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In vitro evaluation of biodegradable microspheres with surface-bound ligands

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Abstract

Protein ligands were conjugated to the surface of biodegradable microspheres. These microsphere–ligand conjugates were then used in two in vitro model systems to evaluate the effect of conjugated ligands on microsphere behavior. Microsphere retention in agarose columns was increased by ligands on the microsphere surface specific for receptors on the agarose matrix. In another experiment, conjugating the lectin *Ulex europaeus* agglutinin 1 to the microsphere surface increased microsphere adhesion to Caco-2 monolayers compared to control microspheres. This increase in microsphere adhesion was negated by co-administration of L-fucose, indicating that the increase in adhesion is due to specific interaction of the ligand with carbohydrate receptors on the cell surface. These results demonstrate that the ligands conjugated to the microspheres maintain their receptor binding activity and are present on the microsphere surface at a density sufficient to target the microspheres to both monolayers and three-dimensional matrices bearing complementary receptors.

Keywords: Caco-2; Microsphere; PLGA; Surface modification; Targeted drug delivery

1. Introduction

The ability to target biodegradable microspheres to specific cell types in vivo would greatly enhance the effectiveness of drug delivery by these vehicles. Targeting of microspheres to selected cell or tissue types would allow for concentration of the administered dose at the site of maximum effect [1]. Many targeted microsphere formulations currently being investigated achieve site specificity through addition of ligands to the microsphere surface that bind selectively to receptors on the surface of the targeted cell type [2–7]. This strategy draws support from the observation that a number of viral and bacterial pathogens are able to selectively bind to M cells, a cell type found in very small numbers in the intestinal epithelium [8]. This binding interaction permits easy host invasion via transcytosis by the M cell and is mediated by ligands on the surface of the pathogen. This observation suggests a clear opportunity for targeting orally administered vaccines to M cells [9]. Similarly, targeted microspheres could potentially be used for targeting tumors [10], sites of inflammation [2], or simply to increase microsphere adhesiveness to the intestinal lining for increasing uptake of released drugs into the circulation after oral administration [3,11,12].

Much of the work done to date investigating the effectiveness of microsphere targeting by surface-bound ligands has used polystyrene microspheres [4,7,12–14]. While effective for demonstrating the possibility of targeting microspheres to specific cell types, these nondegradable particles are inappropriate for drug delivery applications. A few techniques for modifying the surface of degradable poly(lactic-co-glycolic acid) (PLGA) microspheres have been described [3,5,6,15,16]. Coupling ligands to the surface of biodegradable microspheres can be difficult, as the particles are commonly made of materials that are chemically (and biologically) inert, aside from the polymer degradation reaction. Protocols for making PLGA microspheres by emulsion techniques commonly use surfactants to stabilize the emulsion. Most commonly, poly (vinyl alcohol) (PVA) is used as the stabilizer. When the emulsion is formed, the hydrophobic backbone of PVA partitions into the organic phase, while the hydrophilic hydroxyl side chains partition into the aqueous phase. Upon removal of the organic solvent, the hydrophilic side chains remain exposed on the surface of the newly formed microspheres. This surface layer is quite durable, remaining present

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even after repeated washing of the microspheres, suggesting that the stabilizer molecules are incorporated directly into the microsphere surface during production [17]. Other functional groups aside from hydroxyls, however, would be more suitable for many surface modification chemistries. Because of the long-lasting presence of stabilizer molecules at the microsphere surface, we have been able to use an alternate stabilizer, poly(ethylene-alt-maleic acid) (PEMA) to produce a microsphere formulation that allows durable binding of ligands to the microsphere surface by carbodiimide chemistry [18]. The carboxylic acid side chains of PEMA, present at the microsphere surface, can be linked to primary amine groups of the desired ligand, forming stable amide bonds. Here, we describe the effect of ligand conjugation on the behavior of the microspheres in two in vitro systems.

