Novel Heavy Tamponade for Vitreoretinal Surgery

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Purpose. The aim of this study was to produce a heavy tamponade with a specific gravity greater than 1.06 g/mL that was optically transparent, could be manufactured using simple processing, could be injected using standard clinical equipment, and would have appropriate biocompatibility.

Methods. Aerosil silica was added to a phenyl trimethicone and mixed via a roller, overhead stirring, and ultrasonics. The refractive index, visible absorbance, and shear viscosity were measured. The injectability of the solutions was evaluated using the Accurus Viscous Fluid Injection system. The tamponade efficiency was assessed using a model eye chamber and compared with that of Densiron 68, Oxane HD, and F_6H_8. The biocompatibility was evaluated in vitro and in vivo in rabbits.

Results. Tamponade agents were produced with specific gravities of 1.10, 1.11, 1.13, and 1.16 g/mL that had good optical clarity. Mixing using overhead stirring was sufficient to produce tamponade agents with shear viscosities in the range 1000 to 5000 mPa·s that were reproducible and stable during storage. The solutions were easier to inject using the Accurus Viscous Fluid Injection system than silicone oil 1000 mPa·s. The 11% silica solution had greater tamponade efficiency than Densiron 68 or Oxane HD. There was no evidence of cytotoxicity in vitro. Silica solution 11% induced cataract earlier than Polydimethylsiloxane 1000 (PDMS 1000). Silica solution 11% and phenyl trimethicone reduced the a-wave value at 1 week after vitrectomy, but recovery was observed at later time points. Silica solution 11% caused inner nuclear layer (INL) nuclei dropdown in inferior retina from 4 weeks postoperation. Polydimethylsiloxane 1000 induced a similar phenomenon in superior retina 12 weeks postoperation.

Conclusions. We have produced a heavy tamponade with good clarity that has appropriate shear viscosity, injectibility, enhanced tamponade efficiency, and biocompatibility similar to that of PDMS 1000.

Keywords: tamponade agent, viscosity, biocompatibility

Silicone oils are used as long-term ocular tamponades in the treatment of retinal detachment.[1] For many years the silicone oils used in vitreoretinal surgery have not changed; however, silicone oil has its limitations.[2] Specifically, the low specific gravity of silicone oil leaves the inferior retina exposed and potentially bathed in inflammatory mediators.[3,4] Recently, heavy silicone oils have become available[5,6] and are made by adding semifluorinated alkanes or alkenes to the oil. These produce silicone oils with a specific gravity only marginally greater than 1 (1.02, Oxane HD [Bausch & Lomb, Kingston-on-Thames, UK]; 1.06, Densiron 68 [Fluoron GmbH, Ulm, Germany]) due to the limited solubility of the semifluorinated compounds in silicone oil. Additionally, such semifluorinated alkanes/alkenes, although providing good clinical outcomes, may be implicated in intraocular inflammation as well as emulsification similarly to standard silicone oils.[7,8] There has been a debate about the clinical advantages of using these existing heavy tamponades, with recent randomized clinical trials demonstrating no difference between heavy and standard silicone oil on clinical outcome.[9,10] It has been hypothesized that a silicone oil with a greater specific gravity would enhance the tamponade efficiency of heavy silicone oils and that this would have the potential to increase their clinical performance.

Silica nanoparticles are easily dispersed in silicone rubbers and are frequently added as reinforcing materials to increase their mechanical strength.[11] Adding solids to silicone oils adds to their specific gravity; however, to maintain the optical clarity of the silicone oil it is necessary to match the refractive indices of the particles and the oil to inhibit light scattering of the dispersion.[12]

In this study we have demonstrated that we can produce optically clear heavy silicone oils by the addition of silica nanoparticles. The resulting oils have a specific gravity significantly higher than that of existing heavy silicone oils. These can be manufactured by simple processing procedures that can control their viscosities and thus can be injected using standard clinical procedures. We have shown that they are

stable during storage over several months and that they are acceptable in terms of in vitro and in vivo biocompatibility.

Materials and Methods

The silicone oil used was 556 cosmetic grade fluid (phenyl trimethicone; Dow Corning, Midland, MI) (viscosity 20 mPa·s), which has a refractive index of 1.46. The silica used was pharmaceutical grade (Aerosil R972 Pharma; Evonik, Essen, Germany). The oil was treated with granular activated carbon (GAC) prior to use: 1 g GAC (GAC 12–40 mesh; Acros Organics, Geel, Belgium) was added to 9 g oil and was roller mixed for 24 hours at room temperature. The tubes were allowed to stand for 72 hours and the GAC filtered off. The silica was dried for 48 hours at 105°C, then allowed to cool to room temperature immediately before addition to the oil.

Multiple batches of Aerosil R972 Pharma silica in 556 cosmetic grade fluid were prepared at a range of concentrations including 10.0%, 11.0%, 12.5%, and 15.0% wt/wt. These fluids have specific gravities of 1.10, 1.11, 1.13, and 1.16 g/mL respectively. The components were added and mixed on a roller mixer for a range of time periods, and it was established that the silica was well dispersed in the oil after 72 hours of rolling. After roller mixing, some samples were overhead stirred using a two-blade, 35-mm-wide, 12-mm-high stainless steel paddle at room temperature at a range of speeds and times including 250, 500, 1000, and 2000 rpm for 1 hour at each speed and at 2000 rpm for 7 hours. A third batch of samples was ultrasonically mixed for 8 minutes at 10 W after roller mixing.

Batch Reproducibility

The shear viscosity of three batches of 11.0% wt/wt of Aerosil R972 Pharma silica in 556 fluid mixed by roller mixing, followed by overhead stirring at 2000 rpm for 7 hours, was used to demonstrate batch reproducibility.

