

Experimental Vitreous Substitutes

Deneysel Vitreus Materyalleri

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ÖZ

Vitreus materyalleri, retina dekolmanı, diyabetik retinopati komplikasyonları, maküla deliği ve gözün arka segmentini ilgilendiren retina kanamaları gibi vitreoretinal cerrahi gerektiren işlemler sırasında tamponlama amaçlı veya güncel deneysel ve klinik çalışmalarda ilaç uygulama şeklinin bir parçası olarak çeşitli amaçlarla kullanılmaktadır. Halihazırda kullanımda olan silikon yağları, ağır silikon yağları ve gazlar, dekolmanın yatışması için gerekli olan ideal iç basıncı elde etmede kısa süreli bir etkiye sahip olup, biyouyumluluk açısından da ancak kısa süreli bir dayanıklılığa sahiptirler. Bu sebeple, bazı kimyagerler, mühendisler ve oftalmologlar son zamanlarda ideal bir vitreus materyalinin dizayn edilmesi ve üretilmesi konusu üzerinde durmaktadırlar. Doğal vitreusun biyokimyasal, biyomekanik ve fonksiyonel özellikleri ile ilgili yapılan son dönem çalışmalar, daha fonksiyonel ve daha iyi biyouyumluluk gösteren, daha uzun süreli kullanılabilir ve daha az yan etki gösteren vitreus materyallerinin geliştirilmesini sağlamıştır. Bu derleme makale, yeni yapay vitreus materyallerinin gelecek vaadeden potansiyelleri ile her aşamada karşılaşılan sorunları incelemektedir.

Anahtar Kelimeler: Vitreus, İntravitreal ilaç, Vitreus materyalleri, Retina dekolmanı.

ABSTRACT

Vitreous substitutes have been used for many purposes such as tamponade during vitreoretinal surgeries for retinal detachment, complications of diabetic retinopathy, macular hole and retinal bleeding involving the posterior segment of the eye or such as a part of drug delivery system in new experimental studies and clinical trials. Current available vitreous substitutes like silicone oils, heavy silicone oils and gases have a temporary effect to obtain ideal internal pressure for reattachment of the detached retina and short-term biocompatibility. For this reason, some chemists, engineers and ophthalmologists have considered about designing and producing an ideal vitreous substitute in recent years. Recent studies on biochemical, biomechanical and functional properties of the natural vitreous enabled the development of both more functional and biocompatible vitreous substitutes with long-term usability and fewer side effects. This article reviews the novel vitreous substitutes and their challenges and promising developments in future.

Key Words: Vitreous, Intravitreal drug, Vitreous substitutes, Retinal detachment.

INTRODUCTION

The search for an ideal vitreous substitute has been ongoing from the 20th century to our present date. Current available vitreous substitutes, such as gases, silicone oil, heavy silicone oil, and hydrogels have many limitations and drawbacks that lead to glaucoma, cataract, uveitis, and retinal toxicity, etc.^{1,2} Moreover, these conventional substitutes lack functional and biochemical properties of the natural human vitreous. Understanding the natural vitreous

structure together with its biochemical, biomechanical and functional properties in detail has encouraged researchers to search for more suitable vitreous substitutes that are not only more functional and biocompatible with long-term use but also have lesser side effects. Current developments with the novel vitreous substitutes in-vivo and in-vitro have been tested experimentally.³⁻⁶ In this article, we both present a short overview of physical properties and biochemical structure of the natural human vitreous body and review the experimental vitreous substitutes and their future directions.

