extremity, the radial nerve transected proximally at the humeral spiral groove, distally prior to the branch to brachioradialis. The radial nerve was selected as it is responsible for an isolated function with no input from other nerves. Three repair techniques were evaluated: n=6 autograft/suture, n=6 ANA/suture, n=7 ANA photosealed in place with light-activated human amnion wraps (PTB). An objective functional outcome test was conceived using an apparatus that accurately measured the degree of wrist extension as a function of time after surgery. Electromyography (EMG) was performed at 0, 120, 240, and 365 days (euthanasia). Histomorphometry and muscle mass retention were analyzed at euthanasia.

RESULTS: Average loss of wrist extension was 88.3 ± 8.2° after radial nerve defect creation. Autograft group animals recovered 82.0° of extension by 7 months. Wrist extension recovery was modestly slower, as expected, in the ANA groups, however, by 8 months the ANA/PTB group recovered 63.0° of extension with no difference in recovery of baseline EMG amplitude as compared to controls (Avance/PTB=75.81% vs. autograft/suture=65.06%). If recovery follows current trajectory, we expect ANA/PTB to demonstrate equivalent outcomes to the autograft/suture group at one year.

CONCLUSION: This radial nerve defect model improves upon existing animal models by allowing for large nerve gap testing in a primate model more analogous to the clinical large nerve gap injury in humans. ANA/PTB group functional recovery lagged modestly behind autograft/suture (by as long as 8 weeks) but is approaching equivalence at 8 months. EMG recovery is similar at 8 months. This preliminary data confirms PTB as a promising technique to improve outcomes of large nerve gap reconstruction in combination with autograft (previously demonstrated) and with acellular nerve allograft.

15

The Role of Burn Tissue and Denatured Small Leucine Rich Proteoglycans in the Activation of the Toll-like Receptor 4 Pathway

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PURPOSE: Hypertrophic scar (HTS), a common and significant consequence of burn injury to deep dermis with prolonged inflammation, causes reduced range of motion, intense pruritis, heat intolerance, and cosmetic problems. It does not respond well to current treatment options. HTS formation is a dynamic, complex process that involves interactions between multiple factors such as fibroblasts, extracellular matrix molecules, inflammatory cells, cytokines, growth factors, and chemokines. Toll-like receptors (TLRs) are innate immune receptors that respond to microbes to initiate innate immunological defense, and detect and initiate tissue repair after injury. They are expressed in immune cells, gingival, synovial and dermal fibroblasts. Activation of toll-like receptor 4 (TLR4), a proinflammatory pathway, has been suggested to be associated with HTS by responding to extracellular matrix (ECM) and endogenous cellular ligands to promote inflammation. Small leucine-rich proteoglycans (SLRPs) such as decorin, biglycan, fibromodulin, and lumican, are molecules involved in wound healing that modify the ECM by altering fibroblast proliferation, collagen organization, and growth factors. We hypothesized that the endogenous molecules released from damaged burn tissue could activate the TLR4 pathway in dermal cells, which may lead to a cascade of fibrogenic growth factors and collagen deposition following the activation of resident fibroblasts. Therefore, in this study, we determined the role of burn tissue and denatured SLRPs in the stimulation of the TLR4 pathway in vitro, to elucidate immunological mechanisms preceding HTS.

METHODS: Burn tissues, including eschar and exudate, were collected from patients (n=8) at the University of Alberta Hospital. A normal skin sample was collected from a patient who underwent abdominoplasty, as a control. Total cells were isolated from solid tissue by collagenase-digestion. HEK-Blue hTLR4 cells, human embryonic kidney cells that were co-transfected with human TLR4, MD-2 and CD14 co-receptor genes and are used to determine activation of the TLR4 pathway, were treated with the solid tissue, exudate, cells, denatured SLRPs decorin and biglycan, and bacterial lipopolysaccharide (LPS) as a positive control. Secreted embryonic alkaline phosphatase (SEAP) assay was used to measure NF-κB activation as an indicator of TLR4 activity.

