Characterization of cell spheroids as promising tool as 3D printable building blocks for vascular network formation

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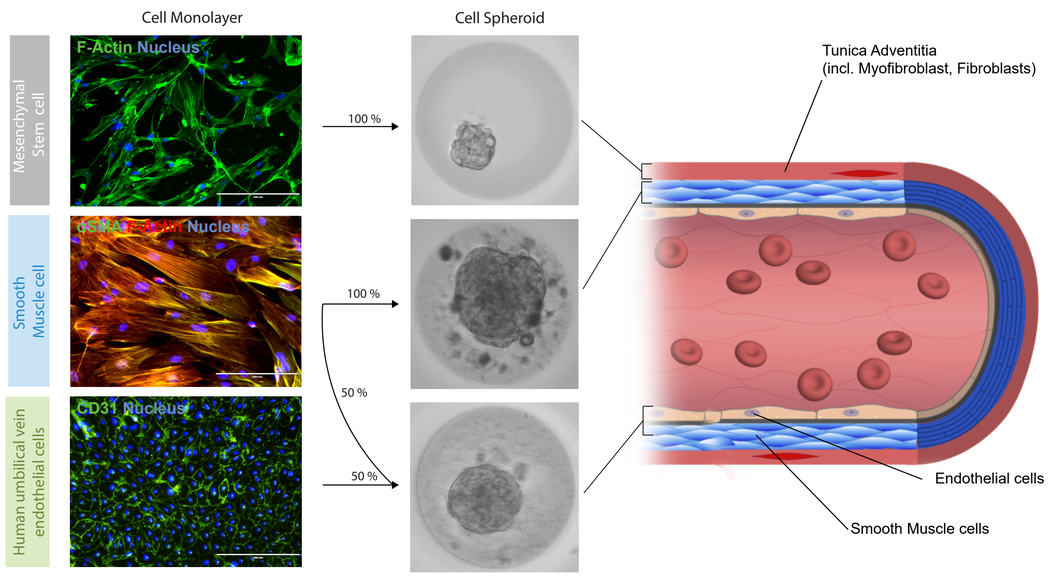
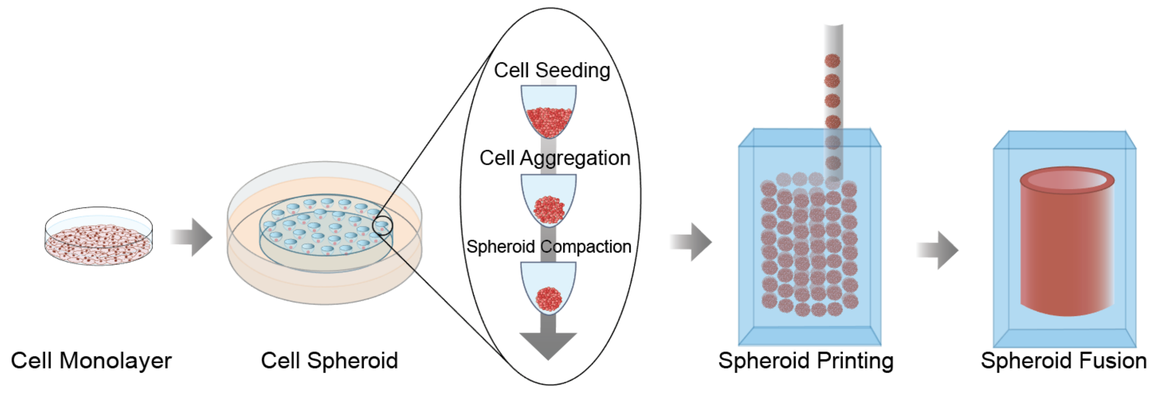
BACKGROUND



Cell spheroids contain cells which can be find in different layers of blood vessels.