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1. Structure and Physical Properties of the Human Vitreous Body A short overview

1.1. General Structure

The human vitreous composes of a heterogeneous gelatinous structure in parts with different densities. Denser part of it is called as 'cortex'. Another anatomically important part of it is called as 'vitreous base' and overlies on ora serrata.⁷ Although the human vitreous body is a gelatinous structure, its vitreous contents gradually shrink and turn into a liquefied structure in aging process. This process is called 'syneresis', which finally progresses to the posterior vitreous detachment. It is thought that the collagen fibers of the vitreous structure are held together by the force of the electric charges, and the pushing effect between the electric charges tends to decrease with aging. Finally, this process leads to a clumping effect in collagen fibers, and the hyaluronan molecules separate from these fibers, then the liquid lacunae are formed.⁸

1.2. Chemical and Organic Composition of The Natural Human Vitreous

The human vitreous body has a number of molecules such

as water (99%), albumin, iron binding protein (transferrin), collagens (type II, V/VI, IX and XI). Also, it has hyaluronic acid, chondroitin sulfate (GAGs), opticin, ascorbic acid, and several types of cells such as hyalocytes, fibroblasts, macrophages.^{9,10} Hyaluronic acid is the major basic component of the human vitreous body. In addition, this molecule gives the three-dimensional shape and viscosity of the human vitreous body through the collagen network.¹¹ In a recent study, it was noted that the level of hyaluronic acid in the vitreous decrease by aging.¹² Ascorbic acid is another important content of the human vitreous body. Ascorbic acid concentration in vitreous is higher than its plasma levels. It is believed that ascorbic acid has the major role in liquefaction of the human vitreous body, and it is also responsible for the inhibition of neovascularization.^{13,14} Different oxygen levels in different parts of the vitreous are achieved by the antioxidant effect of ascorbic acid, therefore, the lower level of free oxygen radicals near the lens prevents early cataract formation.^{15,16} The biochemical composition of human vitreous is presented in Table-1.

1.3. Functions of the Human Vitreous Body

Hyaluronic acid is one of the major factors responsible for

Table 1. Biochemical composition of the vitreous.

Contents (Subgroups)	Molecule	Action
Protein	Albumin	Binding protein
	Alpha-1 Antitrypsin	Protease inhibitor
	Alpha-1 Acid glycoprotein	Acute phase protein
	Immunoglobulin (IgG)	Protective effect
	Iron binding Protein (Transferrin)	Binding protein
	Collagens	Vitreous structure
	Type II (70 %)	
Glucosaminoglycan (GAG)	Type IX (25 %)	
	Type V/IX (< 25 %)	
	Type IV (< 10 %)	
	Hyaluronan (Hyaluronic acid)	Extracellular matrix components
	Versican	
	Heparan sulfate	Responsible for viscosity
		Supplies adequate space between the collagen fibrils
Metabolites	Amino acids	Maintain metabolic activities
	Lactic acid	Inhibition of neovascularization
	Ascorbic acid	
		Induces hyalocyte proliferation
	Glucose	Antioxidant effect
	Lipids	Maintain metabolic activities
	Prostaglandins	Maintain metabolic activities
Cells		Regulation of cells
	Hyalocytes	Matrix production and maintenance
	Fibrocytes	
	Macrophages	Matrix and cell degradation

sustaining internal tension within the eye. More hyaluronic acid contents give more viscoelastic property that contributes to its shock-absorbing property to the vitreous body. Hence, the human vitreous sustains mechanical stress due to constant motion or trauma.^{17,18} Furthermore, the human vitreous body has a barrier function specific for biochemical macromolecules, and it forms the important part of the blood-ocular barrier.¹⁹

2. Why Do We Need an Artificial Vitreous?

Main contents of the human vitreous body, especially hyaluronic acid and ascorbic acid are essential for physiologic and pathologic alterations in the vitreous. One of the recent studies revealed the lower levels of hyaluronic acid in the human vitreous with macular holes or diabetic retinopathy by aging.⁸ Another study reported a positive correlation between cataract formation, liquefaction and aging.²⁰ As mentioned in the literature, ascorbic acid sustains the oxygen radicals like an anti-oxidant. Also, ascorbic acid is probably responsible for oxygen regulatory effect of the vitreous. Holekamp et al.²¹ demonstrated that oxygen exposure to the lens increases in vitrectomized eyes after the vitreoretinal surgeries, and that might be an explanation for cataract formation induced by vitreoretinal surgeries. Physiological changes in the vitreous by aging that causes breaking its structural proteins down into smaller parts might contribute the pathological changes such as thickening internal limiting membrane that leads to tractional force on the retinal surface especially vitreomacular traction.^{22,23} Internal tamponading would be used in the surgeries in particular with complicated retinal detachments, proliferative diabetic eye diseases, and macular hole.²⁴ Hence, removal of the natural human vitreous body requires refilling of its cavity with a suitable substitute that is able to tamponade and maintain the concentration of the contents such as oxygen, ascorbic acid.

