INTRODUCTION

The new SARS-CoV-2 virus causing the ongoing pandemic was isolated from a patient with pneumonia in Wuhan, the capital of Hubei Province, Central China in late 2019. As a highly contagious disease, COVID-19 is commonly manifested by fever (87.9%), dry cough (67.7%), fatigue (38.1%), vomiting (5.0%) and diarrhea (3.7%). In some cases, it can lead to more severe complications of the respiratory system such as dyspnea and/or hypoxemia or acute respiratory distress syndrome, with a slight chance of being lethal. On January 30th 2020, based on the high number of affected people and the global spread of SARS-CoV-2, the World Health Organization (WHO) confirmed the outbreak as a public health emergency of international interest. On March 11th 2020, WHO declared the COVID-19 outbreak a pandemic while on February 25th 2020, the first patient with COVID-19 was identified in Zagreb, Croatia.

Coronaviruses are single-stranded RNA viruses with an approximate diameter of 0.08-0.12 μm, with four known types  α, β,  δ  and  γ. SARS-CoV-2 is an enveloped β-coronavirus with an estimated genome of 30 kb strands of RNA. It enters host cells by binding to the Angiotensin-Converting Enzyme 2 (ACE-2) receptor. This receptor is found in various tissues, including the conjunctiva and corneal epithelia. Ocular manifestations are not common in patients, but recent publications described how conjunctivitis could be the only symptom of COVID-19. When the virus enters a cell, it firstly releases the viral envelope, after which the RNA is also released. Although this RNA virus originates from animals (bats are considered to be the potential source), it spreads relatively quickly and easily from an infected person to a healthy person. It is estimated that one infected person could infect 2-4 people in the situation of absence of physical distancing. The incubation period is evaluated to be extended to 14 days with a median of 3 to 5 days. Respiratory droplets are divided into large droplets (>5 μm in diameter) and
small droplets (≤5 μm in diameter). Large droplets can only be transmitted over short distances as they usually fall rapidly to the surfaces that are touched, they are then transferred by hands onto the eyes, nose and mouth.12 The diameter of large droplets produced by sneezing is around 100 μm.13 Small droplets can evaporate and remain suspended in air for some time and could be inhaled.

Because tissue banking is a very important health service, the European Centre for Disease Prevention and Control (ECDC) accordingly provides information on the current risks which remain theoretical but cannot be completely excluded. The Ministry of Health of the Republic of Croatia issued a decree to temporarily stop harvesting allogeneic femoral heads, amniotic membranes and to suspend cultivation of autologous limbal cells and keratinocytes. The temporary stop of tissue harvesting was in place from March 23rd to May 4th 2020. The COVID-19 pandemic has raised a new challenge for the management of quality control in tissue banking. Therefore, the aim of this article is to establish preventive actions and to reduce the possibility of contamination.

DISCUSSION

Quality management

The Tissue and Cell Bank (Tissue Bank, TB) is a hospital unit of University Hospital Centre “Sestre milosrdnice” in the Republic of Croatia that has laboratories with cleanrooms and laboratory equipment. It also has the human resources required for donor evaluation, testing, processing, preservation, storage and distribution of human tissues for transplantations. The TB should be focused on good laboratory practice, good manufacturing practice and good clinical practice in accordance with national law, international standards, guidelines and recommendations. Our Department of Transfusion and Regenerative Medicine has established a unit for Quality Management (QM) that deals with aspects such as quality control in all segments of work in tissue banking, whilst traceability and document management are guaranteed. The main goal for QM in the field of tissue banking and cell therapy is to reduce the risk of disease transmission to tissue recipients.14

Protection of healthcare personnel

During the first two months of the pandemic, healthcare personnel (clinical transfusiosologist, molecular biologist, molecular and processing biotechnologist, laboratory technicians) were working in two-week shifts. After that, routine work was continued with the full number of employees, working preferably at a physical distance of one meter or more, away from people to reduce the risk of spreading the virus with adequate protection according to the ECDC.15 It is necessary to prevent the SARS-CoV-2 virus from spreading between employees in offices by cancelling non-essential meetings and holding teleconferences. In case laboratory staff members develop symptoms of COVID-19, they should leave the workplace, self-isolate at home, contact a doctor and behave according to available information published by the epidemiology service of the Croatian Institute of Public Health.4

