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Retina

Minimally Invasive Subretinal Perfluorocarbon Liquid (PFCL) Removal

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Purpose: Perfluorocarbon liquid (PFCL) is widely used in retinal surgery; however, it can be complicated by subretinal retention that can affect/threaten fovea. A number of different approaches have been explored to remove retained PFCL. Here, we investigate the use of microneedles with internal diameters as small as 49 G.

Methods: An in vitro model was used to examine the rate of aspiration of a fixed volume of 250 µL PFCL, whereby 30, 41, 43, and 49 gauge needles were examined. An ex vivo porcine model was then used to confirm whether subretinal PFCL could be consistently aspirated without blockage. Finally, this technique was used during pars plana vitrectomy (PPV) in 10 patients presenting with subfoveal or parafoveal retained PFCL.

Results: Mean aspiration of 250 µL PFCL required 0.22, 0.33, and 0.78 minutes for the 30, 41, and 43 gauge needles at 200 mm Hg extraction pressure. Microneedle aspiration of 250 µL required maximal vacuum settings of 650 mm Hg to obtain a measurable average time to aspiration of 3.34 minutes. Ex vivo aspiration of subretinal PFCL required 400–650 mm Hg vacuum, where this was repeatable without any needle blockage. In 10 patients treated with PPV and microneedle subretinal PFCL aspiration, all cases were successful. Mean visual acuity change was of LogMAR −0.08 (±0.12).

Conclusions: We recommend the clinical use of microneedles as a minimally invasive surgical option when considering direct retained PFCL removal.

Translational Relevance: We confirmed the flow properties of microneedles for PFCL aspiration in vitro and ex vivo before successfully applying this to treat retained PFCL in patients.

Introduction

Perfluorocarbon liquids (PFCL) are synthetic fluorochemicals with all hydrogen atoms replaced with fluorine.[1] In modern retinal surgery, PFCL is commonly used to facilitate retinal detachment repair in numerous ways including giant retinal tears, proliferative vitreoretinopathy (PVR), severe proliferative diabetic retinopathy (PDR), and more recently myopic macular hole retinal detachment.[2–5] However, the use of PFCL is not risk free, and it is known that retained PFCL bubbles can enter the subretinal space at the macula and threaten the fovea, albeit a relatively rare occurrence in 1% to 11% of cases.[6,7]

A number of techniques to remove retained bubbles of PFCL can be broadly divided into direct and indirect techniques. Direct techniques include the use of small-gauge instruments including 50 G glass pipettes,[8] 49 G cannula,[6] 41 G cannula,[9] 39 G cannula,[10] and a 36 G retinal translocation needle[11] to aspirate the retained PFCL via an adjacent retinotomy. However, such techniques can lead to sight-threatening complications such as submacular hemorrhage, macular hole, and submacular fibrosis secondary to retinal pigment epithelium and photoreceptor disruption.[6,8] Indirect methods include posture[12] or a surgical approach involving the creation of a macular detachment to indirectly displace PFCL bubbles, which is then assisted by head posturing after

surgery,[13] direct subretinal aspiration during surgery,[14] or with intraoperative head posturing and vibration.[15] However, in all these reports, variable visual recovery has been reported and given the heterogenous reports of PCFL toxicity on photoreceptors in different animal models, it is unclear what the optimal surgical technique and patient characteristics to balance risk and benefit. We therefore proposed to explore direct methods of retained PFCL removal using active aspiration and a passive approach with a combination of in vitro, ex vivo and finally in vivo techniques in patients presenting with retained subretinal PFCL.

Methods

In Vitro Injection of PFCL Microdroplets

Perfluoron (perfluro-n-octane; Alcon, Geneva, Switzerland) was used to examine PFCL microdroplet formation to understand the size and volume of microdroplets clinically found in the subretinal space after surgery that may affect the fovea or threaten the fovea within the macula. Hamilton gas tight syringes were used to inject 0.5, 1, and 2.5 µL onto a 48-well culture plate under video microscopy (Leica S9D with Flexacam C3; Leica, Wetzlar, Germany). Another experimental design was the use of indocyanine green dissolved in double distilled water (ddH_2O) at 5%, which was used to contrast between PFCL droplets and water. PFCL droplets of 0.5, 1, and 2.5 µL were injected at the base of a 1.5 mL Eppendorf tube filled with 1 mL of the diluted ICG-ddH_2O mixture with goal of forming a distinct round droplet.

