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Emulsification Tendency of Silicone-Phenylsilicone Copolymer

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Purpose: We compared the emulsification tendency of silicone-phenylsilicone copolymer (DPC; 5%-phenylated, specific gravity 0.984) with that of silicone oil (SO; specific gravity 0.966) and fluorosilicone oil (FSO; specific gravity 1.256), all of which are used clinically as intraocular tamponades.

Methods: We investigated the tendencies of emulsification in SO, FSO, and DPC. Each was placed in a separate glass container with equal amounts of albumin solution (1 mg/mL) or 1 γ-globulin solution (1 mg/mL) and shaken. We also investigated the toxicity of DPC in the rabbit eye. Following vitrectomy, we injected DPC into the vitreous cavity and assessed the retinal damage histologically.

Results: The SO and DPC, because their specific gravities, are closer to water, tended to become less emulsified than did FSO. We found that DPC did not cause any severe histological damage in the rabbit retina.

Conclusion: Highly phenylated DPC is slightly heavier than water and may be used instead of FSO to treat inferior retinal detachment. **Jpn J Ophthalmol 2001;45:53–59** © 2001 Japanese Ophthalmologiscal Society

Key Words: Emulsification, intraocular tamponade, retinal detachment, silicone oil, vitreous.

Introduction

Silicone oil (SO; dimethylsiloxane, Figure 1A) is widely applied clinically as an intraocular tamponade following vitrectomy.[1–5] However, SO often becomes emulsified if left in the eye for a long period of time.[6,7] Emulsified SO is considered toxic to the ocular tissues.[8–10] Furthermore, SO has a specific gravity of <1, and therefore exerts no tamponade effect on the lower retina. Fluorosilicone oil (FSO; methyl-3,3,3-trifluoroprophyl siloxane, Figure 1B) is also used as an intraocular tamponade[11] and has a specific gravity >1; however, it is reported to become emulsified much more readily than SO.[12] The present study compares the emulsification tendency of SO and FSO to that of a silicone-phenylsilicone copolymer (DPC; dimethylsiloxane-phenylmethylsiloxane copolymer, Figure 1C), which has a specific gravity closer to that of water than do conventional SO and FSO.

In addition, the effects of DPC on rabbit eye tissue are examined histologically, to establish its efficacy as an intraocular tamponade. The purpose of this study is to evaluate this new silicone oil (DPC) as an intraocular tamponade following vitrectomy.

Materials and Methods

Materials

Fifty-centistoke (cs) SO, 1,000-cs SO, 50-cs DPC (5% phenylated), 30,000-cs DPC (5% phenylated),

Received: February 9, 1999

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Jpn J Ophthalmol 45, 53–59 (2001)

Published by Elsevier Science Inc.

0021-5155/01/$–see front matter
PII S0021-5155(00)00292-6

Jpn J Ophthalmol
Vol 45: 53–59, 2001

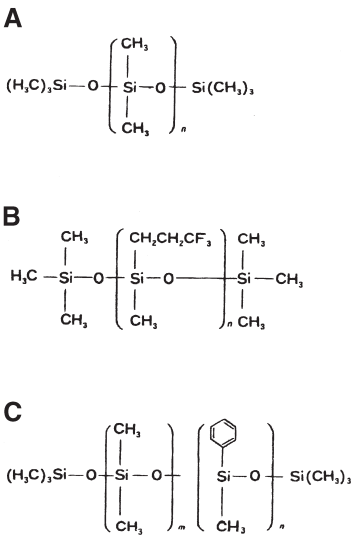

Figure 1. Structure of three clinically used intraocular tamponades: (**A**) silicone oil, (**B**) fluorosilicone oil, and (**C**) silicone-phenylsilicon copolymer (5% phenylated).

and 1,000-cs FSO were used in the present study. Following the Petrarch Systems instruction chart7, 50-cs DPC and 30,000-cs DPC were mixed at a weight ratio of 57:43 to yield 1,000-cs DPC. The oils were mixed with approximately 20 mg/mL of activated charcoal and allowed to sit for 1 day to remove impurities before the activated charcoal was removed using filter paper. The albumin and globulin used for interfacial tension measurement and the emulsification experiment were dissolved in a phosphate buffer (0.01 M, pH 7.4) prior to use.

