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Human Vitreous Collagen Fragments Dimension As a Function of Vitrectomy Cut Rate

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Purpose: To study the dimensions and distribution of human vitreous collagen type II fragments collected after vitrectomy performed at varying cut rates and to evaluate if increasing the cut rate produces smaller collagen fragments, thus reducing retinal traction and/or viscosity.

Methods: Fluid was collected during core vitrectomies performed for macular surgery at cut rates from 1000 to 16,000 cuts per minute (CPM) and immediately refrigerated. Protein fractions were separated by molecular weight (MW; >100 kDa, 50–100 kDa, 50–30 kDa, 30–10 kDa, and <10 kDa) through centrifugal filters. The Human Collagen II ELISA Kit colorimetric assay was then used to measure the COL2A1 in unfiltered and filtered samples.

Results: Vitreous samples collected after vitrectomy performed at 16,000 CPM contained a higher concentration of protein with MW over 100 kDa than at any other cutting frequency ($P < 0.01$). No significant differences were found in fractions collected with a MW between 50 and 100 kDa. Collagen type II fragments over 100 kDa were significantly more represented than smaller fragments at each cut rate. The proportion of smaller (50–100 kDa) collagen fragments compared with those over 100 kDa was higher at 2000 CPM than at higher cut rates.

Conclusions: Vitreous samples collected at different cut rates do not contain a significantly different proportion of collagen type II fragments of the tested MW. The extreme variability of vitreous flow through the cutter port may explain the uncertain predictability of collagen fragment MWs.

Translational Relevance: Increasing the cut rate does not produce vitreous fragments of proportionally smaller dimension. It is necessary to achieve an invariant instantaneous flow through the cutter port in order to decrease retinal traction during vitrectomy.

Introduction

Pars plana vitrectomy (PPV) is a common surgical procedure performed for a variety of indications, including retinal detachment, vitreous hemorrhage, vitreomacular interface syndrome, and macular holes. More than 225,000 PPVs are performed yearly in the United States.[1] The human vitreous is a 98% water content gel that is rich in proteins, hyaluronan, and glucose amino glycans and has a collagen (mostly type II)[2] structure that requires cutting for its excision. Vitreous gel removal is accomplished through the simultaneous aspiration generated by Venturi or volume pumps and cutting by means of reciprocating guillotine blades. Because aspiration pressure may reach 650 mmHg and the inner vitreous cutter bore diameter varies between 0.3 and 0.5 mm (27

and 23 gauge, respectively), the acceleration of fluid through the vitreous cutter port is significant. Significant traction is also exerted on the retina while severing vitreous strands still attached to it.[3,4]

The maximum cut rate of vitreous cutters has been rising exponentially in the past 20 years, from 100 cuts per minute (CPM) to 20,000 CPM, to reduce the risk of iatrogenic retinal tears.[5] The prevailing hypothesis suggests that reciprocating blade motion at higher frequency reduces the length of vitreous fragments and therefore the pulling on the retina between two consecutive cuts.[6–8] Indeed, raising the cut rate consistently and reliably reduces the collagen fragment length and therefore traction over the retina only if the liquid column flows through the cutter port at an invariant velocity. This assumption has recently been challenged by experimental evidence clearly showing the pulsatile progression of fluid through the entire cutter shaft[9] and turbulence close to the port[10] due to the fluid dragging of the reciprocating inner blade.

The purpose of present paper was to study the molecular weight (MW) distribution of human vitreous collagen type II fragments collected during vitrectomy surgery at various cut rates and to evaluate if increasing the cut rate produces a higher proportion of smaller fragments. The study also measured the overall vitreous proteins within the collected samples and fractionated the results as a function of cut rate.

Materials and Methods

Vitreous Sample Collection

The vitreous of 15 consecutive patients undergoing standard 25-gauge three-port PPV (R-Evolution CS800; Optikon 2000, Inc., Rome, Italy) for macular pucker or macular hole surgery was collected. Inclusion criteria were age > 18 years, fellow eye vision greater than 20/40 Snellen equivalent, and ametropia less than ± 3 diopters in spherical equivalent. Exclusion criteria included known genetic alterations with specific regard to collagen inheritable conditions including, but not limited to, amyloidosis, Marfan syndrome, Stickler syndrome, Ehler–Danlos syndromes, Alport syndrome, or osteogenesis imperfecta, as well as the presence of any trace of blood in the vitreous.

Each eye was made pseudophakic at the time of PPV with an intraocular lens implant in the capsular bag without complications.

