Vitrectomy-Assisted Biopsy: An In Vitro Study on the Impact of Cut Rate and Probe Size

Erlend Ulltang,[a] Jens Folke Kiilgaard,[b] Nazanin Mola,[c,d] David Scheie,[e] Steffen Heegaard,[b,e] Jørgen Krohn,[a,f]

[a] Department of Ophthalmology, Haukeland University Hospital, Bergen, Norway
[b] Department of Ophthalmology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[c] Department of Pathology, Haukeland University Hospital, Bergen, Norway
[d] Department of Clinical Medicine, Section for Pathology, University of Bergen, Bergen, Norway
[e] Department of Pathology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[f] Department of Clinical Medicine, Section of Ophthalmology, University of Bergen, Bergen, Norway

Short Title: Ulltang et al.: Vitrectomy-assisted biopsy

Corresponding Author:
Jørgen Krohn
Department of Ophthalmology
Haukeland University Hospital
N-5021 Bergen
Norway
Tel: +47 55974115
Email: jorgen.krohn@helse-bergen.no

Number of Figures: 3 panel figures

Keywords: Pars plana vitrectomy, vitrectomy-assisted biopsy, cut rate, 23-gauge vitrectomy, 25-gauge vitrectomy, digital image analysis

Abstract

***Purpose*:** To optimize the technique of performing vitrectomy-assisted biopsy of intraocular tumors by comparing the cytohistological findings in specimens obtained with different vitrectomy probes and cut rates.

***Methods*:** Vitrectomy-assisted biopsies were taken from fresh porcine liver. For each sampling, the vacuum level was 300 mmHg. The following parameters were compared: cut rate (60, 600, and 6000 cpm), probe type (standard and two-dimensional cutting (TDC)), probe diameter (23-gauge and 25-gauge). The specimens were assessed by automated whole-slide imaging analysis and conventional light microscopy.

***Results*:** Seventy-two biopsies were analyzed for the number of hepatocytes, total area of tissue fragments, and total stained area of each microscope slide. For all probe types, these parameters were significantly and positively correlated with cut rate. TDC probes led to significantly higher scores than standard probes, independent of cut rate. There were no significant differences in results when using 23-gauge or 25-gauge standard probes. Light microscopic examination demonstrated well-preserved cells sufficient for cytohistological analyses in all investigated cases.

***Conclusions*:** The higher the cut rate, the larger is the amount of aspirated cellular material. There were no significant differences between 23-gauge and 25-gauge biopsies. Cut rates up to 6000 cpm did not adversely affect the cytohistological features of the samples.

Introduction

Biopsy of intraocular tumors is increasingly used to secure the diagnosis and provide material for histo- and immunohistochemical, molecular, and genetic analyses. Genetic testing of uveal melanoma tissue provides additional prognostic information on the risk of developing metastatic disease, which is important for patient counselling and to ensure appropriate inclusion in future clinical trials [1, 2]. Patients at high risk for metastatic disease can be monitored more closely and receive treatment at an early stage of the disease [3, 4].

Biopsy with a vitreous cutter has proved to provide sufficient material for histopathological and genetic testing of both anterior and posterior segment tumors [3, 5]. Ocular complications are rare, with intraocular hemorrhages and retinal detachment being the most commonly reported [3, 6, 7]. To reduce the risk of complications, the biopsy should be done with as little manipulation of the vitrectomy probe as possible. Still, it is important to obtain enough tissue for analysis, which may be especially difficult in small tumors and tumors in the anterior eye segment considering the limited time and space available for the procedure. The method of using a vitrectomy probe for tissue sampling is well known and described [7–9]. However, the optimal settings for vitrectomy-assisted biopsies have not been thoroughly investigated. In particular, there is no consensus on the most appropriate cut rate for tissue sampling. Furthermore, vitrectomy probes ranging from 20 to 27 gauges have all been reported to be safe and effective for biopsy of intraocular tumors [3], but direct comparisons between various probe types are lacking.

Here, we report the results of an experimental study evaluating the efficacy of vitrectomy-assisted biopsy and comparing the cytohistological results of biopsies obtained by different vitrectomy probes and cut rates in an in vitro porcine model.

