Silicone Oil Microdroplets and Protein Aggregates in Repackaged Bevacizumab and Ranibizumab: Effects of Long-term Storage and Product Mishandling

Lu Liu,1 David A. Ammar,2 Lindsey A. Ross,3 Naresh Mandava,2 Malik Y. Kabook,2 and John F. Carpenter1

PURPOSE. To quantify levels of subvisible particles and protein aggregates in repackaged bevacizumab obtained from compounding pharmacies, as well as in samples of bevacizumab and ranibizumab tested in controlled laboratory experiments.

METHODS. Repackaged bevacizumab was purchased from four external compounding pharmacies. For controlled laboratory studies, bevacizumab and placebo were drawn into plastic syringes and incubated at −20°C, 4°C, and room temperature (with and without exposure to light) for 12 weeks. In addition, mechanical shock occurring during shipping was mimicked with syringes containing bevacizumab. Particle counts and size distributions were quantified by particle characterization technology. Levels of monomer and soluble aggregates of bevacizumab were determined with size-exclusion high-performance liquid chromatography (SE-HPLC).

RESULTS. Repackaged bevacizumab from the compounding pharmacies had a wide range of particle counts (89,006 to 602,062 particles/mL). Bevacizumab sampled directly from the original glass vial had particle counts of 63,839 ± 349/μL. There was up to a 10% monomer loss in the repackaged bevacizumab. Laboratory samples of repackaged bevacizumab and placebo had initial particle counts, respectively, of 283,675 ± 60,494/μL and 492,314 ± 389,361/μL. Freeze-thawing of both bevacizumab and placebo samples led to >1.2 million particles/μL. In all repackaged samples, most of the particles were due to silicone oil. SE-HPLC showed no significant differences for repackaged samples incubated in the laboratory under various conditions, compared with bevacizumab directly from vial. However, repeated freeze-thawing caused a more than 10% monomer loss.

CONCLUSIONS. Bevacizumab repackaged in plastic syringes could contain protein aggregates and is contaminated by silicone oil microdroplets. Freeze-thawing or other mishandling can further increase levels of particle contaminants.

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Bevacizumab (Avastin; Genentech Technology, Inc., South San Francisco, CA) is a recombinant human monoclonal antibody that inhibits endothelial cell growth and subsequent vascularization. It was approved for intravenous (IV) treatment of metastatic colorectal cancer by the United States Food and Drug Administration (FDA) in 2004 and subsequently has been approved for IV treatment of non–small cell lung cancer, metastatic breast cancer, glioblastoma, and metastatic kidney cancer. Because of its antivascular activity, bevacizumab has also been used by ophthalmologists for the off-label treatment of wet age-related macular degeneration (AMD). This practice has grown rapidly because repackaged bevacizumab has been very effective in treating AMD and because the cost per dose of bevacizumab is substantially lower than that of ranibizumab (Lucentis; Genentech), which is an FDA approved anti-VEGF agent specifically packaged and sold for the treatment of wet AMD.

The apparent safety and efficacy of intravitreal bevacizumab have been supported by published peer-reviewed reports and have led the National Eye Institute (NEI) to undertake a large randomized double-masked multicenter clinical trial (Comparison of AMD Treatments Trials [CATT]) to compare bevacizumab with ranibizumab. However, reports of sustained elevation of intraocular pressure (IOP) and inflammation after the intravitreal use of bevacizumab and ranibizumab have been increasing. Recently, we proposed that some increases in IOP could be due to particulate matter present in bevacizumab, which for off-label use is typically repackaged in plastic syringes. In support of this hypothesis, our investigation documented that there were both protein aggregates and particles ≥1 μm in repackaged bevacizumab obtained from three external compounding pharmacies.

Many factors (e.g., storage time, type of syringe, freeze-thawing, and mechanical shock during shipping) that could affect particle and protein aggregate levels and sizes in repackaged bevacizumab have not been investigated. Furthermore, determining the ranges of values for these critical product characteristics in samples purchased from different external compounding pharmacies is important for the field. In the present study, we addressed these problems with repackaged bevacizumab. First, we quantified the levels of subvisible particles and protein aggregates in bevacizumab in plastic syringes purchased from four external compounding pharmacies. Second, we performed controlled laboratory experiments with bevacizumab repackaged in the same types of plastic syringes as those used by the external compounding pharmacies. We tested the effects of storage at room temperature (with and without exposure to light), −20°C, and 4°C on particle counts and protein aggregates and the effects of repetitive freeze-
thawing. In addition, we studied the potential for freeze-thawing of samples in shipping containers used by the external compounding pharmacies and the effects of mechanical shock due to handling mimicking that occurring during shipping.

We used a particle characterization technique (MicroFlow Imaging [MFI]; Brightwell Technologies, Ottawa, ON, Canada) to count and size particles ≥1 μm and size-exclusion high-performance liquid chromatography (SE-HPLC) to quantify levels of monomeric and aggregated bevacizumab. Also, we conducted analyses and experiments that documented that most of the particles were due to silicone oil microdroplets. Finally, for comparison to results with repackaged bevacizumab, we also quantified the subvisible particles present in ranibizumab samples.

In this article, we report that bevacizumab repackaged in plastic syringes was contaminated by silicone oil microdroplets. Freeze-thawing and mechanical shock can further degrade the product and increase levels of particle contaminants. Although we cannot directly link silicone oil contaminants and bevacizumab aggregates with sustained elevation of IOP and inflammation at this time, based on current studies, mishandling of repackaged bevacizumab should be and can be avoided.

**MATERIALS AND METHODS**

Bevacizumab was purchased from the University of Colorado Hospital Inpatient Pharmacy. It was supplied as 25 mg/mL × 4 mL in preservative-free, single-use glass vials. Unless otherwise indicated, for the controlled laboratory studies, 0.3-mL insulin syringes (BD Ultra-Fine Short; Cat. No. 08290-282438; BD Biosciences, Franklin Lakes, NJ) were used. All chemicals used were of reagent grade or higher.