Stability With Storage

Samples of 11.0% wt/wt of Aerosil R972 Pharma silica in 556 fluid, mixed by roller mixing followed by overhead stirring at 2000 rpm for 7 hours or ultrasonically, were stored at 37°C, and the shear viscosity was measured after 4, 12, and 24 weeks. Additionally the shear viscosity of the overhead-stirred samples was measured after storage for 56 weeks.

Optical Clarity

Samples of 11.0% wt/wt of Aerosil R972 Pharma silica in 556 fluid, mixed by roller mixing followed by overhead stirring at 2000 rpm for 7 hours, were produced and diluted with a solution of 556 oil mixed with polydimethylsiloxane fluid (PDMS, 1000 mPa·s; Fluoron GmbH) at 80:20, 60:40, 40:60, and 20:80 ratios of 556 oil to PDMS. For each compound solution, 5.0 g 11% silica in 556 oil was mixed with 2.5 g of the 556 oil/PDMS oil solution. Duplicate batches of each compound solution were produced.

Refractive Index and Visible Absorption

Refractive index (RI) was measured using an Abbe 60 High Accuracy Refractometer (Bellingham and Stanley, Tunbridge Wells, UK), and visible absorption was measured using a GENESYS 10 UV Scanning spectrophotometer (Thermo Scientific, Waltham, MA). Duplicate measurements were made from each solution. All measurements were made at room temperature.

Shear Viscosity

All shear viscosities were measured at 25°C over the shear rate range 0.1 to 1000/s using the Advanced Rotational AR500 Rheometer (40-mm stainless steel cone and plate; TA Instruments, Eschborn, Germany). Duplicate samples were measured from each test solution.

Injection

The Accurus Viscous Fluid Injection system (Alcon, Fort Worth, TX) was used in conjunction with the original viscous fluid injection pack. The program "viscous fluid injection" was chosen, and the injection pressure was set to 80 psi, the maximum for this type of machine and representing the injection settings in routine vitreoretinal surgery. The standard 20-gauge metal cannula, provided as standard with every silicone oil injection pack, was used. The 23-gauge metal cannula was cut so as to have the same length as the 20-gauge cannula so that the different lumen size was the only variable factor. All injection tests were performed in duplicate, and fresh oils were used for each run to avoid aeration effects. The time for each increment of 1 mL of the various silicone oils to be injected was measured using a stopwatch. The injection times for a total of 9 mL for each silicone oil were measured. The 11.0% wt/wt of Aerosil R972 Pharma silica in 556 fluid, mixed by roller mixing followed by overhead stirring at 2000 rpm for 7 hours, was compared with Polydimethylsiloxane 1000 (PDMS 1000) and Polydimethylsiloxane 2000 (PDMS 2000; Fluoron GmbH).

Tamponade Efficiency

A model eye chamber was constructed to give a spherical polymethylmethacrylate (PMMA) chamber of 21-mm diameter.[13] Prior to each experiment, the internal surface of the model eye chamber was rendered hydrophilic by coating it in protein using 0.5% bovine albumin in phosphate-buffered saline, which was subsequently used as an aqueous substitute. One drop of methylene blue was added to each 100-mL batch of aqueous substitute to aid visualization of the tamponade–aqueous interface. The experiments were carried out at room temperature. Each tamponade agent was introduced into the chamber via 1-mm-diameter port at the inferior pole of the chamber. Excess aqueous substitute escaped passively via a cannula secured to a 1-mm-diameter port at the superior pole of the chamber. Each tamponade agent was introduced in 0.5-mL increments, until no more could be introduced. Following each incremental addition of tamponade agent, a high-resolution photograph was taken. The height of the tamponade bubble after each increment was measured using ImageJ (National Institutes of Health [NIH], Bethesda, MD). The filling experiments were run in triplicate. The 11.0% wt/wt of Aerosil R972 Pharma silica in 556 fluid mixed by roller mixing followed by overhead stirring at 2000 rpm for 7 hours was compared with Densiron 68 (Fluoron GmbH), Oxane HD (Bausch & Lomb), and F6H8 (Fluoron GmbH).

In Vitro Cytotoxicity

Cytotoxicity tests were carried out using methods adapted from International Organization for Standardization (ISO; available in the public domain at www.iso.org) 10993-5. Extracts were prepared as follows: 40-g aliquots of either the 11% silica in 556 oil or 556 oil alone were added to sterile 50-mL polypropylene centrifuge tubes followed by 10-mL aliquots of sterile culture medium (Medium 199 [Life Technologies, Paisley, UK] containing 10% fetal calf serum [FCS; Lonza Group

Ltd., Basel, Switzerland] and 2% antibiotic [Sigma-Aldrich, Poole, UK]). This ratio of oil to extraction vehicle was chosen over standard ratios as it models the oil-filled eye more accurately. Negative control samples containing 10 mL medium and 2 g polyethylene (1-mm-thickness high-density polyethylene sheet; Goodfellow Cambridge Ltd., Huntingdon, UK) were also prepared. The samples were placed on a roller mixer at 37°C and removed after 5 days. The samples were centrifuged for 1 hour at 1125*g*. Approximately 6 mL of the aqueous phase was extracted by pipette and stored at 5°C for no more than 24 hours. One hundred microliters of the extract was added to three replicate wells in a 96-well plate. L929 cells (No. CCL1; American Type Culture Collection, Manassas, VA) were cultured in 25-cm^2 culture flasks in Medium 199 containing 5% FCS and 1% antibiotic. Fifty microliters of the cell suspension (1×10^5 cells/mL) was pipetted directly onto the extracts in 96-well plates. Control wells with culture medium only (vehicle control) and positive controls containing 5% dimethyl sulfoxide (Sigma-Aldrich) in culture medium were included. The plates were incubated for 24 hours at 37°C, 5% CO_2. One hundred microliters of thiazolyl blue tetrazolium bromide (MTT; Sigma-Aldrich) solution (0.5 mg/mL MTT made up in a 1:1 mixture of PBS and double-strength Medium 199) was added to each well, and the cells were incubated for a further 6 hours. The extracts were aspirated, and 100 μL isopropanol was added to each well. The plate was incubated for a further 10 minutes, then read at 570 nm using a spectrophotometer (Biokinetics Reader Microplate; BioTek UK, Potton, UK). A background reading was taken at 690 nm and subtracted from all experimental readings. Optical density (OD) values were normalized to the blank. Data were analyzed in SPSS version 18.0 (PASW Statistics for Windows; SPSS, Inc., Chicago, IL). Statistical analysis was performed using a one-way ANOVA followed by Dunnett's T3 post hoc test ($P \leq 0.05$).

