IOCBE 2024 Conference
The 1st International Online Conference on Bioengineering
16–18 October 2024 | Online
MDPI

Bioengineering of MSC-based 3D constructs with different types of cell organization

Natalia Trufanova[1], Oleh Trufanov[2], Galina Bozhok[3], Ruslana Oberemok[1], Olena Revenko[1], Oleksandr Petrenko[1]

[1] Department of Cryobiochemistry, [2] Department of Cryomicrobiology, [3] Department of Cryoendocrinology, Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

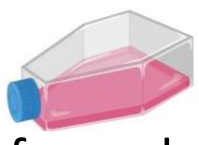
n.a.trufan@gmail.com

INTRODUCTION & AIM

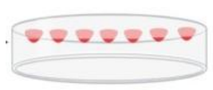
Mesenchymal stromal/stem cells (**MSCs**) possess unique biological properties, including self-renewal, differentiation, and secretory potentials. However, a standard 2D culture does not replicate MSCs' natural microenvironment, compromising their features**.** Engineering **MSC-based constructs** that support various **3D cell organizations** and analyzing cell behavior under such conditions are crucial for biomedical applications, offering relevant model systems and aiding in the development of therapeutic agents.

This study **aimed** to evaluate the impact of cultivating MSCs in **spheroids**, **alginate microspheres** (**AMSs**), and **blood plasma scaffolds** on viability and metabolic, and functional activity.

METHOD

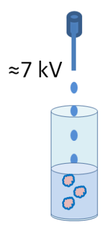


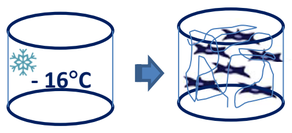
Human adipose tissue-derived MSCs (obtained with adult donors' informed consent) were used.



Spheroids were formed by the **"hanging drop"** method.

AMSs were generated by **electrospraying** of MSCs dispersed in 2% sodium alginate into 2% calcium chloride, were allowed to cross-link for 5 minutes, then washed out with Hank's Balanced Salt Solution and transferred to culture medium.



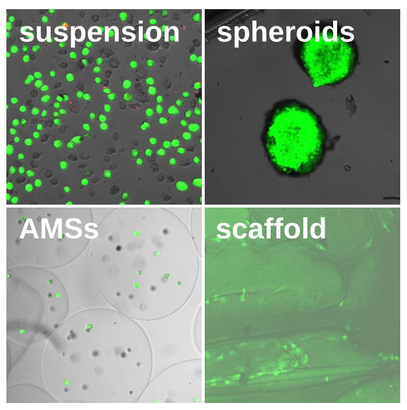


Scaffolds were prepared from diluted **blood plasma** through **cryogelation** and being seeded with cells.

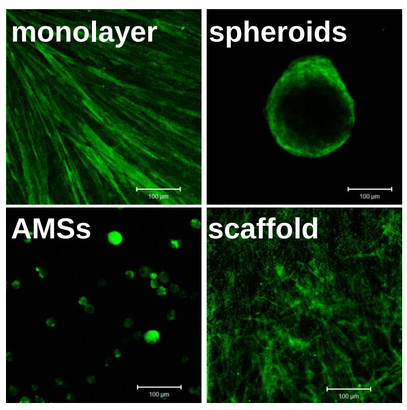
All constructs were **cultured** at 37 °C, 5% CO2, and 95% humidity in alpha-MEM supplemented with 10% fetal bovine serum, 50 µg/ml penicillin, and 50 µg/ml streptomycin. **Viability**(6-CFDA/ annexin V-Cy3 staining), **metabolic activity** (resazurin test), **actin filaments** (Phalloidin-FITC), **cell spreading**, and induced **differentiation** were examined.

RESULTS & DISCUSSION

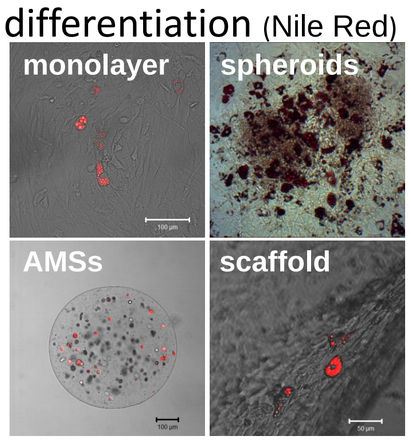
Viability (6-CFDA/ annexin V-Cy3)



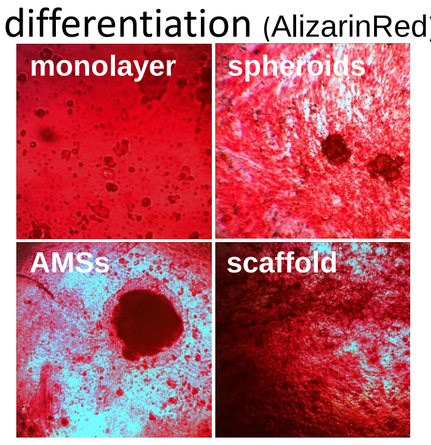
F-actin (Phalloidin-FITC)



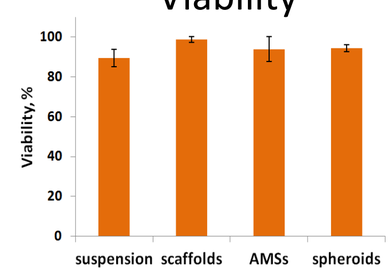
Adipogenic differentiation (Nile Red)



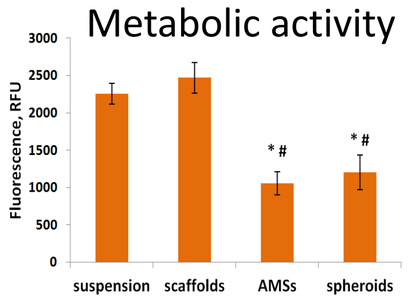
Osteogenic differentiation (AlizarinRed)



Viability



Metabolic activity



Note: *p<0.05 compared to suspension; #p<0.05 compared to scaffold

MSCs exhibited high viability in all constructs but displayed distinct morphologies (spindle-like in scaffolds, round in spheroids, and AMSs). Actin filament development was most pronounced in cells within scaffolds but in all 3Dconstructs was less than in monolayer. Metabolic activity was reduced in AMSs and spheroids compared to scaffolds, and monolayer. All groups have the ability for induced differentiation.

CONCLUSION

The cultivation of MSCs within a macroporous adhesive scaffold promotes fibroblast-like morphology and high metabolic activity. A spheroid and AMS culture results in round-shaped cells with lower metabolic activity, which can reflect a natural-like quiescence state. This study highlights the importance of 3D culture systems in maintaining MSC properties and suggests that constructs' design significantly influences cell functionality, crucial for advancing biomedical applications and therapeutic strategies.

NATIONAL RESEARCH FOUNDATION OF UKRAINE

This study was supported by National Research Foundation of Ukraine (project № 2021.01/0276)