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Anomalous posterior vitreous detachment: a unifying concept in vitreo-retinal disease

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Abstract Posterior vitreous detachment (PVD) is the consequence of changes in the macromolecular structure of gel vitreous that result in liquefaction, concurrent with alterations in the extracellular matrix at the vitreo-retinal interface that allow the posterior vitreous cortex to detach from the internal limiting lamina of the retina. Gel liquefaction that exceeds the degree of vitreo-retinal dehiscence results in anomalous PVD (APVD). APVD varies in its clinical manifestations depending upon where in the fundus vitreo-retinal adhesion is strongest. At the periphery, APVD results in retinal tears and detachments. In the macula, APVD causes vitreo-macular traction syndrome, results in vitreoschisis with macular pucker or macular holes, or contributes to some cases of diabetic

macular edema. At the optic disc and retina, APVD causes vitreo-papillary traction and promotes retinal and optic disc neovascularization. Unifying the spectrum of vitreo-retinal diseases into the conceptual framework of APVD underscores that to more effectively treat, and ultimately prevent, these disorders it is necessary to replicate the two components of an innocuous PVD, i.e., gel liquefaction and vitreo-retinal dehiscence. Pharmacologic vitreolysis is designed to mitigate against APVD by chemically breaking down vitreous macromolecules and weakening vitreo-retinal adhesion to safely detach the posterior vitreous cortex. This would not only facilitate surgery, but if performed early in the natural history of disease, it should prevent progressive disease.

Introduction

Invisible [32, 37] *by design* (Fig. 1), vitreous was long unseen as an important part of the eye. In recent years, however, the roles of vitreous in ocular physiology [9] and the pathobiology of vitreo-retinal diseases [27] have been increasingly appreciated. Thus, the contribution of vitreous to blindness is being treated with ever-evolving therapeutic modalities, for the most part surgical. The future, however, will see the use of pharmacologic agents for therapy and prevention. The success of such pharmacologic therapies will depend upon a better understanding of the biochemical composition and organization of vitreous, how this structure changes with age, and how anomalies in “normal” aging lead to vitreo-retinal disor-

ders via the mechanism of anomalous posterior vitreous detachment (APVD).

Vitreous biochemistry

Vitreous biochemistry is complex and has been extensively reviewed elsewhere [3, 33, 35]. Composed mostly (98%) of water, vitreous contains two major macromolecules—collagen and hyaluronan (HA). Collagen is an important structural protein in vitreous, and the collagen fibrils of vitreous consist of more than one collagen type. Recent studies [4] of pepsinized forms of collagen confirmed the presence of collagen type II, a hybrid of types V/XI, and type IX collagen in a molar ratio of

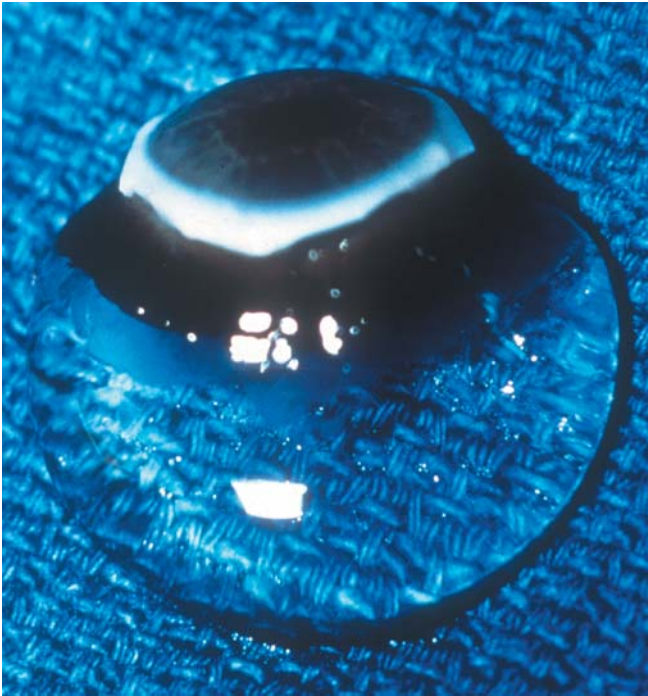


Fig. 1 Human vitreous. Specimen from a 9-month-old child with the sclera, choroid, and retina dissected off the vitreous body. In spite of this specimen being on a surgical towel exposed to room air, the gel state is maintained

75:10:15, respectively. These are organized into fibrils with type V/XI residing in the core, the type II component surrounding the core, and type IX on the surface of the fibril. The fibrils are 7–28 nm in diameter [1] but their length in situ is unknown.

