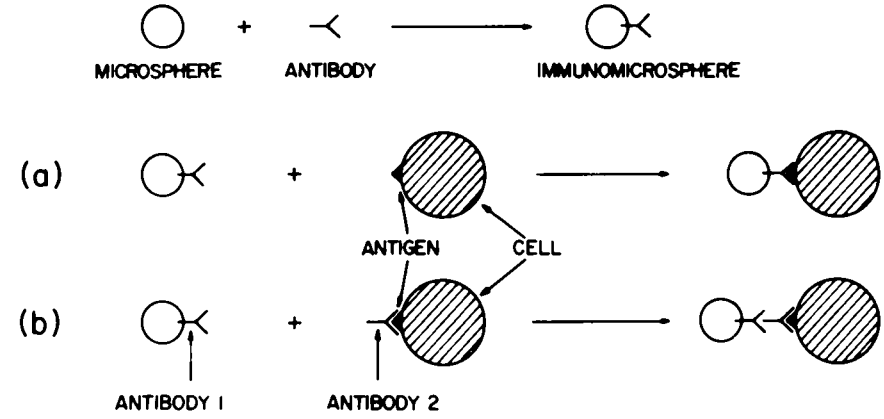

Fig. 1. Schematic representation of direct (a) and indirect (b) labeling of cells by means of immunomicrospheres.

(1) labeling of human RBC by the indirect procedure, antibody 1 was goat antirabbit IgG (GαRIgG) and antibody 2 was rabbit antihuman RBC (RαHRBC). Figure 2 represents SEM photomicrographs of human RBC treated with PGL microspheres. The experimental cells are covered with microspheres, while the control cells are free of microspheres. Similar results were obtained with PA microspheres.

(2) Direct labeling of mouse tumor thymocytes with anti-Thy 1.2 derivatized fluorescent PGL microspheres (Fig. 3). The EL4 tumor line cells bear cell surface Thy 1.2 antigens, while the BW5148 tumor line cells (control) do not. Under a fluorescent microscope, only the EL4 cells fluoresced (the single fluoresced cell among the BW5148 line is most likely a macrophage), indicating the high specificity of this system.

(3) Direct labeling of normal mouse splenocytes bearing cell surface immunoglobulins (B cells) with goat antimouse immunoglobulin (GαMIg) derivatized fluorescent PA microspheres (Fig. 4). Again the B cells fluoresced under the microscope and the T cells (control) did not.

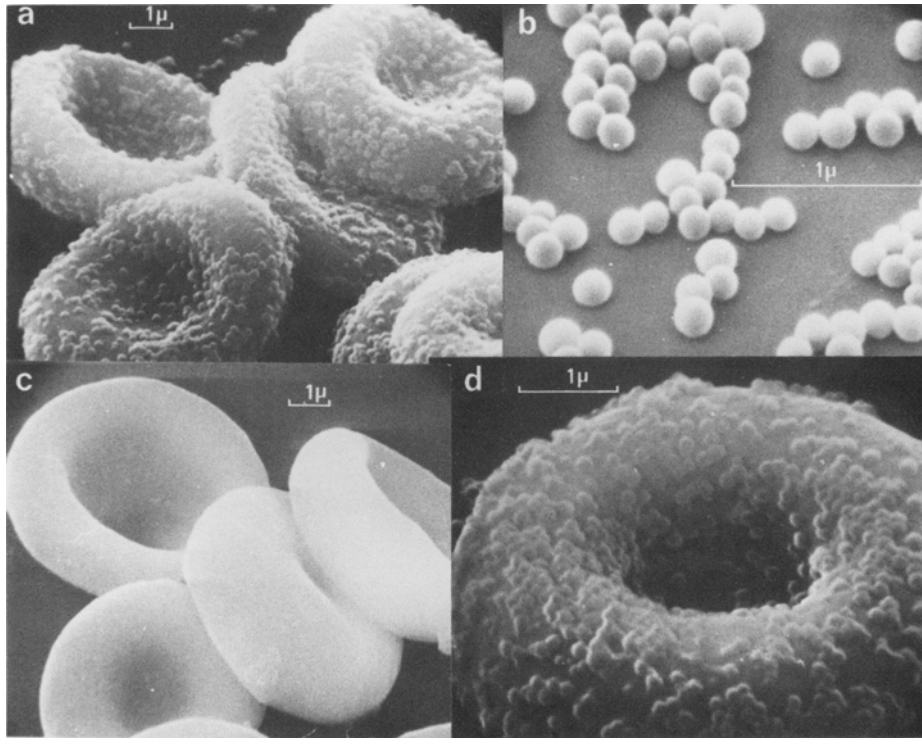

Fig. 2. SEM photomicrographs of human RBC sensitized with RαHRBC labeled with GαR IgG derivatized PGL microspheres: (a) and (d) experimental cells, (b) PGL microspheres, (c) control cells.

