Effects of Emulsification, Purity, and Fluorination of Silicone Oil on Human Retinal Pigment Epithelial Cells

Thomas R. Friberg, Thierry C. Verstraeten, and David K. Wilcox

When silicone oil is used as a vitreous substitute, reproliferation of vitreoretinal membranes beneath the oil occurs frequently. Nevertheless, the effects of various properties of silicone oils such as purity, viscosity, fluorination, or emulsification on cellular proliferation have not been established. Human retinal pigment epithelial (RPE) cells were grown to confluence on filters, and then covered with silicone oil. The cellular monolayers were fed from below. At 72 hr and 14 days a proliferation index was determined by measuring \(^3\)H-thymidine incorporation into the cells. An assay for the enzyme gamma-glutamyl-transpeptidase (\(\gamma\)GTP) was also done to assess cell polarization under some oils. A total of 14 different oils were studied. At 72 hr, emulsified oil was associated with significantly less proliferation than unemulsified oil, a difference that disappeared at 2 weeks. Neither fluorination nor viscosity had a significant effect on RPE proliferation. In addition, RPE proliferation indices were not significantly different from one another when purified oils were compared with most commercial-grade oils. However, a very contaminated oil was associated with a significantly higher proliferation index compared with several purified or medical-grade oils. Invest Ophthalmol Vis Sci 32:2030–2034, 1991

Vitreous replacements, particularly silicone oils, play an increasingly important role in vitreoretinal surgery. Many cases of failed retinal reattachment, often from proliferative vitreoretinopathy (PVR), have been salvaged by placing silicone oil in the vitreous cavity to achieve long-term retinal tamponade.\(^1\)\(^2\) The retinal pigment epithelium (RPE) is linked closely to the development of PVR,\(^3\)\(^4\) and often the RPE is exposed directly to the silicone oil filling the globe. This occurs when the overlying retina is absent after local retinectomy or when RPE cells are present in the vitreous after retinal detachment. How the physical or chemical properties of the silicone oil influence the proliferation of RPE cells is unclear, however. We therefore evaluated the effect of various silicone oils on these cells. To avoid possible confounding factors present in in vivo models, we chose an in vitro model for the study.

Materials and Methods

Human RPE cells were harvested from donor eye-cups and grown on 0.45-\(\mu\)m semipermeable nitrocellulose membranes (Millicell; Millipore, Bedford, MA) at densities of 10–20,000 cells per insert.\(^6\)\(^7\) The cells were fed from below by immersing the insert into Dulbecco’s modified Eagle’s medium and Ham’s F-12 in a 1:1 ratio, supplemented with insulin, transferrin, selenium, and 5% fetal calf serum (Fig. 1). At confluence, as assessed by Giemsa staining, silicone oil was layered over the RPE cells on the apical side while control wells contained Hank’s balanced salt solution (HBSS).

Proliferation of the RPE cells was assessed by measuring the incorporation of \(^3\)H-thymidine in an acid-soluble fraction. The cells were incubated with 2 \(\mu\)Ci of \(^3\)H-thymidine per ml of medium for 48 hr and prepared for scintillation counting. The data were analyzed statistically using a paired student t-test to compare values between paired vitreous substitutes. The proliferation index was calculated by dividing the counts per minute incorporated into the RPE from wells containing oil by the counts per minute incorporated into the control wells. The experiment was done in triplicate.

Various emulsified or unemulsified silicone oils were studied (Table 1). Emulsification was achieved by mixing five parts silicone oil with one part HBSS after placing each liquid in a separate syringe joined by a stopcock. By repeatedly injecting the contents of
Retinal pigment epithelial cells were grown to confluence on nitrocellulose inserts. Emulsified or nonemulsified oil was placed directly on the apical surface of the RPE monolayer. The cells received nutrition from the basal side, as the insert was immersed in growth medium. $V_h$ was always larger than $C_h$, keeping the oils against the RPE cells by a hydrostatic pressure differential.

One syringe into the other for 5 min, a homogeneous-appearing emulsion was produced. For another 5 min, the emulsion was forced through an 18-gauge needle linking the syringes in place of the stopcock. The resultant emulsion was then placed into a test tube and allowed to stand overnight. The emulsion rose to the top of the aqueous phase, was removed, and placed onto the RPE cell inserts. The unused emulsion was set aside for the duration of the experiment to make certain that it remained grossly stable. The emulsions were also inspected using photomicrographs to assess oil droplet size, which ranged from 1–150 μm or more.

