The use of Nanofibrillar Cellulose Hydrogel Towards Culture Expansion of MSCs

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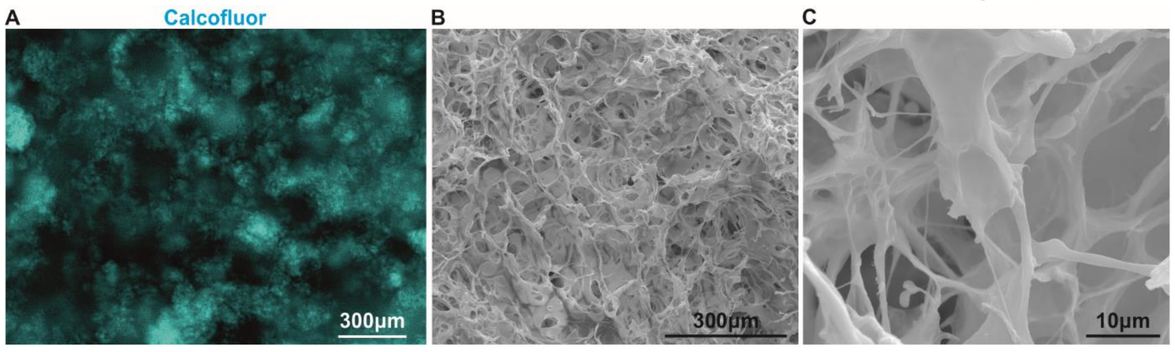
Both transplantation of MSCs and manufacturing of MSC-derived EVs require large cell numbers, long cultivation time, and consequently large volumes of cell culture medium. *In vitro* culture expansion is a necessary step in order to obtain sufficient quantities of cells for the intended therapeutic application. However, it is well known that during initial and extended *in vitro* 2D culture expansion, MSCs accumulate chromosomal aberrations, potentially lead to a loss of multipotency and induction towards cellular senescence.

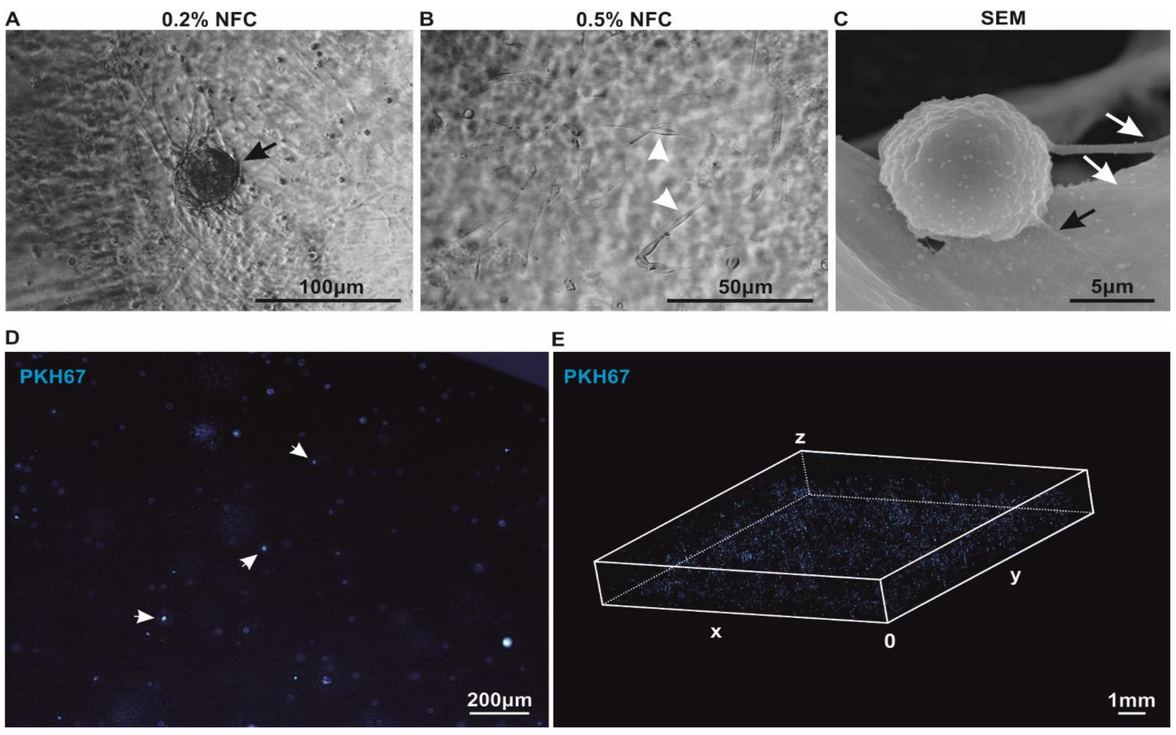
To overcome these limitations, different 3D cultivation methods, more closely resembling the endogenous niche, have been applied to MSCs. In this study, we examined the biocompatibility of multipotent human adipose tissue-derived and bone marrow derived mesenchymal stem cells (adMSCs and bmMSCs) with the commercially available NFC hydrogel GrowDex®.

