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Authors

Rangchian, Aysan
Hubschman, Jean-Pierre
Kavehpour, H Pirouz

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Time dependent degradation of vitreous gel under enzymatic reaction: Polymeric network role in fluid properties

Aysan Rangchian^{a,b,*}, Jean-Pierre Hubschman^c, H. Pirouz Kavehpour^{a,b}

^a*Department of Bioengineering, University of California, Los Angeles*

^b*Department of Mechanical and Aerospace Engineering, University of California, Los Angeles*

^c*Retina Division, Stein Eye Institute, University of California, Los Angeles*

Abstract

The viscoelastic behavior of vitreous gel is due to the presence of biopolymers in its structure. Fluid properties of the vitreous is mainly the result of interactions between the characteristics of collagen type II and Hyaluronic Acid networks. Having a better understanding of the structure of each component and their changes during aging and various diseases such as diabetes can lead to better monitoring and treatment options. We study the effects of collagenase type II on 44 samples of porcine vitreous using an in situ rheological experiment in comparison with 18 eyes in a control group injected with Phosphate Buffered Saline Solution. We analyze the behavior of each component over time in both groups. We focus on the changes of viscosity and elasticity of the collagen network within the vitreous. The results of the

*Corresponding Author

420 Westwood Plaza, Room 46-147G, Complex Fluid & Interfacial Physics Laboratory
Box 951597 Los Angeles, CA 90095

Phone: (310) 825-6494 Fax: (310) 206-4830

Email address: aysanrangchian@ucla.edu (Aysan Rangchian)

analysis in this study show that the changes in the fluid properties of the vitreous after collagenase injection is driven by the structural alterations of the collagen network. Creep compliance values of the collagen network are significantly higher in the first group compared to the control group one hour and twenty-four hours after the injection. In contrast, creep compliance of the HA network shows no statistically significant change one hour after the injection in both groups. The results of the reported analysis of individual components in this study support the previous findings on the alterations within the vitreous structure in its entirety.

Keywords: Biopolymers, Enzymatic reaction, Rheology, Viscoelasticity, Vitreous humor

1. Introduction

1 Vitreous humor is a clear gel that occupies approximately two thirds
2 of the volume of the eye globe. Studying the structure of the vitreous gel
3 (Sebag, 1989c), its roles in the functions of the eye (Sebag, 1989a) and in the
4 pathology of vitreoretinal diseases (Sebag, 1989b), were brought to attention
5 in the late 1900s. Prior to which it was thought vitreous does not play an
6 important role within the eye globe (Foulds, 1987). It is now clear that the
7 vitreous has many roles in protecting the structure and providing the means
8 for the functions of the eye (Foulds, 2014). In addition, vitreous humor
9 supports the lens (Jin et al., 2019) and regulates the intraocular oxygen
10 (Holekamp et al., 2014). The vitreous gel is attached to the retina which
11 results in a direct connection between the changes in its network and the
12 pathology of many vitreoretinal diseases (Sebag, 2008; Sharif-Kashani et al.,

2011).

The network of the vitreous gel has two main biopolymers, collagen and hyaluronic acid (HA) (Sebag, 1989c; Yadav et al., 2015). Collagen has high tensile strength and HA provides a network to support the collagen fibrils within the vitreous (Friess, 1998; Lee et al., 2001; Yadav et al., 2015). It is hypothesized that alterations in the structure of the vitreous gel are due to the characteristic variations of its components. Therefore, a more detailed study of each one of the two networks is helpful to understand the behavior of the vitreous gel in its entirety. A better understanding of the vitreous structure and its properties can lead to improvement of the current intraocular drug delivery methods (Huang et al., 2018; Jin et al., 2019) and surgical treatment options, creation of a better engineered substitute (Morozova et al., 2016), and development of new methods of treatments. Several studies use the rheological properties of the vitreous humor to develop a better substitute with similar viscoelastic behavior (Swindle et al., 2008; Thakur et al., 2020).

