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Generation of osteogenic and vasculogenic implants by perfusion culture of adipose tissue-derived cells

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INTRODUCTION: Vascularization of engineered tissues of clinically relevant size is central for cell survival and successful engraftment, and could be accelerated by addition of endothelial cells. In the context of bone tissue engineering, we hypothesized that osteogenic-vasculogenic constructs could be generated by co-culturing osteoprogenitors together with endothelial progenitors, starting from adipose tissue-derived cells as a single cell source.

METHODS: Lipoaspirates from healthy patients were digested with collagenase for 45-60 minutes and the stromal-vascular fraction (SVF) was isolated by centrifugation. SVF cells were analyzed by FACS and their clonogenic capacity was determined by colony forming unit-fibroblastic (CFU-f) assay. The in vitro differentiation potential was determined after a culture with osteogenic or adipogenic medium for 3 weeks and subsequent staining with alizarin red, Von Kossa or by light microscopy. 3.10^6 cells were seeded and cultured for 5 days in hydroxyapatite porous disks (Engipore, Finceramica, Faenza, IT; 8mm-diameter x 4-mm thick) by using a 3D perfusion bioreactor. A similar amount of the same cells was expanded for 5d in 2D culture on plastic dishes, and statically loaded onto similar scaffolds. After the in vitro expansion, cells in 2D and 3D cultures were harvested by enzymatic digestion and counted; their phenotype was determined by FACS analysis. 2D and 3D generated constructs were subcutaneously implanted in nude mice for 8 weeks. Constructs were then harvested and paraffin-embedded. 7 µm sections were stained either with Hematoxylin/Eosin (H&E) or with antibodies for human BSP, CD34 or CD31.

RESULTS: $5.9 \pm 3.5 \times 10^5$ cells were obtained per 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 and adipogenic lineages in vitro. Phenotypically, freshly isolated SVF contained cells of both mesenchymal (positive for CD90, CD44 and CD105) and endothelial (positive for CD34 and CD31) lineages. After 5 days of culture, the number of cells starting from 1.5×10^5 clonogenic cells was twice higher in 2D cultures ($4.8 \pm 4.1 \times 10^5$) than in 3D perfusion cultures ($2.7 \pm 2.2 \times 10^5$). The phenotype of the cells was similar in both culture conditions, with 65 ± 26 % (n=3) and 72 ± 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 7 ± 3 % and 9 ± 6 % (n = 3). In vivo, an abundant bone formation was observed in constructs generated under 3D perfusion, whereas 2D-generated constructs did not produce bone. BSP expression was found in the newly formed bone. A negative safranin-O staining excluded the probability of an endochondral bone formation. Polarized light microscopy revealed stratification in the newly formed bone. 27-43 % of the blood vessels inside the 3D-generated implants were of human (donor) origin and not from the host (mouse), as demonstrated by a positive immunostaining of these vessels with human-specific anti-CD31 and anti-CD34 antibodies. These blood vessels of human origin were functional and connected to the vasculature of the host, as proved by the presence of erythrocytes in their lumen. Similar results were found in constructs generated with 2D-expanded cells.

DISCUSSION & CONCLUSIONS: In conclusion, direct perfusion of freshly harvested adipose-derived cells through porous ceramic for a short time generated osteogenic constructs with intrinsic vasculogenic capacities. It remains to be tested whether the extension of this approach to larger constructs could actually accelerate the vascularization of the graft and thereby enhance survival of the implanted cells in the construct core.

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