2. Methods

2.1. Materials

PLGA with a 50:50 lactide:glycolide ratio and inherent viscosity of 0.59 dL/g in hexafluoroisopropanol was from Birmingham Polymers (Birmingham, AL). Poly(ethylene-alt-maleic acid) (PEMA) was from Polysciences (Warrington, PA). *R*-Phycoerythrin-labeled goat antibodies specific for mouse IgG and rabbit IgG were from Molecular Probes (Eugene, OR). *Ulex europaeus* agglutinin 1 (UEA 1) and Vectashield with DAPI were from Vector Laboratories (Burlingame, CA). Rabbit antibody specific for goat IgG was from Zymed (South San Francisco, CA). Mouse serum IgG was from Rockland Immunochemical (Gilbertsville, PA). Paraformaldehyde was from Fisher Scientific (Fairlawn, NJ). Biotin–phycoerythrin conjugate (biotin-PE) was from Fluka (Buchs, Switzerland). Streptavidin, bovine serum albumin (BSA), fluorescein isothiocyanate conjugated to BSA (FITC-BSA), rabbit antibody specific for UEA 1, mouse antibody specific for BSA, goat antibody specific for mouse IgG, biotin–agarose beads, *N*-hydroxysuccinimidyl-agarose beads, L-fucose, guanidine HCl, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and ethanolamine were each from Sigma (St. Louis, MO). Vitrogen® bovine collagen was from Collagen Corp. (Palo Alto, CA). All other cell culture reagents and media components were from Gibco (Grand Island, NY).

2.2. Microsphere preparation

Microspheres were prepared using a double-emulsion technique. Four hundred milligrams of PLGA was dissolved in 2 ml dichloromethane in a glass tube. One hundred microliters of a 75 mg/ml solution of FITC-BSA in water was added to the polymer solution while gently vortexing the tube. The polymer solution was then sonicated for 15 s at 40% amplitude with a TMX 400 sonic disruptor (Tekmar, Cincinnati, OH) to create the primary emulsion. Four milliliters of an aqueous 1% w/v solution of PEMA was added to the tube and the sonication step was repeated. Immediately after the second sonication, the emulsion was poured into 100 ml of an aqueous 0.3% w/v PEMA solution, under rapid stirring with a magnetic stirrer. The resulting microspheres were stirred in this solution for 3 h in order to evaporate away the dichloromethane. The microspheres were then washed 3 times with Milli-Q water, resuspended in 4 ml Milli-Q water and lyophilized to dryness.

2.3. Microsphere morphology characterization

Microspheres were fixed to aluminum sample stubs with double-sided carbon tape, and sputter coated with gold for viewing by scanning electron microscopy. Micrographs were analyzed with Scion Image software (Scion Corporation, Frederick, MD) to determine microsphere size distribution.

2.4. Conjugation of ligands to microsphere surface

The technique used to conjugate ligands to the microsphere surfaces was a slightly modified version of a manufacturer's protocol for protein conjugation to carboxylated polystyrene microspheres (Polysciences Technical Data Sheet 238C). Briefly, microspheres were washed twice with 0.1 M sodium bicarbonate buffer, pH adjusted to 9.0. Microspheres were then washed 3 times with 0.02 M sodium phosphate buffer, pH=4.8. Microspheres were then resuspended to 20 mg/ml in phosphate buffer (this and all further microsphere concentrations are based on the initial microsphere mass, assuming no loss during the various conjugation steps). Microspheres were diluted down to 10 mg/ml with 2% w/v EDC in phosphate buffer. This suspension was incubated for 3 h at 25 °C on an end-to-end shaker to activate carboxylic acid groups on the microsphere surface. After incubation, microspheres were washed 3 times in phosphate buffer and resuspended to 10 mg/ml in borate buffer (0.2 M boric acid, pH adjusted to 8.5) with the desired protein ligand at a concentration of 320 μg/ml. Microspheres were incubated with ligand overnight at 25 °C on an end-to-end shaker. Microspheres were then centrifuged at 10,000×*g* for 5 min and supernatant removed. Microspheres were resuspended to 10 mg/ml in borate buffer, and 4 μl of 0.25 M ethanolamine in borate buffer was added for each milligram of microspheres. Microspheres were incubated for 30 min at 25 °C to quench any unreacted sites. Microspheres were then washed twice with Milli-Q water, resuspended in 4 ml Milli-Q water, and lyophilized to dryness.