HA is the other major structural macromolecule of vitreous. Although HA is present throughout the body, it was first isolated in 1934 from bovine vitreous. HA is a long, unbranched polymer of repeating glucuronic acid β -1,3-*N,N*-acetylglucosamine disaccharide moieties linked by β 1–4 bonds [1]. It is a linear, left-handed, threefold helix with a rise per disaccharide on the helix axis of 0.98 nm [43]. The sodium salt of HA has a molecular weight of $3\text{--}4.5 \times 10^6$ in normal human vitreous [1].

As described by Mayne [19], vitreous is a dilute meshwork of collagen fibrils interspersed with extensive arrays of HA molecules. The collagen fibrils provide a scaffold-like structure that is “inflated” by the hydrophilic HA. If collagen is removed, the remaining HA forms a viscous solution; if HA is removed, the gel shrinks [8] but is not destroyed. Comper and Laurent [8] proposed that electrostatic binding occurs between negatively charged polysaccharides and positively charged proteins in vitreous. Bishop [4] has proposed that to understand how vitreous gel is organized and stabilized requires an understanding of what prevents collagen fibrils from aggregating and by what means the collagen fibrils are

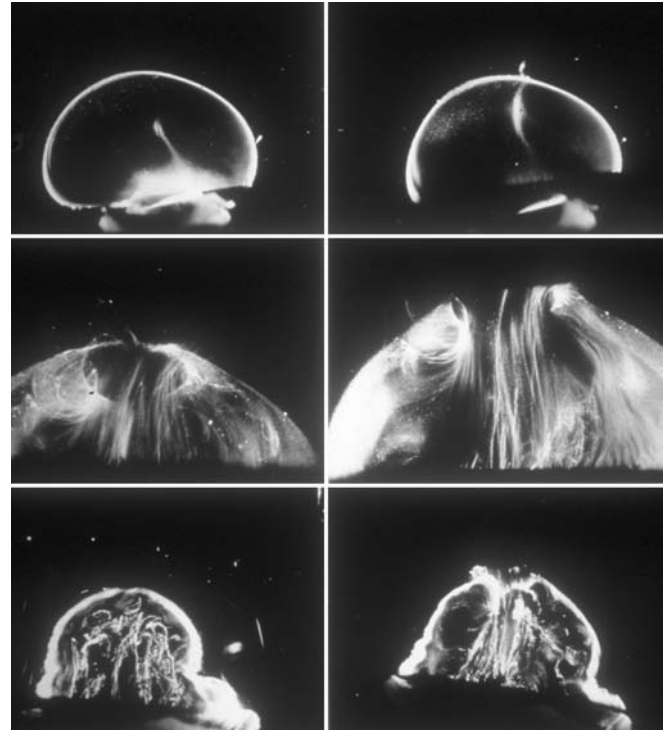


Fig. 2 Aging changes in human vitreous structure. *Top panel*: dark-field slit microscopy of the posterior and central vitreous in a 33-week-old human embryo shows considerable light scattering arising from the vitreous cortex, due to densely packed collagen fibrils. In the central vitreous is the remnant of the hyaloid artery oriented towards the pre-papillary posterior vitreous cortex. This structure is destined to become Cloquet’s canal. *Middle panel*: vitreous structure in adults is characterized by macroscopic fibers with an antero-posterior orientation, inserting into the vitreous base. *Bottom panel*: in old age there is aggregation of the fibers into tortuous structures with adjacent pockets of liquid vitreous that ultimately form lacunae (left side of photograph on right, bottom panel)

connected to maintain a stable gel structure. Studies [24] have shown that the chondroitin sulfate chains of type IX collagen bridge between adjacent collagen fibrils in a ladder-like configuration spacing them apart. Such spacing is necessary for vitreous transparency, since keeping vitreous collagen fibrils separated by at least one wavelength of incident light minimizes light scattering, allowing unhindered transmission of light to the retina for photoreception. Bishop proposed that the leucine-rich repeat protein opticin is the predominant structural protein responsible for short-range spacing of collagen fibrils. Concerning long-range spacing, Scott [23] and Mayne et al. [20] have claimed that HA plays a pivotal role in stabilizing the vitreous gel via this mechanism.