Materials and Methods

Study Design

The study was conducted at the Departments of Ophthalmology at Haukeland University Hospital, Bergen, Norway, and at Rigshospitalet, University of Copenhagen, Copenhagen, Denmark. At both centers, vitrectomy-assisted biopsies were obtained from fresh ex vivo porcine livers using an identical sampling procedure. The biopsies obtained at Haukeland University Hospital were prepared for smear analysis, and those obtained at Rigshospitalet were prepared for microscopy by cytospin. The following variable vitrectomy parameters were compared with respect to the cytohistological results: cut rate (60, 600, and 6000 cuts per minute (cpm)), vitrectomy probe type (standard and two-dimensional cutting (TDC)), probe diameter (23-gauge and 25-gauge), and whether the probe was filled with fluid or air at the start of the procedure.

Vitrectomy Instrumentation and Biopsy Technique

The biopsies were performed with the Eva vitrectomy system (DORC International, Zuidland, The Netherlands) using either a 23- or 25-gauge standard probe or a 23- or 25-gauge TDC probe. A large portion of fresh porcine liver was placed in a container filled with saline. The vitrectomy system was first primed, and for half of the 23-gauge standard probe biopsies, air was aspirated so that the probe and tubing was filled with air at the start of the biopsy procedure. The vitrectomy probe was then inserted through the liver capsule and deep into the hepatic parenchyma. Each sampling was performed

in vacuum mode with the vacuum level set at 300 mmHg. The probe was held still during an aspiration time of 30 seconds to reduce unintended variations associated with the sampling procedure. Each vitrectomy probe was used for a maximum of 6 consecutive biopsies and then discarded.

Sample Preparation

For the biopsies prepared at Haukeland University Hospital, the cutter portal was placed directly on a glass slide. All the aspirated material was expressed by using an air-filled syringe connected to the vitrectomy aspiration tube to gently blow the material through the probe onto the slide. Once the content was expelled on the slide, a second glass slide was placed lightly on and parallel to the sample slide, and pulled lengthways to thinly spread the sample. After air-drying, both slides were stained with hematoxylin-eosin (HE) and further processed for cytohistology.

For the biopsies prepared at Rigshospitalet, the aspirate was withdrawn and flushed via the aspiration tube into an empty syringe. Portions of approximately 0.1 ml aspirate were prepared for cytospin by centrifugation at 1500 rpm for 5 minutes (Shandon Cytospin 4; Thermo Electron Corporation, Cheshire, UK) onto glass slides. The slides were subsequently air-dried and stained with HE.

Cytohistological Evaluation and Quantification Techniques

The HE-stained samples were digitized using an automated whole-slide scanner (Scan Scope XT; Aperio Technologies, Vista, CA) with a 40x objective magnification and a scanning resolution of 0.247 μm/pixel (Fig. 1a,b). Automated whole-slide imaging analyses with respect to the number of hepatocytes, the total area of cell clumps and tissue fragments (mm^2), and the total stained area (mm^2) of each slide were performed

with Aperio algorithms and ImageScope software v.6 (Aperio Technologies, Vista, CA). The automated analyses enable quantification of specific colored tissue types and cellular structures by color thresholding. The Nuclear algorithm detects the nuclear staining of individual cells and classifies them based on their staining intensity. The output is the number of detected cell nuclei in each class (Fig. 1c). The Color Deconvolution algorithm visually highlights stained areas in different colors depending on staining intensity, and outputs the respective total area (mm^2) of the slide (Fig. 1d,e).

In addition to the automated imaging analyses, the HE-stained samples were examined by conventional light microscopy. For each vitrectomy setting combination, the following cytohistological features were evaluated: (1) the amount of diagnostic cellular material present; (2) the degree of cellular degeneration and trauma; (3) the retention of cellular architecture [10].

Statistical Analysis

Continuous variables were given as mean ±standard deviation (SD) and assessed by nonparametric analyses using the Kruskal–Wallis test for comparison between three groups and the Mann–Whitney U test for comparison between two groups. In the statistical analyses, each slide was considered as a statistical unit. The data were analyzed using IBM SPSS Statistics v.25 (SPSS Inc., Chicago, IL). Two-tailed *p*-values <0.05 were considered statistically significant.