Degeneration of the vitreous network leads to many retinal conditions. This degradation process can be due to many reasons such as aging and diseases (Skeie et al., 2015). The structure of the vitreous gel changes over time which causes a separation of the liquid part in the vitreous (i.e. water and HA) from the collagen fibers in the form of bundles (Sebag, 2008). This process is one form of the liquefaction of the vitreous. Liquefaction of the vitreous gel can alter vitreoretinal interface and lead to posterior vitreous detachment (PVD), vitreomacular traction (VMT) syndromes (Patronas et al., 2009), and eventually cause more serious diseases such as retinal tear (RT) or retinal detachment (RD) (Los et al., 2003). Diseases such as diabetes can

38 also change the vitreous structure in different ways and lead to retinopathy
 39 (Fong et al., 2004). These conditions can be treated by Pars Plana Vitrec-
 40 tomy (PPV), a surgical procedure to remove the vitreous humor and replace
 41 it with a fluid as a substitute (Los et al., 2003). Recently an enzymatic
 42 intravitreal injection, Jetrea (Ocriplasmin, Thrombogenics, Inc), has been
 43 found to induce pharmacologic vitreolysis by means of proteolysis of the vit-
 44 reoretinal connections (Haller et al., 2015). In addition, studies introduced
 45 intravitreal injection of certain gases to exert mechanical forces and remove
 46 the adhesion of the vitreous gel to the retina (McDonald et al., 1994; Ro-
 47 drigues et al., 2013). Further investigations are essential to fully characterize
 48 and improve the effects of the mentioned treatments on the vitreous gel.

49 Understanding the fluid properties of a polymeric network can shed light
 50 on the characteristics of its structure. Rheology is an indirect method to
 51 measure the properties of fluid. Prior investigations show that the vitre-
 52 ous humor is a viscoelastic gel (Nickerson et al., 2008; Sharif-Kashani et al.,
 53 2011; Silva et al., 2017). Similar to the molecular structure of the vitre-
 54 ous, its viscoelastic properties are mainly due to the presence of two main
 55 components; collagen and HA network. The viscoelastic properties of each
 56 component within the vitreous gel were studied previously using shear rheo-
 57 logical methods (Sharif-Kashani et al., 2011; Tram and Swindle-Reilly, 2018).
 58 In addition, the enzymatic degradation of the vitreous gel was studied us-
 59 ing the same method where the vitreous gel was dissected (Filas et al., 2014;
 60 Huang et al., 2018). It should be noted that microrheology can provide access
 61 without the need for dissection (Lee et al., 1992, 1994; Watts et al., 2013).
 62 However, because only the localized characteristics are reported, accuracy

63 of the technique needs improvement (Waigh, 2016). Furthermore, it was
 64 shown that using *in situ* rheological experiments, we can measure the effects
 65 of enzymes on the fluid properties of the vitreous gel over time without any
 66 major alterations to the structure of the eye globe (Rangchian et al., 2019).
 67 The described technique was previously evaluated using known Newtonian
 68 and Non-Newtonian fluids (Connelly et al., 2016). Using this method, it is
 69 possible to further investigate the changes in the structure of the vitreous
 70 humor and relate it to each one of the separate components. We analyzed
 71 the data from the eyes in two groups of injections over time to quantify the
 72 alterations in the properties of each network individually (i.e. collagen and
 73 HA). This is the first *in situ* study to analyze the characteristics of the two
 74 main components of the vitreous gel over time. We investigated the changes
 75 in the creep compliance and retardation time of each component as well as
 76 the steady state viscosity of the vitreous gel. We would like to emphasize the
 77 reported values in this study are an indication of the fluid properties of the
 78 individual components of the vitreous gel and the main purpose is to show
 79 the variations over time and due to the enzymatic degradation as opposed
 80 to the exact values of the properties.

