of the midface. Disruption of this developmental process leads to severe craniofacial anomalies, such as frontonasal dysplasia (FND). Using induced pluripotent stem cells (iPSCs) derived from FND subjects with a heritable \( ALX1 \) L165F gene variant, we leveraged a novel method of cranial neural crest cell (CNCC) differentiation to study \( ALX1 \) function and interaction with \( PAX3 \), delineating a transcriptional regulatory pathway crucial to CNCC differentiation.

**METHODS:** Wildtype and \( ALX1^{-/-} \) iPSCs were differentiated into the CNCC lineage through the addition of epithelial growth factor into DMEM F12 medium. Subsequent cells were assayed for their CNCC properties and ability to multi-lineage differentiate into adipocytes, Schwann cells, chondrocytes, and osteoblasts. We then accessed surface marker expression, and sensitivity to apoptosis between wildtype and \( ALX1^{-/-} \) CNCCs using FACS and migration assay. Differential gene expression in iPSC and \( alx1^{-/-} \) zebrafish embryos was analyzed using qPCR. For \textit{in vivo} analysis of CNCC migration, wild-type, \( alx1^{-/-} \) and \( pax3 \) injected zebrafish embryos were analyzed using \textit{sox10:KAEDE} transgenic line.

**RESULTS:** Through analysis of CNCC markers CD90, CD73, CD105, CD57, cellular morphology, gene expression data, we demonstrate that our protocol was able to differentiate iPSCs into CNCCs. Both \( ALX1^{-/-} \) and wildtype cells were capable of differentiating into adipocytes, Schwann cells, chondrocytes, and osteoblasts. However, after CNCC differentiation, while wildtype cells were able to further engage in the CNCC lineage through CD57 down-regulation, \( ALX1^{-/-} \) NCCs maintained a high level of CD57. \( ALX1^{-/-} \) neural crest cells were also more sensitive to apoptotic stress and experienced migratory impairment. qPCR analysis of \( ALX1^{-/-} \) iPSCs and \( alx1^{-/-} \) zebrafish embryos revealed an overexpression of \( PAX3 \) during CNCC differentiation and embryonic development. Conversely, overexpression of \( PAX3 \) into control CNCCs impaired migration in iPSC model. Overexpression of \( pax3a/b \) mRNA in zebrafish embryos phenocopied the craniofacial anomalies seen in \( alx1^{-/-} \) mutants, and injection of a dominant-negative \( alx1 \) (\( alx1\text{DN} \)) variant impaired migration of CNCCs to the central midface.

**CONCLUSION:** Studies of FND using complementary iPSC and zebrafish models revealed that \( ALX1 \) down-regulates \( PAX3 \) to modulate CNCC migration and cell maturation. Disruption of \( ALX1 \) resulted in unsuppressed \( PAX3 \) expression, which caused NCCs to be unable to persist in a progenitor state, more sensitive to apoptosis, and unable to properly migrate. Discordant neural crest cell differentiation and migration resulted in a decreased contribution of NCC to the frontonasal prominences and midface deformities. These studies revealed requirement of \( ALX1 \) in transcriptional regulation of midface development. This study elucidated the molecular and cellular basis of FND pathogenesis, advancing craniofacial malformations from description of affected anatomy to fundamental understanding.

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Near-Infrared Tissue Oximetry Predicts Flap Necrosis in a Rat Dorsal Skin Flap

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**PURPOSE:** The application of technology to predict and prevent perfusion related complications promises to revolutionize plastic surgery by improving patient care and preventing the downstream sequelae of flap necrosis. Although a myriad of devices have been developed toward this goal, their widespread application has been limited by cost, the need for potentially unwieldy machinery, the use of intravenous dyes, and questionable efficacy in human trials. The objectives of this study are to assess the potential efficacy of a novel, handheld, dye-less device utilizing near-infrared spectroscopy in quantifying tissue oxygenation, predicting the risk for flap necrosis, and preventing perfusion-related complications.

