

Video Article

Whole Vitreous Humor Dissection for Vitreodynamic Analysis

Karthik Murali¹, Amir H. Kashani², Mark S. Humayun¹

¹Biomedical Engineering, University of Southern California

²Ophthalmology, University of Southern California

Correspondence to: Amir H. Kashani at ahkashan@med.usc.edu

URL: <https://www.jove.com/video/52759>

DOI: [doi:10.3791/52759](https://doi.org/10.3791/52759)

Keywords: Medicine, Issue 99, Vitreous Humor, Dissection, Vitreous core, Vitreous cortex, Medicine, Eye, vitreodynamics, drug delivery, diffusion

Date Published: 5/24/2015

Citation: Murali, K., Kashani, A.H., Humayun, M.S. Whole Vitreous Humor Dissection for Vitreodynamic Analysis. *J. Vis. Exp.* (99), e52759, doi:10.3791/52759 (2015).

Abstract

The authors propose an effective technique to isolate whole, intact vitreous core and cortex from post mortem enucleated porcine eyes. While previous studies have shown the results of such dissections, the detailed steps have not been described, precluding researchers outside the field from replicating their methods. Other studies harvest vitreous either through aspiration, which does not maintain the vitreous structure anatomy, or through partial dissection, which only isolates the vitreous core. The proposed method isolates the whole vitreous body, with the vitreous core and cortex intact, while maintaining vitreous anatomy and structural integrity. In this method, a full thickness scleral flap in an enucleated porcine eye is first created and through this, the choroid tissue can be separated from the sclera. The scleral flap is then expanded and the choroid is completely separated from the sclera. Finally the choroid-retina tissue is peeled off the vitreous to leave an isolated intact vitreous body. The proposed vitreous dissection technique can be used to study physical properties of the vitreous humor. In particular, this method has significance for experimental studies involving drug delivery, vitreo-retinal oxygen transport, and intraocular convection.

Video Link

The video component of this article can be found at <https://www.jove.com/video/52759/>

Introduction

The goal of this method is to detail a technique to isolate a whole, intact vitreous body, with the vitreous core and cortex intact, from a cadaver eye, for the purposes of vitreodynamic analysis. As the field of vitreous physiology has grown, multi-disciplinary researchers, such as fluid mechanics researchers, are investigating the physical and biomechanical properties of the vitreous¹. To this end, it is essential to detail a technique to isolate the whole, intact vitreous body to aid multi-disciplinary researchers.

Sebag *et al.*² and others³ performed elegant whole vitreous dissections on human cadaver eyes and showed illustrations of the results. However, the technique used was not described in detail and non-experts would not be able to replicate the method independently. Other studies have harvested vitreous from cadaver eyes using simpler methods such as aspiration or partial dissection, both of which do not result in a whole, intact vitreous body. Gisladdottis *et al.*⁴ and Xu *et al.*⁵ investigate permeability in vitreous humor harvested from cadaver eyes. However, since no method of vitreous extraction was described, it was assumed that they aspirated the vitreous humor with a syringe. Watts *et al.*⁶ went one step further by describing a method of isolating rabbit vitreous humor with a surgical technique. However, this method results in an isolation of just the vitreous core and not the vitreous cortex. Skeie *et al.*⁷ later organized the vitreous into 4 unique regions and elegantly described a method to dissect out each part for analysis. This technique however, does not result in an intact vitreous as a whole.

The current technique was developed to facilitate biophysical experiments that are currently only performed in cadaver eyes. Previous methods, as described above, are limited because 1) none completely isolate the whole vitreous body, 2) harvested vitreous core and cortex are homogenized, 3) vitreous anatomical structure is not maintained, or 4) dissection techniques are not adequately detailed for replication by researchers in other fields. In addition, due to the opacity of sclera and choroid, visualization of the vitreous body is limited in the intact eyeball. This limits the precision and feasibility of measurements that can be made inside the whole eye. In addition, the anatomical structures surrounding the vitreous can confound the study of biochemical and physical properties of the vitreous.

