MR sequence that is intrinsically robust to motion and has enhanced bone vs. soft-tissue contrast.

**Methods:** Pediatric patients under 11 years old presenting for craniosynostosis or other craniofacial abnormalities were eligible for the study. Participants all had undergone a head CT scan as part of routine clinical care. Each patient also underwent the 5-minute GA-VIBE MRI sequence, performed as a stand-alone scan or as an add-on to a clinically indicated MR scan. The imaging data was processed to develop 3D-reconstructed images. The 3D images created from MRI and the gold standard CT 3D reconstructions were randomized and presented to three blinded reviewers for assessment (pediatric neurosurgeon, craniofacial plastic surgeon, neuroradiologist). For each image set, the reviewers individually noted whether they recommended that a second scan should be performed “to allow for clinical diagnosis” and/or “pre-surgical planning” on five-point Likert scales. 54 reviews were returned for analysis (9 patients x 2 imaging methods x 3 reviewers).

The presence or absence of the six primary cranial sutures was recorded. For each imaging method (CT and MRI), there were 162 assessments of suture closure and imaging quality in the regions of interest (9 patients x 6 sutures x 3 reviewers).

**Results:** Following IRB approval, nine patients underwent an MRI after a clinical head CT scan. Subject age ranged 3 weeks to 9 years (median age 1.6 years). Median time after CT was 47 days (range: 5 - 193). None of the patients were sedated for CT whereas 5 of the 9 MR scans were performed under sedation per routine clinical care. In 51 of the 54 cases, the reviewer stated that both MRI and CT were acceptable for diagnosis and surgical planning. In one review (Patient 5), the CT was considered unacceptable and the corresponding MRI was acceptable; and in two subjects (Patient 4 and Patient 5) the MRI was deemed unacceptable by one reviewer and the corresponding CT was acceptable.

Reviewers reported clear imaging of the cranial sutures on 98% of CT reviews (159/162 independent assessments) and 97% of MRI reviews (157/162). Sensitivity to detect suture closure from MRI was 100% and specificity was 95%.

**Conclusions:** The 3D reconstructed images using the GA-VIBE sequence in comparison to CT created clinically acceptable cranial images with ability to detect suture patency. Future directions include reducing the scan time, improve and automate motion correction and post-processing for clinical utility.

---

**Fat Grafting Mitigates Radiation-induced Scalp Fibrosis And Decreases The Abundance Of Profibrotic Engrailed1-positive Fibroblasts In The Overlying Skin**

Mimi R. Borrelli, MBBS, MS, Sandeep Adem, MS, Nestor M. Deleon Diaz, Dung Nguyen, MD, Arash Momeni, MD, PhD, Michael T. Longaker, MD, MA, Derrick C. Wan, MD

**Stanford, Menlo Park, CA, USA.**

**Purpose:** Radiotherapy (RT) is used to treat thousands of patients every year, however it can result in significant damage to collateral healthy soft tissue within the radiation field. Radiation-induced soft tissue fibrosis may be severe and debilitating, but despite the frequency of this complication, there are few effective treatment strategies. Fat grafting is emerging as a popular technique able to address volume deficiencies and regenerate fibrotic scar tissue. The mechanisms of improved tissue quality after fat transfer remains poorly understood. We recently identified the Engrailed1(En1)-positive fibroblast lineage in dorsal skin and scalp of mice responsible for the majority of collagen production in acute and chronic fibrosis. Here we asked whether grafted fat could alter the composition of this profibrotic fibroblast subtype in the irradiated scalp skin of recipient mice.

