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Abstract book

CONGRESS ON ADVANCED REGENERATIVE TECHNOLOGIES

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The CRISPR-Cas system is a popular genome editing tool, characterized by its simplicity of design and the ability to modify multiple genes. Despite impressive advances in genome editing and the development of new and improved CRISPR-based tools, several significant challenges still hinder the widespread implementation of this technology in medicine, including the inefficiency of delivery methods.

To date, viral vectors remain the most efficient method for cell transduction *in vitro* and *in vivo*, but are unsuitable for clinical use due to immunogenicity and the potential risk of insertional mutagenesis. This has led to a growing interest in non-viral methods, in particular extracellular vesicles (EVs), which offer low toxicity and the ability to transport nucleic acids and proteins. In our work, we used the transfection of paternal cells as a method of loading vesicles with the CRISPR system. For this purpose, HEK293T cells were transfected with a two-color reporter array (pX330x6(x7)/Green for sgRNA targeting Myocd1 and SP-Cas9-VPR-Tomato). Exosomes were isolation by PEG precipitation. The obtained vesicles were characterized by Nanoparticle tracking analysis (NTA) and demonstrated a size ranging from 120 to 170 nm. The vesicles expressed typical markers: Alix, TCG101, CD63 and CD81. Moreover, we found that the vesicles produced by transfected HEK293T cells were loaded with Cas9 on both the protein and RNA levels, and also contained the RNA of the Myocd1 gene, which was targeted by the Cas9-VPR system for targeted activation of its expression. Vesicles did not demonstrate the cytotoxicity in cultured human cardiac fibroblasts and mesenchymal stem cells, however characterized by different consumption efficiency depending on cells origin. qPCR revealed significant overexpression of Myocd1 in cells treated with vesicles for 6 hours.

Thus, vesicles loaded with the CRISPR-Cas system by transfection of producer cells are a promising and less toxic method of delivering the CRISPR-Cas9-VPR system.

A035

The role of trehalose in increasing the efficiency of human cord blood cell cryopreservation

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Background. Cryopreservation is the only reliable method of long-term storage of human cord blood nucleated cells (NCs), including hematopoietic progenitor cells. Due to their high regenerative capacity and lower risk of immunological complications, these cells are considered a promising resource for the treatment of a wide range of pathologies, including in regenerative medicine. Traditional freezing protocols use the penetrating cryoprotectant dimethyl sulfoxide (DMSO). However, cell damage inevitably occurs both during freezing and thawing due to the induction of, among other things, osmotic and oxidative stress, which disrupt the structure of membrane lipids and proteins. In addition, it is known that DMSO is toxic, both for the cells themselves and for the recipient organism, which stimulates the search for optimal approaches to reduce its concentration. Disaccharides such as trehalose, due to their low toxicity, are widely used as a natural impermeable cryoprotectant and stabilizer of membranes and other cellular structures during dehydration, which makes them possible to use in cryopreservation practice.

Purpose. The aim of our work was to evaluate the effectiveness of trehalose as an additional cryoprotectant to enhance the survival and viability of human cord blood nucleated cells both immediately after cryopreservation and after *in vitro* transfusion simulation.

Materials and methods. Cryopreservation of nucleated cells was performed using DMSO at concentrations of 5 % and 7.5 % with the addition of trehalose in the concentration range from 10 mM to 200 mM. Cell survival, viability, and the number of cells with excess reactive oxygen species were assessed by flow cytometry using appropriate test systems.

Results. Our studies have shown that the survival and viability of cord blood cells after thawing and, especially, after *in vitro* transfusion simulation (a test that allows detecting delayed damages), decreased, and their values correlated with the DMSO concentration: in solutions with a cryoprotectant concentration of 5 %, the yield of viable cells (an indicator that takes into account the number of remaining cells and their viability) was only 42.6 ± 2.7 % immediately after thawing and 33.3 ± 2.8 % after *in vitro* transfusion simulation. Trehalose is characterized by pronounced properties of stabilizing biological membranes and intracellular structures, which allows it to be considered as an additional cryoprotectant. To determine the most effective concentration of trehalose, we conducted a wide concentration screening in the range from 10 mM to 200 mM. Since oxidative stress is known to be a key factor in cryopreservation damage through increased production of reactive oxygen species (ROS), we used the fluorescent probe DCF to assess intracellular ROS levels. It was shown that the addition of trehalose at a concentration of 100-150 mM to the cryoprotective medium reduces the number of DCF^{+}-cells both immediately after thawing and after *in vitro* transfusion simulation at the tested DMSO concentrations, indicating the effectiveness of trehalose in neutralizing oxidative damage. And this reduction in DCF^{+}-cells directly correlates with an increase in the survival and viability of human cord blood cells when trehalose is added at the same concentrations of 100 and 150 mM. It should be noted that the addition of trehalose to a cryoprotective medium containing 5 % DMSO increases the yield of viable cells by 20 % after *in vitro* transfusion simulation. These results are consistent with those obtained when cryopreserving cells with 7.5 % DMSO without the use of additives, which is promising for obtaining cryoprotective mixtures with a reduced DMSO concentration.

Conclusions. The findings indicate that trehalose increases the efficiency of cryopreservation of human cord blood nucleated cells, contributing to their survival and viability after thawing. Determination of optimal cryoprotective media may be useful for the development of new and improvement of existing protocols for long-term preservation of human cord blood cellular components for use in regenerative medicine.

A036

Вибухова травма кінцівок: клініко-хірургічні особливості, ускладнення та результати лікування у порівнянні з вогнепальними пораненнями та іншими відкритими ушкодженнями кінцівок

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Актуальність. Повномасштабна війна в Україні супроводжується значним зростанням кількості вибухових ушкоджень кінцівок, що характеризуються множинними переломами, великими дефектами м'яких тканин та високим ризиком інфекційних ускладнень. На відміну від вогнепальних або побутових травм, вибухові ураження мають комбінований механізм (ударно-хвильовий, фрагментаційний, термічний, бароакустичний) і вимагають спеціалізованої хірургічної тактики.

Мета дослідження. Визначити клініко-хірургічні особливості перебігу, ускладнень і результатів лікування вибухових травм кінцівок у порівнянні з вогнепальними та іншими відкритими ушкодженнями.

Матеріали та методи. Ретроспективно проаналізовано 359 історій хвороб пацієнтів із відкритими травмами кінцівок (2023-2025 рр.). Розподіл: вибухові – 278 (77,4 %), вогнепальні – 67 (18,7 %), інші відкриті – 12 (3,3 %). Оцінювались: вік, стать, локалізація, ступінь пошкодження (Gustilo-Anderson), кількість операцій, застосування VAC-терапії та антибактеріальних спейсерів, тривалість госпіталізації, частота ускладнень та ампутацій. Статистична обробка виконана у Microsoft Excel; значущість $p < 0,05$.