81 **2. Materials and methods**

82 Fresh porcine eyes were harvested and shipped on dry ice, and sent within
 83 the same day by Sierra Medical Supplies (Whittier, CA, USA). In total we
 84 tested 44 eyes in the first group and 18 eyes in the control group. We followed
 85 the same protocol used in the previous publication (Rangchian et al., 2019)
 86 and here we provide a summary. A small triangular incision was made on

87 the pars plana of the eye to access the vitreous gel. This incision provided an
 88 access to the vitreous for testing and injections. A stress-controlled rheometer
 89 (TA instruments, AR 2000) was used with a 0.87mm diameter cylindrical
 90 probe. The rheological procedure used in this study is creep flow, one of the
 91 commonly used methods to understand flow characteristics of fluids. In this
 92 test a constant torque/shear (τ) is applied and the caused deformation (γ) is
 93 recorded. Creep compliance (J), which is an indicator of the elasticity of the
 94 fluid, is derived from the deformation using $J(t)=\gamma(t)/\tau$. After the incision
 95 was made, we secured the eye on a 3D printed cube (ABS polymer) with a
 96 half sphere cut out and a rough surface to avoid extra movements during the
 97 experiment. The probe was fully inserted into the eye to run the test close
 98 to the center of the vitreous cavity. We applied a torque of 1 μ Nm with zero
 99 normal force over 6 minutes on each eye. This first test was performed before
 100 any injections and we refer to it as the pre-injection result (T_0). Immediately
 101 after the pre-injection test, the eye was injected with 50 μ L of collagenase
 102 type II (group 1) or the same volume of PBS in group 2. Collagenase was
 103 chosen to look at the differences on the collagen network more than any
 104 other biopolymeric networks within the vitreous. We recorded the time of the
 105 injection and repeated the test one hour (T_1) after the injection to capture the
 106 changes during a short period of time and to monitor the initial conditions.
 107 The scleral opening was protected during the first hour by inserting a small
 108 capped plastic vial on top of the opening to reduce the alteration of the
 109 vitreous. Subsequent to this test, eyes were individually stored at 4 °C in
 110 a container filled with PBS to minimize evaporation of the water content of
 111 the vitreous gel. Eyes were brought back to the room temperature (25°C)

112 15 minutes before the last experiment to prevent alterations of the results
113 due to temperature changes. The last test was 24 hours after the injection
114 (T_{24}) to observe changes over a longer period of time. We were limited to
115 repeat the test beyond 24 hours as vitreous would naturally degrade. The
116 schematic steps of the experiment are shown in Figure 1.

117 The creep curves in this study are highly nonlinear with many local min-
118 ima, hence we used Python non-linear regression model to improve the ac-
119 curacy of the fittings. Non-linear regression model is an optimization tech-
120 nique to solve highly nonlinear problems using the least square minimization
121 approach. We used the viscoelastic discrete spectra model with two Voigt-
122 Kelvin elements in series (Ferry, 1980) to analyze the fluid characteristics of
123 the elements present in the vitreous humor network (Sharif-Kashani et al.,
124 2011).

125 To perform statistical analyses, R-studio was used which is an integrated
126 development environment used for statistical computing. Mixed ANOVA
127 analyses are used in all of the statistical calculations. In these analyses
128 the injection types are the between subjects (collagenase type II or PBS)
129 and the three time points that the experiments are repeated are the within
130 subjects factor (Statistics, 2015). The significant differences reported by the
131 ANOVA analyses are further investigated using paired or unpaired t-tests
132 (depending on the repeated measure or between subjects analyses) adjusted
133 by Bonferroni correction. These results are also compared to the Welch's
134 t-test. The range of p values are reported as the p values are not exactly
135 the same. However, both tests were in agreement on the existence of the
136 significant differences.

