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Posterior vitreous detachment (PVD) • Anomalous PVD
Liquefactant • Interfactant

Key Concepts

1. Vitreous and the vitreoretinal interface are comprised of molecules that dictate structure and function as well as underlie the changes that result in posterior vitreous detachment (PVD) in most cases and anomalous PVD in some individuals.
2. Pharmacologic vitreolysis, which is the molecular manipulation of vitreous to induce liquefaction via *liquefactant* agents and dehiscence at the vitreoretinal interface via *interfactant* activity, is destined to replace the surgical cure of various pathologies of anomalous PVD.
3. The failures and success of different pharmacologic vitreolysis agents under development hold important lessons for future progress that may include combination therapy but should also emphasize improved drug delivery to the target tissues.

I. Introduction

Vitreous constitutes about 80 % of the volume of the human eye. It is an extended extracellular matrix that is composed of collagen, hyaluronan, and other extracellular matrix molecules but is 98 % water. In both health as well as disease, the molecular composition and organization of vitreous dictate the structure and function of the vitreous body as well as the status of the vitreoretinal interface. During aging, there is

reorganization of vitreous macromolecules resulting in gel liquefaction and structural changes that destabilize the vitreous body. Molecular changes at the vitreoretinal interface, whose components are the retinal inner limiting membrane (ILM), the posterior vitreous cortex, and the intervening extracellular matrix (ECM), usually weaken vitreoretinal adhesion and allow the collapsing destabilized vitreous to separate from the retina, a condition called posterior vitreous detachment (PVD) [see chapter II.C. Vitreous Aging and Posterior Vitreous Detachment]. When there is insufficient weakening of vitreoretinal adherence, anomalous PVD occurs with various pathologic effects on both vitreous and retina [see chapter III.B. Anomalous PVD and Vitreoschisis]. To date, these pathologies have been treated with surgery.

Throughout the history of medicine, therapeutics have evolved as a direct result of advances in our basic understanding of disease pathogenesis. This has led to less invasive yet increasingly effective therapeutics. At first, when little is known, little, if anything, is done. As recognition and understanding of anatomic pathology increases, surgery is employed. With increasing knowledge about the cause(s) of disease, surgery is supplemented with and eventually replaced by pharmacotherapy. When we fully understand a disease, we develop ways to prevent it, obviating the need for both surgery and pharmacotherapy. Consider, for example, that the treatment of severe infections by surgical drainage of an abscess has been to a great extent replaced by the use of antibiotics. Peptic ulcers, for many years a surgical disease believed to be caused by stress, had high mortality. Antacids, antihistamines, and proton pump inhibitors as well as good gastroscopies made huge improvements. The breakthrough came with the discovery of *Helicobacter pylori* as the underlying cause in the vast majority, and now, antibiotic therapy has largely eliminated surgery in many settings. Cervical cancer, once treated with hysterectomy, was increasingly recognized to arise from subtle dysplastic changes in the epithelium. A population level screening program and development of less invasive biopsies and surgical procedures made a huge difference in survival. Recognition of a papilloma virus as the underlying cause has led to widespread vaccination programs, which may prevent the vast majority of cases with this disease.

This type of evolution is now occurring in the discipline of vitreoretinal diseases and surgery. Laser surgery for exudative maculopathies due to diabetic retinopathy, vein occlusions, and age-related macular degeneration (AMD) has been largely replaced by intravitreal anti-VEGF pharmacotherapy. Another recent development is “*pharmacologic vitreolysis*,” a term that was coined in 1998 to refer to the use of drugs to alter the molecular structure of vitreous and the vitreoretinal interface [23]. The objective of this therapy is to pharmacologically induce an innocuous PVD and thus mitigate any untoward effects of vitreous on the retina that

cause or worsen pathology. Furthermore, a variety of physiologic effects are introduced, which are, in some settings, very beneficial.

The foregoing chapters of this book are witness to the expansion of our knowledge about vitreous and its role in disease. Various predisposing genetic factors and congenital anomalies, contributing systemic diseases, anomalous aging effects, and ocular conditions have been described. A few good examples are chapters I.C. [Hereditary Vitreo-Retinopathies], III.A. [Congenital Vascular Vitreo-Retinopathies], I.E. [Diabetic Vitreopathy], III.K. [Vitreous in Retino-Vascular Diseases and Diabetic Macular Edema], III.B. [Anomalous PVD and Vitreoschisis], III.G. [Vitreous in AMD], III.I. [Role of Vitreous in the Pathogenesis of Retinal Detachment], and II.B. [Myopic Vitreopathy]. The surgical management of these conditions has also been discussed at length (see chapters V.A.1. to V.A.6. and V.B.1. to V.B.9.). However, owing to the significantly expanded fund of knowledge related to vitreous and its role in retinal disorders, nonsurgical therapies are being developed, initially to augment but eventually to replace surgery. The following reviews these approaches to the future. Of note is the fact that of the seven major pharmacologic vitreolysis modalities that have been undertaken to date, one has been approved for clinical use, one is currently under development, and five have either failed or been disbanded. All seven research and development programs will be critically reviewed below and presented in greater detail in the remaining chapters of this section.

II. Classification of Approaches to Pharmacologic Vitreolysis

The first retrievable publication on pharmacologic vitreolysis appeared 65 years ago in the British Journal of Ophthalmology [17]. Since this and other initial approaches all used enzymes as adjuncts to surgery, the term “*enzymatic vitreolysis*” was prevalent in the early literature [see chapter VI.B. History of pharmacologic vitreolysis]. However, in 1998, the term “*pharmacologic vitreolysis*” replaced the older term, so that vitreolytic agents could be grouped according to more than just an enzymatic mechanism of action [23]. The following presents two different classifications based upon the actions of the various pharmacologic vitreolysis agents.

A. Chemical Action of Pharmacologic Vitreolysis Agents

In 1998, pharmacologic vitreolysis was viewed in terms of the chemical activity of the various agents under development [23]. Thus, the two broad classes of drugs were

Table VI.A-1 Classification of pharmacologic vitreolysis agents by chemical activity

Enzymatic	Nonenzymatic
Substrate specific	
Chondroitinase	Urea/vitreosolve
Hyaluronidase	RGD peptides
Dispace	
Nonspecific	
tPA	
Plasmin	
Ocriplasmin	
Nattokinase	

Table VI.A-2 Pharmacologic vitreolysis classification based on biologic action

Liquefactants (agents that liquefy the gel vitreous)
Nonspecific:
tPA, plasmin, ocriplasmin, nattokinase, Vitreosolve ^a
Substrate-specific:
Hyaluronidase
Interfactants (agents that weaken vitreo-retinal adhesion)
Nonspecific:
tPA, plasmin, microplasmin, nattokinase, Vitreosolve ^a
Substrate-specific:
Dispace, chondroitinase, RGD peptides ^a

tPA, plasmin, microplasmin, nattokinase, and vitreosolve are believed to be both liquefactants and interfactants

tPA tissue plasminogen activator

^aNonenzymatic agents

enzymatic or *nonenzymatic* agents (Table VI.A-1). Within the larger enzymatic group of agents, there was further sub-division into *substrate-specific* and *nonspecific* agents.

Although logical, this classification of the various approaches to pharmacologic vitreolysis based on chemical activity did not seem useful from the clinical standpoint. Thus, an alternative approach was sought a decade later.

B. Biological Action of Pharmacologic Vitreolysis Agents

In 2009, the various pharmacologic vitreolysis agents under development were reclassified in terms of their biological activity. Central to the biological classification system is the fact that posterior vitreous detachment (PVD) results from concurrent liquefaction of the gel vitreous and weakening of vitreoretinal adhesion [see chapter II.C. Vitreous aging and posterior vitreous detachment]. Thus, these two components of PVD were considered important in classifying agents for pharmacologic vitreolysis [25]. Those agents that primarily liquefy the gel vitreous are referred to a *liquefactants*, while those that induce dehiscence at the vitreoretinal interface are called *interfactants*. As can be seen in Table VI.A-2,

however, the chemical and biological classification systems are not mutually exclusive, since there can be further subdivisions based upon chemical activity (enzymatic vs. nonenzymatic) and substrate specificity. Of further note is that several agents are considered to have both liquefactant and interfactant effects.

The following will consider the various agents that are being developed or already approved for use in clinical pharmacologic vitreolysis. Of considerable interest are those agents whose development has been discontinued, as there are lessons to be learned from their failure or discontinuation.

III. Pharmacologic Vitreolysis Agents

A. Agents with Failed or Discontinued Development

1. Hyaluronidase (Vitrase®)

[See chapter VI.F. Hyaluronidase]

This substrate-specific liquefactant targets hyaluronan, which is a glycosaminoglycan composed of repeating disaccharide units of *N*-acetyl glucosamine glycosidically linked to glucuronic acid [see chapter I.F. Vitreous biochemistry and artificial vitreous]. Hyaluronidase (Hase) has a number of natural biological functions including fertilization and preserving mobility in human body tissues. Hase can also enhance pathologic processes such as bacterial virulence and the spread of cancer cells [7, 18].