In youth, the intricate biochemical organization of vitreous results in a transparent gel (Fig. 1) which has little or no light scattering properties (Fig. 2, top panel). In the adult, there are macroscopic fibers with an antero-posterior orientation and insertions into the vitreous base

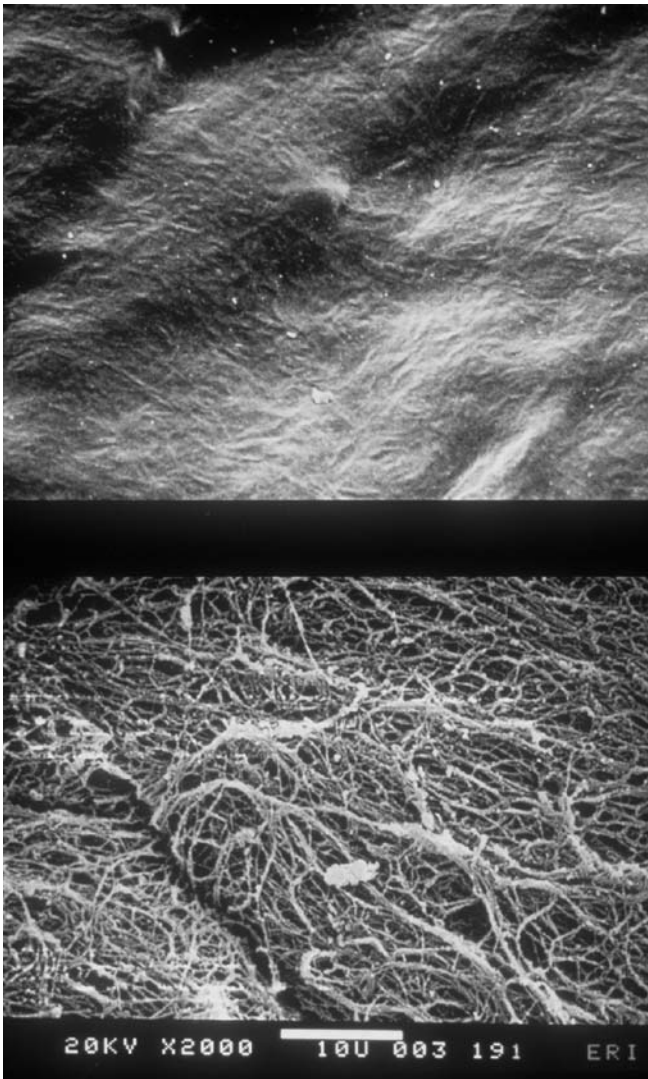


Fig. 3 Scanning electron micrographs of human vitreo-retinal interface. Collagen fibrils in the posterior vitreous cortex (*below*) appears quite different from the type IV collagen in the internal limiting lamina of the retina (*above*)

and posterior vitreous cortex (Fig. 2, middle panel). These fibers are composed of bundles of collagen fibrils [38].

Vitreo-retinal interface

The outermost “shell” of the vitreous body is known as the vitreous cortex. Here the concentration of both collagen and HA is higher than elsewhere in the vitreous body. Figure 3 shows the appearance of the posterior aspect of the posterior vitreous cortex (lower panel). The densely packed collagen fibrils are composed mostly of type II collagen, although type IX and a hybrid of types V/XI are also present in vitreous fibrils. There is quite a

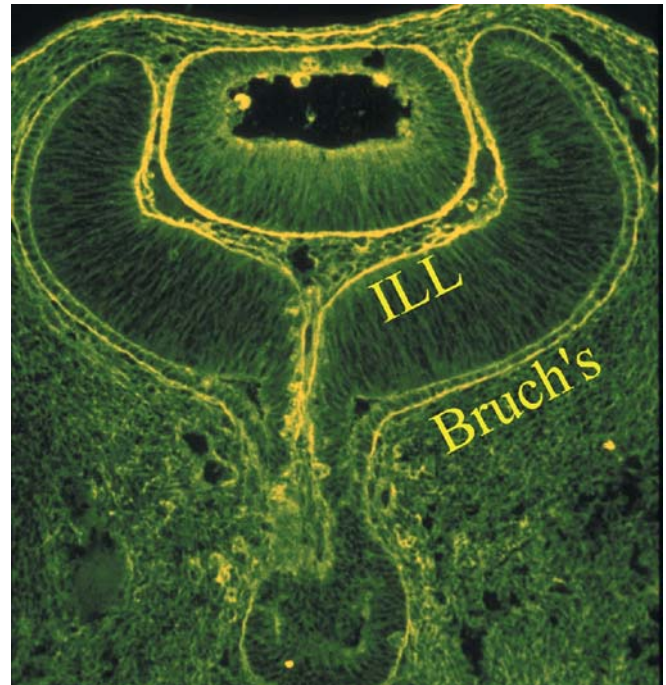


Fig. 4 Photomicrograph of human eye during early embryogenesis. Immunofluorescent antibodies to basement membrane constituents demonstrate the continuity of the internal limiting lamina (ILL) and Bruch's membrane. Before the optic vesicle invaginates, these two planes are continuous as a sphere. Their embryologic identity suggests that they are both coded for by the same gene(s) and are composed of very similar components (photograph courtesy of Greg Hageman, PhD)