NFC forms dense mesh-like structures with large pores.



Calcofluor staining (A) and scanning electron microscopy (SEM, B-C) show the nanofibrillar fibres are evenly distributed with a dense mesh-like structure with large pores each comprising of different sizes.

3D NFC hydrogels are biocompatible with MSCs



Within 0.2% MSCs displayed both a round and a typical fibroblast-like morphology, while several larger neurosphere-like clusters were observed (A, arrow). In contrast, cultivation of MSCs in 0.5% NFC resulted in a homogenous cell population with some bipolar morphology (B, arrow). SEM revealed that MSCs interact and bind to the NFC as evidenced by membrane protrusions onto the matrix (C). High content laser scanning microscopy was used to visualise larger numbers of living MSCs within the NFC, showing cells that are isotropically distributed within the matrix over large areas of the well.

PUBLICATIONS:

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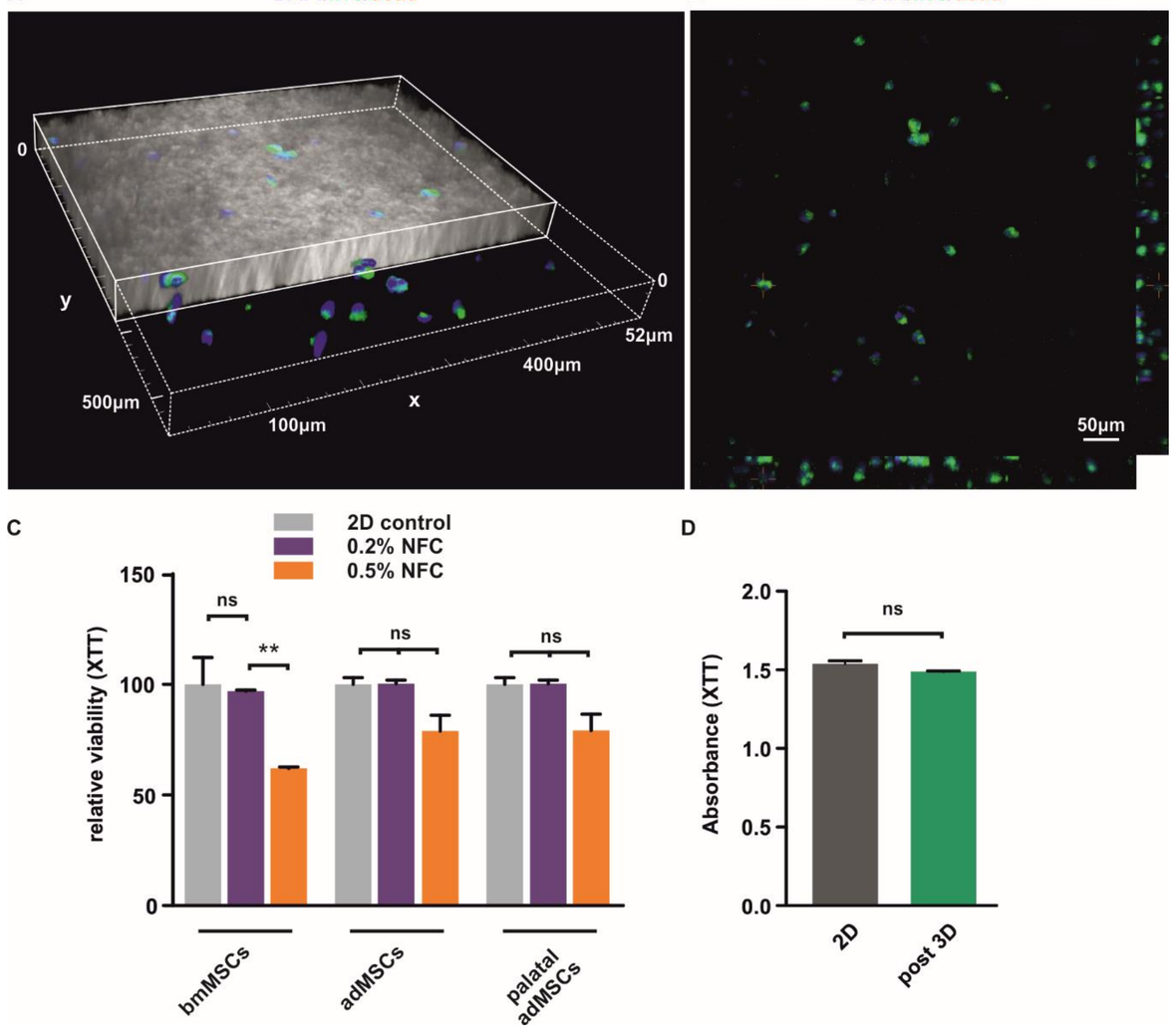
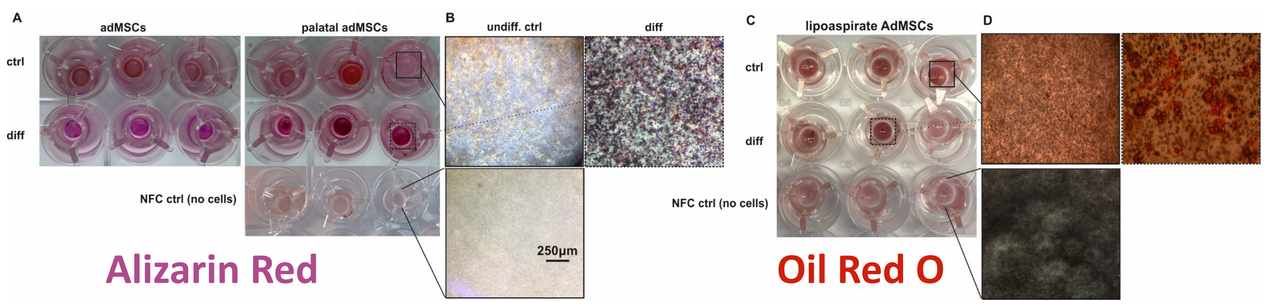
- BHATTACHARYA, M., MALINEN, M. M., LAUREN, P., LOU, Y.-R., KUISMA, S. W., KANNINEN, L., LILLE, M., CORLU, A., GUGUEN-GUILLOUZO, C., IKKALA, O., LAUKKANEN, A., URTTI, A. & YLIPERTTULA, M. 2012. Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. *Journal of Controlled Release,* 164, 291-298.
- LOU, Y.-R., KANNINEN, L., KUISMA, T., NIKLANDER, J., NOON, L. A., BURKS, D., URTTI, A. & YLIPERTTULA, M. 2014. The Use of Nanofibrillar Cellulose Hydrogel As a Flexible Three-Dimensional Model to Culture Human Pluripotent Stem Cells. *Stem Cells and Development,* 23, 380-392.

