9th Annual Translational to Clinical (T2C) Regenerative Medicine and Wound Care Conference
March 17-19, 2016
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CRISPR-Mediated Genome Editing Offers a Novel Therapy for DMD-Associated Cardiomyopathy in Live Mice

Mona El Refaey, Li Xu, Renzhi Han

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Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy with a worldwide incidence of one in 3500 live male births. It is caused by the lack of dystrophin, a critical muscle protein that connects the cytoskeleton and the extracellular matrix (ECM). Cardiomyopathy develops in at least 90% of patients and alone can shorten the life expectancy of DMD patients by at least 2 years and up to 40% of DMD patients eventually die from heart failure. Recently, RNA-guided, nuclease-mediated genome editing based on type II CRISPR (clustered regularly interspaced short palindromic repeat)/Cas (CRISPR-associated) system, has been emerged to alter the genome. In this study, we hypothesize that CRISPR-mediated genome editing could offer a novel therapy for DMD-associated cardiomyopathy in live mice. Our preliminary studies demonstrated that in-frame deletion of the genomic DNA covering exon 23 restored functional dystrophin expression in mdx mice. Two gRNA target sites were chosen from intron 20 and 23. Co-transfection of the two gRNA with cas9 plasmids into mouse C2C12 cells resulted in the detection of a small PCR product as predicted, indicating successful CRISPR-mediated genome editing. Moreover, RT-PCR confirmed that the deletion resulted in the expression of a truncated dystrophin transcript. DNA sequencing confirmed that the transcripts from C2C12 cells treated with gRNA/cas9 were formed due to successful deletion of exons 21-23.

Then, we injected the adenoviral vectors carrying GFP-2A-cas9 and gRNAs into the gastrocnemius (GA) muscles of newborn pups. Three weeks after adenovirus transduction, dystrophin expression was restored in the muscles positive for GFP. Immunofluorescence staining also demonstrated that neuronal nitric oxide synthase (nNOS), which is normally located to the sarcolemma in healthy muscles via interaction with dystrophin-glycoprotein complex, was also restored at the sarcolemma of GFP-positive muscle fibers. These data provide the proof evidence of restoration of dystrophin in live mice.
MG53 promotes wound healing and reduces scar formation following dermal injury

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Cell membrane repair is an important aspect of physiology, and disruption of this process can result in pathophysiology in a number of different tissues including wound healing, chronic ulcer and scarring. We previously identified a novel TRIM (tripartite-motif) family protein, named MG53, as an essential component of the cell membrane repair machinery. Here we report the functional role of MG53 in modulation of wound healing and scarring. Although MG53 is absent from keratinocytes and fibroblasts, remarkable defects in skin architecture and collagen over-production are observed in mg53⁻/⁻ mice; and these animals display delayed wound healing and abnormal scarring. Recombinant human MG53 (rhMG53) protein, encapsulated in hydrogel formulation, facilitates wound healing and prevents scarring in rodent models of dermal injuries. In vitro study shows that rhMG53 protects against acute injury to keratinocytes and facilitates migration of fibroblasts in response to scratch wounding. During fibrotic remodeling, rhMG53 interferes with TGF-β-dependent activation of myofibroblast differentiation; the resulting down-regulation of alpha smooth muscle actin (α-SMA) and extracellular matrix proteins contributes to reduced scarring. Overall, these studies establish a tri-functional role for MG53 as a facilitator of rapid injury repair, a mediator of cell migration, and a modulator of myofibroblast differentiation during wound healing. Targeting the functional interaction between MG53 and TGF-β signaling may present a potentially effective means for promoting scarless wound healing.

Funding: This work was supported by NIH grants to J.M. (R01-AR061385 and R01-1HL069000), NSF grants to J.G. (1006734 and 1160122), and the OSU Lockwood Early Career Development Award to P.L. and H.L.
HUMAN STEM CELL DERIVED BRAIN ORGANOIDS AS MODELS OF BRAIN DISEASES

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Abstract: Induced pluripotent stem cells derived from patient cells have emerged as complementary tools to study human neurological disorders. Our laboratory has generated human iPSC-derived brain organoids which exhibit a remarkable level of development that morphologically resembles a nearly complete human embryonic brain at ~5 week in utero, but after ~12 weeks in culture in vitro. These organoids express a complex milieu of markers characteristic of nearly all types of neurons in the human embryonic brain, as well as cells that are astrocytic, oligodendritic, microglial, and vascular in lineage. This conclusion is based on comparing the whole genome transcriptomic expression profile of the brain organoid to a universal human brain reference standard and to the developing human brain atlas at the Allen Institute’s BrainSpan database. Our analysis supports the formation of all the major regions of the brain including the retina, cortex, midbrain, brain stem and the spinal cord in a single brain organoid and is the first time this milestone in human brain organogenesis has been achieved. Using developmental RNA transcriptomics, immunohisto-chemistry, and 3D whole brain imaging we can assess the earliest impact of a genetic mutation or environmental factor during brain development and follow downstream consequences in the brain organoids at different stages of their development. These organoids represent the next generation of stem-cell derived tools for understanding mechanisms of disease and a platform for toxicological screens and drug discovery. We can use this tool for biomedical-related research on Post Traumatic Stress Disorder (PTSD), Gulf War Illness (GWI), and exposure to noxious environments.

Background: Induced pluripotent stem cells (iPSC), derived from patient blood or skin cells, are useful in vitro models to study genetic, molecular, and cellular abnormalities associated with human disorders.

Hypothesis: Brain organoids may serve as new human model systems to study susceptibility to PTSD and CNS response to noxious environments underlying Gulf War Illness (GWI).

Methods: We follow brain organoid responses to genetic or environmental factors using transcriptomics, immunohisto-chemistry, and 3D whole brain imaging.

Results: Our laboratory has independently generated human iPSC-derived brain organoids which exhibit a remarkable level of development that morphologically resembles a nearly complete human embryonic brain at ~5 week in utero development, but after ~12 weeks in culture in vitro. These organoids express a complex milieu of markers characteristic of nearly all types of neurons in the human embryonic brain, as well as cells that are astrocytic, oligodendritic, microglial, and vascular in lineage.

Discussion: We plan to use these brain organoids to study neurodevelopmental, neurodegenerative and neurotoxic factors affecting human mental health.

Conclusion: We have engineered brain organoids with all the major regions of the brain including the retina, cortex, midbrain, brain stem and the spinal cord.

Sponsor: Ingram Autism Fund and the OSU Wexner Medical Research Fund
FULLY-PASSIVE BRAIN IMPLANTS FOR WIRELESS NEUROPOTENTIAL MONITORING

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Brain implant technology has a strong potential to improve the individual's well-being. However, current implants lack the safety and reliability required for unobtrusive, long-term monitoring of neuropotentials. In this work, we aim to establish a novel technology for safe and reliable brain implants. Our objectives entail: (a) fully-passive implants (no battery, no energy harvester), (b) wireless operation for minimum impact to the individual's activity, (c) extremely simple on-board electronics that generate minimal heat, and (d) tiny footprint to minimize trauma.

The proposed system is comprised of the brain implant and an exterior interrogator. The interrogator sends a carrier (2.4GHz) to activate the implant. That, in return, detects brain signals (f_{neuro}) and mixes them with the carrier. To do so, we employed an anti-parallel diode pair mixer. The latter captures both positive and negative legs of the 2.4GHz carrier, allowing for high-efficiency harmonic mixing at 4.8GHz. Subsequently, the mixed signal (4.8GHz±f_{neuro}) is transmitted to the exterior interrogator, and demodulated to retrieve f_{neuro}.

A proof-of-concept sensor prototype was fabricated and measured. Currently, it occupies 8.7mm×10mm×0.76mm. The implant was immersed inside a phantom that served to emulate the dielectric properties of head tissue. A function generator was used to generate the brain signals. The system was demonstrated to detect emulated neuropotentials lower than 20µVpp at f_{neuro}>10Hz. This is a 25 times improvement in sensitivity as compared to previous implementations. Concurrently, the system is fully-passive and wireless.

For the first time, we present comfortable and carefree brain implants. This is a game changing capability for several applications (e.g., epilepsy monitoring, prosthetics control, early seizure detection, trauma assessment). Exterior interrogators can be placed within a baseball cap for inconspicuous monitoring. Data can then be transmitted to a nearby cell phone. Future work will focus on in-vivo acquisition of brain signals.

Sponsor: National Science Foundation
Home Oxygen Therapy-Related Burns: An Outcome Comparison Based on Location of Intubation

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Home oxygen therapy-related burns (HOTRB) can occur in chronic lung disease patients that smoke during treatment. Burn injuries are often small, involving only the face with patients undergoing unnecessary intubation for airway protection. Our study, focuses on the location (designated burn center (OSU) vs. non (OSH)) of intubation of HOTRB patients and outcomes. A retrospective review of all HOTRB patients at our burn center from 2006-2015 was performed. Data collected included demographics, length of stay (LOS) outcomes, complications and details of location of intubation. A total of 79 patients presented with HOTRB. Patients undergoing intubation versus non-intubation had an average a longer LOS of 9.7 vs. 2.6 days (p<0.0001), longer ICU LOS of 5.75 vs. 0.6275 days (p<0.0001) and a greater number of days on a ventilator 4.5 vs. 0.0 days (p<0.0001). No patient in our study demonstrated inhalation injury on bronchoscopy. Most common complications from intubation resulted in ventilator acquired pneumonia and tracheostomy. The location of intubation over the time of our study demonstrated an increasing trend with the percentage of patients being intubated at OSH vs. OSU of HOTRB admissions (29% vs. 8.6%, p<0.048, 2006-2015). In the first 5 years, 4.3% more patients were intubated at OSH (11.9% vs. 6.9%, p=0.52, 2006-2010). The most recent 5 years this increased to 37% (47.3% vs. 10.3%, p<0.029, 2010-2015). Our study displays the morbidity associated with HOTRB injuries demonstrating longer hospital admissions, ICU stays and ventilator associated complications. No study to date has compared these populations based upon location of intubation. Our data reveals an increasing number of HOTRB patients that are being intubated at outside hospitals that are not designated burn centers compared to our institution. Indications and algorithms for intubation of HOTRB patients need developed, evaluated and disseminated to community personnel to provide best practice for this population.
Bio-inspired Fluorescent Dipeptide Nanoparticles for Targeted Cancer Cell Imaging and Real-time Monitoring of Drug Release

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Peptide nanostructures are biodegradable and are suitable for many biomedical applications. However, to be useful imaging probes the limited intrinsic optical properties of peptides must be overcome. Here we show the formation of tryptophan–phenylalanine dipeptide nanoparticles (DNPs) that can shift the peptide’s intrinsic fluorescent signal from the ultraviolet to the visible range. The visible emission signal allows the DNPs to act as imaging and sensing probes. The peptide design is inspired by the red shift seen in the yellow fluorescent protein that results from \(\pi-\pi\) stacking and by the enhanced fluorescence intensity seen in the green fluorescent protein mutant, BFPms1, which results from the structure rigidification by Zn(II). We show that DNPs are photostable, biocompatible and have a narrow emission bandwidth and visible fluorescence properties. DNPs functionalized with the MUC1 aptamer and doxorubicin can target cancer cells and be used to image and monitor drug release in real time.
Bio-inspired Adhesive Hydrogels from Sundew combined with Adipose Derived Stem Cells for Wound Healing

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The potential to harness the unique physical, chemical, and biological properties of the sundew (Drosera) plant’s adhesive hydrogels has long intrigued researchers searching for novel wound healing applications. However, the ability to collect sufficient quantities of the sundew plant’s adhesive hydrogels is problematic and has eclipsed their therapeutic promise. Inspired by these natural hydrogels, we asked if sundew-inspired adhesive hydrogels could overcome the drawbacks associated with natural sundew hydrogels and be used in combination with stem cell based therapy to enhance wound healing therapeutics. Using a bio-inspired approach, we synthesized adhesive hydrogels comprised of sodium alginate, gum arabic, and calcium to mimic the properties of the natural sundew-derived adhesive hydrogels. We then characterized and showed that these sundew-inspired hydrogels promote wound healing through their superior adhesive strength, nanostructure, and resistance to shearing; when compared to other hydrogels in vitro. In vivo, sundew-inspired hydrogels promoted a “suturing” effect to the wound sites; which was demonstrated by enhanced wound closure following topical application of the hydrogel. In combination with mouse adipose derived stem cells (ADSCs), and compared to other therapeutic biomaterials, the sundew-inspired hydrogels demonstrated superior wound healing capabilities. Collectively, our studies show that sundew-inspired hydrogels contain ideal properties that promote wound healing and suggest the sundew-inspired-ADSCs combination therapy is an efficacious approach for treating wounds without eliciting noticeable toxicity or inflammation.
Bio-inspired Self-assembled Peptide Hydrogel for Wound Healing.

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Present trends in designing and fabricating biomaterials for wound healing focus on developing biocompatible composites that offer appropriate chemical, physical, and biological cues mimicking the native microenvironment. To develop a preferable self-assembled peptide hydrogel for wound healing, using the principles unlocked from the naturally occurring nano-networks, we first designed a peptide sequence, i.e., RIEIRIGIRIEIR, to explore the approaches to tune the self-assembly procedures. As expected, an expected scaffold consisting of self-assembled peptides was successfully developed without noticeable cytotoxic activities against common mammal cells at a peptide concentration up to 200 \textmu g/ml. This preliminary data imply here that the synthesized peptide hydrogel possesses a notable cytocompatibility toward common mammal cells. In addition to this assay, the wound healing potential the nanocomposites consisting of ADSCs and peptide hydrogels were evaluated in a full thickness skin incision mouse model. While respective mice treated with 100 \textmu g/ml or 200 \textmu g/ml peptide hydrogel exhibited similar wound closure rate, the addition of ADSCs demonstrated a significant improvement on accelerating the wound closure. In this study, a bio-inspired peptide hydrogel with stiffness-tunable feature was successfully developed. Overall, this peptide construct demonstrates a good biocompatibility toward multiple mammal cells and shows a great potential to be applied for wound healing.
Population growth dynamics of neural stem cells inform on the developmental neuropathology of DNA repair dysfunction

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DNA repair mutants show paradoxical neuropathology, characterized by cerebellar dysmorphia with relatively preserved forebrain development. We utilized mathematical modeling, neuroanatomical, and transgenic mouse techniques to provide novel insights into this conundrum. CCNA2 ablation in neural progenitors, which phenocopies DNA repair neuropathologies, reveals that extrinsic population dynamics supersedes intrinsic cell cycle signals for brain growth. Specifically, our data show a dependency of DNA repair on CCNA2 and that CCNA2-/- neural progenitors have longer cell cycle. Nevertheless, CCNA2-/- forebrains grow faster than CCNA2-intact brains. Our data was consistent with that of prior studies which suggested that progenitors propagate along an auto-inhibitory growth curve. We used this knowledge to formulate and parameterize a mathematical model whereby logistic growth instructs the progenitor cell as to the cell-types of the progeny. Our data suggested that due to its position in the logistic growth curve, the CCNA2-null developing forebrain has the capacity to undergo compensatory growth. This delayed growth corresponded to modifications in cell-cycle length of CCNA2-null forebrains, imposing an effective developmental delay in the stem cell niche. We propose that such a mechanism may explain how even acute in utero exposure to teratogens induces developmental delay manifested in newborns even months after exposure.
Deterministic transfection and genetic manipulation of organotypic brain slice cultures for ex vivo imaging

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The organotypic brain slice culture has been used to great effect for many studies, from The organotypic brain slice culture has been used to great effect for many studies, from electrophysiology to cell fate determination. However, a major limitation of this technique is the difficulty and expense of reliable genetic manipulation using transfection and virus methodologies. To overcome this problem, we combined cutting-edge nano-engineering techniques with established brain slice culture methodologies. We used the technique of nanochannel electroporation (NEP) to deliver defined quantities of plasmids for co-expression of different genes within a single cell, which is not possible with probabilistic transfection methodologies such as lipid-mediated transfection. Using the NEP methodology, we transfected organotypic brain slices with defined mixtures of plasmids encoding different fluorescent proteins. We were able to transfect a variety of cell types including neurons. Using live confocal imaging, we observed a very high degree of co-transfection with plasmids encoding both green and red fluorescent proteins. We further transfected a plasmid encoding channelrhodopsin and used an optogenetic approach to stimulate specific neurons within slice cultures. Our novel methodology greatly expands the utility of the organotypic brain slice culture, and reduces the need for costly and time-consuming reagents such as viruses for genetic manipulation. The flexibility of our NEP methodology opens the door to many experiments that would otherwise be prohibitively costly and time consuming, and allows investigators to easily perform experiments that would not be possible using standard methodologies.
The proneural gene PHOX2B generates astrocytes critical to autonomic integration

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The pro neural transcription factor PHOX2B has been demonstrated to play key developmental roles in the generation of autonomic circuits. However, its contribution to other cell lineages has been less well-appreciated. Using transgenic mouse approaches, we have discovered a small population of astrocytes located in the ventrolateral medulla (an area critical of autonomic integration of respiration), are derived from the PHOX2B progenitor domain. Furthermore, by genetic ablation of this population, we discovered that these astrocytes play critical functions in respiratory drive that is not compensated for by other neighboring astrocytes. This work, for the first time, demonstrates that integrated units of glia and neurons are developed from the same progenitor pool. These findings implicate that crucial neural circuit development occurs early during embryogenesis, perhaps at the stages of progenitor cell specification.
Implementation of fluorescent microscopy in neuropathology using automated image analysis

Behiye Kaya¹, Fazly Abas¹, Brad Elder¹, Vinay Puduvali¹, Jessica Winter¹, Metin Gurcan¹, and Jose Otero¹

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Local tumor recurrence is seen in the majority of patients diagnosed with glioblastoma, although patients are treated with multimodal aggressive treatment. Neuropathology of glioma recurrence represents significant visual challenges to pathologists because of the reactive and inflammatory morphological changes of brain tissue following chemotherapy and radiation, which can mimic tumor recurrence. Tumor-specific fluorescent antibodies, which can be recognized using optical imaging such as confocal and epifluorescent microscopy, can provide rapid and accurate differential diagnosis of recurrent glioma versus reactive gliosis. However, the presence of well-documented autofluorescence in brain biopsy specimens has hindered the utility of multiplexed immunofluorescent approaches. This study overcomes the visual chal
Surface Topography During Neural Stem Cell Differentiation Regulates Cell Migration and Cell Morphology

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We sought to determine the contribution of scaffold topography to the migration and morphology of neural stem cells by mimicking features of scaffolds found in vivo. We mimicked two types of CNS scaffolds encountered by neural stem cells during development in vitro by constructing different diameter electrospun polycaprolactone (PCL) fiber mats, a substrate that we have shown to be topographically similar to brain scaffolds. We compared the effects of large fibers (made to mimic blood vessel topography) with those of small diameter fibers (made to mimic radial glial process topography) on the migration and differentiation of neural stem cells. Neural stem cells showed differential migratory and morphological reactions with laminin in different topographical contexts. We demonstrate, for the first time, that neural stem cell biological responses to laminin are dependent on topographical context. Topography induced cell morphology changes were inhibited by nocodazole, but not by cytochalasin-D or ROCK-inhibitor, suggesting that microtubule function is necessary for topography-induced morphology changes. We propose that the physical structure of distinct scaffolds induces unique signaling cascades that regulate migration and morphology in embryonic neural progenitor cells.
Whole-mount visualization of brain structures using clearing histological techniques

Summer Fair\textsuperscript{1}, Catherine Czeisler\textsuperscript{1}, Behiye Kaya\textsuperscript{1}, Patrick Gygli\textsuperscript{1}, and Jose Otero\textsuperscript{1}

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In these experiments, we adapted myelin clearing protocols during histological preparation to visualize specific structures in the mouse brain. The goal of this project was to visualize astrocyte networks and autonomic neural networks using specific transgenic reagents capable of identifying these structures. The reagents utilized included transgenic mice expressing GFP under the control of the astrocyte-specific ALDH1L1 promoter to visualize astrocytes. These images are compared to standard confocal images of cryosectioned brain. In a second phase, we utilized a unique tracer mouse that, after crd-mediated recombination, shows tdTomato expression in neuronal somata and GFP at synaptic terminals. This tracer mouse was intercrossed with PHOX2B-cre mice so as to induce recombination in the autonomic lineage. We demonstrate that these histological preparations are capable of visualizing key structures without the need of cryosectioning.
Bacteria and Host Interactions in Wound Biofilm

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Biofilm associated chronic wound is a significant burden for patient and healthcare system. Treatment of biofilm infection designed based on in vitro culture rarely promote the chronic wound healing successfully. The principal limitation of applying results from in vitro biofilm to clinical wound biofilm treatment is that the controlled culture condition cannot represent the real environment of wound biofilm. To understand the structural details of how bacteria survive and thrive in wound, and also compare the structural differences of wound biofilm and in vitro biofilm, we applied electron microscopy techniques in structural study of porcine wound biofilm and in vitro biofilm. Wound biofilms were collected from porcine model that established in our lab. In vitro biofilms were cultured on agar at 37 °C for 48 hours. Biofilm specimens were chemically fixed, stained and embedded in resin. Our Electron Microscopy results disclosed huge difference in vitro and wound biofilm. In vitro biofilm forms organized layer on agar plates. The extracellular polymeric substances (EPS) build up intercellular networking which may facilitate the nutrient and information transfer, promote biofilm grow and disperse. Wound biofilm seldom forms large aggregation on the surface of wound; instead bacteria actively interact with phagocytes, fibroblast, collagen and adipose cells. Bacteria niches in collagen and adipose tissues have various numbers of microorganism aggregation ranged from a few tens to a few hundreds. Occasionally isolated bacteria were found inside intact tissue, which may present a living state of bacteria that explore new niches and escape the chase of immunization cells. We concluded that bacteria and host interaction is the main scenario of wound biofilm. In vitro results can be applied to wound biofilm in limited local aggregation, but not to the whole wound environment. Effective wound biofilm treatment will need research work established on real wound model.
Correlative Microscopy Application in Regenerative Medicine Research

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Various types of microscopes extended human insights and contributed to tremendous discoveries. While every microscopy has its own unique advantages, because of the reverse relationship of spatial resolution and the overall size of specimen within the field of view, no single technique can cover research needs from exploring the whole chunk of tissue to viewing details of microbial cells. Correlative microscopy, an ongoing project at Center for Electron Microscopy and Analysis (CEMAS), demonstrated that by combining the power of X-ray Computed Tomography (X-ray CT), Scanning Electron Microscopy (SEM) and Scanning Transmission Electron Microscopy (S/TEM), we are able to cross the boundary of the association between resolution and field-of-view, and obtain multiscale structural information in several regenerative medicine researches, which include spinal cord injury, wound biofilm and heart valve et al. X-ray CT is a non-destructive three-dimensional (3D) imaging method. In our experiment, X-ray CT produces 3D maps of specimens with dimensions up to several centimeters at low to medium resolution (up to 1μm for micro X-ray CT and up to 50nm for nano X-ray CT). Once the areas of interest are recognized and labeled in 3D maps, excessive material can be removed by plasma FIB, ultramicrotomy or simply using a razor blade. The trimmed specimen will undergo continuing microscopy investment using SEM at intermediate resolution (~5nm for serial section SEM tomography). In cases that high resolution images are needed, such as cell-cell interaction, FIB Lift-out method or ultramicrotomy can be utilized to make grids that are suitable for S/TEM study. In summary, correlative microscopy overcomes barrier of different microscopy techniques. By combine techniques that have overlapping resolving powers, we are able to accurately locate and pick out areas of interest from sample blocks. The area of interest will be investigated at higher resolution, while we have the overview of the whole specimen.
IN VIVO INCUBATION SITE AFFECTS THE PRODUCTION OF TISSUE ENGINEERED INTESTINE

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Hypothesis: The hypothesis of this study was that in vivo incubation sites will impact the quality of the tissue engineered intestine produced.

