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Перспектива розвитку біобанкінгу індукованих плюрипотентних стовбурових клітин: від нерозв'язаних бар'єрів до стабільного виробництва

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Induced Pluripotent Stem Cells Biobank Perspective: From Unresolved Barriers to Stable Production

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Any biomedical research work is carried out by analyzing a large amount of data and biological samples collected from all over the world. Therefore, the quality of these data and the analyzed biological samples is absolutely essential. Researchers may call upon biobanks to continually respond to the needs of the research community, as their role is to provide high quality biological material through standardized procedures and methods.

Despite laboratory studies and investigations on induced pluripotent stem cells (iPSCs), stable large-scale production of iPSCs is still evolving. One of the major barriers to large-scale processing is that operations in iPSC production are complex and performed manually, which can potentially lead to variabilities in cell quality due to fluctuations in manual handling. To meet the growing demand for iPSC models, such large-scale production is possible if done in a harmonized and quality controlled (QC) environment.

Over the past few years, we have implemented standardized operating procedures (SOPs) to obtain induced pluripotent stem cells from human fibroblasts of patients with neurodegenerative diseases (such as Parkinson's disease). Using QC points and validated methods such as immunofluorescence and quantitative PCR, we tested and characterized the generated iPSCs to ensure that the characteristics of pluripotency and dedifferentiation were present in these cells. The presentation will show a case study and discuss the important QC steps involved in the generation of IPSCs in a standardized environment.

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Кріоконсервування тканинно-інженерних конструкцій в кріопакетах

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Cryopreserving Tissue Engineered Scaffolds Within Cryobags

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Cell seeded tissue engineered scaffolds are viewed as a promising approach to substitute damaged tissue and promote tissue regeneration. Due to the limited shelf-life of cells, sufficient storage methods have to be ensured in order to provide an on-demand availability of these artificial substitutes. In this regard, the cryopreservation of cell seeded scaffolds aims to enhance the storage duration, even though also this preservation method is influenced by limiting technical or biological parameters such as heat transport. It is assumed that the utilization of cryobags improves the heat transfer during freezing and thawing compared to currently applied storage containers due to the flat geometrical structure as well as the thinner material thickness.

In this study, cryobags were applied to improve heat transport. Therefore, scaffolds were fabricated by electrospinning polycaprolactone and polylactic acid at a mass concentration ratio of 100:50 mg/ml and seeded with SaOS-2 cells at a density of 5×10^4 cells/cm. The seeded cells were cultivated for 3 days prior to cryopreservation. For cryopreservation 4 different cryoprotective solutions were investigated: 10% (v/v) dimethyl sulfoxide (DMSO) and 10% (v/v) ethylene glycol (EG) as separate agents as well as their combination with 0.3 M sucrose. Samples were placed within cryobags and cryopreserved in air as previously described by our group [Mutsenko, 2020] with a controlled rate freezer at a cooling rate of 1 K/min to –100°C before being plunged into liquid nitrogen and stored in a freezer at –150°C. Thawing was performed in a water bath for 15 s at 37°C. The samples were recultivated for 24 h prior to fluorescence microscopy to determine the post-thaw cell viability.

The post-thaw cell morphology remained unchanged. In regard of the post-thaw viability, EG alone and in combination with sucrose enabled the highest cell viability of 80 ± 11% and 79 ± 7%, respectively. DMSO yielded in a post-thaw viability of 77 ± 6% and 78 ± 7% in combination with sucrose. Comparatively, the utilization of cryobags enabled a higher post-thaw viability with regard to multiwell plates.It has to be noted, that the post-thaw viability can be influenced by possible rupture of the cryobags during thawing and will yield in a reduced viability.

Concluding, current cryopreservation methods can enable the long-term storage of cell-seeded electrospun scaffolds. In view of the utilized cryoprotective agents, 10% EG can serve as an alternative to the current 'gold standard' 10% DMSO; the latter being known to induce cell toxic effects above 4°C. Furthermore, the utilization of cryobags can have a beneficial effect on post-thaw viability.