a three wavelength NIRS device designed to detect the oxidation state of cytochrome aa3, which is a direct measure of cellular ischemia. The device lessened StO2 variation using an established ischemia and reperfusion model, but did not adequately reflect cytochrome aa3 oxidation state. The objective of this study was to compare a broadband NIRS device using white light (> 1000 wavelengths) to the standard two wavelength NIRS device (ViOptix Inc, Freemont CA) commonly employed for flap monitoring by measuring StO2 variability during ischemia and reperfusion and corresponding cytochrome aa3 oxidation – a direct measure of cellular ischemia.

**METHODS:** The two NIRS devices, ViOptix and the broadband NIRS device, were applied to the hands of human volunteers (n = 20) and a blood pressure cuff was placed around the upper arm to occlude arterial and venous flow, as previously described. Measurements were obtained from both devices at baseline, during induced ischemia, and during reperfusion at 30-second intervals. StO2 variability was measured during ischemia and reperfusion between both devices, as was the correlation of broadband-reported cytochrome aa3 oxidation state to StO2 changes.

**RESULTS:** The StO2 measurements from both devices proportionally decreased during ischemia with similar variability. The broadband device reported cytochrome aa3 changing from an oxidized to reduced state during ischemia, which began even during baseline cuff placement before StO2 decreased. Once the ischemia and reperfusion reporting phases began, cytochrome aa3 oxidation state correlated with StO2 readings from both devices.

**CONCLUSIONS:** The ViOptix and broadband NIRS devices performed similarly with regard to ability to measure StO2 as a surrogate marker of tissue ischemia. However, the use of broadband white light wavelength NIRS monitoring also provided the capability of directly measuring cellular ischemia through cytochrome aa3 oxidation state. Interestingly cytochrome aa3 oxidation-reduction state was sensitive even to minor changes in tissue perfusion. Future work will evaluate clinically critical differences in cellular oxidation reporting in a flap model.

**Carrie A. Kubiak, M.D., Daniel C. Ursu, Ph.D., Jana D. Moon, B.S., Parag G. Patil, M.D., Theodore A. Kung, M.D., Paul S. Cederna, M.D., Stephen W.P. Kemp, Ph.D.**

**University of Michigan, Ann Arbor, MI, USA**

**PURPOSE:** There have been many advancements in the field of neuroprosthetics for functional restoration following limb loss. However, devices that provide prosthetic users with intuitive, simultaneous motor control and somatosensory feedback have not yet been realized. Critical to the evolution of this ideal bioprosthetic device is the development of a reliable, biologic human-machine interface that facilitates transmission of both efferent motor signals for device control, and afferent somatosensory information. The Composite Regenerative Peripheral Nerve Interface (C-RPNI) is a novel biologic interface that demonstrates promise in this role. The C-RPNI is a surgical construct composed of a transected, mixed peripheral nerve implanted between a composite free graft consisting of de-epithelialized glabrous skin and skeletal muscle. The purpose of the present study was to investigate the viability and bidirectional signal transduction capabilities of the C-RPNI.

**METHODS:** C-RPNIs were surgically implanted on the end of transected common peroneal nerves of thirty F344 rats using de-epithelialized dermal grafts harvested from glabrous skin of isogenic donor rat hindpaws and free skeletal muscle grafts from the animal’s contralateral limb. Thirty animals underwent endpoint testing at three months (n=15) and at six months (n=15). Electrophysiologic testing was performed to determine both the in vivo efferent and afferent signal transduction capabilities of C-RPNIs following electrical stimulation. C-RPNI constructs were also harvested for histologic evaluation at both three- and six-month study endpoints.

**RESULTS:** C-RPNIs were surgically implanted on the end of transected common peroneal nerves of thirty F344 rats using de-epithelialized dermal grafts harvested from glabrous skin of isogenic donor rat hindpaws and free skeletal muscle grafts from the animal’s contralateral limb. Thirty animals underwent endpoint testing at three months (n=15) and at six months (n=15). Electrophysiologic testing was performed to determine both the in vivo efferent and afferent signal transduction capabilities of C-RPNIs following electrical stimulation. C-RPNI constructs were also harvested for histologic evaluation at both three- and six-month study endpoints.

**RESULTS:** C-RPNIs were surgically implanted on the end of transected common peroneal nerves of thirty F344 rats using de-epithelialized dermal grafts harvested from glabrous skin of isogenic donor rat hindpaws and free skeletal muscle grafts from the animal’s contralateral limb. Thirty animals underwent endpoint testing at three months (n=15) and at six months (n=15). Electrophysiologic testing was performed to determine both the in vivo efferent and afferent signal transduction capabilities of C-RPNIs following electrical stimulation. C-RPNI constructs were also harvested for histologic evaluation at both three- and six-month study endpoints.

**CONCLUSIONS:** The ViOptix and broadband NIRS devices performed similarly with regard to ability to measure StO2 as a surrogate marker of tissue ischemia. However, the use of broadband white light wavelength NIRS monitoring also provided the capability of directly measuring cellular ischemia through cytochrome aa3 oxidation state. Interestingly cytochrome aa3 oxidation-reduction state was sensitive even to minor changes in tissue perfusion. Future work will evaluate clinically critical differences in cellular oxidation reporting in a flap model.

**36**

**Viability and Signal Transduction with the Composite Regenerative Peripheral Nerve Interface (C-RPNI)**
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

Malini Chinta¹, Shamik Mascharak, BA¹, Mimi R. Borrelli, MBBS, MSc¹, Alessandra L. Moore, MD¹, Rachel E. Brewer¹, Jan Sokol¹, Gabriela Kania², Evelyn Garibay¹, Deshka Foster, MD¹, Heather desJardins-Park, AB¹, Bryan Duoto¹, Oliver Distler², Geoffrey C. Gurtner, MD¹, H. Peter Lorenz, MD¹, Derrick C. Wan, MD¹, Howard Y. Chang, MD, PhD¹, Michael T. Longaker, MD, MBA¹

¹Stanford University, Palo Alto, CA, USA, ²University Hospital Zurich, Zurich, Switzerland

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.