In Vitro Aspiration and Injection of PFCL Droplets With Microneedles

For all experiments, the Constellation Vision System (Alcon, Fort Worth, TX, USA) was used. For all experiments, 25 G Vitrectomy packs were used with the Viscous Fluid Control Pack (BL7600; Alcon) that included a 10 mL syringe, syringe plunger, syringe cap, tubing set with syringe coupler, and 20, 23, and 25 gauge cannulas. The needles and microneedles used included a 30 G needle, 25 G/38 G Polytip (MedOne Surgical Inc., Saratosa, FL, USA), Tochigi Seiko 0.11 mm prototype microneedle (Tochigi Seiko Co. Ltd., Tochigi, Japan), and the Tochigi Seiko 0.05 mm microneedle (Tochigi Seiko Co. Ltd.). Eppendorf tubes 1.5 mL were used with a 5% solution of ddH_2O and indocyanine green and then 0.5 mL of PFCL was added. The needle of interest was then immersed into the PFCL with the Eppendorf fixed horizontally under video microscopy to allow timing and measurement of the end point of aspirating a total of 0.5 mL. All experiments were repeated triplicate to estimate the flow rate at fixed extraction pressures to quantify.

Ex Vivo Model of Subretinal PFCL Aspiration

To confirm the feasibility of microneedle aspiration of PFCL subretinal space, we used an ex vivo pig eye model akin to Gatto et al.[16,17] Pig eyes were harvested from freshly terminated animals three to six months of age from abattoirs within the vicinity. The eyes were delivered on ice with the extraocular muscles, conjunctiva, eyelids and tissues intact and arrived at the laboratory within two to four hours of termination. The pig eyes were mounted onto a polystyrene phantom head and washed with ddH_2O. Using the Constellation Vision System (Alcon), a 25-gauge three-port vitrectomy was performed on each pig eye. A vitrectomy contact lens (Hoya, Tokyo, Japan) was used as the posterior viewing system. A posterior vitreous detachment was performed and the posterior hyaloid membrane was peeled with end gripping forceps where necessary to clear posterior vitreous where necessary. The included 10 mL VFC syringe attached to the Polytip 25/38 G (MedOne Surgical Inc.) was used to inject bubbles of PFCL in the subretinal space before targeting a new area of retina overlying the PFCL bubble and aspiration attempted with the following microneedles: Tochigi Seiko 0.11 mm or Tochigi Seiko 0.05 mm needles (Tochigi Seiko Co., Ltd.) before the R retinal pigment epithelium PE suspension was injected. Various extraction pressures were trialed until a fixed pressure was used.

Ex Vivo Imaging

Imaging was carried out using a Cirrus 5000 (OCT) System (Zeiss, Oberkochen, Germany). The pig eyes, while mounted in the phantom head, were imaged. The cornea was hydrated with balanced saline solution (BSS) and viscoelastic. The corneal epithelium was removed if causing impairment of visualizing the retinal structures. The scanning sequences used included the macula cube and five raster scans. The globes were rotated within the simulated orbit as necessary to image the region of interest and the phantom head was manipulated and rotated as necessary to optimize imaging as needed.

In Vivo PFCL Microneedle Aspiration

The Tochigi Seiko 0.05 mm microneedle (Tochigi Seiko Co. Ltd.) was attached to a 10 mL syringe from

the Viscous Fluid Control Pack (Alcon). Both the microneedle and 2–3 mL of the syringe were filled with BSS. Using a contact lens (Hoya contact lens quartz type 5Q; Hoya Co. Ltd.) under high magnification.

Ethics

This study was approved by the Institutional Review Board of Yokohama city university (Approval no. F240600064) and conducted in accordance with the principles of the Declaration of Helsinki.

Results

In Vitro Characterization of PFCL Microdroplets

In all conditions it was found that PFCL droplets evaporated rapidly because of the low boiling point associated with perfluoro-octane. Video microscopy permitted visualization of the droplet formation and rapid evaporation. Furthermore, it was found that on the surface of a 48-well cell culture plate, the PFCL droplets behaved as a Newtonian liquid and spread rapidly into a flat conformation (Fig. 1) This did not permit measurement of the diameter of a discrete droplet, as would be seen clinically in subretinal PFCL retention. We therefore attempted depositing droplets on a hydrogel surface immersed in liquid; however, this also failed to form a discrete droplet. Eventually, we used indocyanine green with ddH_2O as described in the Methods section.