In recent years, the body of vitreous science has grown tremendously and there is reason to believe that the whole vitreous body has different properties than its individual parts. There is growing interest in investigating the physical, biomechanical, and chemical properties of the vitreous for vitreodynamics research, which has applications in clinical medicine such as drug delivery, intravitreal oxygenation⁸ and vitrectomy. Pharmacological vitreodynamics, which uses pharmacologic agents to manipulate the vitreous, can be used to improve vitrectomy outcomes⁹. Biomechanical properties are used to model vitreous fluid flow, which can be used to improve intravitreal drug delivery technologies^{10–12}. Physical properties of various segments of the vitreous are crucial to understanding vitreo-retinal oxygen transport¹³. The proposed vitreous dissection technique can be used to study various properties of the intact vitreous humor. It enables bench-top experiments to be done on whole, intact vitreous bodies with better visualization.

In summary, current methods for study of the vitreous are either not adequately described, or result in an incomplete isolation the vitreous core and cortex. Therefore, there is a need to perform experiments in a transparent eye model while retaining the anatomy of the vitreous that exists in the cadaver eye.

Protocol

All enucleated eyes were obtained from an abattoir and all experiments were performed in accordance with institutional biosafety laws.

1. Secure enucleated eye down on a surface. Do this by placing tissue pins through the excess tissue around the eye and securing it down into a Styrofoam board.
2. Dissect and detach perilimbal conjunctiva from eye.
 1. Use fine forceps (0.3 forceps) and microscissors (Westcott scissors) to incise the conjunctiva at the limbus and bluntly dissect it off the sclera. Cut the conjunctiva along the limbus as the blunt dissection proceeds to allow further dissection.
 2. Remove conjunctiva all the way around the eye (360 degrees) to expose as much sclera as possible.
NOTE: It is helpful to leave a small amount of conjunctiva to facilitate fixating the eye with the surgical pins or a forceps during the rest of the procedure.
3. Create a full thickness scleral flap along one side of the eye (scalpel blade 69).
 1. Make a ~5 mm scleral incision parallel to the limbus and 3 mm posterior to the limbus by gently cutting into the sclera with a scalpel (scalpel blade 11) until darkly pigmented choroid is visible. Be careful not to incise the choroid itself.
 2. Carefully dissect along the plane between the sclera and choroid, either with scissors (using blunt dissection) or with the scalpel, until it is possible to enlarge the potential space between these tissues by pushing the choroid gently inward.
 3. Make another scleral incision perpendicular to the first scleral incision with sharp microscissors, creating a T-shaped incision.
 4. Then continue to bluntly dissect in a circumferential fashion to remove the scleral tissue from the underlying choroid and gently push the choroid away from the sclera as mentioned above.
 5. Enlarge the scleral flap as needed.
 1. Gently blunt dissect the choroid away from the sclera throughout the process.
 2. Enlarge the flap until the incision made perpendicular to the limbus reaches the optic nerve and the incision made parallel to the limbus is at least one-third of the eye circumference (45°).
 3. Use the scleral flap as a source of leverage for manipulating the eye during blunt dissection. The counter-traction on the flap makes it easier for blunt dissection.
6. Repeat the previous step on the other scleral flap.
4. Cut away the scleral flaps to expose a large area of choroid.
5. Continue the incision made in step 3 around the eye's circumference (360 degrees).
6. Remove the remaining choroid-retina tissue.
 1. Within the exposed region, use a cotton-tip to gently brush off the choroid-retina tissue remaining. Alternatively, gently grab the tissue with forceps and peel it off the underlying vitreous body.
 2. If necessary make an incision into the choroid starting from the optic nerve. Then peel away the choroid gently to expose the retina and vitreous cortex.
7. Continue blunt dissecting the choroid away from the sclera and then peel away the choroid to obtain the whole, intact vitreous.
8. Use the scleral rim attached to the cornea to position the whole vitreous in desired location. At this point, the vitreous is attached to the anterior sclera and lens.
9. Blunt dissect the vitreous from the inside of the sclera, around the lens, removing all sclera.
10. Use a blunt tool to scoop the lens away from the vitreous if need be. Use the sample for various vitreodynamic experiments.
 1. Place sample in a glass beaker with known surface area exposed to air. Place oxygen sensitive probe at the edge of the sample. Use a micro-manipulator to move the probe to a known distance (r) into the sample.
 2. Use Fick's laws of diffusion to obtain the theoretical diffusion equation. With the data collected in step 10.1, obtain the experimental diffusion coefficient/reaction term *etc.*

