ABSTRACT INTRODUCTION: Early vascularization in the center of tissue-engineered osteogenic cell-scaffold constructs of clinically relevant sizes is critical for cell survival and successful engraftment. The implants are generally seeded only with osteogenic cells and thus avascular, and the furniture of cell nutrients and oxygen fully relies on the invasion of the implant by blood vessels from the host. Thus, many cells die in the interior of the graft before they can be reached by this new vasculature. This issue can be addressed by implanting osteogenic cells together with endothelial cells, capable to organize an intrinsic vascular network, thereby accelerating the vascularization process. Here, we aimed at generating such a vasculogenic-osteogenic construct starting from a single cell source, namely adipose tissue-derived cells.

METHODS: Lipoaspirates from healthy patients, with body mass index ranging from 16.8-25.1 kg/m², were obtained after informed consent following a procedure approved by the Local Ethical Comity of the University Hospital Basel. They were digested with type II collagenase for 30-45 minutes and the stromal-vascular fraction (SVF) was isolated from the differentiated adipocytes by centrifugation. Freshly isolated SVF cells were first analyzed by FACS for the expression of the proteins described in the results section, and their clonogenic capacity was determined by colony forming units-fibroblastic (CFU-f) assay. The in vitro osteoblastic potential was determined by culturing the cells with osteogenic medium for 3 weeks, with subsequent fixation of the cells and staining with either alizarin red or Von Kossa staining. 3.10⁵ cells were seeded and cultured for 5 days in hydroxyapatite porous disks (Engipore, FineCeramica Faenza, IT; 8mm-diameter x 4-mm thick) by using a perfusion bioreactor. A similar amount of the same cells were statically loaded on H&E-stained sections for scaffolds cultured 5 days under perfusion in the bioreactor. 27% of pore space was filled with lenticular bone tissue, homogenous, abundant bone formation inside the implant was observed with constructs generated by loading SVF cells or cells expanded for 5 days on 2D tissue culture plastic. This will have to be further confirmed in future experiments.

RESULTS SECTION: We obtained 5.9 ± 3.5x10⁵ cells/ml of lipoaspirate (n=7), of which 5.2 ± 0.9 % (n=7) showed clonogenic capacities. These cells were able to differentiate towards the osteoblastic lineage in vitro. Phenotypically, freshly isolated SVF contained cells of both mesenchymal (positive for CD90, CD44 and CD105) and endothelial (positive for CD34, VEGFR2 and CD31) lineages. Culture on plastic, most of the cells expressing CD34, VEGFR2 and CD31 were lost at the first passage whereas expression of CD44, CD90 and CD105 gradually increased to reach 100 % of cells. After 5 days of culture, the number of cells starting from 1.5x10⁴ clonogenic cells was much higher in 2D cultures (4.8 ± 3.8 x 10³ cells, n=5) than in 3D cultures inside the scaffolds (0.63 ± 0.41 x 10³ cells, n=3). The phenotype of cells was similar in both culture conditions, with 59 ± 34 % (n = 2) and 73 ± 29 % (n=3) of the cells expressing the mesenchymal marker CD90 in 2D and 3D respectively. The proportion of CD34+/CD31- cells - of the endothelial lineage - were respectively 6 ± 0.1 % (n = 2) and 9 ± 6 % (n = 3). After 8 weeks of in vivo implantation in mouse, and as shown in the picture below, a homogenous, abundant bone formation inside the implant was observed on H&E-stained sections for scaffolds cultured 5 days under perfusion in the bioreactor. 27% of pore space was filled with lenticular bone tissue, as the ones shown with black arrows. So far, no in vivo bone formation was observed with constructs generated by loading SVF cells or cells expanded for 5 days on 2D tissue culture plastic. This will have to be further confirmed in future experiments.

DISCUSSION: In conclusion, direct perfusion of freshly harvested adipose-derived cells through porous ceramic for a short time generated osteoinductive constructs with intrinsic vasculogenic capacities. The number but also the homogeneity of blood vessels of human origin inside the construct is not optimal yet. However, some preliminary experiments suggest that the survival of more cells of the endothelial lineage for a prolonged period is possible by using specific expansion media. The use of such culture media should likely result in a higher and more homogenous blood vessel formation. It also remains to be tested whether the extension of the approach to larger constructs could accelerate graft vascularization and thereby enhance survival of the implanted cells in the construct core.

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