Hase has been used in ophthalmology to facilitate the periocular distribution of retrobulbar injection of anesthetics. The first use of Hase for pharmacologic vitreolysis was by Pirie [17] who employed it in the rabbit vitreous. Foulds then reported that Hase induces significant vitreous liquefaction [see chapter VI.B. History of pharmacologic vitreolysis]. Much later, ovine testicular Hase was developed for pharmacologic vitreolysis. Preclinical studies demonstrated efficacy in animal models, so clinical studies were undertaken, including a phase 3 trial to assess the efficacy of Hase in accelerating the clearance of vitreous hemorrhage [see chapter VI.F. Hyaluronidase as a vitreous liquefactant]. While Hase was able to clear vitreous hemorrhage more rapidly than saline, the phase 3 results did not provide sufficient evidence to demonstrate efficacy and warrant approval as a commercial product.

The underlying reasons for this failure convey important lessons. Firstly, study subjects with vitreous hemorrhage were predominantly patients with proliferative diabetic retinopathy. It is therefore relevant that none of the preclinical animal studies were performed in diabetic animals, although it is well known that diabetes alters vitreous on a biochemical [27, 28] as well as structural [22] level [see chapter I.E.

Diabetic vitreopathy]. Thus, to extrapolate from nondiabetic animal studies to the diabetic human vitreous is fraught with hazard, especially when dealing with a biochemical phenomenon like pharmacologic vitreolysis, for in diabetes, the substrate of vitreous molecules (especially collagen) is not the same due to glycation and the formation of advanced glycation end products.

Another explanation for the failure of Vitrase relates to the fact that HASE is not an interfactant, only a liquefactant (Table VI.A-2). Because HA does not play a significant role in mediating vitreoretinal adhesion [see chapter II.E. Vitreoretinal interface and inner limiting membrane], the action of HASE is limited to gel liquefaction. While HASE will liquefy gel vitreous, it will not induce vitreoretinal dehiscence and PVD [8, 36]. In proliferative diabetic retinopathy, this will result in persistent traction upon neovascular complexes that have grown into the posterior vitreous cortex with recurrent vitreous hemorrhage and vision loss. This is particularly hazardous in patients with type I diabetes who are younger and have more firm and extensive vitreoretinal adherence. In these individuals, HASE will risk inducing traction retinal detachment, as was the experience of this author during the clinical trials. Thus, not only is Vitrase® not approved for use as an intravitreal injection, it should not be used off-label for pharmacologic vitreolysis, except perhaps in the setting of existing PVD.

2. Chondroitinase

[See chapter VI.H. Chondroitinase]

Chondroitinase is an enzyme complex that cleaves and fragments chondroitin-sulfate-containing glycosaminoglycan (GAG) side chains of proteoglycan “core” molecules [see chapter I.F. Vitreous biochemistry and artificial vitreous]. Given the importance of GAGs at the vitreoretinal interface [see chapter II.E. Vitreoretinal interface and inner limiting membrane], chondroitinase has potential to be a potent interfactant. Chondroitinase specifically cleaves and degrades only bonds between and within the disaccharide *N*-acetylgalactosamine glucuronic acid. As formulated for human clinical trials, chondroitinase is a biological agent that consists of a mixture of two enzymes, Chondroitinase I and II. Chondroitinase I (110 kDa) is a lyase that attacks chondroitin sulfate in endolytic fashion, breaking the polymeric structure into oligosaccharides and disaccharides. Chondroitinase II (112 kDa) has no activity against the intact polymer, but can digest tetrasaccharides produced by the Chondroitinase I-catalyzed reaction. The combined activity of these two enzymes results in more rapid degradation than the use of Chondroitinase I alone.

In human, primate, and pig eye sections exposed to chondroitinase, adhesion between the collagen of the vitreous cortex and the retina, optic disc, and lens was markedly reduced [11, 12]. This effect was greater with chondroitinase

than trypsin, hyaluronidase, dispase, heparinase, and others. Follow-on studies demonstrated that exposure of the human vitreoretinal interface to chondroitinase resulted in complete disruption of the adhesion, a process Russell and Hageman termed “dis-insertion.” Unlike known proteolytic enzymes, chondroitinase resulted in complete, rather than incomplete, separation of the vitreous from the primate retina, but did not liquefy vitreous [1].

The results of phase I/II FDA testing are described in chapter VI.H. [Chondroitinase as a Vitreous Interfactant – Vitreous Dis-insertion in the Human]. Although this was primarily a safety study that found no untoward effects, there was some evidence of efficacy. Nonetheless, there have been no further clinical trials with chondroitinase. In the words of the principal investigator Dr. Stephen Russell, “*Despite the initial discovery and testing of chondroitinase as a pharmacologic vitreolysis agent that began nearly three decades ago, contractual restrictions in the initial years and intellectual drift in more recent years have limited the peer-reviewed publication of most of the animal (monkey) and human studies that evaluated this promising agent.*” Moreover, the procurement process employed for chondroitinase was expensive, far more than a recombinant enzyme. Also, during the 1980s, the chondroitinase project was handled by four different corporate development teams: Storz, American Cyanamid, American Home Products, and Bausch and Lomb. By the time a decision was made for a Phase III trial, none of the early advocates of chondroitinase were managing the project. Since chondroitinase did not originate in their pipeline and the phase I/II data (not powered for efficacy) appeared to be insufficient [see details in chapter VI.H. Chondroitinase as a vitreous interfactant: vitreous disinsertion in the human], chondroitinase did not undergo further study [21]. While this may have made sense in a corporate context, the scientific and clinical perspectives appear to suggest that further research and development is warranted, perhaps with a less expensive recombinant form of chondroitinase.

3. Nattokinase

Nattokinase is an enzyme extracted from natto, a Japanese food made from soybeans fermented by the bacteria *Bacillus subtilis natto*. This popular breakfast food in Japan has a cheese-like smell, a nutty/salty flavor, and a sticky consistency. The dietary supplement of nattokinase is purified from natto and made into tablets and capsules, so it does not have the strong smell or taste as the food. It is a popular supplement because nattokinase is thought to dissolve abnormal blood clots.

Medical research with this substance is limited and relatively recent. Indeed, the first nattokinase publication listed on PubMed dates back no earlier than 1987. This study describes nattokinase as an enzyme with a molecular

weight=20,000 and strong fibrinolytic activity, especially to the plasmin substrate H-D-Val-Leu-Lys-pNA (S-2251) [30]. A more recent study [38] identified that nattokinase promoted the release of tissue plasminogen activator from vascular endothelial cells and HeLa cells. This finding may explain nattokinase's ability to promote pharmacologic vitreolysis [see chapter VI.C. Pharmacologic vitreolysis with tissue plasminogen activator].

The first and, to this author's knowledge, only study of nattokinase in the eye demonstrated the efficacy of this serine protease to induce PVD in rabbits, as determined by scanning electron microscopy [31]. Clinical examination, ERG testing, and light microscopy identified toxicity at a dose ten-fold greater than the dose that was able to induce PVD. Remarkably, PVD was induced within 30 min after injecting nattokinase. In recent years, there have been no further publications on the subject of nattokinase pharmacologic vitreolysis. The chairman of Ophthalmology at Kumamoto University in Japan, Professor Hidenobu Tanihara, describes that this is primarily due to the fact that the producers of nattokinase are producers of food, not pharmaceuticals (personal communication, 2014).

4. Vitreosolve®

Vitreosolve® is a nonenzymatic agent for pharmacologic vitreolysis that is comprised of urea, which "unravels" collagen fibers and purportedly induces both liquefaction and vitreoretinal dehiscence, making it both a liquefactant and interfactant. Following animal studies, this drug has been evaluated in Phase III clinical trials conducted at multiple sites in the United States, India, and Mexico. The objective of the clinical trials was to evaluate the safety and efficacy of intravitreal injections of Vitreosolve® to induce a PVD (as determined by ultrasound) in patients with nonproliferative diabetic retinopathy (NPDR). This prospective, randomized, double-masked, placebo-controlled study enrolled approximately 400 patients with NPDR in the United States and India and 150 in Mexico. The study design involved monthly intravitreal injections for 3 months. The results showed that while Vitreosolve® did induce a PVD in 50 % of cases, PVD also occurred in 36 % of controls ([13]; V. Karageozian, MD, personal communication, 2013). This difference was not statistically significant and all clinical research with Vitreosolve® has been terminated.

While this is disappointing, there may be valuable lessons to be learned. The importance that clinical trials be designed with sufficiently large subject groups to assure statistically significant results is underscored. Although the Vitreosolve® trials involved over 500 subjects, these were apparently not sufficiently large numbers, perhaps suggesting a lack of drug potency, as well as a surprisingly high efficacy (36 %) of placebo injections. Secondly, no preclinical studies were done with Vitreosolve® in diabetic animals. Studies have

previously shown that the vitreous gel and vitreoretinal interface in diabetic patients are quite different from nondiabetic individuals [see chapter I.E. Diabetic vitreopathy]. Thus, future investigators are well advised to research the effects of agents for pharmacologic vitreolysis (or any drug to be injected intravitreally in diabetic patients) in appropriate models of the ultimate disease application, in this case to induce PVD in subjects with diabetic vitreopathy and diabetic retinopathy. As mentioned above, this shortcoming was also a factor in the failure of the liquefactant Vitrase® (hyaluronidase) to induce beneficial effects in diabetic patients and obtain FDA approval.