Repackaged bevacizumab in insulin plastic syringes was purchased from four external compounding pharmacies (designated CP1–CP4) on two different occasions. For each compounding pharmacy, the details of the syringe configurations and the shipping materials used are described in the Results section.

Ranibizumab was obtained from the residual solution remaining in the vials after clinical administration at the University of Colorado Hospital Eye Center. The residual solution was collected on the day of administration, stored at 4°C in the dark, and used in laboratory experiments within 24 hours.

**Observations on Shipping Containers Used for Repackaged Bevacizumab**

On arrival of the repackaged bevacizumab from each of the four compounding pharmacies, observations were made of the inside and outside of each shipping box; the orientation, weight, and surface temperature of the gel packs; the wrapping materials in which the syringes were placed; the relative position of syringes, gel packs, and wrapping materials; and the type of syringe. A thermocouple (Dual thermometer, Fisher Scientific, Pittsburgh, PA) was attached to the surface of a gel pack to determine the temperature on arrival.

**Testing of the Potential for the Formulation to Freeze in Syringes in Shipping Containers**

First, the gel packs obtained from the respective shipping containers (Styrofoam boxes) used by CP1 and CP3 were placed in a −20°C freezer overnight. Then, the gel packs were placed in the respective Styrofoam shipping boxes, in the orientation observed on arrival of the packages.

A thin wire thermocouple was placed on the surface of a gel pack in each box, the box was closed with the lid, and the temperature on the surface of the gel pack was recorded for 45 hours.

To test the potential for repackaged bevacizumab to freeze during shipping, we inserted a thin wire thermocouple into 0.05 mL of placebo solution (60 mg/mL trehalose · 2H2O, 5.8 mg/mL NaH2PO4 · H2O, 1.2 mg/mL Na2HPO4, and 0.4 mg/mL polysorbate 20; pH 6.2) contained in 0.3-mL insulin syringes. The syringes were in direct contact with the surface of gel packs that had just been removed from the −20°C freezer. The gel packs and syringes were placed in the Styrofoam shipping boxes that were obtained from CP1 and CP3. The temperature of the placebo in the syringes was recorded with the dual digital thermometer as a function of time. In another experiment, to avoid potential nucleation of ice by the thermocouple immersed in the solution inside the syringes, we attached the thermocouple to the outside surface of the syringes at the level of the solution inside. The syringes were placed in contact with the gel packs in the Styrofoam shipping containers, as just described, and the temperature was recorded as a function of time. At various times, the syringes were taken out of the box for visual inspection to see whether the placebo had frozen. We also tested the temperature on the surface of the syringes (containing 0.05 mL placebo), which were wrapped with additional multilayers of bubble packing material and placed horizontally or vertically between the gel packs.

**Analytical Methods for Particles and Protein Monomer and Aggregates**

Particle characterization technology (MicroFlow Imaging [MFI]; DPA4100 Flow Microscope, Series B; Brightwell Technologies) was used to capture digital images of particles, quantify particle counts, and determine size distributions (≥1 μm). The flow cell (100 μm) was used in SP3 mode after calibration with a 10-μm polystyrene microsphere standard (cat. no. 4210A; Duke Scientific Corp., Palo Alto, CA). Ultra-pure water (0.22 μm filtered; Millipore, Billerica, MA) was used to check the background counts during MFI. The total volume of sample dispensed into the flow cell was 0.3 mL, and 0.15 mL was run into the cell before the start of data acquisition. The particle count for placebo was subtracted from that of the bevacizumab samples. Then, if applicable, to obtain the particle counts before sample dilution, the particle counts were multiplied by the dilution factor (see below) used in preparation of bevacizumab samples for analysis.

SE-HPLC was used to fractionate and quantify monomer and soluble aggregates in the bevacizumab samples. The samples were centrifuged (10 minutes at 14,000g) to pellet any insoluble material (potentially including protein precipitates). The supernatants were analyzed by using the SE-HPLC mode on an asymmetric flow field flow fractionation system (Eclipse AFF; Wyatt Technology Corp., Santa Barbara, CA) that connected with a multangle light-scattering detector (Dawn EOS; Wyatt Technology Corp.) and a UV detector (Gold Module 166; Beckman Coulter, Fullerton, CA). The mobile phase (0.182 M KH2PO4, 0.018 M K2HPO4, and 0.25 M KCl [pH 6.2]) was run at a flow rate of 0.5 mL/min through a gel column (TSK-GEL G3000SWxl; Tosoh Bioscience, Tokyo, Japan), and elution was monitored at 280 nm. The volume of injection was 100 μL, and the running time was 30 minutes.

The sum of the peak areas (monomer and the earlier eluting oligomers) of the chromatogram for samples directly from the glass vial was calculated (ASTRA V software; Wyatt Technology Corp.) and served as the control value for the repackaged samples. The percentage of monomer and the earlier-eluting oligomer in repackaged samples was obtained via dividing the respective peak area by the summed peak area for the control sample and multiplying by 100.

**Effects of Preanalysis Dilution on Particle Counts**

The volume of repackaged bevacizumab obtained from compounding pharmacies was either 0.05 or 0.1 mL, and 0.05 mL was used in our controlled laboratory studies in insulin syringes, as described in the next section. Analysis by MFI requires at least 0.3-mL samples to give accurate particle counts. To be able to obtain data for samples from individual syringes, it was necessary to dilute the bevacizumab formulation (from repackaged syringes) and samples diluted 1:10, 1:20, 1:30, 1:40, and 1:50 with placebo.

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Analysis of Repackaged Bevacizumab from Compounding Pharmacies

Particle counts and size distributions (≥1 μm) of the repackaged bevacizumab samples were determined by MFI after 1:30 dilution with placebo. Monomer and soluble aggregates in the diluted bevacizumab samples were measured by SE-HPLC. The solution in the syringe was expelled by pushing the plunger, and the sample was collected in a 2-mL cryogenic tube and then diluted with the appropriate volume of placebo. Shipments of syringes with repackaged bevacizumab were obtained on two separate occasions. For the first shipment, the samples were analyzed on receipt. For the second shipment, a set of syringes from each compounding pharmacy were analyzed immediately on receipt. Another set was stored at 4°C to within 14 days of the 3-month expiration date, which was used by all the external compounders for repackaged bevacizumab.