In Vitro Aspiration of PFCL Droplets With Microneedles

With all needles tested, it was possible to extract 250 µL of PFCL without blockage or impairment. This represents a large volume unlikely to be encoun-

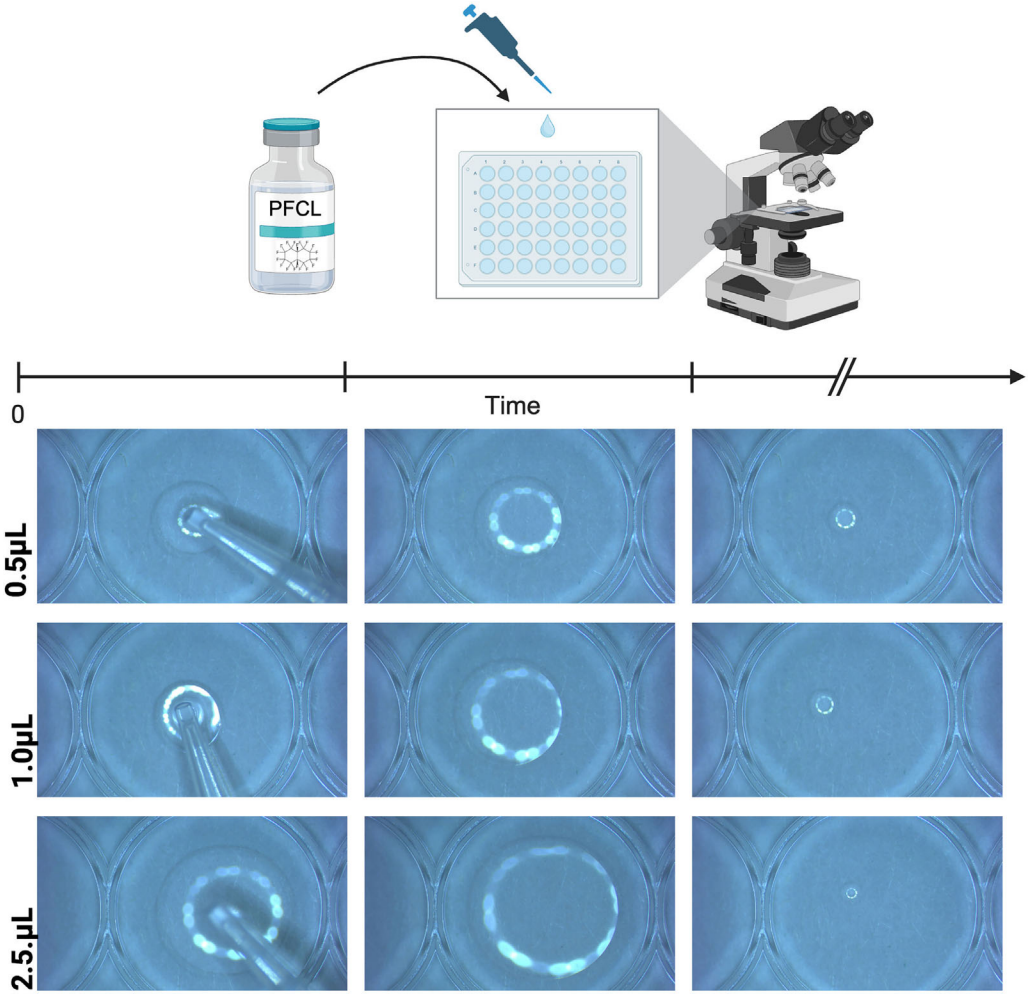
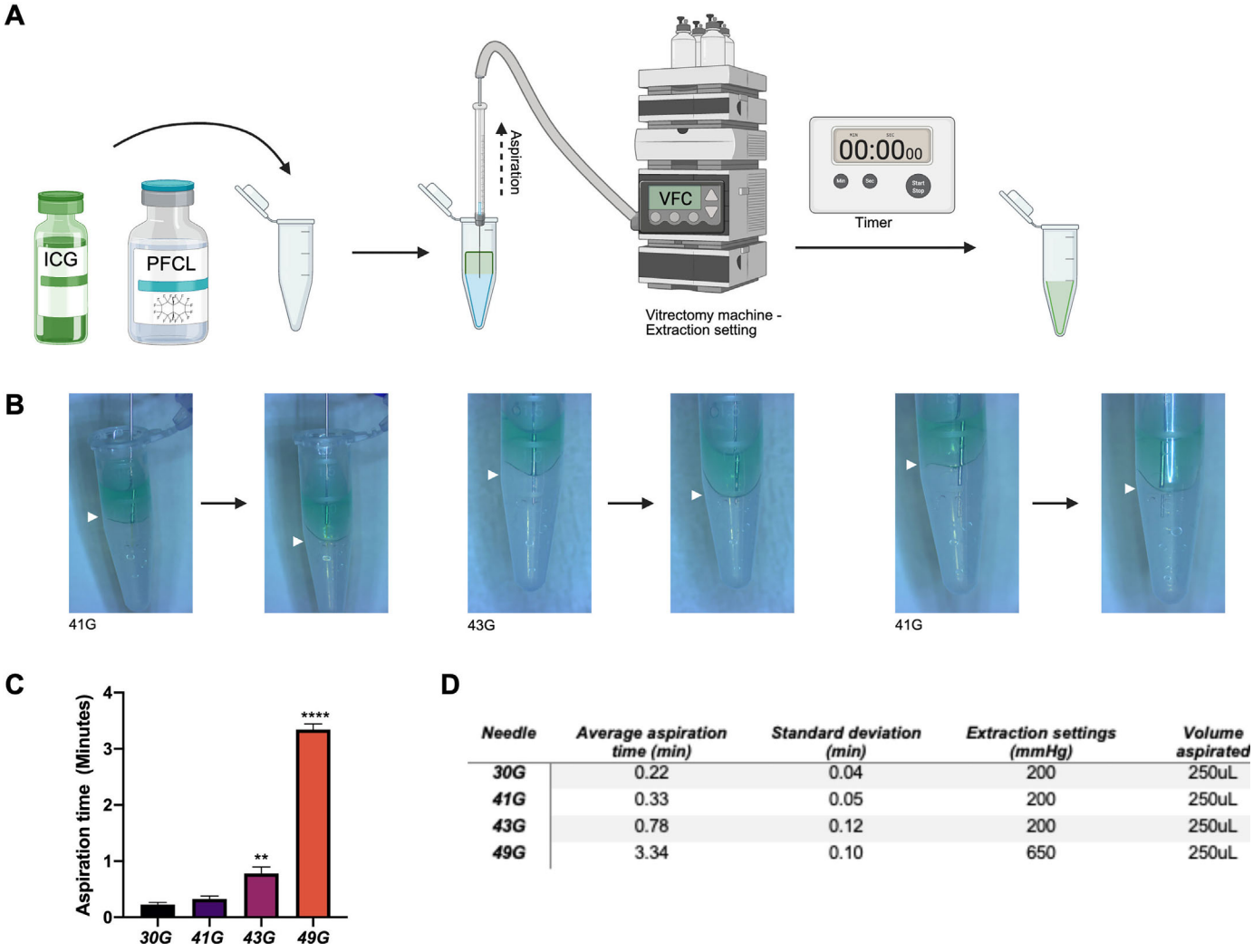