Viscosity and Specific Gravity Measurement

The viscosity of each oil was measured at 37°C using a Brookfield viscometer. Specific gravity was measured using a scale hydrometer, during which time the sample oil was maintained at 37°C in a constant temperature bath. Each sample was measured three times and the average was recorded.

Interfacial Tension Measurement

Interfacial tension between 1,000-cs oils and protein solutions (1 mg/dL of albumin solution and 1 mg/dL of 1 γ-globulin solution) was measured by the Wilhelmy plate method using a Kyowa CBVP A-3 surface tensiometer. The samples were preheated in a constant temperature bath at 37°C for 10 minutes prior to measurement. Measurement was performed three times on each sample and the average was recorded.

Emulsification Experiment

Each oil sample was placed in a 20-mL glass vial (inner diameter 2.8 cm) with an equal amount of protein solution (1 mg/dL of albumin solution or 1 mg/dL of 1 γ-globulin solution). The vial was then vacuum-sealed. The following three shaking experiments were performed to induce emulsification:

1. Sealed vials containing albumin solution (1 mg/mL) or 1 γ-globulin solution (1 mg/mL) in addition to either 50-cs SO, 1,000-cs SO, 50-cs DPC, 1,000-cs DPC, or 1,000-cs FSO were shaken vertically in an SR-Iiw Taiyo Reciproshaker for 30 minutes at room temperature. Shaking frequency was 300/minute; amplitude was 4 cm. Following shaking, the degree of emulsion was observed macroscopically.
2. Sealed vials containing albumin solution (1 mg/mL) or 1 γ-globulin solution (1 mg/mL) in addition to 1,000-cs SO or 1,000-cs DPC were shaken horizontally by the BR-40L Taitec Bioshaker in a constant temperature bath maintained at 37°C for 30 minutes. Shaking frequency was 208/minute; amplitude was 2 cm. Following shaking, the degree of oil emulsification was observed macroscopically. The water solution was removed using a pipette and observed under a phase-contrast microscope (Olympus BP-II, Tokyo).
3. Sealed vials containing albumin solution (1 mg/mL) or γ-globulin solution (1 mg/mL) in addition to 1,000-cs SO or 1,000-cs DPS were shaken perpendicular to the oil surface at 37°C for 30 minutes. Shaking frequency was 120–160/min and the amplitude was approximately 5 cm. Following shaking, the degree of emulsion was observed macroscopically. The same shaking experiment was repeated after allowing the sealed vials containing protein solution and oil to sit for 1 week at room temperature.

Animal Experiment

Nine white rabbits weighing 1.5–2.0 kg were used in the following procedures. Following lens aspiration and vitrectomy of the right eye of each rabbit, the intraocular fluid was replaced simultaneously with air, and 1,000-cs DPC was injected up to the back of the iris. The same technique was employed on the left eye (the control eye), except that BSS Plus™ was injected instead of DPC. The anterior portion of the eye was examined using a slit-lamp microscope, and the fundus was examined using a

binocular indirect ophthalmoscope at 1 and 3 days and 1, 2, 4, and 8 weeks after surgery. Both eyes of three rabbits were enucleated 2, 4, and 8 weeks after surgery, fixed in 2% glutaraldehyde, postfixed in 1% osmium, dehydrated in ordinary ethanol, and embedded in epon resin. Sections (1-μm thick) were stained with hematoxylin and eosin for light microscopic observation; ultrathin sections were double-stained with uranyl acetate and lead citrate for electron microscopic observation. The sections were examined histologically and compared with respect to changes in the chorioretinal region and iris. These rabbits were maintained and used in this study in accordance with ARVO guidelines.

