Mucoadhesive Microdiscs Engineered for Ophthalmic Drug Delivery: Effect of Particle Geometry and Formulation on Preocular Residence Time

Young Bin Choy,[1] *Jung-Hwan Park,*[2] *Bernard E. McCarey,*[3] *Henry F. Edelhauser,*[*,3] *and Mark R. Prausnitz*[*,1]

Purpose. To test the hypothesis that mucoadhesive microdiscs formulated in a rapidly dissolving tablet can increase preocular residence time.

Methods. Microparticles smaller than 10 μm in diameter were fabricated by emulsification with poly(lactic-co-glycolic acid) as a core material and, in some cases, poly(ethylene glycol) as a mucoadhesion promoter. To examine the effect of particle geometry, microparticles were also cut to have flat surfaces (i.e., microdiscs) and were compared with spherical particles (i.e., microspheres). In vitro mucoadhesion of microparticles was tested on a mucous layer under shear stress, mimicking the human blink. The resultant microparticles were also formulated in two dosage forms, an aqueous suspension and a dry tablet, to test the effect of formulation on the retention capacity of microparticles on the preocular space of rabbits in vivo.

Results. Mucoadhesive microdiscs adhered better to the simulated ocular surface than did other types of microparticles. When a dry tablet embedded with mucoadhesive microdiscs was administered in the cul-de-sac of the rabbit eye in vivo, these microdiscs exhibited longer retention than the other formulations tested in this study. More than 40% and 17% of mucoadhesive microdiscs remained on the preocular surface at 10 minutes and 30 minutes after administration, respectively. Fluorescence images from the eye surface showed that mucoadhesive microdiscs remain for at least 1 hour in the lower fornix.

Conclusions. This study demonstrated that mucoadhesive microdiscs formulated in a dry tablet can achieve a prolonged residence time on the preocular surface and thus are a promising drug delivery system for ophthalmic applications. (*Invest Ophthalmol Vis Sci.* 2008;49:4808–4815) DOI:10.1167/iovs.08-2515

From the [1]School of Chemical and Biomolecular Engineering and Center for Drug Design, Development and Delivery, Georgia Institute of Technology, Atlanta, Georgia; [2]Department of BioNano Technology, Kyungwon University, Seongnam, Gyunggi-do, Korea; and [3]Emory Eye Center, Emory University, Atlanta, Georgia.

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*Each of the following is a corresponding author: Mark R. Prausnitz, School of Chemical and Biomolecular Engineering and Center for Drug Design, Development and Delivery, Georgia Institute of Technology, Atlanta, GA 30332-0100; prausnitz@gatech.edu.

Henry F. Edelhauser, Emory Eye Center, Emory University, 1365 Clifton Road, NE, Suite B2600, Atlanta, GA 30322; ophthfe@emory.edu.

Topical drug administration is widely used to treat various eye disorders but is nonetheless limited by low drug bioavailability because of rapid clearance by blinking, tear drainage, and absorption into the conjunctival vasculature.[1,2] Approximately 75% of the applied dose is known to be lost almost instantly through nasolacrimal drainage on topical administration with eyedrops; thus, less than 5% of the applied drug is ultimately bioavailable in the anterior segment of the eye. To maintain therapeutic drug levels, frequent administration or large doses are often required, which can reduce patient compliance, cause local side effects, and lead to undesirable systemic exposure. These problems could be addressed by a drug delivery system that remains on the preocular surface and slowly delivers drug for a prolonged period.

Various attempts have been made to increase drug residence time on the preocular surface. Drug solutions formulated with viscosity enhancers, such as carboxymethyl cellulose, hydroxypropyl cellulose, poly(vinylpyrrolidone), and polyvinyl alcohol, slowed drug clearance and increased drug bioavailability in rabbits.[3–5] However, despite their prolonged residence time, the rate of drug release was not controlled, which often caused a rapid drug efflux out of the polymer network. Gels, ointments, and ocular inserts can increase residence time and also provide sustained release from a drug depot typically in the conjunctival sac.[6–8] However, discomfort, ocular irritation, and blurred vision are often associated with such systems, which can adversely affect patient compliance.

Across medicine, biocompatible microparticles are widely used for controlled-release drug delivery because of their ease of fabrication, simplicity of administration, and possible use in localized and targeted delivery.[9] Drug molecules encapsulated in microparticles are released by drug diffusion, polymer degradation, or both, resulting in sustained release. In addition, microparticles can be easily formulated into a variety of dosage forms, such as suspensions, tablets, and gels, which makes them applicable to many different localized or systemic treatments. Various therapeutic agents, such as acyclovir, dexamethasone, piroxicam, and vancomycin, have been delivered topically to the eye through microparticles and exhibit enhanced targeting and drug bioavailability.[10,11]

Microparticles designed to have increased residence time at the preocular surface would further improve drug retention on the eye and, hence, would be more effective for the therapy of ocular diseases, such as glaucoma, keratoconjunctivitis sicca, and dry eye disease. Microparticles have been made using mucoadhesive polymers to enhance their adhesion to the mucous layer on the preocular surface.[12–15] Microparticles formulated using numerous mucoadhesive polymers, such as chitosan, pectin, hyaluronic acid, sodium carboxymethylcellulose, and polyacrylic acid, have achieved slower preocular clearance and increased drug bioavailability.[14–17]

In this study, we proposed ophthalmic drug delivery using microparticles engineered to optimize three design parameters: mucoadhesion, geometry, and dosage form. We hypoth-

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esized that mucoadhesive microdiscs formulated in a rapid-dissolving tablet can increase preocular residence time. Although previous studies have examined the use of mucoadhesive microparticles, this is the first study to consider microparticle geometry (i.e., in particular a flat, microdisc geometry) and to embed microparticles in a rapidly dissolving tablet formulation for ophthalmic delivery. These attributes were designed to promote mucoadhesion by increasing contact area between the microparticles and the preocular surface and by reducing convective forces of tear fluid flow that carry particles away.