Figure 1. In vitro injection of PFCL microdroplets. *Upper panel* shows schematic of PFCL droplets using an Eppendorf 2.5 µL pipette under videomicroscopy. In vitro still images taken from videomicroscopy recordings of PFCL droplet formation on a 48-well cell culture plate, spreading diffusely and evaporating rapidly.



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Needle	Average aspiration time (min)	Standard deviation (min)	Extraction settings (mmHg)	Volume aspirated
30G	0.22	0.04	200	250uL
41G	0.33	0.05	200	250uL
43G	0.78	0.12	200	250uL
49G	3.34	0.10	650	250uL

Figure 2. In vitro aspiration of PFCL using microneedles attached to a vitrectomy machine. (**A**) A 5% ICG solution and PFCL is added to an Eppendorf before the microneedle is immersed in the PFCL and aspirated with various extraction settings and timed to remove 250 µL. (**B**) Still images from video microscopy of PFCL extraction covered by a layer of 5% ICG-ddH_2O using 41G, 43 G and 49 G microneedles. *White arrow heads* indicate the meniscus between PFCL and 5% ICG-ddH2O, where the first image of each needle gauge is marked at 750 µL before 50 µL is aspirated. The second image and *white arrow head* show that the meniscus has fallen to the 500 µL marker, confirming 50 µL has been aspirated. (**C**) As the internal gauge of the needle becomes smaller, the length of time to aspirate 250 µL increases significantly when using 43 G and 49 G compared to a 30 G standard needle. The extraction pressure of 200 mm Hg was used for all needles except 49 G, which was increased to 650 mm Hg to obtain a feasible flow rate to measure in the experimental set up. (**D**) The average time to aspirate 250 µL of PFCL is shown in the table with SD ($n = 6$). NB: Extraction settings were increased for the microneedle to make it feasible take measurements as the nitrogen gas canister supplying the vitrectomy machine would otherwise deplete prematurely.