Representative Results

Following the protocol will lead to a successful vitreous dissection with the core and cortex (**Figure 3**) intact. This is evident from the residual pieces of retina adhered to the vitreous cortex. Intact whole vitreous humor can be used in several ways for specific vitreodynamic experiments. In our case, the diffusion rate of oxygen in intact vitreous humor and its corresponding time constant was studied (**Figure 2**). Vitreous that was dissected (core and cortex) using our method was placed in a glass beaker with a known exposed surface area. This was compared to vitreous that was harvested using a previously published method that only isolates vitreous core⁶. All other factors were kept consistent across the experimental and control groups. The vitreous surface was exposed to air, which has a known oxygen tension (~160 mmHg). The oxygen tension within the vitreous was low (<10 mmHg). Based on the rate of vitreous oxygen consumption determined by Shui *et al.*¹⁴ for vitreous in the gel state, we can use the equation for 1 dimensional oxygen transport (in steady or unsteady state forms). By measuring the oxygen tension a known distance within the vitreous surface, we can experimentally validate the diffusion coefficient. The oxygen tension was recorded with an oxygen probe such as Oxford, and the error rate was $\pm 10\%$. A sample was collected every 30 sec. The presence of vitreous cortex affects the diffusion rate of oxygen into the mid- vitreous. In the presence of vitreous cortex, the diffusion of oxygen occurs over a longer time interval.

The physical properties of the vitreous core and cortex have an impact in health and disease. For example, localized, supplemental intraocular oxygenation has been proposed as a treatment for retinal ischemia⁸. Understanding the different diffusion coefficients for oxygen through vitreous cortex core will enable researchers to predict the actual rate of oxygen delivery to the retina.

