Since the outbreak of respiratory coronavirus disease (COVID-19) caused by the coronavirus SARS-CoV-2, there is an ongoing discussion about whether the virus could be transmitted through corneal transplantation from donor to recipient. This is of particular concern to eye banks and surgeons while at the same time there is a global challenge of decreasing donation. The purpose of this review was to summarize the current opinions in the scientific community to address some of these concerns and to provide guidance in evaluating the risk for potential virus transfer by corneal transplants.

A literature search was done in PubMed.gov up to February 4, 2021 for relevant articles using keywords such as “COVID-19”, “coronavirus”, and “SARS-CoV-2” in conjunction with “cornea processing”, “cornea transplantation”, “eye banking” and “donation”. The publications also include letters to the editor or preliminary reports that were not peer-reviewed. Further, guidelines from health authorities and eye banking associations were reviewed. Studies have shown that SARS-CoV-2 RNA can be detected in ocular swabs and/or fluid of patients with COVID-19. However, the risk of SARS-CoV-2 virus transmission through these ocular tissues or fluid of patients is judged differently. To date, per literature and official guidelines, no evidence of viable virus in ocular tissue and no cases of transmission of SARS-CoV-2 via tissue preparations have been reported.

Key words: Cornea, cornea processing, COVID-19, eye banking, SARS-CoV-2, virus transmission

SARS-CoV-2 virus
SARS-CoV-2 belongs to the family of coronaviruses. It is one of several human pathogen coronaviruses that are known. Especially SARS-CoV, MERS-CoV, and SARS-CoV-2 can cause life-threatening diseases. It is a single-stranded positive-sense RNA virus with a genome of ca. 30 kb in length. Genetically, SARS-CoV-2 is about 70% similar to SARS-CoV and it utilizes the same cell entry to infect human cells, the angiotensin-converting enzyme II (ACE2) receptor. The SARS-CoV-2 glycoprotein spike binds to ACE2 receptors at a 10- to 20-fold higher affinity than SARS-CoV.[9] In corneal and conjunctival epithelial cells, expression of the ACE2 gene has been detected, but to a lower extent compared to other tissues. Other studies have shown that ACE-2 enzyme seems only present in the retina and aqueous humor.[39,40] It is currently unclear if sole ocular contact with SARS-CoV-2 can manifest in an infection.[7]

Virus detection in ocular secretion of patients with COVID-19
In several studies, SARS-CoV-2 RNA has been detected in tear film and/or conjunctival swabs of patients with COVID-19 by reverse transcription polymerase chain reaction (RT-PCR).[7-11] The studies have in common that a positive detection in ocular swabs, other than in nasopharyngeal swabs, applied only to a low number of cases within the investigated groups. When publications mentioned ocular manifestations associated with COVID-19, conjunctivitis was mainly noted in a small number of patients.[39] Table 1 provides an overview of the literature cited.

Based on the results, most authors see an indication that virus transmission through tears is likely low and conjunctival secretions of patients without conjunctivitis are not an infectious route for SARS-CoV-2.[11,12] On the other hand, limited evidence for a potential ocular route of transmission...
Conjunctival and nasopharyngeal swabs

Ocular conditions

38

Review Articles

Conjunctival swabs, tear fluid, sputum

Samples

Sputum samples

Review examining the current evidence for the hypothesis that ocular surface can be a site of infection with SARS-CoV-2

Similar results were published by Casagrande et al., who investigated corneal discs from patients who died of COVID-19. In 55% of the samples the PCR resulted positive for virus RNA with low viral loads compared to the corresponding blood samples. However, histologically the corneal structure was preserved regardless of positive or negative PCR results. No SARS-CoV-2 spike protein could be detected by immunohistochemistry in corneal cells in any of the samples, nor could the virus be isolated in any of the corneal discs. Another study investigated tissue samples of central nervous system of COVID-19 deceased patients. The samples comprised branches of the trigeminal nerve which innervates the cornea and conjunctiva amongst others. Virus RNA was detected in both types of samples but only at low levels and few patients.

Current statements of national bodies and eye bank associations on risk of SARS-CoV-2 virus transmission

Several official recommendations can be consulted for guidance. National authorities, such as the US Food and Drug Administration (FDA) and German Federal Institute for Vaccines and Biomedicines (Paul-Ehrlich-Institute, PEI) as well as eye banking associations state a very low risk of potential virus transmission by transplantation and propose precautionary measures. An overview of current statements is provided in Table 2.