Results

Viscosity and Specific Gravity Measurement

Viscosity and specific gravity averages for 50-cs oil at 37°C were 52 cs and 0.947 for SO, and 51 cs and 0.975 for DPC. In the case of 1,000-cs oils at 37°C, viscosity and specific gravity averages were 1,070 cs and 0.9666 (SO), 1,079 cs and 0.984 (DPC), and 1.135 cs and 1.256 (FSO). The specific gravity of the 1 mg/mL albumin solution at 37°C 5a was 1.002.

Interfacial Tension Measurement

Interfacial tension between the 1,000-cs oils and phosphate buffer at 37°C was 33.6 dyne/cm (SO), 31.1 dyne/cm (DPC), and 29.2 dyne/cm (FSO). Interfacial tension between the oils and 1 mg/mL albumin solution was 18.8 dyne/cm (SO), 16.7 dyne/cm (DPC), and 15.8 dyne/cm (FSO). Interfacial tension between the oils and 1 mg/mL γ-globulin solution was 18.0 dyne/cm (SO), 16.1 dyne/cm (DPC), and 15.1 dyne/cm (FSO).

Emulsification Experiment

Macroscopic oil droplets were not observed following 30 minutes of vertical shaking (300/min, amplitude 4 cm) at room temperature in 1,000-cs SO or 1,000-cs DPC with protein solution (1 mg of albumin or 1 mg/mL of γ-globulin). On the other hand 1,000-cs FSO was totally emulsified by this shaking (Figure 2). Droplet formation was observed in approximately two thirds of the 50-cs SO, whereas particulate droplets were observed only at the interface in the water solution of the 50-cs DPC (Figure 3). No macroscopic oil drop formation was observed after 1 week of horizontal shaking at 37° (208/min, amplitude 2 cm) in the 1,000-cs SO or DPC with the protein solution.

However, phase-contrast microscopy of the water solution revealed scattered microscopic droplets displaying Brownian movement in all specimens (Figure 4). The formation of relatively large droplets was observed after 1 minute of shaking (120–160/min, amplitude 50 cm) in all 1,000-cs oils and protein solutions. Shaking immediately after sealing the vial resulted in greater oil droplet formation in SO than in

Figure 2. When 1,000-centistoke (cs) silicone oil (SO), 1,000-cs silicone-phenylsilicon copolymer (DPC), and 1,000-cs fluorosilicone oil (FSO) were shaken vertically with 1 mg/mL of 1 γ-globulin solution in completely filled vial, 1,000-cs SO (left) and 1,000-cs DPC (center) were not emulsified; only 1,000-cs FSO (right) was totally emulsified.

Figure 3. When 50-centistoke (cs) silicone oil (SO) or 50-cs silicone-phenylsilicone copolymer (DPC) was shaken horizontally with 1 mg/mL of albumin solution or 1 γ globulin solution in completely filled vial, 50-cs SO (left) was almost totally emulsified. However, 50-cs DPC (right) was only slightly emulsified.

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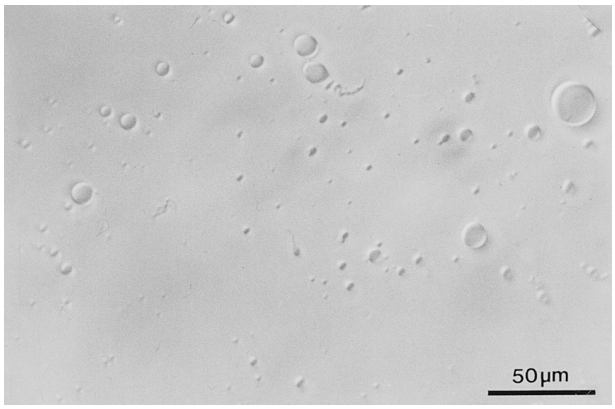

Figure 4. Ten minutes after 1,000-centistoke silicone oil was shaken horizontally with 1 mg/mL of albumin solution in completely filled vial for 1 week, macroscopic oil droplets were not observed. However, when water layer was removed, using pipette, and observed under phase-contrast microscope, fine droplets were seen dispersed in the water.