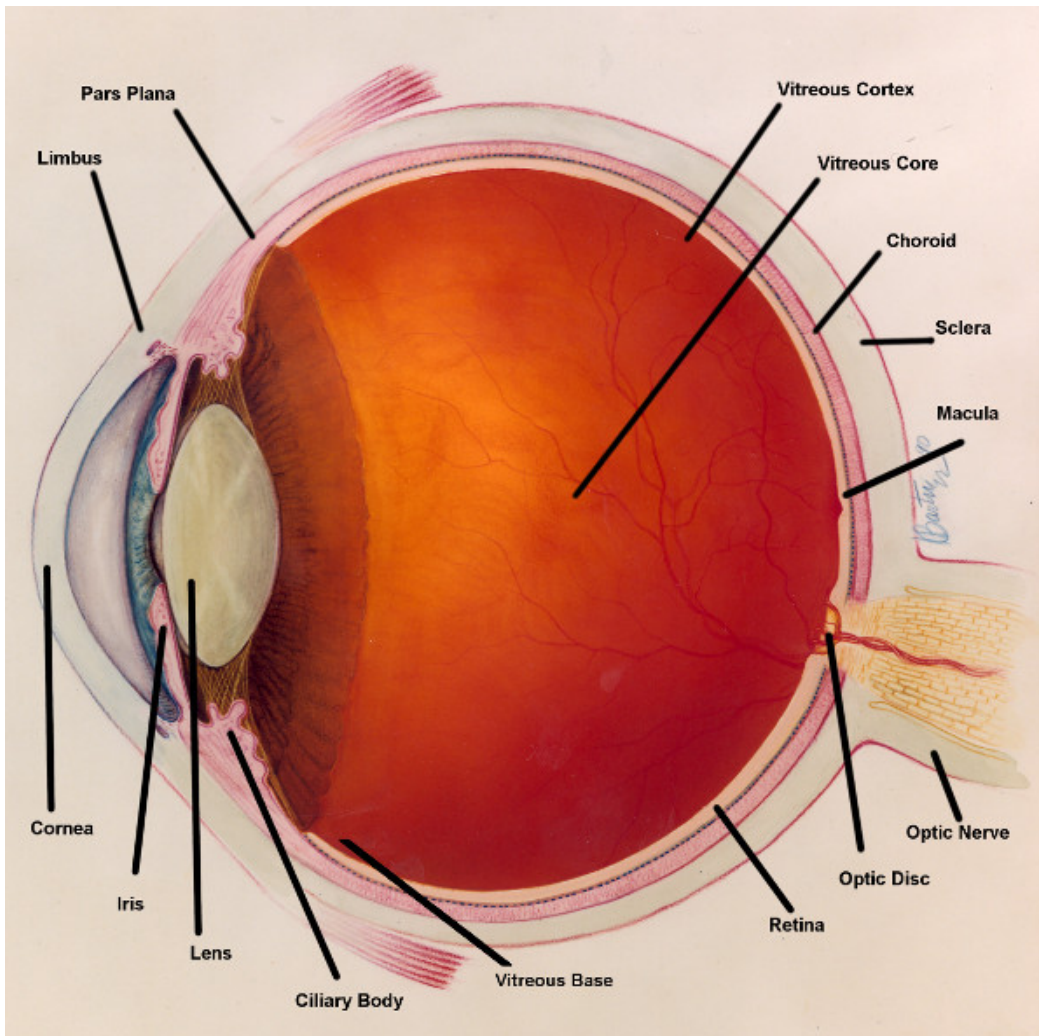


Figure 1: Cross sectional view of the eye displaying relevant anatomical structures (modified from National Eye Institute, National Institutes of Health). The cornea is the transparent tissue that forms the anterior most section of the eye and provides a significant portion of the eye's optical power. The limbus is the junction of the cornea, conjunctiva and sclera. The sclera extends from the limbus to the posterior most aspect of the eye where it meets the optic nerve. The conjunctiva is a thin epithelium that lines the very outside of the eye and forms the boundary between the external environment and the sclera. The internal components of the eye consist of the anterior chamber, lens, iris, ciliary body, posterior chamber, posterior cavity, retina and choroid. The vitreous core comprises the central region of the vitreous body, within the posterior cavity of the eye. The vitreous cortex is located around the vitreous body and is attached to the retina at several points including the pars plana, retinal vessels, optic disc and macula. The vitreous base is a three dimensional zone which extends from ~2 mm anterior to the ora serrata to 3 mm posterior to the ora serrata. The choroid is a vascular tissue layer that is located between the sclera and retina and provides blood supply to the outer retina. The retina is the neurosensory layer of the eye that subserves the light sending capacity of the eye. The sclera is the white opaque layer that provides bulk of the structural support to the eye wall.