Results

Smear Preparations

Based on 48 vitrectomy-assisted biopsies performed at Haukeland University Hospital, a total of 92 smear slides were available for analyses. The automated imaging analysis of

the smear slides showed a statistically significant positive correlation between the number of hepatocytes and the cut rate of the vitrectomy probe. For all specimens obtained by 23- and 25-gauge standard probes (24 and 12 biopsies, respectively), the mean (±SD) number of hepatocytes per slide was 41322 (±37681), 132559 (±126724), and 130600 (±115179) for a cut rate of 60, 600, and 6000 cpm, respectively (p=0.001). Similarly, the total area of cell clumps and tissue fragments as well as the total stained area, were significantly and positively correlated with the cut rate. The mean total area of cell clumps and tissue fragments per slide was 2.0 mm^2 (±2.6), 6.3 mm^2 (±8.4), and 4.4 mm^2 (±4.8) for a cut rate of 60, 600, and 6000 cpm, respectively (p=0.013). The mean total stained area per slide was 37.8 mm^2 (±38.0), 131.4 mm^2 (±97.9), and 155.9 mm^2 (±135.2) for a cut rate of 60, 600, and 6000 cpm, respectively (p <0.0001) (Fig. 2a–c).

For specimens obtained by 23-gauge TDC probes (12 biopsies), the mean number of hepatocytes per slide was 98154 (±54049), 162344 (±102500), and 498799 (±282638) (p=0.004), the mean total area of cell clumps and tissue fragments per slide was 7.9 mm^2 (±3.9), 24.8 mm^2 (±11.0), and 43.6 mm^2 (±21.1) (p=0.001), and the mean total stained area per slide was 120.4 mm^2 (±97.8), 169.0 mm^2 (±86.9), and 452.0 mm^2 (±222.2) (p=0.013) with a cut rate of 60, 600, and 6000 cpm, respectively (Fig. 3a–c). The quantity of each of these cytohistological components was significantly higher for specimens obtained by the TDC probes compared to those obtained by the standard probes, independent of cut rate.

There were no statistically significant differences between the use of 23- and 25-gauge standard probes regarding the number of hepatocytes, total area of cell clumps and tissue fragments, and total stained area per slide. Neither was there any clear and systematic difference between the quantities of these cytohistological components

depending on whether the 23-gauge standard probes were filled with fluid (12 biopsies) or air (12 biopsies) at the beginning of the biopsy procedure.

Cytospin Preparations

Based on 24 vitrectomy-assisted biopsies performed at Rigshospitalet, a total of 36 cytospin slides were available for analyses. The automated imaging analysis of the cytospin slides revealed a positive correlation between the cytohistological components and the cut rate of the vitrectomy probe, although this was only statistically significant for the area of cell clumps and tissue fragments. For all specimens obtained by 25-gauge standard and 25-gauge TDC probes (12 and 12 biopsies, respectively), the mean number of hepatocytes per slide was 1229 (±2264), 3287 (±4401), and 4193 (±5371) (p=0.23), the mean total area of cell clumps and tissue fragments per slide was 0.2 mm^2 (±0.4), 0.3 mm^2 (±0.3), and 0.7 mm^2 (±0.7) (p=0.02), and the mean total stained area per slide was 3.8 mm^2 (±4.0), 4.4 mm^2 (±4.8), and 7.1 mm^2 (±6.8) (p=0.27) for a cut rate of 60, 600, and 6000 cpm, respectively.

Conventional Light Microscopic Evaluation

Conventional light microscopy revealed that cellular material was present in sufficient quantity and quality to establish the presence of normal hepatic cells and tissue fragments in all the HE-stained samples. An estimate of the amount of cellular material present on each slide corresponded well with the results of the automated whole-slide imaging analysis, showing a positive correlation with cut rate. No differences were noted between the different vitrectomy settings regarding the degree of cellular degeneration and trauma, and retention of cellular architecture.

Discussion

It is generally assumed that vitrectomy-assisted biopsy provides a larger sample of tissue than fine-needle aspiration biopsy [5–7, 9], but a reduction in cell viability and damage of cellular structures by the vitreous cutter have been considered a problem. Most authors recommend a relatively low cut rate (between 80–600 cpm) when performing vitrectomy-assisted biopsies [3, 6, 8, 11]. This is probably due to the belief that a low cut rate reduces the risk of cell damage by decreasing the shear stress on the tissue and also provides larger pieces of tumor tissue. Only a few studies have evaluated the effect of various cut rates on the diagnostic yield and quality of the biopsies. Ratanapojnard et al. examined specimens from bacterial, fungal, and leukocyte suspensions and found that although the leukocyte viability was markedly diminished, the clinical utility of all specimen types were well preserved with cut rates up to 1500 cpm [12]. In a rabbit uveitis model, Huang et al. found no difference in loss or damage of lymphocytes sampled at various cut rates up to 600 cpm [13]. Lymphoma cells are very fragile and can easily degenerate during vitreous sampling [14, 15]. By evaluation of cultured B-cell lymphoma cells obtained by a 20-gauge vitrectomy probe and cut rates between zero (aspiration only) and 2500 cpm, Jiang et al. reported a reduced number of viable cells and an increased damage of cell morphology at cut rates above 600 cpm [16].

