Vacuum coatings based on miramistin and their biological properties

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Abstract
This paper provides the first studies of genotoxicity and the ability of miramistin-based coatings to stimulate regenerative processes in eukaryotic cells. The coating testing in the Allium cepa system revealed the absence of pathological mitoses beyond the level of normal spontaneous mutation and an increase in the proliferating activity of the meristematic tissue cells in comparison with the control.
Keywords: electron-beam deposition, miramistin, polymer coatings, antibacterial and antifungal properties

Introduction
The problem of drug resistance is steadily increasing every year around the world [1]. This calls for complex use of various combinations of medicinal compounds: antibiotics, metal nanoparticles (Ag, Cu, etc.), antiseptics in medical practice. This is due to the fact that over the past few decades, not a single new antibiotic molecule has been produced. In this regard, miramistin is of interest as an alternative to standard topical drug compounds. Miramistin is an antiseptic with high antifungal and antimicrobial activity. At present, the open literature provides no comprehensive comparative analysis of the results of in vitro and in vivo studies of the antibacterial and antifungal miramistin activity. The aim of these studies is to study the genotoxicity of miramistin-based coatings and its (miramistin) ability to stimulate regeneration processes in eukaryotic cells.

Materials and methods
The technique of coatings vacuum deposition by a low-energy electron beam has been described in a series of papers, in particular [2]. Miramistin powders of (Tocopharm Co., Ltd, China) were used as a target material. The effective thickness was monitored directly during deposition using quartz crystal microbalance (QCM).
Biotesting was performed to study the miramistin genotoxicity and its ability to stimulate regenerative processes in eukaryotic cells. The common onion (*Allium cepa* L., Schtutgarter Riesen sort) was chosen as a model [3]. It should be emphasized that the results of studies on onion cells can be extrapolated to cells of other organisms, including humans [4]. Moreover, onion cells are currently used to test antibacterial drugs. Before the Allium test, miramistin-based coatings were deposited on substrates of a given size. The geometric thickness of the coating was measured using a scanning electron microscope (Quanta 200 F). This helped to determine the mass of the deposited layer. To obtain the aqueous solution of an antiseptic (1 mg/l), the substrate was placed in a vessel with distilled water, shaken vigorously for 1 minute. Distilled water was used as the control. Slides for cytogenetic analysis were made according to the S. B. Tedesco and H. D. Laughinghouse protocol [5]. In each case, 10 − 30 root-tips were analyzed. The examination of the squashed slides was carried out at a karyological station equipped with a Leica DMR microscope (Germany). At least 10,000 cells were scanned for each version. The mitotic index (MI) was calculated according to R. Sehgaletal [6]. Phase indices (prophase, metaphase, anaphase, telophase) in the cells of

the meristematic tissue were determined according to [7]. Cells with pathology of mitosis (PM) were expressed as a percentage of abnormal dividing cells of the total number of dividing cells.

Results and Discussion

The use of the drug component to inhibit the growth of pathogenic microorganisms and fungi should not be accompanied by toxic tissue damage. Pathological mitosis (PM) in cells of the body is one of the reasons for mutations and the aneuploidy development. The test results revealed the absence of differences in the experimental versions in terms of the «mitosis pathology» indicator (Fig. 1, a). The determination of the correlation between PM taking into account prophase and PM excluding prophase revealed a high positive value of 0.99.

The possibility of delaying cell division and violating chromosome segregation at any stage of mitosis due to damage to cellular structures such as the spindle apparatus, kinetochores and cell membranes under the influence of aneugenic factors has been studied. To achieve this, the calculation of various types of mitotic index has been performed, and the fractions of dividing cells for each phase have been determined (phase indices (PI)). The absence of noticeable differences between the values of phase indices has been established (Fig. 1, b).

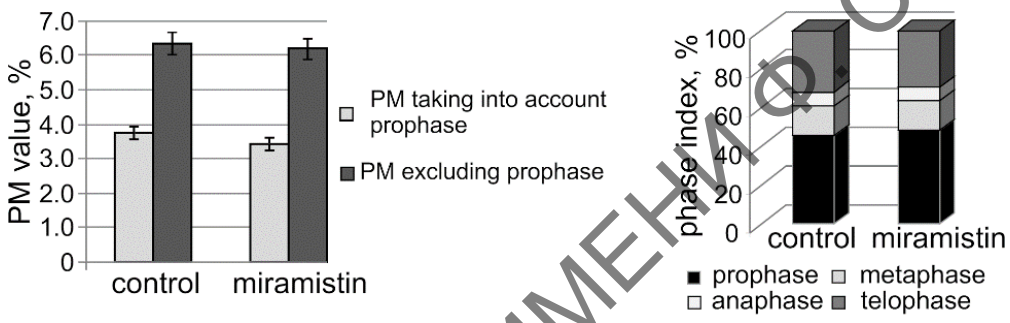

Fig. 1. The miramistin effect on the pathology of mitosis (PM) (a) and on the phase index (PI) (b)

The proportion of cells distribution at different stages of mitosis corresponded to the generally accepted distribution under physiological conditions for plant cells. At the same time, cells at the prometaphase stage were also referred to the metaphase stage. The obtained values of the metaphase-prophase index indicate the absence of mitosis delay in metaphase. The value of this parameter was in the range of 0.32 – 0.33. It should be noted that the pathology “forwarding/lagging chromosomes”, “polar deviation”, “chromosome losses”, “c-anaphase” are found both in the experimental version and in the control one (Fig. 2).

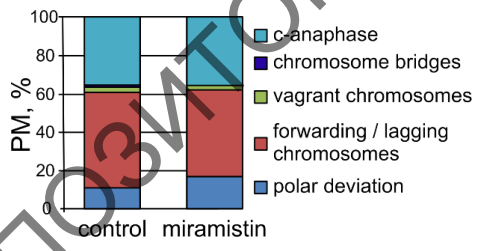

Fig. 2. Composition and spectrum of mitosis pathologies in the root meristem Allium cepa

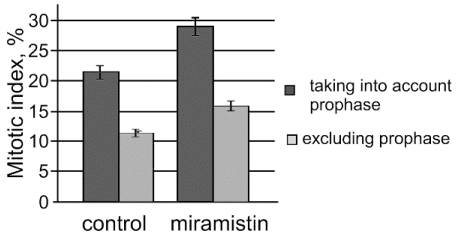

Fig. 3. The influence of miramistin on the mitotic index (MI)

The mitotic index is used as an indicator of adequate cell proliferation. The index indicates the normal mitosis behavior, inhibition of the cell division process or an increase in the mitotic activity of tissues. All this proves the mitotic or mitotic stimulating effect of the factor under study. In the analyzed versions, a rather high mitotic activity of the cells of the formative tissue was observed in the experiment (Fig. 3), but in the case with miramistin, MI was 1.4 times higher.

Conclusions

The miramistin-based coating testing with the *Allium cepa* system showed the absence of pathological mitoses above the level of normal spontaneous mutation and an increase in the proliferating activity of the formative tissue cells compared to the control.

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