Development of biodegradable/implantable microcarriers (MCs) for cultivation of human adipose stem cells (hASCs) for cell therapeutic applications (9 D-LAB)

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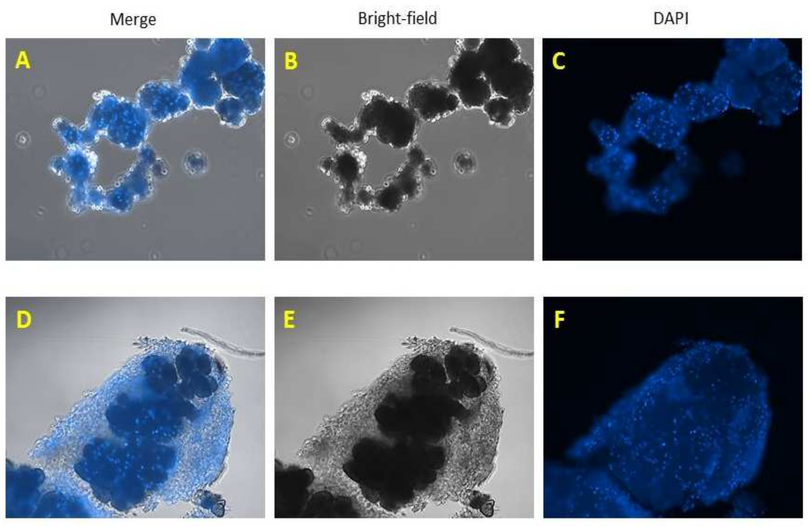
Introduction

Human adipose stem cells (hASCs) are very promising candidates for cell-based therapies. Efficient expansion of clinical grade hASCs can be achieved in a microcarrier-based cultivation system. The specific goal of the project is to develop one of the first biodegradable/implantable suitable microcarrier (MC) for therapeutic use of hASCs.

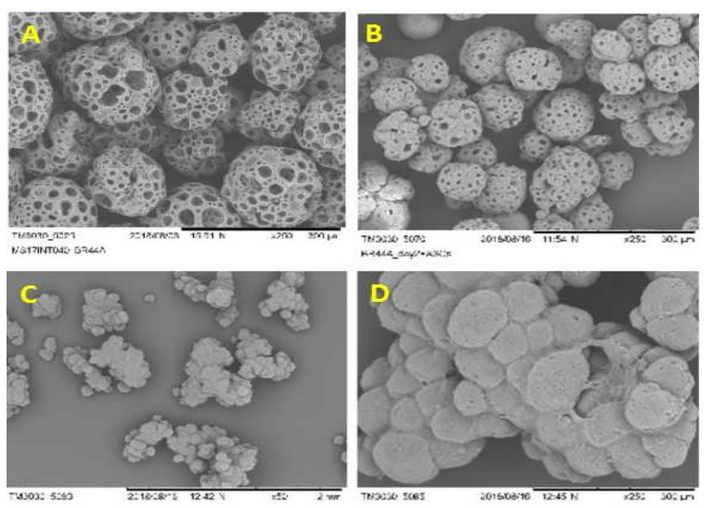
Methods & Results

hASCs were isolated from discarded human adipose tissue and cultured in xeno- and serum-free conditions. Several types of MCs manufactured by Micro-Sphere SA were examined to test their ability to promote attachment on their surface and growth of hASCs in serum-free culture conditions. hASCs cells were analyzed for the expression of typical markers (FACS analysis) and by quantitative RT-PCR to monitor the expression of some marker genes. The growth of the cells on MCs was monitored by immunohistochemical methods and the cell attachment by Scanning Electron Microscopy (SEM). The best performing MCs prototypes were also tested in respect to their biochemical engineering characteristics. These included the measurement of the sedimentation velocities and the determination of the suspension criteria.