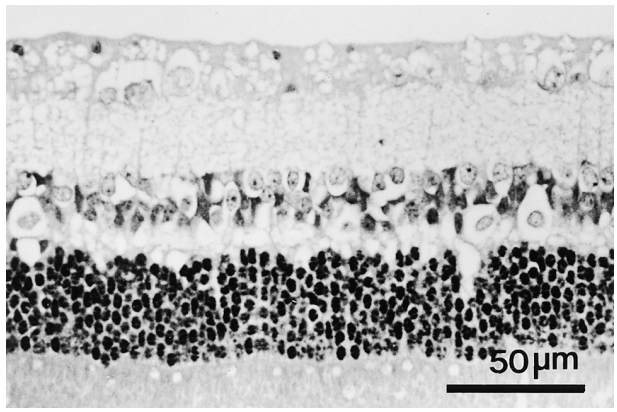

Figure 5. Light microscopy revealed slight vacuolar changes in inner layers of retina 8 weeks after silicone-phenylsilicone copolymer was injected into vitreous cavity.

DPC. In contrast, DPC formed oil droplets more readily than SO when shaken after being allowed to sit at room temperature for 1 week after sealing.

Animal Experiment

All rabbit eyes injected with 1,000-cs DPC and BSS plus™ had clear corneas with a clearly visible fundus each time examined. Light microscopy revealed slight vacuolar changes in the inner retinal layers of the eyes containing DPC and BSS plus™ at 2, 4, and 8 weeks after extraction. However, no such changes were observed in the control eyes (Figure 5). The sensory retina, retinal pigment epithelium, choroid, and iris did not change remarkably in any of the specimens. Electron microscopy revealed no rupturing in the inner limiting membrane of the 1,000-cs DPC-injected eyes after 8 weeks, and there was no vacuolar change or Müller cell degeneration. However, slight protuberance of the nerve cells, swollen mitochondria, and free ribosomes were observed (Figure 6). Macrophages and vacuoles were also observed in the inner limiting membrane, suggesting that DPC had migrated into the cell (Figure 7).

Discussion

In ophthalmology, the term *SO emulsification* refers not to the formation of fine dispersed droplets in suspension, as in ordinary emulsions, but to the formation of relatively large accumulated droplets that contact each other (Figure 8). Two types of mechanisms, thermodynamic[13,14] and hydrodynamic,[13,15,16] have been proposed to explain SO emulsification.

The thermodynamic mechanism implies that emulsification occurs when the interfacial tension decreases as surface-active substances inside the organism attach to the SO surface. Two surface-active

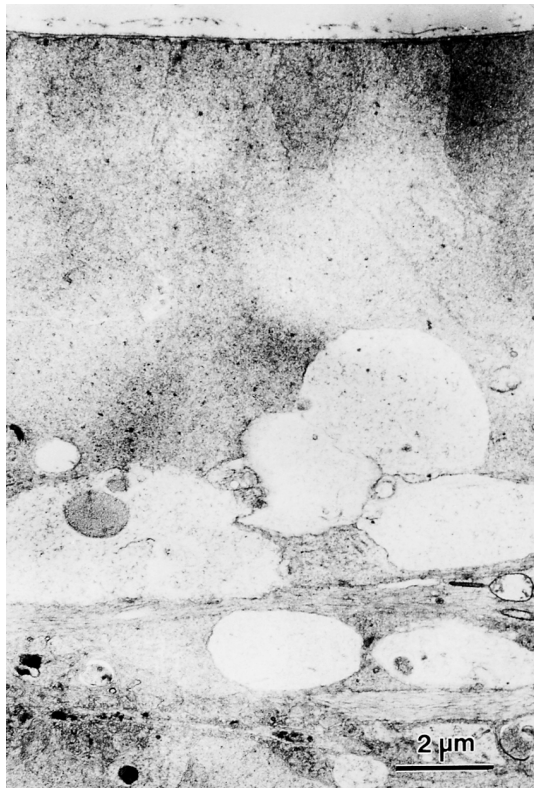

Figure 6. Electron microscopy revealed no rupturing in intraretinal membrane and no vacuoles or Müller cells in 1,000-centistoke silicone-phenylsilicone copolymer-injected eyes after 8 weeks.