**METHODS:** Twenty-four Sprague-Dawley rats underwent elevation of a dorsal, 10cm x 3cm cranially-based random pattern skin flap using the modified McFarlane technique. Rats were divided into 1 of 3 treatment groups: control, single-dose topical nitroglycerin, and two-dose topical nitroglycerin applied immediately post-operatively and again twelve hours later. Tissue oxygenation was measured intra-operatively following flap elevation and at 24-hours post-operatively. On post-operative day seven, the animals were euthanized, and flap survival was ascertained clinically and histologically. The Pearson product-moment correlation coefficient was used to correlate tissue oxygenation to distance from flap pedicle. Statistical analyses were
performed using chi-squared tests and one-way ANOVA. ROC curves were used to evaluate the ability of intra-operative tissue oxygenation in predicting clinical flap necrosis.

RESULTS: Tissue oxygenation was negatively correlated with distance from the flap pedicle ($r = -0.798$) with a statistically significant decrease in mean tissue oxygenation in distal tissues ($p < 0.001$). Necrotic tissue on post-operative day seven demonstrated a significantly lower intra-operative tissue oxygenation (32.1%) compared to healthy tissue (58.6%, $p < 0.001$). As a predictor for tissue necrosis, intra-operative tissue oxygenation demonstrated an area under the ROC curve of 0.969. Control rats demonstrated more tissue necrosis than those receiving a single or two doses of topical nitroglycerin (51.3% vs. 28.8% vs 18.8%, respectively; $p=0.035$). Near-infrared spectroscopy demonstrated a significant increase in tissue oxygenation between the intra-operative and 24-hours post-operative timepoints in ischemic tissue receiving nitroglycerin (+17.8%, $p<0.001$).

CONCLUSIONS: Near-infrared tissue oximetry effectively detects clinically relevant differences in tissue oxygenation and is a strong predictor for flap necrosis in a rat model. The application of topical nitroglycerin ointment results in a measurable increase in tissue oxygenation that correlated to flap survival. Further translational studies are warranted in order to define the role of this technology in patient care.

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IGF-1 Nanoparticles Improve Functional Recovery After Nerve Repair

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PURPOSE: Insulin-like growth factor 1 (IGF-1) is a potent mitogen with well-described trophic and anti-apoptotic effects on neurons, myocytes, and Schwann cells (SCs). Local delivery of IGF-1 is limited by its short half-life. The aims of this study are to (1) encapsulate IGF-1 into biodegradable nanoparticles (NPs) that stabilize IGF-1 in its bioactive state and enable sustained release at target tissue sites that persist throughout the regenerative period; and (2) assess the efficacy of locally delivered IGF-1 NPs in augmenting axonal regeneration while also reducing denervation-induced muscle atrophy and SC senescence to thereby improve functional recovery following nerve injury.

METHODS: (1) NP Fabrication: IGF-1 was first complexed with dextran sulfate to create hydrophobic ionic paired (HIP) complexes, which were then encapsulated in biodegradable NPs. Varying ratios of HIP:polymer were evaluated to maximize loading efficiency and release kinetics. In vitro NP release kinetics were evaluated and mitogenic activity of released IGF-1 was compared to native IGF-1. (2) The effects of locally-delivered IGF-1 NPs on denervated muscle and Schwann cells were assessed in a rat median nerve transection-without-repair model. The effects of IGF-1 NPs on axonal regeneration, muscle atrophy and reinnervation, and recovery of forepaw function were assessed in a model in which chronic denervation is induced prior to nerve repair; functional recovery was assessed weekly with stimulated grip strength testing prior to sacrifice at 15 weeks for histologic analyses.

RESULTS: (1) Fabrication of uniform NPs with an encapsulation efficiency of 83.2% was achieved. NPs composed of 1:5 PEG$_{5k}$-PCL$_{40k}$ yielded optimal release of IGF-1. Near-zero-order release of IGF-1 can be achieved for at least 70 days and released IGF-1 exhibits comparable bioactivity to native IGF-1. (2) IGF-1 treated animals recovered significantly more forceful grip strength compared to negative controls. IGF-1 NP treatment limits muscle atrophy during denervation and improves functional recovery of forelimb grip strength.

CONCLUSION: Encapsulation of bioactive IGF-1 with sustained release for over 70 days was achieved. IGF-1 NP treatment in vivo limits muscle atrophy during denervation and improves functional recovery of forelimb grip strength.

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9-cis-Retinoic acid Reduces Postsurgical Lymphedema Through Rxrα Signaling Pathway

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