Direct contact studies were performed based upon the studies reported in Friberg et al.[14] ARPE-19, an established but nonimmortalized human RPE cell line (CRL-2302; American Type Culture Collection) were cultured in a 1:1 (vol/vol) mixture of Dulbecco's modified Eagle's medium: Nutrient Mixture F-12 (DMEM/F12; Sigma-Aldrich) containing 2 mM L-glutamine (Invitrogen) and supplemented with 10% fetal bovine serum (Biosera Europe, Boussens, France). Cells were maintained under standard conditions and used at passages 15 to 20. Cells were seeded onto polycarbonate cell culture inserts (Millicell-PCF; Merck Millipore, Watford, UK) placed in 24-well plates at 1×10^4 cells per sample in 400 μL medium; 600 μL medium was added to the outer well. After 24 hours, the medium from inside the insert was replaced by 250 μL of the prototype oil or Densiron 68 or 400 μL fresh medium. Cells were cultured for a further 7 days. Fixation was performed by replacing the medium in the outer well with 600 μL 10% neutral buffered formalin (Sigma-Aldrich) and incubating the samples at 37°C for a further 2 hours. The inserts were inverted, and gentle pipetting was used to remove the oil from the inside of the wells. Cells were stained with Alexa Fluor 488 phalloidin and 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen) and visualized using fluorescence microscopy.

In Vivo

Animal Preparation. Three- to four-month-old New Zealand White (NZW) rabbits (2.8–3.1 kg) were kept in a temperature-controlled room with 12-hour light/dark cycle. Rabbits were divided into four groups according to the material injected into the vitreous: sham control, PDMS 1000, 556 fluid, 11% silica in 556 fluid (11.0% wt/wt of Aerosil R972 Pharma silica in 556 fluid mixed by roller mixing followed by overhead stirring at 2000 rpm for 7 hours). A sham group was included to induce only surgical trauma to the eye with no tamponade agent injected. Only the right eye was operated for each rabbit. All the experimental and animal handling procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Faulty Committee on the Use of Live Animals in Teaching and Research at The University of Hong Kong (CULATR No. 1884-09).

Pars Plana Vitrectomy. Anesthesia was induced in the rabbits with intramuscular injection of xylazine (5 mg/kg) and ketamine (45 mg/kg). Amethocaine (1%) was then applied onto the cornea for topical analgesia. The pupil was dilated with topical application of 1% tropicamide eye drops. Standard three-port pars plana vitrectomy (PPV) was performed with an operating microscope (Leica Microsystems AG, Heerbrugg, Switzerland) and vitrectomy machine (Alcon). Three small incisions (each less than 2 mm) were made, and the vitreous was removed with a mechanical cutter. The surgery was illuminated with a light pipe. The vitreous was removed as much as possible under constant infusion of PBS while avoiding lens damage. After fluid–air exchange, the tamponade agent (1.2–1.5 mL) was injected to replenish the vitreous cavity. The sclerotomies were then closed using 7–0 coated Vicryl sutures (Ethicon; Johnson & Johnson, Sint-Stevens-Woluwe, Belgium). The operation was concluded by subconjunctival injection of gentamicin (2 mg/kg) and application of topical antibiotic ointment (Maxitrol; Alcon) to prevent inflammation. The left eye was not operated on.

For rabbits in the sham group, only three surgical incisions were made with no vitreous removed. The wound was then closed as described above.

Postoperational Treatment and Follow-up Clinical Observation. The rabbits were maintained under infrared immediately after vitrectomy surgery until they regained consciousness and were monitored every day thereafter. Antibiotic ointment (Maxitrol; Alcon) was applied onto the cornea on a daily basis for the first 3 days postoperation. The binocular indirect ophthalmoscope (BIO; All Pupil Indirect; Keeler, Winsor, Berks, UK) was used to check the corneal and lens opacities as well as any appearance of retinal breaks or detachments. Rabbit eyes with retinal detachment and cataract were excluded. Tono-Pen XL (Mentor, Norwell, MA) was used to measure the intraocular pressure (IOP) by averaging five readings for each measurement. The IOP measurements were performed at 1, 2, 4, 8, and 12 weeks postoperation.

Electroretinographic Tests. In order to assess the retinal function after operation, flash electroretinographic tests (ERG) were performed on the operated eyes at 1, 2, 4, 8, and 12 weeks after the surgery. All the ERG recordings were performed at approximately 14:00 in order to eliminate any influence of potential circadian fluctuations. Both the photopic and scotopic ERGs were performed. Anesthesia was applied by intramuscular injection of xylazine (5 mg/kg) and ketamine (45 mg/kg) before each visual adaptation was performed. Rabbits were light adapted for 10 minutes before photopic flash ERG, and dark adapted for 20 minutes before scotopic flash ERG. The corneas were further anesthetized with 1% amethocaine. Pupils were then dilated with 1% tropicamide eye drops. Corneal contact lens electrodes (Ocular Instruments, Bellevue, WA) were used as the active electrode for ERG recording. Gel 4000 (Bruschettini, Genova, Italy) was applied between the cornea and the contact lens electrode for corneal protection and better conduction. A reference electrode was placed at the upper eyelid with fur shaved, and a ground electrode was placed at the contralateral ear. A bright stimulation flash with visibly white diffuser, stimulus strength of 2.4 cd-s/m^2, and stimulus duration of 10 μs (GRASS, West Warwick, RI) was adopted. The time interval of each flash was set to 20 seconds

