

# Stromal Vascular Fraction from Adipose Tissue and Cell Sheet Engineering to build Vascularization Units for Tissue Engineering and Regenerative Medicine

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Vascularization holds the gold key for the effective survival and engraftment of complex engineered tissues and organs for Tissue Engineering and Regenerative Medicine. The lack of adequate vascularization post-transplantation often results in cell necrosis and ultimate failure and rejection of the engineered construct. Herein, we propose a strategy capable of surpassing this obstacle. Harnessing easy accessible adipose tissue stromal vascular fraction (SVF) as a source for cells with intrinsic angiogenic potential, and cell sheet technology we were able to engineer cell sheets with high angiogenic potential. SVF was isolated from the adipose tissue of healthy human subjects after enzymatic digestion and  $2 \times 10^5$  nucleated cells/well were seeded on 24 well plates for cell sheet formation. To further boost cells' angiogenic potential, hypoxic conditions of 5% of oxygen were provided to some of the cells while the rest was cultured in typical normoxia, for up to 8 days of culture, in basal medium. Flow cytometry analysis demonstrated the presence of a heterogeneous population of mesenchymal progenitors, endothelial and hematopoietic cells. Furthermore, the proliferation of SVF cells was evaluated through dsDNA quantification, which showed higher numbers for cells in hypoxic conditions, at earlier time points. Immunocytochemistry against CD31 and CD146 revealed the presence of an interconnected and highly branched network of vessel-like structures, more prominent for cells in hypoxia after 5 days of culture and quite similar for both conditions after 8 days, in the absence of any specific media supplementation. In vivo testing using the cell sheets detached from the wells and HIF expression analysis are currently underway. Taken together, the great potentiality of cell sheet technology with SVF cells cultured in hypoxia opens new exciting perspectives and may represent tremendously valuable vascularization units for tissue engineering strategies.