5. RGD Peptides

The rationale for using RGD peptides in pharmacologic vitreolysis derives from their interaction with integrins. Many adhesive proteins present in extracellular matrices contain the tripeptide arginine-glycine-aspartic acid (RGD) as their cell recognition site. Identified nearly three decades ago [20], the RGD sequences of each of the adhesive proteins are recognized by at least one member of a family of structurally related receptors, integrins, which are heterodimeric proteins with two membrane-spanning subunits. These transmembrane receptors mediate the attachment of a cell to other cells or the extracellular matrix (ECM). Integrins are also known to play a critical role in cell signaling [10]. Therefore, in addition to transmitting mechanical forces across otherwise vulnerable membranes, integrins are involved in the regulation of cell cycle, shape, and motility. The specific binding complex "RGD" (a term referring to the amino acid sequence arginine-glycine-aspartate) is important as it is present in various ECM components including laminin, fibronectin, and certain collagens, thereby playing a potentially important role at the vitreoretinal interface [see chapter II.E. Vitreoretinal interface and the ILM]. Indeed, immunolocalization studies have identified integrins on the surface of the ILM [2]. Because synthetic RGD peptides compete for integrin-binding sites, they could disrupt integrin-ECM interaction with subsequent loosening of interface attachments, in this case at the vitreoretinal interface. Thus, it may be possible to disrupt vitreoretinal adhesion and induce PVD via pharmacologic vitreolysis with RGD analogues.

Studies [16] in a rabbit model found that after 24-h incubation of intravitreal RGD peptides, limited vitrectomy (30-s core vitrectomy with attempted PVD induction at low aspiration ≤ 30 mmHg) resulted in a significantly greater extent of PVD in treated eyes compared with controls. Although retinal edema was noted in half of the treated eyes, no toxicity was detected on clinical examination, electron microscopy, or TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) apoptosis assay. Despite the solid rationale and these encouraging initial findings, no further studies have been published in the past 12 years. The reasons are unknown.

B. Agents Under Development

1. Dispase (Vitreolysin™)

[See chapter VI.G. Dispase]

Dispase is being developed under the proprietary name Vitreolysin™ as a purified 35.9 kDa extracellular neutral protease produced by *Paenibacillus polymyxa*. It selectively cleaves the attachment of the posterior vitreous cortex to the inner limiting membrane (ILM) by degrading type IV collagen and fibronectin, which contribute to the attachment between these two structures [see chapter II.E. Vitreoretinal interface and the ILM]. The low affinity of Vitreolysin™ for type II collagen allows it to induce a posterior vitreous detachment (PVD) without disrupting the macromolecular structure of the vitreous body. This *direct* enzymatic action on the posterior vitreous cortex-ILM interface prevents vitreous liquefaction and resultant vitreoretinal traction which is a common way to induce PVD with many *indirectly* acting enzymes [6]. There are several other advantages of Vitreolysin™, such as lack of a known natural inhibitor, which insures sustained enzyme activity even in complex vitreoretinal diseases where several enzyme inhibitors circulating within the serum may leak into the eye. Vitreolysin™'s activity can be quenched by its own titratable autolysis that ensures fine control on its degradation and removal. Vitreolysin™ is a highly stable molecule with a long shelf life in lyophilized form. Early preclinical studies also revealed high rates (80–100 %) of PVD induction at picomolar concentrations.

Whereas early studies demonstrated retinal and optic nerve toxicity in animal models, these effects were attributable to bacterial contaminants. Other studies [15] employed commercially available dispase and found efficacy in young pigs with no evidence of toxicity. Synthesized using recombinant technology, Vitreolysin™ pharmacologic vitreolysis has not been associated with untoward effects [see chapter VI.G. Pharmacologic vitreolysis with dispase (vitreolysin™)]. To date, however, there have been no presentations or publications of human studies investigating the safety and efficacy of pharmacologic vitreolysis with Vitreolysin™.

C. Agents Approved for Pharmacologic Vitreolysis

1. Ocriplasmin [see chapter VI.E.1.

Pharmacologic vitreolysis
with ocriplasmin: basic science studies
and VI.E.2. Pharmacologic vitreolysis
with ocriplasmin: clinical studies]

Ocriplasmin (des-kringle 1–5 plasmin) is a truncated recombinant form of plasmin. Formerly called microplasmin, this recombinant 27 kDa serine protease contains the catalytic

domain of plasmin but none of the 5 kringles located as extensions towards the C-terminal end of the larger of its two chains [37]. These kringles normally serve as binding sites for substrates, regulatory molecules, and inhibitors [33, 37]. As a result, ocriplasmin has a more potent effect on thrombin than is observed with plasmin but binds to its primary inhibitor with less affinity. Ocriplasmin has a well-characterized enzyme activity profile that is stable for several days in an acidic environment. Highly active in neutral pH, inactivation follows second-order kinetics (autolysis), so that when Ocriplasmin is placed in a vitreous substrate, only 25 % of the activity remains at 24 h. However, as a member of the plasminogen family of proteins, ocriplasmin regulates a number of pathways including proinflammatory cytokines and matrix metalloproteinases that help to extend its mode of action and define its secondary clinical manifestations. For maximal effect, it requires injection close to the site of intended activity, which can also limit any adverse effect on adjacent structures such as zonules. Nonetheless, preclinical studies found efficacy without toxicity [see chapter VI.E.1. Pharmacologic Vitreolysis with Ocriplasmin: Basic Science Studies].

The efficacy and safety of ocriplasmin versus placebo for the treatment of vitreo-macular traction (VMT) in humans has been demonstrated in a clinical trial program that includes eight phase 2 studies, two phase 3 studies, and two phase 3b studies. The pivotal phase 3 Microplasmin Intravitreal Injection–Traction Release without Surgical Treatment (MIVI-TRUST) study demonstrated superiority of a single intravitreal injection of 125 µg ocriplasmin versus placebo in pharmacologic resolution of VMA and total induction of PVD. Pharmacologic closure of full-thickness macular hole (FTMH, ≤400 µm and with VMA) was achieved in a higher percentage of patients with FTMH at baseline in the ocriplasmin group compared with placebo and was highest among patients with small FTMH (≤250 µm). On this basis, ocriplasmin was approved by the US Food and Drug Administration for the treatment of symptomatic vitreo-macular adhesion (sVMA) in October 2012 and by the European Medicines Agency for the treatment of vitreo-macular traction (VMT), including when associated with macular hole less than or equal to 400 µm, in February 2013. The details of all study results are reviewed elsewhere in this book [see chapter VI.E.2. Pharmacologic vitreolysis with ocriplasmin: clinical studies]. The following is the story of one of the patients in this clinical research program.

a. Case Study

SC is a 62-year-old white woman who presented with the chief complaint of distortions and vision loss in the right eye of 3 months' duration. Visual acuity was 20/50+3. Three-dimensional threshold Amsler grid testing (3D-TAG; [5, 11, 19, 34]) demonstrated a cylindric central visual field defect with 1.16 % volume lost relative to the hill of vision