tered in clinical practice, however, provides an indication of the reliability of the microneedles to extract PFCL given the potential for small bore needles to be blocked by the passage of any substance. The time to aspiration was found to be consistent with a low standard deviation (SD) (Fig. 2C). Further we found compared to the control needle of 30G, there was a significantly increased time to aspirate 250 µL of PFCL when using the 43 G and 49 G needles. However, notably, the low flow rate expected with the 49 G needle led to a constant extraction pressure of 200 mm Hg to be too slow to measure accurately. This was due to the need to hold the microneedle in situ without excessive movement that would result in damage to the microneedle. Therefore an extraction pressure of 650 mm Hg made this possible with an average aspiration time of 3.34 (SD ± 0.10) minutes. These results confirmed that even very small gauge needles can reliably aspirate relatively large volumes of PFCL without any impairment. No needle blockages

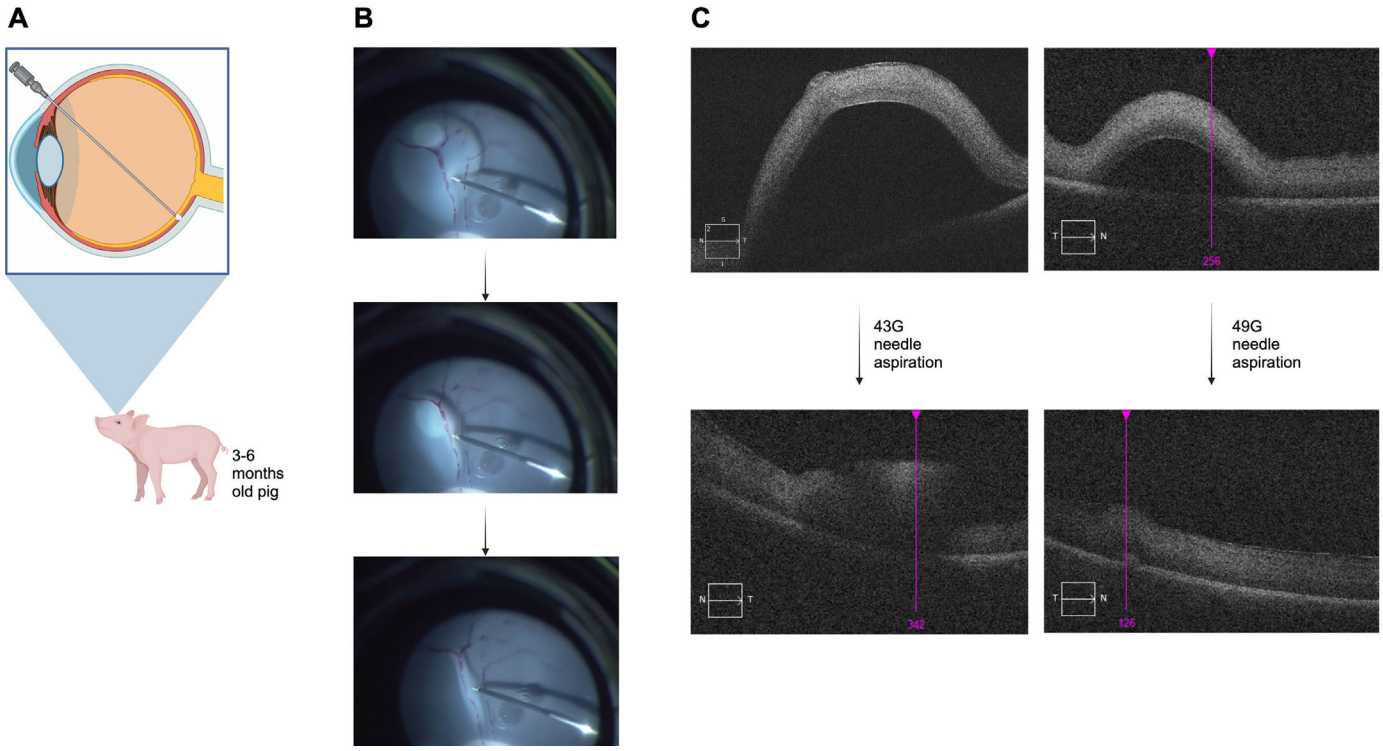

Figure 3. Ex vivo porcine model of retained subretinal PFCL. (**A**) Schematic demonstrating three- to six-month-old pig eye freshly harvested. (**B**) PFCL is injected into the subretinal space using a 41 G cannula before being aspirated with microneedles. Intraoperative images showing the removal of subretinal PFCL in three still images starting at the first top image and ending at bottom third image. (**C**) SD-OCT images depicting subretinal PFCL before removal with a 43 G microneedle (left images) and a 49 G microneedle (right images).

occurred throughout all experiments. Furthermore, to test the reliability of the needles when manipulating PFCL, 250 µL of PFCL aspirated was then injected into a 15 mL Flacon tube to determine whether needle blockages or damage can occur if the reverse procedure was performed with the same needle. In all cases, there were no blockages in 0/6 (0%). This was in stark contrast to injecting anti-VEGF in a separate related study (data not shown), where specific anti-VEGF agents had a higher propensity to block microneedles.

Ex Vivo Model of Subretinal PFCL Aspiration

