Engineering Vascularized Human Adipose Tissue
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Introduction
Due to complications of currently available therapies, the clinical impetus to engineer adipose tissue for the correction of soft tissue defects is high. Unfortunately, poor survival of in vitro-engineered adipose tissue after transplantation is still a major hurdle for clinical use. The main reason for this poor survival is postulated to be insufficient vascularization. In this study, we aim to improve the in vivo-vascularization of engineered adipose tissue by pre-forming vascular structures within in vitro-engineered adipose tissue constructs that can integrate with the host vascular system upon transplantation.

Materials and Methods
Different percentages of human adipose-derived stromal cells (ASC) (n=3 donors) and human umbilical vein endothelial cells (HUVEC) were combined in spheroid co-cultures of 2.5*10^5 cells to obtain the optimal combination of ASC/HUVEC for the generation of prevascularized adipose tissue constructs. In vitro-adipogenic differentiation of ASC in these constructs was assessed by oil red o staining and by gene expression of fatty acid binding protein 4 (FABP4). After seven days of culture, prevascularized and non-prevascularized constructs (each n=12) were transplanted subcutaneously in nude mice (n=6). Upon seven days of transplantation, constructs were retrieved and cross-sections were stained with anti-human CD31 to determine the number and relative area occupied by human vascular structures post-transplantation. Significant differences between groups were determined with the Mann Whitney test. Values with P≤0.05 were considered significant.

Results
Only when 20% ASC and 80% HUVEC were combined and cultured in a 1:1 mixture of endothelial cell and adipogenic medium, prevascular structures were formed in the constructs. Moreover, the ASC accumulated lipid and expressed FABP4. Transplantation of these prevascularized ASC/HUVEC constructs in nude mice resulted in a significantly higher amount of vessels (37±17 vessels/mm²) within the constructs compared with non-prevascularized constructs composed only of ASC (3±4 vessels/mm²). Moreover, a subset of the pre-formed human vascular structures (3.6±4.2 structures/mm²) anastomosed with mouse vessels and delivered blood to the constructs.

A. B.
Fig. 1. Anti-human CD31 (brown) stained sections of [A] 80% HUVEC/20%ASC construct and [B] ASC construct, taken at day 7 post-transplantation. Magnification 200x

Discussion and Conclusions
These results indicate that pre-formed vascular structures within engineered adipose tissue constructs can integrate with the host vascular system and improve the vascularization upon transplantation.

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Disclosures
The authors have nothing to disclose.