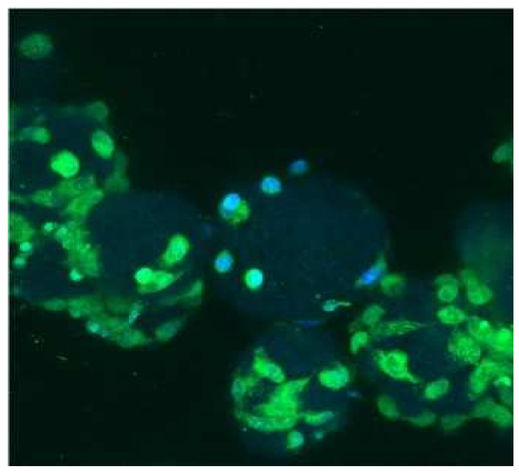
Results



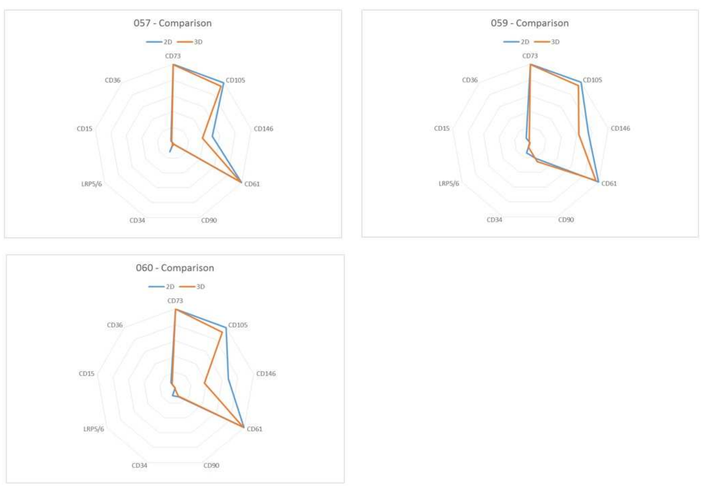
DAPI stained samples of hASCs cultured on the BR44 MCs in US-10 medium in static conditions. **A-C**: after 1 day. **D-F:** after 4 days in culture



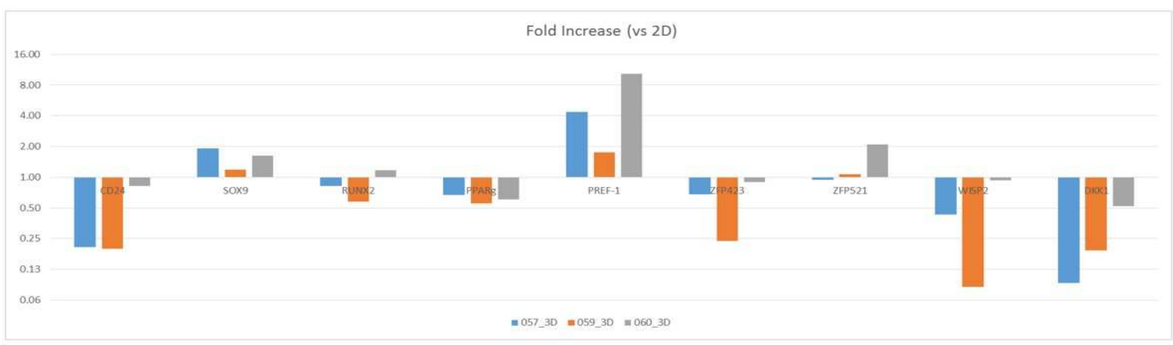
SEM images to confirm the presence of hASCs after culturing BR44 MCs in US-10 in static conditions. A: BR44 alone. **B:** BR44 + cells after 1 day. **C & D:** BR44 + cells after 4 days of culture



Confocal laser-scanning microscopy analysis of BR44 MCs/hASC complexes stained with DAPI after 1 day of culture



Flow cytometry evaluation 2D vs 3D:marker profile. 2D: hASCs cultered on T75 flask in US-10 medium. **3D:** hASCs cultered on the BR44 MCs in US-10 medium. Data from 3 different patients.



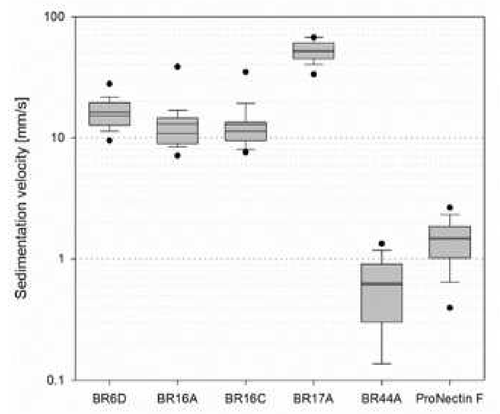
Pro-differentiation 2D vs 3D (MCs BR44)