difference in appearance between the posterior vitreous cortex and the inner surface of the internal limiting lamina (ILL; upper panel) due to the fact that the latter is comprised mostly of type IV collagen.

Interestingly, the embryologic origin of the ILL is the same as Bruch's membrane [39] (Fig. 4). This fact raises some intriguing considerations regarding the pathophysiology of intraocular neovascularization, where sprouting vasculogenic cells migrate and proliferate on tissue planes.

The composition and organization of the molecular components of these tissue planes can influence the process of neovascularization. In the case of choroidal neovascularization new vessels grow in and around Bruch's membrane. In proliferative diabetic vitreoretinopathy new vessels grow in and around the ILL. Given the identical embryologic origin of these two interfaces, it would be of value to explore the similarities and differences between these two extracellular matrix tissue planes in an attempt to elucidate what role, if any, these interfaces play in the pathogenesis of retinal and optic disc neovascularization, the most devastating ocular affliction in young individuals, and choroidal neovascularization, the most common cause of blindness in elderly individuals.

Whereas it was previously believed that the vitreous collagen fibrils inserted into the ILL, it is now considered more plausible that an intervening extracellular matrix serves as a “glue” between the two interfaces [39].

Age-related vitreous degeneration (ARVD)

Throughout life, there are substantial alterations in the vitreous body. After the fourth decade of life there is a significant decrease in the gel volume and an increase in the liquid volume of human vitreous [2, 25, 35]. Post-mortem studies of vitreous structure [26] led to the concept that alterations in the normal HA/collagen association results in the simultaneous formation of liquid vitreous and aggregation of collagen fibrils into bundles of parallel fibrils seen as macroscopic fibers (Fig. 2). In the posterior vitreous such age-related changes form large pockets of liquid vitreous, recognized clinically as “lacunae” (Fig. 2—bottom right photo). By the ages of 80–90 years more than half the vitreous body is liquid [2].

During youth, there is strong adhesion between the posterior vitreous cortex and the ILL of the retina, primarily at the vitreous base and at the posterior pole [28, 29]. Rather than focal adherences at the disc, fovea, and along retinal blood vessels, vitreo-retinal adhesion at the posterior pole appears to be more “fascial” than focal (Fig. 5), consistent with the concept that the two tissues are held together by an extracellular matrix “glue.” With age, there is weakening of vitreo-retinal adhesion, most likely due to biochemical alterations at the vitreo-retinal interface. Studies [22] using lectin probes have identified that one component of the extracellular matrix at the vitreo-retinal interface [galactose β (1,3)-*N*-acetyl-glucosamine] is present in youth but absent in adults. This difference and others may play a role in the observed weakening of the vitreo-retinal interface during aging. The presence of such “adhesive” molecules may also provide opportunities for intervention with pharmacologic agents that target these molecules (Fig. 5).

Posterior vitreous detachment (PVD)

PVD results from weakening of the vitreous cortex/ILL adhesion, in conjunction with liquefaction within the vitreous body. Weakening of the vitreous cortex/ILL adhesion at the posterior pole allows liquid vitreous to enter the retro-cortical space via the pre-papillary hole and perhaps the pre-macular vitreous cortex as well (Fig. 2—middle panel). Volume displacement from the central vitreous to the pre-retinal space causes the observed collapse of the vitreous body. For PVD to occur without complications, two different processes must occur concurrently and to a similar extent: weakening of vitreo-retinal adhesion and vitreous liquefaction. There must be