To examine whether it is clinically feasible to remove subretinal PFCL clinically in a reliable fashion, we used an ex vivo porcine model to simulate subretinal PFCL removal with microneedles. In all cases with the 43 G and 49 G needles (repeated with three eyes each), it was possible to remove PFCL without any difficulty using 200 mm Hg and 400–650 mm Hg extraction pressure, respectively. Using a posterior viewing system it was possible to confirm gross removal of the PFCL directly from the subretinal space and then by SD-OCT immediately after surgery (Fig. 3). Because of the ex vivo model using cadaveric tissues, it was not possible to form microbubbles as seen clinically. An intraoperative video is provided in Supplementary Information (Supplementary Video S1).

In Vivo PFCL Microneedle Aspiration

Finally, to confirm the clinical utility of microneedles for subretinal PFCL removal, we demonstrate its effect in 10 patients with subfoveal or parafoveal subretinal PFCL bubbles. Six of these patients were male, and seven eyes initially presented with rhegmatogenous retinal detachment (RRD) with or without proliferative vitreoretinopathy (PVR), and in 1 case a giant retinal tear. The mean number of retained subretinal PFCL bubbles was 2.44 with a range of 1 to 5. The retained subretinal PFCL remained in situ for a median time of 14 days and 6 eyes had subfoveal involvement. Following surgical removal of the retained PFCL using a microneedle approach, it was found that there was an average improvement of LogMAR −0.08 (±0.12). No postoperative retinal holes or subretinal hemorrhage was observed in any of the cases. These findings are presented in the Table.

To illustrate retained PFCL aspiration with a microneedle, we describe case 4 in greater detail,

Table. Characteristics of Patients Presenting With Retained Subretinal Perfluorocarbon Liquid After Vitrectomy Surgery

Case	Age	Sex (F/M)	Disease	Number of PFCL Drops	Duration of PFCL (Days)	Location of PFCL	Change VA (logMAR)
1	62	F	PDR	2	—	Parafoveal	−0.12
2	32	M	PVR RRD	5	28	Parafoveal	−0.20
3	78	M	PVR	5	28	Parafoveal	−0.02
4	53	F	PVR RRD	3	84	Parafoveal	0.00
5	56	F	GRT	1	7	Subfoveal	−0.20
6	61	M	RRD	1	4	Subfoveal	−0.19
7	53	M	RRD	1	7	Subfoveal	0.21
8	63	M	PVR RRD	2	14	Subfoveal	−0.10
9	63	M	PDR	2	21	Subfoveal	−0.10
10	45	F	PVR RRD	3	14	Subfoveal	−0.02

