Evolution of Vitreous Substitutes

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Ever since man discovered vitreous his quest for the discovery of a perfect substitute started. Due to various pathologies at times it becomes necessary to remove the vitreous and replace it with artificial substitutes. But despite the far advanced research, man has not been successful so far in finding a perfect substitute for vitreous which lacks complications.

Anatomy of Vitreous

The vitreous is a gelatinous structure that fills the space between the lens and the retina. The human vitreous humour occupies 2/3rd of the eye by volume, weighs approximately 4 grams and has a density of 1.0053-1.0089g/cm³. The vitreous is firmly attached to the retina at three places: the strongest attachment is anteriorly at the vitreous base, followed by the optic nerve head and retinal vasculature.

It is composed of rod like collagen and inter-fibrillary Hyaluronic acid and is composed of approximately 98-99% water with pH of 7.0-7.4. The concentration of hyaluronic acid increases from the anterior portion near the lens to posterior vitreous near the retina and it forms a three-dimensional structure with collagen.

The vitreous is acellular with a viscosity of 4.200 cm³/g with Refractive Index of 1.3345-1.3348. The viscosity is highest in posterior vitreous and decreases anteriorly. Though the exact function of vitreous in unknown it is important in maintaining transparency of the media for maximum photon transmission to the retina. It may also maintain lens transparency by reducing the effects of reactive oxygen molecules on lens proteins. Vitreous also helps in circulating nutrients and solutes throughout the eye and helps as a shock absorber.

Role of Vitreous Substitutes

With advancing age the vitreous undergoes liquefaction resulting in its transformation from a formed gel form to fluid form. These collagen fibers condense to form free floating bundles which is referred to as ‘floaters’. which may interfere with vision.

In case of Retinal Detachments (RD), the Vitreous undergoes inflammatory changes and acts as a source of traction resulting in non-resolution of RD. In cases of bleed into the vitreous it acts as an unwanted reservoir of blood cells which may not resolve with time. In such cases the need for removal of Vitreous is warranted. The ideal vitreous substitute would mimic the native vitreous in both form and function while being easily manipulable during surgery. It should have similar viscoelastic properties and be able to maintain the intraocular pressure within a physiologic range and support the intraocular tissues (including the retina) in proper position. At the same time, the substitute should allow movement of ions and electrolytes and maintain the concentration of certain substances (oxygen, lactic acid, ascorbic acid, etc.) comparable to that in the native vitreous.

Like native vitreous, the ideal vitreous substitute should be clear. It should also be permanent, requiring only a one-time implantation. A self-renewing substitute is desirable, because interaction with products released by the surrounding retina can lead to degradation of the substitute. Also, it should not induce any toxic reactions and should...
remain biocompatible for long-term use. Once injected, it should not biodegrade or disperse into small particles that the body can resorb or respond to immunologically. For practical reasons, the ideal substitute should be easily available, stable during storage, injectable through a small syringe, and available at a reasonable cost.

It has been a great challenge to develop one substitute that can match all of these ideal characteristics. Rather than match all the complexities of the vitreous body, the substitutes in clinical use have been created with the intention of acting as retinal tamponades.

**History**

In 1895, Deutschmann injected rabbit vitreous into the vitreous of a human to successfully treat retinal detachment. Intraocular air was first used for treatment of retinal detachment by Ohm in 1911; Rosengren revived its use in 1938. The use of intraocular air and gas for retinal reattachment declined with the advent of Gonin’s discovery that scleral buckling or indentation beneath the retinal break resulted in a high success rate of retinal reattachment.

In the 1950s, Paul Cibis began using silicone oil as a dissecting tool for proliferative vitreoretinopathy (PVR) detachments and also noted its tamponading effect. Further development of silicone oil was delayed by the improvement of techniques of vitreous surgery for managing PVR. As use of the vitrectomy procedure increased, aqueous substitutes for infusion during lengthy vitrectomy were developed. Long-acting gases for ocular tamponade after vitrectomy were developed in the 1970s.

In the 1980s heavier-than-water immiscible perfluorocarbon liquids were investigated for tamponade of inferior retinal tears and reattachment of the retina, especially for giant retinal tears.

In 2001, semiflourinated alkanes were developed that were heavier than water and immiscible but possessed a lesser specific gravity than the perfluorocarbons, which allowed mechanical rotation of the partially flattened retina without gross mechanical damage to the retina.

**Classification of Vitreous Substitutes**

A classification of vitreous substitutes based on their physical properties divides them into aqueous miscible (such as balanced salt solution (BSS), hyaluronic acid solutions, and other viscoelastic substances) and aqueous immiscible substances.

