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Ambient storage of bioengineered MSC-based 3D constructs

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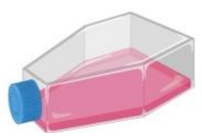
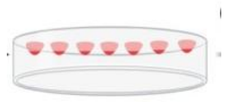
INTRODUCTION & AIM

Bioengineered mesenchymal stem cell (MSC)-based 3D constructs hold immense promise for regenerative medicine and biomedical research. Realizing their full potential necessitates effective preservation methods. While cryopreservation is the current gold standard, it presents challenges as reduced viability and demanding cold chain logistics. Ambient storage provides such benefits as simpler, less expensive transportation and avoidance of cryopreservation-induced damage. Ambient storage needs for research to search for effective solutions and to optimize preservation protocols. This study investigates novel approaches for ambient storage of MSCs in spheroids, alginate microspheres (AMSs), and macroporous scaffolds, aiming to develop effective technology for short-term storage.

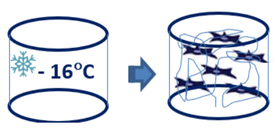
METHOD



Human adipose tissue-derived MSCs (obtained with adult donors' informed consent) were used.

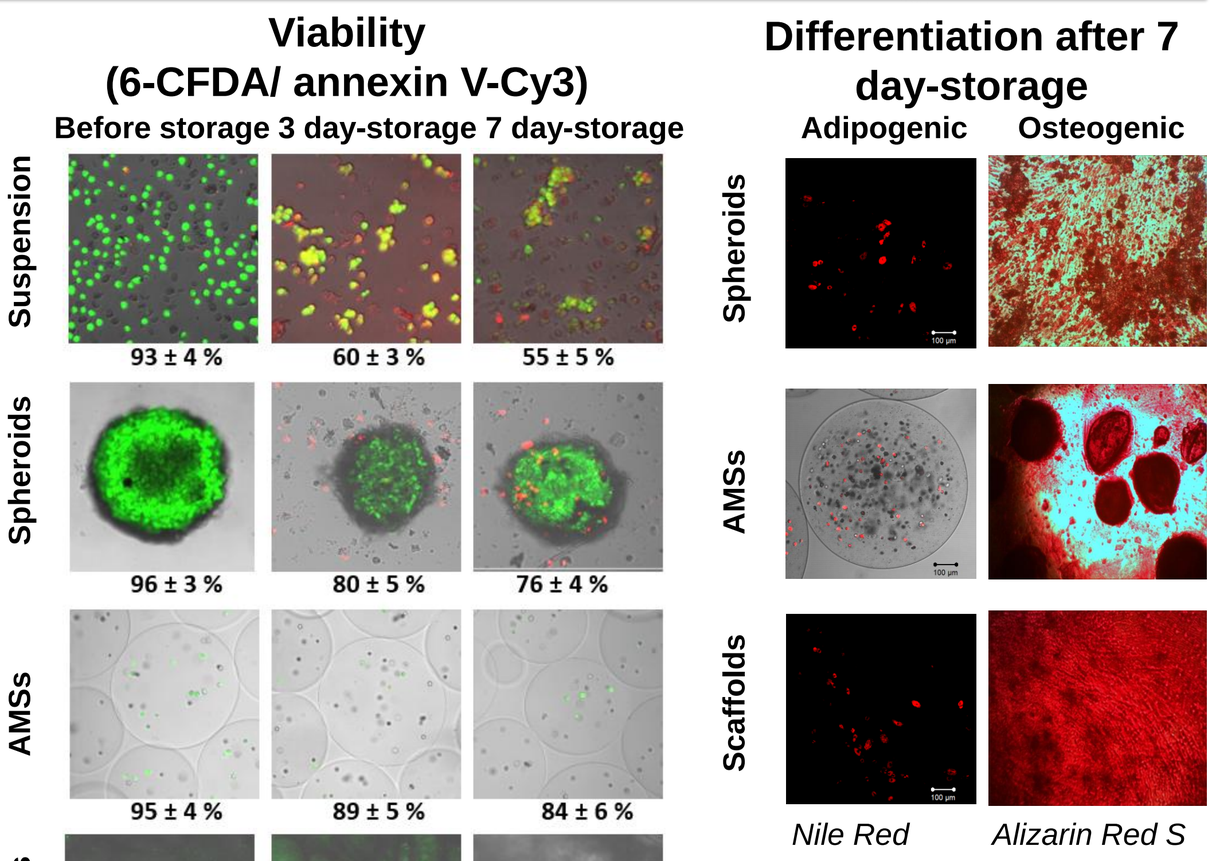


Spheroids were formed by the "hanging drop" method (3 000 cells per drop). **AMSs** were obtained by electrospraying of MSCs suspended in 2% sodium alginate into 2% calcium chloride, where cross-linked for 5 minutes, then washed out with Hank's Balanced Salt Solution. **Scaffolds** were generated by blood plasma cryogelation, then sterilized and seeded with cells. All constructs were cultured for 3 days, then stored in complete medium at 22 °C.



Viability/apoptosis (6-CFDA / annexin V-Cy3), metabolic activity (resazurin), differentiation potential, and reactive oxygen species (ROS) levels (DCFH-DA) were assessed before and after storage.

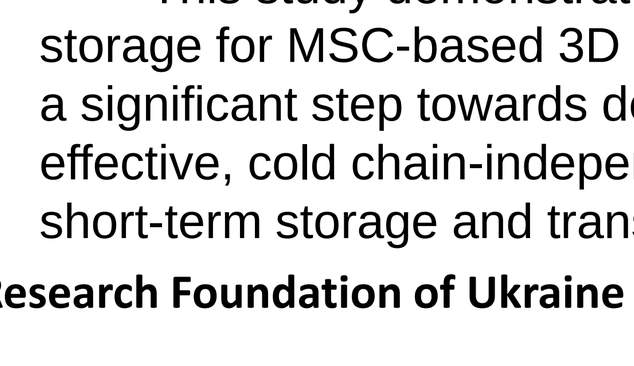
RESULTS & DISCUSSION



Ambient storage of MSCs in suspension resulted in significant viability loss.

Cells within all 3D constructs demonstrated preserved viability, metabolic activity, differentiation ability for up to 7 days of storage.

Resazurin-test before and after storage[&]



*- p <0,05 compared to suspension
#- p <0,05 compared to the index in the correspond group before storage(p <0,05)
& - samples were recultivated for 3 days before the resazurin test

Basal metabolic activity was decreased in spheroids and AMSs, and these constructs maintained unchanged levels of annexin-positive cells and ROS throughout storage. Annexin-positive cell number increased minimally in scaffolds and notably in suspension, with ROS rise in suspension after 7 day-storage.

CONCLUSION

This study demonstrates the feasibility of ambient storage for MSC-based 3D constructs and represents a significant step towards developing a safer, cost-effective, cold chain-independent solution for their short-term storage and transportation.

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