How Should an Ideal Vitreous Substitute Be?

Biologically:

- 1- Nontoxic to retinal tissue
- 2- Similar viscoelastic properties to nature vitreous
- 3- Maintained intraocular pressure within physiologic range
- 4- Should need long time to be degraded (long time resistance to biodegradation)

Physiologically:

- 1- Well-supporting intraocular tissues (agreeable tampon effect)
- 2- Transparent to facilitate visualization
- 3- Effortless to inject and easy to extract

- 4- Allowing ions and electrolyte exchange and maintained concentration of basic substances in the vitreous such as oxygen and ascorbic acid.

The classification of the experimental vitreous substitutes is given on Table-2.

3. Experimental Vitreous Substitutes

3.1. Synthetic Polymers

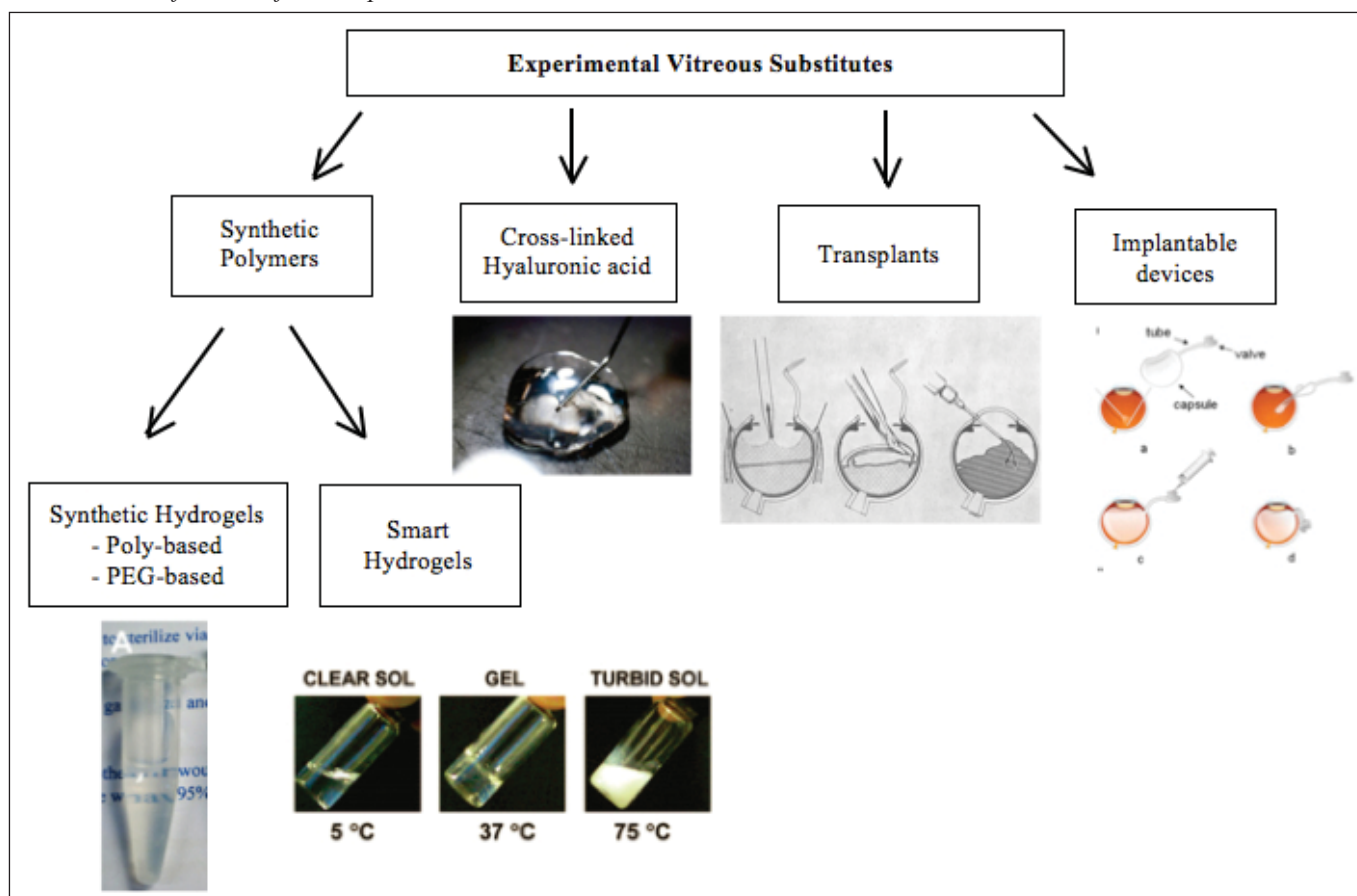
3.1.1. Synthetic Hydrogels

a) Poly-Based Hydrogels

These materials have hydrophilic properties that are arranged in gel network by cross-linking or photoinitiation. Poly (1-vinyl-2-pyrrolidinone), polyacrylamide, poly(2-hydroxyethyl acrylate) are the examples of the synthetic hydrogels.^{25,26} These examples of synthetic hydrogels were exactly unsuccessful due to some complications such as toxicity and inflammation. Most of these polymers give rise to irritation of retinal tissues, inflammation and opacification problems in short time following the surgery.^{26,27} In the past, some scientists attempted to inject these polymers with their cross-linking formation ex vivo. Nonetheless, the fragmentation problem occurred due to injection of the polymer with a small gauge needle.^{24,28} Correspondingly, this issue provoked an alternative way of the injection of soluble form followed by gelation in-situ via cross-linking under air oxidation. This cross-linking method using disulfide cross-linkers allowed injection of acrylamide polymers into the eye in aqueous form without fragmentation problem. Cross-linked copolymers exhibited vulnerable rheological and biocompatible properties. On the other hand, remained un-reacted monomers can lead to severe inflammation addition to toxic and carcinogenic effect.