2.5. Detection of ligand conjugation

Microsphere samples were analyzed for fluorescence on a FACScalibur flow cytometry system (Becton Dickinson, San Jose, CA). Aliquots of microspheres conjugated to BSA, UEA 1, and goat-anti-mouse antibody were suspended at 5 mg/ml in a 1:500 dilution of primary antibody specific for the conjugated protein. Microspheres were incubated for 1 h at room temperature. Microspheres were then washed 3 times with PBS and resuspended to 5 mg/ml in a 1:50 dilution in PBS of PE-conjugated secondary antibody specific for the primary antibody used in the first incubation. Microspheres were again incubated for 1 h at room temperature, and then washed 3 times

with PBS before analysis by flow cytometry. Microspheres with encapsulated FITC-BSA, but not incubated with antibodies, were used as controls to set compensation on the cytometer for detection of FITC fluorescence on detector channel FL2 (PE fluorescence). As a control for non-specific antibody adsorption to the microspheres, separate microsphere aliquots were incubated with primary and secondary antibodies as described above, but the primary antibody was specific for a protein other than the one conjugated to the microspheres.

For microspheres with streptavidin conjugated to the surface, aliquots were suspended at 5 mg/ml in PBS with 5 μg/ml biotin–PE. Microspheres were incubated for 1 h, washed 3 times with PBS, and then analyzed by flow cytometry. To measure non-specific binding of biotin-PE, control microspheres with BSA conjugated to the surface were also incubated with biotin-PE as described and analyzed by flow cytometry.

2.6. Agarose bead preparation

Fifteen milliliters of a 4% suspension of *N*-hydroxysuccinimidyl-agarose beads were washed with 30 ml of ice-cold Milli-Q water on a sintered glass filter. The beads were then transferred to a glass bottle and resuspended in 6 ml of a 1 mg/ml solution of mouse IgG in 0.05M sodium bicarbonate buffer (pH = 8.5). The beads were incubated on an end-to-end mixer for 6 h at 4 °C. After this incubation, 3 ml of 1 M ethanolamine in bicarbonate buffer was added to the beads, which were then incubated for 1 h to quench any remaining unreacted sites on the beads. The mouse IgG-conjugated agarose beads were then washed 5 times with cold PBS (15 ml/wash), and then stored in PBS as a 4% suspension at 4 °C until use.

2.7. Retention of microspheres on agarose columns

A 4% suspension of either biotin–agarose or mouse IgG–agarose (1.5 ml) was added to glass columns (1 cm diameter) and allowed to settle overnight. After washing the column with 2 ml PBS, 1 ml of a 5 mg/ml suspension of microspheres with surface conjugated BSA, streptavidin, or goat antibody specific for mouse IgG was carefully added to the top of the agarose bed, followed by 20 ml PBS. After the PBS was eluted, 15 ml of 6 M guanidine HCl (pH adjusted to 7.5) was run through the column. Eluate was collected in roughly 1-ml fractions, and the fluorescence intensity of each fraction was measured on a Perkin Elmer LS 55 luminescence spectrometer (Shelton, CT) with excitation and emission wavelengths of 488 and 520 nm, respectively. Fluorescence intensity of each fraction was converted to microsphere concentration by comparison to standard curves generated for each microsphere–ligand pair in both PBS and 6 M guanidine HCl.

2.8. Caco-2 culture

The method for growing Caco-2 cells on porous filters has been described elsewhere [19,20]. Briefly, Caco-2 intestinal epithelial cells of subclone BBe1 (ATCC, Rockville, MD) were propagated in 75-cm^2 flasks. The cells were grown in DMEM (4.5 g/l glucose, 10 mM HEPES), supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% nonessential amino acids, and 40 μg/ml human transferrin. Cells were split 1/8 when they reached confluency (typically 6–7 days), and the medium was changed every 2 or 3 days. Polycarbonate Transwell filters (6.5 mm diameter, 3 μm pore size) (Corning Costar, Acton, MA) were inverted and placed in open Petri dishes. The filters were coated with 1 mg/ml collagen, 15 μl per filter. The filters were left in a sterile hood overnight to dry, and then placed in PBS until use. The collagen-coated Transwell filters were inverted in Petri dishes and seeded with 100 μl media containing 2.5×10^5 cells. The Petri dishes were covered and incubated overnight at 37 °C in a 5% CO_2 incubator to allow cell attachment. Filters were then returned to normal orientation in 24-well plates. 500 μl media was added to the lower (apical) chamber, and 125 μl media added to the upper (basolateral) chamber. Medium was changed every 2 days, and cell monolayers were grown for 21 days before use in microsphere adhesion experiments. Transepithelial electrical resistance was measured periodically with an EVOM™ voltmeter/ohmmeter (World Precision Instruments, Sarasota, FL).

