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Combined cryoprotector for long-term preservation of biomaterial based on an cerium dioxide inorganic nanozyme

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Currently, cryoprotectors are used in scientific research, medicine, agriculture, as well as for the conservation of endangered and rare animal species. The main technical difficulties of the cryopreservation process are: formation of ice crystals, cell dehydration, osmotic shock and oxidative stress. At the moment, there are single-component cryoprotectors (dimethyl sulfoxide, glycerin, propylene glycol) and combined (cryoprotector + antioxidant), which are the most effective. The use of antioxidants in combined cryoprotectors is due to the development of intracellular oxidative stress at the stages of freezing and thawing of cell cultures. At the same time, the antioxidants in use are not replenishable, what causes their introduction in sufficiently high concentrations (over 1 mM). Thus, the search for new effective antioxidants for combined cryoprotectors is an urgent task.

As an agent with high antioxidant activity, we selected an inorganic nanozyme based on cerium dioxide. We have developed a scheme for the synthesis of cerium dioxide nanoparticles stabilized by dimethyl sulfoxide, which has pronounced antioxidant activity in nanomolar concentrations and is able to prevent the formation of ice crystals. At the moment, we have carried out the analysis of the cytotoxicity of the synthesized nanocomposite on human mesenchymal stem cell cultures and mouse fibroblast culture of the NCTC L929 line, which did not reveal a significant decrease in cell viability at concentrations above 500 μM. The results of the cryoprotective efficiency of the nanocomposite were also obtained. The experiments were carried out on a mouse fibroblast cell line of NCTC L929 line with the following nanocomposite concentrations: 1 mM, 500 μM, 100 μM and 50 μM, and a negative control group without cerium dioxide (DMSO only) was also present in the experiment. Cell survival after cryopreservation in the presence of nanocomposite was analyzed using a 0.4% trypan blue solution. The increase in the survival rate of mouse fibroblasts at the concentration of 1mM nanocomposite was 30% higher compared to the control value, where no nanocomposite was used. The results of the other groups (500 μm, 100 μm and 50 μM) show that the survival of cells after cryopreservation demonstrates a dose-dependent nature. Thus, it can be concluded that the synthesized nanocomposite is promising for its use as an effective cryoprotector.

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