months. Electrical stimulation of the dermal side of the C-RPNI evoked afferent signals (CSNAPs) in the proximal peroneal nerve at both three and sixth months. The average peak-to-peak CSNAP amplitude was 391.6 ± 145.0 µV at three months and 267.1 ± 143.8 µV at six months. The average conduction velocity with an average conduction velocity was 11.6 ± 3.0 m/sec at three months and 9.6 ± 2.4 m/sec at six months.

CONCLUSIONS: C-RPNI constructs remained viable with preserved innervation for six months following implantation. Recorded efferent motor signals and evoked afferent signals remained robust over time. The C-RPNI facilitates bidirectional signal transduction of both efferent motor signals and afferent sensory signals. This confirmation of bidirectional signal transduction in the C-RPNI validates the potential role of the C-RPNI in human-machine interfacing.

Machine Learning Analysis Of Connective Tissue Networks Enables Objective Characterization Of Skin Fibroses

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PURPOSE: Clinical evaluation of dermal fibroses relies on histopathological analysis, which is inherently observer-dependent. Visual analysis is subjective and may preclude detection of subtle phenotypic changes in early-stage or less-severe disease. We present an image processing algorithm which enables objective quantification of multiple parameters of connective tissue architecture. We then classify histologic specimens by their respective dermal fibrotic pathologies, solely using machine learning analysis of their collagen networks.

METHODS: Ninety-five human specimens were obtained from the following diagnoses: normal skin, scar, striae distensae (stretch marks), hypertrophic scar, keloid, and scleroderma. Mouse dorsal skin and scar specimens were also obtained. Formalin-fixed, paraffin-embedded histologic specimens were stained with Picrosirius-Red, imaged by polarization microscopy, and analyzed using our image processing algorithm in Matlab 2017a. In brief, this algorithm employs color deconvolution, adaptive filtering, and skeletonization of individual collagen fibers followed by quantification of parameters such as fiber length, branching, and randomness. A neural network was trained on connective tissue parameters (using 70% of images), validated (15% of images), and finally tested (15% of images) on histological images of human specimens.

RESULTS: Using our image processing algorithm, 26 connective tissue parameters were identified and quantified. To validate the algorithm, mouse unwounded skin and scar specimens were compared. Using unsupervised hierarchical clustering, these specimens clustered by specimen type (normal skin vs scar) based on four clusters of fiber parameters. The algorithm was then applied to human specimens (unwounded skin, striae distensae, “normal” scars, hypertrophic scars, and keloid). These human specimens were differentiated by five parameter clusters due to the larger degree of variation in connective tissue architecture. The trained neural network classified pathologies with an overall accuracy of 86% (ROC curves > 95% for all specimens), demonstrating high sensitivity and specificity. The neural network also differentiated normal human skin from preclinical scleroderma with a 91% overall accuracy (ROC curves > 95%), demonstrating that our algorithm detected early-stage disease prior to the onset of clinical symptoms.

CONCLUSIONS: We present an automated machine learning analysis pipeline for objective characterization of dermal collagen networks. Using a trained neural network, we classify human fibrosis specimens into disease categories based on quantitative analysis of their connective tissue properties alone. The ability to objectively characterize dermal fibroses and to detect preclinical disease has significant implications for clinical diagnosis and management as well as basic research. We intend to expand the use of this technology to fibroses in both skin and other organs,
with the goal of establishing a standardized approach for histologic analysis of fibrosis.

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Mechanisms of Disruption in Craniofacial Ossification Caused by fgfr1a Mutations

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PURPOSE: Mutations in the FGFR1 gene have been implicated in craniosynostosis in human patients, though the molecular mechanisms of pathogenesis are not well understood. FGFR1 is highly conserved between humans and zebrafish, allowing these molecular pathways to be studied using zebrafish as an animal model.

METHODS: Four fgfr1a zebrafish mutants were generated using the CRISPR/Cas9 system in the same genetic region as the mutation associated with Pfeiffer syndrome. The four mutants are a 12-nucleotide in-frame deletion (fgfr1a del12bp), two frameshift deletions of 13- (fgfr1a del13bp) and 17-nucleotides (fgfr1a del17bp), and a 14-nucleotide rearrangement (fgfr1a del13 + 1bp). Whole mount staining with Alizarin red was performed to examine ossification of bones. qRT-PCR and RNAscope in situ hybridization analyses were used to identify differentially expressed genes. Pentachrome staining was performed to evaluate cranial cartilage formation and organization of the endochondral growth plate.

RESULTS: Two phenotypic changes are evident in the in-frame deletion mutant (fgfr1a del12bp) - flattening of the skull contour with a smaller distance between the top of the head and eyes, and decreased sutural space along the midline sutures between the two halves of the calvaria. These findings suggest changes in intramembranous ossification of the skull bones, with likely increased ossification in the mutant calvaria, resulting in more rapid overlap of frontal and parietal bones along midline sutures.

Endochondral ossification is also affected in mutants, as evidenced by qRT-PCR results demonstrating upregulation of the cthrc1b gene in all mutants except one, and of the nppc gene in the in-frame deletion mutant (fgfr1a del12bp). These two genes have been identified as regulators of endochondral ossification in human and mouse models. qRT-PCR results were supported by RNAscope in situ hybridization analysis for cthrc1b. More cells expressed cthrc1b, and intensity of the detected signal was stronger in the palatoquadrate cartilage (a cranial endochondral cartilage) of the fgfr1a del12bp mutant.

Pentachrome staining of the same cartilage in one of the frameshift deletion mutants (fgfr1a del17bp) demonstrates disruption of the neatly organized endochondral growth plate as well as enhanced ossification, indicated by a decreased number of chondrocytes and shorter length of the growth plate.

CONCLUSIONS: Mutations in the fgfr1a gene in zebrafish appear to disrupt normal craniofacial physiology through effects on both intramembranous ossification of the cranial bones and endochondral bone development. Additional analysis will be performed to better characterize the molecular mechanisms leading to the observed phenotype. Because fgfr1a is highly conserved in zebrafish and humans, we believe this knowledge will translate to better understanding of human craniosynostosis.

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A Simple Way to Reduce Opioid Over-Prescribing by Plastic Surgery Residents

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