2.9. Microsphere adhesion study

Microspheres with surface-conjugated BSA or UEA 1 were suspended in Caco-2 medium at 1 mg/ml. Fucose (500 mM) was added to an aliquot of the UEA 1-conjugated microsphere suspension, in order to inhibit lectin-specific binding of microspheres to the Caco-2 monolayers. 600-μl aliquots of the microsphere suspensions were added to 24-well plates. Caco-2 monolayers on Transwell filters were placed in the wells, and 125 μl of fresh media was added to the basolateral chambers. Plates were covered and incubated at 37 °C in 5% CO_2 for 1 h. After incubation, filters were transferred to new 24-well plates, and media in the basolateral chamber was removed. Filters were moved to wells containing 500 μl 2% paraformaldehyde in PBS, and 125 μl of paraformaldehyde solution was added to basolateral chambers. Filters were incubated for 30 min and then transferred to wells containing PBS. Filters were cut out of their supports with a scalpel and mounted on microscope slides in Vectashield with DAPI.

2.10. Microsphere counting

Cell monolayers were viewed with a Zeiss Axiovert 200 inverted microscope (Thornwood, NY) with fluorescent filters. Monolayers were viewed with a 20× objective, and 5 digital images were captured for each filter. Microspheres per unit area were determined for each filter by visual counting of microspheres in each image. Monolayer filter order was randomized for both the image capture and microsphere counting steps, which were performed by

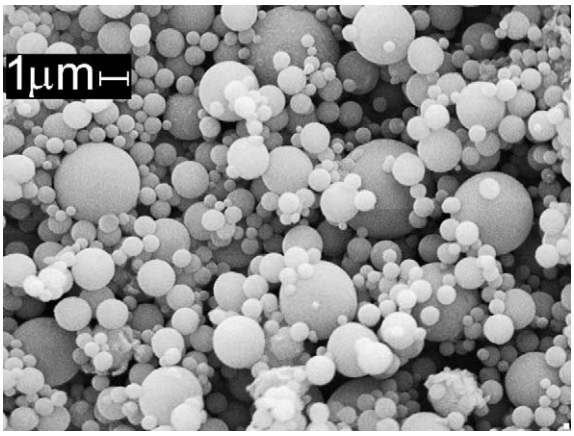

Fig. 1. Scanning electron micrograph of PLGA/PEMA microspheres with encapsulated FITC-BSA.

operators unaware of which microsphere type each individual filter was incubated with.

3. Results

Scanning electron microscopy indicated that the PLGA/PEMA microspheres had smooth, unbroken surfaces (Fig. 1), and a mean diameter of 813 nm. Each of the selected protein ligands was successfully conjugated to the surface of the PLGA/PEMA microspheres, as shown in Fig. 2. The presence of the ligand was indicated by the rightward shifts in the fluorescence histograms for microspheres incubated with fluorescent labels specific for the particular ligand. For ligands detected with labeled antibodies (Fig. 2A, C, D), controls for nonspecific antibody adsorption to the microsphere surface (black lines) had nearly equal fluorescence intensity as microspheres not incubated with labeled antibodies at all (gray lines). For microspheres conjugated to streptavidin (Fig. 2B), the BSA-conjugated control for nonspecific adsorption of the biotin-PE label (black line) shows a significant increase in fluorescence intensity compared to unlabeled control (gray line). In this case, however, the fluorescence intensity of the streptavidin-conjugated microspheres was still significantly greater than that for the BSA-conjugated control.