Controlled Laboratory Studies of Repackaged Bevacizumab

Sample Preparation. In a laminar flow hood, bevacizumab was drawn (0.05 mL, unless otherwise noted) into the 0.3-mL insulin syringes (cat. no. 328458; BD Biosciences) through the rubber closure of the vial. The 0.3-mL insulin syringes were used in all experiments unless otherwise indicated. For comparison, bevacizumab placebo (60 mg/mL trehalose • 2H2O, 5.8 mg/mL NaH2PO4 • H2O, 1.2 mg/mL Na2HP04, and 0.4 mg/mL polysorbate 20 [pH 6.2]) was prepared in the same type of syringe.

Three-Month Incubation Study. Syringes with 0.05 mL of bevacizumab were incubated at room temperature (with and without exposure to laboratory fluorescent lights), −20°C, and 4°C for 0, 1, 2, 4, 8, and 12 weeks. Particle counts and size distribution (≥1 μm) in the incubated bevacizumab samples were determined by MFI. Monomer and soluble aggregates in the bevacizumab samples were measured by SE-HPLC.

Repeated Freeze-thawing. Samples of 0.05 mL bevacizumab in syringes were frozen at −20°C and thawed at 4°C, 1, 5, and 10 times. MFI and SE-HPLC were used to characterize the freeze-thaw samples.

Effect of Syringe Type during Freeze-thawing. To compare 1.0-mL syringes with 0.3-mL syringes, we drew the bevacizumab into 1.0-mL tuberculin syringes (cat. no. 309602; BD Biosciences) through a dispensing pin (Mini-Spike Dispensing Pin, product code: DP1000SC; B. Braun Medical, Inc., Bethlehem, PA) which entered the rubber closure of the vial. The MFI and SE-HPLC were used to characterize the freeze-thaw samples.

Mechanical Shock of Syringes Containing Repackaged Bevacizumab. Syringes containing 0.05 mL bevacizumab were placed between unrozen gel packs in one of the Styrofoam shipping boxes obtained from CP3. To mimic the mechanical shock to which the samples could be subjected during shipping, the box was tossed horizontally at a height of 1.2 m and for a distance of approximately 2 m. This process was repeated 20 times. MFI and SE-HPLC were used to characterize the samples after the tossing.

Characterization of Particles Measured with MFI. To gain insight into the identity of the component material of these particles, we conducted experiments to confirm that most of the particles counted by MFI in the bevacizumab samples from plastic syringes were silicone oil. First, we prepared samples in syringes and incubated them for 3 weeks at 4°C and −20°C. We prepared a sufficient number of syringes so that, after incubation, pooling of the expelled bevacizumab provided enough sample volume that analysis could be performed without dilution. This method allowed us to observe in images from the MFI the full population of particles that were present without dilution (see the Results section, Fig. 6, for representative images) and to observe many more particles per image than noted after dilution. In addition, samples of bevacizumab or placebo were freeze-thawed in the plastic syringes and the results compared to those of the samples of placebo freeze-thawed in cryogenic vials, which are free of silicone oil. We directly drew 0.3 mL of bevacizumab or placebo into 0.3-mL insulin syringes. Placebo (0.3 mL) was pipetted into 2 mL cryogenic vials. Also, we prepared a suspension of microdroplets of silicone oil (160 μg/mL; 1000 cSt; Dow Corning, Midland, MI) in placebo with a vortex mixer (Vortex Genie2, cat. no. 12812, Thermo Fisher Scientific, Inc., Pittsburgh, PA). The suspension of silicone oil microdroplets (0.3 mL) was pipetted into cryogenic vials.

To confirm that the spherical objects in the images were not air bubbles, we tested the effects of degassing. Samples (bevacizumab or placebo in plastic syringes, placebo in cryogenic vials, and placebo spiked with silicone oil in cryogenic vials) were frozen at −20°C and thawed at 4°C. For comparison, another set of samples was kept at room temperature. After that, each of the sample types either with or without freeze-thawing, was degassed under vacuum (20 in. of mercury, i.e., 20 in. HgV) for 5 hours to remove any potential microbubbles of air before MFI analysis. For comparison, another set of each of the sample types was kept at room temperature without degassing. These studies allowed comparison of particles found in placebo samples taken from plastic syringes and cryogenic vials, as well as in an authentic silicone oil sample, with the particles found in the repackaged bevacizumab taken from plastic syringes.

Particle Counts of Ranibizumab. The particle counts and size distributions of the ranibizumab samples were obtained by MFI for the following preparations: removed directly from the glass product vials with a plastic tip on a pipette; drawn without filtration from the vial into a 1-mL tuberculin syringe and then expelled for measurement; and according to a preparation for administration as described in the ranibizumab prescribing information. In this case, the ranibizumab formulation was withdrawn from the vial through a filter needle (Nokor filter needle with 5-μm wall, 19-gauge × 1.5 in., reference No. 305208; BD Biosciences) attached to a 1-mL tuberculin syringe, and then, after removal of the filter, the solution was expelled for measurement. For all methods of sample handling, after the sample was obtained, the ranibizumab was diluted 1:30 with ranibizumab placebo (10 mM histidine HCl, 1% o-tolualdehyde dehydrate, and 0.01% polysorbate 20 [pH 5.5]), and then the particles were counted.