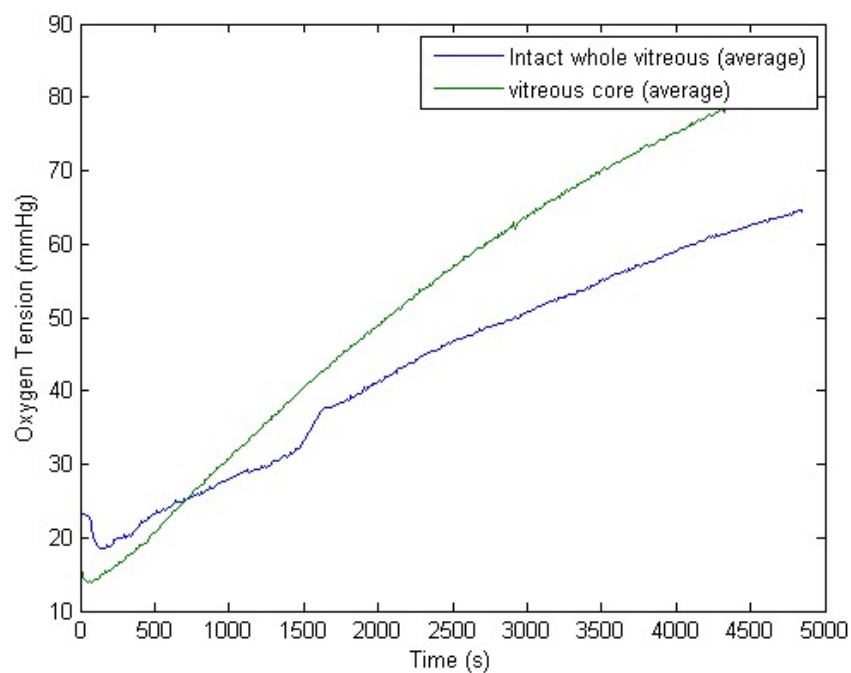


Figure 2: Plot of oxygen tension of vitreous humor against time. Plot of oxygen tension in vitreous core (green line) compared to intact whole vitreous (blue line). An oxygen sensing probe was placed in the middle of the sample and the sample was exposed to air which has an oxygen tension of 160 mmHg.



Figure 3: Examples of isolated whole vitreous body. Top picture is an example of the whole intact vitreous body still attached to the scleral rim. Middle picture is an example of the whole intact vitreous body without sclera but with some remaining retinal tissue. Bottom is an example of whole, intact vitreous body.

Discussion

There are two critical steps that must be carefully performed during vitreous dissection. Step 3, creating a full thickness scleral flap, is crucial to the entire dissection. Care should be taken to not cut into the choroid when creating the full thickness scleral flap. The other critical step is dissecting away the sclera from the choroid. This step must be carefully done to prevent creating multiple holes in the choroid from which the vitreous can spill out. There is a way to modify the protocol and still dissect intact whole vitreous humor. Step 6 can be delayed until step 8. The choroid can be removed at the end after the vitreous tissue is positioned in the desired location.

The limitation of this procedure is that during dissection, there are only visual cues to suggest the success and accuracy of the intact vitreous dissection. Since the vitreous core and cortex are both transparent and undistinguishable to the naked eye, this can be challenging. By noticing the adhesion of the retina onto the vitreous it is possible to determine if successful dissection of the vitreous cortex has occurred. Otherwise, pharmacological substances, such as Kenalog, can be used to improve visualization¹⁵ of the vitreous but cannot readily allow one to distinguish between vitreous cortex and vitreous core. Another limitation of this technique is that it is most effective for the dissection of gel vitreous. Human vitreous gel is a non-regenerative tissue that undergoes liquefaction with age. This liquefaction prevents us from completely dissecting the whole, intact vitreous from the eye. Thus, our technique extends only to vitreous of animals, such as cows, pigs or rabbits, or eyes of young humans who do not have significant amounts of liquefaction. The vitreous humor in these animals tends to be completely in a gel state.

However, currently, there is no established method, that has been described in detail, for dissecting whole intact vitreous from cadaver eyes. Currently, the only vitreous dissection techniques that have been well detailed involve the dissection of unique but separate regions of the vitreous⁷ or the dissection of just the vitreous core⁶.

The applications of dissected intact vitreous humor are under appreciated and under-utilized. Surgical experience with the vitreous as well as clinical, histopathological and biochemical studies suggests that the chemical and structural properties of the vitreous can vary significantly^{16,17}. Therefore, it is necessary to preserve the structure of the whole vitreous body to study the function of the vitreous in ocular physiology and anatomy. Intact vitreous can be explanted to a transparent globe for better visualization during experiments. They can then be used for a variety of mechanical/chemical tests to improve the measurements of physical/biomechanical properties of the vitreous humor. For example, we suggest that the dissected whole intact vitreous can be used in lieu of saline, viscous substitutes, or vitreous core in the cited rheology experiments^{6,18}.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors acknowledge the following funding sources, Whittier Foundation, Harrington Foundation, National Institutes of Health and Research to Prevent Blindness.