When ligand-conjugated microspheres were eluted from agarose columns, a significant quantity of the loaded microspheres were retained on the column, regardless of the particular microsphere ligand or agarose-bound receptor (Figs. 3 and 4). The highest fraction of microspheres recovered for any of the trials was approximately 55%. The fraction of microspheres retained increased markedly when the microsphere ligand was matched to the appropriate agarose-bound receptor molecule. For the streptavidin-conjugated microspheres, total retention was roughly 50% on the agarose–mouse IgG column, but increased to 95% when the micro-

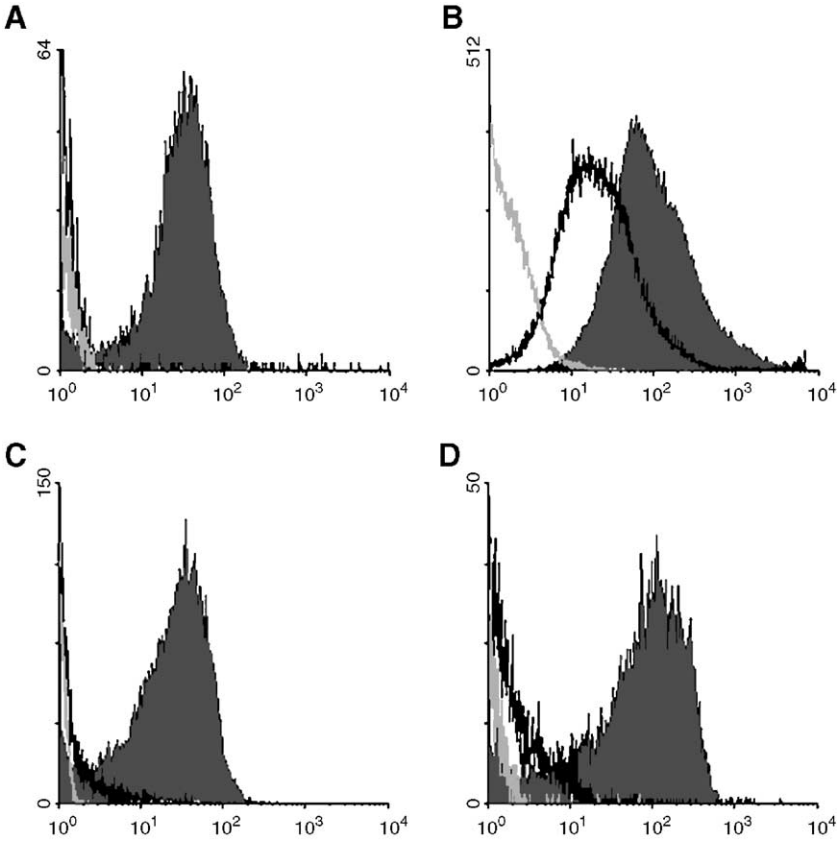

Fig. 2. Detection by flow cytometry of (A) BSA, (B) streptavidin, (C) goat-anti-mouse IgG antibody, and (D) UEA 1 conjugated to microspheres. Data are histograms of PE fluorescence. Gray line: microspheres before incubation with fluorescent label. Black line: controls for nonspecific binding of fluorescent label to microspheres. Solid fill: microspheres incubated with fluorescent labels specific for conjugated protein.

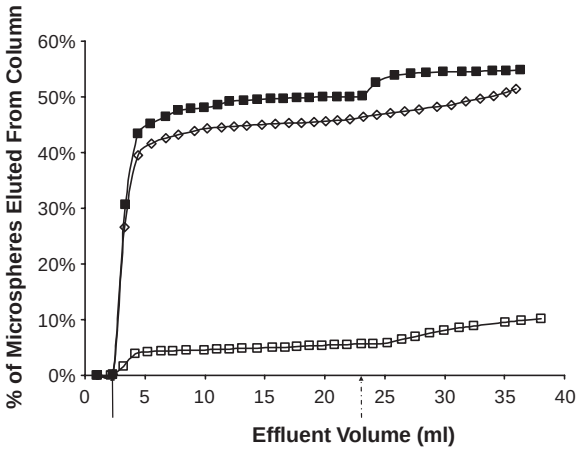

Fig. 3. Retention of streptavidin-conjugated microspheres on agarose columns. □=streptavidin-conjugated microspheres, agarose–biotin column. ■=strepta-streptavidin-conjugated microspheres, agarose–mouse IgG column. ◊=BSA-conjugated microspheres, agarose–biotin column. Solid arrow: addition of microspheres to column. Dotted arrow: switch in elution buffer to 6 M guanidine HCl.