National bodies

According to FDA’s updated Information for Human Cell, Tissue, or Cellular or Tissue-based Product (HCT/P) Establishments, there have been no reported cases of transmission of COVID-19 via these products. Routine screening measures are already in place to evaluate clinical
Evidence of infection in HCT/P donors. FDA does not recommend using laboratory tests to screen asymptomatic HCT/P donors.\(^{[17]}\)

Moreover, the German PEI proposes as precautionary measures to exclude potential tissue donors with confirmed SARS-CoV-2 infection within a certain timeframe before donation.\(^{[18]}\) These recommendations remained unchanged also after the first update provided by the European Centre for Disease Prevention and Control (ECDC) on “Coronavirus disease – 2019 (COVID-19) and supply of substances of human origin in EU/EEA”.\(^{[19]}\)

### Table 2: Overview of current statements of national bodies and eye bank associations on risk of SARS-CoV-2 virus transmission

| Association/National authority | Country/ Region | Statement | Latest Update |
|-------------------------------|----------------|-----------|---------------|
| Food and Drug Administration (FDA) | USA | FDA continues to monitor the coronavirus disease 2019 pandemic caused by the virus, SARS-CoV-2. Respiratory viruses, in general, are not known to be transmitted by implantation, transplantation, infusion, or transfer of human cells, tissues, or cellular or tissue-based products (HCT/Ps). To date, there have been no reported cases of transmission of COVID-19 via these products. | Jan 4, 2021 |
| Paul-Ehrlich Institute (PEI) | Germany | A transmission of respiratory viruses by implantation, transplantation, infusion or transfer of human cells or tissues has not yet been described. The potential for transmission of SARS-CoV-2 through tissue preparations is currently unknown, but no cases of transmission of SARS-CoV-2 via tissue preparations have been reported. Precautionary measures proposed: exclusion of potential tissue donors 1) upon contact with people with confirmed SARS-CoV-2 infection within 14 days before the donation, and 2) with confirmed infection within 14 days after completion of the recovery. | May 4, 2020 |
| European Centre for Disease Prevention and Control (ECDC) | Europe | Suggestion to allow donation of persons who have lived in or visited areas of sustained community transmission of the virus only if tested negative for virus RNA within 72 hours before procurement. Based on the current knowledge of ACE2 and TMPRSS2 distribution, the absence of evidence for infectivity of viral RNA detectable in blood, cells, tissues and organs and any reports of transfusion and transplantation-transmitted cases, the risk of COVID-19 transmission through SoHO remains theoretical. | First update, April 29, 2020; Second update, December 10, 2020 |
| Global Alliance of Eye Bank Associations (GAEBAS) | Global | There is no evidence that coronaviruses can be transmitted by human tissue or cell transplantation and therefore measures in this response are precautionary. There have been no reported cases of transmission of SARS-CoV-2, MERS-CoV, or any other coronavirus via transplantation of human ocular tissue. | November 12, 2020 |
| All India Ophthalmological Society (AIOS) | India | Guidelines by an expert panel on how to restart eye banking and eye collection services during the COVID-19 pandemic. Since May 2020, the Eye Banking activities to be resumed through hospital cornea retrieval program (HCRP) and to be from a hospital which is declared as non-COVID | May 11, 2020 |
| Eye Bank Association of India (EBAI) | India | Currently there is no evidence to suggest the spread of coronaviruses by blood transfusion or tissue transplantation. Retrieval of corneas from home settings is allowed with all precautions being taken to prevent spread of infection to technicians and to the recipient of corneas. Corneas may be utilized for therapeutic as well as optical purposes. | December 28, 2020 |
| European Eye Bank Association (EEBA) | Europe | Referring to GAEBAS and ECDC. The presence of viruses capable of reproduction after PVP iodine disinfection procedure seems very unlikely. | Jan 10, 2020 |
| European Association of Cell and Tissue Banks (EATCB) | Europe | Referring to GAEBAS and ECDC. | March 23, 2020 |

**Eye Bank Associations**

In accordance with national bodies, eye banking associations including the Global Alliance of Eye Bank Associations (GAEBAS) confirm no evidence at present that coronaviruses can be transmitted by tissue transplantation.\(^{[20]}\)

Following a national transplant specific guidance for COVID-19 published by the Eye Bank Association of India (EBAI), a recent paper highlights the consensus-based guidelines by an expert panel of the All-India Ophthalmological Society (AIOS) and the EBAI on how to restart eye banking and eye collection services during the pandemic.\(^{[21, 22]}\) These
guidelines apply to all eye banks across the country and should help ophthalmologists and eye banking staff to resume eye banking while safeguarding themselves.[12]