Microparticles were made of poly(lactic-co-glycolic acid) (PLG) and poly(ethylene glycol) (PEG), which were used as the core material[11] and the mucoadhesion promoter,[18] respectively. Mucoadhesiveness of PEG is known to be generated by hydrogen bonds with mucins that contain hydroxyl, carboxylic acid, and sulfate groups.[19] These materials are also widely established as safe for various medical applications.[20,21]

Two different shapes of microparticles—spheres and discs—were compared to examine the effect of particle geometry. We were interested in studying microdiscs because their flattened surfaces can increase the frictional contact area with the preocular mucous surface and can reduce hydrodynamic resistance when aligned parallel to the direction of tear flow.[22] The diameters of microparticles were also controlled to be smaller than 10 μm to avoid possible eye irritation and to allow safe clearance through the lacrimal canals, which measure 300 to 500 μm in diameter.[2]

Finally, microparticles were formulated in aqueous suspension and as a rapidly dissolving dry tablet. We were interested in studying the dry tablet formulation because tablet dissolution would increase local viscosity, which should facilitate initial contact between mucoadhesive microparticles and the preocular mucous surface, allowing particles to become anchored before exposure to the full hydrodynamic shear of tear fluid flow. Dry tablets were prepared by embedding microparticles in mannitol, which has been widely used in ophthalmic formulations.[23]

Materials and Methods

Materials

PLG (50:50; lot number LP-353; average MWt 15 kDa; IV 0.15–0.25 dL/g) and PEG (average MWt 6 kDa) were purchased from Lakeshore Biomaterials (Birmingham, AL) and Acros Organics (Morris Plains, NJ), respectively. Polyvinyl alcohol (PVA; 87%–89% hydrolyzed; MWt 31–50 kDa), mannitol, Nile Red, and type 2 mucin from porcine stomach were obtained from Sigma Chemical (St. Louis, MO). Methylene chloride and acetone in high purity were purchased from Fisher Scientific (Pittsburgh, PA). Hanks buffered saline solution (HBSS) was obtained from Mediatech (Manassas, VA). Optimal cutting temperature (OCT) compounds, generally used as embedding media for frozen tissue specimens, were obtained from Sakura Finetek USA (Torrance, CA) and used as the microparticle embedding media for cryosectioning. Proparacaine HCl (0.5% ophthalmic solution) was purchased from Bausch & Lomb (Tampa, FL). Anesthetics for subcutaneous injection to the rabbits (ketamine, xylazine, and acepromazine) were obtained from Fort Dodge Animal Health (Fort Dodge, IA), Lloyd (Shenandoah, IA), and Boehringer Ingelheim (St. Joseph, MO), respectively.

Fabrication of Microparticles

Four different kinds of microparticles were prepared: microspheres of PLG (PLG MS), microspheres of a blend of PLG and PEG (PLG/PEG MS), microdiscs (cut microspheres) of PLG (PLG MD), and microdiscs of a blend of PLG and PEG (PLG/PEG MD). To make PLG and PLG/PEG microparticles, a polymer solution was prepared by dissolving either 200 mg PLG or a mixture of 200 mg PLG and 20 mg PEG in 2 mL methylene chloride. Two milligrams of the fluorescent tracer Nile Red was also dissolved in these solutions to label the microparticles.

Each resultant solution was then dispersed in 15 mL water containing PVA (2% wt/vol) and was sonicated at 100 W for 10 seconds to obtain oil-in-water emulsion droplets of appropriate size (ultrasonic converter, CV33; power supply, VC505; Sonics & Materials, Newtown, CT). The emulsion was stirred overnight to evaporate the methylene chloride and to obtain solid microparticles. The solid microparticles were filtered (nylon net filter, 11-μm pore; Millipore, Billerica, MA) to remove particles larger than 10 μm, suspended in OCT compound, and frozen at −75°C for 2 hours. The distribution of PEG inside the microparticles was expected to be fairly homogeneous because of the quick polymer-phase inversion of small microparticles (<10 μm) during solvent evaporation.[24]

To prepare microspheres, the frozen OCT compound was thawed at room temperature without further treatment, washed thoroughly with DI water, and freeze dried (VirTis Advantage, Gardiner, NY) for 2 days. To fabricate microdiscs, a piece of the frozen OCT compound embedded with microparticles was sectioned into 1 μm–thick films on a cryostat (HM 560 Cryo-Star; Microm International, Waldorf, Germany), which were then thawed, washed with DI water, and freeze dried.

Preparation of Microparticle Formulations

Microparticles were formulated in two different dosage forms, an aqueous suspension and a dry tablet. Preparing each of the four microparticle types in each of these two dosage forms yielded eight different microparticle formulations: PLG MS suspension, PLG MS tablet, PLG MD suspension, PLG MD tablet, PLG/PEG MS suspension, PLG/PEG MS tablet, PLG/PEG MD suspension, and PLG/PEG MD tablet. Suspensions were prepared in 100 μL HBSS with concentrations of 10 mg/mL and 5 mg/mL microparticles for the in vitro and in vivo mucoadhesion tests, respectively. To fabricate a dry tablet used for in vivo tests, a homogeneous mixture of 20 mg mannitol and 0.5 mg microparticles was hand pressed in a 3 mm–diameter bore formed in a 1 cm–thick acrylic sheet (Goodfellow, Oakdale, PA). In this way, each of the eight formulations used for the in vivo study was prepared to contain 0.5 mg microparticles for single use.