Fifteen eyes of 15 patients were randomized to receiving core vitrectomy at various cut rates (1000, 2000, 4000, 7500, and 16,000 CPM); therefore, each of the five different cut rates was used in three different patients. Balanced saline solution (BVI Medical, Waltham, MA) was used in the infusion bottle, and the aspiration generated by the Venturi pump was set at 500 mmHg in all cases. Although the R-Evolution features a double pump (Venturi effect and peristaltic) that can be seamlessly switched at the surgeon's will during surgery, we elected to use only the Venturi pump throughout the study because it is the one used by most vitreous cutter machines.

The entire fluid-catch bag contents and aspiration line rinsing after core vitrectomy were collected, immediately stored at 4°C, and then sent to the laboratory within 1 hour. The remainder of vitreous surgery progressed at different cut rates as needed.

The study received internal review board approval, is registered at Clinicaltrials.gov (NCT04570007), and followed the tenets of the Declaration of Helsinki.

Vitreous Samples Analysis: Sample Collection and Cut-Off Filtration

Fluid was collected during core vitrectomy performed for macular surgery at cut rates between 1000 and 16,000 CPM. The post-surgery samples were passed through Amicon Ultra Centrifugal Filters (Nominal Molecular Weight Limits (NMWL) 100,000 and 50,000; MilliporeSigma, Burlington, MA) and Centriprep Centrifugal Filters (NMWL 30,000 and 10,000; MilliporeSigma) to obtain five fractions separated for MW (>100 kDa, 50–100 kDa, 50–30 kDa, 30–10 kDa, and <10 kDa). Proteins were detected in each fraction, demonstrating that protein fractionation did occur. The protein concentrations of each fraction and the unfiltered samples were measured using the bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, MA). The procedures were carried out following the manufacturer's protocols.

Human Collagen II Enzyme-Linked Immunosorbent Assay

The Human Collagen II Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Novus Biological, Littleton, CO) colorimetric assay was used to measure the amount of collagen type II alpha 1 chain (COL2A1) in unfiltered and filtered vitreous samples. ELISA tests were carried out following the manufacturer's instructions, and the concentrations of COL2A1 were calculated according to the standard curves prepared on the same ELISA plates.

Statistical Analysis

The results are expressed as mean ± SD from at least three independent experiment sample. Analysis of

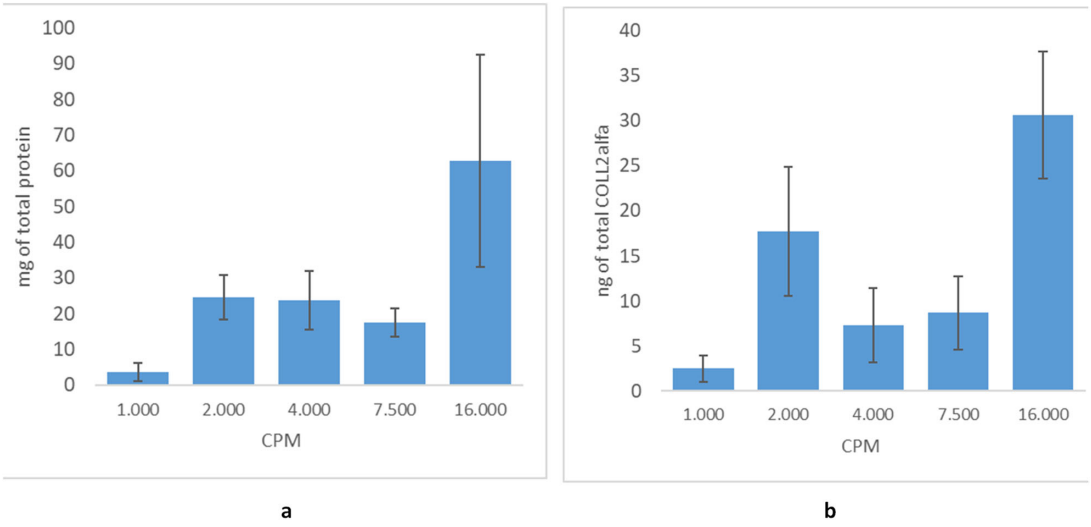

Figure 1. Average total protein (a) and collagen (b) content as a function of cut rate.

variance (ANOVA) was used for continuous variables with Bonferroni correction for multiple comparisons. Pearson's correlation coefficient was used to investigate the correlation between cut rate and collagen fragment MW. $P < 0.05$ was considered statistically significant.

Results

The overall protein and COL2A1 content found in the vitreous samples are reported in Figure 1.

The total protein content varied in the range of 5 to 50 mg, whereas COL2A1 was between 2.5 and 27 ng. Samples collected at 16,000 CPM contained significantly more proteins and specifically more COL2A1 than all other groups of cut rates.