The European Eye Bank Association (EEBA) and European Association of Cell and Tissue Banks (EATCB) refer to guidance provided by the ECDC (1st update, April 2020): corneal transplants are usually disinfected with Povidone (PVP) iodine and then stored in organ culture at 30–37°C for at least 14 days. The presence of viruses capable of reproduction after this procedure seems very unlikely. These data and the absence of known ocular transmission cases indicate that the risk of COVID-19 cases entering the eye donor pool and subsequent transmission is theoretical.[23,24]

Risk of virus transmission through donation and processing of corneal tissue

In general, virus presence in the donor cornea and complication in the recipient cannot be completely excluded. Exemplarily, Broniek et al. investigated corneal tissue and corneal preservation fluid of postmortem donors for presence of human herpesviruses (HHV-1 and HHV-2) and human adenovirus (HAdV). HHV-1 or HAdV DNA was detected in three out of 57 corneal tissue samples and clinical follow up of the recipients of these corneas showed complications in two cases where the recipient was older and immunosuppressed.[25]

These types of DNA virus differ from coronaviruses; thus, no direct translation can be made but the small number of positive samples seems to be comparable to the detection levels of SARS-CoV-2 virus RNA found in the recent studies.

Our literature search revealed that very few studies discuss the question whether SARS-CoV-2 virus could be transmitted through cornea donation and induce a COVID-19 disease (see overview of publications in Table 3). To our knowledge, no case of SARS-CoV-2 transmission through ocular tissue transplantation has been reported to date. A study by Bayyoud et al. reported no SARS-CoV-2-RNA detection in the cornea, conjunctiva or aqueous humor of five COVID-19 positive postmortem donors.[26] The authors acknowledge the small number of patients and a possibility of false-negative testing. Nevertheless, they conclude that the risk for SARS-CoV-2 infection or transmission via corneal or conjunctival tissue seems very low.

Table 3: Overview of publications discussing potential virus transmission through cornea donation

| Author [Reference] | Samples | Patients | Investigations | Main result |
|--------------------|---------|----------|----------------|-------------|
| Broniek G, 2017[26] | 57 paired samples (corneal tissue remaining after trepanation and corneal preservation fluid) | 57 postmortem donors | PCR for viral DNA | Viral DNA was detected in 3 out of 57 corneal tissue samples: HHV-1 DNA in 1 cornea and 1 preservation sample of the same donor (1.8%), and adenovirus DNA in 2 cornea samples (3.5%) |
| Bayyoud T, 2020[26] | 10 corneas | 5 postmortem donors, COVID-19 patients | PCR for virus RNA in ocular tissues and intraocular fluid | No SARS-CoV-2-RNA was detected in conjunctiva, anterior chamber fluid and corneal tissues (endothelium, stroma and epithelium). |
| Casagrande M, 2021[15] | 11 Corneas (1 per patient). As reference: conjunctival swabs, throat swabs, blood samples, aqueous humor and vitreous humor samples | 11 deceased COVID-19 patients | PCR for genomic and subgenomic virus RNA Immunohistochemistry of SARS-CoV-2 spike protein | SARS-CoV-2 genomic RNA was detected in the cornea of 6 of 11 eyes (55%) of patients with viremic coronavirus disease. Subgenomic SARS-CoV-2 RNA was present in 4 of these 6 eyes (67%). Infectivity or presence of viral structural proteins could not be detected in any eye. |
| Sawant OB, 2020[26] | 132 corneal and scleral tissue samples | 33 postmortem donors, 10 out of 33 were patients with COVID-19 | PCR for virus RNA in ocular tissues Immunohistochemistry for SARS CoV-2 envelope and spike protein | Small prevalence of SARS-CoV-2 in ocular tissues from COVID-19 donors: In 20 eyes from 10 COVID-19 donors: 3 conjunctival, 1 anterior corneal, 5 posterior corneal, and 3 vitreous swabs tested positive for SARS-CoV-2 RNA. SARS-CoV-2 spike and envelope proteins detected in epithelial layer of corneas that were procured without Povidone-Iodine disinfection. No protein detection in samples of disinfected eyes. |
| Miner JJ, 2020[27] | 25 corneas | 25 postmortem donors | For 7 out of 25: PCR for virus RNA in tissue samples that were inoculated with SARS-CoV-2 virus, others with HSV-1 and ZIKV | No SARS-CoV-2 replication in human cornea |
| Thaler S, 2020[29] | Discussing the importance of corneal organ culture in donors with possible SARS-CoV-2 infections and a potential routine testing of the medium. | | | |

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Salz, et al.: Risk of SARS-CoV-2 transmission from donor cornea
In a recent publication Miner et al. investigated whether a clinical isolate of SARS-CoV-2 virus could replicate in human donor corneal tissue.[28] In seven independent donor samples they could not detect any evidence of SARS-CoV-2 replication, whereas herpes simplex virus (HSV-1) used as control could replicate in portions of the same donor corneas. Based on their results the authors suggest that the human cornea does not support a SARS-CoV-2 infection despite expression of ACE2 receptor in the human corneal epithelium.