Table. Numbers of Eyes With Cataract in Every Group at Every Time Point; Data Presented as the Number of Cataract Eyes per Total Number of Eyes in the Given Group

	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks
Sham control	0/12	0/9	0/9	0/5	0/5
PDMS 1000	0/10	0/7	1/7	3/3	3/3
556 fluid	0/10	0/7	0/7	2/3	3/3
11% silica fluid	0/11	2/8	2/8	3/4	3/4

for retinal function recovery. All the instruments were grounded to ensure minimal noise. Amplitudes of each a-wave and b-wave were obtained after averaging 20 to 30 recordings.

Histology. Rabbits were killed by intravenous administration of sodium pentobarbital (100 mg/kg) at 1, 4, and 12 weeks after the vitrectomy. Eyes were enucleated and immediately fixed with 4% paraformaldehyde solution. Approximately 5 mm of temporal and nasal sides of the eyeball were cut after fixation, leaving an approximately 10-mm-wide central part with the optical nerve attached for embedding in paraffin wax. Each eyeball was positioned with its cornea facing the left side of the paraffin block to ensure orientation consistency. Samples were cut along the sagittal plane into 7-μm sections which were subsequently stained with hematoxylin and eosin (H&E). The anatomical position of each section was determined by referencing its relative position to the edge of the optic nerve. Sections from the same anatomical position of each sample were selected and compared. Histological images of superior and inferior retinae were captured under the ×20 objective with an optical microscope (Nikon Eclipse 80i; Nikon, Tokyo, Japan). Images were captured from every adjacent field of view under the objective. Thicknesses of inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL) were measured using SPOT imaging software (Diagnostic Instruments, Sterling Heights, MI). Cell nuclei number of the inner and outer nuclear layer were also counted and expressed as cells per field. The entire retina was separated into six anatomical positions: the superior central, superior midperipheral, superior peripheral, inferior central, inferior midperipheral, and inferior peripheral. Subretinal layer thicknesses and nuclei counts in every anatomical position were averaged and compared. All quantification was repeated in triplicate in a masked manner. Data, expressed as mean ± SD, were analyzed by one-way ANOVA followed by Bonferroni posttest (Prism version 5.0; GraphPad Software, Inc., San Diego, CA). Bonferroni posttest was performed only when the means of the groups were significantly different ($P < 0.05$).

Results

The solution of 11% silica in 556 fluid appeared transparent to the naked eye, and all further experiments used this solution. However, complete optical clarity measured using spectrophotometry was achieved in the solution composed of 11.0% silica, 82.6% 556 fluid, 6.4% PDMS (1000 mPa·s). It was possible to incorporate the aerosol silica into the 556 fluid at all concentrations evaluated using simple roller mixing over a 24-hour time period. The shear viscosities of these mixtures (Supplementary Fig. S1) increased with the concentration of silica added. For the 12.5% silica in 556 fluid, the peak shear viscosity was approximately 20 times that of the commonly used clinical grade silicone oil 5000. Overhead stirring of the mixtures resulted in a large reduction in the peak shear viscosities for each silica concentration (Supplementary Fig. S2) comparative to currently used clinical silicone oils. From these data, the 11% silica in 556 fluid was chosen for further study. The higher the stirring speed and the longer the stirring time, the lower the initial and peak shear viscosity of the solution (Supplementary Fig. S3). Increasing the amount of energy put into the system using ultrasonic mixing produced a further reduction in the shear viscosity of the solution over the whole range of shear rates. Multiple batches of the 11% silica in 556 fluid produced with overhead stirring at 2000 rpm for 7 hours showed good reproducibility in terms of shear viscosity across the range of shear rates from 0.1 to 1000/s (Supplementary Fig. S4). The storage of these samples up to 56 weeks at 37°C was demonstrated to have very little influence on the shear viscosity (Supplementary Fig. S5a). At each time point the samples were briefly inverted prior to application into the rheometer (Advanced Rheometer AR500; TA Instruments). Similar results were observed following ultrasonic mixing and storage up to 24 weeks (Supplementary Fig. S5b). It is important that any new tamponade agent can be used clinically with existing instruments if possible. The length of time taken to inject the 11% silica in 556 fluid was demonstrated to be less than that for either PDMS 1000 or PDMS 2000, two of the lower-viscosity clinical grade tamponade agents (Supplementary Fig. S6), with either a 20- or 23-gauge cannula.

The height of the tamponade bubble was measured in a spherical model chamber (Supplementary Fig. S7a) with dimensions similar to those of an eye. As the specific gravity increases, the height of the bubble for a particular volume is reduced; thus a greater proportion of the bubble surface makes contact with the internal surface of the spherical chamber, increasing the tamponade efficiency. Four materials were compared: 11% silica in 556 fluid (specific gravity 1.12), Densiron 68 (specific gravity 1.06), Oxane HD (specific gravity 1.03), and F_6H_8 (specific gravity 1.70). The bubble height for Densiron 68 and Oxane HD was very similar, but that for the 11% silica in 556 fluid was significantly lower for each volume added (Supplementary Fig. S7b). The height of the F_6H_8 bubble was still lower, but this fluid is not used as a long-term tamponade.

In compliance with the ISO 10993 standard, the in vitro tests of the 556 fluid alone and the 11% silica in 556 fluid showed no evidence of cytotoxicity (Supplementary Figs. S8, S9). Full results are shown in Supplementary Appendix 1.