The main finding of the present study was the significant positive correlation between all the cytohistological parameters and the cut rate of the vitrectomy probe. This correlation was even stronger for specimens obtained by the TDC probes, which can be explained by the probe design. The TDC probes facilitates cutting both forward and backward of each stroke, actually doubling the predetermined cut rate. In addition to an increased number of hepatocytes, higher cut rates also led to more cell clumps and

tissue fragments. This was a rather unexpected finding in view of the common belief that a low cut rate allows the probe port to be filled with more tissue between each cut, thereby producing larger pieces of tissue. A likely explanation is that, due to the elasticity of solid tissue, a low cut rate allows the tissue to retract from the probe port between each cut. We also found a positive correlation between the total stained area of each slide and the cut rate. As all smears were made in the same way by the same person and all the aspirated material was used for the smear preparation, the total stained area may reflect the amount of the aspirate. Because higher cut rates lead to lower aspiration flow, a positive correlation between aspirate volume and cut rate was unexpected. However, as opposed to ordinary vitrectomies where the material to be aspirated is mostly gelatinous or liquid, a vitrectomy probe placed within solid tissue can only remove the adjacent tissue and limited amounts of blood and plasma.

Contrary to the standard probes, the port of the TDC probes is always open, with a constant aspiration flow independent of cut rate, so that the tissue is continuously sucked into the probe port during cutting. The combination of a constant aspiration flow and a high actual cut rate may thus explain the significantly higher cytohistological scores obtained by the TDC probes. Also for the cytospin slides, which were prepared by using just a fixed portion and not the entire aspirate, there was a positive correlation between all the cytohistological scores and the cut rate. Although this was statistically significant only for the area of cell clumps and tissue fragments, the results indicate that a high cellular yield is not only due to a large aspirate volume, but also to a high cut rate per se.

The success rate of intraocular biopsy is highly dependent on tumor size [3, 17]. For all the vitrectomy probes used in the study, the distance from the distal end of the probe to the cutter port is only 0.22 mm, making it possible to biopsy even small tumors. The

strong positive correlation between the diagnostic yield and cut rate suggests that high-speed cutting is particularly advantageous in small tumors, due to the limited space to orient and maneuver the probe. We found no statistically significant differences in any of the examined cytohistological parameters between samples obtained by 23- and 25-gauge standard probes. Analogous results have been found with conventional fine-needle aspiration biopsy of other organs, where the diagnostic yield has proved to be similar between 22- and 25-gauge needles [18, 19]. There was also no clear difference in outcomes between standard vitrectomy probes filled with fluid or air prior to the biopsy procedure. This may indicate that our fixed aspiration time was sufficient to remove all the air from the system before harvesting the sample.

Evaluation by conventional light microscopy revealed a sufficient quantity and quality of the cellular material to establish the presence of normal hepatic cells and tissue fragments in all the samples, and no major differences were found between those obtained with different vitrectomy probes or cut rates. These results differ from those reported in previous studies on leukocytes and lymphoma cells, showing a reduction in the number of viable cells and an increase in cell damage with higher cut rates [12, 16]. However, for tissues and solid tumors with less vulnerable cells, we believe that the risk of cell damage is low and outweighed by the benefits of high cut rates to optimize the diagnostic yield of the samples.

Over the last decades, genetic testing has become increasingly important in the management of uveal melanoma. Many studies have shown that small-gauge vitrectomy-assisted biopsy yields sufficient material for various types of cytogenetic analyses and gene expression profiling [5, 7, 20]. A large sample size also reduces the risk of misclassification due to tumor heterogeneity [5]. In the present study, no attempts were made to compare the yield and quality of RNA and DNA from the tissue samples.