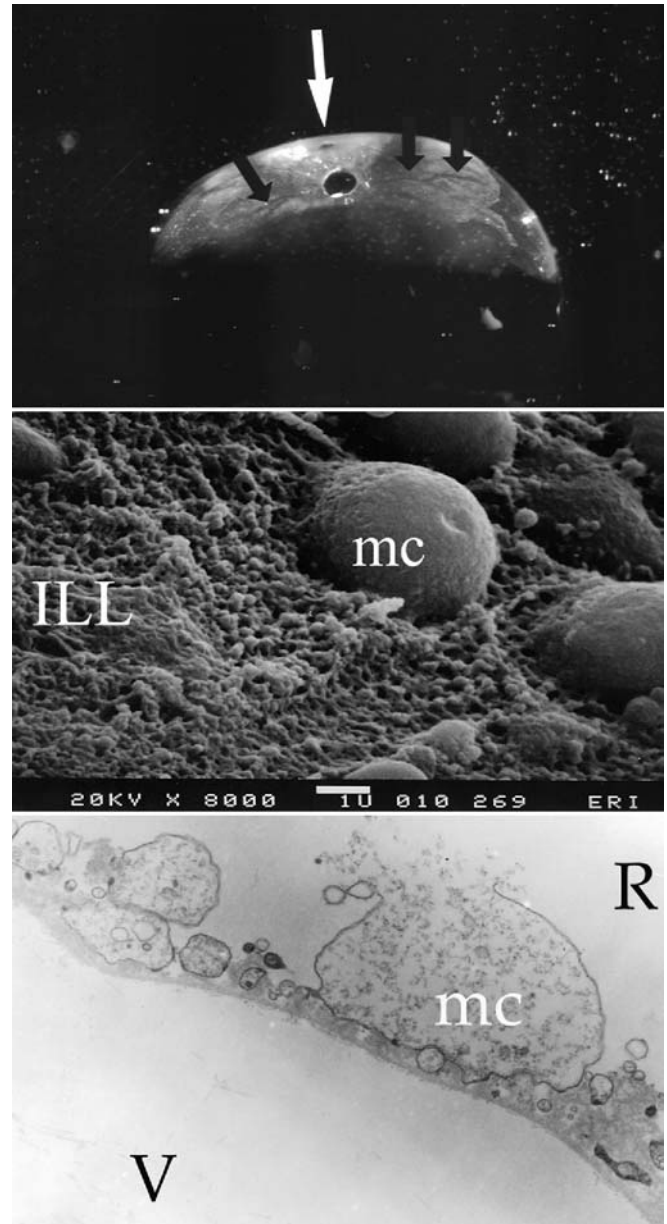


Fig. 5 Human vitreo-retinal interface in youth [adapted from Sebag J: age-related differences in the human vitreo-retinal interface. *Arch Ophthalmol* 109:966–971, 1991]. *Top panel:* dark-field slit microscopy of posterior vitreous in a 14-year-old boy after dissection of the sclera, choroid, and retina. A cap of tissue adheres to the vitreous with a hole corresponding to the optic disc, linear structures resembling retinal vessel patterns (*black arrows*), and the foveal imprint (*white arrow*). *Middle panel:* scanning EM of tissue shown above. Mueller cell (MC) endplates inserted onto the internal limiting lamina (ILL) of the retina. [*bar* = 1 μ m]. *Bottom panel:* transmission EM identifies the tissue as the internal limiting lamina (ILL) of the retina (R) attached to the posterior vitreous cortex (V), with the broken inner segments of Mueller cells adherent to the posterior aspect of the ILL.

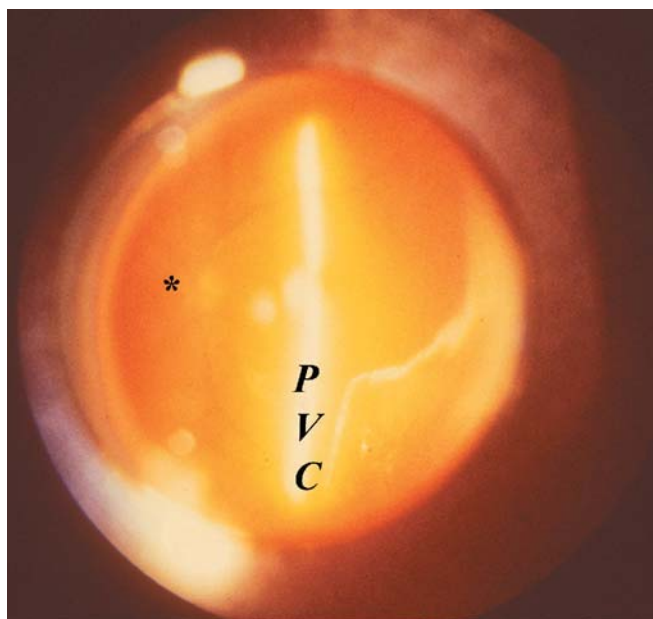


Fig. 6 Posterior vitreous detachment. Vitreous liquefaction (synchysis) in conjunction with dehiscence at the vitreo-retinal interface allows liquid vitreous to dissect the posterior vitreous cortex (PVC) away from the internal limiting lamina of the retina and optic disc (*asterisk*). Collapse (syneresis) of the vitreous anteriorly can be seen in the *upper right hand corner* of this clinical photograph taken by preset lens biomicroscopy. The *bright, vertically oriented, white linear area* is the reflection of the illuminating light beam off the fundus [photo courtesy of C.L. Trempe, MD]