Characterization of Microparticles

The size and shape of microparticles were examined using a scanning electron microscope (LEO 1530; Carl Zeiss SMT, Peabody, MA). A droplet of an aqueous suspension of microparticles was placed on a small piece of silicon wafer attached to the top of a scanning electron microscope sample holder. Samples were dried with desiccant overnight and sputter-coated with gold. Microparticles were imaged at 2 to 10 kV. A Coulter counter (Multisizer 3; Beckman Coulter, Fullerton, CA) equipped with a 50-μm aperture was used to determine the size distribution of microparticles. Microparticles were suspended in electrolyte (Isoton; Beckman Coulter) with a dispersant (Dispersant IA; Beckman Coulter) to prevent aggregation. At least 5000 particles were counted for each sample.

Nile Red was used as a fluorescent tracer to facilitate measuring the amount of microparticles remaining on the preocular surface over time. Although the initial loading of Nile Red (1% wt/wt) was known, the amount of Nile Red actually entrapped in microparticles was also measured by dissolving 4 to 5 mg Nile Red–loaded microparticles in 10 mL acetone with strong agitation and then measuring the Nile Red concentration in the solution using calibrated fluorescence spectroscopy (Photon Technology International, Birmingham, NJ).

A differential scanning calorimeter (DSC, STA409; Netzsch, Exton, PA) was used for thermal analysis of microparticles. Briefly, 5 to 15 mg pure PLG or pure PEG or a microparticle formulation was placed in an aluminum pan with hermetic sealing and heated from 25°C to 150°C at a rate of 10°C/min. The locations of endothermal peaks were determined to identify the peak shift associated with the presence of PEG in microparticles made of a blend of PLG and PEG.

In Vitro Mucoadhesion Study

Microparticle adhesion to the mucous layer was examined under a condition mimicking the shear force applied to the preocular surface during the blink of a human eye (150 dyne/cm^2).[25] A mucous layer was prepared on a hydrophilic membrane measuring 1 cm × 1 cm (cellulose nitrate membrane, 0.45-μm pore size; Whatman, Florham Park, NJ) that was first soaked for 2 hours with an aqueous mucin solution prepared in HBSS (0.1% wt/vol).[15] Then, 20 μL of a 10 mg/mL suspension of microparticles was applied as a single drop at the center of the membrane. After 30 seconds, the membrane was placed on a stress/strain-controlled rheometer (MCR 300; Anton Paar, Ashland, VA) to apply continuous shear stress in the range of 140 to 160 dyne/cm^2 for 60 seconds, which was performed using parallel surfaces separated by 50 mm and filled with HBSS. Peltier temperature control was used to maintain the temperature of HBSS at 33°C to 34°C during the experiment because the temperature of tear fluid is known to be approximately 2° to 3°C lower than the body core.[25] After applying the shear stress, the microparticles remaining on the membrane were dissolved in acetone under strong agitation and quantified using calibrated fluorescence spectroscopy (Photon Technology International). Experiments were performed in triplicate for each type of microparticle.

In Vivo Mucoadhesion Study

In vivo studies were performed using male New Zealand White rabbits (Myrtle's Rabbitry, Thompsons Station, TN) weighing 3.4 to 3.6 kg and without any ocular defects. All experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Georgia Institute of Technology. Rabbits were housed singly in a standard cage without any restriction of food and water.

To administer the microparticle formulation, each rabbit was removed from the cage and placed in a soft cloth restraint bag (Four Flags Over Aspen, Janesville, MN) opened just enough to expose its head. Each rabbit lay on its stomach during this whole procedure. A total of 100 μL microparticle suspension (5 mg/mL) was added topically to the rabbit eye by four consecutive administrations of 25 μL each at 1-minute intervals, which collected to a large extent almost instantly in the lower cul-de-sac because of gravity. Alternatively, a tablet was placed in the lower cul-de-sac, and the eyelid was closed manually for 5 minutes so that the tablet could completely dissolve in the tear fluid. The same dose of microparticles (0.5 mg) was administered using suspensions and tablets. No anesthesia was required for these procedures, which were well tolerated by the rabbits. After administration, the rabbit was placed back in the cage and allowed to move freely until samples were collected.

Microparticles remaining on the preocular surface were collected 10 minutes, 30 minutes, or 60 minutes after administration of the suspension or complete dissolution of the tablet. Only one sampling time was used per animal. The preocular surface was wiped thoroughly using a cellulose surgical sponge (Ultracell Medical Technologies, North Stonington, CT) while the eye was locally anesthetized with topical administration of 25 μL of 0.5% proparacaine HCl ophthalmic solution. The surgical sponge containing microparticles was then submerged in acetone for 1 hour, and the acetone was assayed with calibrated fluorescence spectroscopy (Photon Technology International) to determine the amount of microparticles collected. For each sample and each time after administration, at least three eyes were selected randomly among eight different eyes (four rabbits were used in this study). The interval between two consecutive sample collections for each eye was a minimum of 48 hours to permit the animals to recover from any experimental stress.

