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APPLICATION OF OZONE AGAINST THE MICROFLORA IN CELL CULTURES GROWTH MEDIUM

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ABSTRACT
The aim of the study is to introduce a modern and effective method that equally well remove bacterial contamination and prevent the development of fungal colonies in cell culture growing media. We chose ozone as an active gas with strong antibacterial and antifungal activity. Ozone is an unstable chemical substance that kills bacteria and fungi. It can be used for decontamination of the growth medium in the art of cell culture growth. Ozone is much cheaper than the antibacterial and antifungal agents that are sold in pharmacies and it does not affect cell lines and does not change the results of the study.

Key words: ozone, cell cultures, bacteria, fungi, growth media.

INTRODUCTION

The cell cultures are present method wherein on artificial growth media in appropriate conditions - temperature, humidity and saturation of CO_2 cells are grown, primarily with an experimental purposes. These cells are separated from their tissues with the aid of trypsin, or other appropriate means. Onto the resulting cell monolayer in vitro can be experimenting new drugs or the effects of physical and chemical factors. There have been successful attempts to treat extensive burns by grafting skin epithelial monolayer on the wound surface. Upon well-timed epithelial graft wounds heal primarily without deforming scar remains and the healing process is faster and without complication.

Cells outside a living organism are very sensitive to changes in environmental conditions, so their development is influenced even by the smallest changes in the parameters as well as the growth medium or in the work of the maintenance equipment. Lowering the temperature by a few degrees severely retarding the growth of the cells and the development of cell colonies and their normal function.

One of the factors that disturb the normal existence of cell cultures and distort the final result is the emergence of dirt from fungi or bacteria and their growth on the medium used for cultivation of the cell monolayer. Typically this pathogenic agent was removed with the aid of anti-bacterial and antifungal agents. The use of antibiotics and chemical substances with antifungal activity, on the one hand increases the cost of the experiments, on the other hand, the change in the composition of the growth medium, can result in erroneous outputs and to incorrect conclusions in their interpretation.

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PURPOSE AND OBJECTIVES

The aim of the study is to introduce a modern and effective method that equally well remove bacterial contamination and prevent the development of fungal colonies growing media used. We chose ozone as an active gas with strong antibacterial and antifungal activity.

To active the target we set ourselves the following tasks:

1. Creating a device for the production of ozone, which is a simple appliance which has a low cost price of manufactured end product.
2. Use of ozone to destruct colonies of fungi or to prevent their occurrence.
3. Application of ozone to prevent bacterial contamination or to remove already arisen growth of pathogenic microflora.

MATERIAL AND METHODS

Experiment with ozone, which is relatively inexpensive and has proven effective action not only against the agents of the inflammatory process, but a number of infectious diseases. For the production of ozone we used a device called "ozonator" created in the Department of Medicine of disasters **(Figure 1).** The ozonator is a simple construction and it is made from an inexpensive materials, which reduces the cost not only of the produced ozonebut also the cost of each experiment.

Figure 1. Ozonator – a device for ozone production

Culture medium on which we impact the ozone are standard media for growing epithelial monolayer *(Minimum Essential Medium Eagle, SIGMA Life Science, Chemie GmbH, Germany).* The culture medium were received in sterile petri dishes with a diameter of 50 mm, made of plastic material. When the cell material was planted, the containers were opened with the aid of a sterile loop made of chrome-nickel fiber, and then the cells were applied to the culture medium. The manipulation was performed in conditions of total sterility into a laminar flow box *(GELAIRE (BSB3) – Flow Laboratories device, Class 100)* **(Figure 2)** in order to avoid contamination with microorganisms.

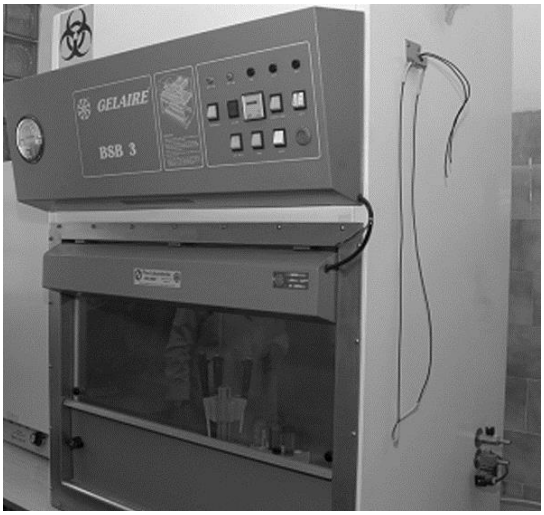

Figure 2. GELAIRE - flow laboratories device, Class 100

Growth medium was placed in a closed glass vessel which through a transparent polyethylene pipe is connected to the source of ozone **(Figure 3).**