sufficient weakening of vitreo-retinal adherence so that when the critical amount of liquefaction has developed, the collapsing vitreous separates away from the retina and PVD occurs without complications (Fig. 6).

Anomalous posterior vitreous detachment (APVD)

Anomalous PVD results when the extent of vitreous liquefaction exceeds the degree of weakening of vitreo-retinal adherence and traction is exerted at this interface. There are various causes for this imbalance between the degree of gel liquefaction and weakening of vitreo-retinal adherence. Inborn errors of collagen metabolism, such as

those present in Marfan's, Ehlers-Danlos, and Stickler's syndromes [44] result in extreme gel liquefaction at an early age with persistent vitreo-retinal adherence. The result is a high incidence of large retinal tears and detachments [18]. Systemic conditions such as diabetes induce biochemical [40] and structural [30] alterations in vitreous. These changes can be detected *in vivo* using advanced optical instrumentation and techniques [41, 42]. The result is diabetic vitreopathy [31], an important contributor to the pathobiology of proliferative diabetic vitreo-retinopathy. Changes associated with myopia can similarly be considered as myopic vitreopathy, where there is excess vitreous liquefaction for the degree of vitreo-retinal adherence, resulting in anomalous PVD and undue traction at the vitreo-retinal interface.

As a result of abnormal traction at the vitreo-retinal interface, there can be deleterious effects upon retina as well as vitreous (Table 1). Effects upon the retina include hemorrhage, retinal tears and detachment, and vitreo-macular traction syndromes, including macular holes and some cases of diabetic macular edema. Proliferative diabetic vitreo-retinopathy (PDVR) can be aggravated by anomalous PVD. Effects upon vitreous involve posterior vitreoschisis [16], where splitting of the posterior vitreous cortex and forward displacement of the vitreous body leaves the outer layer of the posterior vitreous cortex still attached to the retina. This can result in macular pucker, macular holes, or complicate proliferative diabetic vitreo-retinopathy [7].

Unifying the spectrum of vitreo-retinal diseases into the conceptual framework of anomalous PVD underscores that to more effectively treat and ultimately prevent these disorders, it is necessary to replicate the two components of an innocuous PVD—gel liquefaction and vitreo-retinal dehiscence. Surgical approaches have been successful in removing the gel vitreous and replacing it with an aqueous solution, effectively achieving liquefaction. However, there are still many challenges concerning the surgical induction of vitreo-retinal dehiscence. It is likely that since the adhesion is on a molecular level, the best way to dissolve this adhesion is similarly via molecular mechanisms, *i.e.*, pharmacologically and not surgically.

Table 1 Anomalous PVD

Traction site(s)	Retinal effects	Vitreous effects
Blood vessels	Retinal hemorrhages Aggravate retinal neovascularization	Vitreous hemorrhage
Macula	Vitreo-macular traction syndrome Diabetic macular edema (diffuse)	Vitreoschisis with Macular pucker Macular holes
Periphery Optic disc	Retinal tears/detachments Vitreo-papillary traction Syndrome Aggravate NVD (PDVR, CRVO)	White without pressure

NVD neovascularization of the optic disc, PDVR proliferative diabetic vitreoretinopathy, CRVO central retinal vein occlusion.

Table 2 Pharmacologic vitreolysis agents

	Non-specific	Substrate specific
Enzymatic	Plasmin Microplasmin Dispase	Chondroitinase Hyaluronidase Matrix metalloproteinases (including collagenases)
Non-enzymatic		

Pharmacologic vitreolysis

Coined in 1998 [34], the term *pharmacologic vitreolysis* refers to the use of exogenous (to the vitreous) agents to alter the biochemical and biophysical states of the macromolecules responsible for maintaining vitreous structure and vitreo-retinal adhesion. The goals of pharmacologic vitreolysis are to induce liquefaction of the gel and promote complete dehiscence of the vitreous from the retina. It is important to note that the success of pharmacologic vitreolysis depends upon inducing these two events simultaneously, or at least insuring that liquefaction does not progress without sufficient vitreo-retinal dehiscence. Uncoupling these two processes, particularly by inducing liquefaction without weakening vitreo-retinal adherence, may worsen matters significantly by provoking rather than preventing or ameliorating anomalous PVD and its untoward sequelae, i.e., vitreo-papillopathies, vitreo-maculopathies, and peripheral retinal traction.