To obtain images of microparticles on the eye, each rabbit was anesthetized with a single subcutaneous injection of a cocktail of 17.5 mg/kg ketamine, 8.5 mg/kg xylazine, and 0.5 mg/kg acepromazine. One or two additional boosters (5.6 mg/kg ketamine, 2.8 mg/kg xylazine, and 0.16 mg/kg acepromazine) were used if needed. Each of the eight microparticle formulations was administered under the same condition as the quantitative analysis described. The images of microparticles on the preocular surface were obtained using a fluorescence stereomicroscope (SZX 12; Olympus America, Center Valley, PA) 10 minutes, 30 minutes, and 60 minutes after administration while the rabbit lay on its side. Locations imaged were cornea, lacrimal caruncle, upper fornix, and lower fornix. Between the intervals of imaging, the rabbit lay on its stomach, and the eye was manually blinked once per 5 minutes as if the rabbit were awake.

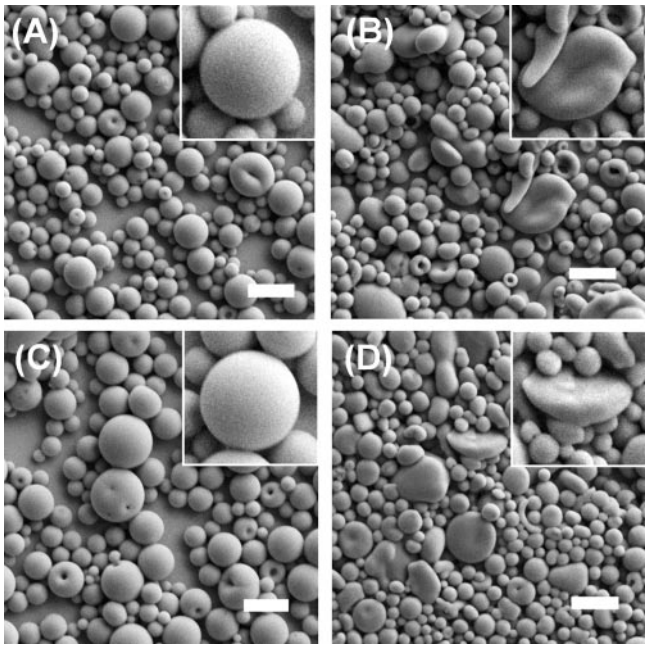

Figure 1. Scanning electron micrographs of microparticles prepared for ocular adhesion studies. (**A**) PLG MS. (**B**) PLG MD. (**C**) PLG/PEG MS. (**D**) PLG/PEG MD. Scale bars, 10 μm.

Statistical Analysis

The percentage of remaining microparticles was assessed based on the amount applied initially for in vitro and in vivo experiments. Mean percentages of remaining microparticles among the different microparticle formulations were statistically analyzed using a generalized linear model ANOVA with $\alpha = 0.05$, followed by pairwise comparisons using a Tukey post hoc test.

Results

Characterization of Microparticles

Four different kinds of microparticles were prepared, as shown in Figure 1. PLG MS was prepared as a control formulation of spherical geometry and no mucoadhesion promoter. PLG MD was prepared to assess the effect of microdisc geometry in the absence of a mucoadhesion promoter. PLG/PEG MS was prepared to assess the effect of a mucoadhesion promoter in spherical microparticles. PLG/PEG MD was prepared to assess the combined effect of a mucoadhesion promoter and microdisc geometry.

The average diameter of all microparticles was less than 10 μm (Table 1), which was achieved by removing larger particles by filtration. Microparticles in this size range should be appropriate for topical ophthalmic delivery.[2] After cutting, the microdiscs exhibited flat surfaces (Figs. 1B, 1D), whereas the uncut particles were spherical (Figs. 1A, 1C). The amount of Nile Red entrapped in microparticles was determined to be 7 to 8 μg/mg microparticles (Table 1).

TABLE 1. Properties of Microparticles Tested for Ocular Adhesion

Microparticle	Mean Diameter (μm)	Nile Red (μg/mg)
PLG MS	4.13 ± 1.76	8.07 ± 0.12
PLG MD	3.59 ± 1.19	7.51 ± 0.17
PLG/PEG MS	4.21 ± 1.93	7.53 ± 0.07
PLG/PEG MD	3.09 ± 1.24	7.01 ± 0.31

Values are mean ± SD.

To further characterize the effect of cutting, particle size distribution was measured using a multisizer. As shown in Figure 2 and Table 1, the cut microdiscs were smaller than the uncut microspheres. Although the 4 μm–diameter microspheres were cut to 1-μm thickness to make microdiscs, the multisizer shows a relatively small change in size because the multisizer measurement depends strongly on particle orientation in the electric field formed in the measuring orifice.[26] If the long axis of a microdisc is oriented perpendicularly to the electric field, that particle appears to the instrument to be of similar size as the original microspheres. If a microdisc is oriented parallel to the electric field, its size will appear to be close to 1 μm. In addition, because of limitations inherent in the cryosectioning method (bluntness of the blade, particle deformability in cryostat), very small microspheres might not have been cut satisfactorily. In this regard, the data make better sense when considering the biased averaging of microdisc size performed by the Coulter method.

DSC analysis was also performed on microparticles to validate the presence of PEG in microparticles made of a blend of PLG and PEG. As control experiments, Figure 3 shows that the T_g (glass transition temperature) of pure PLG appeared at 52°C, and the T_m (melting temperature) of pure PEG appeared at 77°C. Microparticles of PLG alone exhibited the same T_g as pure PLG. However, microparticles composed of a blend of PLG and PEG exhibited a higher T_g of 54°C, which is attributed to the presence of PEG in the microparticles. This is consistent with a previous report in which a similar transition of T_g was observed with a film made by solvent casting of PLG (75:25) and PEG (10 kDa).[27] The lack of PEG-melting endotherm for the PLG/PEG microparticles could be explained by the high miscibility and small mass fraction of PEG in microparticles, probably smaller than the initial loading of 10% wt/wt, because of partial PEG diffusion out of the particles during the solvent evaporation step of fabrication.