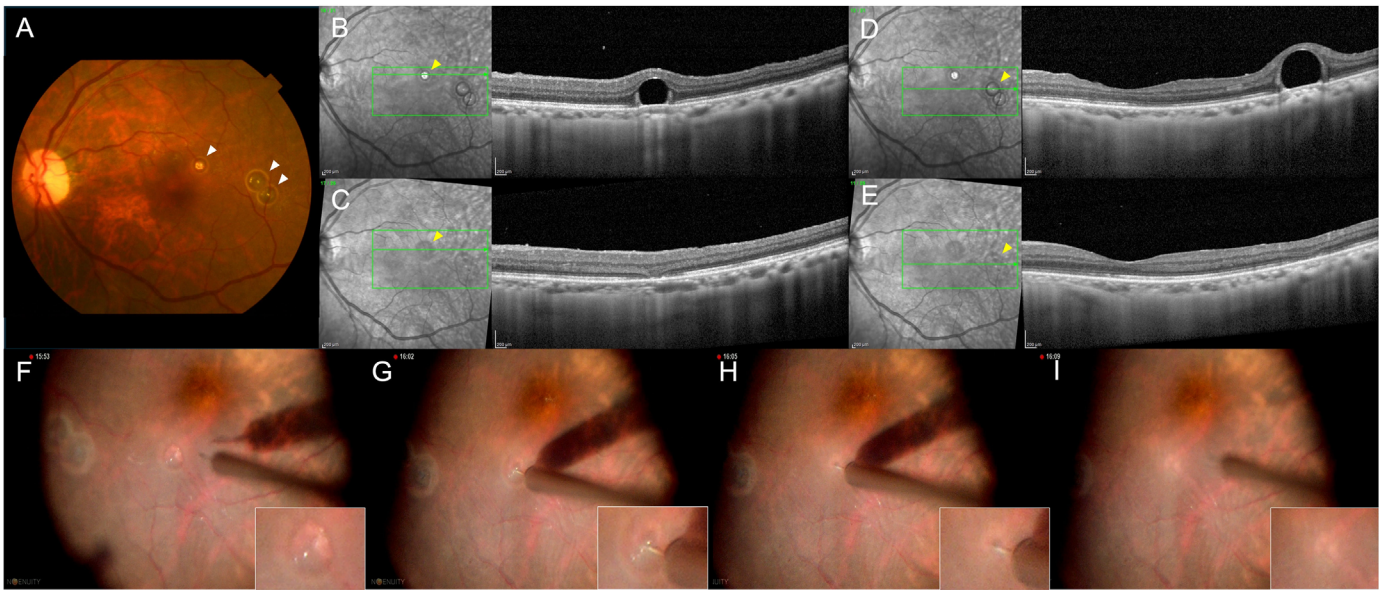

Figure 4. In vivo subretinal PFCL aspiration. (**A**) The fundus photograph reveals three distinct retained PFCL droplets (*white arrowheads*). (**B**–**D**) Preoperative (**B, D**) and postoperative (**C, E**) infrared and SD-OCT images are shown. **B** corresponds to **C**, and **D** corresponds to **E**, with *yellow arrowheads* marking the same locations. SD-OCT confirms complete removal of PFCL, with no changes observed except for EZ irregularity caused by retained PFCL. (**F**–**I**) The series of surgical images includes an enlarged view of the PFCL droplet in the lower-right corner. The 49 G microneedle is directed toward the center of the PFCL droplet (**F**, **G**). Once puncture is confirmed, suction is applied using active aspiration system (**H**). Successful aspiration of PFCL droplet is confirmed (**I**).

who was a 53-year-old woman presenting with PVR secondary to RRD involving the macula in her left eye. Her best-corrected visual acuity was 20/35. We performed 25-gauge pars plana vitrectomy and PFCL assisted retinal detachment repair. One month after surgery, the patient's best-corrected visual acuity was 20/50, and the retina remained attached. However, residual subretinal PFCL droplets were observed (Figs. 4A, 4B, 4D). After two months, we performed a second surgery to remove the retained PFCL using a 49 G microneedle. (Figs. 4F–I). Initially, the VFC was filled with BSS and then set to "inject" mode to prime and confirm no blockage at the microneedle tip. Subsequently, VFC was set to "extract" mode, with the maximum aspiration pressure set at 650 mm Hg. The 49 G microneedle was advanced toward the center of the PFCL droplet (Figs. 4F, 4G) and the target subretinal space entered before aspiration is activated (Fig. 4H and Supplementary Video S2). Complete aspiration of the PFCL droplet was confirmed (Fig. 4I). At the end of surgery, fluid-air exchange is completed.

SD-OCT confirms complete removal of PFCL, with no changes observed except for EZ irregularity caused by retained PFCL. (Figs. 4C, 4E.) Changes in the retinal layer structure caused by microneedle puncture are not detectable even on SD-OCT volume scans (Supplemental Fig. S3).