Computer Automated Amsler Grid Test for Perimetry

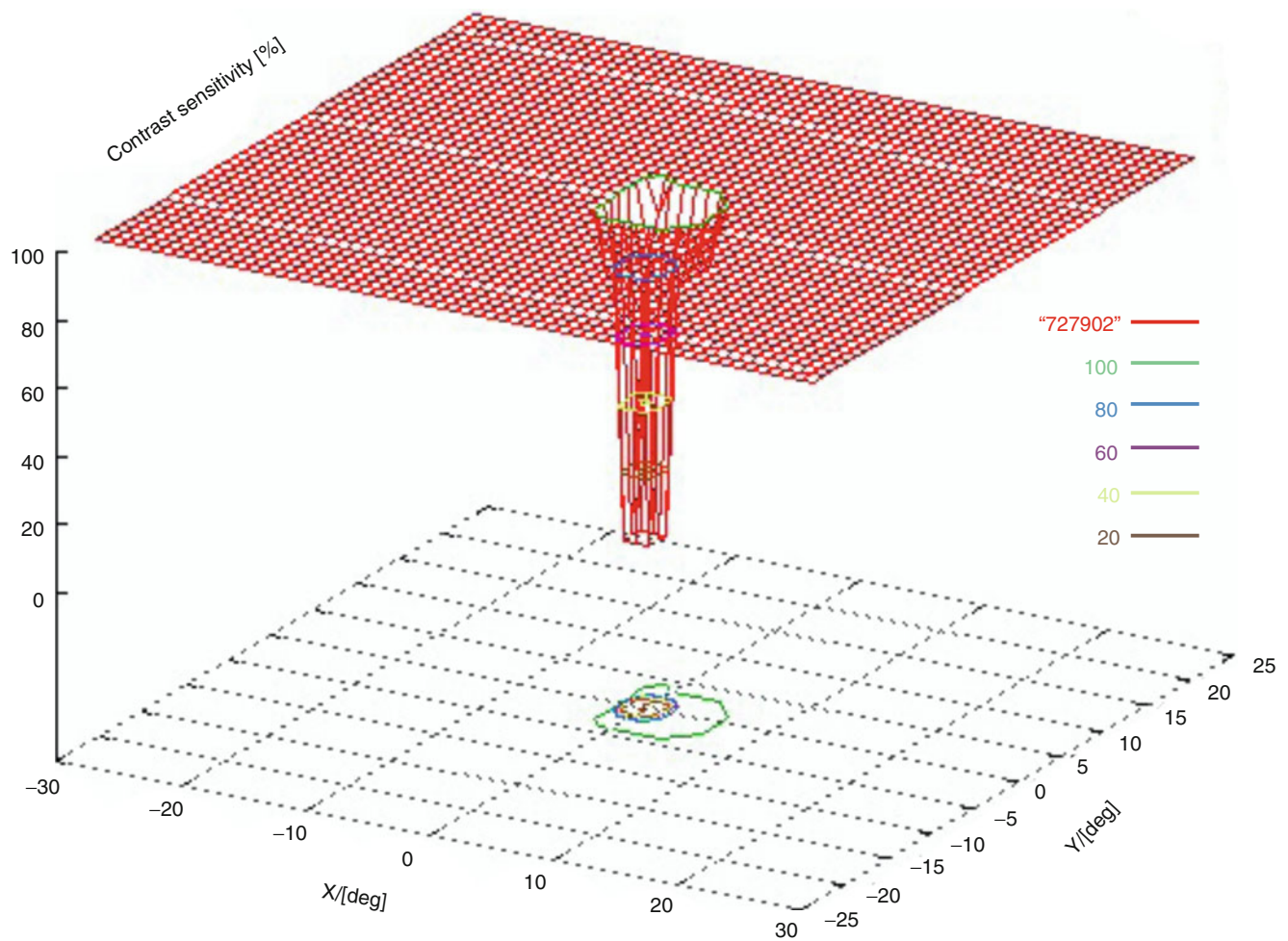


Figure VI.A-1 3D threshold Amsler Grid plot demonstrates the central visual field abnormality on presentation. The x- and y-axes display the area of distortion as drawn by the patient onto a touch screen computer monitor.

This was repeated five times at contrast levels ranging from 5 to 100 % contrast. The vertical z-axis stacks the five sets of test results, thus creating a 3-dimensional representation of the central visual field abnormality

(Figure VI.A-1). Combined optical coherence tomography/scanning laser ophthalmoscopy (OCT/SLO) demonstrated anomalous PVD with persistent adhesion of the posterior vitreous cortex to the fovea and an underlying macular cyst (Figure VI.A-2). This constituted focal, isolated vitreo-macular traction (VMT), according to the new IVTS classification system [3] and made the patient eligible for pharmacologic vitreolysis. The patient elected to consider enrolling in the phase II FDA trial of microplasmin pharmacologic vitreolysis (MIVI III) [see chapter VI.E.2. Clinical studies of ocriplasmin pharmacologic vitreolysis].

Six weeks later, she returned with decreased vision and increased distortions. Visual acuity measured 20/200 (previously 20/50). 3D-TAG detected a worsening of the central visual field abnormality, which increased from 1.16 % volume lost to 2.24 % volume lost (Figure VI.A-3). Combined OCT-SLO demonstrated a full-thickness macular hole (Figure VI.A-4), which on 3D OCT imaging clearly

demonstrated a membrane attached to both the edge of the elevated macular hole and the temporal margin of the optic disc (Figure VI.A-5), constituting vitreo-papillary adhesion (VPA), a finding present in 87.5 % of all eyes with macular holes [29, 35]. The linear distance of vitreo-macular adhesion (VMA) was 515 μm in the horizontal and 525 μm in the vertical axis. Central macular volume (central \equiv 1 mm Early Treatment Diabetic Retinopathy Study region) measured 0.32 ml (normal = 0.16 mL; [4]). The patient enrolled in the MIVI III study and received an injection of 125 μg of ocriplasmin (masked at the time of injection).

One week after injection, the distortions were much reduced and vision had subjectively improved. Visual acuity was 20/40 (pre-injection VA = 20/200). 3D-TAG demonstrated diminution of the central visual field abnormality with a reduction from 2.24 to 0.66 % volume lost (Figure VI.A-6). Combined OCT/SLO demonstrated PVD with closure of the macular hole and no evidence of cysts



Figure VI.A-2 Combined OCT/SLO imaging of the patient demonstrating vitreo-foveal adhesion with an underlying central cyst. By the IVTS classification, this constitutes focal, isolated vitreo-macular

traction (VMT) [see chapter III.D. Vitreo-Macular Adhesion/Traction & Macular Holes - Pseudo, Lamellar & Full-Thickness]

(Figure VI.A-7). There was, however, elevation of the fovea, which is seen following surgical closure of macular holes. During the ensuing 15 months, the foveal elevation slowly disappeared (Video VI.A-1), with complete restoration of normal foveal anatomy and attainment of VA=20/20 (Figure VI.A-8). At 6 months after injection, the macular volume was decreased to 0.21 mL (pre-injection=0.32 mL), and 3D-TAG measured an absolute percent volume lost of 0.65 %. At 15 months, macular volume improved to 0.15 uL, within the normal range. The patient is still very happy.

b. Case Selection

Retrospective analyses of the clinical trial data have resulted in a very interesting set of observations. Certain pre-injection factors were found to be associated with a higher success rate of inducing PVD and relieving VMT. If the extent of VMA was less than 1,500 μm , the success rate increased from 27.1 to 34.7 %. The aforementioned case has about 500 μm of VMA. The absence of a premacular membrane (PMM) with pucker (so-called ERM) was associated with a success rate of 37.4 %. The case study above had no PMM. If the patient had VMA <1,500 μm , had no PMM, and was phakic, the success rate increased to 44.7 %. The aforementioned patient was phakic. If in addition to these factors the patient were younger than 65 years of age, the success rate was an impressive 68.4 %. The case study was a 62-year-old woman.

The resolution of macular holes following a single injection of ocriplasmin was similarly associated with certain clin-

ical features. If the macular hole were smaller than 250 μm in diameter, the closure rate was 58 %. In similar fashion to the release of VMT, the closure rate for macular holes increased if the patient was younger than 65, had no PMM with pucker ("ERM"), and had VMA less than 1,500 μm in extent. In such cases, the closure rate was a striking 80 %. It is interesting to note that the case study described above meets these criteria, perhaps explained the very favorable response to pharmacologic vitreolysis with ocriplasmin.

IV. Future Considerations

A. Enhancing Drug Delivery

Preclinical studies with ocriplasmin demonstrated that for maximal drug effect, the drug should be injected close to the site of intended action [see chapter VI.E.1. Pharmacologic Vitreolysis with Ocriplasmin: Basic Science Studies]. Presently, all drugs are injected into the mid-vitreous without very much concern or control over the direction of drug delivery. Further, it is not known whether or not following injection, a drug could be directed posteriorly by having the patient lie supine for a period of time or whether iontophoresis forces could be employed to direct drug distribution posteriorly. Of course, the former maneuver would only be effective if the specific gravity of the drug solution is greater than vitreous. Special needles have been discussed, such as