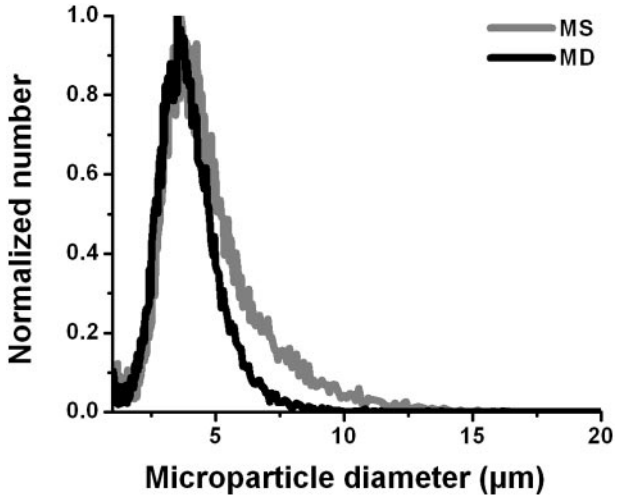

FIGURE 2. Size distribution of PLG MS (*gray curve*) and PLG MD (*black curve*) measured by a multisizer.

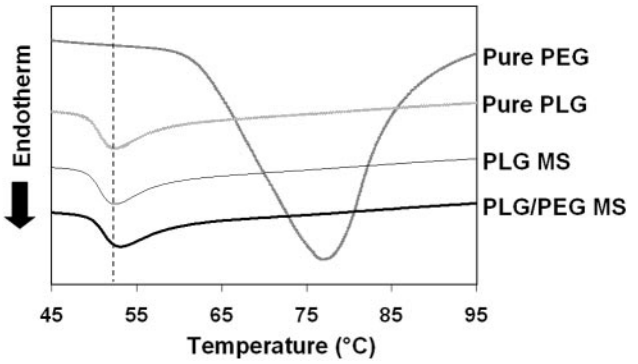

FIGURE 3. Differential scanning calorimetry thermograms of PLG MS and PLG/PEG MS. Thermograms from pure PLG and PEG are also plotted for comparison.

Microparticles were formulated in two different dosage forms, an aqueous suspension and a dry tablet, to examine the effect of the formulation on microparticle adhesion to the preocular surface. For ophthalmic application, a tablet should be small and dissolve rapidly in tear fluid to minimize possible discomfort on the sensitive eye surface. Figure 4 shows the fluorescence images of a dry tablet containing microparticles and the resultant microparticle suspension after tablet dissolution in HBSS. The fluorescence signal observed from the tablet indicates the presence of embedded microparticles labeled with Nile Red (Fig. 4A). The cylindrical tablet measured 3 mm in diameter and 3 mm in height, corresponding to a volume of 21 μL, and was small enough to be applied in the lower cul-de-sac of the eye. Mannitol, a highly water-soluble and biologically inert material, was used as the embedding medium of microparticles, which enabled the tablet to fully dissolve in HBSS within 5 minutes and to release the individual microparticles (Fig. 4B).

In Vitro Mucoadhesion of Microparticles

With this collection of microparticle formulations, we determined the effect of mucoadhesion and microparticle geometry on preocular residence time using an in vitro model of the preocular surface. This test was carried out for 60 seconds under a shear stress (140–160 dyne/cm^2) that mimicked the blink of a human eye because most particle clearance is expected during blinking.[28]

Under these conditions, 15% of PLG MS, with spherical geometry and no mucoadhesion promoter, remained on the model preocular surface, as shown in Figure 5. Neither cutting the particles into microdiscs nor adding a mucoadhesion promoter significantly changed the extent of particle adhesion ($P > 0.10$). However, the combination of the microdisc geom-

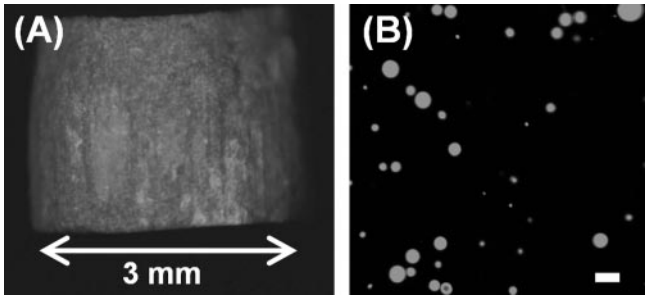

FIGURE 4. Fluorescence micrographs of (**A**) red fluorescent PLG MS embedded in a dry mannitol tablet and (**B**) PLG MS suspension after dissolution of a tablet for 5 minutes in HBSS. Scale bar, 10 μm.

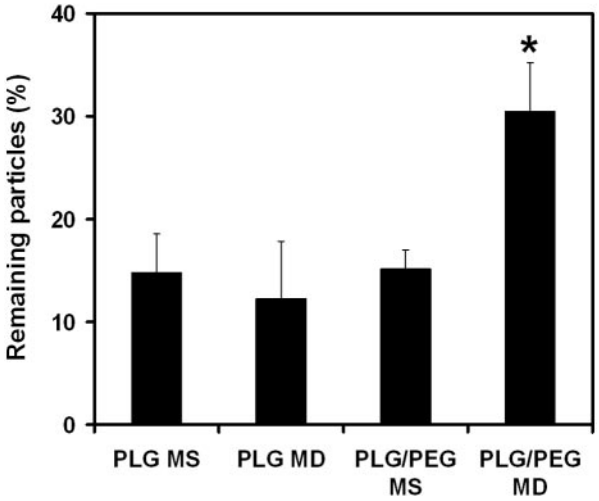

FIGURE 5. In vitro mucoadhesion of microparticles. The percentage of microparticles remaining on a model mucous membrane was measured after applying a shear stress similar to that of a human blink (140–160 dyne/cm^2) for 60 seconds. *PLG/PEG MD was significantly different from the other types of microparticles ($P < 0.05$). Data points represent the mean ± SD of three measurements.

etry and the mucoadhesion promoter doubled the percentage of microparticles remaining to 30% ($P < 0.05$). This can be explained by the increased force of adhesion of microparticles to the model preocular surface enabled by the mucoadhesion promoter and the reduced fluid mechanical force acting to remove microparticles enabled by the microdisc geometry.