The 556 fluid and the 11% silica in 556 fluid were compared with PDMS 1000 and a sham operation following injection into the vitreous cavity of rabbits after vitrectomy. During the postoperational monitoring and observation, no visible intraocular inflammation or hemorrhage was observed at either 1 week or 12 weeks postvitrectomy in all experimental groups. The cornea of all rabbits remained clear.

Cataracts were observed in eyes of all silicone oil groups (Table). No cataract was observed (0%) in eyes from the sham group up to 12 weeks postoperation. For eyes with PDMS 1000 as tamponade agent, 1 of 7 rabbits (14.3%) started to develop cataract in the operated eye 4 weeks after vitrectomy. From 8 weeks postvitrectomy, the remaining 3 rabbits developed cataract in the operated eye (100%). For the 556 fluid group, no instance of cataract was observed (0%) until 8 weeks after vitrectomy, when 2 out of the 3 remaining rabbits developed cataract in their operated eye (66.6%); and at 12 weeks postvitrectomy, cataract was observed in all 3 remaining rabbits (100%). For rabbits in which 11% silica fluid was used as tamponade agent, 2 of 8 rabbits developed cataract (25%) at 2 weeks after vitrectomy, and from 8 weeks postoperation, cataract was identified in 3 out of the 4 remaining rabbits (75%).

The IOPs of the operated eyes for the sham group, the PDMS 1000 group, the 556 fluid group, and the 11% silica fluid group ranged from 6.4 to 13.4, 6.4 to 20.4, 5.0 to 13.0, and 5.6

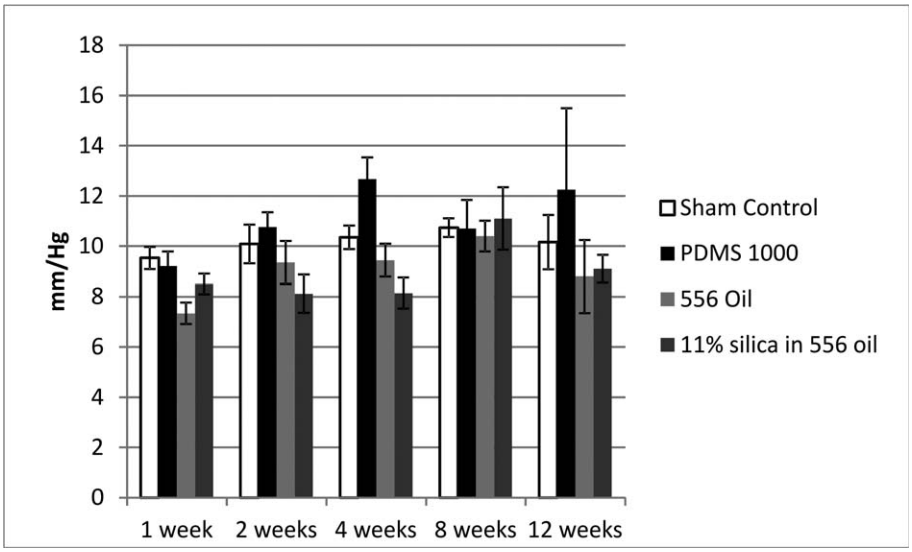

Figure 1. Postoperative IOP measurements presented as mean ± SEM (*n* varied as follows: sham 1 week [12], 2 weeks [9], 4 weeks [9], 8 weeks [5], 12 weeks [5]; PDMS 1000 1 week [10], 2 weeks [7], 4 weeks [7], 8 weeks [3], 12 weeks [3]; 556 oil 1 week [10], 2 weeks [7], 4 weeks [7], 8 weeks [3], 12 weeks [3]; 11% silica in 556 oil 1 week [11], 2 weeks [8], 4 weeks [8], 8 weeks [4], 12 weeks [4]). Statistical analysis using ANOVA with Bonferroni posttest demonstrated no significant differences at $P < 0.05$ except for PDMS 1000 between 1 and 4 weeks.

to 13.4 mm Hg, respectively. No significant difference was identified between the IOP of all eyes in the silicone oil groups and those in the sham group (Fig. 1).

Postoperative retinal functions were represented by ERG. At 1 week after vitrectomy, the a-wave amplitude of both photopic and scotopic ERG for the 556 fluid group and 11% silica fluid group was significantly smaller than that for the sham group (Fig. 2A). A significant difference appeared only at 8 weeks postoperation, when the value of photopic b-wave of the PDMS 1000 group was compared with that of the sham group (Fig. 2B). All other a-wave and b-wave values in the silicone oil groups appeared to be normal when compared with the sham group, and no significant difference was observed (Supplementary Fig. S10).

At 1 week after vitrectomy, the histology images of retinal samples in all groups were in regular order. There was no significant difference in morphology between the silicone oil groups and sham group in either the superior or inferior retina (Supplementary Fig. S11). This was confirmed with the quantitative result of subretinal layer thickness measurement (Supplementary Fig. S12) and nuclei count (Supplementary Fig. S13) at 1 week, with no significant difference observed when the silicone oil groups were compared with the sham group at all six retinal positions (superior-central, superior-midperipheral, superior-peripheral, inferior-central, inferior-midperipheral, and inferior-peripheral).

At 4 weeks after vitrectomy in 3 out of 4 samples of the 11% silica fluid group, in the middle peripheral area of inferior retina (which consisted of approximately one-fourth the length of the entire inferior retina), the bipolar cell nuclei in INL appeared to drop down to the OPL layer, and the OPL appeared to be unclear (Fig. 3). This observation was confirmed by retinal thickness measurement, with the OPL in the 11% silica fluid group significantly thinner compared with that in the sham group (Fig. 4A).