spheres were introduced onto an agarose–biotin column (Fig. 3). Microspheres with BSA conjugated to the surface were retained at a level of approximately 45% on the agarose–biotin matrix, indicating that the high level of retention for streptavidin-conjugated microspheres on the agarose–biotin column is due to specific interaction of microsphere-bound streptavidin with biotin coupled to the agarose matrix. Similar results were obtained with microspheres conjugated to goat antibody specific for mouse IgG (Fig. 4). On the agarose–biotin matrix, approximately 80% of the microspheres were retained on the column, whereas nearly 95% were retained on the agarose–mouse IgG column.

Changing buffer from PBS to 6 M guanidine HCl during elution did not result in release of additional BSA- or streptavidin-conjugated microspheres from either the agarose–biotin or agarose–mouse IgG columns (Fig. 3). For the goat antibody-conjugated microspheres, the change in buffer was

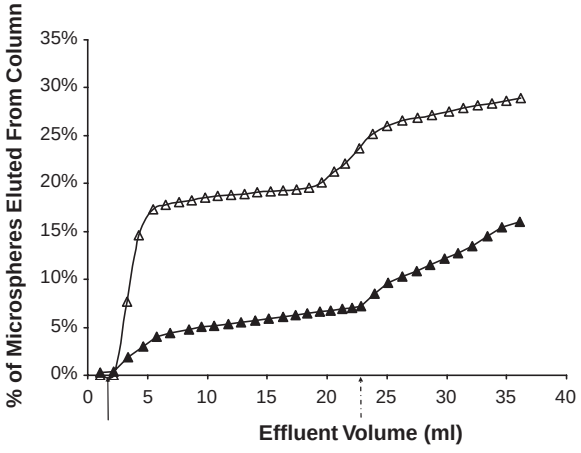

Fig. 4. Retention of goat anti mouse IgG-conjugated microspheres on agarose columns. △=agarose–biotin column. ▲=agarose–mouse IgG column. Solid arrow: addition of microspheres to column. Dotted arrow: switch in elution buffer to 6 M guanidine HCl.

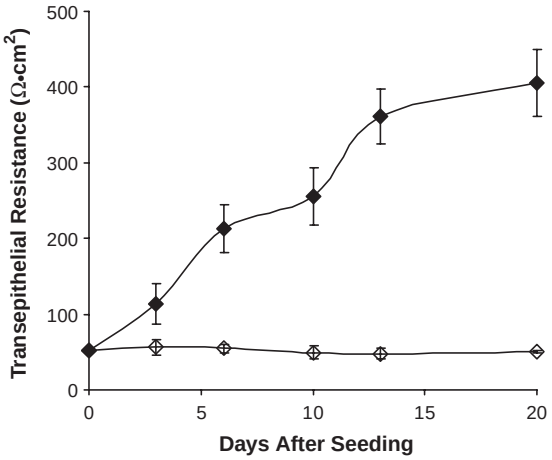

Fig. 5. Transepithelial electrical resistance measurements for Caco-2 cells grown on Transwell filters. ♦=filters with cells. ◊=blank filters. Data are means±standard deviation for wells in each group ($n=12$ for filters with cells, $n=4$ for blank filters).

followed by elution of approximately 10% of the microspheres from the agarose–mouse IgG column (Fig. 4). These microspheres showed a similar two-step elution profile on the agarose–biotin column, but the increase in elution rate started before the switch of elution buffer to 6 M guanidine HCl, suggesting that it was caused by factors other than the change in elution buffer.