CD24 ↓
PPARγ ↓
Runx2 ↓
ZFP423 ↓
DKK1 ↓

Pro-stemness/anti-differentiation 2D vs 3D (MCs BR44)

Sox9 ↑
Pref-1 ↑
ZFP521 ↑
WISP2 ↓

RT-qPCR evaluation of the expression levels of some key genes involved in cell stemness or cell differentiation. hASCs grown in standard 2D or in 3D (microcarriers BR44) cell culture systems. Fold increase/decrease relative to 2D expression levels, data from 3 different patients.



Comparison of the sedimentation velocities. The sedimentation velocities were measured for five MC-prototypes and compared with those of a commercially available polystyrene-based MC (ProNectin F).

MC type	N_{S1U} / N_{S1}	u_{tip}	Re	P/V	τ_{nt} LSS
[-]	[rpm]	[m/s]	[-]	[W/m³]	[*10⁻³ Pa]
BR6D	69 / 76	0.15 / 0.17	2845 / 3134	1.70 / 2.21	6.79 / 7.48
BR16A	99 / 124	0.22 / 0.27	4082 / 5113	4.62 / 8.84	9.76 / 12.24
BR16C	98 / 118	0.21 / 0.26	4041 / 4865	4.50 / 7.67	9.66 / 11.65
BR17A	116 / 136	0.25 / 0.30	4783 / 5608	7.30 / 11.46	11.45 / 13.44
BR44A	75 / 90	0.16 / 0.20	3092 / 3710	2.07 / 3.42	7.31 / 8.92
ProNectin	49 / 63	0.11 / 0.14	2020 / 2598	0.70 / 1.34	4.80 / 6.19

Overview of the different calculated biochemical engineering parameters

N_{s1} is the impeller speed required to just fully suspend all particles

N_{s1u} is determined as the lower limit of N_{s1} meaning that some particles are still located at the bottom of the flask, but none of them are at rest

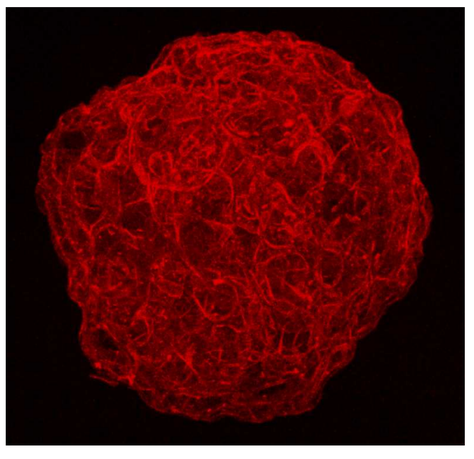
U_{tip} gives an indication about the max. Expected velocity in a stirred bioreactor

Re (Reynolds number) is used to describe the fluid flow in a bioreactor

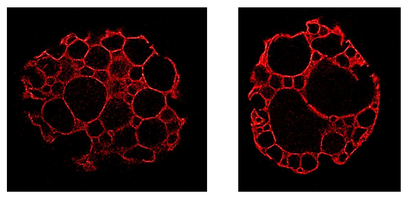
P/V, the specific power input, represents an important parameter in terms of process scale-up and the estimation of hydrodynamic stresses

t_{nt} gives an idea of the hydrodynamic stress acting on the cells

Checking the distribution of the bovine gelatin on the surface of the microcarrier BR44



BR44 microcarriers were labelled with the CF633 dye (Biotium) which reacts with free amino goups. After washing away the dye in excess, the carriers were examined under a confocal microscope.
This representative microphotographs clearly demonstrate that the porcine gelatin is distributed homogeneously among the highly porous MC



Conclusions

Only a specific combination of components & manufacturing parameters resulted in a MCs prototype with the desired functionality. The MC prototype BR44 allows to hASCs to stick to its surface, to grow in a defined serum-free cell culture medium (UrSuppe-10, US-10), and to maintain their stemness. Biochemical engineering investigations of the prototype BR44 showed suspension parameters similar to those found by the commercial non-biodegradable MC ProNectinF (Pall SoloHill). The system is now mature for testing a scale-up of hASCs production in stirred single-use bioreactor under defined serum-free conditions.

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