# Availability of extracellular matrix biopolymers and differentiation state of human mesenchymal stem cells determine tissue-like growth in vitro


***Abstract***

To explore the space-filling growth of adherent mesenchymal stem cells (MSC) into tissue-like structures in vitro, human bone marrow derived MSC were exposed to fibronectin-coated, millimeter-sized, triangular channels casted in poly(dimethyl siloxane) carriers. The results revealed that the threedimensional (3D) growth of MSC differs in dependence on differentiation status and availability ofextracellular matrix (ECM) proteins: Massive 3D structure formation was observed for MSC under pro-osteogenic stimulation but not for undifferentiated MSC nor for MSC under pro-adipogenic stimulation; boosting cellular matrix secretion and addition of soluble ECM proteins caused extensive 3D tissue formation of undifferentiated MSC. The reported findings may contribute to bridge the gap between in vitro and in vivo analyses and guide the application of MSC in tissue replacement approaches.


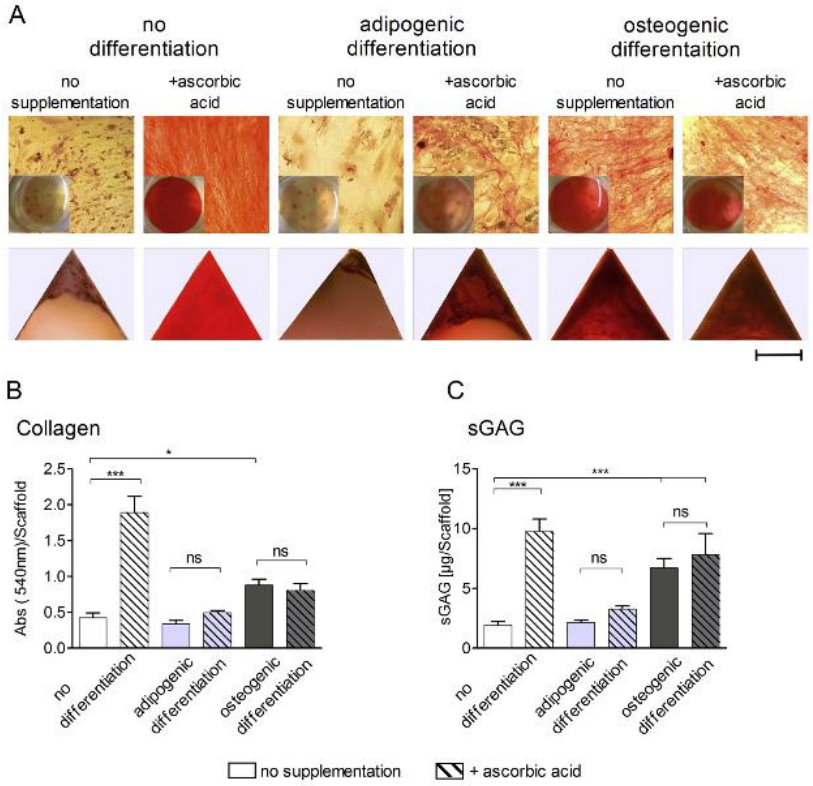


**Fig. 3.** ECM production of MSC. (A) Light microscopy images of collagen staining (Direct red) of MSC cultures under standard, adipogenic or osteogenic culture conditions, with or without ascorbic acid supplementation. The upper panel shows collagen distribution on top of the whole PDMS carrier. The insert shows an overview of the whole carrier with six channels. The lower panel shows collagen distribution inside the 3D tissue (one corner of a triangular channel). Scale bar 200 µm. (B) Quantification of total collagen (absorbance of extracted Direct red dye) and sulfated glycosaminoglycans (sGAGs, quantified by colorimetric assay of extracted GAGs) from the whole PDMS carrier (including 6 channels) after 24 days of cultivation. Data represent 2 independent experiments with 4 different MSC donors. Statistical significance by ANOVA: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns – not significant. Error bars indicate S.E.M.