Computer Automated Amsler Grid Test for Perimetry

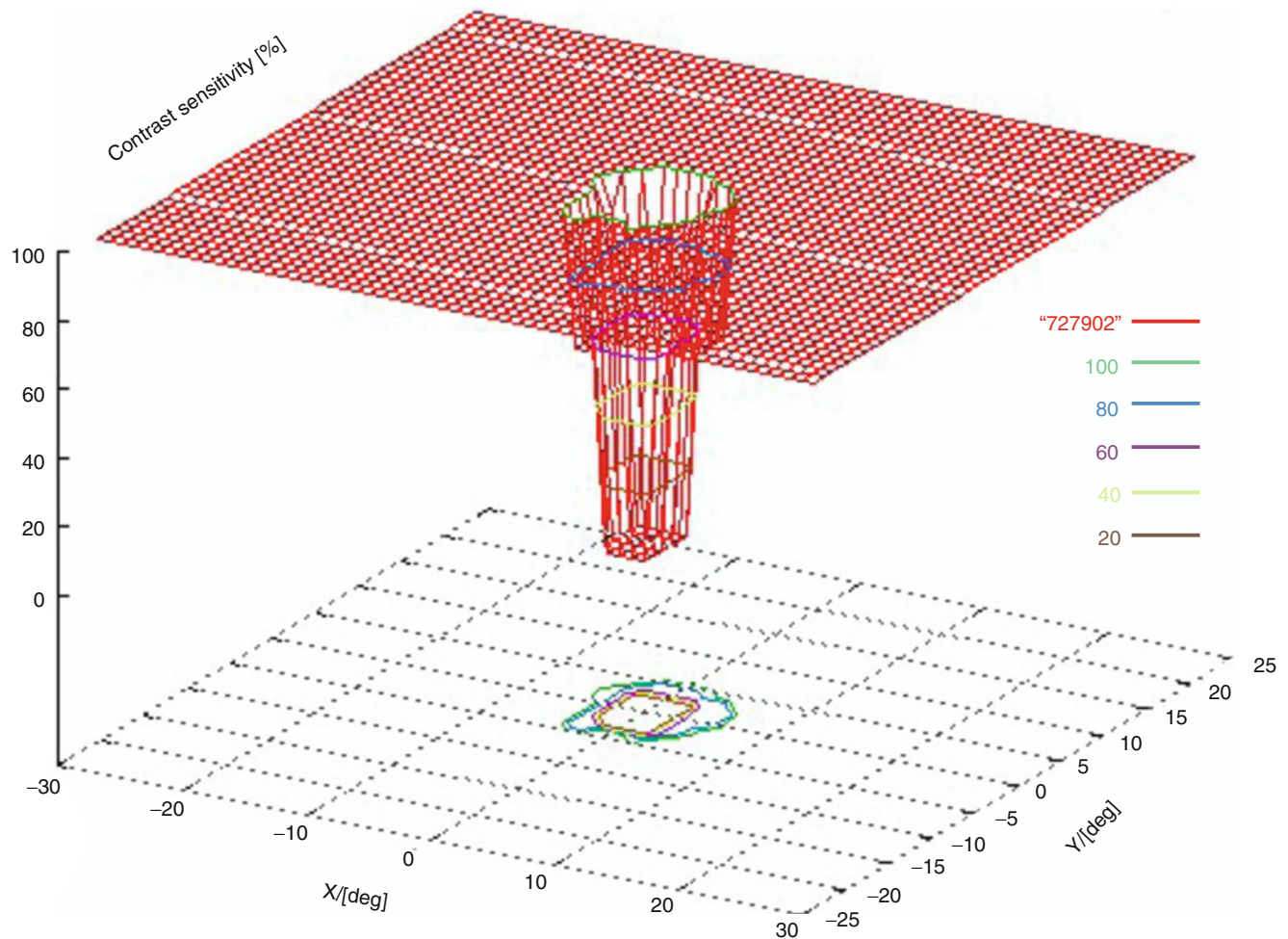


Figure VI.A-3 3D threshold Amsler Grid plot demonstrates the central visual field abnormality 6 weeks after presentation. Visual acuity was reduced to 20/200 and the central visual field abnormality worsened

from 1.16 % volume lost to 2.24 % volume lost, as a percent of the total hill of vision (Reprinted with permission from Tozer et al. [32])

the “quadraport” tip, but none has been adopted for routine use. Clearly, this is an area that would benefit greatly from enhanced intravitreal drug delivery systems [see chapter IV.E. Principles and Practice of Intravitreal Application of Drugs].

A potentially important aspect that has not been considered in this or any clinical setting of intravitreal drug therapy is vitreous structure. It is well known that vitreous structure is very different at different ages (Figure VI.A-9) and in different ocular conditions and disease states [see chapters II.C. Vitreous Aging and Posterior Vitreous Detachment; II.B. Myopic Vitreopathy; I.E. Diabetic Vitreopathy]. These and other influences create topographic heterogeneity in vitreous structure that could influence drug distribution and delivery to the target following intravitreal injection. In the elderly, this is particularly relevant since there can be profound variability of vitreous structure from one part of the vitreous body to another. It is plausible, if not probable, that drug distribution would be very different if an injection were placed

into a lacuna of liquid vitreous as opposed to an injection into the adjacent gel vitreous (Figure VI.A-10). There is currently no way to control for this variable. Thus, future approaches should strive to enhance drug delivery for pharmacologic vitreolysis as well as other forms of intravitreal pharmacotherapy by achieving therapeutic drug levels at the desired site of action taking into consideration the particular biochemical and structural attributes of the eye being treated. This type of individualized therapeutic approach, however, will require enhanced ways to evaluate the biochemical composition and structure of vitreous and adjacent tissues, ideally on a molecular level.

Indeed, one of the most significant limitations facing ophthalmology today is the lack of adequate diagnostic approaches to evaluate both the structure and function of the eye on more fundamental levels. While OCT imaging of the posterior pole has certainly been a very useful diagnostic tool from a structural standpoint (Videos VI.A-2 and



Figure VI.A-4 Combined OCT/SLO imaging of the patient 6 weeks after presentation. The macular cyst is now a full-thickness macular hole, classified as primary (initiated by VMT), small ($<250\mu\text{m}$)

full-thickness macular hole [see chapter III.D. Vitreo-Macular Adhesion/Traction & Macular Holes – Pseudo, Lamellar & Full-Thickness] [3]

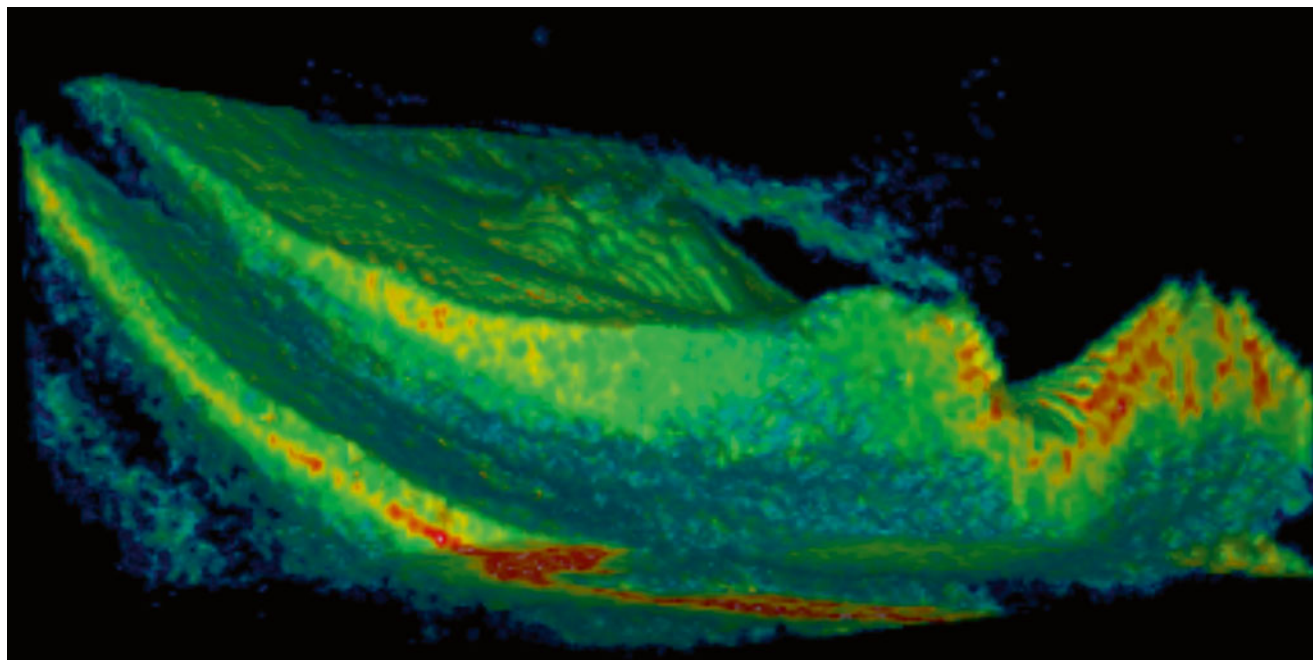


Figure VI.A-5 3D Combined OCT/SLO imaging demonstrates the “erupted volcano” appearance of the macular hole in this patient with the central macula being lifted anteriorly and retracted nasally by a

vitreous membrane (posterior vitreous cortex) attached to the temporal margin of the optic disc