The property of surface tension of the immiscible substances helps to seal retinal breaks either temporarily or on a long-term basis. They can be subdivided into gases (such as air, sulfur hexafluoride (SF6, perfluoro-propane (C3F8)), and liquids, including those that are lighter than water - polydimethylsiloxane (PDMS) (silicon oil) and those that are heavier than water such as fluorinated silicone oils, perfluorocarbon liquids (PFCL) and semifluorianted alkanes (SFA). They can also be categorized as:

1. **Gas-Based Substitutes** (air, expansile gases).
2. **Perfluorocarbon Liquids**.
3. **Silicone Oils**.
4. **Semi-Fluorinated Alkanes**.
5. **Combination of Semi-Fluorinated Alkanes and Silicone Oil**.
6. **Natural and Semi-Synthetic Polymers**.
7. **Experimental Substitutes**.

**Gas Based Substitutes**

**Air Based Substitutes**

Air was first used by Ohm in 1911 to repair retinal detachments. Inexpensive and readily removable and completely absorbed by the eye. But disadvantage is it is very short acting and lasts for only few days in the eye. Hence its use is limited to Pneumatic Retinopexy and nowadays at the end of small gauge vitrectomies as it helps seal the ports better than fluid.

**Expansile Gas-Based Substitute**

Expansile gases have been used since 1970s. The most commonly used gases are SF6, which is five times heavier than air, and C3F8, which is six times heavier than air. Both gases are colorless, odorless, and nontoxic. They were approved for use by the U.S. Food and Drug Administration in 1993 for pneumatic retinopexy.

Advantages are that these have the highest surface tension of 70 dynes/cm2 and last longer than air. These gases diffuse into the blood stream and the vitreous cavity gets filled by aqueous humor, so a second surgery to remove this vitreous substitute is not required.

Disadvantages are the unpredictable rise of IOP in the immediate post-op period which can result in retinal vascular occlusion. Gas expansion to dangerous levels can occur if the patient rapidly ascends to a higher altitude. Other disadvantages are post-op positioning of patient, cataract formation and endothelial damage.

Properties of various gases has been depicted in (Table 1).

**Perfluorocarbon Liquids**

PFCLs are fluorinated, synthetic, carbon containing compounds that are clear, colorless, and odorless. PFCLs are approximately twice as dense as water and possess an extensive capacity for transporting and releasing both O2 and CO2. They were originally designed as blood substitutes with good oxygen carrying capability.
Their use as an intraoperative tamponade has changed the surgical management of proliferative vitreoretinopathy because the retina can be flattened and stabilized intraoperatively, facilitating epiretinal membrane removal and traction release. The most commonly used PFCLs are perfluorodecalin (PFD), perfluorohexyloctane (F6C8), perfluoroxypropylhydrophenanthrene, and octafluoropropane.

**Advantages**

The high specific gravity of PFCLs makes them effective for the intraoperative repair of complex retinal detachments. It acts as surgical third hand during removal of proliferative membranes and stabilizes macula while disecting the anterier PVR membranes5. Use of PFCL to unroll the flap of a giant retinal tear has greatly helped in increasing success rate of surgeries for such large retinal tears. With a refractive index near water, PFCLs allow for the use of a conventional contact lens for visualization during vitreous surgery. Retinal laser can also be done through the PFCL. Because of low viscosity, PFCLs allow for easy intraoperative tissue manipulation, injection, and removal. Anterior and posterior segment complications are uncommon because PFCLs are usually removed intraoperatively. Studies in animals have shown a potential neuro-protective effect on the ischemic retina, likely due to PFCLs' high oxygen solubility.

**Disadvantages**

Currently, PFCLs are for the most part limited to the intraoperative use as a result of their toxicity over longer periods. Their presence intravitreally in rabbit eyes over more than 2-4 days has been shown to lead to irreversible cell damage to the inferior retina, most likely as a result of mechanical damage to cells and near total emulsification by 6 days postsurgery. Iatrogenic retinal breaks and subretinal migration of PFCLs are known to occur by forced injection, lack of release of full traction intraoperatively.

**Silicone Oils (SO)**

SO has been in use as a vitreous substitute since the 1960s, but was not approved by the FDA until 1994. It is similar to silicon rubber, but shorter polymer chains and a lack of chemical cross-linking result in a liquid form. It is a hydrophobic substance with a specific gravity slightly less than water (0.97 g/mL) (8) and a refractive index of 1.4 (slightly higher than the vitreal index of 1.33) (Table 2).