Four of the commercial oils were purified by an extraction method described previously to remove low molecular weight components. The resultant purified oils had a higher viscosity than the original product. An oil known to contain 5428 ppm of impurities, as measured by optical emission spectroscopy, was also evaluated as an extreme example of a contaminated oil. It contained multiple impurities including: arsenic, 55 ppm; tin, 13 ppm; chromium, 27 ppm; and iron, 17 ppm. Commercial versus purer oils were compared. We also compared RPE proliferation of monolayers exposed to low viscosity oils with those subjected to high viscosity oils. These oils were of similar grade and fluorination status. Finally, we compared fluorinated oils with nonfluorinated oils of similar viscosity and grade.

Cell polarity was measured by assaying gamma-glutamyl-transpeptidase ($\gamma$GTP) activity after 3 days of incubation. This enzyme is located preferentially in the apical surface of the RPE cells, and a ratio of $\gamma$GTP activity in the apical versus the basal layer provides a measure of cell polarity. This assessment, also run in triplicate, was done on RPE monolayers exposed for 3 days to several medical-grade or purified oils.

### Results

#### Emulsification

Incubation of RPE monolayers under emulsified oils resulted in a significantly lower proliferation index compared with unemulsified oil at 72 hr ($P < 0.05$). At 2 weeks, there was no significant difference between emulsified and unemulsified oils in their effect on the RPE monolayers (Fig. 2).

### Table 1. Silicone oils studied

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<tbody>
<tr>
<td>A</td>
<td>Petrarch commercial fluorosilicone, 1000 cs (centistokes)</td>
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<tr>
<td>B</td>
<td>Petrarch commercial silicone, 1000 cs</td>
</tr>
<tr>
<td>C</td>
<td>Dow Corning medical silicone, 1000 cs</td>
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<tr>
<td>D</td>
<td>Silicone oil B after purification and fractionation, 2618 cs</td>
</tr>
<tr>
<td>E</td>
<td>Silicone oil A after purification and fractionation, 1486 cs</td>
</tr>
<tr>
<td>F</td>
<td>Dirty oil, manufacturer not stated, 5000 cs silicone</td>
</tr>
<tr>
<td>G</td>
<td>Fluorinated Wacker, 5000 cs</td>
</tr>
<tr>
<td>H</td>
<td>Dow Corning medical grade silicone, 5000 cs</td>
</tr>
<tr>
<td>I</td>
<td>Petrarch commercial fluorosilicone, 10,000, cs</td>
</tr>
<tr>
<td>J</td>
<td>Dow Corning medical silicone, 12,500 cs</td>
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<tr>
<td>K</td>
<td>Fractionated purified Petrarch silicone oil, 14,815 cs</td>
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<tr>
<td>L</td>
<td>Silicone oil 1 after purification and fractionation, 14,689 cs</td>
</tr>
<tr>
<td>M</td>
<td>Adatomed 5000 cs silicone</td>
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<tr>
<td>N</td>
<td>Wacker silicone, 1000 cs</td>
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Fig. 2. Proliferation indices for nonemulsified versus emulsified silicone oils at 3 (top) and 14 (bottom) days. The oils from left to right are identified in Table 1 as B, N, D, I, E, C, and M.
COMMERCIAL vs PURIFIED OIL AT 3 DAYS

Fig. 3. Proliferation indices for commercial grade oils compared to their more pure counterparts at 3 (top) and 14 (bottom) days. See Table 1 for oil identification.

Purification

Removal of low molecular weight components from four commercial-grade silicone oils by fractionation did not significantly alter RPE proliferation compared with the nonfractionated oils at either 3 or 14 days (Fig. 3).

Fluorination

There was no significant difference between proliferative response to fluorinated versus unfluorinated oils of similar viscosity and grade at either 3 or 14 days (Fig. 4).

Viscosity

With regard to the proliferation index, there were no significant differences between monolayers exposed to low viscosity and high viscosity oils of similar grade, with or without fluorination, at either 3 or 14 days (Fig. 5).

Contaminated Oil

When a very impure fluorinated oil was compared with several so-called “medical-grade” oils, we found significantly greater proliferation ($P < 0.05$) after exposure to the contaminated oil at 14 days. At 3 days, some oils had proliferation indexes similar to that of the impure oil (Fig. 6).

Polarity

Contaminated oil, oil F, caused an apparently greater decrease in RPE polarity compared with several other oils at 3 days as seen graphically (Fig. 7). The difference, however, was not statistically significant because the standard deviations of polarity results were large.