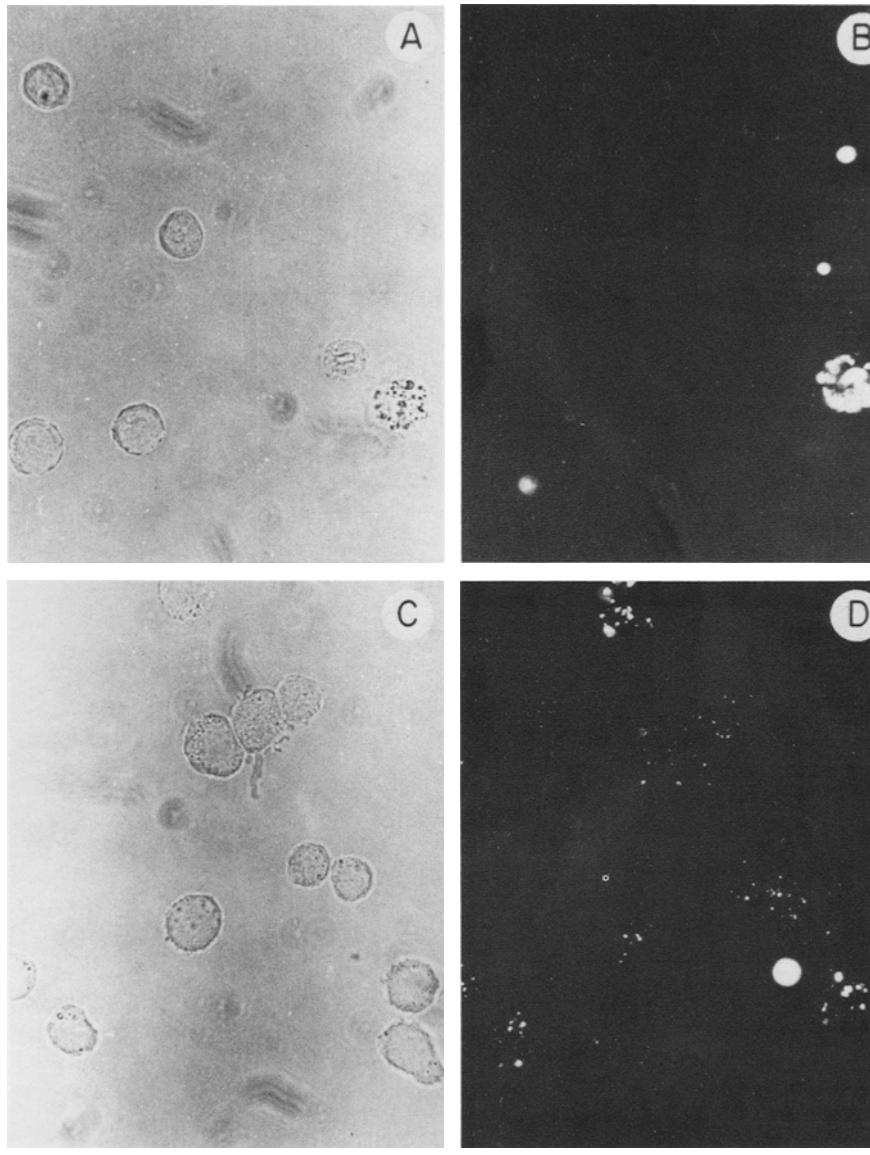

Fig. 3. Light and fluorescence micrographs of a mouse tumor line of thymocytes, BW5147 (A,B) and EL4 (C,D) mixed with rhodaminated PGL microspheres bound to monoclonal anti-Thy 1.2.

Synthesis and Binding of the Agarose–Polyaldehyde Microsphere Beads.

Agarose–polyaldehyde microsphere beads were prepared as previously described (*7*). Briefly, agarose in water was heated to 95°C until the gel melted into a clear solution. The temperature was then decreased to 70°C and appropriate polyaldehyde microspheres solution, PA or PGL, heated to 70°C were added. The