SO is effective as a short or long-term tamponade for complex retinal detachments, and is the only substance currently accepted for long-term vitreous replacement with lesser complication rates9. Recommendation to remove SO ranges from 6-8 weeks to 6-30 months. Currently used in 1000, 1300 and 5000 centistokes forms.

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**Table 1: Properties of the commonly used gases as tamponading agents**

<table>
<thead>
<tr>
<th>Gases</th>
<th>Mol wt.</th>
<th>Typical dose in pneumatic retinopexy</th>
<th>Largest size</th>
<th>Expansion</th>
<th>Longevity (days)</th>
<th>Nonexpansile conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>29</td>
<td>0.8ml</td>
<td>immediate</td>
<td>No expansion</td>
<td>5-7</td>
<td>---</td>
</tr>
<tr>
<td>SF6</td>
<td>146</td>
<td>0.5ml</td>
<td>36 hours</td>
<td>doubles</td>
<td>10-14</td>
<td>18</td>
</tr>
<tr>
<td>C3F8</td>
<td>188</td>
<td>0.3ml</td>
<td>3 days</td>
<td>quadruples</td>
<td>38-55</td>
<td>14</td>
</tr>
</tbody>
</table>

**Table 2: Comparing the properties of various Vitreous substitutes**

<table>
<thead>
<tr>
<th>Tamponade agent</th>
<th>Chemical composition</th>
<th>Specific gravity (g/cm³ @ 25°C)</th>
<th>Viscosity</th>
<th>Surface tension (mN/m)</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>SF6</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>C3F8</td>
<td>-</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Silicone Oil (1000 cSt)</td>
<td>PDMS 100%</td>
<td>0.97</td>
<td>1000</td>
<td>21</td>
<td>1.4</td>
</tr>
<tr>
<td>Silicone Oil (5000 cSt)</td>
<td>PDMS 100%</td>
<td>0.97</td>
<td>5000</td>
<td>21</td>
<td>1.4</td>
</tr>
<tr>
<td>C8F18</td>
<td>C10F18-100%</td>
<td>1.76</td>
<td>2.7</td>
<td>16</td>
<td>1.27</td>
</tr>
<tr>
<td>Densiron 68</td>
<td>F6H8-30.5% PDMS (5000cSt) - 69.5%</td>
<td>1.06</td>
<td>1349</td>
<td>19.13</td>
<td>-</td>
</tr>
<tr>
<td>Oxane HD</td>
<td>RMN3-11.9% OXANE 3700-88.1%</td>
<td>1.02</td>
<td>3300</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Advantages

The major advantages of SO are its high surface tension, ease of removal, low toxicity and transparency. Because it is immiscible with water, the high surface tension and low specific gravity create a tamponade effect on the superior retina, with a 70% success rate in preserving anatomical integrity. It is preferable to use a SO tamponade if post-operative air or high altitude travel is planned, or with difficulties in post-operative positioning in children or adults with physical impairment, which make it a more versatile replacement than air or gas.

Disadvantages

Because of low specific gravity, tamponade of the inferior retina is difficult. Emulsification has been shown to be a problem with SO, although in several studies less than 5% of eyes showed oil emulsification after surgery. Corneal abnormalities have been reported with SO, although the Silicon Study showed a 27% incidence of corneal abnormalities at 24 months, which did not differ significantly from SF6 gas. Emulsified droplets can adhere to a silicone IOL. Post-operative cataracts are common, and if the oil is left in the eye after the surgery, cataracts often develop. Other complications of SO include anterior chamber migration, corneal decompensation and band keratopathy, glaucoma from pupillary block or overfilling at rates of 2-40%, and hypotony from underfilling.

Another drawback to SO is that patients must be followed closely for possible complications and, in the majority of cases, the SO needs to be removed. The removal process can cause complications; therefore, careful clinical judgment is required when selecting patients for SO removal. Recurrent retinal detachment has been reported in 5-67% cases after silicon oil removal. Silicon oil removal can be complicated by visual acuity loss, hypotony, and corneal abnormalities. The phenomenon of “sticky silicone oil” adherent to the retina has been reported to complicate removal in approximately 12% of cases by Veckeneer et al.

Semifluorinated Alkanes (SFA)

SFAs also known as partially fluorinated alkanes (PFAs) or fluorinated alkanes, are the first internal tamponade agents that can be used beyond the intraoperative setting unlike PFCLs and can be left in situ for longer duration to tamponade the inferior retina since they are heavier than water. They were investigated in the early 2000s. SFAs have a specific gravity of 1.35 g/mL and a refractive index of 1.3. The low specific gravity of SFAs (compared to PFCLs) is thought to produce less retinal damage.