At 12 weeks after vitrectomy, the same phenomenon of "INL nuclei dropdown" appeared in both the PDMS 1000 group and the 11% silica fluid group. In 2 out of 3 samples of the PDMS 1000 group, the nuclei dropdown was observed in the central and midperipheral part of the superior retina. In 2 out of 3 samples in the 11% silica fluid group, the nuclei dropdown was observed in the midperipheral part of the

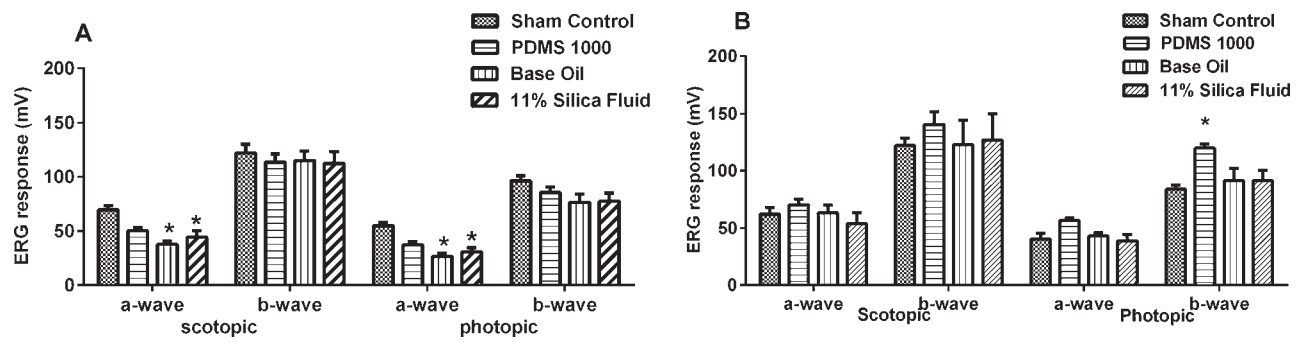

Figure 2. The scotopic and photopic electroretinographic response indicating the retinal functions of different groups at (**A**) 1 week and (**B**) 8 weeks postoperation. Both a-wave and b-wave responses were recorded and compared (two-way ANOVAs were performed; $^*P < 0.05$ after correction for multiple comparison).

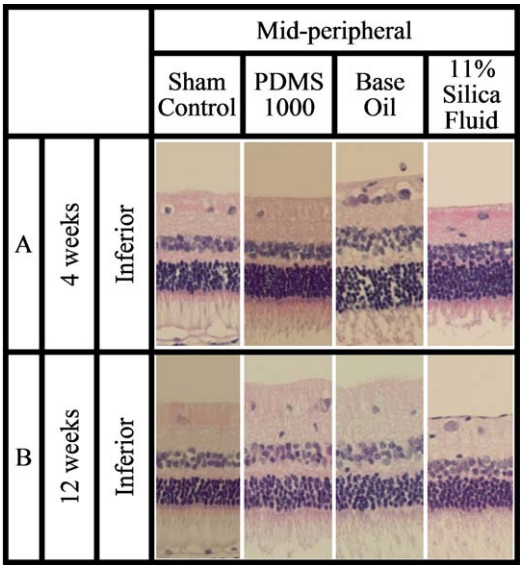

FIGURE 3. Representative retinal sections at inferior-midperipheral retina in animals killed at (**A**) 4 weeks and (**B**) 12 weeks postoperation. The thickness of IPL, INL, OPL, and ONL was measured.

inferior retina (Fig. 3). Again, the result was confirmed by the thickness measurement showing that compared with the sham group, the OPL was significantly thinner in inferior midperipheral for the 11% silica fluid group (Fig. 4B).

The result at 12 weeks postoperation also showed that the nuclei number in ONL of the superior retina in the PDMS 1000 and 556 fluid groups was significantly larger than that in the sham group (Supplementary Fig. S13), which correlated with the retinal thickness result (Supplementary Fig. S12). Yet the significant thickness increase in superior INL and even IPL for PDMS 1000 and 556 fluid cannot be correlated to the nuclei number result. Also, the difference between the nuclei number in ONL of the inferior retina for the 11% silica fluid and the sham group was significant (Supplementary Fig. S13), while the difference in thickness of OPL between the 11% silica fluid and sham control was significant (Fig. 4B).

For a full comparison of data in all time points with various treatments please refer to Supplementary Appendix 1.

DISCUSSION

The use of silicone oil tamponades in the treatment of complex retinal detachments is well documented and accepted in clinical practice. The role of the tamponade is to seal retinal breaks and to oppose the detached retina and to re-place it against the underlying retinal pigment epithelium. From a materials science point of view, the important properties of the silicone oils to achieve this are their interfacial tension with the remaining aqueous, viscosity, and specific gravity.[15]

Silicone oils are generally polydimethylsiloxanes, a polymer with a –Si-O- backbone and methyl (-CH_3) pendant groups that render the polymer hydrophobic. This property permits the tamponade to exclude aqueous from the retinal break. The 556 fluid used in this study is a poly phenylmethylsiloxane with similar hydrophobic properties. Thus as long as the tamponade is positioned over the retinal break, similarly to the standard oil, the tamponade should seal retinal breaks and its buoyancy should oppose the detached retina back onto the underlying retinal pigment epithelium. Standard silicone oils have a specific gravity just below 1; therefore they float and are most efficient at tamponading superior retinal tears. Heavy tamponades have a specific gravity that is greater than 1 and so sink below any remaining aqueous; thus they are more appropriate for tamponading inferior retinal tears.[5] The difference in specific gravity between the tamponade agent and the remaining aqueous has an influence on the shape of the tamponade bubble due to the buoyancy forces acting on it. The very small difference in specific gravity of the normal silicone oil and aqueous results in the silicone oil bubble having a near-spherical shape within the vitreous cavity. The existing heavy tamponade agents in clinical practice have specific gravities that are only slightly higher than 1 (Densiron 68 is 1.06 g/mL, and Oxane HD is 1.02 g/mL); therefore, although they sink, they also have a near-spherical shape and thus their tamponade efficiency is low. Increasing the specific gravity further, so that the difference from the aqueous is greater, causes the bubble to be less spherical, and thus a smaller volume is needed to tamponade the same surface area of retina, increasing the tamponade efficiency.[15–17] In this study the addition of Aerosil R972 silica to the fluid significantly increased the specific gravity because of the higher density of the solid material, and this caused a significant change in the shape of the bubble in the model eye chamber in comparison with Densiron 68 and Oxane HD. This holds the promise of a novel tamponade agent potentially with better tamponade properties than any existing oil-based tamponade. A particular advantage of increasing the specific gravity by adding a solid material is the opportunity to tailor the specific gravity by varying the amount of material added, whereas with the semifluorinated alkanes/alkenes, the maximum specific gravity is governed by their solubility in the silicone oil.