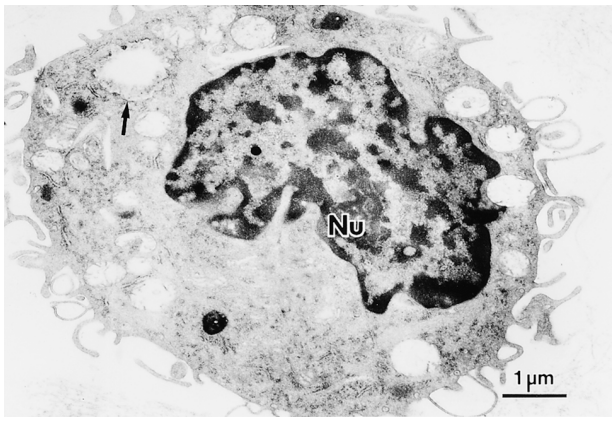

Figure 7. By electron microscopy, macrophages and vacuoles were also observed in intraretinal membrane, suggesting that silicone-phenylsilicone copolymer had been incorporated into cell.

substances in the eye most likely involved in emulsification are proteins and phospholipids.[16,17] The presence of large SO droplet aggregates, that form only in the presence of protein in vitro, as well as the localization of phospholipids exclusively in the hyaloid membrane[13] despite a high concentration of proteins in the intraocular fluid, suggest that albumin, as well as other soluble proteins present in relatively large quantities in the intraocular fluid, are contributing to SO emulsification.

The hydrodynamic mechanism implies that emulsification occurs due to deformation of the oil surface induced by external mechanical energy, such as shaking. This is explained by the Raleigh-Taylor instability theory, which applies when acceleration occurs on the boundary surface between two fluids as a heavier fluid attempts to flow beneath a lighter one.[18]

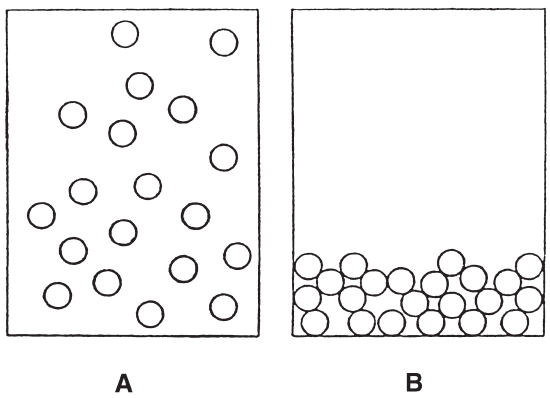

Figure 8. Classification of silicone oil emulsification. (**A**) Formation of fine dispersed droplets in suspension that do not contact each other. (**B**) Formation of relatively large accumulated droplets that contact each other.

Therefore, in the case of equal surface tension, the larger the difference in specific gravity between the two fluids, the greater the instability of the boundary surface.[19] According to this theory, DPC should exhibit less emulsification due to mechanical shaking than SO or FSO because the specific gravity of DPC is closer to that of water, while its interfacial tension is close to that of SO and FSO (Table 1). In the present experiment, although DPC showed the least emulsification tendency when shaken immediately after the vial was sealed, it tended to emulsify more readily than SO when shaken after being allowed to sit for 1 week after the vial had been sealed. This may suggest that the hydrodynamic mechanism of emulsification dominates initially (density difference between SO and water is greater than that between DPC and water). However, as the amount of protein attached to the oil surface increases, the interfacial tension decreases, due to protein denaturation. As a result, the thermodynamic mechanism becomes dominant, causing DPC to emulsify more readily than SO.