Methods: Intestinal stem cell (ISC)-containing crypts from pups were seeded onto tubular polyglycolic acid (PGA) scaffolds and implanted into the dam at different intraperitoneal locations.

Results: Intestine harvested from 2-5 day old Lewis rat pups was harvested and subjected to enzymatic digestion. The resulting mixed cells were then filtered through different sized sieves (200, 70, and 25 μm). Concentrated ISC-containing crypts were located in the size range of 25-70 μm, and were used to seed tubular PGA scaffolds (5 mm dia. and 10 mm length). Scaffolds were implanted into the dam of the pups, with each dam receiving 4 scaffolds as follows: 1) one wrapped with omentum, 2) one attached to the peritoneal surface of the anterior abdominal wall, 3) one wrapped around the native intestine, and 4) one inserted subcutaneously in the left groin. After 4 weeks of in vivo incubation, scaffolds (n=12 from each location) were explanted, examined histologically for the presence of neomucosa, and quantification of neomucosal villous height performed. Ten of 12 scaffolds in Groups 1 and 2 (83%), 12 of 12 scaffolds in Group 3 (100%), and 4 of 12 scaffolds in Group 4 (33%) were positive for neomucosa. Average villous heights were 378 ± 38 μm, 357 ± 48 μm, 412 ± 45 μm, and 115 ± 33 μm for each of the respective groups (p < 0.05, one way ANOVA, Group 3 vs. Groups 1, 2, and 4).

Discussion: Cell-seeded scaffolds require nutrients to develop into TEI. Scaffolds in Group 3 had the highest incidence of mucosal development, with the longest villous height, which may be due to their ability to obtain nutrients from both peritoneal fluid and from neovascularization derived from the outer surface of the native intestine.

Conclusion: The greatest quantity and quality of TEI neomucosa was observed when seeded scaffolds were wrapped around native small intestine during in vivo incubation.
THE PRODUCTION OF TISSUE ENGINEERED INTESTINE USING EXPANDED ENTEROIDS

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**Background:** Short bowel syndrome is a life-threatening condition with few solutions. Tissue engineered intestine (TEI) is a potential treatment, but donor intestine is a limiting factor.

**Hypothesis:** Epithelial surrogates known as enteroids can serve as a potential donor source when paired with *ex vivo* culture and expansion.

**Methods:** Mouse jejunal biopsies were processed for enteroid culture, expanded *ex vivo*, seeded onto scaffolds, implanted for four weeks, and processed histologically for detection of TEI.

**Results:** Enteroid cultures were able to be established from diminutive intestinal biopsies. Crypts obtained from jejunal biopsies produced 192 ± 71 enteroids. A single passage of the enteroids produced 685 ± 58 enteroids. Only one passage was necessary to obtain enough enteroids for scaffold seeding. TEI was produced in 8/9 scaffolds seeded with expanded enteroids. Histologic examination demonstrated crypt domains and overlying blunted villi. Periodic Acid Schiff staining and immunofluorescence demonstrated normal epithelial lineages including goblet cells, Paneth cells, enterochromaffin cells, intestinal stem cells, and enterocytes. Immunofluorescence was also able to identify intestinal subepithelial myofibroblasts and smooth muscle cells in the underlying stroma despite their absence in the enteroids used as a starting material.

**Discussion:** Enteroid cultures can be established from a minimal biopsy, comparable to that which a short bowel syndrome patient could provide. Moreover, those same enteroids can be grown *ex vivo*, expanded, and used to grow TEI with many of the major components of native intestine.

**Conclusion:** Expanded enteroids show promise as a solution to the limited intestinal donor source for TEI production in patients with short bowel syndrome.
DECELLULARIZATION TO PRODUCE BIOLOGICAL SYNOVIAL EXTRACELLULAR MATRIX SCAFFOLDS

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The objective of this study was to produce decellularized synovium (syn ECM) that could serve as a living scaffold when seeded with viable cells. Four methods that are rapid, inexpensive, and have been successfully applied in similar tissue types were compared.

Methods
Synovium was harvested from equine stifles. Fresh-thawed synovium was randomly assigned to control (no processing) or 1 of 4 decellularization methods in triplicate: 1) 0.1% peracetic acid solution (1X); 2) method 1 but repeated (2X); 3) 1% Triton X/DNAse, and; 4) method 3 with 2M sodium chloride solution replacing the Triton X. Synovium from each group was cut with a biopsy punch and tested for morphology (histologic, scanning electron microscopy) and decellularization (cell content, DNA, DNA fragmentation). Data was analyzed with ANOVA for method and time and Tukey’s post-hoc test with significance set at P<0.05.

Results
The PAA procedure (1X) had no loss of villous matrix integrity but had significantly greater retention of cells, DNA (P=6.5¹⁸), and DNA size > 200bp. The 2X PAA procedure also had no loss of matrix integrity, but had fewer cells than 1X, (p=4.9⁷) lower retention of DNA, and small DNA size (<200bp). TritonX/DNAase and 2MNaCl/DNAase left little to no discernable synovium, no cells, low (140-143ng/mg) and small (<200bp) residual DNA.

Discussion/Conclusion
The 0.1% PAA, performed twice, was considered to have the best scaffold potential due to the low cellularity, low DNA content and retained villous architecture.
CHARACTERIZATION OF LIVING SYNOVIAL EXTRACELLULAR MATRIX SCAFFOLD FOR GENE DELIVERY

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Cartilage injury and degeneration is a leading cause of disability in humans and horses. There is currently no treatment to reverse the loss of chondrocyte function. Regenerative cells, including synovial stromal cells (SSCs) and anabolic agents, combined with scaffolds for targeted delivery are a promising option. The synovium offers the advantage of containing highly metabolic cells that readily proliferate and have been shown to secrete joint restorative substances. Synovial cells can also be genetically engineered to secrete growth factors. Our hypothesis was that decellularized synovium (synECM) seeded with synoviocytes-BMP-2/GFP (BMP2-SMSCs) would show good scaffold migration, engraftment, and living cell counts while producing significant levels of BMP-2. Synovium from equine stifles were harvested and decellularized. Synoviocytes, from digested synovium, were either transduced with Ad-BMP-2/GFP (SMSCSs-BMP2/GFP) or left as control cells (SMSCs). The synECM was seeded and incubated using a 30% fetal bovine serum gradient. On day 3, 7 and 14 the explants were examined for cell growth, CD-90 expression, viability, morphology and the supernatant tested for BMP-2, hyaluronic acid (HA) and proteoglycan (PG). Increasing cell counts, cell migration into the scaffold and evidence of cell differentiation support that synECM seeded with SMSCs or BMP2-SMSCs produced a living synovial scaffold. Significant levels of BMP-2 concentrations were produced by the BMP2/GFP-SMSCs, followed by an increase in both HA and PG indicating gene production and enhanced synovial function. The results of this study suggest that synECM seeded with normal and genetically altered SMSCs may have potential for treatment in cartilage healing.
PROBIOTIC BIOFILMS ENHANCE PROTECTION OF INTESTINES FROM NECROTIZING ENTEROCOLITIS

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Background
Probiotic administration is beneficial in the prevention of necrotizing enterocolitis (NEC). However, current regimens require probiotics to be administered daily to achieve their beneficial effects.

Hypothesis
Stimulating probiotics to produce biofilms on the surface of prebiotic-loaded microspheres prior to administration will enhance protection from NEC with only a single dose.

Methods
Neonatal rats were delivered via cesarean section, given a single enteral prebiotic/probiotic treatment, then subjected to repeated stress (hypoxia/hypothermia/hypercaloric feeds) to induce experimental NEC.

Results
69% of untreated stressed pups developed NEC. Compared to untreated pups exposed to experimental NEC, 65% of pups treated with microspheres alone (p=0.656), 58% of pups treated with prebiotic-loaded microspheres alone (p=0.699), and 59% of pups treated with the conventional free-living form of Lactobacillus reuteri alone (p=0.488) developed NEC. This is in contrast to pups who received a single dose of L. reuteri administered in the form of a biofilm. Only 32% of pups treated with L. reuteri grown on unloaded microspheres (p=0.009) and 33% of pups treated with L. reuteri grown on prebiotic-loaded microspheres (p=0.005) developed NEC. There was no statistically significant difference in NEC incidence or severity in pups that were exposed to NEC and treated with L. reuteri in the presence of either unloaded or prebiotic-loaded microspheres (p=0.723). No breast fed unstressed control pups developed NEC.

Discussion
Although prebiotic-loaded biocompatible microspheres had similar efficacy to unloaded microspheres in the reduction of NEC incidence and severity in this study, we are currently examining the efficacy of incorporation of improved prebiotic preparations into microspheres, in order to further improve protection of the intestines from NEC.

Conclusion
A single dose of Lactobacillus coupled with biocompatible microspheres to promote biofilm formation significantly reduces the incidence and severity of experimental NEC.

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INJECTION OF LIVING DENTAL PULP CELL PARTICLES FOR THE TREATMENT OF EQUINE LAMENESS CONDITIONS

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Historical data in many species demonstrate anti-inflammatory and tissue healing actions of autologous cell therapy for the treatment of tendonitis, suspensory desmitis, and osteoarthritis (OA). Dental pulp has recently served as a source of pluripotent stem cells with multipotent differentiation potential. Sterile dental pulp cells (DPCs) recovered from fresh perished foals was processed to produce minimally manipulated, unexpanded allogeneic living cells suspended in extracellular matrix. We hypothesized that injection of DPC would improve pain and lameness in horses. We used a prospective, randomized, blinded, controlled clinical trial using natural-occurring cases for tracking outcomes of lameness, inflammation, pain, and client satisfaction. Forty client-owned horses with confirmed OA (n=20), desmitis (n=14) or tendonitis (n=6) were assigned to receive 2 ml intra-articular (n=20 OA) or intraligamental (n=20) injection of control vehicle (n=20) or 10 x 10^6 dental pulp cells (n=20). Horses were first acclimated to the treadmill and force plate, had baseline measurements performed, and were then injected on study Day 0. Horses were exercised for 2 weeks, evaluated by clinical parameters, lameness score, gait analysis, edema (score and circumference), pain on flexion or pressure, and clients’ scores for pain and discomfort before, and through 45 days after cell injection. Treated horses showed persistent decrease in both lameness and induced lameness after flexion (OA) or pressure (desmitis, tendonitis) compared to baseline or control (P<0.05). Cells induced mild edema on day 1 at the injection site (P<0.05). Client assessment of lameness and comfort were improved from before to 31 or 45 days after cell injection (P<0.05). Clinical improvement with tendonitis and desmitis was greater than OA. Dental pulp cells can be considered as an effective and safe treatment option for equine lameness due to OA, desmitis or tendonitis. This study was funded, in partial, by Dental Stem Cell Development Co. LTD.
REDUCING NECROTIZING ENTEROCOLITIS INCIDENCE AND SEVERITY WITH STEM CELLS

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Background: Necrotizing enterocolitis (NEC) is a leading cause of death in premature infants. Although stem cell therapy is promising, no study has compared different stem cell efficacies.

Hypothesis: Stem cells derived from different sources will have different potencies, enabling the identification of a type of stem cell that will more effectively treat NEC.

Methods: Rat pups subjected to experimental NEC were treated with stem cells from amniotic fluid, bone marrow, and fetal gut, and intestine examined histologically for NEC.

Results: The incidence of histological and clinical NEC in control pups was in the range of 60-67%, as it has been historically in our lab. Preliminary results showed a reduction in the incidence of NEC to 15-20% in pups treated with amniotic fluid-derived mesenchymal stem cells (AF-MSC), and a reduction to ~30% in pups treated with bone marrow-derived mesenchymal stromal cells (BM-MSC). However a large enough sample size has not yet been collected to confirm significance. Data collection is ongoing, as is the determination of incidence and severity data for treatment with amniotic fluid-derived neural stem cells (AF-NSC) and fetal enteric nervous system-derived neural stem cells (ENS-NSC).

Discussion: These preliminary results suggest that AF-MSC may be more effective at reducing incidence and severity of NEC than BM-MSC. As data collection continues, this relationship and the relationship of these cells to AF-NSC and ENS-NSC will be definitively determined.

Conclusion: Stem cells reduce the incidence and severity of NEC in an established animal model, with different stem cell types likely having varying efficacies.

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EXOSOMES SECRETED BY NEURAL STEM CELLS PROTECT THE INTESTINES FROM ISCHEMIA/REPERFUSION INJURY

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Background: Alterations in the enteric nervous system (ENS) are associated with many intestinal disorders including necrotizing enterocolitis (NEC). We have previously reported that transplantation of neural stem cells (NSC) protects the ENS from NEC. The aim of the current study was to determine whether extracellular particles (exosomes) released from stem cells mediate the therapeutic efficacy of NSC therapy, and to determine whether different types of stem cells produce exosomes with variable efficacy.

Methods: Enteric NSC and mesenchymal stem cells (MSC) were prepared from mouse neonatal intestine and bone marrow (BM) respectively. NSC-derived exosomes or BM-MSC-derived exosomes were isolated from condition medium. Exosomes were labeled with the red fluorescent cell linker dye PKH-67 and then injected intravenously by tail vein to mice which had been subjected to segmental intestinal ischemia to the distal ilium only (70 minutes), followed by reperfusion. Exosome distribution was tracked using XENOGEN fluorescence scanning 6h or 24h after exosome delivery. Intestinal histologic injury and ENS integrity were assessed 24h after I/R injury.

Results: NSC-derived exosomes localized exclusively to injured intestine 6h after administration, whereas BM-MSC-derived exosome were distributed to multiple organs as well as injured and non-injured intestine. I/R injured intestine from animals that received NSC-derived exosomes had significantly higher fluorescence intensity compared to those treated with BM-MSC-derived exosomes. Immunohistochemistry of intestinal histologic sections confirmed that NSC-derived exosomes target myenteric ganglion cells of the ENS. Administration of either NSC-derived exosomes or BM-MSC-derived exosomes decreased intestinal histologic injury, with NSC-derived exosomes achieving the best results.

Conclusions: Both NSC-derived exosomes and BM-MSC-derived exosomes protect the intestines from I/R injury. NSC-derived exosomes specifically target the ENS during intestinal I/R injury. Stem cell-derived exosomes may represent a novel, non-cell based therapy to protect the intestines from injury in the future.
CATHEPSIN K INHIBITION RENDERS EQUINE BONE MARROW NUCLEATED PROGENITOR CELLS HYPO-RESPONSIVE TO LPS AND UNMETHYLATED CPG STIMULATION IN VITRO

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Cathepsin K (CatK) is an important enzyme regulating bone degradation and immune response. VEL-0230 is a highly selective and potent inhibitor of CatK that has been proposed as a therapeutic for equine osteo-inflammatory conditions. Bone marrow is the body's resource for progenitor cells of the lymphoid cell lines and the osteoblastic and osteoclastic cell lines responsible for bone formation and turnover. Our study aimed to investigate the effect of CatK inhibition on Toll like receptor (TLR) 4 and TLR9 signaling pathways in equine whole bone marrow nucleated cells (BMNCs). This cellular fraction was chosen to include both the lymphoid and non-lymphoid cells (myeloid progenitors, mesenchymal stem and other progenitor cells) since equine immune (myeloid and lymphoid) and non-immune cells, such as chondrocytes and synovial fibroblast-like cells showed significant inflammatory response when stimulated with Lipopolysaccharides (LPS) in vitro. Equine BMNCs were isolated and exposed to VEL-0230 at a concentration of 0, 1, and 10 μM in cell culture media with and without LPS (1 μg/ml) and unmethylated CpG (5 μg/ml). Subsequent analyses of cell viability, cytokine secretion by stimulated BMNCs; specifically IL-1β, IL-6, and TNF-α, and BMNCs surface markers’ expression and Major histocompatibility (MHC) II molecule were performed. Cathepsin K inhibition promoted BMNCs viability and reduced cell apoptosis. Moreover, CatK inhibition significantly decreased cytokine secretion and MHC II molecules expression of either naïve or stimulated BMNCs. In conclusion, CatK inhibition in horses did affect BMNCs other than mature osteoclasts rendering them hype-responsive to both TLR4- and TLR9-induced inflammation, predicting a proteolytic activity for CatK within the MyD88 pathway and/or the following proteolytic events required for the cytokines secretion. This study was funded, in partial, by Feestride Therapeutics, MI, USA.
LOCALIZATION OF CATHEPSIN K IN EQUINE DECALCIFIED BONE SECTIONS AND EFFECT OF ITS INHIBITION ON STEM AND PROGENITOR CELLS DIFFERENTIATION IN VITRO.

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Cathepsin K (CatK) is an important enzyme regulating bone degradation and immune response. Selective inhibition of CatK is a promising therapeutic potential for diseases associated with excessive bone loss and osseous inflammation, such as osteoarthritis, periodontitis, and osteoporosis. In equine, stress-related injuries are common and accompanied by excessive bone resorption and periostitis. VEL-0230 is a highly selective inhibitor of CatK that significantly decreased bone resorption and increased bone formation biomarkers. We conducted this study with the objectives to 1: investigate the tissue localization of CatK in equine decalcified bone sections, and 2: determine the effect of CatK inhibition on chondrogenic, osteogenic, and osteoclastogenic differentiation-related gene expression. Six healthy exercising horses were used in this study. Bone biopsies were harvested from tuber coxae and proximal phalanx and processed for immunostaining against CatK in decalcified bone sections. Additionally, sternal bone marrow was collected and cultured in monolayer to select/deselect bone marrow derived-mesenchymal stromal cells for cell differentiation assays under the effect of 0, 1, 10, or 100 μM of VEL-0230 and subsequent gene expression analysis was performed. All cells morphologically characterized as osteoclasts and 65.4±7.4% of active bone lining osteoblasts stained positive for CatK. Gene expression analysis revealed a significant, VEL-0230-concentration dependent, up-regulation of the differentiating cells ability to form cartilage (Collagen I, Collagen II and Aggrecan), bone (Collagen I, Alkaline phosphatase, Osteocalcin and Osteopontin), and resorb bone (Tartrate-resistant acid phosphatase and CatK). These results suggest that CatK inhibition may have anabolic effect on bone and cartilage regeneration and may be explained as a feedback response to CatK depletion. In conclusion, the use of CatK inhibition over other current systemic, local, or regional treatments to moderate bone resorption and inflammation in equine osseous disorders may offer advantages that would require further study. This study was funded by Feestride Therapeutics, MI, USA.
EFFECT OF HYALURONAN, ALONE OR IN COMBINATION, TREATMENTS ON CHALLENGED SYNOVIAL CELLS

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We have reported that lipopolysaccharide (LPS) challenged synovial fibroblasts lose cell morphology, cell number, and increase inflammatory products. We have also demonstrated that high molecular weight hyaluronan (HA) was protective against these LPS effects. We hypothesized that a combination of HA and other anti-inflammatory therapies [Chondroitin 4 (CS4), CS6, in 10% solution of N-Acetylglucosamine; HA-CS-NAG] would provide an additive or synergistic effect. The response to 2 hour LPS challenge (20ng/ml or 50ng/ml) and the influence of pre- (24hr) and sustained (24hr) HA treatment alone or in combination (HA-CS-NAG) showed that 2hr LPS challenge reduced the loss of cell viability, attachment, contraction and death. Cells treated with HA alone or HA-CS-NAG were significantly protective against the negative effects of LPS and that HA-CS-NAG had the greatest anti-inflammatory effect. Our work demonstrated that HA and HA combined with Chondroitin 4 (CS4), CS6, and N-Acetyl glucosamine can protect synovial cells from the toxic and pro-inflammatory effects of LPS Use of a combination product may have more profound anti-inflammatory effect supporting clinical use. Supported, in part, by ArthroDynamic Technologies Incorporated.
ADVANCING THE STATE-OF-THE-ART OF COMPUTER-AIDED TISSUE ENGINEERING SCAFFOLD DESIGN