Experimental studies showed that they could be tolerated intra-ocularly for 2-3 months. A higher interface tension theoretically provides a better chance for bridging giant retinal tears.

Disadvantages are emulsification and cataract formation.

Silicone Oil/SFA Combinations

Recently, mixtures of SFAs and silicone oil as tamponade agents have been investigated and approved for clinical use. By combining the two liquids, the solution gets the advantage of the high specific gravity of the SFAs and the high viscosity of silicone oil in order to produce a vitreous substitute with a good tamponade effect and minimal emulsification.

The various techniques described are,

Double Fill

Double fill (DF) is a combination of Silicone oil and SFAs, with the goal of having the light silicone oil support the superior retina while the heavier SFA supports the inferior retina, resulting in fewer complications than each liquid alone keeping the percentage of oil as low as possible to avoid toxicity.

Advantage is that it can be used in large inferior retinal breaks, it has ability to act as a single large tamponading agent with reduced dispersion.

Disadvantages are that they are not good in providing simultaneous tamponade as much as described theoretically, there are some occasions of forming an egg shaped bubble with superior dissolved SFA in silicone oil with inferior pure SFA.

Heavy Silicone Oil

Heavy silicone oil (HSO) is an internal tamponade agent that is heavier than water and is created by combining SO and a PFA in such a way as to create a homogenous solution. A multicentered, prospective, randomized controlled clinical trial (n = 700) comparing the standard SO tamponade to the HSO tamponade in the treatment and prognosis of eyes with PVR of the lower retina is currently underway, but the intermediate results published shows no significant difference between silicone oils and Heavy Silicone oils.

Advantages

The newest HSOs are more viscous, more stable, and consequently better tolerated than their predecessors, resulting in longer times until removal. Removal with limited complications has occurred after 1-4 months for Oxane HD and Densiron 68 and up to 3 months with HWS 46-3000. Studies have shown the tamponade success rate of Densiron is from 85.2-87.6%. Several studies of HSO have shown good anatomical success rates (54-81%) at 3 months or more and stable visual outcomes with good intraocular tolerance and no significant emulsification.

Disadvantages

HSO can be challenging to remove because it is heavier than water. Currently, it is being removed using strong
active aspiration through a long 18-gauge needle just above the optic disc, which increases the risk of iatrogenic damage to the optic nerve. A novel removal technique “from a distance” using a shorter (7.5 mm) and smaller (20G needle potentially reduces the risk of entry site tears, postoperative hypotony, or other iatrogenic damage.

Complications of HSO include cataract, intraocular inflammation, emulsification, and elevated intraocular pressure. Some oils (such as HWS 46-3000) have been associated with a very high frequency (~100%) of posterior subcapsular cataract formation.

**Natural and Semisynthetic Polymers**

Natural polymers such as hyaluronic acid are an obvious choice for vitreous substitutes given their native presence in the vitreous humor, but have not gained popularity despite good biocompatibility because of their high degradation tendency. Hyaluronic acid has been investigated as a vitreous substitute since the early 1970s\(^1\). Collagen has been used clinically in patients with complicated retinal detachment. Modified collagens such as methylated collagen have been investigated for use as vitreous replacements. More recently, a natural crustacean product, chitosan, looks promising as a natural polymer substitute.

Currently used for visco-dissection of epiretinal membranes and short term vitreous substitutes, their main advantage lies in the fact that they are natural materials with mild, transient inflammatory reaction with no cataract formation.

Disadvantages being that they are lighter than water so this limits their utility as inferior retinal tamponades and also they have a rapid biodegradation. Also collagen gels when injected via cannula are fractured, thus destroying their function as structural gel.

**Experimental Substitute**

**Hydrogels**

Hydrogels, also known as “swell gels,” are three-dimensional polymers that swell in aqueous solutions without dissolving. They were the first biomaterials synthesized for human use. Their use is still in animal model stage.

Possible uses include retinal tamponade with possibility of drug delivery, with major advantage of being injected in aqueous form which transforms into a gel intra-ocularly due to disulfide linkage.

Polyvinyl alcohol-methacrylate (PVA-MA) is an injectable aqueous solution containing a photoinitiator that can form a hydrogel in situ when irradiated with the proper wavelength of UV radiation applied via an optical fiber. However, the current radiation time required is not realistic for surgical application. A new type of modified poly(acrylamide) gel that appears to have solved the problem of shearing on injection has been developed and is made soluble by reducing disulfide cross-linked bridges. The soluble gel can then be injected through a small-gauge needle, and consequently undergoes gelation in situ upon exposure to oxidation by air.