Caco-2 cells grown on Transwell filters formed differentiated monolayers within 3 weeks after seeding. Transepithelial electrical resistance reached approximately 400 Ω cm^2 in this time period (Fig. 5), which is consistent with other reports for the resistance of well-differentiated monolayers of Caco-2 cells [19]. The lectin UEA 1 has been used for labeling the apical surface of Caco-2 monolayers [21], making it a suitable molecule for evaluating the ability of microsphere–ligand conjugates to target surface receptors on monolayers of Caco-2 cells. UEA 1 binds to carbohydrates with terminal fucose residues, allowing the UEA 1-carbohydrate binding interaction to be inhibited through the addition of fucose to the incubation medium. BSA-conjugated microspheres adhered to the Caco-2 monolayers at a density of 710±205 microspheres/mm^2

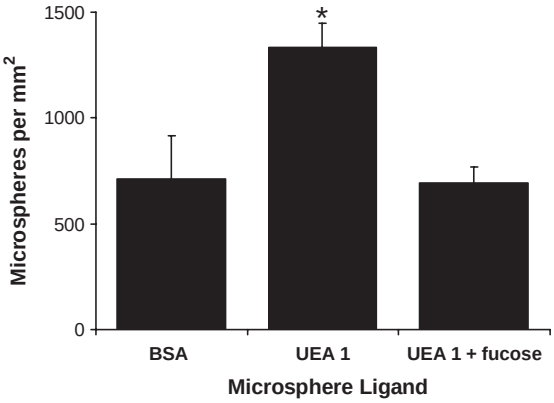

Fig. 6. Effect of microsphere surface ligands on adhesion to Caco-2 monolayers. *$P<0.02$ for two-tailed Student's t-test comparison of BSA and UEA 1-conjugated microspheres.

(mean ± S.D. for 3 filters) (Fig. 6). Microspheres with UEA 1 as the surface-bound ligand showed nearly double the adherence density, 1330 ± 118 microspheres/mm^2. Incubating the UEA 1-conjugated microspheres with monolayers in medium containing 500 mM fucose inhibitor negated the targeting effect of the ligand, as the adhesion density was reduced to 690 ± 78 microspheres/mm^2. This adhesion density was essentially equal to that of the BSA-conjugated microspheres, indicating that the observed increase in adhesion density for UEA 1-conjugated microspheres was due to specific interactions of the UEA 1 with receptors on the apical surface of the epithelial cell monolayers.

4. Discussion

A variety of protein ligands were covalently coupled to PLGA/PEMA microspheres by carbodiimide conjugation. Successful ligand conjugation was verified by flow cytometry of microspheres incubated with ligand-specific fluorescent labels. For the control sample of UEA 1 conjugated microspheres incubated with nonspecific antibody (Fig. 2D, black line), a slight increase in fluorescence is seen as compared to the other samples incubated with antibody nonspecific for the conjugated ligand (Fig. 2A, C). The nonspecific control antibody used with the UEA 1-conjugated microspheres was specific for BSA, making it likely that the observed slight increase in fluorescence is due to anti-BSA antibody binding to FITC-BSA entrapped at the surface of the microspheres. This control is significant with regard to the BSA-conjugated microspheres (Fig. 2A) as well, because it demonstrates that the level of anti-BSA antibody binding to FITC-BSA is relatively low, and that therefore, the substantial rightward shift in fluorescence seen in Fig. 2A is due to antibody binding to surface-conjugated BSA, and not to entrapped FITC-BSA.

When the microspheres were loaded onto agarose columns, a significant fraction of the microspheres was retained in the column, regardless of the particular combination of microsphere-coupled ligand and agarose-bound receptor. This result is not surprising, as the agarose beads form a tight matrix that could be expected to physically impede the flow of microspheres no matter the particular ligand/receptor pair being evaluated in a given trial. The addition of denaturing 6 M guanidine HCl to the columns did not result in any additional elution of BSA-conjugated microspheres from the agarose–biotin column or streptavidin-conjugated microspheres from the agarose–mouse IgG column. This observation supports the explanation that much of the microsphere load placed on each column becomes physically trapped within the column.