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Background: Conventional methods for fabricating tissue engineering scaffolds result in stochastic porosity that lacks repeatability in fabrication and presents issues involving uneven degradation, tissue ingrowth, and stress-distribution. **Hypothesis:** Additive manufacturing will enable the fabrication of highly complex structures possessing external geometry derived from patient data and porous internal architecture to optimize scaffold efficacy. **Methods:** Scaffolds were designed in MATLAB utilizing the gyroid triply periodic minimal surface as the foundation for pore architecture and additively manufactured by mask projection stereolithography. **Results:** Due to their mathematical complexion, TPMS-derived structures are extremely manipulable and computationally efficient. Using a custom algorithm, we show that gyroid-derived porous scaffolds feature an exceptional range of achievable porosity (approx. 2 to 98%), continuous curvature, and uniform mass distribution. Therefore, the pore and strut dimensions can be easily tailored on a per case basis. Our algorithm produces CAD models with features accurate to within 1.0% of a specified value. Furthermore, we demonstrate the ability to embed anatomical shapes with gyroid porosity and we have manufactured scaffolds with struts as thin as 125 microns by additively manufacturing poly(propylene fumarate) (PPF). **Discussion:** This novel design methodology elucidates a high level of CAD-driven surgical planning. We design and manufacture patient-specific porous implants beginning with a 3D CT scan that allow for optimal tissue ingrowth, mechanical properties, and degradation characteristics while ensuring an accurate fit which promotes osseous integration and an outstanding aesthetic restoration. **Conclusion:** Our approach integrates with current CAM methods and advances the state-of-the-art of tissue engineering scaffolds by offering the researcher and clinician more power of design. **Sponsor:** United States DoD: AFIRM II, Award No. W81XWH-14-2-0004.
Oxygenated Fluorinated Methacrylamide Chitosan Hydrogel Dressings Enhance Wound Healing through Improvement in Targeted Metabolic Processes

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Oxygen plays an essential role in wound healing; however, current oxygen therapies are limited. We have created a unique fluorine-functionalized hydrogel that enhances oxygenation. We hypothesize, that our novel hydrogel will deliver beneficial levels of oxygen when applied in vivo on wounds and accelerate wound healing. Pentadecafluoro methacrylamide chitosan (MACF) was formed into hydrogel sheets and applied to a porcine excisional wound model. Wound closure, histology and metabolomics analyses were performed. We have previously shown that MACF hydrogels sustain delivery of oxygen up to 5 d in vitro. In this study we created larger clinically relevant hydrogel dressings (2.5 x 2.5 cm) that delivered oxygen to wounds between dressing changes (2-4 d). Gilman results showed significantly faster closure for MACF as compared to Dermagel™ (p<0.0001). Histological analyses revealed enhanced re-epithelialization and re-granulation, supporting the benefits of local oxygen delivery from MACF + O2 hydrogels. Metabolomics bioinformatics is in process, and we expect significantly different metabolic profiles for our treatment versus controls. So far, hydroxyproline synthesis was significantly upregulated in MACF + O2 vs Dermagel™ at day 14 (p<0.01). Interestingly, by day 21, the hydroxyproline amounts in wounds treated with these treatments were similar (p>0.05). These results demonstrate the striking benefits of MACF biomaterial oxygen delivery to a wound, with enhanced wound healing in a full thickness excisional pig model over 21 d. Excitingly, we have also explored the potential of metabolomics for understanding and revealing underlying key pathways that are positively altered by interaction with biomaterials.
Precision Multimodal Imaging in a Dog Translational Model of Osteoarthritis

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Osteoarthritis (OA) is characterized by degradation and loss of articular cartilage, and remodeling of underlying bone. Currently, conventional radiography is the standard method for diagnosis and evaluation of severity of OA. Thus, refined imaging techniques and modalities have been studied to detect molecular properties and metabolic changes in the knee. Magnetic resonance imaging (MRI) can image all relevant joint tissues within the knee and to visualize cartilage morphology and composition. Nuclear medicine imaging with radiotracers enables imaging of active metabolism and visualization of bone turnover changes seen with osteophyte formation, subchondral sclerosis, subchondral cyst formation and bone marrow lesions as well as sites of synovitis. It has been used to assess inflammation and infection from knee prosthesis. Multimodal imaging such as PET-CT and PET-MR imaging combine functional imaging with high-resolution anatomical imaging. The goal of our research was to serially assess an in vivo dog model of OA combining several imaging modalities. Post-traumatic OA was created via anterior cruciate ligament transection (ACLT). PET/CT and MRI were performed, analyzed and co-registered. Regions of interest from different knee structures were assessed for 2-18F-fluoro-2-deoxy-D-glucose (18F-FDG) uptake values. 18F-FDG was upregulated after ACLT in comparison with the knees that did not undergo ACLT. This research combined innovative multimodal imaging techniques to provide novel metabolic and inflammation information in the knee in an ACLT canine model of OA. 18F-FDG uptake appeared to be a potential imaging biomarker for an early OA diagnosis and could advance the development of precision therapies in an OA translational large animal model.
Microwave-activated Phase-transitional Nanodroplets for Real-time Monitoring and Augmentation of Minimally Invasive Microwave Ablation

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The difficulty in real-time monitoring in percutaneous ablation is a key obstacle in improving the outcomes of minimally invasive thermal ablation. The ablated images are often limited by inadequate spatial resolution and contrast with the currently available real-time imaging methods. Here we show a unique “small-to-big” nanodroplet, which enables intra-operative delineation of thermal ablation margins in real time both in vitro and in vivo via a novel phase-transition strategy called microwave-induced droplet vaporization (MWDV). Simultaneously, this nanodroplet acts as a synergistic agent, by significantly augmenting the efficiency of percutaneous ultrasound (US)-guided microwave ablation (MWA) both ex vivo and in vivo by combining with US. This strategy resolves other critical issues, such as incomplete ablation associated with the currently available minimally invasive thermal ablation procedures. The nanodroplet provides an excellent platform for percutaneous US-guided MWA to enable accurate imaging of ablation margins, synergistically.
A Portable Navigation System for Real-time Wound Assessment

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Introduction: Wound assessment is critical in establishing a diagnosis, guiding treatment course, and predicting effective wound healing while optimizing wound healing outcomes. Currently, there are only a limited number of methods offering quantitative wound assessment and associated vascular or microvascular tissue perfusion in the therapeutic guidance of acute, subacute, and/or chronic wounds and/or injuries. To overcome this limitation, our multidisciplinary care and research team has developed a portable navigation technique for real-time, non-invasive, and quantitative wound assessment and tissue perfusion. This innovative technology has great applications to wound assessment, traumatic or oncologic injuries, medical therapies where poor or inadequate perfusion can lead to adverse outcomes, reconstructive surgery, amputation/residual limb procedures or limb salvage, as well as acute, subacute, and chronic wound care.

Methods: The portable wound navigation system consists of a handheld piece, a wearable goggle, and a contrast agent. Laser speckle and multispectral images of the wound are acquired for reconstruction of tissue oxygenation and perfusion maps. The images can be projected to a google glass to guide the therapeutic intervention at the point of care, injury, or wound assessment.

Results: We have developed a wearable surgical navigation system and demonstrated image-guided surgery in ex-vivo tissue models. We have also developed and clinically tested a multimodal imaging system that integrates multispectral imaging and laser speckle imaging for biological tissue characterization.

Conclusions: Our preliminary experiments have demonstrated the technical feasibility of wound assessment using the proposed portable navigation system. Further engineering integration and clinical validation efforts are necessary in rapidly deploying this system within clinical trials for applications to various medical problems where rapid and real-time wound assessment and image-guided therapy would be of great benefit to the patient.
IN VITRO DEGRADATION OF 3D PRINTED POLY(PROPYLENE FUMARATE) SCAFFOLDS

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Background: Biodegradable, tissue engineered, poly(propylene fumarate) scaffolds are a promising approach to facilitate the regeneration of host tissue across critical-sized craniofacial bone defects.

Hypothesis: We hypothesize that by altering the geometry and molecular weight, the scaffold degradation rate can be tailored to coincide with the rate of bone formation. Methods: Scaffold degradation was accelerated at pH 12 and 37 °C. Mechanical properties, mass loss and pH levels were assessed throughout the 30 day period. Results: Low molecular weight (MW)/low feature dimension (FD) scaffolds exhibited a 66.2% mass loss and decrease in average yield stress from 737.44±125.56 kPa to 270.16±63.67 kPa. Low MW/high FD scaffolds experienced 36.5% mass loss and a decrease in average yield stress from 689.54±96.96 kPa to 245.80±32.46 kPa. High MW/low FD scaffolds exhibited 22.1% mass loss and a decrease in yield stress from 331.03±35.65 kPa to 199.55±87.00 kPa. LMW/LFD scaffolds experienced an initial pH drop to 10.86 and rose to 11.89 by Day 30. LMW/HFD pH levels initially dropped to 11.11 with a more rapid ascent, while HMW/LFD pH dropped very little. Discussion: We have shown that feature dimensions and molecular weight both correlate with degradation rate. The reduction of pH drop over time for all samples indicates the degradation rate slows down as mass is lost. With these positive in vitro results, future work should validate PPF degradation in vivo. Conclusion: The results indicate that scaffold architecture and molecular weight can be adapted to accommodate for specific degradation profiles for tissue engineered bone segmental defects. Sponsor: United States DoD: AFIRM II, Award No. W81XWH-14-2-0004.
IDENTIFICATION OF NOVEL STAPHYLOCOCCUS AUREUS BIOFILM ASSOCIATED PROTEINS WITH NEUTROPHIL KILLING FUNCTION

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S. aureus is a notorious pathogen commonly isolated from chronic burn wounds, with 20–50% of infections being associated with drug resistant (MRSA/VRSA) strains of the bacterium. This makes them a clear problem in both inpatient care and wound clinics. The proficiency of this pathogen to persist is conferred in part by its ability to form robust biofilms. This mode of growth allows for increased tolerance to antimicrobial treatment and immune cell defenses, thus necessitating continued sharp debridement coupled with multiple antiseptic and antibiotic treatments. S. aureus biofilm infections such as those found in chronic burn wounds, have been shown to be associated with a robust influx of neutrophils. These cells undergo necrotic cell death, resulting in a hyper-inflammatory response. The mechanisms underlying this response however, are not fully understood. The goal of this study is to identify S. aureus biofilm proteins causing neutrophil lysis and define the underlying mechanisms triggering cell death. Comparative analyses of the effect of planktonic and biofilm cultures on neutrophil function (reactive oxygen burst assays) and viability (LIVE-DEAD) indicate the presence of a biofilm-associated protein(s) that rapidly kills neutrophils by a process resembling neutrophil extracellular trap formation (NETosis). We are currently utilizing a combination of genetic and biochemical techniques to identify the factor(s) and define the mechanism for killing. A genetic screen of the NARSA transposon mutant library has revealed a role for the accessory gene regulatory network (agr) in expression of the protein(s). Mutants of the agr locus show a loss of biofilm-associated killing activity. This phenotype was found to be independent of known agr regulated toxins including PVL, LukED and LukGH/AB as well as biofilm structural components (PIA/eDNA). We are currently utilizing size exclusion chromatography and mass spectrometry (ESI-LC-MS/MS) for protein identification, enabling the demonstration of direct killing function in vitro. Once identified, mutagenesis studies will be conducted to define domains and residues crucial for mediating neutrophil cell death. Ultimately, therapies targeting these factors will allow for normal functioning of neutrophils and thereby eliminate the advantage provided to the pathogen during infection.
THE HOST IMMUNE RESPONSE TO AGGREGATED PSEUDOMONAS AERUGINOSA

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The opportunistic bacterial pathogen \textit{Pseudomonas aeruginosa} is one of the most common causes of nosocomial infections. It is frequently isolated from burn and surgical site wounds and is often lethal in immunocompromised individuals. During infection, \textit{P. aeruginosa} forms aggregated communities known as biofilms. A hyper-biofilm forming clinical variant of \textit{P. aeruginosa}, known as a rugose small-colony variant (RSCV), is frequently isolated from patients during chronic infection. RSCVs exhibit increased antibiotic tolerance and reduced immunogenicity, but it is not currently understood how this phenotype promotes persistence and host immune evasion. Because neutrophils play a key role in host immunity during \textit{P. aeruginosa} infection, we began by investigating the effects of the RSCV aggregation on neutrophil function. Confocal microscopy was utilized to determine that bacterial aggregation inhibited neutrophil phagocytosis. In other systems, microbial inhibition of phagocytosis stimulated NET formation. To observe the impact of \textit{P. aeruginosa} aggregation on NET formation, we measured neutrophil reactive oxygen species (ROS) production and quantified NET-associated elastase during infection with different \textit{P. aeruginosa} strains. We determined that RSCV aggregates stimulated high ROS and elastase production by neutrophils relative to non-aggregated stains. After determining that \textit{P. aeruginosa} aggregation altered host immune cell function, the broader impact of these effects was investigated \textit{in vivo} using a porcine burn wound model. Bacterial persistence and wound healing was evaluated during infection with non-aggregated or the aggregated RSCV strain of \textit{P. aeruginosa}. While the non-aggregated strain was almost completely cleared from the host after 3-4 days, the aggregated strain persisted in the wound and inhibited healing. We hypothesize that \textit{P. aeruginosa} aggregation is inhibiting typical immune cell function promoting bacterial persistence during infection. Future studies will investigate the interactions between \textit{P. aeruginosa} aggregates and other host cell types including macrophages and epithelial cells.
ANTI-PSL ANTIBODIES AS NOVEL THERAPEUTICS AGAINST PSEUDOMONAS AERUGINOSA BIOFILMS

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*Pseudomonas aeruginosa* is a Gram-negative bacterium that causes acute and chronic infections. After colonization, the bacteria often persist, due to their ability to form biofilms. An important aspect of these biofilms is the extracellular matrix composed of DNA, proteins, and polysaccharides, which is critical for antimicrobial tolerance and host immune evasion. Since no effective treatments exist for these infections, we sought to identify new therapeutics by developing a monoclonal antibody (mAb) selection strategy using phage libraries constructed from healthy individuals and patients convalescing from *P. aeruginosa* infections. This strategy led to the identification of mAbs that bound three distinct epitopes (class I, II and III) on the matrix component Psl. Psl is critical for initial adherence and structural stability of biofilms, making it an ideal therapeutic target. We first evaluated the impact of the mAbs on biofilm initiation and found these antibodies reduced attachment and aggregation of bacteria to a surface, thus potentially deterring biofilm formation. However, none of mAbs disrupted established biofilms. Since anti-Psl mAbs mediate opsonophagocytic killing, we evaluated the effect of neutrophils and mAbs on established biofilms. In combination with human neutrophils, all anti-Psl mAbs significantly reduced biofilm biomass (class I > class III > class II). Using fluorescent versions of these mAbs, we observed discrete staining of established biofilms, with the class I mAb staining the surface and class II and III mAB staining the center and base of the biofilm, respectively. Therefore, mAb epitope accessibility correlated with opsonophagocytic activity with neutrophils, which suggests that different forms of Psl exist in distinct regions of mature biofilms. These mABs also stained infected porcine burn wounds, indicating that these mABs recognize biologically relevant forms of Psl during chronic infection. Overall, these data suggest a potential role for anti-Psl mAbs in the therapeutic treatment of *P. aeruginosa* biofilm infections.
CHARACTERIZATION OF FETAL SKIN FIBROBLASTS: IMPLICATIONS FOR REGENERATION/SCARRING

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Wound healing generally leads to scarring in mature skin, but wounds created at early stages of development heal scarlessly. The exact mechanisms leading to scarless healing are not fully understood, but unique characteristics of fetal fibroblasts are believed to be important. We hypothesize that alterations in fetal skin fibroblasts that result from normal skin development drive scar formation. To date, most studies in this area have used cultured fibroblasts to study this process, but we took a unique approach to examine uncultured fibroblasts harvested directly from fetal skin. To identify proteins that might regulate scarless and fibrotic healing, shotgun proteomics was performed on dermal fibroblasts harvested from collagen1a1-green fluorescent protein (coll-GFP) mice, which contain GFP+ fibroblasts. Embryonic day 15 (E15) skin, which heals scarlessly, and embryonic day 18 (E18) skin, which heals with a scar, were enzymatically digested, then fluorescence-activated cell sorting (FACS) was used to isolate GFP-positive dermal fibroblasts. Protein isolated from E15 and E18 fibroblasts was subjected to proteomics analysis. Established fibroblast markers, such as vimentin, were highly expressed in both cell types. Initial analysis revealed a number of differentially expressed proteins between fibroblasts isolated from E15 and E18 fetal skin, suggesting that fibroblasts change during the process of fetal skin development. Follow-up studies are now being performed to validate differentially expressed proteins, which will eventually be screened for their ability to affect regeneration/scarring in skin wounds. One candidate protein of particular interest is fetuin A. This protein has previously been associated with inflammation and was strongly up-regulated in E18 fibroblasts, suggesting that it may play a role in scar formation. Overall, these data suggest that proteomics analysis of uncultured fibroblasts from coll-GFP fetal skin may be a useful tool to dissect the mechanisms involved in scarless and fibrotic healing.
EFFECTS OF IL-33 ON WOUND INFLAMMATION

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Inflammation is important for preventing infection in skin wounds, but excessive inflammation can impair healing and increase scarring. Many signaling molecules regulate the inflammatory process; however, very little is known about the effects of the alarmin IL-33. This IL-1 family member has been shown to promote inflammation in various pathologies, and is increasingly suggested to play a role in wound healing. The goal of this study was to determine the role of IL-33 in inflammation after injury. Full-thickness wounds were generated in fetal or adult murine skin, collected at various times after wounding, and immunohistochemically stained for IL-33. Fetal wounds created at embryonic day 15 (E15) lack an inflammatory response and heal scarlessly, while wounds created in E18 or adult skin heal with robust inflammation and scarring. In fetal wounds, the peak number of IL-33-expressing keratinocytes and the duration of IL-33 expression were greater in E18 wounds compared to E15 wounds. In adult wounds, IL-33 staining increased in keratinocytes at the wound margin beginning at 24 hours, peaked at 48 hours, and remained elevated until 7 days. This demonstrates that IL-33 is temporally and spatially associated with inflammation during wound healing. To determine the effect of IL-33 on fetal wound healing, recombinant murine IL-33 was injected into E15 wounds. The addition of IL-33 delayed closure and disrupted scarless healing. To examine the role of IL-33 in inflammation, excisional wounds were generated in adult IL-33 knockout mice (IL-33/-) and wild-type mice. Wounds from IL-33/- mice collected at 48 hours showed reduced neutrophil infiltration by immunohistochemical staining compared to wild-type mice. Ongoing studies are examining the effects of IL-33 on other markers of inflammation and scarring in fetal and adult wounds. These data suggest that IL-33 may be a good therapeutic target to dampen inflammation and minimize scar formation.
Compromised membrane repair is a pathogenic mechanism contributing to the progression of human heart failure.

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A conserved plasma membrane repair mechanism exists to counteract membrane damage and restore membrane barrier function in order to maintain normal cellular homeostasis. This response can involve various mechanisms including activation of signaling pathways that trigger vesicular trafficking to the site of injury followed by vesicular fusion with the damaged portion of the membrane to patch the membrane disruption. Previous studies indicate that compromised repair capacity can exacerbate cardiac injury while increasing membrane repair capacity can reduce cardiac pathology. In our studies, membrane repair assays on cardiac and non-cardiac cell lines demonstrated that this process is dependent on activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling axis through the downstream target Akt1. One mechanism found to increase membrane repair following PI3K/Akt1 activation is elevated exocytotic and endocytotic activity. Further studies indicate that the PI3K/Akt1 pathway is relevant to membrane repair in native hearts. Thick slices of myocardium from explanted human and mouse hearts were probed using multi-photon microscopy to determine the membrane repair capacity. These studies indicate decreased repair capacity in failing human myocardium as well as in mouse hearts following transaortic constriction. This membrane repair response requires PI3K/Akt1 signaling. PI3K or Akt1 inhibition prevents membrane rescaling in non-failing human or mouse myocardium. The compromised membrane repair observed in failing myocardium can be ameliorated by PI3K or Akt1 agonists. Our results indicate that failing cardiomyocytes exhibit compromised membrane repair and that increased PI3K/Akt1 signaling can increase repair capacity thereby demonstrating potential as a heart failure treatment.
Wound Healing Processes in Metastasis and Post-Metastatic Cancer: Review of Recent Developments.

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In his seminal 1986 NEJM paper “Tumors: Wounds That Do Not Heal” Harold Dvorak wrote, “I will suggest that tumor stroma generation is wound healing gone awry.” Dvorak himself demonstrated the key role of VEGF in tumor angiogenesis. In subsequent decades, the common roles of additional growth factors including EGF, FGF, TGFβ, and PDGF, as well as Hedgehog signaling and Wnt signaling, in wound healing and tumorigenesis have been extensively elucidated. Indeed, working around these commonalities to avoid increased probability of tumorigenesis has been a significant source of challenges in developing regenerative medicine therapies. While the proliferative stage of tumorigenesis has previously received significant attention in this regard, wound healing-related processes are now increasingly emerging as pivotal in the EMT (epithelial-mesenchymal transition), metastasis, and later stages of cancer progression. A few of these latter developments in tumor biology are summarized here for researchers in regenerative medicine.
GROWTH FACTORS AND OTHER BIOACTIVE MOLECULES FOR TISSUE ENGINEERED BONE GRAFTS

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Background: Bone marrow-derived human mesenchymal stem cells (BM-hMSCs) have the potential to differentiate toward an osteogenic lineage and, therefore, are of interest in bone tissue engineering.

Hypothesis: We hypothesize that optimal combinations of growth factors will induce proliferation and differentiation of BM-hMSCs to an osteogenic lineage that will secrete bone-like extracellular matrix.

Methods: Scaffold-seeded BM-hMSC proliferation and differentiation were assessed following culture with different combinations/concentrations of growth factors. Cells were cultured on 2D and 3D poly(propylene fumarate) scaffolds. Results: Initial studies were performed on 2D, thin film scaffolds. Proliferation was assessed at D1, D3, and D7 using combinations of 1X and 10X concentrations of the growth factors FGF-2, PDGF-bb, and EGF. Greatest proliferation was observed with the combination of 1X of each of the three growth factors. Differentiation studies compared BMP-4, -6 and -7 and commercially available “osteogenic media”. 1X BMP-7 and “osteogenic media” were found to be the most effective means of differentiating BM-hMSCs to an osteogenic lineage. Preliminary results on 3D scaffolds showed bone-like ECM deposition after use of the best-performing growth factor regimens.