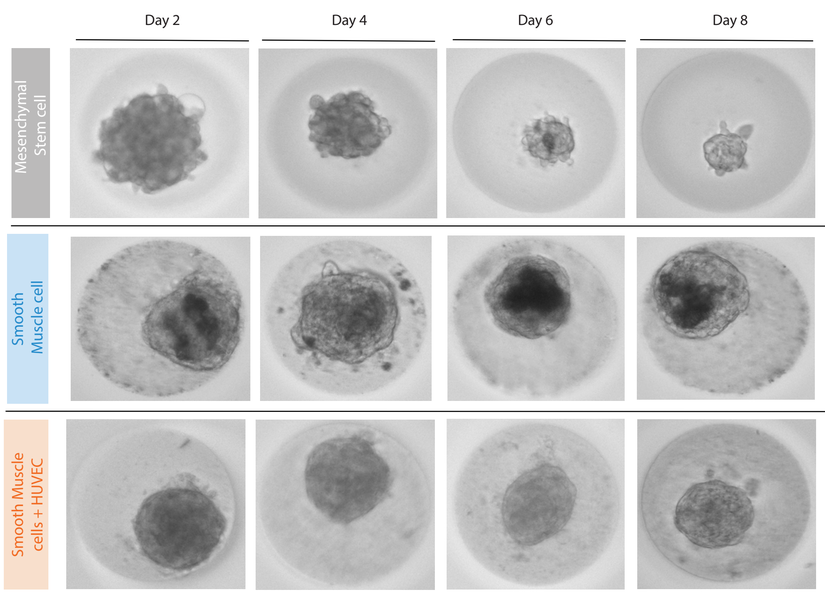
WORK FLOW



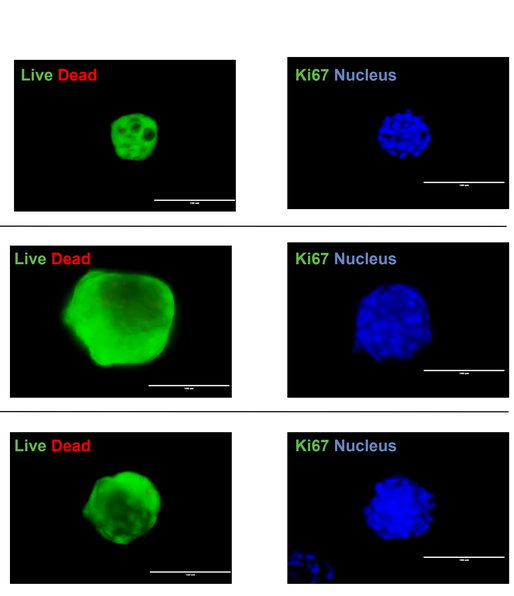
Multicellular spheroids of different cellular compositions are cultured in agarose microwell array platforms. After cell seeding cells aggregate to spheroids and become more compact over time. Finally the cell spheroids can be used as building blocks in a 3D printing approuch in suspension hydrogels where they are able to fuse with each other.