Computer Automated Amsler Grid Test for Perimetry

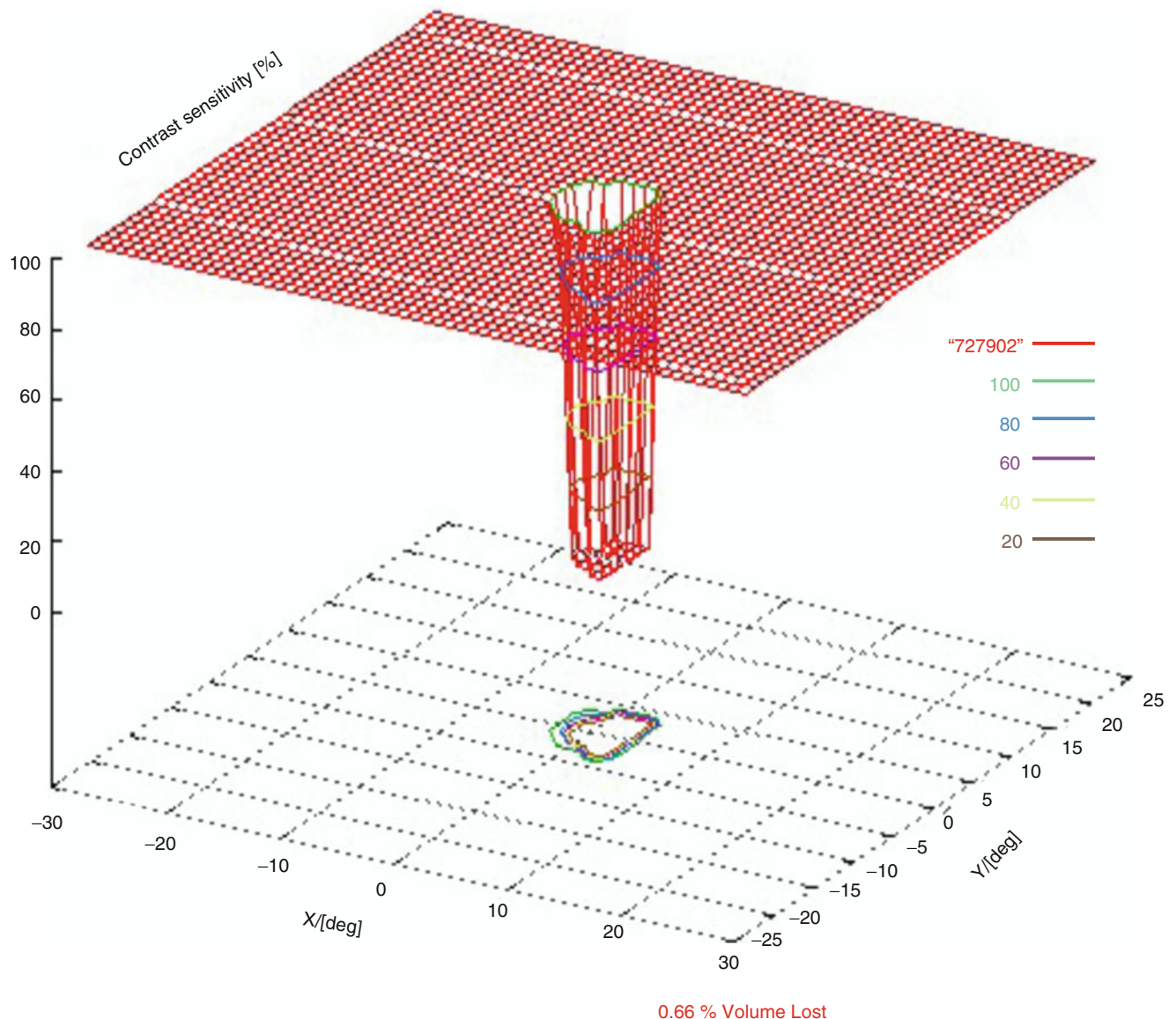


Figure VI.A-6 3D threshold Amsler Grid testing demonstrating a marked reduction in the area of the central visual field abnormality at all contrast levels 1 week after pharmacologic vitreolysis. Quantitatively,

the degree of abnormality decreased from 2.24 % (pre-injection) to 0.66 % volume lost (Reprinted with permission from Tozer [32])

VI.A-3), it does not represent a paradigm shift, which is vital to progress. The fundamental reason for our lack of powerful and innovative diagnostic approaches is surprisingly the ophthalmoscope, the very instrument that spawned our field. When introduced by Helmholtz in 1857 (based on earlier designs by Purkinje), the ophthalmoscope revolutionized ophthalmology. While imaging technologies have since advanced, our concept of disease has not substantially changed for more than a century. We are still “laden” with the notion that until structural abnormalities are detected, there is no disease. Thus, for example, we await the appearance of visible abnormalities such as retinal microaneurysms and microhemorrhages before diagnosing diabetic

retinopathy. Although the disease actually begins long before the development of such structural changes, these early changes are not diagnosed by today’s imaging technologies. What is needed, therefore, is a new generation of diagnostic technologies that evaluate the eye on a molecular level—nanotechnologies, for lack of a better term. These new approaches are already in development [see chapters IV.A. Vitreous Physiology; II.F. To See the Invisible - The Quest of Imaging Vitreous] and will have great impacts on our understanding of disease pathogenesis, as well as our ability to diagnose abnormalities early in the natural history of disease when there is a greater likelihood of reversibility and more potential for preventing further progression.



Figure VI.A-7 Combined OCT/SLO imaging of the patient 1 week after pharmacologic vitreolysis demonstrating release of vitreo-macular traction and detachment of the posterior vitreous cortex away from the retina.

There is closure of the macular hole with elevation of the foveal region, an appearance that is very similar to what is often seen following surgical closure of macular hole (Reprinted with permission from Sebag [26])

B. Improving Vision Outcome Measures

As a corollary to the aforementioned needs for enhanced imaging on a molecular level and for methodologies to assess ocular physiology in health rather than just pathology in disease, there is a similar need for improved measures of visual function. This will not only enable earlier diagnosis in some cases but also provide improved outcome measures of therapy. At the present time, there is great emphasis on visual acuity. However, this is only one aspect of visual function. Contrast sensitivity, another very important component of the phenomenon of vision, has recently been shown to be severely reduced (on average by over 60 %) in many patients with floaters [see chapter V.B.8 Floaters and Vision – Current Concepts and Treatment Paradigms]. Remarkably, this normalizes in every single case (now a series of over 50 patients) within a week of minimally invasive vitrectomy. Recent studies [14] have quantified similar reduction of contrast sensitivity in patients with macular pucker, which also normalized after vitrectomy with membrane peeling.

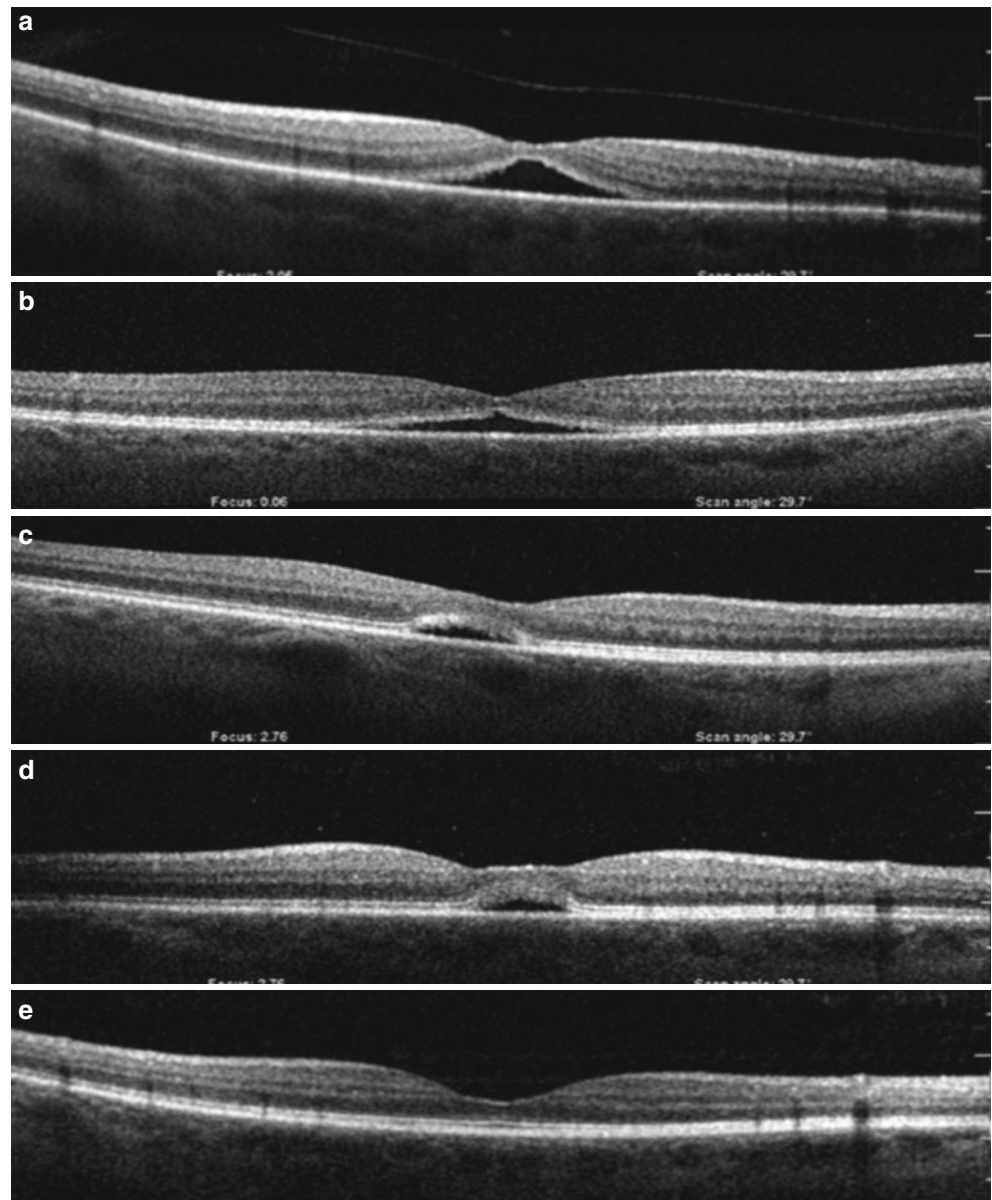
Metamorphopsia is a significant complaint of patients with vitreo-maculopathy [see chapters III.B. Anomalous PVD and Vitreoschisis; III.C. Pathology of Vitreomaculopathies; III.F. Vitreous in the Pathobiology of Macular Pucker; V.A.2. Vitreomaculopathy Surgery]. Nevertheless, we do not routinely quantify this aspect of vision. The case study described above features the use of Three-dimensional Threshold

Amsler Grid testing to quantitate central visual dysfunction induced by anomalous PVD with vitreo-macular traction (VMT) and macular hole. This prototype diagnostic technology was in fact able to quantitate worsening of central vision during disease progression and improvement following pharmacologic vitreolysis. This type of diagnostic assessment would be very useful as a quantitative measure of vision to supplement visual acuity in two regards: it could help determine the degree of disease severity [11] and thus be useful for case selection. As described above, it could also find great utility as an outcome measure of the efficacy of pharmacologic vitreolysis as well as other therapies of eye disease. Lastly, it could help new drug development.