RESULTS

Observations on Shipping Containers Used for Repackaged Bevacizumab

The shipping systems for the repackaged bevacizumab varied among the compounding pharmacies (Table 1). In particular, the weight and orientation of the gel packs used as cold media, and the relative position of individual syringes to the gel packs varied greatly (Table 1). The materials wrapped around the syringes of the repackaged bevacizumab were also different. In some cases (CP1, -2, and -4), the syringes were first packed individually in a transparent plastic bag, which was then placed in a large brown or silver plastic ziplock bag. In one case (CP3), the syringes were initially packed individually in small black plastic bags, which were then placed inside a large transparent ziplock plastic bag. Samples from CP3 had an additional layer of bubble wrap packing materials outside the large ziplock bag. On arrival, after overnight shipping, the surface temperature of the gel packs from all four compounding pharmacies was 1°C.

The plastic syringes used by the four compounding pharmacies to repack bevacizumab were also different (Table 1). CP2, CP3, and CP4 used 0.3-mL insulin syringes with permanently attached needles. The needle lengths were ½ in. (8 mm) on syringes obtained from CP3 and CP4 and ½ inch (12.7 mm) on those obtained from CP2. Only one compounding pharmacy (CP1) used 1-mL tuberculin syringes with a Luer-Slip fitting to which a new needle must be attached before intravitreal injec-
tion. A black rubber cap on the Luer-Slip fitting was used to seal the 1-mL syringe.

Testing of Potential for Formulation to Freeze in Syringes in Shipping Containers

After the gel packs equilibrated in the −20°C freezer, they were taken out and placed into their original shipping boxes. The surface temperatures of the gel packs remained below 0°C for up to 5 hours (Fig. 1A). Then, the temperature remained at 1°C for 20 to 40 hours, depending on the weight and initial temperature of the gel packs when they were placed into the shipping boxes. The temperature of the placebo in the syringes in contact with the gel packs—removed from the −20°C freezer and placed in the shipping container—cooled below 0°C, and the solution froze in less than 10 minutes (Fig. 1B). To avoid potential nucleation of placebo due to the thermocouple directly contacting the placebo solution, the thermocouple was attached to the outside surface of syringes. The sample temperatures dropped as low as −17°C (data not shown) and the samples froze within 30 minutes. Also, we tested placebo-containing syringes wrapped with an additional multilayer of bubble packing material. In syringes placed horizontally between the gel packs (CP3; Table 1), the placebo solution froze within 30 minutes. The temperature of samples placed vertically between the gel packs (CP1; Table 1) dropped to −12°C, but the solution did not freeze within 30 minutes (data not shown). Clearly, at this degree of supercooling, freezing would be expected to occur ultimately during actual shipping. The results presented in Figure 1 demonstrate that there is potential for repackaged bevacizumab to undergo freeze-thawing during the shipping process. However, unless temperature monitoring equipment is used during actual shipping from compounding pharmacies, the temperature profiles during the shipping of repackaged bevacizumab cannot be assessed.

Effect of Preanalysis Dilution on Particle Counts

Compared with undiluted bevacizumab formulation, the particle number and size distribution of samples diluted 1:30, 1:40, and 1:50 with placebo did not show substantial differences from values obtained without dilution (data not shown). In contrast, the particle number and size distribution of samples diluted 1:10 and 1:20 did differ from the values obtained without dilution, but due to the variability of the results for individual replicates, the differences were not statistically significant (P > 0.05) when comparing the mean particle counts for each dilution to that for the undiluted sample by Student’s t-test. We chose a 1:30 dilution for all repackaged bevacizumab samples, unless otherwise indicated.

Analysis of Repackaged Bevacizumab from Compounding Pharmacies

The repackaged bevacizumab obtained in the first batch from the compounding pharmacies (no less than duplicate samples tested, except for CP2) had a wide range of particle counts (89,006 ± 56,406 /mL to 602,062 ± 18,349 /mL; Fig. 2A). For comparison, bevacizumab sampled directly from the original glass vials had a particle count of 63,839 /mL. Particle counts also varied greatly in the same batch of repackaged bevacizumab from CP3 (Fig. 3). Representative particle images of the CP3 and CP4 samples (Fig. 2) showed that there were some large size particles (e.g., 111 μm) in the CP3 samples and various sizes of dark spherical particles in the CP4 samples that were later confirmed to be silicone oil microdroplets.

### Table 1. Details of the Repackaged Bevacizumab

<table>
<thead>
<tr>
<th>Compounding Pharmacy</th>
<th>Total Weight of Gel Packs (kg)</th>
<th>Orientation of Gel Packs</th>
<th>Relative Position of Repackaged Bevacizumab</th>
<th>Syringe Reference Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1</td>
<td>0.34</td>
<td>Vertical</td>
<td>Between two gel packs</td>
<td>309602</td>
</tr>
<tr>
<td>CP2</td>
<td>1.14</td>
<td>Vertical</td>
<td>Between three gel packs</td>
<td>328440</td>
</tr>
<tr>
<td>CP3</td>
<td>0.79</td>
<td>Horizontal</td>
<td>Between two gel packs</td>
<td>328438</td>
</tr>
<tr>
<td>CP4</td>
<td>0.57</td>
<td>Horizontal</td>
<td>Under two gel packs</td>
<td>309501</td>
</tr>
</tbody>
</table>

Data show the weight and orientation of gel packs, the relative position of repackaged bevacizumab to gel packs, and the reference number of plastic syringes used by the four external compounding pharmacies to repack bevacizumab.
The same samples of repackaged bevacizumab were analyzed by SE-HPLC for monomer levels and soluble aggregates (Figs. 4, 5). Representative chromatograms are shown in Figure 4, with the peaks for high-molecular-weight (HMW) species 1 and 2 labeled (HMW1 and HMW2) in the Figure 4 inset. The weight-averaged molecular mass of the species in the chromatogram peaks was estimated with an online light-scattering detector. Based on analysis of the SE-HPLC results for all samples studied by this method (data not shown), we determined that the HMW1 species were dimers to tetramers and the HMW2 species were composed of higher-order oligomers ranging from octamers to decamers.