In Vivo Mucoadhesion of Microparticles

We next sought to validate these in vitro findings using the rabbit as an in vivo model. The same four types of microparticles were formulated as an aqueous suspension and a dry tablet and were administered topically to the rabbit eye. The percentage of microparticles remaining on the preocular surface was determined after 10, 30, and 60 minutes, as shown in Figure 6. Retention of any of the four microparticle types, when administered as a suspension, was relatively poor, with just 1% to 10% remaining after 10 minutes and even less remaining at later times. We believe this poor retention can be partially explained by the coadministration of eyedrop carrier fluid to the preocular space, which is known to expedite tear drainage and thus to remove microparticles more rapidly.[29,30]

To reduce the effect of rapid removal of microparticles by excess tear drainage, we formulated the same dose of microparticles into rapidly dissolving mannitol-based tablets. These small cylindrical tablets dissolved in tear fluid on the preocular surface within 5 minutes (data not shown). This tablet formulation had no significant effect on PLG MS relative to the suspension formulations ($P > 0.1$). PLG MD tablets increased microdisc retention from 1.7% to 10.5% at 10 minutes and from 0.9% to 3.9% at 30 minutes compared with suspension, but this was not statistically significant ($P > 0.1$). The tablet formulation of PLG/PEG MS also made nonsignificant increases in mucoadhesive microsphere retention relative to PLG/PEG MS suspension ($P > 0.1$).

Finally, PLG/PEG MD tablets gave the best result, with 41% remaining at 10 minutes and 17% remaining at 30 minutes. The use of PLG/PEG MD tablets increased microparticle retention by 8-fold at 10 minutes and 17-fold at 30 minutes compared with a PLG MS suspension. Retention of PLG/PEG MD tablets was significantly greater at 10 minutes than for all other formulations except PLG/PEG MS tablets and at 30 minutes compared with all kinds of suspensions ($P < 0.05$). This shows that the combination of mucoadhesion promoter, microdisc geometry, and tablet formulation provided the greatest increase in preocular residence time.

As a companion to these quantitative results, we next imaged the distribution of microparticles on the preocular surface using the same eight microparticle formulations at the same three time points, as shown in Figure 7. Four different regions of the eye were imaged: cornea (CN), lacrimal caruncle (LC), upper fornix (UF), and lower fornix (LF). Microparticles applied as a suspension were not seen much in any region of the eye. Some microparticles observed at the LC could be attributed to accumulated microparticles draining toward the lacrimal duct. Similarly, tablets embedded with PLG MS and PLG MD also disappeared rapidly, probably because of the absence of a mucoadhesion promoter (Figs. 7B, 7D).

More extensive microparticle retention was observed with PLG/PEG MS and PLG/PEG MD tablets (Figs. 7F, 7H) at all regions except the CN. The UF showed moderate accumulation of microparticles that mostly disappeared 1 hour after administration. The LF showed extensive microparticle retention, especially for the PLG/PEG MD, which persisted for at least 1 hour. Extensive accumulation in the LF can be explained because the goblet cells in the lower cul-de-sac are known to be mainly responsible for the production of mucin on the preocular surface.[13,25] Therefore, the local mucin concentration should be higher in the LF than in other locations on the ocular surface, which would promote mucoadhesion of PLG/PEG microparticles. The LF was also the site of tablet administration, which would further help localize mucoadhesion at that location.

The absence of microparticles on the CN could be explained by the locally vigorous hydrodynamics and lack of mucin. During blinking, tear fluid on the CN experiences the strongest shear and highly dynamic mixing.[25] This, combined with the thin film thickness of the tear fluid (<10 μm),[31] makes microparticle adhesion to the CN surface difficult.

DISCUSSION

Because of the rapid clearance of drug from the preocular surface, topical delivery to the eye would benefit from a

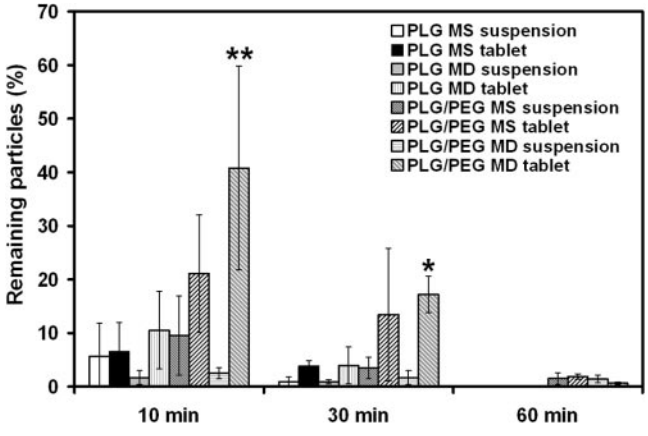

FIGURE 6. In vivo residence time of microparticles on the rabbit eye. The percentage of microparticles remaining on the preocular surface of rabbits was measured for eight different microparticle formulations. **At 10 minutes, the PLG/PEG MD tablet was significantly different from all other formulations ($P < 0.05$) except the PLG/PEG MS tablet. *At 30 minutes, the PLG/PEG MD tablet was significantly different from all suspensions ($P < 0.05$). Data points represent the mean ± SD of three to five measurements.