C. Combination Therapy

The theoretical superiority of combination therapy over monotherapy has previously been postulated for pharmacologic vitreolysis [24]. Initially, combination pharmacologic vitreolysis involved the use of intravitreal drug and gas injection. While hyaluronidase alone was unable to induce PVD in rabbits [8], the addition of perfluoropropane gas injection was able to induce PVD [12]. A similar phenomenon was observed in rabbits where the effect of plasmin was augmented by sulfur hexafluoride gas [9]. Thereafter, combination therapy involved the use of two different drugs.

Figure VI.A-8 Time course of normalization in foveal anatomy following successful closure of macular hole with pharmacologic vitreolysis. Closure of the macular hole after pharmacologic vitreolysis was followed by persistent elevation of the central macula with gradual resolution over the ensuing 15 months. There was ultimately complete restoration of normal macular anatomy with visual acuity of 20/20 and normal 3D threshold Amsler Grid test results. (a) one week, (b) one month, (c) six months, (d) nine months, (e) fifteen months post-injection



Studies on diabetic rats found that neither hyaluronidase nor plasmin alone achieved effective pharmacologic vitreolysis, yet the combination of the two agents induced a total PVD in 8/10 (80 %) eyes. The fact that other studies were able to induce PVD with plasmin alone yet this one did not might relate to the fact that this study employed diabetic animals whose vitreous is very likely very different from nondiabetic animal models [see chapter I.E. Diabetic vitreopathy]. A plausible explanation for why the combination of hyaluronidase and plasmin yielded superior results to either agent alone could be that the liquefactant hyaluronidase induced gel liquefaction while the interfactant properties of plasmin induced sufficient dehiscence at the vitreoretinal interface to induce total PVD in a much higher percentage of cases.

Future clinical development should consider combination therapy as a means of increasing the efficacy of pharmacologic vitreolysis. It is, in fact, unlikely that a single agent will meet the needs of all patients at all ages with different systemic conditions and a variety of ocular diseases at various stages of severity. Rather, the future will most likely see the use of a mixture of agents whose selection will be based upon the results of basic and clinical experimentation to determine which combination will have the highest likelihood of inducing a salubrious PVD in different clinical settings. It is furthermore likely that the concentrations of the various ingredients will be varied depending upon various clinical criteria. What seems quite important, however, is that vitreoretinal dehiscence should

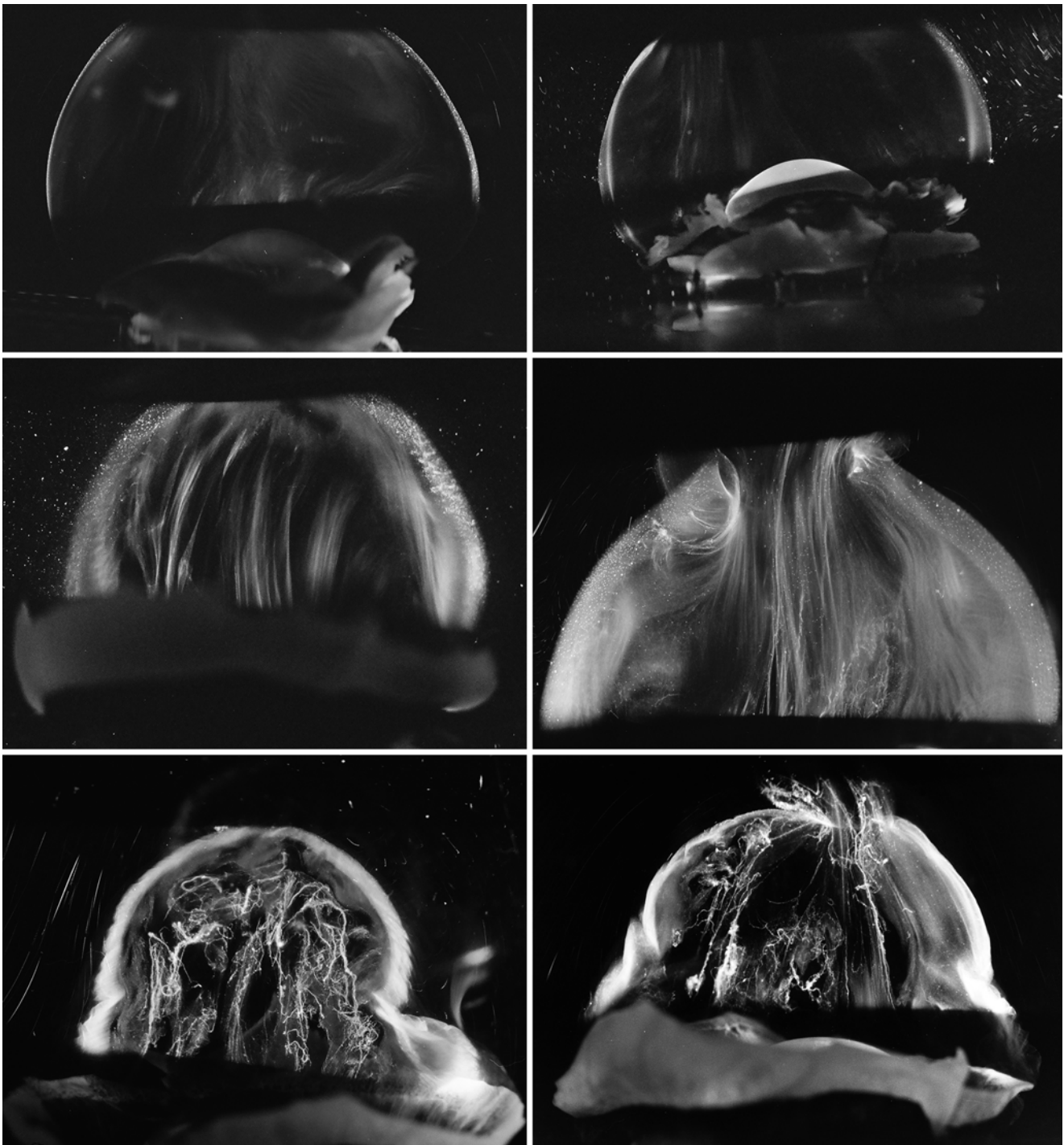


Figure VI.A-9 Age-related differences in human vitreous structure. Dark-field slit microscopy of fresh unfixed whole human vitreous with the sclera, choroid and retina dissected off the vitreous body, which remains attached to the anterior segment. A slit lamp beam illuminates from the side, creating a horizontal optical section with an illumination-observation angle of 90° , maximizing the Tyndall effect. The anterior segment is below and the posterior pole is above in all specimens. *Top row:* The vitreous bodies of an 11-year-old girl (*left*) and a 14-year-old boy (*right*) demonstrate a homogeneous structure with no significant light scattering within the vitreous body, only at the periphery where the vitreous cortex is comprised of a dense matrix of collagen fibrils.