137 3. Results

138 3.1. Fitting parameters

139 The viscoelastic discrete spectra model (Ferry, 1980; Sharif-Kashani et al.,
140 2011) is used (Equation 1) to analyze the behavior of the vitreous gel as a
141 result of the interaction of its two components.

$$J(t) = \sum_k J_k(1 - r^{(-t/t_k)}) + t/\eta_m \quad (1)$$

142 There are 5 parameters in this equation. Viscosity of the vitreous gel (η_m) in
143 the steady state region (i.e. the linear segment of the creep curve). Each one
144 of the components within the network of the vitreous gel (i.e. collagen and
145 HA) has two parameters related to their fluid properties, creep compliance
146 (J_k) and retardation time (t_k). The changes in creep compliance (J_k) and
147 retardation time (t_k) values of each component lead to changes in the fluid
148 properties of the vitreous gel.

149 3.2. Creep curve fitting

150 The result of each experiment for one eye is a creep curve. As explained
151 earlier, the experiment is repeated at three time points (T_0, T_1, T_{24}), produc-
152 ing three curves for each eye. Samples of all three curves for one eye from
153 each group are shown in Figure 2 a & b. Viscosity of the gel at the steady
154 state was calculated for each one of the eyes using the slope of the linear seg-
155 ment of the creep curve between $t=240$ s and $t=300$ s. Using the calculated
156 viscosity, we modeled the entire creep curve to find the rest of the parameters
157 for each experiment. The fit of the pre-injection curve provided the values
158 for J_1 , t_1 , J_2 , and t_2 . The calculated value of t_2 (i.e. retardation time of HA

159 network) from the first fit (T_0) was kept constant for T_1 and T_{24} modeling.
160 The hypothesis is that the effect of time is the same in both groups on the
161 retardation time of the HA network, hence the value of t_2 should not change
162 significantly between the two groups.

163 3.3. Collagen parameters

164 The statistical results are provided in this section comparing collagen
165 parameters between and within the two groups over time. At each time
166 point the average value for elasticity of the collagen network is compared
167 between the groups and p values of unpaired t-tests are provided (Figure 3).
168 In addition, statistical comparison of each group over time (paired t-test) is
169 shown in Table 1. The same analysis was done on the retardation time of
170 the collagen network (Figure 4 and Table 2).

171 3.4. HA parameters

172 The same properties, creep compliance J_2 and retardation time t_2 , can
173 be calculated for the HA network. Average values of the creep compliance of
174 the HA network is shown in Figure 5 as well as the p values from unpaired
175 t-test analyses between the two groups at each time point. P values from the
176 paired test for each group over time is reported in Table 3. The effects of
177 collagenase and PBS injections on the retardation time of the HA network
178 are minimal (Filas et al., 2014). Due to the high non-linearity of the data
179 and fittings, we hypothesized that the changes of t_2 values over time are not
180 significant. This hypothesis was validated by calculating the t_2 values at T_1
181 and T_{24} using the previously fitted parameters.

182 4. Discussion

183 Vitreous gel has a complicated fluid structure which is due to its polymeric
184 network and molecular structure (Meyer and Palmer, 1934; Sharif-Kashani
185 et al., 2011). HA network and collagen fibrils are the main components
186 of this structure with distinguishable fluid characteristics. Aging can alter
187 the structure of the vitreous gel where it becomes more liquid-like. This
188 happens mainly as a result of the cross linking of collagen fibrils (Swann,
189 1987) that may lead to a pulling force on the retina at the vitreoretinal
190 interface. This pulling can cause a full PVD but in some cases the vitreous
191 gel remains partially attached to the retina and causes point forces at certain
192 locations which could result in RT or RD. If a patient becomes symptomatic,
193 PPV surgery can help removing the force (Rodrigues et al., 2013; Hikichi
194 et al., 1995; McDonald et al., 1994; Writing et al., 2010). As PPV is an
195 invasive course of treatment, there are many studies that focus on alternative
196 options such as pharmacologic vitrectomy, and pharmacologic or gas induced
197 vitreolysis (Rodrigues et al., 2013; Soman and Banerjee, 2003; Shah and
198 Trese, 2016).