SE-HPLC analysis showed that there was substantial monomer loss in the repackaged bevacizumab in batch one from CP2 and -3, whereas the levels in samples from CP1 and -4 were similar to that for bevacizumab taken directly from the drug product vials (Figs. 5A–C). For the repackaged bevacizumab in batch 2, the monomer level in samples from all the external compounding pharmacies was about the same as that measured in the bevacizumab taken directly from the drug product vials. In the samples in batches one and two, the levels of HMW1 ranged from approximately 2% to 4% and the levels of HMW2 from approximately 0.2% to 0.4%, respectively (Fig. 5).

Compared with the samples of repackaged bevacizumab obtained in the first batch, the range of particle counts was narrower among all the samples in the second batch when they were tested immediately on arrival (Fig. 2B). However, particle counts varied substantially between the samples taken from individual syringes in the second batch of repackaged bevacizumab from CP1 (Fig. 3). When we tested the second-batch samples that were stored at 4°C until 14 days before the 3-month expiration date, there was an increase in the total particles per milliliter in samples from CP2 and CP4 (Fig. 2C). There were no substantial changes in the levels of monomer or high-molecular-weight bevacizumab in the stored samples (Figs. 5D–F).

**Controlled Laboratory Studies of Repackaged Bevacizumab**

**Three-Month Incubation Study.** Laboratory samples of repackaged bevacizumab and placebo had initial (after 2 hours at 4°C, used as time-0 data) particle counts, respectively, of 283,675 ± 60,494/ml and 492,314 ± 389,561/ml (Table 2). In
contrast, bevacizumab sampled directly from the original glass vial had particle counts in the range of 20,000 to 60,000/mL (Figs. 2A, 2B), which indicates that the bevacizumab was contaminated when repackaged in plastic syringes. Particle counts (Table 2) and size distributions (data not shown) did not change substantially during 12 weeks of incubation at 4°C.

**Figure 4.** Representative SE-HPLC chromatograms of bevacizumab directly from the original glass vial and repackaged bevacizumab ordered from CP2 and CP3 (first batch).

**Figure 5.** The percentages of monomer, HMW1, and HMW2 of bevacizumab directly from the original glass vial and repackaged bevacizumab from the four CPs. (A–C) First batch; (D–F) second batch. Sample tested on receipt (n = 3); sample tested within 14 days of the 3-month expiration date (n = 2). The sum of the peak areas (monomer, HMW1, and HMW2) of the chromatographs of samples directly from glass vials served as the control for the repackaged samples. The percentage of monomer, HMW1, and HMW2 in repackaged samples was obtained by dividing the respective peak areas by the summed peak area for the control sample and multiplying by 100.
Within 1 week, light exposure at room temperature (RT) caused clogging of the needle in the syringes containing bevacizumab such that the solution could not be expelled from the syringe by operating the syringe plunger. The sample had to be collected by removing the syringe plunger and then carefully cutting the syringe barrel short enough so that a pipette fixed with a plastic tip could be used to remove the sample. Exposure to light at RT did not cause syringe clogging in the placebo samples.

Freeze-thawing of both the bevacizumab and placebo samples immediately led to >1.2 million particles per milliliter. The particle counts in the frozen bevacizumab samples remained constant during subsequent incubation followed by thawing, whereas the values for the placebo samples increased up to 6 million after 12 weeks of frozen storage at −20°C followed by thawing. The reason that particle counts were higher in the freeze-thawed placebo than in the bevacizumab samples is unclear, but it could be due to variations in silicone oil content in the individual syringes.

SE-HPLC data showed that there were no significant differences in the percentages of monomer in the repackaged samples obtained by dividing the respective peak areas by the summed peak area for the control sample and multiplying by 100. SE-HPLC data can show us the change of concentrations of monomer and high-molecular-weight species in different samples. RT, room temperature.