²⁹⁻³¹ Poly (vinyl alcohol methacrylate) is a newer polymer that becomes into gel form by photoinitiator. The photoinitiator induces cross-linking under the effect of irradiation. It was reported that more irradiation leads to more cross-linking, i.e., more gelation. As a disadvantage, those in situ gels were firmer than nature human vitreous.³² As a result, poly (vinyl alcohol methacrylate) was a tolerable vitreous substitute in macaques; however, further in vivo and in vitro studies with this substitute are needed to better understand the biocompatibility and biomimicry.

b) Polyethylene Glycol (PEG)-Based Hydrogels

Polyethylene Glycol (PEG)-based hydrogels are an aqueous solution of poly (ethylene oxide) with a molecular weight of 400kDa viscoelastic materials with similar optical and physical properties compared to nature vitreous. PEG-based hydrogels were studied firstly in in-vivo rabbit models. Eventually, it was shown that there was no significant change in electrophysiological tests and histological examinations over 6 weeks.³³ Annaka and associates³⁴ published an

Table 2. Classification of The Experimental Vitreous Substitutes^{43,44,50-52}.

experimental study in which they described an injectable PEG-based vitreous substitute made of thermosensitive hydrophobically modifying poly (ethylene glycol). The nontoxic polymer had thermosensitive properties that showed rapid gelation at body temperature then becoming flower-like micellar aggregates. As a result, this study indicated that the gel supplied tampon effect adequately in the vitreous cavity, also allowed the light to reach the photoreceptors at a similar rate as the natural human vitreous. Moreover, the intraocular pressures were in normal range.