Discussion: We have developed growth factor regimens for the proliferation and differentiation of BM-hMSCs to an osteogenic lineage. Current and future studies will focus on the use of these regimens with 3D-printed, porous scaffolds for bone tissue engineering. Conclusion: A strategy to produce well-characterized tissue engineering bone grafts has been developed by optimizing growth factors for proliferation and differentiation. Sponsor: United States DoD, AFIRM II, Award No. W81XWH-14-2-0004.
Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen, is the predominant agent of chronic infections, in which *P. aeruginosa* exists in a biofilm state of growth as a multi-cellular community of adherent cells encased in a matrix. Small colony variants of *P. aeruginosa* exhibit hyper-biofilm forming phenotype. We measured viability and persistence of a small colony strain and its isogenic parent in a chronic burn wound porcine model. Colony forming unit analysis of biopsies collected on Days 7, 14, and 35 showed that the small colony strain exhibited persistence until Day 35 in the wound environment relative to the parental strain. Histological analysis of the biopsies revealed delayed wound healing in wounds infected with the small colony strain relative to the parental strain. We also observed impairment of keratinocyte migration as measured by Keratin 14 immunohistochemistry in wounds infected with the small colony strain relative to the parental strain, which may account for the delayed wound healing. In addition, we isolated revertant colonies, which indicates that the chronic wound environment exhibits a unique physiological pressure on *P. aeruginosa*. Overall, we determined that the small colony strain persisted longer than the parental strain. This persistence may be attributed to its hyper biofilm forming capability. In order to determine whether the persistence was due to biofilm formation, we will conduct a follow up experiment with a small colony strain deficient in biofilm formation. This will then elucidate whether the persistence is dependent upon hyper biofilm forming phenotype. In addition, we are performing whole genome sequencing of several paired wild type and revertant isolates to determine the genetic alterations responsible for the revertant phenotype.

Sponsor: Dr. Daniel J Wozniak
Dermal Regenerate and Spray Skin in Restoring Full Thickness Soft Tissue Defects

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Background: Treating full thickness defects secondary to large burns, traumatic and war-related injuries requires novel strategies to restore the functional, aesthetic and protective properties of skin.

Methods: We used a dermal regenerate (DRT) with spray skin technology in addition to STSG to treat full thickness soft tissue losses in two trauma patients.

Results: The two patients were 29 and 36 years of age, with 600 and 1190 cm² wounds, respectively. In the first stage, wounds were debrided and DRT applied. In the second stage, wounds were treated with intraoperative application of spray skin and 6:1 meshed STSG. In both patients, complete re-epithelialization of donor sites was achieved by 2 weeks and recipient wound sites at 4 weeks, respectively. In patient 1, matching pigmentation of recipient wound sites to native skin occurred at 6 months follow-up, whereas patient 2 showed evidence of progressing re-pigmentation nearly matching native skin at 3 months follow-up.

Discussion: The staged used of DRT and spray skin limited donor site morbidity, decreased donor site burden and time to complete healing. It also allowed for a greater mesh ratio, and resulted in matching pigmentation.

Conclusion: We show that staged use of DRT and spray skin provides a safe and effective method for restoring large soft tissue defects.
Animal or human tissue is the source of cells for almost all cell-based assays or autogenous/allograft cell therapies. The culture expanded cells from each tissue source represent the progeny of a heterogeneous population of tissue resident progenitors. Each progenitor gives rise to a clone of progeny, and can therefore be defined as a "colony founding unit" (CFU). The prevalence and biological performance of individual CFUs vary widely between clones and are likely to reflect important aspects of the underlying health or state of the source tissue. This provides an opportunity to make objectively informed choices about which CFU subtypes or clones that should be included or excluded from a specific cell product or cell-based assay.

We have developed a robust, automated robotic platform, (Figure 1), comprising of an automated inverted microscope, high precision motion control (0.1 μm repeatability), high precision fluid handling, control and analysis software that enables:

1) Fast and accurate montage image acquisition
2) Automated image analysis to identify colonies or cells of interest
3) Selection of cells using fluid handling technology to remove and transfer cells to another culture for more specific analysis.

Current state of the art for cell therapy involves preferential expansion of cells in interest. (Figure 2). Our process involves use of imaging and robotics to perform early selection to pick cells of interest and transfer them for subculture. The transferred cells do not have to compete with other cells, resulting in a more pure cell population in a shorter period of time. Use of this technology results in savings in cost, increased quality and performance, and increased control and documentation.

We have demonstrated the capability of this system to image cells, efficiently remove cells, viably transfer cells to another culture surface, and show that these cells maintain their proliferative capabilities following transfer. We observe and quantify heterogeneity in colonies using quantitative image analysis.
WOUND MACROPHAGE-DERIVED ONCOSTATIN M INDUCES S100A9 IN CUTANEOUS WOUND EPITHELIUM

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Wound macrophages play multifaceted role in cutaneous wound healing. Emergent line of evidence argues in favor of a strong macrophage-keratinocyte cross-talk. This work builds on previous observation that human wound-site macrophages and wound fluid is rich in Oncostatin M (OSM). OSM, unlike other macrophage-derived cytokines exclusively involved in inflammation, primarily functions by its effect on keratinocytes. A diverse and networked antimicrobial defense system in keratinocytes defends against skin infections. Antimicrobial peptides/proteins (AMPs) like β-defensins and S100 proteins represent a key innate defense mechanism of the human skin. We sought to test the significance of OSM on antimicrobial defense system of the cutaneous wound epithelium (WE). Laser capture microdissection and proteomics studies identified S100A9 as being highly expressed in the human chronic cutaneous WE. Importantly, OSM present in human chronic wound fluids potently induced (p<0.001; n=4) the expression of S100A9 in human keratinocytes. Additionally, OSM induced β-defensins, establishing a cause and effect link between wound-site OSM and keratinocyte AMPs. OSM repressed keratinocyte SOCS3, a negative regulator of JAK-STAT signaling. In turn, OSM-induced S100A9 was potentiated in keratinocytes with repressed SOCS3. OSM receptor knockdown abrogated (p<0.05; n=4) OSM-induced S100A9 expression suggesting that OSM-mediated suppression of SOCS3 drives JAK-STAT signaling leading to S100A9 induction. S100A9 over-expression in human keratinocytes induced (p<0.05; n=5) expression of specific β-defensins and increased the killing of S. aureus. The ability to fight S. aureus infection was compromised in S100A9-null mice. Finally wound fluid from human chronic wounds with poor infection outcomes was associated with low (p<0.05; n=7) OSM and S100A9 in wound fluid. Taken together, this work upholds the critical significance of wound macrophage-keratinocyte cross talk in endowing the WE with antimicrobial defenses. Wound macrophage derived OSM enables such cross-talk adding a new dimension to the significance of wound inflammation.

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NOVEL MECHANISMS OF COLLAGENASE SANTYL® OINTMENT (CSO) IN WOUND MACROPHAGE POLARIZATION AND RESOLUTION OF WOUND INFLAMMATION

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Collagenase Santyl® Ointment (CSO) is an FDA-approved wound debridement agent. This work builds on our previous observation that CSO promotes wound macrophage (wM) polarization to an M2 phenotype. Thus, in addition to its widely recognized role in debridement, CSO may support resolution of wound inflammation. The objective of the present study was to further characterize the mechanisms by which the active ingredient of CSO (CS-API), influences wM polarization. wM isolated from subcutaneously implanted polyvinyl alcohol sponges in C57bl6 mice were sorted using anti-CD11b tagged magnetic microbeads. The wM were treated with CS-API (250 ng/ml) for 24h ex vivo. The expression of M1 markers (NOS2, IL-12 and CD74) and M2 markers (IL-10, arginase and CD206) was determined using quantitative PCR. CS-API induced (p<0.05; n=5) the expression of genes representing M2 markers. At the same time, genes representative of M1 phenotype were significantly downregulated (p<0.05; n=5). Taken together, these results demonstrate the ability of CS-API to direct wM to M2 phenotype. To identify the transcriptional regulation of CS-API mediated M2 polarization of wM, cell signaling was characterized in murine RAW 264.7 macrophages. Treatment of such macrophages with CS-API for 1h attenuated (p<0.05; n=5) transactivation of the pro-inflammatory transcription factor, NF-κB. In the same experimental setting, CS-API significantly induced the activity of STAT6 (p<0.05; n=5), a transcription factor involved in macrophage M2 polarization. In summary, this work presents first evidence demonstrating that the active ingredient of the widely used clinical debridement agent CSO potently induces M2 polarization and resolution of wound inflammation via regulation of STAT6 and NF-κ B pathways.

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Wound healing process in the tumor micro-environment

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There is evidence that the most common cancer treatments like surgery, chemotherapy, and radiotherapy produce necrotic cells death. The necrotic cells send signals to the immune cells to initiate the wound healing process. In this work, we simulate the wound healing process after a treatment, which only kills cells without changing the tumor's inflammatory environment. More specifically, we develop a stochastic model for cells dynamics after a treatment, which kills epithelial cells. We assume after the treatment: there is a wound that needs to be healed. We denote the fitness of cancer cells over the normal cells by $r$. Briefly, if two cells; one cancerous and one normal, receive proliferation signals to fill out an empty location, then the probability that the cancer cell divides is $r$ times the normal one. The model shows that if $r > 1.01$, then a single cancer cell would outcompete 1000 normal cells to heal a wound. Although, tumor cells close the wound, however a high level of proliferation signals is needed to develop a fast growing recurrence. Implying epithelial tumor cells alone cannot cause a fast growing recurrence.
ROBOT-ASSISTED MECHANICAL THERAPY PROTECTS AGAINST SKELETAL MUSCLE INJURY AFTER STROKE

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Background: Mechanistic study of post-stroke rehabilitative therapies remains limited. The current work addresses the development of a Robot-Assisted Mechanical Therapy (RAMT) device and its facilitation of reproducible, objective study of post-stroke mechanical physiotherapy.

Methods: Male Wistar rats (n=26) were subjected to the intraluminal thread method of ischemic stroke after which they received daily RAMT (RAMT+) or none (RAMT-, control) for up to 14 days post stroke. The effects of RAMT on post-stroke function were quantified, and stroke-affected muscle was evaluated to identify molecular targets of stroke injury.

Results: In comparison with controls, RAMT+ rats benefited from daily RAMT, experiencing improved perfusion in the stroke-affected gastrocnemius muscle as shown with laser speckle flowmetry (LSF). Automated gait analysis with TreadScan indicated improved post-stroke gait in RAMT+ rats, and ANY-maze behavioral testing software demonstrated superior locomotor and sensorimotor recovery with RAMT. Following assessment of functional recovery, evaluation of stroke-affected gastrocnemius muscle identified novel targets of RAMT protection against ischemic stroke injury.

Discussion: Mechanical therapy improves tissue perfusion and offsets inflammation. However, research on associated molecular targets is limited. Current stroke therapy guidelines lack evidence-based research, and methods for studying post-stroke physiotherapy are scarce. RAMT allows for reproducible, objective, pre-clinical study of post-stroke mechanical therapy.

Conclusion: Stroke adversely affects signal transduction in skeletal muscle. Mechanical rehabilitative therapy is successful in reversing such ill effects and restoring hindlimb function.
ANTIHYPOXAMIR FUNCTIONALIZED GRAMICIDIN LIPID NANOPARTICLE RESCUES AGAINST ISCHEMIC MEMORY AND ACCELERATES WOUND CLOSURE

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Peripheral vasculopathies cause severe wound hypoxia inducing hypoxamiR miR-210. Elevated miR-210, persisting in wound-edge tissue as ischemic memory, is aimed at keeping the tissue-at-risk alive by minimizing the oxygen cost of survival. Thus, miR-210 suppresses oxidative metabolism and inhibits cell proliferation in the ischemic wound tissue. While on one hand, high miR-210 is a survival factor for the tissue-at-risk, at the same time it is in conflict with healing that requires oxidative metabolism and cell proliferation. Laser capture microdissection (LCM) of human ischemic wound-edge epithelium revealed elevated miR-210 expression that was tightly associated with inhibition of epidermal cell proliferation as evident by low Ki67. In murine ischemic wound, administration of naked anti-miR-210 was ineffective in neutralizing hypoxia-induced elevated miR-210 in the wound tissue. The objective of the present work was to develop a novel antihypoxamiR functionalized lipid nanoparticles (LNPs) capable of inhibiting excessive miR-210 and improving cutaneous wound healing. To inhibit miR-210 in murine ischemic wound-edge tissue, we report the formulation of antihypoxamiR functionalized gramicidin lipid nanoparticles (AFGLN). AFGLN had an average diameter of 150 nm and a zeta potential of +10 to -10 mV at pH of 5 and 8, respectively. The encapsulation efficiency of these LNPs was found to be 80.12%. A single intradermal delivery of AFGLN encapsulating LNA-conjugated anti-hypoxamiR-210 (AFGLN_miR-210) lowered miR-210 level in murine ischemic wound-edge tissue. AFGLN_miR-210 treated ischemic wound showed improved re-epithelialization with the presence of characteristic hyperproliferative epithelium as would be expected in healing wounds. In repTOP1⁹⁷mitoIRE mice, AFGLN_miR-210 rescued keratinocyte proliferation as visualized by in vivo imaging system (IVIS). ⁴¹P NMR studies showed elevated ATP content at the ischemic wound-edge tissue following AFGLN_miR-210 treatment indicating recovering bioenergetics necessary for healing. Consistently, AFGLN_miR-210 improved ischemic wound closure. The nanoparticle based approach reported herein is effective for miR-directed wound therapeutics warranting further translational development.
Role of Proopiomelanocortin in Wound Healing

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Proopiomelanocortin (POMC) is a precursor polypeptide that is eventually cleaved to form several critical regulatory hormones for the body. Hormones that are produced as a result of the cleavage of POMC include β-endorphin, adrenocorticotropic hormone (ACTH), and α-MSH. POMC has been shown to play a role in energy maintenance and production of adrenal hormones. Recent research has reported that dysregulation and mutations of the POMC gene and protein could lead to serious clinical conditions such as obesity and adrenal hormone insufficiency. Our lab has previously published data indicating that wound fluid contains factors that can significantly increase the production of POMC. TNF-α is a pro-inflammatory cytokine that has the ability to increase the expression of neutrophils with specific antigen CD177, which as our data suggests show high expression of POMC. We sought to test the significance of POMC in cutaneous wound healing. Wound fluid was collected from human subjects, and the fluid showed a significant increase in POMC mRNA expression when compared to the plasma control. Additionally, the presence of CD177 positive PMN was most prevalent from 6-48h post-surgery. Mice having heterozygous deletion of POMC were used for the study. Knockdown of POMC was confirmed in the heterozygous mice when compared to wild type mice. Full-thickness splinted excisional wounds were developed on the back of mice. Wound closure was significantly impaired (p<0.05; n=5) in POMC+/− mice as compared to wild-type (POMC+/+). Such impairment was associated with increased production of the pro-inflammatory cytokines, IL1β and TNF-α which in turn attenuated wound angiogenesis in POMC+/− mice. This work provides evidence establishing a critical role of wound-site neutrophil POMC in resolving wound inflammation favoring wound angiogenesis and improved healing outcomes. Further studies are needed to understand the regulatory pathway POMC proceeds through and the roles POMC’s downstream peptide hormones play in a wound environment.
Apex-1 promotes c-jun DNA binding in Nox-4 mediated HE tumor progression

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Abstract

Tumor forming endothelial cells have highly elevated levels of Nox-4 that release H2O2 oxidants into the nucleus, which is generally not compatible with cell survival. We sought to identify compensatory mechanisms that enable tumor forming endothelial cells to survive and proliferate under these conditions. Ape-1/ref-1 (Apex-1) is a multifunctional protein that promotes DNA binding of redox sensitive transcription factors, such as AP-1 and repairs oxidative DNA damage. A validated mouse endothelial cell (EOMA) tumor model was used to demonstrate that Nox-4- derived H2O2 causes DNA oxidation that induces Apex-1 expression. Apex-1 functions as a chaperone to keep transcription factors in a reduced state. In EOMA cells Apex-1 enables AP-1 binding to the monocyte chemoattractant protein-1 (mcp-1) promoter and expression of that protein is required for endothelial cell tumor formation. Intraperitoneal injection of the small molecule inhibitor E3330, which specifically targets Apex-1 redox sensitive functions, resulted in a 50% decrease in tumor volume compared to mice injected with vehicle control (n=6 per group) indicating that endothelial cell tumor proliferation is dependent on Apex-1 expression. These are the first reported results to establish Nox-4 induction of Apex-1 as mechanism promoting endothelial cell tumor formation.
MRP-1 dependent GSSG efflux as a critical survival factor for oxidant-enriched tumor forming endothelial cells

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Abstract

Endothelial cell tumors are the most common soft tissue tumors in infants. Tumor forming endothelial (EOMA) cells are able to escape cell death fate despite excessive nuclear oxidant burden. Our previous work recognized peri-nuclear Nox-4 as a key contributor to EOMA growth. The objective of this work was to characterize the mechanisms by which EOMA cells evade oxidant toxicity and thrive. In EOMA, compared to that in the cytosol, nuclear GSSG/GSH ratio was five-fold higher. Compared to those in healthy murine arterial endothelial cells (MAE), GSSG/GSH was over twice in EOMA in a situation. Multidrug resistance-associated protein-1 (MRP-1), an active GSSG efflux mechanism, showed two-fold increased activity in EOMA compared to MAE. Hyperactive YB-1 and Ape/Ref-1 were responsible for high MRP-1 expression in EOMA. Proximity ligand assay demonstrated MRP-1 and YB-1 binding. Such binding enabled the nuclear targeting of MRP-1 in EOMA in a leptomycin-B sensitive manner. MRP-1 inhibition as well as knockdown trapped nuclear GSSG causing cell death of EOMA. Disulfide loading of cells by inhibition of GSSG reductase (bischloronitrosourea) or thioredoxin reductase (auranofin) was effective in causing EOMA death as well. In sum, EOMA cells survive a heavy oxidant burden by rapid efflux of GSSG, which is lethal if trapped within the cell. A hyperactive MRP-1 system for GSSG efflux acts as a critical survival factor for these cells making it a productive target for EOMA therapeutics.
NON-INVASIVE HIGH-RESOLUTION VISUALIZATION AND QUANTIFICATION OF DYNAMIC BLOOD FLOW IN GATED HUMAN SKIN MICROVESSELS

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Background: Cutaneous microvasculopathy drives a wide range of peripheral vascular disease and their complications including non-healing wounds. High-resolution quantitative assessment of gated individual peripheral microvessel and its function is therefore of outstanding interest. Current approaches to study the most fine branched peripheral microvessel rely on invasive procedures. Imaging modalities such as laser speckle contrast analysis (LASCA) and clinical SPY imaging technologies are non-invasive but lack the resolution to gate a single peripheral branched microvessel. Methods: We adopted LASCA imaging principles (785 nm laser with CCD recorder) with two major improvements. First, we employed high magnification optics featuring 20 micron/pixel, as opposed to 100 micron/pixel in standard laser speckle imaging. Second, we retooled the LASCA algorithm by writing a new program code based on universal Matlab platform. Results and Discussion: The first improvement was insufficient to visualize or gate microvessels. Post-processing of image using the second improvement described above helped visualize and gate the primary, secondary, tertiary and quaternary microvessels. In addition to enumeration of microvessels, blood flow in gated vessel of interest was quantified. For studies involving changes in blood flow over time, dynamic (100 fps) quantitative imaging was performed on a region of interest. We employed an extended moving window averaging technique specifically designed to enhance contrast to noise ratio. This work presents the first distinct image and dynamic blood flow data from the gated human foot microvasculature. Interestingly, reactive hyperemia noted in humans after focal cold stimulation was not evident in mice arguing in favor of a discrepant microvascular physiology in these two species. In mice, cold-induced vasoconstriction was evident in tertiary and quaternary microvessels where primary and secondary microvessels remained unaffected. Conclusion: Taken together, this work offers a novel powerful technological advancement to non-invasively interrogate human microvascular anatomy as well as function that are likely to markedly influence cutaneous wound healing outcomes.
Silencing hypoxia Induced microRNA-1 improves keratinocytes survival and migration in murine ischemic wound

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Abstract
Chronic wounds are commonly associated with peripheral vasculopathies are primarily responsible for wound ischemia. Limitations in the ability of the vasculature to deliver O₂-rich blood to the wound tissue leads to, among other consequences, hypoxia. Thus, hypoxia is a subset of ischemia. Studies during the last five years have identified that hypoxia may serve as an inducer of several hypoxamir. Better recognized as myomiRs, miR-1 is induced under ischemic conditions in skin. In this study we show that hypoxia induced miR-1 targets Dll-1 and facilitates Bax dimerization on mitochondrial membrane. miR-1 represses mitochondrial respiration and associated downstream functions which in turn causes keratinocyte growth arrest, cell death and compromises wound closure. Intradermal delivery of antisense oligonucleotides facilitates keratinocytes proliferation and migration under ischemic conditions. Thus, LNP conjugated anti-miR-1 based system for optimum delivery to wound keratinocyte may be effective in improving ischemic chronic wound outcomes.
AN EQUINE PERICARDIAL COLLAGEN MATRIX (ePCM) DRESSING STRENGTHENS ANTIMICROBIAL DEFENSES IN HUMAN KERATINOCYTES

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A biologically stabilized, acellular, equine pericardial collagen matrix (ePCM) is clinically used as wound dressing. For the care of chronic wounds such as diabetic ulcers, ePCM is used as a single application where the dressing remains embedded in the wound until closure. Preliminary observations support that in patients, ePCM improves wound closure. The mechanism of action of ePCM in wound healing remains unclear. Initial studies from our group characterized human keratinocyte growth, proliferation and differentiation on ePCM in vitro. Our studies indicated that human keratinocytes attached to ePCM and acquired a differentiated phenotype compared to those growing on glass surfaces. This provided first cues suggesting that ePCM may serve as a scaffold for cells within the wound microenvironment. Interestingly, the antimicrobial peptide (AMP) defense system was significantly upregulated in cells adhered to ePCM compared to those on glass. AMPs kill a wide variety of microbes including bacteria. Such upregulation of AMPs in keratinocytes adhered to ePCM could provide effective defenses against bacterial colonization and wound infection.

Most bacteria are able to attach and grow on biological surfaces leading to infection. In keeping with this, scanning electron microscopy imaging identified that ePCM by itself was a suitable substrate for robust bacterial growth of the primary wound pathogen Pseudomonas aeruginosa (PA01). This further suggests that effective containment of wound infection in vivo is likely to be contributed by bolstered epithelial antimicrobial defense system caused by ePCM. Our observation demonstrating that an acellular collagen matrix may modify keratinocyte antimicrobial defenses draws attention to the far-reaching influence of biological dressings above and beyond their direct physico-chemical influence on the wound milieu.