### Table 2. Summary of Contents of Repackaged Bevacizumab Tested in Laboratory Controlled Experiments

<table>
<thead>
<tr>
<th></th>
<th>Bevacizumab (1000/mL)</th>
<th>Placebo (1000/mL)</th>
<th>Monomer (%)</th>
<th>Bevacizumab HMW1 (%)</th>
<th>HMW2 (%)</th>
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</thead>
<tbody>
<tr>
<td><strong>3-Months Incubation Study</strong></td>
<td></td>
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<tr>
<td>Time 0</td>
<td>284 ± 60</td>
<td>492 ± 389</td>
<td>98.0 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT (No light exposure)</td>
<td>361 ± 238</td>
<td>159 ± 161</td>
<td>101.2 ± 0.4</td>
<td>2.0 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>RT (With light exposure)</td>
<td>374 ± 123</td>
<td>49 ± 30</td>
<td>100.0 ± 1.1</td>
<td>2.3 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>4°C</td>
<td>191 ± 84</td>
<td>296 ± 384</td>
<td>98.8 ± 0.8</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>−20°C</td>
<td>1201 ± 223</td>
<td>1819 ± 1273</td>
<td>98.0 ± 0.5</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
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<tr>
<td>Week 2</td>
<td></td>
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<tr>
<td>RT (No light exposure)</td>
<td>473 ± 510</td>
<td>659 ± 512</td>
<td>100.6 ± 1.3</td>
<td>1.9 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>RT (With light exposure)</td>
<td>434 ± 472</td>
<td>61 ± 51</td>
<td>99.9 ± 0.8</td>
<td>2.7 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>4°C</td>
<td>128 ± 37</td>
<td>14 ± 22</td>
<td>100.6 ± 0.2</td>
<td>1.7 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>−20°C</td>
<td>948 ± 277</td>
<td>1447 ± 863</td>
<td>98.4 ± 1.5</td>
<td>1.7 ± 0.7</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT (No light exposure)</td>
<td>108 ± 60</td>
<td>428 ± 528</td>
<td>101.1 ± 6</td>
<td>2.3 ± 0.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>RT (With light exposure)</td>
<td>213 ± 68</td>
<td>283 ± 197</td>
<td>98.3 ± 2.3</td>
<td>2.9 ± 0.2</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>4°C</td>
<td>109 ± 29</td>
<td>134 ± 170</td>
<td>100.9 ± 0.8</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>−20°C</td>
<td>1285 ± 241</td>
<td>3714 ± 140</td>
<td>102.1 ± 1.5</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Week 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT (No light exposure)</td>
<td>294 ± 156</td>
<td>380 ± 82</td>
<td>98.2 ± 1.9</td>
<td>2.7 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>RT (With light exposure)</td>
<td>713 ± 593</td>
<td>1342 ± 1521</td>
<td>99.3 ± 5.1</td>
<td>3.9 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>4°C</td>
<td>151 ± 40</td>
<td>216 ± 158</td>
<td>96.8 ± 5.1</td>
<td>1.8 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>−20°C</td>
<td>1410 ± 270</td>
<td>4045 ± 2249</td>
<td>98.6 ± 0</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Week 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT (No light exposure)</td>
<td>129 ± 2</td>
<td>1038 ± 18</td>
<td>100.7 ± 1.9</td>
<td>3.3 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>RT (With light exposure)</td>
<td>789 ± 34</td>
<td>474 ± 7</td>
<td>99.3 ± 4.1</td>
<td>4.6 ± 0.3</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>4°C</td>
<td>275 ± 5</td>
<td>322 ± 6</td>
<td>99.4 ± 0.4</td>
<td>1.9 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>−20°C</td>
<td>1346 ± 7</td>
<td>5996 ± 50</td>
<td>98.9 ± 2.1</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td><strong>Repeated Freeze-Thawing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cycle</td>
<td>1101 ± 271</td>
<td>1819 ± 1273</td>
<td>98.0 ± 0.5</td>
<td>1.7 ± 0.7</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>5 cycles</td>
<td>2143 ± 471</td>
<td>1782 ± 265</td>
<td>84.7 ± 13.3</td>
<td>3 ± 0.6</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>10 cycles</td>
<td>2527 ± 2009</td>
<td>2382 ± 934</td>
<td>85.1 ± 8.2</td>
<td>5 ± 0.3</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td><strong>Effect of Syringe Type during Freeze-Thawing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 mL</td>
<td>1963 ± 10</td>
<td>97.9 ± 1.3</td>
<td>1.8 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>1 mL</td>
<td>452 ± 1</td>
<td>98.3 ± 0.4</td>
<td>1.8 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
<tr>
<td><strong>Mechanical Shock of Syringes with Repackaged Bevacizumab</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tossed 20 times</td>
<td>1278 ± 34</td>
<td>95.1 ± 0.4</td>
<td>3.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

Data represent average ± SD of triplicate samples. The sum of the peak areas (monomer, HMW1, and HMW2) of the chromatograph for samples directly from the glass vial served as the control for the repackaged samples. The percent of monomer, HMW1, and HMW2 in repackaged samples was obtained by dividing the respective peak areas by the summed peak area for the control sample and multiplying by 100. SE-HPLC data can show us the change of concentrations of monomer and high-molecular-weight species in different samples. RT, room temperature.
controlled laboratory study, multiple freeze-thaw cycles of repackaged bevacizumab and placebo samples caused the total particle counts to increase (Table 2). There was not a significant monomer loss of bevacizumab in samples of the repackaged protein freeze-thawed once (Table 2). However, repeated freeze-thawing caused approximately 10% monomer loss. The percentages of HMW1 slightly increased after multiple freeze-thaw cycles (Table 2), whereas those for HMW2 were unchanged.

Effect of Syringe Type during Freeze-thawing. In our 3-month incubation studies, we selected the 0.3-mL plastic insulin syringes because three of the four compounding pharmacies used this syringe. We also tested the 1-mL tuberculin type of syringe used by one of the compounding pharmacies because freeze-thawing significantly increased particle counts in bevacizumab repackaged in 0.3-mL insulin syringes. Interestingly, freeze-thawing of bevacizumab contained in the 1-mL tuberculin syringe showed substantially lower particle counts, particularly in the large size range (e.g., 2–10 μm) than that observed for the sample freeze-thawed in a 0.3-mL syringe (Table 2).

Mechanical Shock of Syringes with Repackaged Bevacizumab. After being tossed 20 times, the bevacizumab repackaged in our laboratory had more than 1 million particles (Table 2). In addition, much higher levels of small and relatively larger particles were observed in the tossed samples compared with the untossed controlled samples (data not shown).

Characterization of Particles Measured with MFI. Visual examination of the digital images of particles in the CP4 samples indicated that nearly all the particles were dark, spherical objects of various sizes (Fig. 2). Because these samples were diluted before MFI analysis, the images show only a small fraction of the particles present in the sample before dilution. To provide images of the actual particle number, we tested samples that had not been diluted before MFI analysis. For this experiment, bevacizumab was incubated in 0.3-mL insulin syringes for 3 weeks at 4°C and −20°C. Compared with the samples incubated at 4°C, the freeze-thawed samples had a dramatically higher number of dark, large-sized particles (>30 μm) that had a circular cross section with an annular appearance (Fig. 6). These properties are characteristic of silicone oil microdroplets shed from syringes.15

To confirm that the particles were not due to micro air bubbles, we prepared samples of repackaged bevacizumab that were degassed before analysis by MFI. Freeze-thawed bevacizumab samples without and with degassing had 173,472 ± 8,459/mL and 383,005 ± 90,282/mL, respectively. Similarly, freeze-thawed placebo samples taken from plastic syringes had 315,255 ± 152,926/mL and 270,153 ± 183,486/mL respectively. Degassing did not result in a significant reduction in the particle counts (Fig. 7A). In some cases, degassed samples even had an increase in particle counts. At least two factors may contribute to this effect. First, the quantity and distribution of silicone oil may vary from syringe to syringe.16–18 Second, we have observed the same phenomena in other studies in which solutions of other proteins were used that were subjected to a degassing protocol (data not shown). It is unclear at this time whether putting protein solutions under a vacuum leads to the formation of new particles or whether there are increased particle counts due to contamination during additional handling.