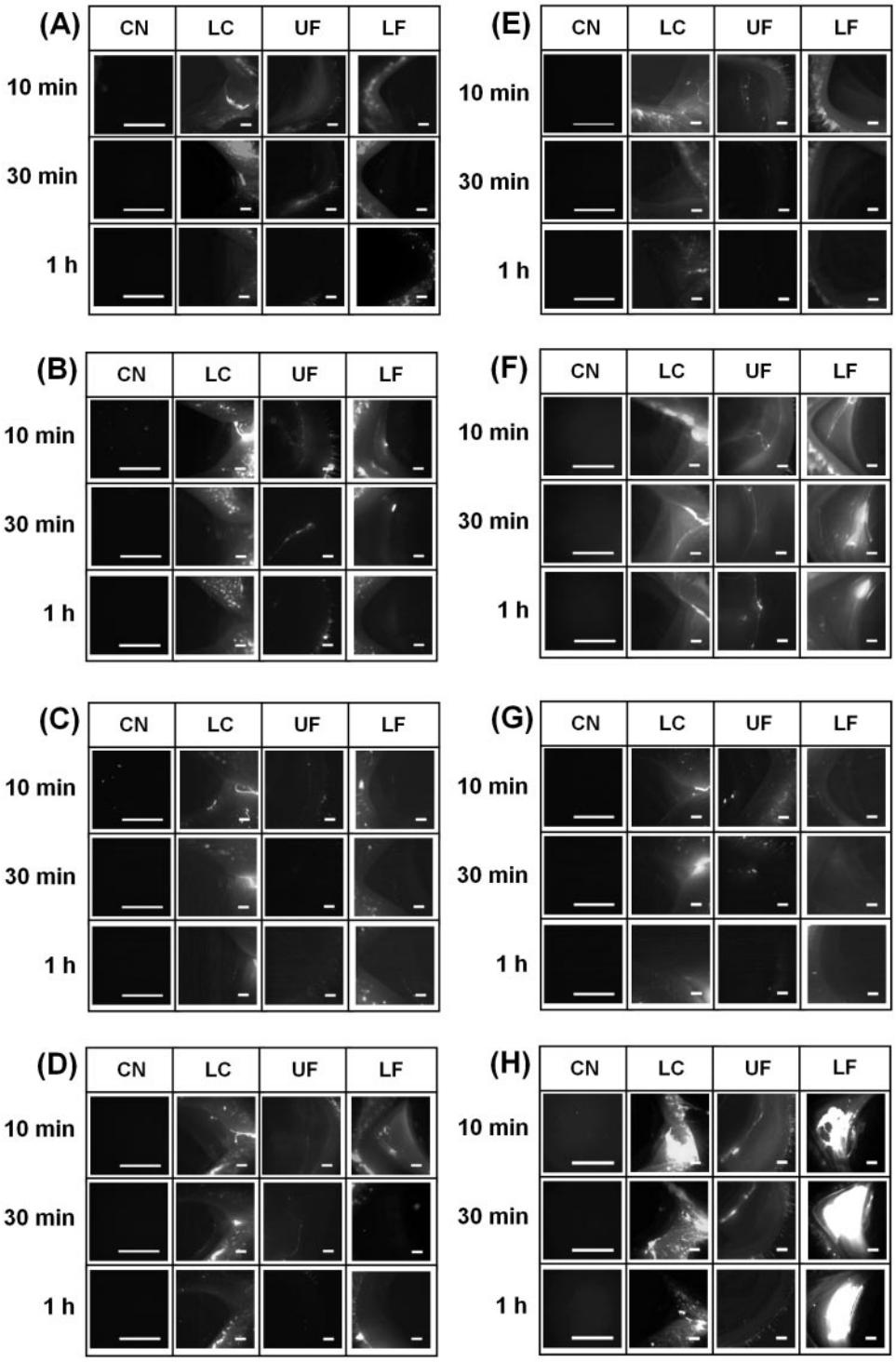

FIGURE 7. Fluorescence micrographs of red-fluorescent microparticles remaining on the preocular surfaces of rabbits as a function of time and location on the eye. Formulations used were (**A**) PLG MS suspension, (**B**) PLG MS tablet, (**C**) PLG MD suspension, (**D**) PLG MD tablet, (**E**) PLG/PEG MS suspension, (**F**) PLG/PEG MS tablet, (**G**) PLG/PEG MD suspension, and (**H**) PLG/PEG MD tablet. Each column presents images from a specific ocular region (CN, LC, UF, LF). Each row presents images at different times after microparticle administration. Scale bars, 500 μm.

delivery system with longer preocular residence time to improve drug bioavailability and efficacy of therapy. To achieve this goal, we designed microparticles considering three design parameters: mucoadhesion, disc geometry, and tablet formulation. With this approach, we achieved microparticle residence times on the preocular surface of up to 1 hour, an order-of-magnitude increase over conventional eyedrops, which have a typical residence time of just 3 to 7 minutes.[32]

The turnover rate of mucin on the preocular surface is known to be much slower than that of the tear fluid.[13] Thus, microparticles formulated with a mucoadhesion promoter, such as PEG,[18] are expected to remain on the eye longer. Considering tear flow and blinking, a disc shape should also help retain microparticles in the preocular space because of the low hydrodynamic resistance and high static frictional adhesion to the eye surface enabled by this geometry. The in vitro test in this study supports these expectations by showing synergetic enhancement of preocular residence time resulting from mucoadhesion and disc geometry. The use of mucoadhesive microdiscs (PLG/PEG MD) increased adhesion to the simulated ocular surface by a factor of 2 (Fig. 5).