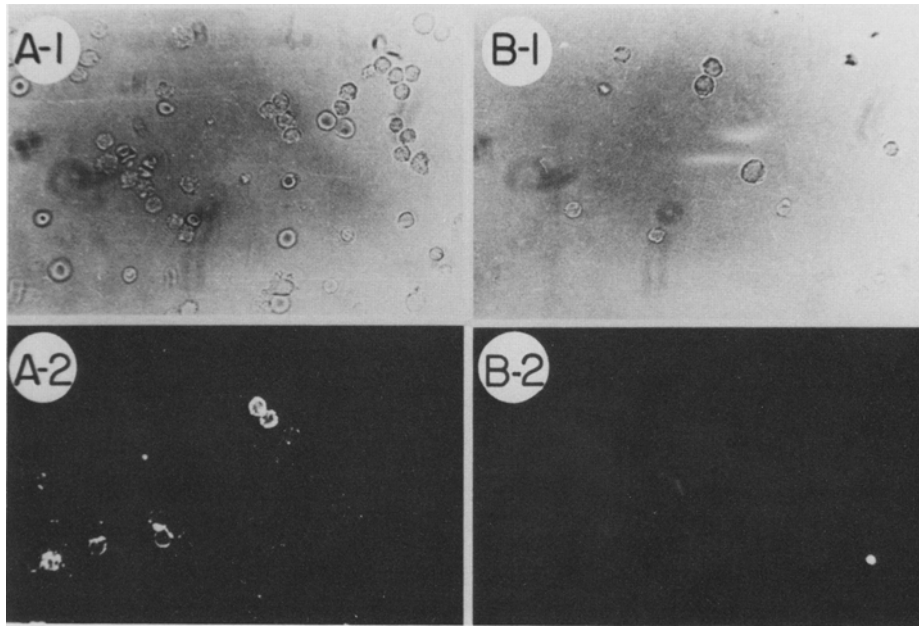

Fig. 4. Light and fluorescence micrographs of mouse splenocytes (A) and thymocytes (B) mixed with rhodaminated PA microspheres bound to GαMIg: 1, light picture; 2, fluorescence picture.

final concentration of both agarose and the microspheres were 4% (*W/V*). The solution was poured into a stirred (300 rpm) peanut oil at 70°C. Ten minutes later, the solution was cooled with ice. The beads formed were purified from the oil by several extractions with ether. Ether was then removed by evaporation. The diameter of the beads obtained ranged from 50 to 250 μm. Fractions containing beads of diameters 50–150 μm and 150–250 μm were obtained by passing the beads through appropriate sieves. Larger beads can be prepared by carrying out the same procedure at a lower peanut oil stirring rate.

Uniform beads of 1-mm diameter size were prepared in a procedure similar to that described by Losgen et al. (*12*). A melted aqueous solution containing agarose (4%, *W/V*) and polyaldehyde microspheres (4% *W/V*) was drawn into a glass syringe apparatus. The apparatus was kept at 70°C while stirring the agarose–polyaldehyde aqueous suspension at 300 rpm. Thereafter, the gel was forced out by the motor driven piston and was injected dropwise into a tall vessel containing an ice-cold solvent mixture of toluene, chloroform, and hexane in the ratio of 10 : 4 : 2, respectively. The beads formed were separated from the solvent mixture in a crude sieve, washed several times with dioxane, and finally cleared of solvents by exhaustive washing with distilled water. The beads are stored in the cold in presence of 0.05% (*W/V*) sodium azide until use.

The agarose–polyaldehyde microsphere beads fulfill the essential requirements of effective immunoadsorbents. Agarose in a concentration of 4% (*W/V*) provides the mechanical strength, the specificity, the high porosity and the biocompatibility of the beads. The polymeric microspheres encapsulated in the agarose matrix are

used for the covalent binding of amino ligands. The binding capacity of these beads towards various amino ligands, e.g., proteins [bovine serum albumin (BSA), dinitrophenol-BSA (DNP-BSA), and rabbit immunoglubulin (RIgG)], antibodies (GαMIg and monoclonal anti-Thy 1.2), lectins [concanavalin A (Con A) and soybean agglutinin], and hormones (bovine insulin) is illustrated in Table 2. Steric requirements may explained the significantly increased (3–6-fold) in the binding capacity of the beads bound to the spacer arm, polylysine–glutaraldehyde. The beads preserved their physical and mechanical properties after the coupling of the ligands. Leakage of bound proteins from the beads to the supernatant under basic, acidic, and physiological conditions was not detected using the method of Lowry et al. (*13*) for protein determination. Furthermore, radioactivity in the supernatant of a solution containing beads bound to radioactive BSA (^{131}I) was not detected during the radioactive iodine lifetime.

The potential use of the agarose–polyaldehyde microsophere beads for various applications, e.g., affinity chromatography, cell fractionation, and hemoperfusion was demonstrated as described in the proceeding publication.

Affinity Separation of Antibodies. The removal and purification of antibodies from the serum of immunized animals was achieved with agarose–polyaldehyde microsphere beads with diameters ranging from 50–150 μm. Figure 5A represents a light microscopy picture of the agarose–polyaldehyde microsphere beads. A

TABLE 2
Binding Capacity of the Agarose–Polyaldehyde Microsphere Beads to Various Amino Ligands[a]

Ligands	Spacer	Binding capacity, mg	
		Agarose–polyacrolein microsphere beads	Agarose–polyglutaraldehyde microsphere beads
BSA	—	2.2	2.4
BSA	Polylysine–glutaraldehyde	14.8	11.5
DNP-BSA	—	1.8	1.5
DNP-BSA	Polylysine–glutaraldehyde	9.0	9.1
RIgG	—	1.0	1.0
RIgG	Polylysine–glutaraldehyde	10.0	5.4
Soybean agglutinin	Polylysine–glutaraldehyde	5.9	—
Con A	—	2.4	—
Con A	Polylysine–glutaraldehyde	11.5	—
Bovine insulin	—	7.2	—
Bovine insulin	Polylysine–glutaraldehyde	22.0	—
GαMIg	—	2.4	—
GαMIg	Polylysine–glutaraldehyde	12.4	—
Anti-Thy 1.2	Polylysine–glutaraldehyde	9.8	—

[a] 1 mL of the beads were shaken with excess quantities of the various ligands in 5 mL for 12 h at room temperature.

