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Original Article

Effects of residual H_2O_2 on the growth of MSCs after decontamination

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ABSTRACT

Introduction: Regenerative therapy is a developing field in medicine. In the production of cell products for these therapies, hygienic management is even more critical than in the production of a chemical drug. At the same time, however, care is required with the use of decontamination agents, considering their effects on cell viability and characteristics. To date, hydrogen peroxide (H_2O_2) is most widely used for decontamination in pharmaceutical plants and cell processing facilities.
Methods: In this study, we examined the effects of residual H_2O_2 in the atmosphere of cell processing units after decontamination on the viability and proliferation of mesenchymal stem cells derived from human bone marrow.
Results: We detected residual H_2O_2 sufficient to affect cell proliferation and survival even more than 30 h after decontamination ended. Our results suggest a longer time period is required before starting operations after decontamination and that the operating time should be as short as possible.
Conclusions: Here we show the effects of post-decontamination residual H_2O_2 on the viability and proliferation of mesenchymal stem cells derived from human bone marrow, which may provide us with important information about the hygienic management of cell processing facilities.

1. Introduction

Regenerative medicine is currently a focus of global attention, and countries worldwide are actively working to create these products as new treatment modalities through pioneering research and novel discoveries of technologies [1]. In the production of cell products for clinical administration, hygienic management is even more important than in the production of chemical agents. Materials and products in cell/tissue production cannot be sterilized chemically or physically, and contaminating pathological microbes can proliferate during cell processing. In Japan, two laws were established in November 2014 [2–5]: the Act on the Safety of Regenerative Medicine and the Act on Pharmaceuticals and Medical Devices (PMD Act) were implemented, and legal regulations both in clinical research and in production of regenerative products were prepared. Under these regulations, sanitary control and prevention of contamination in cell processing facilities (CPFs) are major components. Although detailed and global guidance and standards of hygiene management are available regarding the production of chemical agents, no well-established guidance exists for the production of cell/tissue products at the bench in CPF [6]. In most situations, guidelines for good manufacturing practices or others for sterile pharmaceutical products and foods are applied for the production of cell/tissue processing, but each individual person at each facility operates production under their own judgment regarding the specific issues in regenerative products.

In the selection of decontamination agents for CPF, the adverse effects on staff and cells must be considered along with antimicrobial effectiveness. In the regenerative therapy field, because we use various cell types and amounts of cells for different conditions, the types and quantities of agents for decontamination should be

Abbreviations: H_2O_2, hydrogen peroxide; CPF, cell processing facility; BSC, biological safety cabinet; MSC, mesenchymal stem cell; HEPA, high efficiency particulate air.

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selected in accordance with the specific factors involved. Formerly, formaldehyde was generally used for atmospheric decontamination in pharmaceutical facilities. However, with the cancer-causing effects of formaldehyde identified [7,8] as well as acute toxicities such as mucosal irritation and cutaneous inflammation [9], it was classified as a probable human carcinogen by the World Health Organization (WHO) International Agency for Research on Cancer. Currently, its use is seriously restricted by WHO and other environmental regulations. Thus, the establishment of proper guidance for the sanitation of CPF atmosphere using decontamination agents other than formaldehyde is an emergent issue.

Hydrogen peroxide (H_2O_2), chlorine disinfectants, and their mixtures now are widely used for decontamination in pharmaceutical plants and CPFs [10,11]. Above all, H_2O_2 is mostly a sterilizing agent for sanitation in CPFs [12]. H_2O_2 is a reactive oxygen species, which can degrade organic compounds. Because it demonstrates broad-spectrum antimicrobial efficacy, including against bacterial spores, viruses, and yeasts, and has insecticidal power, it is applied to sterilize various surfaces [13], such as surgical tools [14], and can also be used as mist for room sterilization [15,16]. However, highly concentrated H_2O_2 should be considered hazardous because it is an aggressive oxidizer and will corrode many materials, including human skin [17]. The American Conference of Governmental Industrial Hygienists (ACGIH) has also classified H_2O_2 as a "known animal carcinogen, with unknown relevance on humans" and has established a permissible exposure limit of 1.0 ppm calculated as an 8-h time-weighted average [18].

In this study, we first examined the effects of residual H_2O_2 after decontamination on the viability and proliferation of cells. Although the effects of low concentration of H_2O_2 in the atmosphere will be different depending on the cell types, in this study, we selected MSCs as cell type, which can be a representative example because they are insulated from the influence of other conditions, such as operation technique, cell lot, and all.