199 There have been many rheological studies to characterize the effects of
200 the aging on the structure of the vitreous. Comparison of the creep results
201 on ovine eyes from three different ages, showed a decrease in both loss and
202 storage modulus with age which is due to the breakdown of the collagen
203 network (Colter et al., 2015). A rheological study on the dissected human
204 vitreous reported a decrease in the viscoelasticity of the vitreous humor which
205 is related to the liquefaction of the eye (Schulz et al., 2019). Another study
206 on the human vitreous at different ages showed higher stiffness and viscosity

207 for the solid phase of the older vitreous and lower viscosity for the liquid
208 phase of the older vitreous gel (Tram and Swindle-Reilly, 2018); however,
209 the discrete retardation analysis did not show significant correlation, which
210 could be due to the dissection of the vitreous as well as the low number of
211 samples tested. These findings are in contradiction with the studies that
212 measure the vitreous properties in its entirety. Our findings in group 1 are
213 in agreement with the aging analysis of the vitreous as one gel; we assume
214 that the similarity is due to the injection of the collagenase which breaks the
215 bonds in the collagen network.

216 The molecular structure of the vitreous gel and the interaction of its
217 networks of biopolymers are very important in understanding of the pathol-
218 ogy of many vitreoretinal diseases. It can also provide information about
219 the possible approaches to slow down the degenerative aging process or to
220 treat the resulting conditions. To be able to invent a substitute for the
221 vitreous gel with better quality, one should be fully aware of its properties
222 and roles (Berkowitz et al., 1991; Soman and Banerjee, 2003; Swindle et al.,
223 2008; Januschowski et al., 2019; Thakur et al., 2020). Previous studies could
224 identify the composition of the vitreous network (Swann, 1987; Meyer and
225 Palmer, 1934; Sebag and Balazs, 1989) but due to the fragile structure of the
226 vitreous gel, reported direct measurements require dissection and are limited
227 (Nickerson and Kornfield, 2005; Nickerson et al., 2008; Filas et al., 2014; Silva
228 et al., 2017; Tram and Swindle-Reilly, 2018).

229 Each one of the networks of biopolymers in the vitreous has its own
230 properties (Sharif-Kashani et al., 2011; Tram and Swindle-Reilly, 2018) and
231 changes in one of the components can lead to alterations in the vitreous

232 characteristics. Microrheological experiments show that the probe can be
233 trapped in one of the components and lead to localized information. For
234 instance if the probe is located in the fibrils of collagen network the results
235 would drastically differ compared to when the probe is in the liquid pockets
236 within the vitreous (Watts et al., 2013). Therefore, better understanding
237 of the properties and structure of the main components on the macro scale
238 are essential to investigate the changes in the characteristics of vitreous. In
239 addition, exploring the behaviour of mentioned components after enzymatic
240 degradation can provide better constraints for the design of new treatments
241 for vitreolysis. There have been no reported *in situ* rheological studies of the
242 vitreous network over time as with the normal shear rheological setup of the
243 experiments, the structure of the eye globe is disturbed. Hence, after the first
244 run of the test, the eye must be discarded. There are reported less invasive
245 microrheological methods both *in vivo* and *ex vivo* however, the spatial size
246 limitation of the experiment and localized characteristics constraint still exist
247 (Pokki et al., 2015).

248 It is important to quantify the time variations on vitreous humor. The
249 ability to repeat the experiment allows us to characterize the degradation due
250 to time and/or different enzymes on the rheological properties of the vitre-
251 ous. There are reported studies on rheological measurement of the dissected
252 vitreous properties over time. Silva and co-workers reported the rheological
253 properties of the dissected gel and liquid parts of the vitreous individually
254 over time (Silva et al., 2017). In our *in situ* experimental setup, we can re-
255 peat the test over time (Rangchian et al., 2019). We measured the viscosity
256 and creep compliance of the vitreous and their changes due to the injection

257 of collagenase over time. In the present study we modeled the individual
 258 components of the vitreous and their changes as a result of collagenase in-
 259 jection in comparison with a control group. The results reported here are in
 260 agreement with the previous studies that used the viscoelastic discrete spec-
 261 tra model to analyze porcine and human vitreous to show two distinguished
 262 components (Sharif-Kashani et al., 2011; Tram and Swindle-Reilly, 2018).