The addition of a solid material could, however, cause light scattering and thus opacification of the tamponade. To

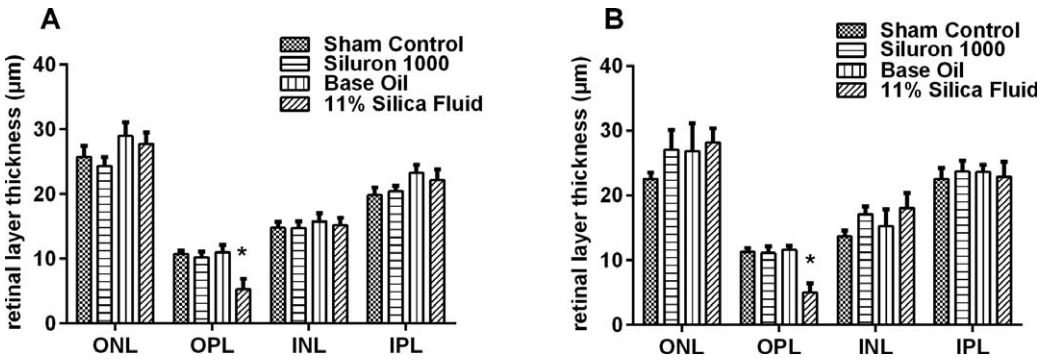

FIGURE 4. The thickness of IPL, INL, OPL, and ONL at (**A**) inferior-central and (**B**) inferior-peripheral retina in animals killed at 4 weeks postoperation (two-way ANOVAs were performed; $^*P < 0.05$ after correction for multiple comparison).

overcome this, either the solid particles must be small enough or the RI of the solid and the fluid need to match. In this study, the Aerosil silica was made up of micron-sized aggregates of silica particles approximately 20 nm in diameter. These aggregates would need to be broken down to the individual 20-nm particles to inhibit light scattering, but this would require considerable energy input.[18] The alternative approach was taken in which a poly phenylmethylsiloxane with a RI of 1.46 was used that matched the RI of the silica. When RI matching is used, the clarity of the solution is independent of the amount of solid added. We showed that although the 11% silica in 556 fluid appeared only slightly turbid to the eye, the solution composition required manipulation by the addition of 20% polydimethylsiloxane to obtain complete optical clarity across all wavelengths examined. It should be noted that these measurements are temperature dependent and were made at room temperature. Increasing the temperature to 37°C slightly reduces the RI of the silicone fluid, improving the optical clarity of the 11% silica in 556 fluid alone.[19]

Adding solid material to the 556 fluid will increase its viscosity. The Aerosil silica, as mentioned above, is made up of aggregates of silica nanoparticles approximately 20 nm in diameter. When these are added to a fluid, the aggregates will bind using hydrogen bonding to form a network structure throughout the whole volume.[20] This results in a very high viscosity solution as was observed after the silica had been incorporated into the 556 fluid using roller mixing alone. This three-dimensional network, however, can be broken down by shearing the solution and thus decreasing the viscosity. We have demonstrated that overhead stirring of the silica/556 fluid solutions can reduce the viscosity in a controlled manner. Since the siloxane has some hydrogen binding capability, once the network has been dismantled the siloxane will bind to the particles and inhibit the reforming of the network structure as demonstrated by the stability of the viscosity of the stored solutions over time. If some network does reform, it is easily broken down again by simple inversion of the solution in its vessel. We demonstrated that the 11% silica in 556 fluid treated by overhead stirring for 7 hours at 2000 rpm produces a solution with a peak viscosity of 2000 mPa·s that could be produced reproducibly over several batches and that remained stable during storage. This solution was chosen for further evaluation in terms of clinical relevance.

We have demonstrated that this prototype solution can be injected using an off-the-shelf Alcon Accurus injection system, and in fact there was less resistance to injection than with either PDMS 1000 or PDMS 2000 even though the peak viscosity of the heavy tamponade prototype was 2000 mPa·s. This suggests that the shear exerted on the fluid by the injection system aids the injection process, and a similar effect would be expected on removal of the tamponade.

The biocompatibility of different silicone oils toward the retina has been considered and studied extensively in in vitro research through to clinical trials, but the results of these studies appear to be controversial.[21–29] Heavy silicone oils have been shown to prevent biological factors that induce inflammation and PVR from accumulating next to the inferior part of the retina.[5,30] On the other hand, some heavy silicone oils have been shown to cause a problem with toxicity to the retina compared with conventional silicone oil because of the components added into the silicone oil. The current generation of heavy silicone oils (such as Densiron 68 and Oxane HD), however, has been associated with much lower complication rates compared with the previous generation (such as fluorinated silicone and perfluorocarbon liquids).[31–33]

The 556 fluid is a cosmetic grade poly phenylmethylsiloxane, and thus it is essential to evaluate its biocompatibility in this application. Its in vitro cytocompatibility was evaluated using a test adapted from the ISO 10993-5 methodology. This adapted method has been used commercially during previous tamponade development. These studies demonstrated no cytotoxicity for either the 556 fluid alone or the 11% silica in 556 fluid. Furthermore, evaluation of the influence of the 11% silica in 556 fluid in direct contact with retinal pigment epithelial cells suggested qualitatively no detrimental effect on the cell growth or morphology in comparison with control cultures and cells grown in contact with Densiron 68. There is understandable concern about the release of nanoparticles and their migration into the retina and other tissues. Since the particles and the oil are both hydrophobic, there is no affinity for the particles to leave the oil and enter the aqueous phase. Furthermore, because of the amorphous nature of the silica particles, if they did enter the aqueous phase they would dissolve as silicic acid, which is ubiquitous as orthosilicic acid in the body. There was no evidence of particles in the cells under light microscopy, but future work will check for this using electron microscopy to determine if any 20-nm particles can be detected.