Discussion

The relative scarcity of subretinal PFCL, let alone fovea involving or threatening, has led to numerous surgical removal techniques with no consensus on the most effective method. We rationalized that exploring alternative minimally invasive techniques may lead to improved removal of particularly small bubbles of subretinal PFCL and create a smaller retinotomy with less potential for complications. To our knowledge, there have been no previous reports characterizing the effect of needle gauge on PFCL removal. In the present study we confirm that such microneedles can be used safely and effectively by using in vitro, ex vivo and finally in vivo investigations.

Previous studies using small gauge glass pipettes of 50 G,[8] and microcannulae of 49 G[6] have shown success in PFCL removal. However, it is known that glass pipettes can fracture during surgical manipulations[18] and therefore are not widely used, which ultimately led to development of the first microfabricated microneedles by our group.[19] As such, in our study, we confirm the size of the microneedles using microscopy and electron microscopy (data not shown and presented in a related study) and also explore PFCL flow through different needle gauges. Further, Joondeph[20] previously described a novel 40 G microneedle and showed no evidence of a retinal wound on time domain OCT. However, images of this microneedle resembles the structure of the established Polytip 25 G/38 G cannula as opposed to having the characteristics of a microneedle. In contrast, we have fully characterised and validated the clinical use of microneedles for endovascular cannulation and now provide complimentary evidence supporting the clinical use of microneedles in retained subretinal PFCL removal.

In the present report, no complications were observed in any of the cases. Nevertheless, retinal holes or subretinal hemorrhage can occur as potential complications and thus require careful attention. It should be noted that retinal tears may be induced if hand tremor or patient movement enlarges the entry site after the puncture; the use of an armrest or similar support has been recommended to mitigate tremor.[21,22] To prevent subretinal hemorrhage, retinal vessels are carefully avoided and the puncture is performed under elevated infusion pressure. In addition, when systemic blood pressure is elevated, it is adjusted and lowered as clinically appropriate.

Cases of spontaneous resolution of retained submacular PFCL have been reported, whereby observation over several months leads to resolution.[23,24] However, in both these cases, the authors did not report the type of PFCL used. In our setting in Japan, only perfluoro-octane is licensed for surgical use and therefore we were unable to compare the properties of other PFCL brands or alternatives such as perfluorodecalin. This is of importance when considering the possible mechanisms of PFCL spontaneous resolution and when to consider a surgical or conservative approach. The high vapor pressure of perfluoroctane has been thought to be the cause of a previously described fatal gas embolism during endoresection surgery[25] and therefore may also contribute to spontaneous resolution where a retinal defect overlying the retained subretinal PFCL defect exists.[24] Laplace laws may also contribute to this phenomenon as described by Chan et al.[26] where it may be possible to use additional pre-retinal PFCL, create a small retinal defect in the overlying retina of a retained PFCL bubble, and this can spontaneously resolve. In the present study, this was attempted in ex vivo porcine eyes using a range of needles with no success (data not shown), however, given that small subretinal bubbles could not be created in our model, we could not confirm whether the published mathematical model is applicable in vivo.

Conclusions

Retained PFCL is a relatively rare complication of retinal surgery but can result in irreversible visual loss without timely removal. We confirm that stainless steel microneedles can be used reliably, safely and effectively to directly remove subretinal retained PFCL. We therefore recommend it as an option, particularly when considering cases where subfoveal or perifoveal PFCL is present and a direct surgical approach is preferred.

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Author Contributions: This project was conceived and initially developed by J.C., S.T. and K.K. Benchtop experiments and ex vivo porcine experiments designed by J.C., S.K. and performed by J.C. Data collection and analysis were carried out by J.C. and S.K. Manuscript draft preparation and finalization undertaken by all authors.

Disclosure: **J. Ching**, none; **S. Tanaka**, none; **K. Shohei**, none; **K. Kadonosono,** 45 G/49 G needle (P)

* JC and ST contributed equally.

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Supplementary Material

Supplementary Video S1. Ex vivo fresh porcine eye vitrectomy model demonstrating intraoperative microneedle aspiration of subretinal PFCL. VFC extract settings were set to a maximum of 650 mm Hg using a linear setting from 0 mm Hg.

Supplementary Video S2. Intraoperative video of In vivo retained subretinal PFCL removal using the microneedle. VFC extract settings were set to a maximum of 650 mm Hg using a linear setting from 0 mm Hg.