2. Materials and methods

2.1. Experimental design

The effects of residual H_2O_2 on cell growth after decontamination were evaluated by exposing human MSCs in a biological safety cabinet (BSC) in an experimental vinyl chamber within a cleanroom. After the decontamination with H_2O_2 vapor, we waited until the atmospheric concentration of H_2O_2 adequately fell down. And then MSCs were exposed in the BSC for some periods, and their proliferation rates were evaluated.

2.2. Construction of a vinyl chamber in clean room

The chamber was composed of vinyl sheets (PVC Film Achilles flare FU-RE04; Achilles Corporation, Tokyo, Japan), including a BSC and H_2O_2 generator (DryDeco mobby MHPE-0411TK02; Taikisha Ltd., Tokyo, Japan) to produce H_2O_2 mist. It was constructed within a clean room to prevent H_2O_2 from leaking out and to keep the atmospheric concentration of H_2O_2 constant. The size of chamber was 9 m^3.

2.3. Measurement of H_2O_2 concentration in water and in atmosphere

Concentrations of H_2O_2 dissolved in water were measured using water analysis products (DIGITALPACKTEST-MULTI DPM-MT and PACKTEST WAK-H_2O_2; Kyoritsu Chemical-Check Lab Corp., Tokyo, Japan) for H_2O_2 (high concentration, 3–700 mg/L; low concentration, 0.05–5 mg/L).

Atmospheric concentrations of H_2O_2 were measured by H_2O_2 sensors (DragerPolytron700, 8317610; Drager Sensor H_2O_2 HC, 6809675, for 30–300 mg/L and Drager Sensor H2O2LC, 6809705 for 3–30 mg/L). We also used a detector tube (gas detector tube measurement system GV-110S and H_2O_2 detector tube No. 32; Gastec Corporation, Kanagawa, Japan) to confirm that H_2O_2 concentration was lower than 1.0 mg/L.

2.4. Cell preparation and cell culture

MSCs derived from human bone marrow were purchased from PromoCell GmbH (Heidelberg, Germany, Cat. C12974, Lot 4031804.5) and cultured in Dulbecco's Modified Eagle Medium (Nacalai Tesque, Kyoto, Japan) containing 10% fetal bovine serum (Nacalai Tesque, Kyoto, Japan) and 1% antibiotic-antimycotic mixed stock solution (100×), including penicillin 10,000 units/mL, streptomycin 10,000 μg/mL, and amphotericin-B 25 μg/mL (Nacalai Tesque). At experiment start, MSCs subcultured in three passages were seeded at a density of 2.2 × 105 cells/100 mm dish and cultured in a highly humidified incubator maintained with 5% CO2 at 37 °C. H2O2 for supplementation in culture medium was purchased form Fujifilm Wako Pure Chemical Corporation (Osaka, Japan), and sodium pyruvate solution was from Nacalai Tesque.

2.5. Cell calculation

To quantify the proliferation rate of cultured MSCs, detached MSCs were stained with trypan-blue solution. Cell numbers were counted using Countess® II FL (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.6. Statistical analyses

Statistical analyses were carried out using standard Student *t* tests, and error bars indicate standard deviation. A P value ≤ 0.05 was considered to represent a statistically significant difference.

3. Results

3.1. Decontamination with H_2O_2 in the working unit in CPF and its concentration in the atmosphere

The effects of residual H_2O_2 on cell growth after decontamination were evaluated using human MSCs in an experimental vinyl chamber installed within a cell processing clean room. As shown in Fig. 1a, two types of H_2O_2 sensors, for high concentration (30–300 mg/L; sensors A and B) and for low concentration (3–30 mg/L; sensor c), were set in the chamber. Sensors A and B were used during generation of H_2O_2, and sensor c was used for concentration monitoring after generation of H_2O_2 was stopped.

In the chamber, H_2O_2 was generated to keep the concentration in the atmosphere at approximately at 200 mg/L for 5 h. During this time, the BSC continued running, and the H_2O_2 of the atmosphere within the cabinet was kept about 200 mg/L, just below the concentration outside the cabinet. For concentrations during and after the generation of H_2O_2, values detected by sensors are shown in Fig. 1. After stopping the generation, the cracking unit was started, and H_2O_2 concentrations at sensors A and B were rapidly degraded to 50 mg/L. However, after that, the decrease rate of H_2O_2 became slower and slower. It was not until 25 h and 40 min after stopping generation of H_2O_2 that the sensitive detector tube showed 1.0 mg/L, which is the time-weighted average threshold limit value of H_2O_2 by ACGIH [18]. At that time point, defined as Time A, we started the clean room ventilation and removed the vinyl sheets.