Routine procedures and preventing contamination of cleanrooms

Before entering the Laboratory for Tissue Engineering with cleanroom laboratory, staff must remove make-up, clothes, footwear, jewelry, wristwatches, cell phones, keys and wash their hands for at least 20-30 seconds. After that, hands should be dried and disinfected for 20-30 seconds, which corresponds to the recommendations of ECDC.15 Hand washing with liquid soap (detergent with bactericidal, fungicidal and virucidal effects as an antiseptic, it also has a prolonged antimicrobial effect) and water is used for hygienic and surgical washing of hands and forearms as a measure of preventing the spread of microorganisms and thus the SARS-CoV-2. After hand washing and disinfection with 70 % ethyl alcohol, it is very important to use personal protective equipment such as a sterile face mask, cap, goggles, gloves, undergarment and overshoes, which are put on before entering a grade C cleanroom. Face masks provide a physical barrier between the mouth and nose of the staff and potential infection in their surroundings. They can block large-particle droplets that may contain viruses and bacteria, keeping them from entering people’s mouths and noses. Face masks may also help to reduce exposure of saliva and respiratory secretions to others. Avoiding talking during the processing should also be a preventive measure. Additional safety is gained by wearing clogs, sterile disposable suits and double sterile gloves in grade B cleanrooms (Figure 1). Additional preventive measures introduced during COVID-19 were a total of 37 intermediate clothing items (cleanroom undergarment, fabric 100% polyester) that were replaced.

The Laboratory for Tissue Engineering (subordinated to the Bank) is organized and built according to the technology of cleanrooms in the manner of an existing HVAC system (Heating, Ventilating and Air Conditioning). The Laboratory is defined with two grade B cleanrooms (ISO class 6), one grade C room (ISO class 7) and one grade D room (ISO class 8). Surrounding cleanrooms with different grades have a pressure differential approximately 10 - 15 Pa, according to GMP. Particle size in the air is strictly controlled by using HEPA filters class H 14 which remove at least ≥99.995% of particles according to the European standard EN 1822-1.16 Our Laboratory for Tissue Engineering is designed and constructed in a way to control and minimize the
introduction, generation and retention of concentrations of airborne particles inside the cleanrooms in line with ISO 14644-1:2015.\textsuperscript{17} Validation of cleanroom cleaning and maintenance of sterility within a particular class of a production space of the mentioned laboratory is a necessary precondition for safe tissue processing and/or cell cultivation. The source of contamination within the microbiologically controlled area of cleanrooms consists of various biological samples (placenta, skin, limbus biopsy and various cell cultures such as keratinocytes, limbal cells, dermal fibroblasts, 3T3 cell line, etc.), laboratory equipment, reagents, consumables and staff.

Additional preventive measures introduced during COVID-19 were the new protocol for routine cleaning and disinfection of cleanrooms. In fact, we used the same disinfectants, but we introduced a new timetable. On the first day a sterile non-ionic detergent with low residues is used for cleaning and maintaining work surfaces in cleanrooms, while a 70% isopropyl alcohol (70% IPA) blended with deionized water was used to remove residues and for daily disinfection of surfaces. Moreover 70 % IPA has bactericide, fungicide and virucide efficacy (viruses with envelopes such as SARS-CoV-2). On the second day a sporicidal agent (0.6 % hydrogen peroxide solutions) is used for disinfection of work surfaces (in spray or in the form of wet wipes), which is effective against bacteria and fungi. Furthermore, our plan is to periodically exchange other sporicidal agents (0.5% sodium hypochlorite solutions) because they are also effective against most microorganisms, including viruses, bacteria and fungi.

Medical examination of allogeneic tissue donors

The donors are examined, their medical documentation is reviewed, and they complete the Medical Questionnaire for a living allogeneic tissue donor which is all administered by a medical doctor associated to the TB. This medical questionnaire is also used for femoral head donors and placental donors which contains questions on whether they have had fever and respiratory symptoms such as cough and shortness of breath in the past 28 days, whether they have been in contact with an infected person or travelled in that period in a COVID-19 focus area. If donors of the femoral head have a test result for SARS-CoV-2 in their medical history, we will make a copy of the findings, and attach it to the donor’s documentation in the TB. The recommended molecular test for SARS-CoV-2 infection is a real-time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR). Placental donors should be pre-tested for the presence of the SARS-CoV-2 virus. The diagnostic molecular test result should not be older than 48 hours at the time of tissue collection. This testing is necessary to avoid possible contamination of clean areas and laboratory staff.

Additional preventive measures introduced for the safety of tissue banking during COVID-19 were that all allogeneic donors are required to leave a contact or cell phone number where they will be available. About 99% of symptomatic patients will develop/present symptoms before day 14.\textsuperscript{19} Therefore, after a minimum of 14 days of tissue storage, the responsible physician will contact the donor to check the onset of symptoms. In case of confirmation and / or suspicion of the disease, the tissue will not be used clinically and will be discarded in accordance with regulations. A telephone check of the donor will be entered into forms related to tissue donor quality control.