3.1.2. Smart Hydrogels

Smart hydrogels are the substitutes that are not only polymeric but also responsive hydrogels to an external stimulus such as temperature, pH, ion or light. Use of physical, noncovalent supramolecular forces such as hydrogen bonds, hydrophobic interactions for the gelation is the main reason to be called as a smart hydrogel. Additionally, these supramolecular forces are non-permanent that makes the solution to gel formation reversible. Therefore, the secondary removing of the gel is possible. Pluronic F127 and WTG127 were the first examples of smart hydrogels. However, they exhibited severe retinal toxicity and short degradation time. Another study with WTG127 revealed that the gel drifted under retinal tear, besides the researchers could not detect the gel in vitreous

cavity after one week.^{35,36} Recently, notable technological advancements were accomplished to cope with the handicaps of pluronic F127. One of these advancements is related to the novel hydrogel with improvement biological and rheological properties by compounding oligoester segments. Loh et al.³⁷ reported the results of a novel biodegradable thermosetting copolymer composing of poly R-3-hydroxybutyrate (PHB), polyethylene glycol (PEG) and polypropylene glycol (PPG). The thermosetting copolymer can be transformed from a solution to gel form as the temperature rises from 4°C to body temperature. Moreover, the copolymer has a very low critical gelation concentration (CGC) ranging from 2 to 5 wt %. The CGC value is much lower according to Pluronic F127 copolymer (15-20 wt %). As a result, the study revealed that the substitute could remain in vitreous cavity at pH 7.4 and 37°C up to 6 months by adjusting its copolymer components. Accordingly, the copolymer smart gel like a drug delivery vehicle into vitreous might be available substitute whose releasing rate could be altered by adjusting its copolymer content. Most recently, Chang et al.³⁸ tested a novel in-situ-forming zwitterionic hydrogel as a vitreous substitute. Zwitterionic hydrogels have shown ultra-low biofouling properties at the surface and have prominent biocompatibility. The copolymer hydrogel has two important compositions as zwitterionic component

and thiol functional group. The study showed that the hydrogel has excellent biocompatibility and remained clear, transparent up to 2 months. In conclusion, smart hydrogels are still being investigated experimentally. So far the results seem promising for the future; however, further studies are still needed.

3.2. Natural Polymer-Based Hydrogels

Hyaluronic acid is the major component of the natural human vitreous. This molecule is a hydrophilic substance demonstrating more compatibility than currently used synthetic tamponading materials such as silicone oils and gases for the ocular tissues.³⁹ On the other hand, increasing the popularity of cross-linking using method has given rise to improve hyaluronic acid gels with further optimization of short biodegradation issue, additionally, the cross-linking method has provided a more suitable substitute that is biologically more similar to nature vitreous. Schramm et al.⁴⁰ published an experimental study of hyaluronic acid with carboxylation or photo-crosslinking with UV light. As a result, the synthesized hydrogels were all clear and transparent with a refractive index similar to human vitreous. The rheologic measurements suggested sufficient viscosity and elasticity for intraocular usage. The UV cross-linked biogels showed no retinal toxicity or induction of apoptosis. In vivo the UV cross-linked biogels remained for up to 6 weeks; moreover, electrophysiology and histology demonstrated excellent tissue biocompatibility. Recently, Barth et al.⁴¹ reported the morphologically and functionally availability of a novel cross-linked HA hydrogel (Healaflo®) as a vitreous substitute. The authors studied in the eyes of twelve pigmented rabbits, and they injected approximately 1 ml of Healaflo® following a combined 25-20 gauge pars plana vitrectomy with posterior vitreous detachment. The rabbits were clinically evaluated with intraocular pressure and full-field electroretinogram. Thereafter, the rabbits were sacrificed at varying time periods between 42 and 105 days. They enucleated the eyes to examine macroscopically, photographed, and worked the enucleated eyes histologically. Consequently, intraocular pressure was slightly elevated (15-25 mmHg) during the postoperative period. Electroretinography recordings showed no toxic effect on rod and cone cells. Histological examination revealed normal morphology with a few Müller cell activation compared to unoperated eyes and no notable DNA fragmentation. In conclusion, biopolymers such as cross-linked hyaluronic acid are promising vitreous substitutes.