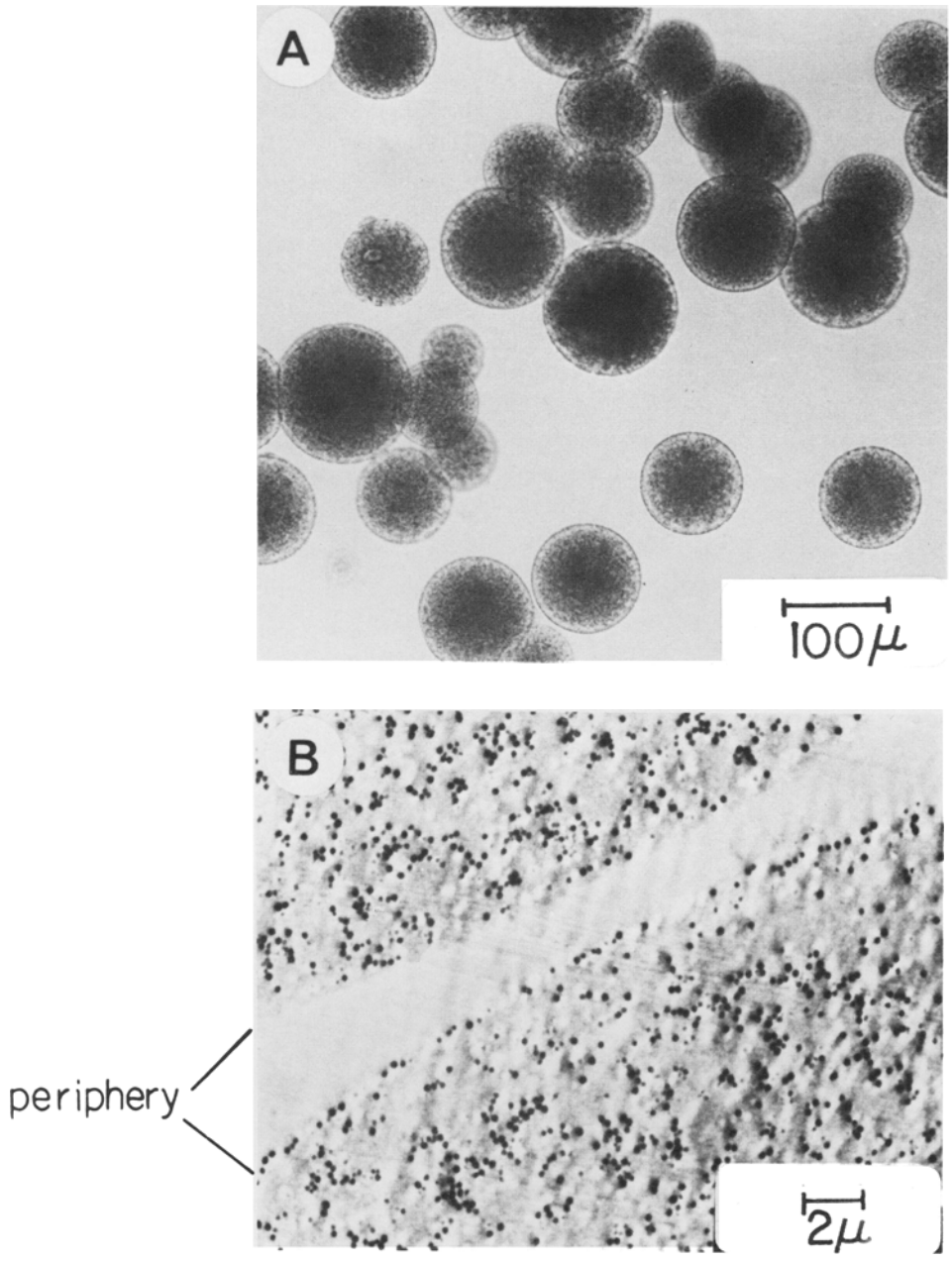

Fig. 5. (A) A light microscopy picture of the agarose–polyacrolein microsphere beads. (B) A transmission electron microscopy picture describing a cross-section of the agarose–polyacrolein microsphere beads.

cross-section photomicrograph showing the "naked" microspheres encapsulated within the agarose is shown in Fig. 5B.

The beads conjugated to the desired protein (immunobeads) were washed successively with PBS, eluting medium (0.2*M* glycine–HCl buffer at pH 2.4), and

again with PBS. The immune serum was then passed through a column filled with the appropriate immunobeads at a flow rate of 1 mL/min (5–10 mL serum for each 1 mL of beads). The immunobeads were then washed several times with PBS. Adsorbed antibodies were then eluted with 0.2*M* glycine–HCl buffer solution at pH 2.4, neutralized with NaOH, dialyzed against PBS, and then analysed by polyacrylamide gel electrophoresis (*14*). The immunobeads, after the treatment with glycine-HCl buffer, were washed several times with PBS and stored cold in presence of sodium azide (0.05%) until reused.

