PURPOSE: Research has not yet demonstrated a reliable solution to overcome the postoperative volume loss in fat grafting. The establishment of an early blood supply is important to prevent tissue necrosis for higher fat engraftment. We have recently developed an ex vivo expansion system named Quality and Quantity control culture system (QQc) which strongly stimulates the vasculogenic potential of endothelial progenitor cells (EPCs) and multiplies their number. We hypothesized that the addition of EPCs, enhanced by the QQ culture, to freshly harvested adipose tissue aids in the establishment of a blood vessel network which leads to better graft survival.

METHODS: C-kit+ Sca-1+ lineage-negative (KSL) cells were isolated as EPC-precursors from C57BL/6 mice and cultured for 7 days in QQc. Adipose tissue, harvested from C57BL/6 mice and weighing 0.25g per graft, was transplanted with 2x10^4 QQc-cultured KSL-cells (group 1, n=18) or with 2x10^4 non-cultured KSL-cells (group 2, n=10), while controls received the adipose tissue alone (group 3, n=18). 5 and 10 weeks later, the grafts were explanted and graft survival was evaluated by weight maintenance. Histological and immunohistochemical assessment of the grafts was performed after 5 weeks. The expression of the angiogenic marker PDGF-B and the adipocyte-specific markers adiponectin and FABP4 in the grafted tissue was analyzed by qRT-PCR after 5 weeks and compared to group 3.

RESULTS: Group 1 demonstrated the highest graft survival after 5 weeks (58.2±23.8%) compared to group 2 (51.1±21.3%, p>0.05) and group 3 (40.3±19.7%, p<0.05). The graft weight remained stable between 5 and 10 weeks after grafting. CD31 immunohistochemistry confirmed a higher vessel density in group 1 (35.0±7.4/mm²) in comparison with groups 2 (28.4±5.5/mm², p<0.01) and 3 (20.8±6.4/mm², p<0.0001). In addition, the lowest percentage of fibrotic tissue was found in group 1 (13.8±10.8%; group 2: 20.9±10.1%; group 3: 21.9±11.4%, p<0.01) and grafts treated with QQc-cultured KSL-cells showed less CD68-positive local inflammation units (31.4±21.9/mm² vs 40.2±26.7/mm² vs 42.8±33.5/mm², p<0.05). Moreover, we observed a significant upregulation of PDGF-B (1.7-fold change, p<0.01), adiponectin (1.9-fold change, p<0.05) and FABP4 (1.9-fold change, p<0.05) in group 1.

CONCLUSIONS: The addition of QQc-cultured EPC to murine fat grafts improves their survival by stimulating neovascularization. This is associated with a higher vessel density, less fibrosis, less inflammatory infiltrates and an increase in adipocyte-specific markers. Since the QQ culture greatly increases the EPC number and their vasculogenic potential, an optimal result can be achieved from an initially small number of cells. In the future, QQc-cultured EPC may lead to a more stable postoperative result in human fat grafting.

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The Effects of Adipose Derived Stromal Cells on SAS Human Head and Neck Squamous Cell Carcinoma

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PURPOSE: Many oncologic surgeries create significant defects with a need for reconstruction. Cell-assisted lipotransfer (CAL) has shown promise for fat graft retention in reconstructive surgery. However, mesenchymal stem cells have been shown in breast and other cancers to promote tumor proliferation and metastasis, thus leading to concern for utilizing CAL following oncologic surgery. In contrast, there is little information regarding the effect of adipose-derived stromal cells (ASCs) on head and neck squamous cell carcinoma (HNSCC). While Rowan et al. showed no increase in Cal-27 and SCC-4 HNSCC tumor growth with ASCs, an increase in micrometastasis was noted when these HNSCC cells were co-injected with ASCs. As a multitude of other HNSCC cell lines exist, there is still much to be examined regarding the effect of ASCs on HNSCC.

METHODS: SAS HNSCC was grown in culture with either regular media, tumor conditioned media, or ASC
conditioned media. Tumor cells were lifted and counted in triplicate every other day for 9 days post plating. For the in-vivo arm, 5x10⁵ GFP labeled SAS HNSCC cells were injected into the subcutaneous plane of the scalp of immunocompromised mice either alone, in addition to a 200uL fat graft, or in addition to a 200uL fat graft and 10,000 ASCs. CT scans were taken weekly for 4 weeks, and subsequently tumors and grafts were explanted, digested, and fluorescence-activated cell sorting was performed.

**RESULTS:** At three weeks, there was significantly greater tumor volume by microCT in mice receiving cancer cells with fat graft and ASCs relative to mice receiving cancer cells only and cancer cells plus flat graft. Additionally, the cancer cells plated with ASC conditioned media show greater proliferation than the cancer cells plated with either regular media or tumor conditioned media.

**CONCLUSIONS:** Our results show that there is increased SAS HNSCC growth when in the presence of ASCs in-vivo, and ASC conditioned media in-vitro. While further studies are necessary, reconstruction using CAL following oncologic surgery for SAS HNSCC can potentially increase residual cancer cell growth.