Support: Harbor MedTech
OSSABAW SWINE AS A POWERFUL MODEL TO ADDRESS CHRONIC WOUNDS COMPLICATED BY UNDERLYING METABOLIC SYNDROME (METSYN)

Comprehensive Wound Center, Davis Heart and Lung Research Institute, Center for Regenerative Medicine and Cell Based Therapies, The Ohio State University Wexner Medical Center

Infected chronic wounds in the MetSyn patients are common and this work recognizes the Ossabaw swine as a powerful pre-clinical experimental model to study mechanistic underpinnings. We have characterized the development of MetSyn using comprehensive metabolic panels in pigs maintained on the modified atherogenic m-Ath diet (N=15) or control diet (N=5) for a year. Within two months of being on diet, sedentary Ossabaw maintained on m-Ath diet showed parameters approaching MetSyn, including abdominal obesity, dyslipidemia and high blood pressure which parallel the situation in the human MetSyn population. Skin perfusion analysis identified onset of peripheral vasculopathy. Following 6-8 month of diet, full thickness (2"x2") excisional wounds were created on the dorsum and followed up to d31 post-wounding. Wounds were left uninoculated or inoculated with a mixed species biofilm infection involving -  Pseudomonas aeruginosa (PA01), Staphylococcus aureus (USA300) and Acinetobacter baumannii (19606). Infected wounds were treated with Tegaderm or patterned electroceutical dressing (PED). We developed PED as a battery operated silk dressing with silver printed in a geometric pattern that generates up to 6V electric potential with related field and current when applied to an open wound. Wounds, where infection was not induced, closed rapidly (d21) primarily supported by adipocyte proliferation. In contrast, wounds challenged by biofilm infection showed delayed closure (d31) resulting in ‘faulty skin’ with defective barrier function as measured by elevated trans-epidermal water loss (TEWL). This observation parallels clinical findings where wound chronicity is strongly linked to presence of infection, especially in people with MetSyn. PED treatment rescued the barrier defect caused by biofilm infection. Most bacteria are negatively charged on the surface. The involvement of electrostatic forces in the assembly of biofilm is recognized. Our observation demonstrating that electroceutical approaches are productive in treating wound biofilm warrant further mechanistic understanding.

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ORAL SHILAJIT SUPPLEMENTATION AND THE HUMAN SKELETAL MUSCLE TRANSCRIPTOME RESPONSE

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The objective of the present study (clinicaltrials.gov NCT02026414) was to observe the effects resulting from oral supplementation of a purified and standardized Shilajit extract. The effects would be observed on skeletal muscle adaptation in adult overweight/class 1 obese human subjects from the US population. Shilajit is a mineral pitch that oozes out of Himalayan rocks. The study design consisted of a baseline visit followed by 8 weeks of 250 mg of oral Shilajit supplementation b.i.d and then an additional 4 weeks of supplementation with exercise. At each visit, blood samples and muscle biopsies were collected for further analysis. Supplementation was well tolerated, without any changes in blood glucose levels or the lipid profiles, after 8 weeks of oral supplementation and the additional 4 weeks of oral supplementation with exercise. Also, no changes were noted in creatine kinase and serum myoglobin levels after 8 weeks of oral supplementation and the additional 4 weeks of supplementation with exercise. The microarray analysis identified a cluster of 17 extracellular matrix (ECM) related probe sets that were significantly upregulated in muscles following 8 weeks of oral supplementation as compared to the expression at the baseline visit. This cluster included tenascin XB, decorin, myofibrin, collagen, elastin, fibrillin 1, and fibronectin 1. The differential expression of these genes was confirmed using quantitative RT-PCR. The study provided maiden evidence that oral Shilajit supplementation in adult overweight/class 1 obese human subjects promoted skeletal muscle adaptation via upregulation of ECM related genes that control muscle mechano-transduction properties, elasticity, repair and regeneration.

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Key Words: Shilajit, skeletal muscle, extracellular matrix, adaptation
DEVELOPMENT OF CHARCOT NEUROARTHRPATHY IN DIABETIC PATIENTS WHO RECEIVE KIDNEY AND KIDNEY-PANCREAS TRANSPLANTS.

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Background: Incidence of Charcot neuroarthropathy in diabetics is low, but greatly increases the risk of foot complications. Incidence among solid-organ transplant patients appears to be higher.

Hypothesis: Charcot neuroarthropathy incidence is higher in diabetic patients who received kidney or kidney-pancreas transplants.

Methods: This retrospective chart review over 11 years included 487 diabetic transplant patients who had kidney or kidney-pancreas transplant. Incidence of Charcot and complications were evaluated.

Results: The incidence of Charcot development in the diabetic patients who had a kidney-pancreas transplant was 18.4% (44/238), which was statistically significantly more than the incidence in kidney transplant patients which was 11.2% (28/249). Neuropathic transplant recipients’ incidence of Charcot was 31.4% in kidney-pancreas, and 20.4% in kidney transplants. 42% of all study patients developed ulceration, while 80% of the Charcot group developed ulceration. The percentage of patients who developed osteomyelitis and amputations was also double in the Charcot group. Peripheral arterial disease was a statistically significant independent risk factor for developing ulceration, osteomyelitis, and subsequent amputation. Type 1 diabetics developed Charcot at a statistically significant higher rate than type 2 diabetics.

Discussion: In Charcot patients the inflammatory pathway is triggered, leading to osteolysis and subsequent fracture. Transplantation increases risk of developing Charcot. ESRD leads to worse neuropathy and microvascular complications, and kidney transplantation alters bone metabolism. Immunosuppression and steroid medications lead to bone metabolism changes.

Conclusion: Incidence of Charcot development after kidney and kidney-pancreas transplantation is high. Charcot development leads to increased risk of foot ulceration, osteomyelitis, and amputation in these patients.
MICROBIAL CERAMIDASES INDUCED IN WOUND BIOFILM INFECTION DISRUPTS SKIN BARRIER FUNCTION

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Cutaneous lipids, 50% of which are ceramides (Cer), play a major role in skin barrier function. Bacterial biofilms are estimated to account for 60% of all chronic wound infections. Biofilm infected wounds may visually appear closed but remain functionally open because of disrupted barrier function of the repaired skin. Such “defectively closed” wounds display high trans-epidermal water loss (TEWL) and are likely to show higher recurrence rate. We have recently reported the biofilm infection compromises skin barrier function. This study is based on the observation that bacterial ceramidase are over expressed around (~500 fold, n=4, p<0.05) in in vivo biofilm model (porcine infected with Pseudomonas aeruginosa and Acinetobacter baumannii mixed biofilm). Such excessive microbial ceramidases deplete host skin ceramides. Lipidomics, using electrospray ionization-mass spectrometry (ESI/MS), showed that long chain ceramides are depleted in biofilm-infected porcine skin with concomitant increase in sphingosine, a breakdown product of ceramide (n=4, p<0.05). We further noted through immuno histochemistry that biofilm-infection caused focal erosion of ceramides in the wound-edge skin (n=3, p<0.05). In vitro functional measurement of keratinocyte intercellular barrier function using the electrical cell impedance system (ECIS) showed reduced trans-keratinocyte electric resistance (TkER) in biofilm. These findings were further elaborated by a concurrent finding that host ceramide synthase 3 (CerS3) is silenced by biofilm-inducible microRNAs (miRs) miR-146a and miR-106b (n=3, p<0.05). As a measure to restore cutaneous ceramide deficiency in biofilm infected wounds, we topicaly applied a repurposed FDA approved ceramide emulsion to biofilm infected wound in mice, restoring barrier function to defectively closed wounds (n=3, p<0.05). Skin aging results in gradual loss of ceramides, and thus loss of cutaneous ceramide in biofilm infection may accelerate premature skin ageing. Hence, this study of biofilm dependent depletion of skin ceramides points towards the testable hypothesis that biofilm infected skin may undergo an accelerated aging phenotype.

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TOCOTRIENOL INDUCES ANAGEN HAIR FOLLICLES FACILITATING CUTANEOUS WOUND HEALING WITH REGENERATIVE PHENOTYPE

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Stem cell niche of the hair follicle is of outstanding interest in the context of cutaneous wound healing outcomes. The objective of this work was to develop a strategy to induce anagen hair follicles and hair follicle neogenesis and test its effect on cutaneous wound healing. Studies screening natural products identified tocotrienol (TCT) as a potent inducer of anagen stage and hair follicle neogenesis. TCT is a natural form of vitamin E primarily found in seeds and oil. Eight week old C57BL/6 mice (with all hairs on the back in telogen phase) were subjected to hair depilation followed by topical TCT-rich Tocovid Suprabvio 5mg/cm2 application on skin thrice per week. After week 4, two 6mm stented excisional wounds were made on the dorsal skin. H&E staining from the dorsal skin collected at time of wounding revealed significant increase in number of hair follicles in the TCT treated group (p<0.01, n=4) compared to a placebo (PBO) group. TCT upregulated β-catenin expression (p<0.05, n=4). Mice were sacrificed at day 10 and wound tissue was harvested. Adult skin is known to heal with scar. Neogenesis of hair follicles in the repairing skin is widely recognized as one marker of regenerative healing. TCT treatment caused hair follicle neogenesis in the repaired skin (p<0.01, n=4). Furthermore, TCT upregulated K15, a marker of follicular bulge stem cells (p<0.05, n=4). TCT enhanced wound angiogenesis as marked by higher CD31 (p<0.05, n=4) and increased perfusion detected by laser speckle imaging. Finally, TCT upregulated the following regenerative wound healing markers: collagen III, matrix metalloproteinase 2, hyaluronic acid synthase and chondroitin sulfate. In summary, we present first evidence demonstrating that topical application of tocotrienol-rich natural vitamin E potently induces anagen hair follicle, wound bed hair follicle neogenesis and cutaneous wound healing displaying markers of regenerative phenotype.
TARGETED REINNERVATION FOR THE AMPUTEE. A MULTI-COHORT ANALYSIS

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Targeted Reinnervation (TR) is a surgical technique in which the native motor nerve of the target muscle is divided, and the amputated nerve is then transferred to a near by motor nerve. Previous studies demonstrate the effectiveness of TR in relieving chronic neuroma pain after amputation. This study seeks to determine the success of TR at the time of amputation in preventing symptomatic neuroma and phantom limb pain, as well as analyze the success of TR in specifically treating symptomatic neuroma and phantom limb pain. We have thus far performed TR on 13 patients. Eleven of these patients underwent TR at the time of amputation. Amputation was necessitated for the following reasons: oncologic disease, skeletal trauma, and chronic osteomyelitis. Two patients underwent TR secondary to painful neuroma following previous amputation. Each subject will be observed on an outpatient basis for one year. Follow up data of at least three months post TR is available for 10 patients. All subjects have denied neuroma pain post amputation. All subjects whom underwent TR at time of amputation initially reported phantom limb pain at one month, but all reported improvement in this pain at or before six months. The two patients whom underwent TR for treatment of symptomatic neuroma report significant improvement in symptoms by two months. TR can treat and prevent painful neuroma and phantom limb pain symptoms that often affect amputees. Furthermore, this series analyzes TR in the oncologic patient, a study cohort where TR has had minimal use. This innovative research will expand the general understanding of TR and provide a baseline for future studies regarding the specific use of TR for preventing neuroma and phantom limb pain.
RESULTS FROM A RANDOMIZED CONTROL TRIAL USING TOTAL OFF-LOADING FOOT BRACE FOR DIABETIC FOOT ULCERS

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Background: Off-loading is the mainstay of treatment for diabetic plantar ulcers. Standard of care includes total contact casts, but it is cumbersome to reapply between debridements.

Hypothesis: This study uses a novel brace (Toad Medical) designed to completely off-load the foot while being easily removable. This may accelerate healing of plantar ulcers.

Methods: Patients were recruited from two hospitals and randomized to either standard therapy or the off-loading brace. Peak plantar force measurements for the first few patients were measured using F-scan. Wound assessment was performed for 3 months, and foot ulcer measurements were analyzed from pictures using planimetry.

Results: A total of 17 diabetic patients were recruited and randomized to either standard therapy (n=7) or the brace (n=10). Due to a protocol deviation, two patients were excluded from data analysis. Reductions in peak plantar pressures at the ulcer sites ranged from 67.3 to 89.4% (P=0.09). While not statistically significant, faster average wound surface area healing in the brace group was seen in Week 2-5 of follow-up (brace vs. control: 36.0% vs. 6.8% healing) and in Week 6-9 (50.7% vs. 17.0%). Difference in the degree of healing between the two groups at Week 12-15 was minimal (brace vs. control: 71.7% vs. 80.3%).

Conclusion: The total off-loading foot brace technology allows minimal plantar pressure for diabetic foot ulcers, effectively allowing healing to occur. Further optimizations in the brace design may lead to better healing of plantar ulcers than seen in current therapy.
Role of Zeb-1 in the regulation of epidermal wound angiogenesis


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Background: Cutaneous wound healing is a cascade of interactive process involving various cellular cues leading to the re-establishment proper skin restoration post injury. A dynamic interaction of endothelial cells and extracellular matrix (ECM) in newly formed granulation tissue is required for setting up the wound angiogenesis. Wounds with increased levels of endothelial cell apoptosis or ECM degrading agents are hard to heal due to lack of proper angiogenesis. Recent reports suggest that zinc finger E-box binding homeobox 1 (ZEB-1) gene directly regulates endothelial cells apoptosis. Hence, we hypothesized that the lack of ZEB-1 expression may lead to improper cutaneous wound healing.

Methods: Two 6-mm diameter full thickness stent wounds were done on the dorsal skin of ZEB1-/- and wild type mice. Digital photography, advanced ultrasound based measurements along with laser speckle perfusion imaging was done for wound closure and perfusion analysis. Results: Quantitative RT-PCR showed reduced levels of Zeb-1 mRNA in ZEB-1-/- compared to wildtype controls. Ultrasound imaging using Vevo 2100 showed that Zeb-1-/- mice exhibited compromised wound closure. The wound perfusion levels were also diminished in Zeb-1-/- mice compared to wild types as indicated by Perimed Laser speckle perfusion imaging (n = 3, p-value = 0.003). Immunohistochemical analysis for blood vessel markers like CD31, alpha smooth muscle actin and collagen IV revealed that the wound angiogenesis was compromised in ZEB1-/- compared to controls. Conclusion: In this study, we observed that reduced levels of ZEB-1 may result in poor angiogenesis and thereby compromise cutaneous wound healing.

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Nanochannel-Based Electrotansfection Assisted in-vivo Reprogramming of skin fibroblasts to hypermetabolic brown-like or beige adipocytes


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Background: Our program on non-viral direct in-vivo reprogramming of cells led to the development of devices that are capable of delivering uniquely designed cargo with the aim to reprogram cells in contact to other cells of interest. In this study our goal was to address the overabundance of white adipose tissue (AT) as commonly seen in the rapidly escalating problems of obesity. Recent studies indicate the existence of beige adipocytes in adult humans, an attractive therapeutic target for obesity and obesity-related diseases, including type 2 diabetes. Our strategy is to reprogram fibroblasts to hypermetabolic brown/beige adipocytes with the overall goal of minimizing white AT.

Methods: Cleanroom-based technologies were used to fabricate nano-channeled platforms for controlled tissue electro-transfection. Briefly, arrayed nanochannel clusters were drilled through silicon using a combination of projection/contact lithography and deep reactive ion etching. Microscale reservoirs were defined on the back side of the platforms by photolithography, and were used to hold the cargo to be transfected into the tissue. By utilizing this device, we conducted the non-viral delivery of PRDM16 and C/EBP-β genes to mouse skin in-vivo by nano-electroporation in a targeted, controlled and safe manner, which is not attainable by any of the existing transfection technologies. During in-vivo reprogramming of dermal fibroblast to brown-like or beige adipocytes, co-stimulation with cold treatment was necessary.

Results: Delivery of PRDM16 and C/EBP-β gene on mouse skin followed by a post cold treatment notably increased the expression of UCP1 suggesting efficient reprogramming of skin fibroblasts to brown or beige adipocytes. Interestingly, gene expression analysis of signature markers of brown or beige adipocyte showed that skin fibroblast reprogrammed toward beige adipocyte lineage. Thermal imaging data indicated increased thermogenic activity of the reprogrammed beige adipocytes on mouse skin. Positron emission tomography result indicates increased glucose uptake by reprogrammed cells which further supports fibroblast to beige adipocyte conversion. Upon delivering of genes on abdominal skin with cold co-stimulation cause induction of beige adipocytes in underlying visceral white fat, consistent with the idea that our vectors controls the beiging process and suggest that could be used to reduce obesity.

Conclusions: This work presents first evidence on in-vivo reprogramming of fibroblast to brown-like or beige adipocytes as a strategy to minimize subcutaneous white AT and visceral fat. This approach has the potential to open novel avenues for the development of new therapeutics for metabolic diseases such as insulin resistance and type 2 diabetes.
Development of Animal Model and Hydrogel Delivery System to Treat Traumatic Optic Neuropathy.

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Traumatic optic neuropathy (TON) is either a temporary or permanent loss of function of the optic nerve as a result of head injury. Recently, we developed a physiologically relevant animal model for indirect TON. Rats were subjected to varying levels of unilateral torsion generated by a novel apparatus designed to induce torsional indirect TON. Functional visual testing, in the form of the flash visual evoked potential (fVEP), was performed, histological analysis was conducted, and threshold statistics were used to assess the data. The threshold values were 33.3 degrees of rotation at an angular velocity of 2190 degrees/s (p<0.05), 39.4 degrees at 1860 degrees/s (p<0.01), and 41.7 degrees at 2190 degrees/s (p<0.001) for irreversible TON induction. Histology further demonstrated that this approach reliably reproduced clinically relevant TON. We hypothesized that an injectable hydrogel cast with the ability to form and contract (with minimal compression) around the damaged optic nerve could locally deliver neuroprotective agents to the site of TON injury, preventing further axonal degeneration. In our current study, injectable hydrogel scaffolds composed of a protein and polysaccharide were prepared and evaluated for gel formation under physiological conditions and the ability to contract upon formation. Collagen gels were evaluated with the addition of several biocompatible polysaccharides including gellan, alginate, iota-carrageenan, kappa-carrageenan, xanthan gum, and pullulan. Impact of salt concentration was investigated for contraction and deswelling. Results indicate formulations prepared with a blend of collagen with alginate, iota-carrageenan, or gellan gum form hydrogels with the ability to contract. These hydrogel formulations have mechanical properties in the range optimal for nerve regeneration. Recently, various neurotrophic factors, methylene blue and erythropoietin, have been identified as possible neuroprotectives. These neuroprotective agents will be incorporated in the injectable scaffolds and evaluated using the animal model developed for TON. This work is sponsored by DOD Grant W81XWH-15-1-0074.
TARGETED REINNERVATION AFTER AMPUTATION DUE TO BURN INJURY

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About 25% of major limb amputees develop pain secondary to neuromas within their residual limb(s). Targeted Reinnervation (TR) is a technique to possibly prevent these symptoms. This study seeks to determine the success of TR in relieving neuroma pain, and represents one of the first reported TRs performed in a burn patient. The subject was a 48 year-old male who suffered significant burns resulting in RUE amputation. Neuroma excision and targeted reinnervation were performed in December 2015. To this date, the patient has completed his 1 month follow-up; this study will follow the subject for a total of 12 months. Prior to surgery, the subject reported radiating pain and a burning sensation of his RUE, with shooting pain to palpation of the residual limb. He also reported phantom limb pains. The patient had difficulty using the residual limb and avoided use of his prosthesis due to the severity of his neuroma pain. Less than 1 month post-TR, the patient reported that his painful neuroma symptoms had resolved. On physical examination, patient’s previously sharp pain to palpation is now a minimal tenderness around his incision sites. The results suggest that targeted reinnervation can provide symptomatic relief and improved functionality to burn patients who have undergone amputation. This is one of the first reported TRs performed in a burn patient, and suggests a potential means of therapy for other burn victims. Ongoing research seeks to determine if TR performed at time of amputation can reliably prevent the development of painful neuromas.
EFFECTS OF PERI-OPERATIVE ANALGESIA AND ANESTHESIA ON POST-OPERATIVE PAIN FOLLOWING AN EXCISIONAL WOUNDING PROCEDURE IN C57BL/6 MICE