Histological examination of the rabbit eyes revealed macrophages on the inner limiting membrane of the DPC-injected eyes 8 weeks after extraction.[20–22] However, the inner limiting membrane showed no sign of collapse, nor were there any apparent changes in the inner retinal layers.

In the present study, we used DPC that had a specific gravity of 0.98 and a phenyl content of 5%. The DPC with higher phenyl content and specific gravity exceeding 1 exists (eg, DPC with 25% phenyl content has a specific gravity of 1.07), and therefore could replace FSO as a tamponade material for the lower retina.

The FSO, which has a specific gravity greatly exceeding that of water, is readily emulsified by mechanical shaking. In contrast, both SO and DPC, with specific gravities similar to that of water, were

Table 1. Physicochemical Properties of 1,000-Centistoke Silicone Oil (SO), Fluorosilicone oil (FSO), and Silicone-Phenylsilicone Copolymer (DPC) at 37°C

Properties	SO	FSO	DPC
Density (g/cm^3)	0.966	1.256	0.984
Density difference* (g/cm^2)	0.036	0.254	0.018
Interfacial tension† (dyne/cm)	18.8	15.8	16.7

*Density difference between oil and 1 mg/dL of albumin solution.
†Interfacial tension between oil and 1 mg/dL of albumin solution.

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emulsified only when the interface of the oils and the water were subjected to extremely greater acceleration. However, when shaken relatively lightly, SO and DPC oil droplet formation was not observed, even after 1 week. Nonetheless, even after only light shaking, microscopic observation of samples of the water solution revealed a large number of dispersed minute particles of emulsified SO and DPC.

Emulsification that is caused by decreased interfacial tension in the absence of external mechanical energy is referred to as spontaneous emulsification, and it involves only extremely minute droplets held in a suspended state. The microscopic suspended oil droplets found in the protein solution containing 1,000-cs SO and 1,000-cs DPC after 1 week of shaking resembled such spontaneous emulsification droplets with respect to size and dispersion. However, the thermodynamic mechanism that arises due to decreased interfacial tension, rather than the hydrodynamics mechanism associated with shaking, appears to be primarily responsible for droplet formation. Conversely, clinical observation of relatively large and nondispersed SO emulsification suggests that hydrodynamic mechanisms that arise during mechanical shaking are primarily responsible for droplet formation. Nevertheless, gentle shaking of oil in vitro demonstrates that microscopic intraocular emulsification may occur due to the thermodynamic mechanism.

Based on this assumption, control of the hydrodynamic mechanism alone (ie, specific gravity and viscosity) may not prevent oil emulsification. The role of the thermodynamic mechanism in this process must, therefore, be investigated further. Theoretically the ideal nonemulsifying intraocular tamponade material would be a clear, nontoxic, hydrophobic liquid that has positive energy for protein absorption on the interface between oil and water.[23] In the field of biomaterials, solid surfaces that repel proteins have already been developed, and could possibly yield new liquids that repel proteins at their interfaces. Hydrophobic liquid materials that repel surface-activating proteins from their surfaces could lead to the development of nonemulsifying intraocular tamponade materials. The DPC, which seems to be less emulsified than SO and FSO, may be useful as a material for a long-term intraocular tamponade.

This study was supported in part by a grant in-aid for scientific research (08672031) from the Ministry of Education, Science, Sports and Culture of Japan, a research grant from Kyoto Foundation for the Promotion of Medical Science, and the intramural research fund of Kyoto Prefectural University of Medicine.

This study was originally published in *Nippon Ganka Gakkai Zasshi* (*J Jpn Ophthalmol Soc*) 1997;101(2):111–117. It appears here in a modified form after peer review and editing for the *Japanese Journal of Ophthalmology.*

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