Background: Unpredictable retention outcomes remain a significant issue in autologous fat grafting procedures. Liposuction cannula variation leads to variability in fat particle size. Recent data suggest that the size of fat particles is closely related to graft healing outcomes, however this remains a point of contention due to potential confounding variables such as tissue trauma with harvest. The aim of this study was to compare autologous fat grafting outcomes with variable fat particle sizes in an animal model which isolated fat particle size as the primary experimental variable. The overall goal of this work is to determine if reducing fat particle size is an effective method for enhancing graft retention in autologous fat grafting.

Methods: The range of fat particle diameter harvested by four common liposuction cannulas was quantified to define relevant small and large particle target diameters. To determine if particle size impacted nutrient and oxygen permeability, small and large particles were incubated in vitro in a spinner flask with an abundance of culture media and VEGF secretion was measured with ELISA. Finally, small and large fat grafts were prepared from subcutaneous mouse fat pads and syngeneically grafted in Balb/CJ mice. Weight and volume retention were evaluated at 1, 4, 8, and 12 weeks. Histological analysis with Masson’s trichrome and perilipin immunofluorescent staining was performed. qRT-PCR was performed for adipogenic, inflammatory and apoptotic genes.

Results: The range of fat particle diameters harvested with four commonly used cannulas was 2-7 mm. In vitro studies showed that 5-7mm particles had significantly increased VEGF secretion normalized to weight, indicating increased tissue hypoxia in these particles compared to 2-4mm. Surprisingly, in vivo comparison in two unique studies showed 2-4mm and 5-7mm fat particles had comparable graft retention \((p=0.5329)\). Masson’s trichrome staining revealed increased extracellular matrix and fibrosis in the 5-7 mm particle group \((p=0.0115)\). Adipocyte survival with perilipin demonstrated comparable viability. Gene expression showed large particles experienced increased inflammation and apoptosis at 1 week after grafting, but overall there were no significant differences between groups.

Conclusions: The ideal fat particle size should be large enough to contain adequate mesenchyme while not so thick as to preclude imbibition. This study suggests that despite changes in hypoxia and VEGF levels, differing fat particles (2-4mm and 5-7mm) can achieve similar graft retention.

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Technique And Morphologic Evaluation Of Component Restoration In The Bilateral Intermediate Cleft Tip Rhinoplasty

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Purpose: The bilateral cleft nose (BCN) deformity is characterized by a broad nasal tip and shortened columella due to structural defects that include cartilage weakness and malpositioning, in addition to nasal vestibular lining deficits. Corrective rhinoplasty to address anomalies of the BCN is stratified into the primary, intermediate, and secondary phases. This study describes a novel intermediate cleft tip rhinoplasty (ICTR) component restoration technique for BCN and includes an evaluation of the chronologic postoperative morphometric changes for surgical patients in comparison with non-operated age-matched controls.

Methods: The ICTR component restoration technique involves repositioning and fixation of the bilateral cleft lower lateral cartilages through composite auricular cartilage grafting to address BCN lining and structural anomalies. Photographic analysis of patients with BCN who had ICTR \((n=12)\) and age-matched controls who did not have ICTR \((n=8)\) were assessed at timepoints T0 = preoperative, T1 = less than 2 years postoperative, T2 = more than 2 years postoperative. Evaluation includes alar symmetry, nasal tip projection, nostril dimensions, nasofacial angle, and facial symmetry. Independent sample T-tests compared morphometric measurements of ICTR patients to control groups at T0 to assess baseline morphology. One-way analyses of variance with posthoc corrections using the Tukey criterion were performed to compare morphometrics over all time points. \(P<0.05\) was considered statistically significant.