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Experimental research procedures utilizing animal models have the potential to cause pain, and it is imperative this be alleviated when possible to ensure humane use of animals in research studies. Previously, analgesics were not used for wound healing studies due to their association with immunomodulation, potentially obscuring research studies at the molecular level. However, current animal research approaches require that analgesia be utilized unless proven unnecessary or detrimental to obtaining data. The goal of this project was to evaluate the effects of perioperative analgesia in the mouse used in an excisional dermal wound healing study. Four groups of 8-week-old C57BL/6 male mice underwent isoflurane anesthesia and received perioperative subcutaneous treatments (0.003mg buprenorphine, 0.125mg bupivacaine, 0.003mg buprenorphine + 0.125mg bupivacaine [B+B], or 0.15mls saline) prior to receiving two 6.0mm full-thickness excisional wounds. Behavioral assessments including nest building, exploratory activity, and hyper-analgesia were used to assess well-being at four, eight and twenty-four hours, followed by euthanasia and tissue collection for histopathological analysis. Nest complexity scoring (NCS) revealed a significant decrease in nesting behavior for all treatment groups. Saline-treated and bupivacaine groups had significantly higher NCS than buprenorphine and B+B treated mice. Exploratory behavior was assessed by open field testing; mice receiving buprenorphine and B+B had increased centerfield passes compared to other treatment groups. All analgesic-treated mice had significantly increased rearing behavior compared to saline-control mice. Hyper-analgesia developing in response to pain is assessed using von Frey hairs. There was no difference between treatment groups at any of the time points. Buprenorphine and buprenorphine/bupivacaine treated mice displayed more exploratory behavior compared to other groups, although both had impaired nesting behavior, potentially due to sedative effects of the drug. Bupivacaine-alone treatment did not alter behavior assessments compared to the saline-control. Postmortem analysis of effects of these compounds on the inflammatory response of wound healing is ongoing.
Myocardial infarction (MI) affects millions of people in the Western countries. Cardiac fibrosis naturally occurs after MI and progresses with time. The increase of cardiac fibrosis leads to gradual decrease of cardiac function. Myofibroblasts, differentiated from cardiac fibroblasts mainly through TGFβ signaling pathway, are responsible for cardiac fibrosis. Therefore, to inhibit cardiac fibrosis, it is essential to prevent TGFβ pathway-induced myofibroblast formation. However, ideal therapeutic approaches to achieve this goal remain to be established. We hypothesized that controlled release of anti-fibrotic agent basic fibroblast growth factor (bFGF) will efficiently inhibit myofibroblast formation. bFGF has been shown to prevent myofibroblast activation in various tissues. It counteracts the profibrotic activity of TGFβ. In this study, we tested whether controlled release of bFGF from a thermosensitive hydrogel can efficiently inhibit cardiac fibroblasts from differentiating into myofibroblasts. The hydrogel was based on N-isopropylacrylamide, acrylate-polylactide, and 2-hydroxyethyl methacrylate, and synthesized through free radical polymerization. The hydrogel had a sol-gel transition temperature ~26°C. The hydrogel solution (20%, w/v) can be readily injected through a 26 gauge needle at 4°C. The bFGF was able to continuously release from the hydrogel during a 28-day period. The released bFGF remained bioactive as it promoted rat cardiac fibroblasts (RCF) proliferation. The release kinetics was dependent on bFGF loading. A higher bFGF loading released greater amount of bFGF. The released bFGF remarkably attenuated myofibroblast differentiation. At the mRNA level, the group injected with hydrogel and bFGF had significant lower expression of myofibroblast markers αSMA and CTGF. At the protein level, the density of αSMA positive myofibroblasts was significantly decreased compared to the control group that injected with hydrogel only. The results demonstrated that this system has potential to control cardiac fibrosis after MI.
Glutamate Oxaloacetate Transaminase Enables Anaplerotic Refilling of TCA Cycle Intermediates In Stroke-Affected Brain

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Ischemic stroke results in excessive release of glutamate that contributes to neuronal cell death. We previously identified that supplemental oxygen (100\% O\textsubscript{2} inhaled) protects against stroke injury by inducing the expression of a glutamate metabolizing enzyme (Glutamate Oxaloacetate Transaminase, GOT) in stroke-affected neurons. This observation led to the new hypothesis tested here that otherwise neurotoxic glutamate can be productively metabolized by GOT during stroke to maintain cellular energetics in the absence of glucose. Under hypoglycemic and normoxic culture conditions, extracellular glutamate rescued cell viability and maintained ATP levels via GOT-mediated metabolism. Correction of hypoxia during ischemic stroke in vivo, increased penumbra volume as measured by perfusion-diffusion mismatch MRI and decreased glutamate levels in stroke-affected SI-cortex. Mass spectroscopy study of [U-\textsuperscript{13}C]propionate metabolism in stroke-affected tissue support GOT-dependent anaplerotic refilling of TCA cycle intermediates when stroke-induced hypoxia is corrected by supplemental oxygen or when GOT is over-expressed in brain. Furthermore, GOT over-expression in partially oxygenated ischemic stroke penumbra attenuated neurodegeneration, reduced lesion volume, and improved post-stroke sensorimotor function in the absence of supplemental oxygen. Taken together, these results support a new paradigm that during stroke GOT can enable the transformative switch of glutamate from potent inducer of cell death to survival factor.

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THE PLASMA miRNA PROFILE IN RESPONSE TO VASCULAR INJURY

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Background: miRNA are short non-coding nucleotides that post-transcriptionally regulate messenger RNA. Recently, they have been discovered to be stable and amplifiable components of circulating plasma that are implicated in inflammation and remodeling. Here, we seek to identify novel circulating miRNA targets that are differentially expressed in response to vascular injury.

Hypothesis: The circulating miRNA transcriptome changes in response to vascular injury.

Methods: Rats underwent sham left external carotid ligation, or left carotid balloon injury. Plasma samples were obtained at day 3 and day 14 after surgery. Carotid arteries were collected, sectioned and H&E stained to measure intimal hyperplasia. High resolution in vivo ultrasound was performed and analyzed to assess wall variation and intimal thickness in response to balloon angioplasty. Plasma miRNA was purified, then quantified via nCounter miRNA Nanostring microarray. Data was normalized to two spike-ins present in each sample using nSolver.

Results: Of the 449 genes on array, 35 were found expressed significantly over background in cell free plasma samples with no indication of cellular contamination by qPCR or cell housekeeping markers. At day 3 after intervention there is an initial significant change (p<0.05) of miRNA sequences after acute injury (miRNA 489 upregulated, miRNA 466c, 376a, 455 downregulated). However, by post procedure day 14, there is a shift in the miRNA profile with a trend towards overall downregulation (miRNA 489, 466c, 376a, 539, 3595, 3593-5p downregulated). This corresponds to a change in histology with an increase in intima that is also detectable by high-resolution, non-invasive in vivo ultrasonography.

Discussion and Conclusion: This work represents first efforts to query the circulating plasma miRNA transcriptome in response to balloon angioplasty. Identification of these targets may further elucidate miRNA regulation of vascular inflammation as well as serve as predictive biomarkers of re-stenosis following balloon injury.

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Biochanin A Protects Against Ischemic Stroke by Inducing Glutamate Oxaloacetate Transaminase

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Glutamate serves multi-faceted (patho)physiological functions in the central nervous system as the most abundant excitatory neurotransmitter and under pathological conditions as a potent neurotoxin. Glutamate oxaloacetate transaminase (GOT) has emerged as a new therapeutic target in protecting against ischemic stroke injury. GOT catalyzes the transfer of the amino group from glutamate to the 4-carbon TCA cycle intermediate oxaloacetate to generate aspartate and the 5-carbon TCA cycle intermediate alpha-ketoglutarate. In search of a small molecule inducer of GCT, we identified Biochanin A (BCA) as the most potent phytoestrogen isoflavone that increases GOT mRNA expression in neuronal cells. Here we hypothesize that BCA induces GOT expression via an estrogen related receptor alpha (ERRα) dependent mechanism to protect neuronal cells against glutamate-induced toxicity. To test this hypothesis, neuronal cells in culture were treated with BCA. BCA treated cells showed significant increase in ERRα mRNA at 6h and GOT mRNA and protein expression at 24h. Cells treated with BCA were significantly protected against glutamate toxicity. Notably, this protection was lost in BCA treated cells when GOT was knocked down. To verify these findings in vivo, C57Bl/6 mice were intraperitoneally injected with either vehicle control (75% DMSO in water) or BCA (5 or 10 mg/kg body weight) daily for 4 weeks. After supplementation, mice were subjected to ischemic stroke using the intraluminal thread method of middle cerebral artery occlusion (MCAO). BCA concentration was significantly increased in the brain of supplemented animals as measured by HPLC. Immunohistochemical analysis confirmed increased levels of cortical ERRα and GOT expression. BCA treatment significantly improved post-stroke sensorimotor function and attenuated stroke-induced lesion volume as measured by 9.4T MRI. In this work we have identified BCA as a natural small molecule inducer of GOT. BCA is a safe, naturally occurring phytoestrogen isoflavone that represents a therapeutic target that lends itself to be translated to clinical study.
SAFETY AND PLATELET FUNCTION PROFILE OF
NATURAL VITAMIN E TOCOTRIENOL IN STROKE SURVIVORS:
OUTCOMES OF THE NUTRITION CLINICAL TRIAL

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Stroke (cerebrovascular disease) is among the top 5 leading causes of death in the U.S. according to the Centers for Disease Control and Prevention. Current standards of care for the treatment and prevention of ischemic stroke are tissue plasminogen activator (tPA; thrombolytic) and antiplatelet drugs (blood thinning), however these therapies are not viable for 1/3 of stroke survivors. Vitamin E encompasses a family of 8 naturally occurring isoforms – tocopherols and tocotrienols. Compared to tocopherols that are abundant in the western diet, biological functions of tocotrienols remain under-studied. For the past 15 years our laboratory has reported on the unique protective properties of tocotrienol vitamin E against acute ischemic stroke. In the pre-clinical setting we have identified multimodal (neuro and vascular) mechanisms of tocotrienol protection against stroke that are not shared by tocopherols. The current work represents first efforts to test tocotrienol vitamin E in a stroke patient population. In double-blinded Phase 1 and Phase 2 clinical trials, 130 healthy subjects and 150 stroke survivors have been enrolled (ClinicalTrials.gov identifiers: NCT01578629, NCT01858311). Participants received daily oral supplementation of placebo (vehicle control), 400mg, or 800mg tocotrienol-rich vitamin E gel capsules (Tocovid Suprabio™). Subject compliance was measured using high-performance liquid chromatography (HPLC) to detect tocotrienol vitamin E levels in whole blood. Hematological outcomes were monitored with three separate platelet aggregometry platforms and 36 blood assessments including complete blood count and lipid profile. Outcomes from aggregometry testing suggest a synergistic interaction between tocotrienol vitamin E and Clopidogrel (Plavix®) in inhibiting platelet function. Furthermore, no adverse events have been attributed to tocotrienol-rich vitamin E supplementation. Taken together, early results support the safe tolerance of up to 800mg tocotrienol vitamin E in healthy subject and stroke patient populations.

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THE ROLE OF PIGMENT EPITHELIUM-DERIVED FACTOR AS A REGULATOR OF WOUND ANGIOGENESIS AND COLLAGEN CONTENT

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Background: The resolution phase of wound healing involves refinement of the capillary bed and remodeling of the extracellular matrix (ECM), two activities that dictate scar formation.

Hypothesis: Pigment epithelium derived factor (PEDF) is a critical wound resolution factor that modulates angiogenesis and collagen maturity.

Methods: Vascular content, collagen maturity, wound breaking strength and external wound closure in dermal excisional wounds was analyzed from PEDF⁺⁻ mice and wildtype littermate controls.

Results: As compared to wildtype, vascularity in PEDF⁺⁻ mice was significantly elevated. Collagen maturity and wound closure were significantly decreased in PEDF⁺⁻ mice.

Discussion: Results demonstrate that PEDF is critical in wound resolution; the absence of PEDF led to increased wound vascularity, decreased collagen maturity, and delayed wound closure.

Conclusion: These findings indicate that PEDF is an important wound resolution factor that regulates both wound angiogenesis and the final ECM composition.

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TARGETED REINNERVATION FOR THE ONCOLOGIC AMPUTEE

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About 25\% of major limb amputees will develop chronic localized symptomatic neuromas in the residual limb. Targeted Reinnervation (TR) is a surgical technique in which the native motor nerve of the target muscle is divided, and the amputated nerve is then transferred to a nearby motor nerve. This neurorrhaphy reestablishes various functions of the amputated nerve to reduce pain symptoms. Previous studies demonstrate the effectiveness of TR in relieving chronic neuroma pain after amputation. This study seeks to evaluate the use of TR performed at the time of amputation as a preventative measure for neuroma and phantom pain. Specifically, this study focused on TR in three oncologic patients. One pediatric (9 yo) patient underwent an above-knee amputation, one adult patient underwent a below-knee amputation, the other an arm amputation. All underwent TR at the time of amputation. Each subject will be followed for one year to evaluate symptoms of neuroma or phantom limb pain, patient satisfaction and functionality after TR. Adult subjects’ progress will be measured by the Patient Report Outcomes Measurement Information System questionnaire, which is not validated for subjects under 18 years old. All three patients denied neuroma pain post-amputation. All subjects initially reported phantom limb pain at their 2-week follow-up, but all three reported improvement in this pain at or before their 6-month follow-up visit, with one patient reporting improvement by 1 month. Primary targeted reinnervation can prevent painful neuroma symptoms that often affect amputees. Performing TR at the time of amputation prevents an additional operation and can accelerate recovery by reducing pain.
Human cerebrospinal fluid microRNA: temporal changes following subarachnoid hemorrhage.

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Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating form of hemorrhagic stroke with 30-day mortality between 33-45%. Delayed cerebral ischemia (DCI) is the chief cause of morbidity and mortality in patients who survive the initial aSAH. DCI accounts for almost 50% of deaths in patients surviving to treatment of the ruptured aneurysm. The mechanisms for brain injury after aSAH and the brain’s response to this injury are not fully understood in humans. MicroRNAs (miR’s) are 22 to 25-nucleotide single-stranded RNA molecules that inhibit the expression of specific messenger RNA targets. In this work, miR profiling of human cerebrospinal fluid (CSF) from eight patients after aSAH was performed daily for ten days with the goal of identifying changes in miR abundance. Using the nanoString nCounter Expression Assay, we identified two specific clusters of miR that were differentially regulated over time. Quantitative rtPCR was performed on select miR’s from each cluster. The first cluster contained miR’s known to be present in blood and decreased in abundance over time. miR’s in this group include miR-92a and let-7b. The second cluster contained several poorly characterized miR’s that increased in abundance over time. miR’s in this group included miR-491. This second cluster of miR’s may be released into the CSF by the brain itself as a result of the initial SAH. Temporal changes in the abundance of specific miR’s in human CSF after aSAH may provide novel insight into the role of miR’s in brain injury and the brain’s response.
Regulation of Cell Membrane Resealing with Tripartite Motif E3 Ubiquitin Ligase Family Proteins

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Human cells and tissues must respond to frequent mechanical stress and the resulting injury. Plasma membrane repair is one of the fundamental processes of normal cellular physiology and is necessary to maintain cell integrity and cellular homeostasis. Previous experimental results showed that mitsugumin 53 (MG53), a novel muscle-enriched protein from the tripartite motif family (TRIM72), is an essential component of the cell membrane repair machinery that protects multiple cell types, including muscle and non-muscle cells. The TRIM protein family, which consists of more than 100 members, is a diverse and poorly studied gene group of E3 ubiquitin ligase proteins that regulate many biological processes, including differentiation, apoptosis, proliferation and various signaling pathways. Our recent results indicate that a subset of TRIMs, including TRIM72/MG53, can regulate membrane repair through modulation of the phosphoinositide 3-kinase (PI3K) signaling pathway. We have also established that TRIM27 (or Ret finger protein), is a novel binding partner for TRIM72/MG53 that forms heterodimers in vitro and, that higher molecular weight oligomers can be a part of TRIM protein function. While MG53 is expressed only in the heart and skeletal muscle at high levels, it is clear that TRIM27 is much more abundant in many other cell types. Our recent findings have resolved that TRIM27 may act as a negative regulator of membrane repair by antagonizing TRIM72/MG53-mediated activation of the PI3K signaling cascade. Resolving the detailed mechanism of TRIM proteins function and determining their role in regulating PI3K signaling pathway during cell damage will be further defined.

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IMMUNE RESPONSE TO SUBCUTANEOUS NANO-PLGA INJECTION IN MICE

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Poly (lactic-co-glycolic acid) (PLGA) is a copolymer that has been used extensively as a material in medical devices, surgical sutures and grafts. Additionally, it is currently used in the form of synthesized micro and nanoparticles for drug delivery systems. Due to its biodegradability and compatibility in various physiological conditions, as well as its ability to serve as potential drug delivery targets to specific tissues and organs, interest in PLGA particles, particularly of the Nano subset, has grown. Not much is understood about the immune response elicited from these particles or the difference to different subsets of particles, particularly in skin. Here, we compare BALB/c mice injected subcutaneously with equivalent doses of Nano PLGA (200-300 nm), Nano SPION-coated PLGA (200-300 nm) or micro (5-20μm) particles. Compared to the Nano SPION or micro particles, Nano PLGA particles evoked significantly reduced infiltration of immune cells into the dermis including neutrophils, macrophages, and T lymphocytes, and exhibited lower mRNA expression of inflammatory cytokines. Use of LI-COR imaging technology to visualize indocyanine-labelled particles confirmed confinement of particles to area of interest, indicating that the reduced inflammation caused by the Nano PLGA particles was not due to more rapid degradation. These studies reveal the low inflammatory potential of Nano PLGA particles, and suggest that this formulation could be a valuable biomedical drug delivery vehicle in skin and in skin wound healing.
DIFFERENTIAL EXPRESSION OF SIALIC ACID RELATED GENES IN SKIN AND ORAL MUCOSAL WOUND HEALING


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Wounds of the oral cavity are known to heal more rapidly and with less scar formation than skin. The current study examined differences in the expression pattern of glycosylation related genes, including sialyltransferases/ sialidases, in skin and tongue wounds. Sialyltransferases/ sialidases catalyze the transfer and hydrolysis of sialic acids, monosaccharides with important roles in protein stabilization, cell site recognition, and ganglioside activity. Gene expression was examined in 1mm excisional skin and tongue wounds in Balb/c mice via microarray. Of the 23 sialic acid glycosylation related genes examined, 17 in the tongue and 16 in the skin were significantly modulated over the course of wound healing. ST3Gal5 and ST8Sia1 were significantly modulated only in skin wounds and ST3Gal2, ST3Gal4, and ST8Sia5 were significantly modulated only in tongue wounds. 64% of the significant sialic acid related genes demonstrated a large difference in the patterns of expression between skin and tongue wound healing including ST3Gal1, ST8Sia2, ST8Sia4, ST3Gal6, ST8Sia6, ST6Gal1, ST6GalNac5, and Neu2. In addition, 29% of the significant sialic acid related genes displayed peak expression levels earlier in the skin than tongue. Additionally, relative changes in the skin were approximately 2 times that of the tongue for ST8Sia4, ST3Gal6, ST6GalNac5, and Neu1 at similar time-points. Following histologic wound closure, >50% of the sialyltransferases remained highly upregulated in the tongue or downregulated in the skin by ≥ 25% of the respective baseline including ST3Gal3, ST3Gal5, ST8Sia1, ST8Sia6, ST6Gal1, ST6GalNac5, and ST6GalNac6. The inability of skin sialyltransferases to be restored to baseline levels soon after histologic closure may suggest reduced sialylation in skin as compared to the tongue. Confirmation of the presence of hypo-sialylation and PCR validation are required to confirm these findings. Overall, skin and tongue wounds demonstrate differences in the pattern, time, and degree of sialyltransferase/ sialidase expression during wound healing.
A WIRELESS ELECTROCEUTICAL WOUND DRESSING DISRUPTS MIXED SPECIES BACTERIAL BIOFILM IN A PORCINE PRE-CLINICAL MODEL

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Keywords: Wound Infection, Dressing, Biofilm

Background: Recently our laboratory reported the first pre-clinical model for chronic wound mixed species biofilm infection. Biofilm infection limits wound healing by compromising the barrier function of the repaired skin. While many attempts have been made to develop biofilm disrupting drugs, current outcomes are not satisfactory. Microbes rapidly acquire drug resistance. Methods: To address this limitation, we developed a wireless electroceutical dressing (WED) and tested its anti-biofilm properties in a long-term (56d) pre-clinical model for chronic wound mixed species biofilm infection. WED consists of a matrix of silver-zinc coupled biocompatible microcells, which in the presence of conductive wound exudate gets activated to generate electric field (0.3- 0.9V). Domestic Yorkshire pigs (N=15) were subjected to full-thickness burn (2“x2”). A clinically relevant mixed-species (Acinetobacter baumannii 19606 and Pseudomonas aeruginosa PAO1) infection was established. On the day of infection, wounds were either treated with placebo dressing or WED twice a week for up to 56 days. Results: SEM (Scanning Electron Microscopy) demonstrated that compared to placebo, WED disrupted biofilm aggregates attached to the wound surface. WED significantly decreased (p<0.05, n=5) PAO1 burden on d35 post-infection. Closure of placebo treated biofilm infected wounds featured disrupted barrier function as indicated by high transepidermal water loss (TEWL). WED corrected (p<0.05, n=7) leakiness of such repaired skin. Next, we tested the efficacy of WED in eradicating already established biofilm infections in porcine burn wounds, resembling the clinically presented chronic wounds with biofilm infections. Wounds were infected for 7d to establish multispecies biofilm. Treatment of biofilm infected wounds with WED showed improved healing outcomes. WED significantly (p<0.05, n=5) improved wound re-epithelialization in biofilm-infected wounds. Conclusions: In summary, this work presents the first in vivo evidence demonstrating that WED is effective in preventing biofilm infection as well as in disrupting established biofilm from cutaneous wounds. (Supported by DoD W81XWH-11-2-0142, NIH NR013898 and unrestricted research gifts).
OUTCOMES OF MULTI-ARTICULATING PROSTHETIC HANDS COMPARED TO HAND TRANSPLANTS

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A correlational study was designed to analyze functional between individuals with hand transplants and individuals wearing advanced prostheses. It was hypothesized that the different interventions may each have unique benefits that should be documented and shared with potential candidates. Participants included 15 amputees fit with multi-articulating prostheses and 5 individuals with hand transplants evaluated with 4 outcome measures. Because of the differences in subject group size and overall sample size, the data was calculated by Cohen’s $d$, to represent group differences. Subjects who utilize electric multi-articulating hands and digits perceived themselves as “less disabled” when compared to hand transplant subjects. ($d=−.96$). This was calculated by the Disabilities of Arm, Shoulder and Hand (DASH) questionnaire. Subjects who utilize electric multi-articulating hands and digits scored slightly higher ($d=−.12$) in their overall “index of function” recorded by the Southampton Hand Assessment Procedure (SHAP). Bilateral hand transplant subjects scored slightly better in manual dexterity, based upon their Box and Blocks Test score, and bilateral prosthetic users scored better in fine motor dexterity based upon the Nine Hole Peg scores. There were similar scores in function and “perception of disability” in the unilateral transradial prosthetic users when compared to unilateral hand transplant subjects at the same level. Early results reveal that the outcomes of hand transplants demonstrate similar outcomes to prosthetic users in activities of daily living, fine motor and manual dexterity.
DETERMINISTIC GENE DELIVERY DRIVES EFFICIENT DIRECT LINEAGE
CONVERSIONS IN VITRO

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Background: Direct lineage conversions can be achieved by overexpressing specific combinations of transcription factors. This requires not only the knowledge of which factors can stably induce a desired cell type, but also the ability to introduce exogenous genes/proteins into cells in a controlled fashion. Current approaches to direct nuclear reprogramming face a number of translational hurdles, including heavy reliance on viral transfection, and a highly stochastic nature, which typically leads to inefficient and potentially unsafe reprogramming outcomes. We overcame these barriers by developing a nanotechnology-based platform (i.e., 3D nanochannel electroporation or 3D NEP) for deterministic gene transduction, which enables precise gene delivery with single-cell resolution.