A major hurdle is making the hydrogels compatible with the immune system. There are few reported incidences where hydrogels incited intravitreal inflammation, fragmentation and phagocytosis and 50% of the hydrogel degraded within 4 weeks\(^1\).

**Smart Hydrogels**

“Smart hydrogels” are a relatively new class of stimuli-sensitive hydrogels that can respond to a variety of signals including pH, temperature, light, pressure, electric fields, or chemicals. They offer the possibility of self-assembly and targeted bioactivity within the eye in response to certain signals.

They have enormous potentials in creating environments which can respond to various stimuli and also can create closed feedback loops for drug delivery. So far glucose, glutathione and pH sensitive hydrogels are undergoing investigations. Their main disadvantage so far has been their biocompatibility.

**Thermo Setting Gels**

Thermo-setting gels are a type of “smart” hydrogel that react to tissue temperature. Originally developed as a vehicle for improving bioavailability of ophthalmic solutions for application to the surface of the eye. The experimental substance WTG-127 (Wakamoto Pharmaceutical, Tokyo, Japan) is a type of thermo-setting gel that has been investigated recently as a vitreal substitute. It can gelate at 36 °C and retains transparency upon gelation.

Experimentally it was found that the gel drifted under retinal tears and also the investigators were unable to confirm its presence intra-ocularly after 1 week.

**Transplants & Implants**

Experimental attempts were made as early as 1946 to transplant human vitreous. In all transplants, the donor vitreous was drawn out through a needle and injected into the recipient posterior chamber, rather than being removed and transplanted as a whole.

The most recent human vitreous transplant study was published in 1976 by Shafer as a case series of 200 human vitreous transplants performed for retinal detachment. Vitreous was obtained via stored eye-bank aspirate stored at 4°C for 2 to 14 months, plated on blood agar and incubated for 48 h at 37°C prior to use. The vitreous was planted using an 18-gauge needle through a pars plana incision with the needle tip just posterior to the recipient lens. Patients received daily antibiotic therapy with tetracycline for 4 days postoperatively.
The retinal reattachment rate was 40% overall. The most frequent post-operative complication was mild vitreous haze that disappeared without treatment after 2–5 days. Uveitis occurred in 3.5% of cases, clearing after 2–9 months of corticosteroid therapy.

Implantable devices may be able to support the retina and control intraocular pressure without the need of a potentially reactive and biodegradable intravitreally injected solution. One device that has been tested in a rabbit model is the capsular artificial vitreous body with a pressure-control valve. It is a thin, vitreous-shaped capsule made of a silicone rubber elastomer with a silicone tube valve system filled with a physiologically balanced solution.

The capsule itself is 0.01 mm thick with a 1-mm diameter silicone tube leading to the pressure-control valve. The silicone rubber capsule has shown good biocompatibility and is easily implanted and removed experimentally in rabbit eyes via a 1.5 mm scleral incision. Over an 8-week treatment period, there was no corneal opacity, intraocular inflammation, cataract formation, or vitreal opacity.

**Future of Vitreous Substitutes**

Many researchers have assumed that as the vitreous liquefies with aging, convection is the predominant way of drug distribution, and therefore drugs applied intravitreally would spread quickly in a uniform fashion. However, vitreous liquefaction is non-uniform, so convection is the dominant way of distribution only in certain regions. Vitreous is isolated by blood–retinal and blood–aqueous barriers, making it difficult to deliver drugs via topical or systemic application. The most direct method of delivery is an intravitreal injection, but there are several disadvantages, including rapid clearance within a few days. A proper vitreous substitute used as a long-term drug delivery system should either reduce or eliminate the need for multiple intravitreal injections.

A fascinating possibility could be the artificial generation of vitreous in vitro, which could solve many problems plaguing artificial substitutes, including biomechanical properties, biocompatibility, and lack of a role as a transport and repository medium.

One way to stimulate vitreous synthesis is to enhance hyalocyte proliferation. It has been demonstrated that ascorbic acid enhances hyalocyte proliferation in a dose-dependent manner at concentrations between 0.1–3 mg/mL by increasing collagen production and mRNA expression of cells in vitro.

**Conclusion**

The vitreous body has distinct biomechanical, biophysical, and biochemical properties that change during development and aging and differ between species. Understanding the structure, properties and characteristics of formed vitreous, evolve as our quest for the ideal vitreous substitute continues.

In India more than patient compliance and comfort, the cost of the substitute plays a major role in selecting the substitute for the patient and has thus far limited our use mainly to expansile gases and Silicone Oils. One day we may be reading this as part of history, when in the future people might be regenerating formed vitreous!

**References**