Use of a tablet formulation also played a key role to increase the residence time of microparticles in vivo. Mucoadhesive microdiscs, when administered in a dry tablet to rabbit eyes in vivo, exhibited the best retention of all and provided an increase in remaining particles of up to 17-fold compared with a PLG MS suspension (Figs. 6, 7). In contrast to aqueous suspensions, dissolution of the mannitol-based tablet probably increased the viscosity of tear fluid at the site of administration, which gave time for particles to react with the mucin better and thereby enhanced microparticle attachment to the preocular surface.

Based on these observations, we propose mucoadhesive microdiscs formulated in a rapidly dissolving tablet as a novel topical drug delivery technology for the eye. We propose that in addition to prolonged residence time on the preocular surface, controlled drug release from microdiscs can be achieved by encapsulating drug within the core material (PLG).[20] Depending on the ratio of lactic acid to glycolic acid, polymer molecular weight, degree of drug loading, addition of other excipients, and other factors, the drug release rate can be tailored to achieve sustained or otherwise controlled release. Previously, suspensions of PLG MS (non-mucoadhesive microspheres), when administered topically to the rabbit eye, showed significant improvement in drug bioavailability because of controlled release.[10,11,16]

In addition to mucoadhesiveness, PEG may play a role in modulating the drug release profile. For example, the release of transforming growth factor β_1 was reported to be more sustained from microparticles blended with PEG.[33] In contrast, different microparticles with water-soluble PEG became more porous because of rapid PEG dissolution in the release media, which accelerated the release of a number of compounds, such as ovalbumin, immunoglobulin, and dextran.[34,35]

Optimal bioavailability might be achieved by matching the duration of drug release from the microparticles to the residence time of microparticles on the preocular surface. Note that PLG microparticles can be formulated for drug release more quickly than the time taken to fully degrade the microparticles, especially for small drug molecules and at high drug loading in the microparticles.[9] In this way, controlled release from PLG microparticles can be achieved according to time scales most useful to topical delivery to the eye (e.g., hours) rather than with microparticles that may biodegrade over much longer times (e.g., days or weeks). Biodegradation time of the microparticles may not be a critical parameter because after they are cleared from the preocular surface through the lacrimal ducts, they will probably be eliminated through the gastrointestinal tract in hours.[36]

In some previous studies, microparticles made solely of mucoadhesive materials, such as pectin and poly(acrylic acid), were shown to achieve residence times of up to 2 hours on the preocular surface.[15,16] Although it is difficult to make quantitative comparisons between studies because of different experimental methods and analytical detection limits, this study showed that mucoadhesive microdiscs in a dry tablet had a similar residence time of at least 1 hour (Fig. 7H) but had the added advantage of a PLG formulation that lent itself to controlled release of encapsulated drugs. This increased residence time was achieved with a small mass fraction of mucoadhesive PEG (10% wt/wt at most). Thus, the addition of mucoadhesion promoter, along with improved optimization of microdisc and tablet formulation, could increase residence time still further.

Microdiscs developed in this study should be distinguished from nanoparticles widely investigated for topical drug delivery to the eye. Such nanoparticles are often used based on their ability to penetrate the cornea through intracellular or paracellular pathways.[37–39] For example, mucoadhesive nanoparticles (PEG-coated nanoparticles) were previously suggested as drug carriers with accelerated transport across the epithelium on the eye.[37] In contrast, microdiscs in this study were envisioned primarily as controlled-release vehicles for release into the tear fluid. Indeed, drug released from microdiscs was intended to transport into the eye, but the microdiscs themselves remained outside.

Microdiscs also differ from ocular inserts. Although each system serves as a preocular reservoir to deliver drug into the tear fluid,[8] ocular inserts remain on the preocular surface as macroscopic devices without dissolution or degradation, which is bothersome to many patients.[40] In contrast, a mannitol-based tablet can rapidly dissolve in the tear fluid, leaving behind microdiscs smaller than 10 μm that are expected to be well tolerated by patients.[2]

Conclusions

This study supported the hypothesis that mucoadhesive microdiscs formulated in a rapidly dissolving tablet can increase preocular residence time. Mucoadhesion can improve microparticle attachment to the mucous layer of the preocular surface. The microdisc geometry can further promote mucoadhesion by increasing the contact area between microparticles and the preocular surface and by reducing convective forces of tear flow that carry particles away. In vitro tests showed that microparticles prepared with a mucoadhesion promoter (PEG) and microdisc geometry can adhere to a model mucous membrane twice as well as microparticles without these design features.

Formulation in a rapidly dissolving tablet can also increase preocular residence time because dissolution of the mannitol-based tablet can increase the viscosity of tear fluid at the site of administration, which gives time for microparticles to react better with the mucin and thereby enhance microparticle attachment to the preocular surface. In vivo tests in rabbits showed that formulation of mucoadhesive microdiscs in a rapidly dissolving tablet can increase preocular residence up to 1 hour, which represents a 1 order-of-magnitude increase over conventional microparticles and eyedrop formulations. Overall, we conclude that mucoadhesive microdiscs formulated in a rapidly dissolving tablet, coupled with encapsulation of drug within the microparticles for controlled release into the tear fluid, is a novel method of topical drug administration for sustained delivery to the eye.

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