Methods: Cleanroom-based methods were used to adapt conventional cell culture systems (i.e., Transwell inserts) into easy-to-operate 3D NEP platforms. These devices were then tested within the context of well-established and newly-developed reprogramming models of induced neurons (iNs), in which overexpression of transcription factors Ascl1, Brn2, and Myt11 results in fibroblastic-to-neuronal conversions. Successful reprogramming was confirmed via expression of specific neuronal markers (e.g., Tuj1, MAP2) and electrophysiological activity.

Results and Discussion: 3D NEP-based gene delivery resulted in superior performance relative to existing non-viral methods. Reprogramming efficiencies were comparable to viral methodologies without the constraints of capsid size and with the ability to control plasmid dosage. Controlled dosage variations allowed us to modulate the phenotype of the reprogrammed cell population. Moreover, our approach allowed easy interrogation of the reprogramming process.

Conclusions: Our newly developed 3D NEP platform was used to transfect primary fibroblasts with different combinations of the \textit{ABM} reprogramming factors, which successfully induced faster and easier to tailor lineage conversions compared to conventional reprogramming/transfection approaches. Ongoing studies are focused on studying the regenerative potential (and underlying mechanisms) of these cells in a mouse stroke model.
Multidisciplinary Approach to Calcaneal Osteomyelitis: A Case Presentation

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Osteomyelitis secondary to diabetic foot ulceration is an unfortunate complication that may require an extended course of long term intravenous antibiotics, operative debridement, amputation, and commonly a combination of these. Calcaneal osteomyelitis comprises approximately 5-6% of osteomyelitis cases. This is a particularly difficult subset to manage given the structural importance of the calcaneus, relatively small amount of soft tissue coverage present, and poor areas of vascularity. When attempting limb salvage of the lower extremity with calcaneal osteomyelitis, there is often the need for more advanced limb salvage techniques required often involving a multidisciplinary approach. In the diabetic population at our wound center, many also have a concurrent PAD diagnosis. We are fortunate to have vascular surgery participating in our center which provides expedited evaluation and treatment of arterial disease. Plastic surgery also participates in our center with flaps, grafts, coverage, and reconstruction.

One such method for limb salvage in calcaneal osteomyelitis is a free vascularized fibular osseocutaneous flap to recreate some of the weight bearing structure of the calcaneus removed in surgery or lost to infection. This osseocutaneous flap has been described in the oral and maxillofacial surgery realm for mandibular reconstruction. It has more recently been described to assist with osseous stability in hindfoot reconstruction after calcaneal osteomyelitis. This type of reconstruction in combination with proper antibiotic therapy guided by operative cultures within the multidisciplinary setting is capable if giving the patient with calcaneal osteomyelitis the best chance possible of a braceable, stable foot.
A COMPUTER ASSISTED APPROACH FOR SURGICAL PLANNING AND 3D PRINTING MANDIBULAR IMPLANTS

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Background: Stateside, over 400,000 craniomaxillofacial bone restoration procedures are performed annually. Autologous bone grafting remains the standard despite high rates of discomfort and donor site morbidity. Hypothesis: Advanced computer-aided design (CAD) techniques and 3D-printing strategies can be used to improve clinical outcomes by facilitating surgical planning, custom implant design, and rapid fabrication. Methods: Custom algorithms were written in MATLAB to identify the defect space, and design a patient-specific implant and cutting guides. Implants and cutting guides were 3D-printed. Results: Patient data derived from CT scans was segmented and reconstructed into a solid model. Novel modeling strategies implemented in MATLAB were successfully used to identify the defect site, construct osteotomy planes, and segment the defect space. Subsequently, the patient’s anatomy was used to design a tailor-made implant to fill the defect space as well as matching guides to assist in the surgical procedure. The implant was 3D-printed out of the biocompatible, bioresorbable, and photocrosslinkable polymer, poly(propylene fumarate) (PPF). In vitro biocompatibility testing yielded positive results for proliferation and differentiation of hMSCs on the implants. A large animal study is ongoing. Discussion: It has been previously shown that computer-assisted reconstructive techniques are superior to manual methods. We expand upon previous work by integrating new advances in medical modeling, 3D printing, and resorbable biomaterials. We anticipate that our combined approach will reduce operating time and costs, while enabling better reconstructive and regenerative outcomes. Conclusion: In this work, an anatomy-preserving mandibular implant is designed directly from CT data, ensuring an accurate fit to promote osseous integration and outstanding aesthetic restoration. Sponsor: United States DoD: AFIRM II, Award No. W81XWH-14-2-0004.
NANOTECHNOLOGY-BASED LOAD-BEARING SCAFFOLDS FOR BONE DEFECTS

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Background: Bone substitute materials are required to repair critical-size osseous defects in a number of different scenarios including traumas, degenerative pathologies, and certain surgical procedures. Although autografts and allografts continue to be a gold standard treatment, widespread use is hampered by limitations such as tissue scarcity, donor site morbidity, the potential for immune rejection and pathogen transmission. A number of biomaterials, including ceramics, polymers, and composites, have been studied in the development of bone tissue engineering (BTE) scaffolds. However, these materials generally present low mechanical stability and irregular degradation rates. Therefore, the need for a suitable scaffolding material for load bearing BTE has yet to be met satisfactorily. Here we describe the development of strong, cement-based scaffolding systems with nanoscale surface and structural modifications for BTE applications.

Methods: Cement-based scaffolds with suitable characteristics for load-bearing BTE applications were manufactured using standard microfabrication techniques. The biocompatibility, bioactivity and strength of the cement was enhanced via nanoscale surface and/or structural modifications that entailed carbonation and/or pozzolanic reactions. Mechanical properties were characterized via compressive tests. Bioactivity was analyzed in a simulated body fluid solution. Scanning electron microscopy and energy dispersive spectroscopy were used to evaluate scaffold morphology and chemistry, respectively. The cytocompatibility was tested using human osteoblast-like cells.

Results and Discussion: All the samples evidenced bioactive behavior. The scaffolds presented average porosities between 70 and 80% with mean pore sizes ranging ~300 μm. The scaffolds presented compressive strengths and elastic modulus values comparable to trabecular bone (~4-2 and 443 MPa, respectively). Cell experiments confirmed a highly cytocompatible response to the nano-modified samples. The cement-based scaffolds supported human osteoblast-like cell adhesion, spreading, and propagation (t =1–28 days). Cell metabolism and alkaline phosphatase activity were found to be enhanced at longer culture intervals (t ≥ 14 days).

Conclusion: Different processing conditions were developed to obtain porous scaffolds from nano-modified cement with suitable morphological, mechanical, and biological properties for applications in load-bearing BTE. The scaffold morphology and mechanical stability were easily tailored by modifying different fabrication parameters. Cell culture experiments confirmed the compatibility of the scaffolds. This work illustrates the possibility of developing strong and biocompatible scaffolds for bone repair applications from easily processable and inexpensive cement materials.
NANOTECHNOLOGY-BASED APPROACHES FOR TRIGGERING AND CONTROLLING ADULT TISSUE REPROGRAMMING

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Background: In vivo cell reprogramming has the potential to enable the use of a patient’s own tissue as a bioreactor to generate specific cells for therapeutic use. Here we report on a powerful yet simple to implement non-viral nanotechnology-enabled approach to controllably transduce and reprogram/remodel naturally-exposed or surgically-accessible tissues.

Methods: Cleanroom- and non-cleanroom-based methods were used to fabricate nanochanneled devices to deliver reprogramming genes into exposed tissue surfaces via nanoelectroporation. Transfection experiments were conducted on adult mice. Fluorescently-labeled DNA and large plasmids encoding for specific reprogramming factors were used as model cargo. Tissue transfection was characterized by fluorescence microscopy and qRT-PCR, and in situ cell reprogramming was evaluated via immunohistochemistry.

Results and Discussion: Labeled DNA and reprogramming plasmids were effectively delivered into adult tissue in a rapid (<1 second) and minimally-invasive manner. High plasmid bioactivity was seen a few hours post-delivery, as indicated by positive reporter gene expression and further confirmation by qRT-PCR. Long-term experiments indicated that depending on the specific plasmid combination, terminally differentiated tissue cells were successfully and controllably reprogrammed into different types of somatic cells (e.g., neurons, endothelial cells, beta cells) that thrived in the skin microenvironment for prolonged periods of time (>10 weeks).

Conclusions: We have demonstrated for the first time that adult and terminally differentiated cells could be successfully reprogrammed in situ into different cell types via nanotechnology-based non-viral approaches. Ongoing experiments focused on evaluating the implementation of such cells (or cellular byproducts) in restorative and regenerative medicine applications have shown promising results in terms of brain tissue repair and regeneration following injury, the revascularization of ischemic tissues and limbs, and the development of cell specific tissue engineering and composite tissue replacement strategies. This technological breakthrough has an exponential number of potential applications to both military and civilian medical research, translation, and future clinical care strategies.
ORGANOID-ON-A-CHIP

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Background: Guided assembly of microscale tissue subunits (i.e., organoids) has found applications in cell therapy, tissue engineering, developmental biology, and drug discovery. As organoid size and geometry are known to influence cellular responses, the ability to control cellular assembly in a high throughput manner could be advantageous for many biomedical applications. Here we present a novel micro/nano device for the assembly and maintenance/culture multicellular organoids. Our platform possesses several advantages over previously developed systems: a more in vivo-like topographical stimulation of cells; better nutrient/waste exchange; and easy integration into standard two-chamber cell culture well systems.

Methods: Our micro-nano device consists of a 3D multilayered structure, with a soft-lithographically fabricated array of through-thickness microwells structurally bonded to a sheet of electrospun micro-nano fibers. The microwells and fibers were manufactured from several polymers of biomedical interest. The ability of the device to allow for controlled cluster formation was tested using different cells of interest, such as pancreatic cells, human hepatocytes, embryonic stem cells, and cardiomyocytes. In addition, the ability of the device to support studies on semi-controlled heterotypic interactions was demonstrated by co-culturing hepatocytes and fibroblasts.

Results and Discussion: Arrays of microwells with different diameters were structurally interfaced with a porous sheet of micro/nano fibers upon which the cells of interest anchored and assembled into 3D organoids. The microwells effectively regulated the spatial distribution of the cells on the platform, as well as cluster size, shape and homogeneity. Organoid geometry was effectively modulated by the microwell shape and size. In comparison, organoid assembly on control surfaces (fibers without microwells or tissue culture plastic) resulted in irregularly shaped/sized cell aggregates. Our micro/nanodevice supported superior organoid functionality compared to control conditions, as evidence by increased expression levels of certain hormones and/or metabolic factors.

Conclusion: In summary, we have presented a method to produce more consistent and functional cell clusters using micro/nanodevices that can be fabricated from a number of polymers of interest. The potential application of these devices in the field of guided assembly of microscale tissue subunits has been demonstrated using a host of relevant cell types. Our platform could potentially be used in high throughput screening of organoids (e.g., for drug discovery and studies in cellular and developmental biology), or incorporated into numerous cell-based therapy studies and applications.
MODULATING MECHANO-TRANSDUCTION AND MIDDLE EAR INFLAMMATION USING MiR-146a

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Background: Otitis Media (OM) is characterized by a severe inflammation of the middle ear. OM is one of the most common infections in children in the US, with an estimated treatment cost of 4 billion dollars per year. The Eustachian Tube (ET) connects the Middle Ear (ME) to the nasopharynx and opening of the ET allows for the ME pressure and gas composition to equilibrate with the atmosphere. However, during OM dysfunction of the ET leads to sub-ambient pressures and hypoxic conditions in the ME. Although these conditions may exacerbate ET dysfunction, the effect of ME pressure and gas composition on inflammatory signaling and mucin secretion is not well understood. The present study explores how sub-ambient pressures and changes in oxygen tension influence the secretion of key pro-inflammatory cytokines and mucin in primary human ME epithelial cells, and the potential application of miR-146a as a potential regulator of inflammation in the ME during OM.

Methods: Human ME epithelial cells (HMEECs) were cultured on transwell inserts at an air-liquid interface to form a polarized epithelium. Cells were subsequently exposed to different permutations of negative pressure (-25 cmH2O), hypoxia (6% O2), and/or hyperoxia (95%O2). Control conditions were defined as atmospheric pressure and normoxic conditions (21% O2). Secretion of key pro-inflammatory cytokines IL-6 and IL-8, and anti-inflammatory cytokine IL-10 was characterized via ELISA. In parallel experiments, the effect of miR-146a on this type of inflammation was assessed via transfection using a Pre-miR miRNA precursor.

Results and Discussion: HMEECs are highly sensitive to oxygen tension and negative pressure. Negative pressures induced the maximum cytokine secretion in comparison with other treatment groups, with a 15 and 10.3 fold increase in IL-6 and IL-8 secretion, respectively. Similarly, hyperoxic conditions induced a 6 and 3.3 fold increase in IL-6 and IL-8 secretion, respectively. Interestingly, although sub-ambient pressure and hyperoxia were strongly pro-inflammatory, hypoxic conditions did have a significant effect on pro-inflammatory cytokine secretion. However, this condition induced significant secretion of the anti-inflammatory cytokine IL-10, which may highlight the injurious effect of oxygen tension in the ME. Overexpression of miR-146a significantly reduced secretion of pro-inflammatory cytokines, with a decrease of 73.7 and 76% for IL6 secretion, and 16.7 and 44.4% for IL8 secretion; during hypoxia and negative pressure, respectively.

Conclusion: Negative pressure dominates the inflammatory response to mechanical injury in HMEECs. Hypoxic conditions induced increased anti-inflammatory cytokine (IL-10) secretion, which may alter secretion of pro-inflammatory cytokines. Regulation of mechanically induced and oxygen tension induced inflammation may lead to the development of new/complementary therapies for Otitis Media - e.g. with the potential delivery of mechanosensitive miRNAs, such as miR-146a, to reduce local inflammation.

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ROLE OF MYELOID CELL-DERIVED PGDF IN TISSUE-ENGINEERED VASCULAR GRAFT NEOTISSUE FORMATION

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Widespread clinical application of the tissue-engineered vascular graft (TEVG) is limited by the development of stenosis. Myeloid lineage inflammatory cells mediate neotissue formation, but the exact mechanisms of this process are not completely elucidated. Platelet-derived growth factor (PDGF) is a potent regulator of angiogenesis, inflammation, and SMC migration and proliferation. In this study, we examined the role of myeloid cell-derived PDGF in the development of TEVG neotissue in a mouse model. Myeloid cell-specific PDGF knockout mice (PDGF KO) were generated by adoptive transfer of disaggregated hematopoietic fetal livers from otherwise nonviable PDGF-/- pups to gamma irradiated C57BL/6 (WT) mice. 19G TEVG scaffolds were implanted as IVC interposition grafts in either PDGF KO or WT mice. After two weeks, grafts from PDGF KO mice had significantly more remaining scaffold polymer and less intimal neotissue development. Evaluation of MAC3⁺ macrophages, the major cell type involved in graft degradation, demonstrated decreased overall number and increased apoptosis in PDGF KO grafts. Analysis of smooth muscle cells, the primary component of the neovessel media, revealed greater proliferation in WT grafts, while collagen content was significantly lower in PDGF KO grafts. In conclusion, myeloid cell-derived PDGF contributes to the inflammation-mediated process of vascular neotissue formation in both an autocrine and paracrine fashion by regulating macrophage apoptosis, smooth muscle cell proliferation, and ECM deposition. We submit that this protein may be a useful target in combination with other signaling events to regulate graft remodeling during the time course of scaffold degradation and neovessel formation.
Laser Capture Molecular Core at the Ohio State University

Emergent technologies in wound research

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Spatially-resolved cell-specific molecular analysis of tissue affected by focal injury is now possible because of current laser capture microdissection (LCM) technology. A variant of LCM is laser microdissection and pressure catapulting (LMPC). In LMPC, the biological material is placed directly on top of a thermoplastic polyethylene naphthalate (PEN) membrane. The membrane acts as a scaffold to allow for catapulting relatively large amounts of intact material. A focused laser beam cuts out an area of the membrane and corresponding biological material. Next, the beam is defocused and the energy used to catapult the membrane and material from the slide. A motorized robotic stage moves the sample through the laser beam path to allow the user to control the size and shape of the area to be cut. The catapulted sample is generally captured in an aqueous media (e.g. RNA or protein stabilizing solution). Under direct microscopic visualization, LMPC permits rapid procurement of histologically defined tissue samples from spatially-resolved (e.g. wound core vs leading edge) regions of the tissue. Previously, our laboratory has developed novel methods that enable the molecular study of micro vessels captured from skin vs chronic wound tissue (Roy et al., PNAS, 2007; 104(36):14472-7). We also developed methodology to perform proteomics study of hyperproliferative epithelium derived from human chronic wounds (Shapiro et al., J Proteomics. 2012 Dec 21;77:433-40). Recently, we performed LCM capture of bacterial biofilm from infected porcine burn wounds (Roy et al., J Pathol. 2014 Aug;233(4):331-43). We evaluated expression patterns of genes known to be induced under biofilm growth conditions. These included rpoS, which is implicated in the morphology and antibiotic resistance of biofilms, and rhlR/aprA previously linked to quorum sensing and biofilms. The expression of rhlR, rpoS and arpR were significantly upregulated in biofilm bacteria laser captured from burn wounds compared to those of planktonic bacteria obtained from wound scrubbed fluid. These developments and the potential of LCM/LMPC technology to illuminate new aspects of wound biology from a novel vantage point will be presented.

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Zinc Binding to MG53 Facilitates Repair of Injury to Cell Membrane

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**ABSTRACT**

Zinc (Zn) is an essential trace element that participates in a wide range of biological functions, including wound healing. While Zn deficiency has been linked to compromised wound healing and tissue repair in human diseases, the molecular mechanisms underlying Zn-mediated tissue repair remain unknown. Our previous studies established that MG53, a tripartite motif (TRIM) family protein, is an essential component of the cell membrane repair machinery. Domain homology analysis reveals that MG53 contains two Zn-binding motifs. Here we show that Zn-binding to MG53 is indispensable to the assembly of the cell membrane repair machinery. Live cell imaging illustrates that Zn entry from extracellular space is essential for translocation of MG53-containing vesicles to the acute membrane injury sites for formation of a repair patch. The effect of Zn on membrane repair is abolished in the \textit{mg53}\textsuperscript{-/-} muscle fibers, suggesting that MG53 functions as a potential target for Zn during membrane repair. Mutagenesis studies suggest that both RING and B-box motifs of MG53 constitute Zn-binding domains that contribute to MG53-mediated membrane repair. Overall, this study establishes a base for Zn interaction with MG53 in protection against injury to the cell membrane.
PROMOTING LIMB HEALTH THROUGH MANAGEMENT OF SOCKET INTERFACE MOVEMENT

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Movement between the prosthetic socket and residual limb can place excessive forces on the soft tissue of the limb that lead to injury. We hypothesize that elevated vacuum suspension, a method of keeping the prosthesis on the residual limb, can be used to quantify and regulate movement between the limb and prosthesis. Two studies were performed. First, a bench-test was executed to correlate vacuum pressure changes to displacement of the socket and investigate differences in the correlation as a function of fit of the socket. Second, clinical data was collected as a series of case studies to determine the translation of the bench test results to the clinic. The results of both studies found that the amount of movement between the socket and residual limb decreased as vacuum pressure setting was increased. The magnitude of the movement differences was impacted by the fit of the socket, suggesting that elevated vacuum suspension can not only be used to measure and control movement between the limb and socket, which will reduce forces that can damage the soft tissues, but also to quantify socket fit, a process which currently lacks objective outcomes. Future work is needed to understand and optimize how socket movement affects residual limb health.
TRACING MYELOID LINEAGE OF MYELOID CELLS IN VIVO USING A DOUBLE FLUORESCENT CRE REPORTER MICE

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Immune cells of myeloid lineage are known to play important roles in tissue repair and regeneration. The Cre/loxP recombination technology has been used for conditional mutagenesis in mice. The technology can be applied to determine the lineage of a specific cell type, like myeloid cells involved in wound healing pathways, which can dedifferentiate and differentiate in the healing of the wound tissue. For this, we have used ROSA mT/mG mice which have a double-fluorescent Cre reporter. These mice express membrane targeted red fluorescent protein Td Tomato (mT) prior to Cre-mediated excision and membrane targeted green fluorescent protein GFP (mG) after excision. Cross breeding these mice with tissue specific Cre recombinase will result in preferential expression of GFP in Cre expressing cells whereas the non cre expressing cells will express Td Tomato and thus fluoresce red. If cre expressing cells de-differentiate and change their fate to other cell types, they will still continue to express GFP thus enabling us to determine their parental lineage. Furthermore, these mice can be utilized to study cells of a specific lineage under fluorescent microscopy without the assistance of staining with antibody conjugated external fluorophore. We have developed Rosa mT/mG- LysM for myeloid lineage and Rosa mT/mG- K14 for keratinocyte lineage in our laboratory. For Rosa mT/mG- LysM, we have crossbred Rosa mT/mG (Gt(Rosa)26Soi/ACTB-tTomato, EGFP/lg) with LysMCre (Lyz2tm4serfho). Rosa mT/mG- K14, was developed by cross breeding Rosa mT/mG with K14 Cre (Tg(KRT14-cre)4lAmc). Verification of GFP expression in expected cells type was done by fluorescent microscopy. These mice serve as an excellent model for lineage tracing of the myeloid cells in wound healing studies.
GENERATION OF MYELOID-SPECIFIC KNOCKDOWN OF MICRORNA-21 AND MICRORNA-210 IN MICE USING CRE-RECOMBINASE TECHNOLOGY