3.3. Transplants

The vitreous transplantation trial firstly was done by Katzin and Blum⁴² as an experimental study. They removed the native vitreous of 24 rabbit eyes and replaced them with the

rabbit donor vitreous. Vitreous haze was the most common complication of the study. Most of the cases occurred within 2 months and persisted for 6 months. Shafer⁴³ published the results of the human vitreous transplantation trials in 1976. The author transplanted 200 human vitreous from eye bank to 200 of eyes with the detached retina. The success rate (reattached retina) was 40% overall. The most common postoperative complication was cloudiness of the vitreous. A mild vitreous haze frequently occurred on the first 7 days after the transplantation and disappeared without treatment. Severe degree of vitreous haze occurred in 7 of the eyes. All of the eyes treated with corticosteroid, and only one of the eyes had persistent haze until 9 months of the transplantation. Studies related to vitreous transplants are still scarce, and further studies are needed to evaluate the success of this method.

3.4. Vitreous Implants

There is a new concept for the artificial vitreous called as the *Novel Foldable Capsular Vitreous Body*. The foldable capsular vitreous body consists of a thin vitreous-shaped capsule, a drain tube, and a valve. The stages of the technique are to implant a foldable capsule into the eye then injecting a medium such as balanced salt solution, silicone oil or hydrogels into the capsular part of the material. The capsular part of the foldable capsular vitreous body is silicone rubber. Intraocular pressure can be controlled adjusting the volume of the injected medium through a tube-valve system. After filling of the capsule, the valve of the system is implanted onto scleral surface similar to the glaucoma valve.⁴⁴ Promising results with animal models lead to clinical trials for the foldable capsular vitreous body by ophthalmologists.⁴⁴⁻⁴⁶ In a recent study, Lin et al.⁴⁷ reported about the evaluation of flexibility, efficacy and safety of a foldable capsular vitreous body in severe retinal detachments. Eleven eyes of the 11 patients with retinal detachment were enrolled into the study. The implantable capsular body was injected through the *pars plana*, and then, the balanced salt solution was injected inside of the capsular body. Consequently, 8 of 11 eyes had reattached retina at 3 months after implantation. There were just slightly contacted but they did not crush on the ciliary body during 3 months. As a result, there were no significant inflammation and increment in intraocular pressure compared to the preoperative stage. It is suggested that the foldable capsular vitreous body can be an available carrier for a slow-releasing drug such as dexamethasone because of the tiny and fine structure of it. Therefore, the foldable capsular vitreous body can be a proper vehicle for intravitreal and episcleral drug delivery system.

Can We Produce The Natural Human Vitreous Using Gene Transcription?

Inducing hyalocyte proliferation is one of the ways to

stimulate vitreous synthesis. Sommer et al.⁴⁸ demonstrated that ascorbic acid increases hyalocyte proliferation rate by inducing collagen synthesis and mRNA expression of the cells in vitro. On the other hand, hyalocyte proliferation must be controlled during the transcription process. It has been reported that hyalocyte proliferation could be regulated by β FGF and TGF- β 1.⁴⁹ Therefore, producing hyalocyte could be controlled by several growth factors such as β FGF and TGF- β 1 in vitro to stimulate vitreous synthesis.

CONCLUSION

Currently available vitreous substitutes have various postoperative problems such as intraocular pressure rising, cataract, emulsification. Moreover, they can not exactly compensate the physiological role of the natural human vitreous. Biocompatibility issue is another crucial problem for available vitreous substitutes. Increased knowledge regarding the physiology and biochemical structure of the natural human vitreous body forced researchers and ophthalmologists to generate new vitreous substitutes. An ideal vitreous substitute should be optically clear, less toxic and more stable inside the eye. The human eye has anti-oxidant enzymes, matrix metalloproteinases and ascorbic acid. Therefore, these enzymes and ascorbic acid could influence the stability of the substitute. Further developments with materials as a vitreous substitute should be focused on these anti-oxidant factors to improve the stability of the materials. Although current experimental studies show that there is still no exactly an ideal vitreous substitute, the results of the studies, so far, are encouraging.

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