There have been a variety of approaches attempted to date. Hyaluronidase was employed as early as 1949 and collagenase in 1973. Table 2 outlines the approaches that are currently being developed. As indicated in this table, the different pharmacologic agents can be broadly grouped as enzymatic and non-enzymatic. Within the enzymatic group there are substrate-specific agents and non-specific agents.

Early observations on the effects of blood upon vitreous laid the groundwork for approaches based upon extracting active agents from blood for pharmacologic vitreolysis. *Plasmin* is a non-specific protease that can be isolated from the patient's own serum for use at surgery. It has been tested in rabbits [49] and several small series of pediatric [6] and adult [46, 50] patients undergoing vitrectomy. To date, this agent has primarily been advocated as an adjunct to vitreo-retinal surgery. There are also other sources of plasmin. Studies [12] in Marburg, Germany have shown that effective intravitreal levels of plasmin can be generated by injecting tissue plasminogen activator (tPA) and breaking down the blood-vitreous barrier with cryopexy or laser photocoagulation. Other studies [47] have used combinations of plasminogen and urokinase. Recently, microplasmin, a human recombinant molecule with a molecular weight of 29,000 kDa representing a portion of the plasmin molecule that lacks the five "kringle" domains but contains the protease domain

of plasminogen, has been shown to separate the vitreous cortex from the retina in pigs [48].

Another relatively non-specific protease currently under investigation is *dispase*. The first investigations [45] used dispase to induce PVD in enucleated porcine and human cadaver eyes, noting no untoward effects upon retinal histology and ultrastructure. Subsequent studies [21] were successful in using this agent *in vivo* to remove cortical vitreous during vitreous surgery in the pig. Since dispase has proteolytic activity against type IV collagen and fibronectin, there is some concern that the ILL of the retina might be adversely affected by this agent. Yet, the histologic studies [47] in porcine and human cadaver eyes found that only the lamina rara externa of the ILL was affected, with lesser effects upon the lamina densa. The subsequent animal studies found that this agent did not alter the ERG *in vivo*, and no neuroretinal ultrastructural abnormalities were detected *post-mortem* [21]. However, a more recent and extensive study [15] in rabbit and human eyes found that this agent causes retinal toxicity, perhaps due to the broad range of proteins subject to the effects of dispase.

A substrate-specific enzyme that has been in development for a number of years is *chondroitinase*. This agent lyses chondroitin sulfate, a molecule that may be important in the maintenance of both the gel state of vitreous [10], as well as vitreo-retinal adhesion; hence, there has been considerable interest in the use of this agent. Indeed, studies [11] have purportedly shown that when used as an adjunct to vitreous surgery, chondroitinase facilitates the removal of pre-macular membranes. Several years ago, a phase I trial using this agent during vitreous surgery in patients was completed in the United States, yet the results still await publication. In this trial, patients with macular holes and others with PDR were treated with chondroitinase during vitreous surgery, with no untoward effects. To date, phase II studies of efficacy have yet to be undertaken.

In addition to facilitating vitreo-retinal surgery as currently performed, pharmacologic vitreolysis could possibly replace vitrectomy, as was proposed with *hyaluronidase* to clear vitreous hemorrhage without vitrectomy. However, in the phase III FDA trial undertaken in the United States, this drug was not found to be effective. This may be due to the fact that the trial included both patients with type I and type II diabetes. In the former group, patients are younger and more likely to have an attached vitreous without a weakened vitreo-retinal interface. Thus, although the enzyme may have decreased the viscosity of vitreous gel and facilitated the outflow of red blood cells, in the younger type I patients the persistent attachment of the posterior vitreous cortex to the retina and to any neovascular complexes arising from the retina and optic disc would cause recurrent vitreous hemorrhage. Indeed, there has been some discussion of using this agent to induce PVD in patients with non-PDR,

since liquefaction of the vitreous body and detachment of the posterior vitreous cortex away from the retina prior to the onset of new vessel growth into the vitreo-retinal interface (see below) will have a far better prognosis than if the vitreous were still attached. However, the results of the phase III FDA trial would suggest that this not the case. Furthermore, on theoretical grounds hyaluronidase is not likely to induce vitreo-retinal separation and PVD, because HA is probably not involved in maintaining vitreo-retinal adhesion. Indeed, studies in experimental animal models have shown contradictory results in this regard, with the most recent studies [14] finding no such effect. On the other hand, combining hyaluronidase with SF₆ has purportedly induced PVD in the rabbit, similar to what has been shown with plasmin and SF₆ [13]. It is plausible that the expanding gas, and not the enzyme, is responsible for these effects, since many years ago Lincoff and Machemer reported similar effects with expanding gas alone.