The binding capacity of some of the immunobeads to the appropriate antibodies and the resultant eluting data are given in Table 3. In all cases antibodies were not detected after the adsorption step, as was determined by the ring test with the antisera. Antibodies eluted from the column and analyzed by polyacrylamide gel electrophoresis were found to contain only IgG. Normal rabbit serum was passed through a column containing the immunobeads. The beads were then treated as described for the isolation of antibodies. Under these conditions, proteins were not eluted from the beads, indicating that nonspecific absorption of proteins onto the beads did not occur. The immunobeads have also been used repeatedly during a period of 6 months without any significant loss of their antibody binding capacity, as shown in Table 4.

Cell Fractionation of Mouse B and T Splenocytes (15). GαMIg or anti-Thy 1.2 derivatized agarose–polyacrolein microsphere beads (1 mL) with diameters of 150–250 μm were packed in a siliconized pasteur pipet plugged with a glass wool. The beads were washed first with PBS to remove sodium azide and then with Hank's solution containing 5% horse serum (HS). Washed, viable cells ($1–2 \times 10^7$) suspended in 1–2 mL of Hank's + 5% HS were filtered through the immunobeads at room temperature, at a rate of 1–3 drops/min. The column was then rinsed with Hank's + 5% HS until the eluate became cell-free. Non-adsorbed cells were recovered by centrifugation at 500*g* for 15 min and then resuspended in 1–2 mL of Hank's + 5% HS. The beads were poured into a glass vial containing approximately 5 mL of Hank's + 5% HS, and the adsorbed cells were then recovered by gently stirring the beads with a pasteur pipet. The supernatant containing the eluted cells was centrifuged at 500*g* for 15 min, and the cells were then resuspended in 0.5 mL of Hank's + 5% HS. Mouse B splenocytes were detected with rhodaminated GαMIg. Mouse T splenocytes were detected with FITC conjugated to anti-Thy 1.2. The immunobeads were washed with PBS and then stored in PBS + 0.05% (*W/V*) sodium azide until reused.

Beads with diameters ranging from 150–250 μm were found to be the most suitable for cell fractionation purposes. By using smaller size beads higher percentage of the cells are retained in the interstices of the beads and the recovery of cells non-adsorbed to the immunobeads is lower. On the other hand, when using larger size beads, the efficiency of the cell fractionation is not as good as with the optimal size beads. Figure 6 is a light microscopy picture demonstrating the labeling of mouse B splenocytes with GαMIg derivatized beads. More quantitative data describing the efficiency of the cell fractionation through the various beads is shown in Table 5. In the control experiment, mouse splenocytes were passed through a column containing GαRIgG derivatized beads. The composition of the B and T cells re-

TABLE 3
Isolation of Antibodies with the Agarose–Polyaldehyde Immunoadsorbents[a]

Beads	Spacer	Antigen	Quantity of bound antigen, mg	Antiserum used	Quantity of bound antibody mg	Quantity of eluted antibody, mg
Agarose–polyglutaraldehyde microsphere beads	—	BSA	1.5	Rabbit	1.6	1.6
Agarose–polyglutaraldehyde microsphere beads	Polylysine–glutaraldehyde	BSA	11.5	Rabbit	14	12.6
Agarose–polyglutaraldehyde microsphere beads	Polylysine–glutaraldehyde	Rabbit IgG	11.0	Goat	14	14
Agarose–polyacrolein microsphere beads	—	BSA	2.2	Rabbit	2.4	2.2
Agarose–polyacrolein microsphere beads	Polylysine-glutaraldehyde	BSA	14.8	Rabbit	16.5	17.0
Agarose–polyacrolein microsphere beads	Polylysine-glutaraldehyde	RIgG	10.0	Goat	12.0	12.0
Agarose–polyacrolein microsphere beads	Polylysine-glutaraldehyde	DNP–BSA	9.2	Rabbit	8.5	8.4

[a]mL beads.

TABLE 4
Quantity of Anti-BSA Eluted from the Agarose–Polyacrolein Microsphere Beads Bound to Polylysine–Glutaraldehyde-BSA During Three Successive Isolations in 6 Months[a]

Time, months	Quantity of rabbit anti-BSA eluted, mg
1	16.5
3	16.0
6	17.0

[a]1 mL beads bound to 14.8 mg BSA.

mained almost constant, 53% and 43% of B and T cells before fractionation compared to 51% and 45% after fractionation. A very pure population of T cells (97–98%) was obtained in the non-adsorbed cell fraction passed through the GαMIg derivatized beads. However, the cells eluted from these immunobeads formed small aggregates that made cell counting difficult. The efficiency of the separation obtained with the anti-Thy 1.2 derivatized beads as shown in Table 5 is also satisfactory, although it is not as efficient as with the GαMIg conjugated beads. In all the experiments described in Table 5 the viability of the fractionated cells was unaffected and the recovery was between 80 and 100%.