263 The slope of the creep curve in the steady state for group 1 is the smallest
 264 for the pre-injection curve with an average value of 6×10^{-5} [1/Pa.s]. One and
 265 24 hours after the injection the slope increases, average values are 9×10^{-5}
 266 and 2×10^{-4} [1/Pa.s] respectively. Group 2 has approximately the same slope
 267 for all three time intervals, the average varying from 4×10^{-5} to 5×10^{-5}
 268 [1/Pa.s].

269 The average values of elasticity of collagen network (J_1) does not differ
 270 significantly between the two groups before the injection with an average
 271 of 0.16 for group 1 and 0.14 Pa^{-1} of group 2. However, results from the
 272 experiments at T_1 and T_{24} in group 1 are significantly higher than the one in
 273 group 2, p values are reported in Figure 2. The average values for J_1 in group
 274 1 are 0.21 Pa^{-1} at T_1 and 0.58 Pa^{-1} at T_{24} compared to 0.11 Pa^{-1} and 0.14
 275 Pa^{-1} for group 2. Moreover, J_1 increases noticeably over time in group 1,
 276 whereas in group 2 the increase is not significant (Table 1). Creep compliance
 277 is directly related to the inverse of elasticity. Therefore, the results suggest
 278 that elasticity of the collagen network has significantly decreased in group
 279 1. This could be explained by having less bonds present in the network of
 280 collagen due to the enzymatic effect of the collagenase. The presence of less
 281 bonds in the network of a biopolymer makes it easier to elastically deform

282 the structure. This result supports the hypothesized changes on the collagen
283 network and confirms that the change in the elasticity of the vitreous is due
284 to the alterations of the collagen component of the gel.

285 It is noteworthy to mention that there are no statistically significant dif-
286 ferences between the average values of the retardation time of the collagen
287 network, t_1 , between the two groups at T_0 , with values of 7.48 s and 6.62 s
288 for group 1 and group 2 respectively, and T_1 with an average value of 6.69
289 s for group 1 and 5.88 s for group 2. Whereas $t_1 = 9.61$ s is significantly
290 higher in group 1 compared to $t_1 = (6.19$ s) for group 2 at T_{24} (Figure 4).
291 The paired t-test results of the comparison of t_1 values for each group are
292 reported in Table 2. It is worth mentioning that in group 1 the t_1 value
293 increases over time; however, the increase from T_0 to T_1 is not statistically
294 significant. The average value of t_1 at T_{24} is noticeably higher than the other
295 two time points in group 1. The increase in the retardation time suggests
296 that there is a change from solid-like behaviour to more liquid-like behaviour
297 in the collagen network. This change can be due to the presence of less bonds
298 in the collagen networks. This data validates the previous findings on the
299 changes of the vitreous network as one gel (Rangchian et al., 2019).

300 There are no statistically significant differences observed for t_1 values in
301 group 2, but the value increases over time. This could have been the result of
302 unavoidable evaporation of the water content of the gel. These results show
303 that we can map the effects of various active and placebo injections on the
304 specific parameters of the vitreous gel.