Discussion

Anomalous PVD is a unifying concept of vitreo-retinal disease that guides therapeutic and preventative efforts to dissolve the vitreous body as well as lyse vitreo-retinal adhesion. Pharmacologic vitreolysis is intended to mitigate against anomalous PVD by chemically breaking down vitreous macromolecules and weakening vitreo-retinal adhesion to safely detach the posterior vitreous. This should not only facilitate surgery, but if performed early in the natural history, it may prevent progressive disease. As described above, a number of pharmacologic vitreolysis agents have been tested to date, but without any significant breakthroughs. Broad-acting agents, such as plasmin, microplasmin, and dispase, have a higher likelihood of inducing both a breakdown in vitreous macromolecules and vitreo-retinal separation. However, there may be untoward side effects if the agent's action is too broad. Dispase is an interesting case in point. Although initial studies showed efficacy and safety, the results of a more recent and extensive study in rabbit and human eyes found that this agent causes retinal toxicity, perhaps due to the broad range of proteins subject to the effects of dispase. This is the reason for the proposal [36] that a combination of highly specific agents may be safer and more effective than a single broad-acting agent. Future studies should determine the relative merits of these two approaches. It may turn out that a substrate-specific approach is more appropriate for some clinical circumstances, while a broad-acting agent is preferable in other situations.

It should be pointed out that there is some question as to the true specificity of the enzymes currently being utilized for pharmacologic vitreolysis. Bishop and colleagues [5] and others [17] have found considerable cross-

reactivity amongst different agents being developed for pharmacologic vitreolysis and surprisingly little true substrate specificity. Thus, future progress may depend upon more purified and specific enzymes than those that are currently available and combining two or more agents to achieve both a breakdown of vitreous macromolecules and dehiscence at the vitreo-retinal interface. As far as hyaluronidase is concerned, there are doubts as to whether it would be able to achieve separation at the vitreo-retinal interface. Without inducing vitreo-retinal separation, pharmacologic vitreolysis with such an agent can actually cause or worsen anomalous PVD, possibly resulting in vitreo-macular traction syndromes, macular holes, and, in the periphery, retinal tears and detachments. Since a more thorough understanding of the mechanisms of action of pharmacologic vitreolysis is needed in order to avoid such untoward events, future studies should investigate vitreous biochemistry more thoroughly and select agents on the basis of these findings before undertaking expensive clinical research and exposing many patients to agents that ultimately fail, such as hyaluronidase.

Throughout the history of medicine, increased knowledge of the pathophysiology of disease has enabled the evolution of therapeutics from surgical to medical strategies, and ultimately to preventative modalities. Advances in our understanding of vitreo-retinal pathobiology and new developments in technology have produced an impressive array of surgical instruments and techniques for conditions that permanently blinded many just a generation ago. However, these exciting developments have engendered complacency with respect to the traumatic nature of surgery, its inherent limitations, and the enormous costs to society. As a result, many have lost sight of the fact that as successful as a surgical approach may seem, it can never be as beneficial as a non-surgical treatment, both in terms of clinical outcome measures and with respect to socio-economic aspects. Furthermore, true prevention is only possible by non-surgical approaches. The need for less invasive approaches to treat and prevent vitreo-retinal disorders may one day be met by pharmacologic methods of altering the biochemical and biophysical states of the vitreous body in an effort to reduce or eliminate its role in disease. Although the day when vitreous surgery will be replaced by non-invasive therapy is still quite far off, pharmacologic vitreolysis holds great promise. Not only will such approaches facilitate and enhance our present methods of treating vitreo-retinal disorders, but these new techniques will very likely usher in an era when fewer patients will need the extensive invasive procedures currently being performed. What's more, the development of a simpler, cheaper, and less-invasive approach to vitreo-retinal disease may some day make therapy available to many of the less-privileged people on earth who cannot presently avail themselves of such advanced and costly care.

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