All proteins from each sample were filtrated, generating five fractions with different molecular weights (Fig. 2). Vitreous samples collected at 16,000 CPM contained a significantly higher concentration of proteins with MWs over 100 kDa compared with any other cutting frequency ($P < 0.01$), whereas no significant difference was found for the fractions collected at MWs between 50 and 100 kDa. Fractions containing proteins with MWs lower than 50 kDa were significantly less well represented at 16,000 CPM compared with 2000, 4000, and 7500 CPM ($P < 0.05$).

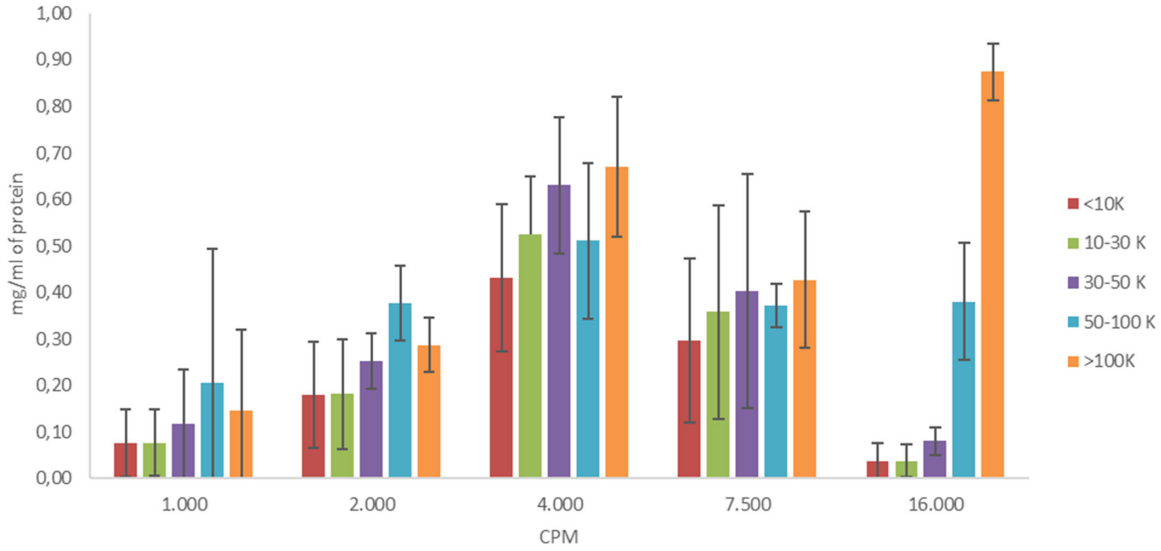

Figure 2. Protein content in each fraction at different MWs determined at each vitrectomy cut rate (CPM).

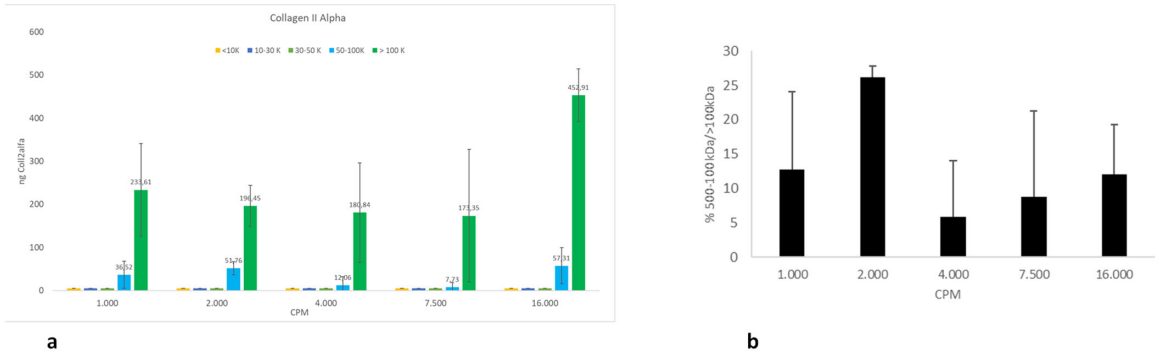

Figure 3. (a) COL2A1 content in different fractions per cutting rate. (b) Ratio of 50- to 100-kDa fraction to the fraction over 100 kDa.

COL2A1 could be detected only in protein fractions of molecular weight greater than 50 kDa (Fig. 3), regardless of cut rate. All cut rates showed a significant prevalence of COL2A1 fragments at MWs greater than 100 kDa compared with fractions in the range of 50 to 10 kDa ($P < 0.01$ in all cases; Fig. 3a).

The ratios of fragments between 50 and 100 kDa to those exceeding 100 kDa (Fig. 3b) did not show significant differences across groups of cut rates.

Discussion

The human vitreous contains more than 1100 unique proteins[11]; collagen accounts for about 15% of the entire protein content, with type II being the most abundant (65%) followed by type IX (20%).[12] The collagen type II alpha helix fibrillary structure interspersed in the viscoelastic fluid matrix made of hyaluronan and glucose aminoglycans represents the "skeleton" of vitreous gel and explains why the simultaneous actions of aspiration and cutting are needed to accomplish vitrectomy.