As we found a high number of intact cells in all the obtained samples, and the amount of cellular material is the main limiting factor in molecular and genetic analyses, it seems reasonable to assume that our results also apply to genetic testing.

The present study has several limitations. First and foremost, our in vitro setting does not fully reflect the in vivo clinical situation. Given the different properties and volumes of the tissue to be sampled, the translatability of our results to intraocular tumor biopsies is unsettled. However, compared to an ocular tumor model, our approach makes it easier to control variables and standardize all the vitrectomy procedures. The liver also offers a homogenous tissue with characteristic cell morphology, well suited for this type of study. Second, in order to reduce the number of variables, the vacuum was set to a fixed value for all the experiments, and other vacuum levels may have provided different results.

In summary, we found that the higher the cut rate, the larger is the amount of aspirated cellular material. Cut rates up to 6000 cpm for standard probes, equivalent to an effective cut rate of 12000 cpm for TDC probes, did not adversely affect the cytohistological features or quality of the samples. No differences were found between the use of 23-gauge and 25-gauge vitrectomy probes. Considering the general advantages of small-gauge vitrectomy surgery, our results suggest that the use of a 25-gauge TDC probe with a high cut rate provides the best diagnostic yield in vitrectomy-assisted biopsies of intraocular tumors.

Acknowledgement

We thank Associate Professor Sabine Leh for sharing resources and advice on automated whole-slide imaging analysis.

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The paper is exempt from ethical committee approval because no human subjects or live animals were used in this study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare. Dr. Heegaard is an Editorial Board Member of Ocular Oncology and Pathology.

Funding Sources

Supported in part by research grants from Fight for Sight Denmark. DORC Scandinavia AB kindly provided the vitrectomy probes used at Haukeland University Hospital, but had no influence on study design or implementation.

Author Contributions

E.U., J.F.K., and J.K. planned and designed the study. J.K. and E.U. drafted the manuscript. E.U., J.F.K., N.M., D.S., S.H., and J.K. contributed to data acquisition and analysis, revision of the manuscript, and approval of the final version to be published.

References

1. Singh AD, Shields CL, Shields JA. Prognostic factors in uveal melanoma. Melanoma Res. 2001 Jun;11(3):255–63.
2. Damato B, Eleuteri A, Taktak AF, Coupland SE. Estimating prognosis for survival after treatment of choroidal melanoma. Prog Retin Eye Res. 2011 Sep;30(5):285–95.
3. Frizziero L, Midena E, Trainiti S, Londei D, Bonaldi L, Bini S, et al. Uveal Melanoma Biopsy: A Review. Cancers (Basel). 2019 Jul;11(8):1075.
4. Staby KM, Gravdal K, Mørk SJ, Heegaard S, Vintermyr OK, Krohn J. Prognostic impact of chromosomal aberrations and GNAQ, GNA11 and BAP1 mutations in uveal melanoma. Acta Ophthalmol. 2018 Feb;96(1):31–8.
5. Bagger M, Andersen MT, Heegaard S, Andersen MK, Kiilgaard JF. Transvitreal Retinochoroidal Biopsy Provides a Representative Sample From Choroidal Melanoma for Detection of Chromosome 3 Aberrations. Invest Ophthalmol Vis Sci. 2015 Sep;56(10):5917–24.
6. Finn AP, Materin MA, Mruthyunjaya P. Choroidal tumor biopsy: A Review of the Current State and a Glance Into Future Techniques. Retina. 2018 Sep;38 Suppl 1:S79–S87.
7. Bagger M, Tebering JF, Kiilgaard JF. The ocular consequences and applicability of minimally invasive 25-gauge transvitreal retinochoroidal biopsy. Ophthalmology. 2013 Dec;120(12):2565–72.
8. Finger PT, Latkany P, Kurli M, Iacob C. The Finger iridectomy technique: small incision biopsy of anterior segment tumours. Br J Ophthalmol. 2005 Aug;89(8):946–9.
9. Sen J, Groenewald C, Hiscott PS, Smith PA, Damato BE. Transretinal choroidal tumor biopsy with a 25-gauge vitrector. Ophthalmology. 2006 Jun;113(6):1028–31.
10. Mair S, Dunbar F, Becker PJ, Du Plessis W. Fine needle cytology--is aspiration suction necessary? A study of 100 masses in various sites. Acta Cytol. 1989 Nov-Dec;33(6):809–13.
11. Grewal DS, Cummings TJ, Mruthyunjaya P. Outcomes of 27-Gauge Vitrectomy-Assisted Choroidal and Subretinal Biopsy. Ophthalmic Surg Lasers Imaging Retina. 2017 May;48(5):406–15.
12. Ratanapojnard T, Roy CR, Gariano RF. Effect of vitrector cutting rate on vitreous biopsy yield. Retina. 2005 Sep;25(6):795–7.