The potential use of the "naked" PGL and PA microspheres as a tool for mapping of cell surface receptors was demonstrated. Several publications have also reported on the use of polymeric microspheres, especially with magnetic properties, for cell separation purposes (*6, 16, 17*); however, we fo und that the use of "naked" polymeric microspheres for cell separation is limited. The microspheres' size is much smaller than that of the cells to be labeled, 0.2 μm compared to approximately 5 μm. During the labeling procedure, each cell can be labeled with hundreds of thousands of microspheres, as shown in previous publications by SEM pictures (*3, 5*). Each microsphere can bind through its aldehyde groups to many receptors. It is extremely difficult to remove the microspheres from the cell surface and to obtain the pure cell population. On the other hand, the agarose–polyaldehyde microsphere beads can be a very useful support for cell fractionation. The cell size is smaller than that of the beads, 5 μm compared with average size of 200 μm. Each bead can be labeled with many cells. However, by a gentle stirring of the labeled beads, the cells detach from the beads' surface and a pure cell population can be obtained.

Hemoperfusion, Removal of Specific Antibodies (18). The standard experimental procedure is shown in Fig. 7. A Travenol roller-type blood pump cycled the immunized blood from the reservoir through the uniform (1 mm size) BSA derivatized beads column at a flow rate of 62 mL/min. Samples were drawn from the reservoir and checked for anti-BSA activity by the quantitative precipitation reaction (*19*) and solid-phase radioimmunoassay (*20*). Formed elements of the blood were counted in the hemocytometer or in the Coulter Counter. The glass column was fitted with a glass disc containing 0.2 mm apertures. All glassware was

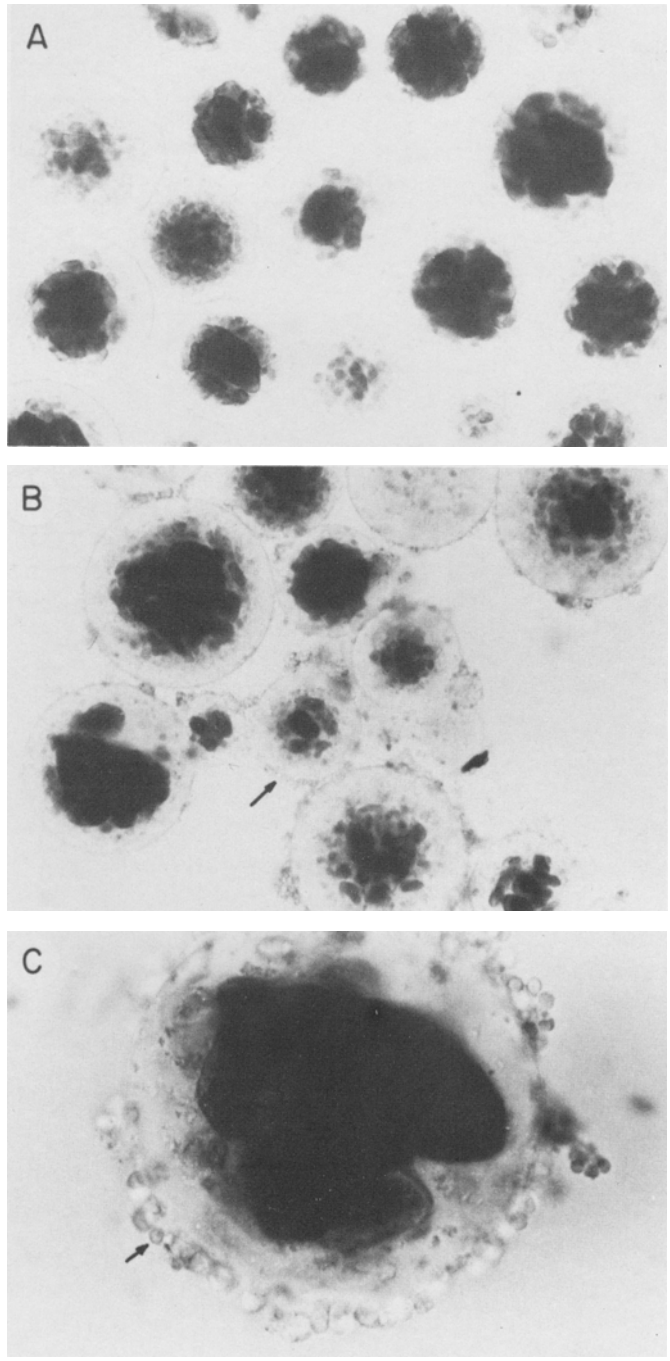

Fig. 6. A light microscopy of mouse spleen cells adsorbed to Gα MIg derivatized agarose–polyacrolein microsphere beads: A-control, immunobeads without attached cells. B and C, immunobeads labeled with B cells, (arrows indicate B cells attached to the beads). C represents higher magnification (×4).