Modified guillotine systems currently represent the most widespread mechanism of action. Rotary and ultrasonic devices have also been proposed,[13] based on the same principle of severing the collagen structure while aspirating its fragments and the highly viscous matrix.

The rush to ever higher cutting rates, from less than the 100 CPM of Machemer's vitreous infusion suction cutter[14] to the 20,000 CPM of today's cutters, is based on the assumption that cutting the collagen mesh at a higher frequency would almost instantaneously release the retinal traction exerted by suction and produce smaller vitreous fragments. This would result in a safer surgery.[15]

In our study, the analysis of vitreous samples did not seem to support this apparently intuitive concept, as increasing the cut rate did not result in a higher proportion of either smaller MW proteins (Fig. 2) or collagen type II fragments (Fig. 3). Indeed, cutting the vitreous at 16,000 CPM yielded both a significantly higher proportion of proteins and collagen II fragments heavier than 100 kDa, as well as a significantly smaller proportion of proteins under 50 kDa MW when compared with all other cut rates.

Interestingly, we were able to detect proteins with MW less than 50 kDa at all tested cut rates (Fig. 2), but collagen type II fragments were present in only the 50- to 100-kDa and >100-kDa fractions, and their ratios did not vary significantly with cut rate (Fig. 3b).

This finding is somewhat puzzling if blade action is regarded as the only variable influencing collagen fragment length, but it can be explained if the large variability of vitreous flow through the cutter port is considered. In order to achieve reproducible and reliably shorter collagen fragments, in fact, not only must the blade motion be reciprocating at a constant pace but the fluid "column" must also flow through the cutter port at a steady rate. In other words, both cut rate and flow rate must be invariant.

This is clearly not the case, though, as flow through the cutter port has been demonstrated to pulsate synchronously with the inner blade as its reciprocating motion drags the adjacent layers of fluid back and forth.[3,4]

It should also be noted that we elected to use the Venturi pump, which is a feature of most commercially available machines. All pressure pumps (such as the Venturi effect pump) create invariant pressure; therefore, according to the Hagen–Poiseuille equation ($\Delta P = \frac{8\eta' LQ}{\pi R^4}$), flow ($Q$) becomes inversely proportional to viscosity and is only dependent on it ($\acute{\eta}$) when the circuit pressure (ΔP), radius (R), and length (L) do

not change. Because the vitreous chamber is replenished with large volumes of balanced salt solution during surgery and the human vitreous itself contains pockets of liquified gel, the viscosity of the fluid entering the cutter port is constantly changing, resulting in changes in flow rate.

Despite the consistently cyclical action of blade reciprocation, the unpredictable variability of fluid velocity entering the cutter port due to viscosity changes and blade dragging results in fragments of extremely variable MW. The increased shear rate created at the cutter port by exceedingly high cut rates may create smaller protein aggregates,[16] thus explaining the reduced proportion of proteins of less than 50 kDa (Fig. 2).

Recently, Lue et al.[17] reported the results of an elegant experiment showing that vitreous traction on the retina did not vary as a function of cut rate but rather was dependent only on aspiration. That study used two distinct machines, both equipped with Venturi pumps, and did not add balanced salt solution to the porcine vitreous. The results strengthen the hypothesis that increasing the cut rate does not significantly reduce the length of vitreous fragments because the fluidics predominates, and reducing the vacuum becomes more efficient.

In summary, our experiment suggests that increasing the cut rate does not necessarily produce human vitreous collagen fragments of significantly different MW, at least not when a Venturi pump is used to generate the vacuum, most likely because the precisely periodic blade cycling is negated by the magnitude of instantaneous variations in flow rate.

This observation raises questions about the assumption that increasing the cut rate per se reduces retinal traction and makes surgery safer, as observed by Lue et al.[15] Flow invariance (that is, fluid velocity invariance though the cutter port) is as important as cut rate and deserves greater attention.

Focusing on both flow invariance and cut rates may therefore prove more rewarding than simply focusing on increasing cut rates.

Pitfalls of the present study are primarily related to the inherent difficulty of assessing the MWs of collagen type II fragments and correlating them to the linear dimension, as well as, to a lesser extent, the uncertainty of antibody ligand specificity. We also examined fluid obtained directly from the vitrectomy bag, but variability in the flow of balanced salt solution through the eye during surgery makes it difficult to make general assumptions about protein and collagen concentrations.

Nonetheless, we believe that the lack of a correlation between cut rate and collagen fragment dimensions clearly points to the need for more accurate fluidics control to reduce retinal traction during vitrectomy and improve safety.

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