Results: No significant differences in any demographic characteristics were found between the two groups. At baseline, ICTR patients had decreased nasal tip projection
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No differences were seen in alar symmetry, nostril dimensions, nasofacial angle, or facial symmetry. ICTR technique resulted in statistically significant improvements in nasal tip protrusion based on nasofacial angle representing lateral view (32.9±4.7 vs 37.5±4.9, p=0.04) and nasal tip projection representing basal view (40.2±9.5 vs 51.7±6.6, p=0.04), in addition to improvements in nostril dimensions (67.7±14.4 vs 81.9±12.8, p=0.02) when comparing timepoints T0 with T2. On the other hand, non-operated control patients did not demonstrate statistically significant changes in any morphometrics.

**Conclusion:** Baseline assessment reveals a greater degree of nasal deformity for patients who received ICTR. Also, this technique improves bilateral cleft nasal tip protrusion that is sustained over time. In contrast, patients who did not receive ICTR did not show improvements in nasal morphology, suggesting that these changes were not secondary to growth.

**Cd146+ Subpopulation Of Adipose-derived Stromal Cells Augmentation Of Fat Grafts Reduce Soft Tissue Atrophy Caused By Radiotherapy**

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**Purpose:** Radiation therapy remains a pivotal component for head and neck cancer treatment, but carries adverse side effects that negatively affect tissue function. The most common detrimental sequelae of radiotherapy include contour deformities, skin retractions, restricted movement, and non-healing wounds. Healing via fibrosis activated by radiation heavily damages vascularity of soft tissue, resulting in increased fibrosis and collagen deposition which negatively impacts tissue functionality and elasticity. Autologous fat transfer enriched with adipose-derived stromal cells (ASCs) have been observed to induce a regenerative effect in irradiated tissue by improving vascularity and dermal structure. Previous studies highlight a subpopulation of ASCs, CD146+, which express significantly higher amounts of VEGF, a signaling factor that promotes growth of new blood vessels and improves vascularity. We hypothesized that fat grafts enriched with CD146+ ASCs, compared to CD146- ASCs, will improve skin and fat quality vascularity and fibrosis.

**Methods:** CD-1 nude immunodeficient mice (n=25) were irradiated at the scalp (30 Gy total) and after 4 weeks, grafted with lipoaspirate and ASCs, which expressed negative hematopoietic markers (CD45, CD235a, CD31) and a positive marker for CD34, isolated via FACS from human patients. The treatment groups were: fat only (n=5), fat enriched with isolated ASCs (n=5), fat enriched with isolated CD146+ ASCs (n=5), fat enriched with CD146- ASCs (n=5), and control saline injection (n=5). MicroCT scans were obtained biweekly for 8 weeks. After 8 weeks of fat grafting, skin and fat grafts were harvested and analyzed by histology. Harvested fat grafts were sectioned and stained with hematoxylin and eosin (H&E) and CD31 immunofluorescence. Harvested mice skin from the scalp was sectioned and stained for Masson’s Trichrome, H&E, Picro Sirius Red, and CD31 immunofluorescence.

**Results:** Fat grafts enriched with CD146+ ASCs were significantly more vascularized than CD146- fat grafts, as exhibited by CD31 immunofluorescence staining. MicroCT scans demonstrated improved fat graft viability after 8 weeks with CD146+ ASC enrichment compared to CD146- ASC enrichment. Skin histology for mice grafted with CD146+ ASC enriched fat also displayed significantly less dermal thickness and collagen deposition compared to CD1 nude mice grafted with CD146- ASC enriched fat. Immunofluorescence of skin grafted with fat enriched CD146+ ASCs also revealed significant revascularization after radiotherapy and less collagen deposition.

**Conclusions:** Fat grafts enriched with ASCs improve fat graft viability and can even vascularize radiation damaged skin. The CD146+ subpopulation of ASCs, which express high amounts of VEGF, can also mediate blood vessel formation when supplemented within fat grafts. This regenerative effect on skin is highly promising for patients with detrimental wound healing impairment and deformities following radiotherapy. Further studies will observe other subpopulations of ASCs that can enhance regenerative effects of fat grafts and bridge the gap of knowledge of ASCs.