The posterior aspect of the lens is visible at the bottom of each image. *Middle row:* Vitreous structure in a 56-year-old (*left*) and a 59-year-old (*right*) subject features macroscopic fibers in the central vitreous body with an anteroposterior orientation. These form when hyaluronan molecules no longer separate collagen fibrils, allowing cross-linking and aggregation of collagen fibrils into visible fibers. *Bottom row:* In old age, the fibers of the central vitreous become significantly thickened and tortuous, as demonstrated in the two eyes of an 88-year-old woman. Adjacent to these large fibers are areas of liquid vitreous, at times forming pockets, called lacunae

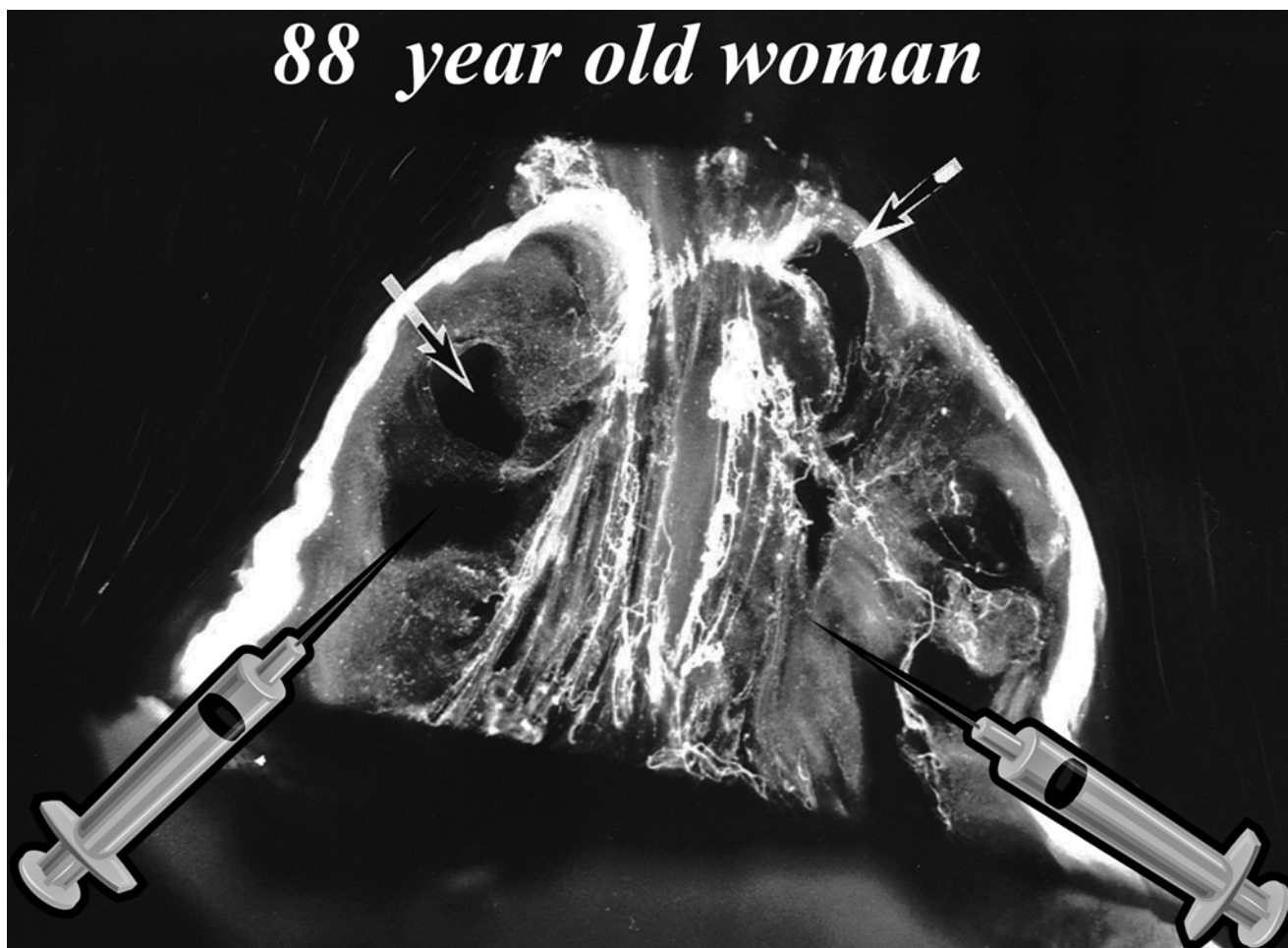


Figure VI.A-10 Heterogeneous vitreous structure in old age. Intravitreal injection of any drug could have variable drug distribution and thus efficacy, depending upon whether the drug is injected into a lacuna of liquefied vitreous or into an area of gel vitreous. The syringe on the lower left is injecting into a lacuna (see arrows at the top indicating other lacunae)

of liquid vitreous. The syringe on the lower right is injecting into gel vitreous. The pharmacokinetics and pharmacodynamics will likely be very different for any drug injected into such very different areas within the vitreous body. Such heterogeneity of vitreous structure is significant in older individuals and less prominent in youth

be induced before gel liquefaction to avoid causing an iatrogenic anomalous PVD [see chapter III.B. Anomalous PVD and Vitreoschisis].

D. Prevention

The foregoing has presented considerable information regarding the use of pharmacologic vitreolysis to treat existing diseases. The true promise of this approach, however, lies with implementation in patients at risk of anomalous PVD who would likely benefit greatly from prophylactic pharmacologic vitreolysis. Alternatively, the future may see the development of ways to inhibit the molecular changes that underlie anomalous PVD and prevent this fundamental cause of various vitreoretinopathies.

1. Prophylactic Posterior Vitreous Detachment

As has been extensively discussed in this book, vitreous plays an important role in a variety of retinal diseases. Thus, while the foregoing has focused on the use of pharmacologic vitreolysis to treat existing vitreoretinal disorders, it is reasonable to consider the induction of PVD as prophylaxis in situations that are known to be high risk. Fellow eyes of rhegmatogenous retinal detachments (especially from giant tears), macular holes, and perhaps macular pucker may be good candidates for prophylactic PVD induction. Two clinical conditions would seem very amenable to this approach—diabetic retinopathy and age-related macular degeneration.

Diabetes has significant biochemical, structural, and physiologic effects upon vitreous [see chapter I.E. Diabetic Vitreopathy]. Vitreous plays a particularly important role in

proliferative diabetic retinopathy as well as in diabetic macular edema (DME). [see chapter III.K. Vitreous in Retinovascular Diseases and Diabetic Macular Edema]. As many studies have shown that PVD is associated with a much better prognosis in diabetic retinopathy, it would seem advantageous to prevent advanced stages of disease and vision loss by inducing a prophylactic PVD. To this end, ocriplasmin is being tested in a multicenter, randomized, double-masked, sham-controlled, ascending-dose trial. This study of 60 patients with vitreo-macular adhesion (VMA) and concomitant advanced DME is complete, but data are not yet published.

Vitreous may also play an important role in the pathogenesis of exudative age-related macular degeneration [see chapter III.G. Vitreous in AMD]. Thus, a clinical trial was undertaken to test the hypothesis that ocriplasmin pharmacologic vitreolysis can induce an innocuous PVD and that this will have salubrious effects on the course of the wet AMD. This phase 2 study was a multicenter, randomized, double-masked, sham-controlled trial of 100 patients with VMA and concomitant exudative AMD who were randomized 3:1 to receive a single intravitreal injection of 125 μ g ocriplasmin or sham treatment. Eyes that had previously undergone vitrectomy or received more than 9 injections of bevacizumab and/or ranibizumab were excluded. The primary end point was pharmacologic resolution of VMA at day 28 after injection. Selected secondary end points included resolution of VMA at all other study visits, PVD status, macular thickness, area of vascular leakage, change in visual acuity (ETDRS letters) from baseline, requirement for additional therapy, and number of bevacizumab and/or ranibizumab injections required over the course of study. The study is complete, but data are not yet published.

2. Anomalous PVD Prevention

With further evolution in our understanding of the mechanisms that underlie vitreous liquefaction and dehiscence at the vitreoretinal interface, new treatment paradigms may be developed to prevent these changes and thus prevent anomalous PVD [see chapter III.B. Anomalous PVD and Vitreoschisis]. Such an approach might not only obviate the untoward effects of anomalous PVD on the retina, optic disc, and macula but also promote healthier intraocular physiology, since age-related macular degeneration and diabetic retinopathy are known to be aggravated by unhealthy vitreous physiology [see chapter IV.A. Vitreous Physiology]. Given that cataracts are at least in part related to dysfunction of intravitreal oxygen physiology, it may also be determined that treatments to prevent vitreous degeneration and promote its various physiologic functions will mitigate against cataract formation [see chapter IV.B. Oxygen in Vitreoretinal Physiology and Pathology].

Abbreviations

AMD	Age-related macular degeneration
APVD	Anomalous posterior vitreous detachment
DME	Diabetic macular edema
ECM	Extracellular matrix
ERG	Electroretinography
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	US Food & Drug Administration
FTMH	Full-thickness macular hole
GAG	Glycosaminoglycans
HA	Hyaluronan
Hase	Hyaluronidase
HeLa	Henrietta Lack's immortal cell line
ILM	Inner limiting membrane
kDa	Kilodalton
MIVI-TRUST	Microplasmin Intravitreal Injection–Traction Release without Surgical Treatment
mL	Milliliter
NPDR	Nonproliferative diabetic retinopathy
OCT/SLO	Combined optical coherence tomography/scanning laser ophthalmoscopy
PMM	Premacular membrane
PVD	Posterior vitreous detachment
PVR	Proliferative vitreoretinopathy
RGD	The amino acid sequence arginine-glycine-aspartate (RGD)
sVMA	Symptomatic vitreo-macular adhesion
tPA	Tissue plasminogen activator
TUNEL	Terminal deoxynucleotidyl transferase nick end labeling
μ L	Microliter
μ m	Micrometer
VA	Visual acuity
VMA	Vitreo-macular adhesion
VMT	Vitreo-macular traction
VPA	Vitreo-papillary adhesion
3D-TAG	Three-dimensional threshold Amsler Grid test

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