**Methods:** Adult (60-day old) En1Cre;R26mTmG transgenic reporter mice (n=9) underwent full body lethal irradiation with 9 Gy for hematopoietic depletion. Mice were then immediately transplanted with 2 million nucleated bone-derived cells from donor NSG (NOD.CB17-Prkdcscid/J) mice via retro-orbital sinus injection. Fluorescence-activated cell sorting (FACS) analysis of peripheral blood was used to assess the success of reconstitution. After 4 weeks, the calvarial region was then irradiated with a total of 30 Gy, fractionated into six 5 Gy doses delivered on alternative days across 12 days. A 4-week recovery period followed to allow for chronic radiation-induced skin fibrosis to develop. The irradiated site was then grafted with 200ul of fresh human lipoaspirate. Graft retention was measured radiographically over the subsequent 8 weeks. The calvarial skin was then harvested for histological assessment of fibrosis as well as for the abundance of En1-positive fibroblasts.
Results: FACS analysis revealed that >90% of the hematopoietic cells (CD45+) in the peripheral blood of En1Cre;R26mTmG mice were membrane tomato (mT-) negative and membrane GFP negative (mG-) 2 weeks after bone marrow transplantation, indicating successful reconstitution. Scalp irradiation induced a significant expansion of the GFP+ profibrotic En1-positive fibroblasts 4 weeks following scalp irradiation. While reduced volume retention of grafted fat was observed over 8 weeks, this was associated with significant remodelling of the overlying skin; histological evidence of decreased collagen as well as a reduction in the profibrotic En1-positive fibroblast subpopulations was observed compared to irradiated but not grafted sites.

Conclusion: Here, we show that fat grafting reduces radiation-induced scalp skin fibrosis by reducing the skin collagen content, remodelling collagen fiber networks, and by altering the composition of dermal fibroblast subpopulations. Specifically, we show that fat grafting reduces the abundance of the profibrotic En1-positive fibroblasts. Greater exploration of how radiation alters the function of this fibroblast subpopulation may lead to refined therapeutics for patients suffering from radiation-induced tissue fibrosis.

Adipose-derived Stromal Cells Within Transplanted Fat Hone To Blood Vessels And Assume A Pericyte Structure

Mimi R. Borrelli, MBBs, MSc, Sandeep Adem, MS, Nestor M. Diaz Deleon, Michael Januszyk, MD, PhD, Dung Nguyen, Arash Momeni, MD, PhD, Michael T. Longaker, MD, MA, Derrick C. Wan, MD

Stanford, Menlo Park, CA, USA.

Purpose: Fat grafting can restore volume defects and improve the texture, quality, and appearance of overlying skin. The adipose-derived stromal cells (ASCs) within transplanted fat are thought to drive these regenerative effects, either by direct differentiation or by paracrine signaling. The survival and differentiation fate of transplanted ASCs post grafting, however, remains incompletely understood. We therefore performed lineage tracing of transplanted human ASCs in vivo using a mouse model of fat grafting.

Methods: ASCs were isolated from human lipoaspirate and transduced to express green fluorescent protein (GFP). Successfully labeled cells were isolated using fluorescence-activated cells sorting (FACS). Next, CD1 nude mice were grafted with fresh human lipoaspirate enriched with GFP+ ASCs (10,000 cells/200ul lipoaspirate/graft). Mice were harvested at 2, 4, 6, and 8 weeks (n=5 mice/group). Grafted fat was explanted and assessed by whole tissue mounting, confocal microscopy, and image reconstruction software to characterize the three-dimensional structure of fat with reference to the GFP+ ASCs.

Results: Transplanted ASCs were evident in whole mount sections through 6-weeks post transplantation. GFP+ ASCs grouped together and honed to blood vessels, and adipocyte size was correlated with proximity to GFP+ ASCs. ASCs co-stained for the known pericytes markers Tie2, smooth muscle actin (SMA), and nerve growth factor 2 (NGF2). Although ASCs became fewer in number with time post grafting, fat enriched with ASCs was more vascular throughout the graft compared to fat not enriched with ASCs.

Conclusions: In this mouse model of fat grafting we show that the transplanted human ASCs may survive and hone to blood vessels, assuming a pericyte-like position. These data suggest a role for ASCs in directing and supporting angiogenesis and neovascularization by direct differentiation. Future work may further profile the exact cytokine profile of transplanted ASCs throughout grafting to determine the mechanism by which they drive their regenerative effects.

In Vivo Comparison Of Adipose Particle Size On Autologous Fat Graft Retention In A Rodent

Xiaonan Yang, MD, PhD1,2, Francesco M. Egro1, Taraneh Jones1, Vincent Nerone1, Jeffrey A. Gusenoff1, J. Peter Rubin1, Lauren Kokai1

1Department of Plastic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, 2Plastic Surgery Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.