Furthermore, the freeze-thawed placebo contained in the syringes had significantly higher particle counts than the samples freeze-thawed in cryogenic vials that were free of silicone oil (Fig. 7B). The representative images (Fig. 8) of bevacizumab freeze-thawed in a 0.3-mL insulin syringe (Fig. 8A), placebo freeze-thawed in a 0.3-mL insulin syringe (Fig. 8B), placebo freeze-thawed in a cryogenic vial (Fig. 8C), and placebo spiked with silicone oil and freeze-thawed in cryogenic vial (Fig. 8D) show that particles in authentic silicone oil samples are similar to the particles found in the repackaged bevacizumab and the placebo taken from the plastic syringes.

Finally, it is important to emphasize that large increases in particle counts were observed when the placebo was freeze-thawed in 0.3 mL plastic syringes and that these particles had the characteristic appearance of silicone oil droplets (data not shown). In these samples, protein could not contribute to the particles. Taken together, the results support the conclusion that the silicone oil used to lubricate
There were not only substantial differences in particle counts that there was a wide range of particle levels in the solution. Syringes from the four compounding pharmacies documented exposure to mechanical shock.

In the plastic syringes is the source of most of the particles observed in the repackaged bevacizumab and of the large increases in particles noted when the samples were purposefully mishandled—for example, by freeze-thawing or exposure to mechanical shock.

**Particle Counts in Ranibizumab Samples by MFI.** The particle counts for ranibizumab taken directly from the product vial (Fig. 9). The particle counts in ranibizumab samples filtered through the 5-μm filter into a tuberculin syringe and drawn into a syringe without filtering were slightly higher than that for the unfiltered sample taken from the vial without exposure to a syringe (Fig. 9). These increases in particle counts were most likely due to the silicone oil microdroplets entering the formulation from the syringe.

**Discussion**

Our studies of repackaged bevacizumab obtained in plastic syringes from the four compounding pharmacies documented that there was a wide range of particle levels in the solution. There were not only substantial differences in particle counts in the samples obtained from the different pharmacies but also between the individual syringes from a given pharmacy. Also, interestingly, there were fewer particles measured in the second batch of syringes purchased, particularly compared to levels observed in the first batch obtained from CP3 and CP4. Furthermore, there was some variation in the amount of monomer protein present in the different samples. In particular, in samples obtained the first time from CP2 and CP3, the level of monomeric bevacizumab was substantially lower than that found in product obtained directly from the vial or in other samples from the different compounding pharmacies. Taken together, these results raise many questions relating to the causes of the differences in sample quality, particularly in subvisible particles counts in the different syringes of repackaged bevacizumab and the potential impacts of variations in product quality on patients.

To address the first question, we consider insights gained from our controlled laboratory experiments with repackaged bevacizumab in plastic syringes. Characterization of the particles in the samples documented that nearly all these particles were silicone oil microdroplets. Furthermore, increases in particle counts due to treatments (e.g., freeze-thawing) were due primarily to increases in levels of silicone oil microdroplets. Silicone oil is used to lubricate the barrel of the plastic syringe. Some of the differences in particle counts between samples could be due to differences in the level of silicone in individual syringes. However, an examination of the particle counts in samples of repackaged bevacizumab removed from the syringes 2 hours after preparation (Table 2) showed clearly that the levels of droplets were relatively low, as was the variation in particles counts among syringes.

Instead it appears that mishandling (freezing or mechanical shock) of the syringes containing repackaged bevacizumab could be responsible for the higher levels of silicone microdroplets noted in some syringes obtained from compounding pharmacies. In controlled laboratory studies, we found that freeze-thawing, even only once, greatly increased the levels of microdroplets in samples of bevacizumab repackaged in plastic insulin syringes. Furthermore, it was clear from studies with the gel packs and shipping systems used by the compounding pharmacies that, depending on how the materials were handled and prepared, repackaged bevacizumab solution in the syringes could freeze-thaw during shipping. For example, if syringes were placed into the shipping container with gel packs that could freeze-thaw during shipping. For example, if syringes were placed into the shipping container with gel packs taken directly from the −20°C freezer, the solution inside could freeze within minutes. By the time the shipping container arrived at the clinical site, however, the temperature would have increased to ~1°C, and the solution would be thawed. Thus, it appears that one important factor that could account for increased particle counts is mishandling leading to freeze-thawing during shipping from the compounding pharmacy to the clinical site. Unfortunately for the receiving physician, without analysis of the solution inside the syringe for subvisible particles, there is nothing to indicate whether the repackaged bevacizumab in a given syringe had been subjected to freeze-thawing.

Compounding pharmacies could develop procedures for preparing gel packs and shipping containers to reduce the risk of accidental freeze-thawing of bevacizumab repackaged in plastic syringes. One suggestion can be made based on the time-temperature profiles that we obtained with the gel packs (Fig. 1). Gel packs could be removed from the −20°C freezer and allowed to warm to ~1°C before being placed into the shipping container with the syringes. With this approach, the bevacizumab would not be subjected to subzero temperatures. Of course, a sufficient mass of gel packs would have to be used to assure that the temperature did not increase too much during overnight shipping. It should be a straightforward re-
quirement that individual compounding pharmacies develop and test packaging and shipping protocols that assure that repackaged bevacizumab is maintained at the appropriate temperatures and that freeze-thawing is avoided.

It is also important to avoid freeze-thawing of repackaged bevacizumab at the clinical site. Obviously, the syringes should be stored in a refrigerator set at a temperature near 4°C and not in the freezer compartment. Even in a refrigerator, solutions can freeze and thaw because some locations in a given refrigerator can be exposed to temperatures below and above 0°C. In these cold spots solutions can freeze, and temperature fluctuations during normal refrigerator operation can result in multiple freeze-thaw cycles of repackaged bevacizumab.

Another factor that caused a large increase in silicone oil microdroplets in the bevacizumab formulation was mechanical shock due to tossing the shipping container and letting it hit the floor. Such handling is not uncommon during routine shipping, but without the use of shock indicators, one cannot know whether a given package has been subjected to such a mechanical shock. We recommend that compounding pharmacies design their shipping system with sufficient padding of the syringes of repackaged bevacizumab to prevent increases in particles in the solution due to mechanical shock.