Figure 3. Effect of ozone on the growth medium

We experimented on three groups with different duration **(Table 1).**

Table 1. *Different duration of ozone effect.*		
No	**Group**	**Duration *(min)***
1	First group – 2 dishes	5
2	Second group – 2 dishes	10
3	Third group – 2 dishes	15
4	Control group	no

Each one of these groups has two vessels full with growth media. Two control Petri dishes were left after seeding without contact with ozone. All media were left for seven days in an CO_2 incubation box (Heareus Holding GmbH, Hanau, Germany) **(Figure 4)** under suitable conditions for growth of the cell cultures **(Table 2).**

Figure 4. Cell cultures CO_2 incubator

Table 2. *Suitable conditions for growth of the cell cultures for mammalian cells*		
No	**Conditions**	**Quantity**
1	Temperature (C^o)	typically, 37°C
2	Humidity (%)	different
3	CO_2 concentration (%)	5% CO^2
4	pH	7.0 – 7.4

RESULTS

In control Petri dishes on the third day there was a sections of fungi while in those treated with ozone none were found. On the fifth day a round shaped bacterial colonies grew, but their nature were not able to clarify. After seven days the fungal growths dominated – they covered almost the entire medium **(Figure 5),** but there were 1 or 2 small circular bacterial colonies. In the vessels treated with ozone **(Figure 6)** there is a total lack of development of the microflora in the three groups.

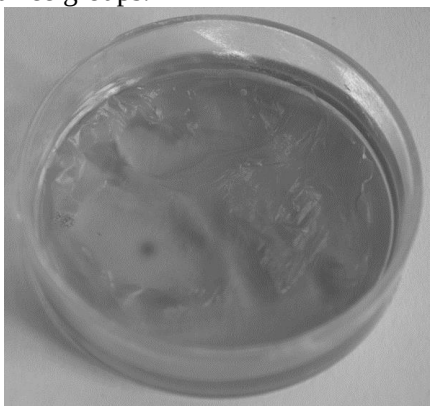

Figure 5. Fungial growths in non threated medium

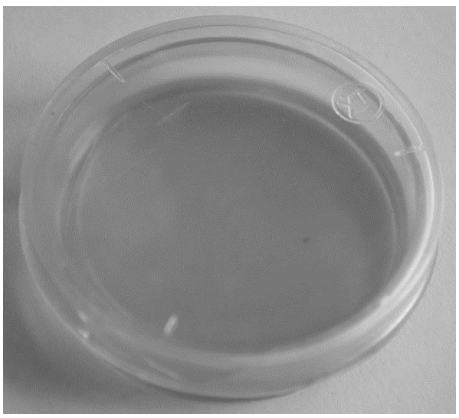

Figure 6. Ozone threated growth medium

DISCUSSION

Different cells need various conditions for their life cycle. They are depending on cell type but biological sterility is a basic condition always. There are much microorganisms such as bacterial, fungi, and viruses that contaminate cell cultures and destroy them (Dominici, 2006; Handbook, 2009).

Ozone is the allotropic form of oxygen, which at room temperature is a gas - almost transparent, with a light blue color and pungent odor

(Housecroft&Sharpe, 2004). It has proven effective against a number of causes of infectious diseases, including that ones used as biological weapons or potential biological weapons (Иванов и кол, 2011, Иванов и кол., 2012). It acts broad spectrum antimicrobial (de Boer, 2006). These properties of ozone are responsible for its disinfecting and sterilizing action. It destroys bacteria, viruses, spores, fungi, cysts and removes dissolved organic material, occurred as a result of oxidation. So we can say that its effect can be used against most types of biological weapons, which was introduced into the common practice of armies around the world (Cartwright, 2008). It is used at the fresh fruit and vegetables to destroy yeasts, molds and bacteria (Banerjee, 1985). It also decreased the content of residual pesticides, herbicides, fungicides and fertilizers and remove accumulated detergents, soaps, polishing agents and waxes on the surface of the agricultural production (Iglesias, 2006). Ozone kills bacteria and destroys hormones in meat, seafood and other meat food products. Ozone could be used for sterilization of plates and cooking utensils, and it also ozonized olive oil.

Ozone is an unstable chemical substance that is a very strong oxidant and reacts with most oxidizable chemicals. Because of this instability, it exists in an atmosphere for 20 minutes, and then is converted to oxygen. This period is variable and is dependent on the ambient temperature. In everyday practice, ozone is used for disinfection of drinking water to disinfect surfaces or biological materials, as well as to counter biological weapons to armies of countries hostile to our country.

CONCLUSIONS

1. Ozone is an antiseptic agent which can be used for decontamination of the growth medium in the art of cell culture growth.
2. Ozone is much cheaper than the antibacterial and antifungal agents that are sold in pharmacies.
3. Unlike antibacterial and antifungal agents, ozone does not affect cell lines and does not change the results of the study.

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