CELL SPHEROID COMPACTION

Cell spheroid morphology during compaction

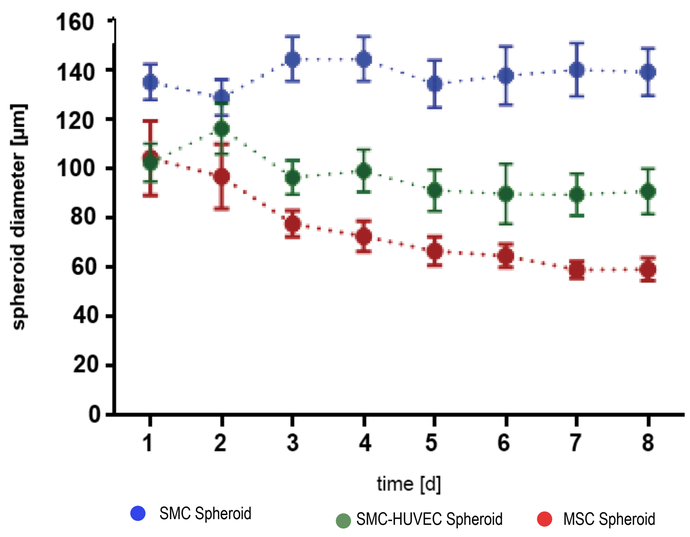


Viability



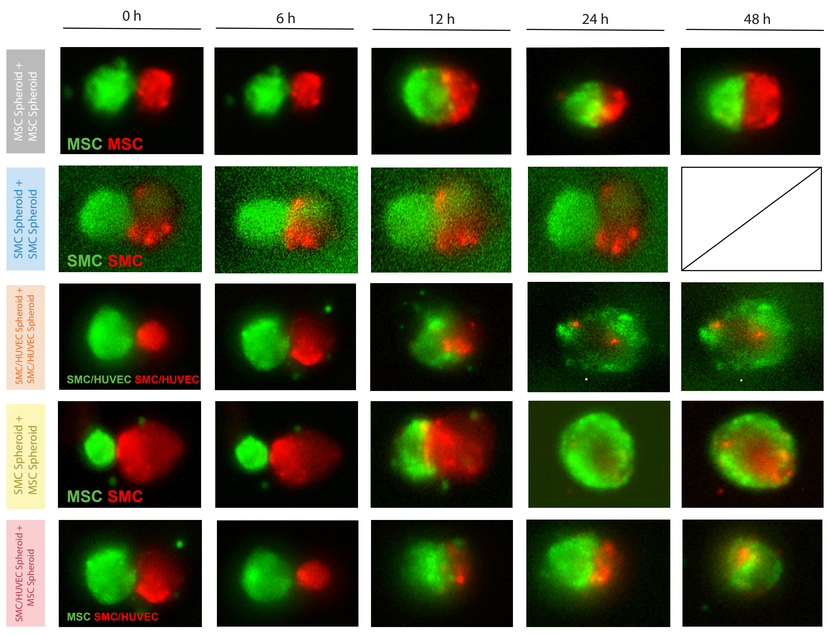
Proliferation

Cell spheroid compaction rate



Three different types of spheroids (100 % SMC, 100 % MSC, 50 % SMC + 50 % HUVEC) were cultivated in agarose multiwell array platforms for 8 days and their compaction was analyzed by using a custom developed MATLAB based program. After 8 days, the viability and possible proliferation was analzed by a Live/Dead and Ki67 staining.

CELL SPHEROID FUSION



Cell spheroids of the same and different cell types were labled with cell tracker.Their fusion in agarose microwell array platforms was analyzed over 48h.

CONCLUSION

Cell spheroid compaction

- each spheroid consists in average of 267 cells
- 100 % SMC spheroids don't become more compact over time and have a final average diameter of 139 µm
- 100 % MSC spheroid reach their final compaction at day 7 with a final average diameter of 60 µm
- 50 % HUVECs + 50 % SMC spheroids reach their final compaction at day 5 with a final average diamter of of 91 µm
- in all spheroid cell types: after 8 days neither dead cells nor proliferating cells could be detected

Cell spheroid fusion

- all fusion experiments took place in cell cultivation medium
- fused MSC spheroids reach their final fusion state after 12 hours but don't mix both cell populations
- fused SMC-MSC spheroids reach their final fusion state after 24 hours and the MSCs seem to cover the surface of the fused spheroid while the SMCs form the core
- SMC/HUVEC-MSC spheroids reach their final fusion state after 48 hours and seemed to be randomly mixed
- SMC spheroids show an on giong fusion even after 48 hours without mixing both cell populations
- SMC/HUVEC spheroid fusion reach their final state after 24 hours and showed a randomly mixed cell population

Using agarose microwell arrays for creating a high number of cell spheroids displays a usefull platform for creating spheroids of predictable size, who don't show cell death after the cultivation and allow spheroid fusion after the transfer to a new cultivation system. These characteristics make spheroids to a promising tool for use in 3D hydrogel suspension printing.

ACKNOWLEDGEMENTS

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