References

1. Siggers, J. H., Ethier, C. R. Fluid Mechanics of the Eye. *Annual Review of Fluid Mechanics*. **44**, (1), 347-372 (2012).
2. Sebag, J. Age-related changes in human vitreous structure. *Graefes Arch Clin Exp Ophthalmol*. **225**, (2), 89-93 (1987).
3. Grignolo, A. Fibrous components of the vitreous body. *AMA Arch Ophthalmol*. **47**, (6), 760-774 (1952).
4. Gisladdottir, S., Loftsson, T., Stefansson, E. Diffusion characteristics of vitreous humour and saline solution follow the Stokes Einstein equation. *Graefes Arch Clin Exp Ophthalmol*. **247**, (12), 1677-1684 (2009).
5. Xu, J., Heys, J. J., Barocas, V. H., Randolph, T. W. Permeability and diffusion in vitreous humor: implications for drug delivery. *Pharm Res*. **17**, (6), 664-669 (2000).
6. Watts, F., Tan, L. E., Wilson, C. G., Girkin, J. M., Tassieri, M., Wright, A. J. Investigating the micro-rheology of the vitreous humor using an optically trapped local probe. *Journal of Optics*. **16**, (1), 015301 (2014).
7. Skeie, J. M., Mahajan, V. B. Dissection of human vitreous body elements for proteomic analysis. *J Vis Exp*. (47), e2455 (2011).
8. Abdallah, W., Ameri, H., et al. Vitreal oxygenation in retinal ischemia reperfusion. *Invest Ophthalmol Vis Sci*. **52**, (2), 1035-1042 (2011).
9. Goldenberg, D., Trese, M. Pharmacologic vitreodynamics: what is it? Why is it important. *Expert Review of Ophthalmology*. **3**, (3), 273-277 (2008).
10. Choonara, Y. E., Pillay, V., Danckwerts, M. P., Carmichael, T. R., du Toit, L. C. A review of implantable intravitreal drug delivery technologies for the treatment of posterior segment eye diseases. *J Pharm Sci*. **99**, (5), 2219-2239 (2010).
11. Balachandran, R. K., Barocas, V. H. Computer modeling of drug delivery to the posterior eye: effect of active transport and loss to choroidal blood flow. *Pharm Res*. **25**, (11), 2685-2696 (2008).
12. Smith, C. a., Newson, T. a, et al. A framework for modeling ocular drug transport and flow through the eye using micro-CT. *Phys Med Biol*. **57**, (19), 6295-6307 (2012).
13. Quiram, P. A., Leverenz, V. R., Baker, R. M., Dang, L., Giblin, F. J., Trese, M. T. Microplasma-induced posterior vitreous detachment affects vitreous oxygen levels. *Retina*. **27**, (8), 1090-1096 (2007).
14. Shui, Y., Hlekamp, N. The gel state of the vitreous and ascorbate-dependent oxygen consumption: relationship to the etiology of nuclear cataracts. *Arch Ophthalmol*. **127**, (4), 475-482 (2009).
15. Burk, S. E., Da Mata, A. P., Snyder, M. E., Schneider, S., Osher, R. H., Cionni, R. J. Visualizing vitreous using kenalog suspension. *J Cataract Refract Surg*. **29**, (4), 645-651 (2003).
16. Spaide, R. Visualization of the Posterior Vitreous with Dynamic Focusing and Windowed Averaging Swept Source Optical Coherence Tomography. *Am J Ophthalmol*. **S0002-9394**, (14), 00537-00536 (2014).

17. Domalpally, A., Gangaputra, S., Danis, R. P. *Diseases of the Vitreo-Macular Interface*. **21**, 21-27 Springer Berlin Heidelberg: Berlin, Heidelberg (2014).
18. Stocchino, R., Repetto, A., Cafferata, C. Experimental investigation of vitreous humour motion within a human eye model. *Phys Med Biol*. **50**, (19), 4729-4743 (2005).