By matching the ligand on the microspheres with the proper receptor on the agarose matrix, however, microsphere retention can be significantly increased, as demonstrated by the substantial increase in the fraction of streptavidin-conjugated microspheres retained on an agarose–biotin column (Fig. 3, open squares). Elution with 6 M guanidine HCl did not cause significant release of streptavidin-conjugated microspheres from this column, which is not surprising given the high affinity of streptavidin–biotin interaction. To evaluate the feasibility of targeting microspheres to the column-bound receptors using a ligand/receptor pair with a more typical binding affinity, such as an antibody–antigen pair, we coupled mouse IgG to agarose and goat-anti-mouse IgG to microspheres. Antibodies have previously been generated for the purpose of targeting microspheres to specific cell types [7], suggesting that this is a reasonable model system which could later be substituted with microsphere-bound antibodies specific for clinically relevant receptor molecules. Fig. 4 shows that retention of the goat-anti-mouse IgG-conjugated microspheres was increased on the agarose–mouse IgG column as compared to agarose–biotin. In contrast to the streptavidin–biotin system, the change in elution buffer from PBS to 6 M guanidine HCl resulted in the steady release of microspheres from the column (Fig. 4, closed symbols). The fraction of microspheres retained on the column after elution with the denaturing buffer was nearly equal to that for the same microspheres on the agarose–biotin column (open symbols), strongly suggesting that the increase in retention of these microspheres on the agarose–mouse IgG column was due to ligand–receptor interactions. Interestingly, the agarose–biotin column also released microspheres during the elution phase. This release began before the switch in elution buffer from PBS to 6M guanidine HCl, however, and tapered off after the passage of 5 ml of buffer through the column, making it unlikely that the release of microspheres was due to disruption of ligand–receptor interaction.

Compared to the agarose columns, the experiment with Caco-2 cell monolayers provides a very different environment for evaluating the ability to target PLGA/PEMA microspheres to a selected surface. Caco-2 monolayers have been incubated with ligand-bearing microspheres before [3,6], with the result being increased association of the targeted microspheres with the monolayers as compared to untargeted microspheres. In these studies, the monolayers were at the bottom of the incubation chambers, so that microspheres settling to the bottom of the chamber during incubation had direct extended contact with the monolayer surface. In the system described here, the cell monolayer is inverted and suspended at the top of the incubation chamber, so that the monolayer only encounters microspheres freely suspended in solution. The results show that conjugating the ligand UEA 1 to the microsphere surface doubles the adhesion density of the microspheres to the apical surface of the Caco-2 cells. This increase was due to specific interaction of the microsphere-bound lectin with receptors on the apical surface of the monolayer, as incubation of the lectin-bearing microspheres with the cells in the presence of the inhibitory sugar L-fucose reduced adhesion density of the microspheres to that of BSA-conjugated control microspheres. Confocal microscopy indicated that particles were adherent to the apical surface of the cells, but not endocytosed in significant numbers (data not shown). The strong dependence of uptake by Caco-2 monolayers on particle size [22] suggests that for microspheres of the size used in this study, a different model system would be more suitable for evaluating the ability of the surface-bound ligands to increase microsphere uptake. The Caco-2 model system is also not fully representative of the

conditions in vivo, in that it eliminates the continually renewing mucus gel layer present along the gastrointestinal tract. However, this model system demonstrates that microspheres freely suspended in solution can be targeted to epithelial cell surfaces with appropriate ligands.

5. Conclusions

The experiments described here demonstrate that PLGA/PEMA microspheres can be conjugated to a variety of protein ligands, which alter the interaction of the microspheres with surfaces expressing appropriate receptors. The model systems investigated here provide two different environments for examining microsphere adhesion under conditions that replicate different aspects of biological recognition. The increased retention of ligand-bearing microspheres in agarose columns suggests that microspheres could be targeted to cells within densely packed environments where microspheres are in constant close contact with receptor-bearing surfaces. Similarly, the increase in adhesion of cell surface receptor-targeted microspheres on Caco-2 cell monolayers shows that microspheres free-floating in suspension can have their interactions with surfaces altered through the use of appropriate surface-conjugated ligands. These model systems are a simplification of the relevant environments in vivo, but the results shown here demonstrate the feasibility of targeting PLGA/PEMA microspheres to selected surfaces. Experiments in vivo should indicate what, if any, modifications to this strategy will be required to enable ligand-receptor mediated targeting of microspheres to increase their effectiveness as drug delivery vehicles.

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