TABLE 5
Cell Fractionation of Mouse Splenocytes with Immuno-agarose–Polyacrolein Microsphere Beads

	Percentage of cells					
			Adsorbed cells		Non-adsorbed cells	
Immunobeads	B cells	T cells	B	T	B	T
GαRIgG beads (control)	53	43			51	45
GαMIg beads	60	40	—	6	0	98
GαMIg beads	58	40	—	6	0	97
Anti-Thy 1.2 beads	57	40	40	54	86	13
Anti-Thy 1.2 beads	59	40	37	54	84	14

[a]Mouse splenocytes ($1–2 \times 10^7$ cells) were passed at a flow rate of 1–3 drops/min through a column containing beads derivatized with either GαMIg or anti-Thy 1.2.
[b]Small aggregates of cells made cell counting difficult.

siliconized before using. Silicon tubing was used throughout the system. The relative high size beads and their uniformity, as shown in Fig. 8, increased the blood compatibility of the formed elements (*12*) and improved the flow properties. In the model system, the beads were covalently bound to BSA and circulating anti-BSA were adsorbed onto the beads from serum or whole blood of an immunized rabbit or goat. In practice and antigen may be covalently bound to the beads and the appropriate circulating antibody (in autoimmune diseases) may be removed. A kinetics study of the adsorption in vitro of rabbit anti-BSA from immune rabbit serum is shown in Fig. 9: 62% was removed in 30 min; 78% in 60 min; 91% in 120 min, and

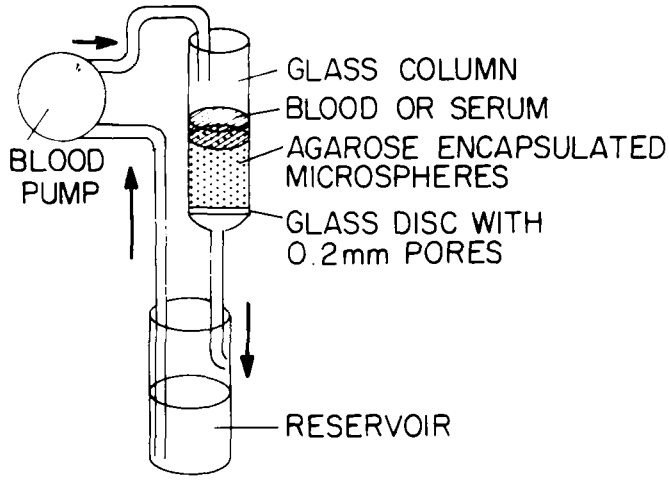

Fig. 7. A schematic diagram of the apparatus employed for the removal of anti-BSA from immunized serum or whole blood.

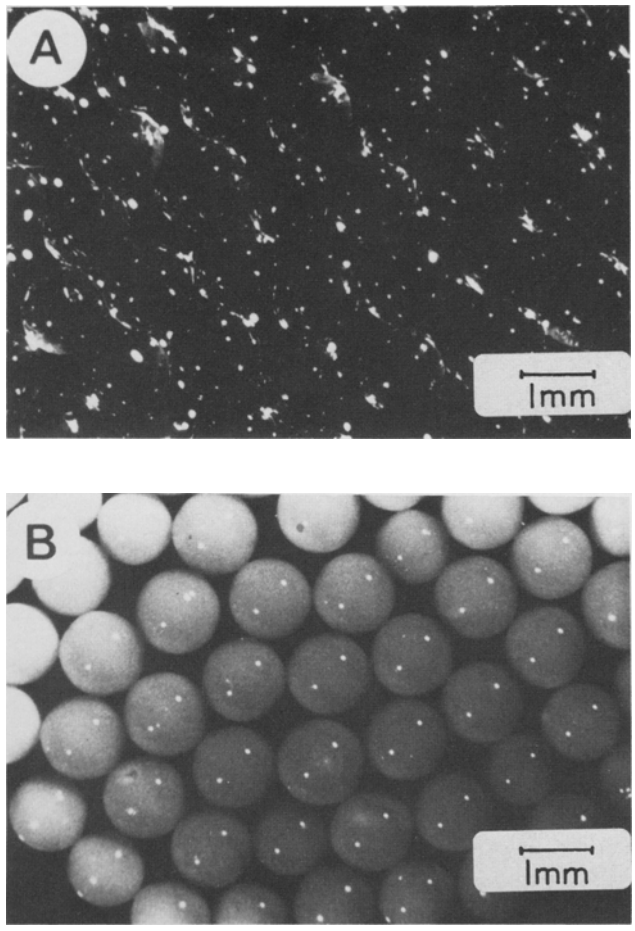

Fig. 8. A light microscopy photomicrograph showing the uniform agarose–polyacrolein microsphere beads, (A) agarose beads, (B) agarose–polyacrolein microsphere beads.

95% in 180 min. The same beads, after the elution of the rabbit anti-BSA, were used for studying the adsorption in vitro of anti-BSA from an immunized goat whole blood with the column at overlood conditions. Figure 10 shows that 37% of the anti-BSA was removed in 30 min; 57% in 60 min; 61% in 120 min, and 65% in 180 min. The blood compatibility of the beads was checked by counting during the experiment the red blood cells, white blood cells (WBC), and the thrombocytes (Table 6). Both the RBC and WBC counts decreased by 4%. The platelet count descreased by 20%.