305 The average value of elasticity of HA network (J_2) does not differ signif-
306 icantly between the two groups at T_0 with 0.024 Pa⁻¹ for group 1 compared

307 to 0.018 Pa^{-1} for group 2 and T_1 , average values of 0.030 Pa^{-1} and 0.012
 308 Pa^{-1} for group 1 and group 2 respectively. However, results from the exper-
 309 iment at T_{24} in group 1, 0.37 Pa^{-1} , is significantly higher than the one in
 310 group 2, 0.014 Pa^{-1} , p values of the unpaired t-tests are reported in Figure 5.
 311 Moreover, J_2 at T_{24} is higher compared to the other two time points in group
 312 1, whereas in group 2 the increase is not significant (Table 3). Therefore, the
 313 results suggest that elasticity of the HA network has decreased 24 hours after
 314 the injection of collagenase. This could be due to unavoidable alteration on
 315 the HA network after the degradation of the enzyme in addition to the effect
 316 of time on the bonds in its network.

317 As it was mentioned earlier, PPV is an invasive surgery with longer time
 318 for the rehabilitation of the vision (Johnson et al., 2015). Enzymatic vitre-
 319 olysis can induce PVD to remove the traction force of the vitreous on the
 320 retina. However, discovering the right enzymatic injection can be difficult.
 321 Jetrea (Ocriplasmin, Thrombogenics) is the first intravitreal drug injection
 322 that was approved by FDA in 2012. Ocriplasmin is a recombinant protease
 323 that has activity against fibronectin and laminin in the vitreoretinal interface
 324 (Kupfermann, 2012; Khan and Haller, 2016). While this method has many
 325 advantages compared to PPV, it is limited to some patients with specific
 326 conditions. In addition, there are many side effects associated with the Je-
 327 trea injection such as lens instability and macular hole enlargement (Johnson
 328 et al., 2015), temporary disturbances or loss of vision (Fahim et al., 2014),
 329 and acute visual loss due to the ellipsoid zone changes (Reiss et al., 2015;
 330 Johnson et al., 2015).

331 Effects of Jetrea should be more extensively studied as a treatment op-

tion, as its roles as a protease may not be limited to the vitreoretinal interface and modify the properties of the vitreous gel (Beebe, 2015) and cause the aforementioned side effects. This study was only on collagenase injection which is primarily active against collagen, but the described method can be used to further investigate the potential effects of an enzyme such as Jetrea on separate components of the vitreous. This can potentially help patients with diabetic retinopathy or abnormal vitreoretinal adherence to have an induced PVD which removes the need for surgical intervention.

We investigated the changes on the components of the vitreous structure due to an active enzyme injection on the specific components of the network over time. Effects of time can be more precisely modeled by adding more time intervals to the experimental procedure (i.e. add time points between 1 hour and 24 hours) and to predict the changes at other time points without the experimental procedure. In future, our method can help finding and evaluating possible enzymatic treatments and to predict the effects of other potential injections over time and their possible roles in the treatment of some vitreoretinal conditions.

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Conflict of interest

The authors have no affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript.

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Tables

Table 1

Mean [Pa^{-1}]	0	1 hour	24 hour
Group 1	0.16 ± 0.01	0.21 ± 0.02	0.58 ± 0.05
Group 2	0.14 ± 0.02	0.11 ± 0.01	0.14 ± 0.02
P values	0 vs 1 hour	0 vs 24 hour	1 vs 24 hour
Group 1	<0.05	<0.05	<0.05
Group 2	>0.05	>0.05	>0.05

Table 2

Mean [s]	0	1 hour	24 hour
Group 1	7.48 ± 0.2	6.69 ± 0.5	9.61 ± 0.6
Group 2	6.62 ± 0.5	5.89 ± 0.4	6.19 ± 0.4
P values	0 vs 1 hour	0 vs 24 hour	1 vs 24 hour
Group 1	>0.05	<0.05	<0.05
Group 2	>0.05	>0.05	>0.05

Table 3

Mean [Pa^{-1}]	0	1 hour	24 hour
Group 1	0.024±0.001	0.030±0.01	0.366±0.1
Group 2	0.018±0.003	0.012±0.001	0.014±0.002
P values	0 vs 1 hour	0 vs 24 hour	1 vs 24 hour
Group 1	>0.05	<0.05	<0.05
Group 2	>0.05	>0.05	>0.05