Sawant et al. evaluated the presence of SARS-CoV-2 RNA and proteins in ocular tissues of COVID-19 positive and negative post-mortem donors.[29] Amongst the 20 eyes of ten positive patients, some swabs were positive for virus RNA, resulting in a positivity rate of 5% for anterior and 25% for posterior corneal surface. Immunohistochemistry for SARS CoV-2 envelope and spike protein revealed positive results in three out of ten samples that were procured without any PVP iodine disinfection. In contrast, no spike protein could be visualized in PVP-treated corneas. The findings show a small prevalence of SARS-CoV-2 in ocular tissues from COVID-19 donors. Hence the authors stress the importance of post-mortem PCR testing, PVP iodine disinfection and donor screening guidelines in eye banking to eliminate the possibility of handling tissues with SARS-CoV-2 for corneal transplantation.

Regarding corneal processing in the eye bank Thaler et al. point out that corneal organ culture, which is mainly used in European eye banks, are alike to culture conditions that are used for in vitro virus analyses. Hence, they suggest considering a routine testing of the organ culture medium used for donor corneas for SARS-CoV-2 prior to transplantation during the pandemic.[30] To the best of our knowledge, no data on virus detection in cornea culture medium have been published so far. In parallel, no study results seem to be available by now concerning SARS-CoV-2 detection in relation to cold storage of cornea, which is mainly used in e.g., India and the USA.

The same work group noted that based on the above findings and the current eye banking associations’ recommendations it should be safe to transplant corneas from donors whose postmortem donor tissue tested negative with a validated test.[31] Regrettably, the validation of RT-PCR testing of postmortem donors is still pending. The impact of the time window for testing and the importance of validated tests to obtain meaningful results is also addressed in a case report by Wille et al. on a postmortem cornea donor.[32]

Discussion

The current literature reveals that there are heterogenous opinions about a potential risk of SARS-CoV-2 transmission through ocular tissue, while the study results are similar. So far, ocular manifestations such as conjunctivitis and detection of virus RNA in conjunctival secretions or tears have occurred rarely and primarily in people with symptomatic COVID-19 and a rather severe course of the disease. At large, the current data suggest the virus is unlikely to bind to the ocular surface to initiate infection.[14]

In general, a positive detection of SARS-CoV-2 RNA in tears and conjunctival secretions of a patient with conjunctivitis does not imply that the virus can replicate in the conjunctiva.[32] According to Peng and Zhou, the absence of SARS-CoV-2 RNA in the ocular samples of the COVID-19 patients without conjunctivitis provides evidence that the virus does not replicate in conjunctival epithelia, indicating that SARS-CoV-2 is less likely transmitted through the conjunctiva.[33] Results published by Miner et al. and Casagrande et al. support this opinion since no SARS-CoV-2 replication or spike protein was detected in human cornea.

With regards to tissue and especially cornea donation, the studies by the work group around Bayyoud and Thaler represent a very important step in better understanding implications of SARS-CoV-2 virus on cornea retrieval, processing, and transplantation. To the best of our knowledge, there is one first hint of SARS-CoV-2 virus RNA in ocular tissue in relation to a potential axonal transport through olfactory tract projections into other neuroanatomical areas.[10] More comprehensive investigations are needed and desirable to collect more robust data, since most studies generally have limitations such as small patient numbers, small sample numbers or type of sample. In parallel, testing methods for postmortem donors need to be validated.

According to eye banking associations, there is no proven evidence at present that coronaviruses can be transmitted by cornea transplantation. Nevertheless, during the COVID-19 pandemic, precautionary measures should be taken during recovery and at the time of transplantation surgery. Currently, the cause of death confirmed as COVID-19 is a contraindication of eye donation. In the future, the guidelines for tissue exclusion may need to be eased if the infection rate in the population increases globally. Desautels et al. note that this may force eye banks to process cornea from positive donors, and that the avoidance of COVID-19-affected tissues could disproportionally affect import-reliant countries that source tissues from surplus nations.[33]

Conclusion

Although transmission of SARS-CoV-2 cannot be completely eliminated, we believe that, overall, the risk of transmission through corneal tissue is very unlikely. Frequent risk assessment and re-evaluation should be done to find an appropriate balance between safeguarding everyone involved in eye banking and transplantation and ensuring care for patients requiring corneal transplants.

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There are no conflicts of interest.

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