Another type of mishandling that can affect the quality of repackaged bevacizumab is exposure of the syringes to light. Surprisingly, we found that even relatively short-term exposure to laboratory lights at room temperature led to clogging of the syringe needle and mechanical failure of the syringe. The solution could not be expelled without excessive use of force. The cause of this clogging is not clear, because in light-exposed repackaged bevacizumab, there was not a substantial increase in particles or protein aggregates compared with samples not exposed to light. However, the practical implications are clear: Care should be taken to minimize exposure of repackaged bevacizumab to light at the compounding pharmacy and the clinical site.

The low level of monomer noted in the samples from one compounding pharmacy are perplexing because, based on SE-HPLC analysis of these samples, we were not able to account for this loss by the formation of soluble protein aggregates. The loss of monomer could be due to precipitation of the protein from solution and pelleting during centrifugation, but a pellet was not observed after centrifugation of these samples. Alternatively, the protein may adsorb onto the silicone oil droplets and/or the interior surfaces of the syringe, due to its amphiphilic character. However, this possibility seems unlikely because the formulation contains a nonionic surfactant, polysorbate 20, at a level that should be sufficient to reduce protein adsorption to interfaces. Unfortunately, MFI is not able to distinguish silicone oil particles from protein-coated silicone oil particles. In our earlier study, using a bevacizumab ELISA, we also found that the bevacizumab protein level was low in a few samples of repackaged bevacizumab from compounding pharmacies. Nothing in our investigations to date explains the cause of the reduction in protein concentration.

Another quality issue is that plastic syringes were not designed and approved to store therapeutic protein products. Rather, the 510K regulatory approval for insulin syringes is specifically for the injection of insulin after removal from a product vial. Thus, repackaging bevacizumab in these syringes is an off-label use of the syringe. Because these syringes were not specifically designed and tested for intraocular use, control of relevant parameters such as the amount of silicone oil applied per syringe may not be as stringent as one would desire for use in injection into the eye. Furthermore, as we have previously noted, during long-term storage in plastic syringes, there is the potential for extractables and leachables to enter into the bevacizumab solution. The types of and levels of these compounds going into solution of repackaged bevacizumab are unknown, as are the potential impact of these compounds on bevacizumab stability and the direct effect on patients.

Each of the compounding pharmacies from which we obtained repackaged bevacizumab appeared to set a 3-month shelf life for the repackaged product. Presumably, this shelf life is based on the time from repackaging bevacizumab in the plastic syringes until the designated expiration date. However, compounding pharmacies repackage drug products under USP
797, which specifically sets a postpackaging storage time of no more than 2 weeks at refrigerated temperatures.

Regarding the potential impact of silicone oil contaminants and protein aggregates on patients, we have suggested that they may play a role in the IOP spikes noted in a few patients. Multiple reports have now been published revealing both acute and chronic IOP elevations that have been associated with single or repeated injections of both bevacizumab and ranibizumab. The elevation in pressure is in many cases resistant to traditional IOP-lowering drops and laser trabeculoplasty and requires filtration surgery. This clinical picture is similar to the IOP spikes noted after silicone oil migrates from the posterior to the anterior chamber after retinal surgeries that employ silicone oil. This further supports the likelihood that silicone contaminants, when injected into the vitreous cavity at the time of anti-VEGF injections, could cause persistent elevations in IOP.

Another proposed mechanism for the IOP spikes from previous reports was the possibility of a direct toxic effect of anti-VEGF agents on trabecular meshwork (TM) cells. Previous work done by members of our group has shown that the anti-VEGF agents themselves are not likely to be the cause of IOP elevations, since only bevacizumab is toxic to TM cells and only when the concentration studied is four times that of the clinical dose. Ranibizumab is not toxic, even at very high doses. Therefore, a direct toxic effect on the aqueous outflow pathway cells is unlikely. Considering our finding of a large number of micrometer-size silicon oil droplets, the simplest explanation of IOP elevation involves physical obstruction of the aqueous outflow pathway (as review by Ichhpujani et al.). However, in samples exhibiting ~10% loss of protein monomer, these protein aggregates may also be responsible.

Although in most reports the IOP spikes after administration of anti-VEGF agents have been linked to bevacizumab, there have been a few cases linked to ranibizumab. Our analysis of ranibizumab found that subvisible particles counts in samples taken directly from the product vial were comparable to those in bevacizumab taken from the vial. Furthermore, drawing the ranibizumab formulation from the vial into a syringe (with or without using a 5-μm syringe filter) resulted in modest increases in particle counts. These increases were most likely due to silicone oil microdroplets from the syringe. Because ranibizumab is stored in a glass vial and is only exposed to the syringe environment for a brief time, the risks of protein aggregation and contamination with silicone oil appears to be much less than for bevacizumab repacked in plastic syringes. However, mishandling of ranibizumab (e.g., leaving the product in the syringe for long periods and/or freezing the product in a syringe) could also lead to degradation that could cause complications in patients. Therefore, as with all therapeutic protein products, it is important that ranibizumab be stored and prepared for administration exactly as described in the instructions for the product.

Further studies are necessary to assess the clinical relevance of subvisible particles in intravitreal protein therapeutics. Both bevacizumab and ranibizumab appear to exhibit relatively low large-particle counts when compared with samples that are freeze-thawed or subjected to mechanical shock. Further studies are necessary to ascertain the ideal shipping mechanism for intravitreal protein therapeutics such as ranibizumab and bevacizumab. It appears that avoidance of freeze-thawing and mechanical shock is effective in decreasing large-particle counts. In addition, the use of filters and silicon-lubricated syringes may contribute to increased particle counts.

Acknowledgments

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References

6. NEI. Comparison of AMD Treatments Trials (CATT); Lucentis-Avastin Trial; 2010.