3D NFC supports high viability of MSCs. Viable MSCs can be easily retrieved from the 3D NFC hydrogel.



To assess the viability of MSCs within the NFC hydrogel, Live/Dead and XTT assays were performed. Laser scanning microscopy and post-hoc image analysis revealed that most cells within the hydrogel stained positively for Calcein (A-B). Moreover, the cells were distributed evenly in all three dimensions of the hydrogel (A). No pyknotic or fragmented nuclei were observed. MSCs cultivated in 0.2% NFC showed no significant changes in cellular viability compared to the 2D control (C). In contrast, all MSCs showed reduced viability in 0.5% NFC. Cells were easily retrieved by enzymatically digesting the NFC using cellulase (GrowDase®). No significant decrease was seen in XTT viability of the retrieved cells when reseeded under standard 2D conditions (D).

3D NFC hydrogels are suitable for osteogenic and adipogenic differentiation of MSCs in 3D



To assess the feasibility of osteogenic differentiation in 3D NFC, MSCs were subjected to osteogenic (A-B) and adipogenic differentiation (C-D) in 0.2% NFC for 21 days and stained for calcium deposition and lipid deposition using Alizarin Red and Oil Red O respectively. MSCs deposit high amounts of calcium and lipids after 21 days of differentiation compared to undifferentiated cells in 0.2% NFC (B&D).

CONCLUSION

We demonstrate that both adMSCs and bmMSCs interact with the NFC, are evenly distributed within all three dimensions of the hydrogel, and are viable in 0.2% and 0.5% NFC hydrogel. Moreover, MSCs were successfully differentiated into osteogenic and adipogenic cells within the 3D hydrogel. Finally, we show that MSCs can be easily retrieved from the 3D hydrogel via an enzymatic digestion of the NFC with a cellulase-mix and that the post-NFC MSCs show similar viability to 2D pre-cultivated MSCs.