Discussion

The search for new effective immunoadsorbents is still continuing. Several publications have reported on the synthesis of beads derivatized with aldehyde groups, suggesting their use in affinity chromatography. An aldehyde-activated

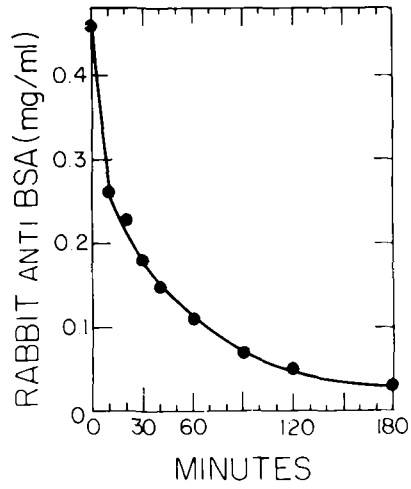

Fig. 9. Kinetics of adsorption in vitro of anti-BSA from an immunized rabbit serum: 100 mL serum were circulated at a flow rate of 62 mL/min through 17 g of BSA-derivatized agarose–polyacrolein microsphere beads.

polyacrylamide support was prepared by Fiddler and Gray (*21*, *22*) from a commercially available aminoethyl polyacrylamide gel. However, polyacrylamide has affinity towards many substances, and therefore meaningfully increased the nonspecific adsorption. Moreover, the porosity of polyacrylamide is low in comparison with agarose, and high molecular weight proteins cannot diffuse through this gel. Guesdon and Avrameas (*23*) prepared aldehyde activated polyacrylamide agarose beads. Unfortunately, the reported binding capacity of these beads towards proteins is low, 1–2 mg protein/mL beads. Wilchek et al. synthesized aldehyde-activated beads by reacting CNBr activated agarose with various polyhydrazides to give hydrazidoagarose. The hydrazidoagarose product was then subsequently converted to the aldehyde derivatized by its coupling with glutaraldehyde (*24*). The main disadvantage of this method is the use of the CNBr

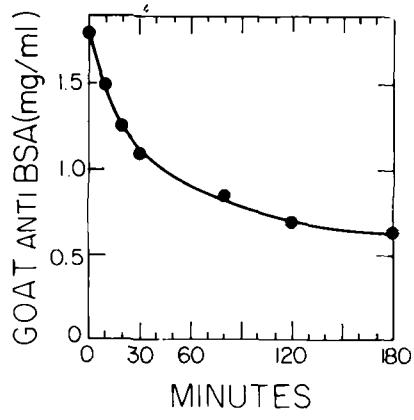

Fig. 10. Kinetics of adsorption in vitro of anti-BSA from an immunized goat whole blood: 110 mL citrated blood were circulated at a flow rate of 62 mL/min through 17 g of BSA-derivatized agarose–polyacrolein microsphere beads.

TABLE 6
Cell Counts of the Formed Elements of Freshly Drawn Citrated Goat Blood Pumped Throught the Agarose–Polyacrolein Microspheres Beads[a]

Time, min	RBC	WBC	Thrombocytes
0	16,990,000	6680	106,000
30	16,635,000	6742	
60	16,120,000	6080	
120	16,270,000	6348	
180	16,289,000	6428	85,000

[a]110 mL citrated blood were circulated at flow rate of 62 mL/min through 17 g of the BSA-derivatized beads.

activation method during the derivatization procedure. This activation method suffers from several disadvantages, such as high toxicity of the reagent CNBr, low yield of the reaction, and instability of the isourea bond formed by the CNBr activation method to hydrolysis and to nucleophilic substitution reactions (*22*). Recently, Wilchek et al. elucidated the mechanism of the CNBr activation method (*25*). Because of their studies, they were able to increase the yield of the reaction between amino ligands and polysaccharide resins and thereby the amount of CNBr required for the activation could be decreased significantly. However, the instability of the isourea bond is still creates in some systems a major difficulty.

Hereby, the synthesis of "naked" and encapsulated polyaldehyde microspheres is described. The formed beads are suggested as alternatives to the immunoadsorbents that are currently in use. The "naked" microspheres may be used for cell labeling purposes. The agarose–polyaldehyde microsphere beads, in the appropriate sizes, can be used for cell fractionation, affinity chromatography, and hemoperfusion. A further research especially related to the removal by hemoperfusion of various poisoning compounds with the the appropriate ligand-beads conjugate is going on in our laboratory.

Acknowledgments

The author is grateful to L. Marcus and M. Offarim for carrying out some of the experiments described in this review. The work describing the labeling of RBC with PGL microspheres has been carried out with Dr. A. Rembaum at Jet Propulsion Laboratory, California, Institute of Science, USA.

The research was supported partially by the G. Schmidt Fund for Industrial Research at the Weizmann Institute of Science and by the Ministry of Energy and Infrastructure, National Council for Research and Development, Israel.

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