Table 1 shows data on harvested femoral heads before and during COVID-19. As shown in Table 1, divergence in the number of collected femoral heads in these two trimesters is significant. It stems from a lower number of available donors due to the pandemic (number of patients that have undergone total hip arthroplasty was smaller). In the period from June 1\textsuperscript{st} to September 30\textsuperscript{th} none of the donors were COVID-19 positive and none of the tissues were lost due to this infection.

| Period | Femoral heads |
|--------|---------------|
| 01 Jan - 23 Mar 2020 | 33 |
| 01 June - 30 Sep 2020 | 24 |
| Total | 57 |

Medical examination of autologous tissue and cell donors

Patients undergoing treatment with an Advanced Therapy Medicinal Product (ATMP), for which autologous cells (limbal or keratinocytes) will be grown in cultures, are checked for fever and respiratory symptoms such as cough and shortness of breath in the past 28 days. In addition, before taking and processing autologous tissues, it is necessary to provide a molecular
rRT-PCR test which cannot be older than 48 hours. This additional measure was to prevent contamination of the TB facilities and safety for our personnel.

Tissue procurement

Tissues must be quarantined until donor testing and data collection procedures are performed. Upon receipt of the collected tissues in our bank, it is necessary to document that the conditions of transport, packaging, labelling, attached documentation and samples meet the standard/recommended procedures. The TB has a Standard Operation Procedure (SOP) and specifications for checking the tissues and samples received. Data of the donor and the collection of tissues, swab and blood samples are reviewed by authorized laboratory workers. Tissues of allogeneic donors with COVID-19 positive epidemiological history would be safely discarded. This also applies to already stored tissues of allogeneic donors monitored for COVID-19-like symptoms within 14 days after surgery. For autologous tissues of COVID-19 positive donors, the tissue would not be processed until the rRT-PCR test is negative.

Tissue processing

Certain risk of COVID-19 transmission through cells and tissues is possible through isolated cells and distribution of such affected cells into specific tissues and organs. The Laboratory for Tissue Engineering, in which human tissues and cells are treated, is constructed, organized and maintained to minimize the risk of contamination, including cross-contamination. Performance, organization and maintenance efficiency must be validated and monitored. The performed installation meets the design requirements and the standards related to cleanrooms and the appropriate controlled environment.

Keratinocyte cultivations are essential in the treatment of patients with burns, and because of that they are recognized as tissues for life-saving transplantations. For safe tissue processing, healthcare personnel should use a Microbiological Safety Cabinet (MSC) class II with installed High-Efficiency Particulate Air (HEPA) filters that can remove particle sizes greater than 0.3 µm (Figure 2). The laboratory staff members should clean and decontaminate work surfaces of the MSC, and all equipment used for tissue processing (with detergent and disinfectants such as 0.5% sodium hypochlorite solutions).

Tissue and cells storage

Information about stability of SARS-CoV-2 on different surfaces has been published and described that the virus was detected after up to 24 hours on cardboard, 72 hours on plastic and stainless steel surfaces under the experimental circumstances tested (controlled relative humidity and temperature). Standard practice according to the Guide to The Quality of Tissue and Cells for Human Applications is that all harvested/collected tissue should be stored in primary and secondary containers before distribution for clinical use. During distribution, we additionally packed tissues in a sterile plastic zip lock bag, as a third protective layer, which was also treated with disinfectant before being placed in the transport container. For long-term preservation of human tissue such as amniotic membranes or femoral heads, they must be stored at -80°C (Figures 3, 4). Keratinocytes and limbal cells are stored in liquid nitrogen at -186°C. According to the ECDC, there is no evidence of virus transmission through contaminated packages that are exposed to different environmental conditions and temperatures.

CONCLUSIONS

We have presented our experience with following standard recommendations for safe tissue banking procedures during COVID-19, and we have also described additional safety measures that we have introduced in this period. These additional measures were: defined clinical criteria for prevention of COVID-19 infection for donor selection, tissue procurement, processing, and protection of tissue bank personnel and monitoring of previously stored tissues for COVID-19. These measures might have contributed to the prevention of COVID-19 spread, contamination of tissue bank facilities, and they have definitely reduced the risk for personnel.

This manuscript provides the necessary information on preventive measures during the COVID-19 pandemic and the procedures of the TB, healthcare and surveillance measures for tissue donors, microbiological environmental monitoring of cleanrooms, SOP for the safe storage of tissues, cells and all laboratory tests prior to their release for clinical use.
The coronavirus SARS-CoV-2 has imposed increased risks in tissue banking with new challenges for cleanrooms and for healthcare personnel. Therefore, it is very important to keep these risks to a minimum, protect employees and keep processes running.

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