13. Huang JS, Russack V, Flores-Aguilar M, Gharib M, Freeman WR. Evaluation of cytologic specimens obtained during experimental vitreous biopsy. Retina. 1993 13(2):160–5.
14. Whitcup SM, de Smet MD, Rubin BI, Palestine AG, Martin DF, Burnier M Jr, et al. Intraocular lymphoma. Clinical and histopathologic diagnosis. Ophthalmology. 1993 Sep;100(9):1399–406.
15. Intzedy L, Teoh SC, Hogan A, Mangwana S, Mayer EJ, Dick AD, et al. Cytopathological analysis of vitreous in intraocular lymphoma. Eye (Lond). 2008 Feb;22(2):289–93.
16. Jiang T, Zhao Z, Chang Q. Evaluation of cytologic specimens obtained during experimental vitreous biopsy using B-cell lymphoma line. Eur J Ophthalmol. 2014 Nov-Dec;24(6):911–7.
17. Singh AD, Medina CA, Singh N, Aronow ME, Biscotti CV, Triozzi PL. Fine-needle aspiration biopsy of uveal melanoma: outcomes and complications. Br J Ophthalmol. 2016;100(4):456–62.
18. Siddiqui UD, Rossi F, Rosenthal LS, Padda MS, Murali-Dharan V, Aslanian HR. EUS-guided FNA of solid pancreatic masses: a prospective, randomized trial comparing 22-gauge and 25-gauge needles. Gastrointest Endosc. 2009 Dec;70(6):1093–7.
19. Madhoun MF, Wani SB, Rastogi A, Early D, Gaddam S, Tierney WM, et al. The diagnostic accuracy of 22-gauge and 25-gauge needles in endoscopic ultrasound-guided fine needle aspiration of solid pancreatic lesions: a meta-analysis. Endoscopy. 2013;45(2):86–92.
20. Nagiel A, McCannel CA, Moreno C, McCannel TA. Vitrectomy-assisted biopsy for molecular prognostication of choroidal melanoma 2 mm or less in thickness with a 27-gauge cutter. Retina. 2017;37(7):1377–82.

Figure Legends

Figure 1 a–e

Whole slide image of a porcine liver smear obtained with a 23-gauge two-dimensional cutting (TDC) vitrectomy probe and a cut rate of 6000 cuts per minute (hematoxylin-eosin stain; bar = 6 mm) (**a**). Magnified view of the region enclosed by the blue rectangle in (**a**) is shown in (**b**) (bar = 200 μm). The same image as in (**b**); analyzed by the Nuclear algorithm (Aperio Technologies, Vista, CA) to quantify the number of hepatocyte nuclei, which are highlighted in blue (weaker staining) and yellow (stronger staining) (**c**); analyzed by the Color Deconvolution algorithm to quantify the total area of cell clumps and tissue fragments (**d**); analyzed again by the Color Deconvolution algorithm (with different settings) to quantify the total stained area, which is highlighted in yellow, orange, and red for regions with weak to strong staining intensity (**e**).

Figure 2 a–c

Box plots illustrating the number of hepatocytes (**a**), the total area of cell clumps and tissue fragments (**b**), and the total stained area (**c**) of each microscope slide obtained with 23- and 25-gauge standard vitrectomy probes, grouped according to cut rate (60, 600, and 6000 cuts per minute). Middle line: the median; bottom and top box edges: the 25th and the 75th percentiles; whiskers: the most extreme values within 1.5 interquartile ranges; circles: outliers; asterisk: extreme values.

Figure 3 a–c

Box plots illustrating the number of hepatocytes (**a**), the total area of cell clumps and tissue fragments (**b**), and the total stained area (**c**) of each microscope slide obtained with 23-gauge two-dimensional cutting (TDC) vitrectomy probes, grouped according to cut rate (60, 600, and 6000 cuts per minute). Box plot details are as described in Figure 2.