Further biocompatibility evaluation was undertaken via an in vivo study in rabbits. Ophthalmic examination can indicate several biocompatibility issues following injection of tamponade agents, such as inflammation, cataract induction, and IOP change.[28,29] The ophthalmic examination performed in our study showed no indication of inflammation or significant change in IOP. Cataract is a common complication of silicone oil use as long-term tamponade agents, for both conventional and heavy silicone oils.[9,28,34] In our study, it was shown that at 8 weeks postsurgery, cataract formation occurred in most of the eyes in all three silicone oil groups. The 11% silica fluid appeared to induce cataract earlier (2 weeks postoperation) than PDMS 1000 (4 weeks postoperation). The 556 fluid on its own induced cataract at a relatively late stage (8 weeks postoperation), which suggested that the addition of silica caused the early formation of the cataract possibly due to the increased specific gravity.

According to previous studies, cataract would not affect ERG results significantly unless the cataract was severe.[35,36] We took this factor into account when analyzing the ERG results; however, since the evidence of cataract was based on BIO observation instead of slit-lamp imaging, the severity of cataract was not categorized by cataract classification.[37,38]

There are contradictory conclusions about the effect of silicone oils on the IOP in the literature.[22,31,39–41] Some studies show that even the vitrectomy surgery itself might cause IOP change.[42,43] For this reason, we included a sham group with surgical trauma induced as a control and compared all the silicone oil groups with this sham group. Intraocular pressure measurements for all eyes were within the normal range, and there was no significant difference between any of the measurements over the entire postoperative period.

The longer-term effect of tamponade agents on retinal function is another important factor in relation to their biocompatibility. As indicated in both scotopic and photopic ERG responses, the photoreceptor functions represented by the a-wave appeared to be significantly affected by both the 556 fluid and 11% silica fluid 1 week after vitrectomy, but then recovered at later time points. From this result, it seems that the heavy tamponade might affect the photoreceptor function for a short period of time (within 1 week) after vitrectomy, and this could be due to either the 556 fluid or the increased specific gravity. On the other hand, the b-wave in ERG response, which represents mainly the bipolar cell and Müller cell function, appeared to be unaffected over the entire postoperative period. In conclusion, the consistency of b-wave value as well as the reversible a-wave change suggested limited or recoverable functional neurological defect caused by the

11% silica fluid. This result was consistent with previous in vivo studies showing no retinal damage after prolonged use of heavy tamponade agents.[22,44]

The most remarkable finding relating to the morphological changes was the phenomenon that the bipolar cell nuclei in INL dropped down to the IPL. It seems that this retinal morphology change happened in a time-dependent manner. It appeared earlier in the 11% silica fluid group (4 weeks postoperation) than in the PDMS 1000 group, which was similar to the situation with cataract formation. In fact, the OPL thickness in the PDMS 1000 group decreased at 4 weeks postoperation, although the decrease was not significant, which might indicate an emergence of morphological change (OPL thickness shrinkage) as well.

Currently, there are only a few studies that discuss the phenomenon of nuclei dropdown.[22,44,45] As shown in our study, the INL nuclei dropdown and OPL shrinkage happened at the inferior retina for the 11% silica fluid group and at the superior retina for the PDMS 1000 group; thus the pressure toward retina might be a possible explanation for the morphological change (pressure that came from the gravity of heavy silicone oil and from the buoyancy of conventional silicone oil).[46] The 11% silica fluid has a greater difference in specific gravity from water than PDMS 1000; this would be expected to cause a higher pressure on the retina, which might explain the earlier influence on the OPL thickness. An alternative explanation might be changes in the intraocular environment caused by the displacement of the aqueous film at the surface of the retina by the tamponade. The aqueous film that was in contact with the retina may be thinner after tamponade agent injection and thus could inhibit the sodium and potassium ionic exchange between the Müller end-feet and the aqueous. The cells may then become hyperpolarized and affected by the excitotoxicity.[47,48] This may affect the INL nuclei dropdown, and this higher specific gravity of the 11% silica fluid could decrease the thickness of the aqueous film more than PDMS 1000. As the INL nuclei dropdown phenomenon was not observed in the control eyes, it may be a sign of late cellular toxicity; whether the nuclei dropdown would lead to other side effects needs to be investigated further. Yet our study showed no indication of functional deterioration correlated with this phenomenon.

In conclusion, this study has demonstrated that a heavy tamponade can be developed with a variable specific gravity, depending on the particular need of the surgery, by the addition of varying amounts of silica particles. The optical clarity of the tamponade is maintained by RI matching the silica and the silicone oil. The viscosity of the solution can be controlled by the mixing protocol using standard laboratory procedures. The properties of the tamponades are reproducible from one batch to another and are stable during storage up to 56 weeks. We have demonstrated that this novel tamponade has several attributes to allow applicability to clinical practice, including injectability using an off-the-shelf Alcon Accurus injection system, good cytocompatibility in vitro, and encouraging in vivo biocompatibility in comparison with 1000 mPa·s silicone oil.

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