Discussion

Silicone oil is associated with several complications when used intraocularly. Emulsification of oil, secondary glaucoma, and particularly corneal decompensation are frequent sequelae of intraocular use. In addition, the presence of silicone oil in the vitreous cavity is sometimes associated with reproliferation of preretinal membranes. If extensive, these mem-

FLUORINATED vs NONFLUORINATED AT 3 DAYS

Fig. 4. Proliferation indices for fluorination versus unfluorination oils of similar grade and viscosity at 3 (top) and 14 (bottom) days. See Table 1 for oil identification.
branes can contribute to the ultimate redetachment of the retina. Both in vitro and in vivo experiments, cellular proliferation was found beneath silicone oil. This proliferation may be triggered by the oil stimulating the release of growth factors which, in turn, act in an autocrine fashion on the RPE layer.

Alternatively, released growth factors may be concentrated in the aqueous phase beneath the oil, or the oil itself may contain mitogens.

Intraocular use of silicone oil in the United States has been somewhat limited by potential complications and by the fact that few oil manufacturers are inclined to seek Food and Drug Administration approval for its ophthalmic use. Consequently, silicone oil is difficult to obtain outside of collaborative studies. Other countries may have fewer restrictions on the use of silicone oil as a vitreous substitute, and therefore distributors and manufacturers inadvertently become sources of oil. Unfortunately, no standards of purity have been universally adopted to characterize silicone oil. Hence, one company’s “commercial grade” is not necessarily less pure than another company’s “medical grade.” Indeed, some clinicians have been misled because oils have been called medical grade merely because they have been used in human vitreoretinal surgery, rather than having passed rigorous tests for purity. Occasionally this...
has led to the intraocular use of oils of dubious quality.

At least two laboratories addressed the potential problem of various impurities contaminating silicone oil. Parel and associates found multiple inorganic impurities and volatiles in various commercially produced silicone oils with spectroscopy and chromatography. Refojo and coworkers suggested that complications from silicone oil may be related to low molecular weight components in the oil, and they devised a method to extract these components using solvent fractionation. Removal of these components, however, does not prevent mechanical emulsification of the oils, which is dependent primarily on interfacial tension. Both laboratories kindly provided oils for our study.

Our data confirmed earlier reports that $^{3}$H-thymidine incorporation is increased over controls in human RPE cells maintained for 1–4 weeks under silicone oil. Moreover, we found (somewhat surprisingly) that emulsification of oil does not lead to enhanced RPE proliferation despite the fact that many of the oil droplets were only a few microns in diameter and potentially could be phagocytosed by the RPE cells in the test wells. At 72 hr, the emulsified oils were associated with significantly less RPE proliferation than unemulsified oils. At 2 weeks, no difference was found. Some might argue that it was unlikely that the oil droplets in the emulsions remained apposed to the RPE cells during the course of the experiment. However, when we removed the silicone oils from the wells to assay for $^{3}$H-thymidine incorporation, the oil grossly adhered directly to the RPE layer, whether or not the well contained emulsified or unemulsified oil.

Our results also demonstrated that most silicone oils tested affected RPE proliferation similarly with respect to $^{3}$H-thymidine incorporation. Removal of low molecular weight components from commercial-grade oil had no significant effect on RPE proliferation, nor did the presence or absence of fluorination or the viscosity of the oil. The statistical power of our observations was low because only four sets of oil were used in these comparisons. However, if the differences between the means for the compared groups was 50%, we would have expected to show statistically significant differences at a power of 0.80.

A very contaminated oil, oil F, contained multiple impurities and caused significantly greater proliferation than the purified or medical-grade oils to which it was compared. Similarly, our data suggest that contaminated oil results in a greater loss of RPE polarity than purer oils. Loss of polarity implies a diminished ability of the RPE cell to perform normal physiologic functions. We did not assay for polarity at 14 days because the original RPE monolayer become multicellular after prolonged silicone oil exposure, making the comparison between apical and basal enzyme activity difficult to interpret.

Although the RPE cell is a key component in the development of PVR, the effects of the silicone oils on other cell types such as fibroblasts or glial cells was not assessed in this study. We therefore urge caution when interpreting our results. Although in our model, most commercial- and medical-grade oils had similar effects on RPE proliferation, this might not be true in vivo. We believe, however, that our in vitro assay is useful in identifying poor quality oils among many potentially useful vitreous substitutes.

**Key words:** retinal pigment epithelium, silicone oil, emulsification, proliferation, in vitro

**References**