RESULTS: HEK-Blue hTLR4 cells treated with solid tissue, exudate, and burn tissue-isolated cells showed higher TLR4 activity compared to untreated cells. Burn site microbiology and days post-burn injury are clinical patient factors that influenced TLR4 activity. Normal skin tissue stimulated TLR4 pathway to some extent, possibly due to
the presence of skin microbiota. Denatured decorin and biglycan caused lower TLR4 activity than controls, possibly due to endotoxin in the recombinant reagents.

CONCLUSION: The results indicate that there may be endogenous molecules released from the burned tissue and cells that are able to stimulate the TLR4 pathway. Stimulation of the TLR4 pathway would lead to production of Type 1 interferons and proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, which may promote fibrosis and HTS formation. We will further explore the mechanism by culturing burn tissues and SLRPs with deep dermal fibroblasts. These studies will help in the development of future therapeutics, providing benefits to patients suffering with HTS and other fibroproliferative disorders.

16

Tissue-Engineered Articular Cartilage Construct in Hand Surgery for Sub-mm Fractional Repair

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PURPOSE: Articular cartilage lesions in large joints have been successfully treated with autologous chondrocyte implantation (ACI) with or without a matrix scaffold (e.g., MACI). However, lesions in small joints of the hand and wrist cannot be easily treated with current ACI techniques. Fractional treatment, either mechanically or by laser, to make perforations into skin has been shown to promote regeneration. The purpose of this study was to develop a new strategy that combines fractional treatment with our method for generating new cartilage matrix using dynamic Self-Regenerating Cartilage (dSRC) to treat cartilage lesions in the joints of the hand and wrist.

METHODS: 10e7 freshly harvested autologous swine chondrocytes were cultured on a rocker for 14 days at 37 degree C to form dSRC. The dSRC was then mixed with collagen gel and placed into mechanically punched holes or laser (wavelength 10.6 μm) drilled holes at the center of a swine articular cartilage disc and capped with collagen gel. The control group consisted of isolated chondrocytes (3x10e7 cells per mL) injected into punched or laser drilled holes. Four groups were tested - (1) dSRC + 0.3 mm laser hole, (2) dSRC + 0.3 mm punch hole, (3) chondrocytes + 0.3 mm laser hole, and (4) chondrocytes + 0.3 mm punch hole. The cartilage discs were cultured in medium at 37 degree C for 8 weeks (n=6/group) then evaluated. In a pilot, in vivo study, the dSRC were encapsulated in collagen hydrogels and placed into 0.3 mm diameter punched and laser drilled holes in swine articular cartilage discs and capped with hydrogel. These discs were implanted subcutaneously in female nude mice. Constructs were harvested at 8 weeks. All constructs were evaluated histologically and immunohistochemically for cartilage formation and integration with native cartilage.

RESULTS: After 8 weeks in vitro, dSRC-treated constructs generated contiguous new cartilage matrix, compared to isolated chondrocyte-filled constructs that showed only pericellular matrix formation. dSRC groups demonstrated intense staining with Safranin-O and Toluidine blue stains indicating high glycosaminoglycan (GAG) production when compared to faint staining of groups treated with isolated chondrocytes. After 8 weeks in vivo in mice, contiguous cartilage matrix was observed in both dSRC filled punched holes and CO2 laser-drilled holes. Immunohistochemical staining further confirmed that the matrix of dSRC group was typical of normal hyaline cartilage, rich in collagen type II and no collagen type I, similar to native cartilage. Results of this study demonstrate that dSRC capped with hydrogels can successfully engineer contiguous articular cartilage matrix in both mechanical and fractional laser created environments.

CONCLUSIONS: Fractional treatment combined with dSRC demonstrates successful hyaline cartilage formation and integration with native cartilage both in vitro and in vivo. Such a strategy could be employed to translate ACI techniques commonly used in the knee to treat cartilage defects or osteoarthritis in the smaller joints of the hand and wrist.

17

Increasing Ischemia Time Diminishes Chimerism After Vascularized Bone Marrow Allotransplantation

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