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microRNAs (miRs) are small non-coding RNA that negatively regulate expression of genes; they are key to the normal homeostasis of cellular function. Besides negative regulation, miRs have been shown to be associated with a wide range of diseases including cancer, obesity, mental disorders, and heart and kidney disease. miR-210 is widely accepted as an important miR during response to hypoxia, whereas miR-21 is involved in inflammatory processes. miRs are also known to regulate gene expression in cells of myeloid lineage, thus affecting their development and function. Lysozyme M (LysM) is a gene which is expressed in the cells of myeloid lineage. Tagging the promoter for LysM to a Cre recombinase gene will make preferential expression of the Cre recombinase in cells of myeloid lineage, which includes monocytes, macrophages, and granulocytes. This principle can be exploited for myeloid-specific knockdown of a gene of interest using the Cre-LoxP recombination system. Using this technology, we have generated myeloid-specific knockdown of microRNAs (miR-21 and miR-210) in our laboratory. In order to study the roles of these miRs in macrophages, we generated myeloid-specific knockdown. For this, we crossbred LysMcre mice from Jackson laboratory (Lyzo2cre/+cre) with miR-210/miR-21 floxed mice developed in our laboratory. The presence of transgenes was verified using PCR-based genotyping approaches. Verification of knockdown of miRs in myeloid-specific cells was determined by quantitative real-time PCR using isolated macrophages.
ANALYSIS OF SINGLE VERSUS STACKED FRACTIONAL CO2 LASER PULSES IN A PORCINE SCAR MODEL

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The mechanism of the salutary effects of fractional CO₂ laser treatment is unknown. It has been hypothesized that depth of beam penetration is important to optimizing treatment outcomes. We assessed the effect of a single laser pulse versus 3 stacked pulses on healing time and resulting inflammation. Burn scars in Red Duroc pigs were treated with fractional CO₂ laser (5% fractional coverage, 70.0 mJ core setting, 396 J/cm² fluence) with a single laser pulse (n = 8) or with 3 stacked laser pulses (n = 8). Immediately prior to laser treatment and at 1 hour, 24 hours, 4 days, and 7 days after laser treatment, we measured transepidermal water loss (TEWL), erythema, histologic characteristics of laser injury, and inflammatory gene expression. Both single pulse and stacked pulse treatments increased scar erythema, which peaked at day 1 post-treatment and returned to baseline levels by day 7. TEWL, which reflects epidermal barrier function, increased 1 hour post-treatment and remained significantly above baseline until day 7 in both treatment groups. Histologic sections showed that treatment with 3 stacked laser pulses resulted in deeper and slightly wider MTZs compared with a single laser pulse. Expression of TGF-β1, TGF-β3 and IL-6 was elevated above baseline in both single and triple pulse groups at one hour post laser treatment, but returned to baseline 24 hours. Stacking of three laser pulses resulted in slightly deeper and wider ablated cavities without an increase in coagulated tissue, delay in the return of barrier function or extend the presence of inflammation or erythema compared to a single pulse treatment. Of particular interest, barrier function did not return to baseline until 7 days after treatment in either group, suggesting that stacked pulses may enhance the delivery of topical agents compared with a single pulse, without significantly slowing healing.
FEMALE RED DUROC PIG AS AN ANIMAL MODEL FOR HYPERTROPHIC SCARRING

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A challenge with scar research is the availability of an in vivo testing model as longitudinal studies in human patients cannot control for burn depth, size and location and rodent models do not naturally form excessive scars. More recently, female, red Duroc pigs (FRDPs) have been shown to form robust scars, which are similar in anatomy to human hypertrophic scars. Our goal was to develop new animal models for excessive scarring and compare them to the most commonly used model associated with deep dermatome excisional wounds. In the first model, standardized full-thickness thermal wounds were created on the dorsum of FRDPs. Wounding for the second model included the application of autologous split-thickness skin grafts following the excision of full-thickness burns. Wounds for the dermatome model were re-created using two different total dermatome settings (TDS): 0.060 in and ≥ 0.075 in. Results showed that burn wounds and autograft wounds healed at much slower rate in comparison to dermatome wounds of both settings. Significant scarring was observed in all wounds of the new models with greater than 25% scar contraction measured at the end of the study. Furthermore, scars resulting from thermal injury and autografts were significantly thicker than dermatome scars. Of note, scars from the autograft model were significantly raised and in some scars the boundary between the non-involved tissue and scar were sharp as seen in human burn patients. These new models provide greater similarities to human hypertrophic scars and can be used to study and improve current anti-scar therapies.
Novel Developments in Amphiphilic Polymers for Wound Care.

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Non-stinging adhesive liquid bandages have been developed to protect skin from moisture and bodily fluids, prevent medical adhesive-related trauma, and act as a barrier to exogenous bacteria and debris. Amphiphilic siloxysilane polymers were synthesized and characterized for properties relevant to use as spray-on bandages, including moisture vapor transmission rate, oxygen transmissibility, elastic modulus, and adherence to skin. Two polymers were evaluated in porcine burn and wound models. The amphiphilic polymers were dissolved in hexamethyldisiloxane, a non-stinging biocompatible solvent with low heat of vaporization. Upon application to the skin, the solvent rapidly evaporates within a minute, resulting in a clear conformal polymer film. The amphiphilic polymers formed water-insoluble films and demonstrated water-barrier properties both in vitro and in vivo. As a representative example, one of the polymers has a moisture vapor transmission rate of 448±129 g/m²/day, which is in the semi-occlusive range favorable for wound healing. The oxygen transmission rate was 2.3 mL/m²/min, which is four times higher than that of skin, making these films breathable. The small strain and large strain Young's moduli for the polymer film was 0.035±0.005 MPa and 0.024±0.004 MPa, respectively, which are lower than skin, imparting flexibility to the bandage. Testing indicates the polymer films remain adherent to skin for 2-4 days. These amphiphilic polymers formed water-insoluble, water-vapor and oxygen permeable, conformal protective films and demonstrated the ability to act as a bandage or coating on skin. This work was sponsored by NSF Grant IIP-1228399 to Rochal Industries.
A randomised controlled trial investigating the effectiveness of a specialised seating programme in reducing pressure ulcer incidence for clients in long term care

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Aim: While guidance is available on most aspects of pressure ulcer prevention and management, there has been little information on addressing these issues in seated patients. The issues most often addressed include the use of pressure-redistributing beds and mattresses, risk assessment, repositioning and management of established pressure ulcers. The role of specialised seating can often be overlooked. This research investigates the effectiveness of a specialised seating programme and its impact on pressure ulcer incidence in long term care.

Method: A mixed methods design was ethically approved and employed. Participants were recruited from three long term care settings before random allocation. The control group continued to use their existing seating while the intervention group was provided with seating tailored to their individual needs following a complex assessment. Participants were observed for pressure care, saturated oxygen levels, posture, function and comfort.

Results / Discussion: Seven of the intervention participants who had red skin areas at the beginning of the trial no longer presented with these at the end of the 12 week trial period. None of the intervention participants developed skin redness. One participant in the control group developed a pressure ulcer in their existing seating and those with redness noted at the beginning of the trial remained following the trial period.

Conclusion: This research demonstrates that prescribed seating can contribute to a reduction in pressure ulcer incidence for patients in long term care. It highlights that the needs of each patient are different, requiring individualised evaluation of seating needs before making recommendations for an appropriate seating system. This research has attempted to address an area which is often overlooked, providing evidence based daytime pressure management through therapeutic seating to compliment therapeutic surfaces at night.
The Clinical Benefits and Cost Effectiveness of Grafix – a Unique Skin Substitute with Antimicrobial Activity

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Diabetic foot ulcer (DFU) infection is a serious complication that increases the risk of hospitalization and amputation and is costly for the patient and the healthcare system: increasing from $5,391/patient to ~$17,000/patient when infection is present in DFUs. The safety and efficacy of a commercial human cryopreserved placental membrane (Grafix) for the treatment of chronic DFUs were demonstrated in a multicenter, randomized, controlled clinical trial. Compared to standard of care, Grafix closed more wounds, faster with fewer wound-related infections and hospitalizations. The average cost savings per healed patient was $20,622 compared to non-healed patients, which is partly attributable to fewer wound-related infections. One property of placental membranes in utero is to protect the fetus from infections. However, different preservation methods can negatively impact tissue functionality. Therefore, we investigated whether Grafix retains any inherent placental antimicrobial activity, which may have contributed to the reduced wound-related infections in the trial. The antimicrobial activity of Grafix in vitro against 6 pathogens associated with chronic wounds – Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter aerogenes – was assessed. Bacteria were incubated for 24 hr with a collagen dressing (control) or Grafix. The bacterial growth inhibition was assessed by counting colony-forming units and expressed in log reductions. We observed a significant log reduction in the growth of all 6 pathogens (1-7 logs). In summary, Grafix retains the ability to reduce bacterial growth indicating that antimicrobial activity is not compromised. This is likely beneficial for the treatment of chronic wounds.
TITLE: Blood Perfusion and Wound Healing Following Alveolar Bone Regeneration

AUTHORS: L. Alsum, B. Erdal, V. Yildiz, D. N. Tatakis, B. Leblebicioglu

ABSTRACT

Objectives: Despite the significant progress in regenerative procedures, the fate of buccal plate is not predictable, especially in maxillary anterior sextant. Recent studies report continuous remodeling and bone resorption even following immediate implant and/or graft placement. This study aims to determine the recovery time and the role of blood perfusion following socket preservation (SP) and guided bone regeneration (GBR) surgery.

Methods: Adult patients scheduled to receive bone regeneration surgery (simple extraction with socket preservation [SP] or guided bone regeneration with bone graft and membrane placement [GBR]) in maxillary non-molar single tooth sites were recruited. Laser Doppler Flowmetry (LDF) was used to determine tissue blood perfusion prior to, immediately after surgery, at 3, 6, 9 days, 1 and 4 months postoperatively. Results were reported as mean perfusion unit (PU)/120 seconds. In addition, wound closure was documented through clinical parameters and by hydrogen peroxide test. Wound healing and possible complications were recorded using specific clinical scales. Wound fluid samples were collected from wound edges using sterile paper strips. Also, ridge dimensions were documented using Cone Beam Computed Tomography (CBCT) obtained immediately after surgery and at 4 months.

Results: Ten patients (50.3 ± 18.7 yrs; 7 males; 5 treated with SP and 5 treated with GBR) completed 4 months follow-up (4.3±0.2 months). Healing was uneventful in all cases. Wound opening, which caused minor graft loss, was detected in 2 cases. Overall, the blood flow decreased immediately following surgery compared to baseline (-54 [-111 to +413]DPU) with no difference between SP and GBR groups (P>0.05). The recovery was complete for most cases by 1 month (+95 [-92 to +469]DPU). In SP, blood flow values were similar to baseline at 4 months follow up, while a hyperemic state continued in 4 GBR cases (80%) even at 4 months. Wound fluid volume increased 2 fold after surgery and returned to baseline values by 1 month. Mean ridge width loss detected at 4 months was 1.04±0.2 mm with negligible differences between SP and GBR groups.

Conclusions: Within the limits of this study, it appears that differences among SP and GBR in overlying flap postoperative blood perfusion are not associated with differences in ridge width changes.

(no table selected)

KEYWORDS: Wound healing, Regeneration, CBCT, outcome.
TITLE: Peri-implant Treatment Modalities and Outcomes

AUTHORS: T. Barriere, L. Aissum, V. Yildiz, D. N. Tatakis, B. Leblebicioglu

ABSTRACT

Objectives: Peri-implantitis is an infectious condition of the tissues around osseointegrated implants with loss of supporting bone and clinical signs of inflammation. The purpose of this study was to determine the prevalence of peri-implant tissue breakdown within a specific institutional patient population. In addition, treatment modalities applied to treat peri-implantitis and the short- and long-term outcomes of such treatments were investigated.

Methods: A retrospective study design was used. OSU College of Dentistry patients diagnosed with peri-implant disease following at least 6 months of loading and the treatment modalities applied to treat this disease were screened by using the college computer system patient records. The maintenance of implant supported dental restorations through these treatments was documented. Surgical treatment modalities and complications were determined by screening individual patient charts.

Results: 20 cases (65±2 yrs; 11 female) with documented peri-implantitis surgical treatment were identified from a total of 486 patients treated with screw-type dental implants (4%). All implants were restored as single crowns with most of them localized at posterior sextants (11 maxillary and 8 mandibular sites). Mean loading time prior to peri-implantitis treatment was 55±9 months (median: 49 [4-94] months). Extraction, due to implant loosening, was performed in one case (5% of peri-implantitis cases). Applied surgical treatment modalities included GBR (15 cases), osseous surgery (1 case) and flap only (4 cases). A common post-operative complication was wound opening and related graft exposure (12 cases). Following surgical treatment, no implants were explanted (postoperative observation period: 3-24 months).

Conclusions: Within the limits of this study, the prevalence of peri-implantitis in an institutional setting is low. Although various treatment options can be used to treat peri-implantitis and appear to stabilize tissue integrity, prospective studies are needed to determine short and long-term outcomes.

KEYWORDS: peri-implantitis, bone graft, Retrospective, outcome.
Delivery of miR-21 to Macrophages Using Lipid Nanoparticles to Promote Wound Healing

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Macrophages are important mediators of wound inflammation and healing. During the initial, inflammatory phase of wound healing, macrophages are of the pro-inflammatory M1 phenotype. As the healing process progresses, wound macrophages transition to the anti-inflammatory M2 phenotype, which aid in the resolution of inflammation and promotion of wound closure. MicroRNA-21 (miR-21) is known to promote the transition from M1 to M2 phenotype in wound macrophages. Delivery of miR-21 to wound macrophages could potentially aid in the healing of chronic diabetic wounds. Here, we used mannosylated cationic lipid nanoparticles (LNPs) to specifically deliver miR-21 to macrophages. Our results indicate that LNPs specifically delivered their payload to macrophages ex vivo. Quantitative PCR (qPCR) analysis of miR-21 expression in these cells further supports that miR-21 was delivered to macrophages. These data have important implications for the development of novel wound healing therapeutics.
Controlled Delivery of Platelet Proteins Accelerates Porcine Wound Healing

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Platelet-rich plasma (PRP) is a therapy used clinically to aid in wound healing due to the presence of therapeutic proteins such as VEGF and PDGF. However, the short half-life of these proteins requires multiple large doses, and the efficacy of this treatment is highly debated among clinicians. We have shown that protecting these growth factors and releasing them in a controlled manner using our heparin-based coacervate delivery system improves wound healing in a porcine excisional wound model. Platelet-derived proteins incorporated into the coacervate were protected and slowly released over 4 weeks in vitro. In an in vivo porcine model, PRP coacervate significantly accelerated the healing response over 10 days. Treatment with PRP coacervate resulted in a 35% increase in reepithelialization compared to control. Additionally, treatment with PRP coacervate doubled the rate of wound contraction compared to all other treatment groups, including that of naked PRP proteins. This accelerated wound closure reduces the risk of infection due to restoration of the skin’s barrier function. After 10 days most of the wounds still exhibited high vascularity as a result of widespread angiogenesis. The only exception was wounds treated with PRP coacervate, which exhibited a vascular density resembling uninjured tissue at the wound edge. This indicates an accelerated maturity of the vessels and the wound bed compared to other treatments. PRP is easy to obtain and use, which explains its wide usage clinically despite the ongoing debate over its efficacy. This study suggests that PRP is ineffective as a therapy without a controlled release mechanism. Here we show that the coacervate delivery system is a simple and effective therapeutic tool to improve the efficacy of platelet-derived proteins for wound healing.
Host cells infiltrate electrospun poly(glycerol sebacate) vascular grafts in a porcine vascular access model.

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Synthetic vascular grafts implanted as hemodialysis access sites display poor patency and require frequent reinterventions. As a potential alternative, we previously developed a solvent-cast, salt-leached poly(glycerol sebacate) (PGS) graft that rapidly remodeled into a living neovessel in rats. In this study, clinically sized composite grafts were constructed of electrospun PGS/PVA cores and a bonded electrospun PCL reinforcing wrap. Cores had a dry porosity of 72±1\%. PCL reinforcement improved the incremental circumferential modulus from 0.20±0.04 to 1.83±0.03 MPa. The suture retention load was increased from 45±7 to 280±40 gf. Composite grafts were implanted in four pigs as femoral-femoral or carotid-jugular arteriovenous shunts (L = 2 cm and ID = 6 mm for two pigs euthanized at 28 d, and L = 2.5 or 4.5 cm and ID = 5 mm for two pigs euthanized at 15 and 14 d). H&E staining indicated a cellular infiltration/reorganization and ECM deposition at 14 d, proceeding radially outwards from the lumen through approximately 2/3 of the graft wall. This degree of early infiltration is markedly greater than that seen with less porous electrospun PGS/PVA grafts recently implanted in mice. Possible endothelial, intimal and medial layers developed around the graft scaffold without causing stenosis. In addition, very preliminary data suggests superior closure of cannulation sites as compared to standard ePTFE grafts, which could have a significant positive impact on dialysis unit process of care/workflow and patient quality of life. Future work will evaluate the phenotypes of the remodeling cells, characterize the neotissue quality, and extend the implants to later timepoints.
Additive Manufacturing for Multimaterial and Multiscale Scaffolds in Tissue Engineering

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In this work, a prototype machine concept was developed for the manufacturing of multimaterial and multiscale scaffolds for tissue engineering. The prototype machine integrates two different manufacturing processes (fused deposition modeling and electrospinning) to create multilayered scaffolds, based on resorbable polymers such as polylactic acid (PLA) and polycaprolactone (PCL). It was found that process temperature, among other parameters, plays an important role in the viability of this kind of hybrid scaffolds. Results indicate the feasibility to manufacture multimaterial and multiscale scaffolds, combining materials of different melting points and physical properties. The hybrid scaffolds have a structure with FDM fibers (diameters between 200 and 300 μm), while layers of much finer fibers (1 to 5 μm in diameter) are electrospun in sequence. Some potential applications of this kind of hybrid scaffold include bone regeneration in periodontal disease and orofacial clefts. In these applications, the scaffold functionality includes a strong structure in order to fill the volume between bone tissue and soft tissue, as well as fine electrospun fibers that can locally deliver growth factors and antibiotic compounds.
Novel method to detect *Pseudomonas aeruginosa* variants on lawn biofilms generated in response to antibiotics

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*Pseudomonas aeruginosa* is an important cause of nosocomial infection, especially in patients with compromised host defense mechanisms. The formation of surface-associated communities called biofilms is one factor that enhances colonization and persistence in environments. Another factor is the ability of *P. aeruginosa* to diversify genetically, generating phenotypically distinct subpopulations. Variant populations could be generated to ensure bacterial survival against conditions such as antibiotics or host immune factors. Selective identification and characterization of these variant populations within biofilms is difficult, therefore we developed a new method. This method is beneficial in selectively determining antibiotic tolerant and resistant variant morphotypes of *P. aeruginosa* formed on lawn biofilms after treatment with antibiotics. Tobramycin antibiotic impregnated carrier materials were placed on Lysogeny Broth (LB) agar with pre-grown lawn biofilms of bioluminescent *P. aeruginosa* generated variant colonies after 4 days of incubation and showed bright luminescence as compared to the lawn biofilm. Antibiotic sensitivity, pigment production and morphological characteristics were studied. Zone of inhibition was monitored using an *in vivo* imaging system (IVS). Our results showed that the variants displayed differences in pigment production, morphology and levels of sensitivity towards tobramycin. The antibiotic stress response selection pressure could lead to the generation of variant populations within biofilms clinically. In conclusion, variant population is easily differentiated, quantified for specific antibiotics and biofilm growth selects for distinctive subpopulations important in infections.

**Keywords:** biofilms, *Pseudomonas*, antibiotic resistance, variants, bioluminescence
BACTERIOSTATIC PROPERTIES OF A MODIFIED CHITOSAN DRESSING

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Chitosan, the deacylated derivative of chitin (found in the shells of crustaceans) is traditionally and widely in use as a topical hemostat in surgical wounds, traumatic injury and to control minor bleeding following debridement of wounds. Antimicrobial properties have been attributed to chitosan but with no clear consensus on mechanism. A modified chitosan dressing (mCSD) with gelling technology is the first application of this advanced biological material for use in chronic wound care. Preliminary observations support that in patients, this dressing improves wound closure. However, the mechanism of action of mCSD in wound healing, particularly as it relates to infection remains unclear. Initial studies from our group characterized biofilm growth on the dressing. Scanning electron microscopy (SEM) based imaging of 48h static cultures of Pseudomonas aeruginosa (PA01), Staphylococcus aureus (USA300) and Acinetobacter baumannii (19606) showed that the chitosan fibers themselves serve as a suitable substrate for robust biofilm formation of all of these primary wound pathogens. This provided first cues suggesting that the chitosan fibers themselves may serve as a scaffold for adhesion and sequestration within the wound microenvironment. Interestingly, in growth curve studies with planktonic PA01, mCSD was found to attenuate the growth kinetics of this pathogen suggestive of a bacteriostatic effect on growth kinetics. Furthermore, following removal of mCSD from the culture, this effect was found to be sustained for atleast a 15h period compared to untreated cultures. Our observations demonstrating that a modified chitosan dressing by itself may have two non-mutually exclusive roles to play in wound infection: i. the adhesion of bacteria to the dressing may serve to sequester pathogens away from the wound surface and ii. bacteriostatic effect on planktonic bacteria may prevent establishment of infection in the wound. Studies are ongoing to address the effect of mCSD on other pathogenic bacteria.
A WIRELESS ELECTROCEUTICAL WOUND DRESSING DISRUPTS MIXED SPECIES BACTERIAL BIOFILM IN A PORCINE PRE-CLINICAL MODEL

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Keywords: Wound Infection, Dressing, Biofilm

Background: Recently our laboratory reported the first pre-clinical model for chronic wound mixed species biofilm infection. Biofilm infection limits wound healing by compromising the barrier function of the repaired skin. While many attempts have been made to develop biofilm disrupting drugs, current outcomes are not satisfactory. Microbes rapidly acquire drug resistance. Methods: To address this limitation, we developed a wireless electroceutical dressing (WED) and tested its anti-biofilm properties in a long-term (56d) pre-clinical model for chronic wound mixed species biofilm infection. WED consists of a matrix of silver-zinc coupled biocompatible microcells, which in the presence of conductive wound exudate gets activated to generate electric field (0.3-0.9V). Domestic Yorkshire pigs (N=15) were subjected to full-thickness burn (2"x2"). A clinically relevant mixed-species (Acinetobacter baumannii 19606 and Pseudomonas aeruginosa PAO1) infection was established. On the day of infection, wounds were either treated with placebo dressing or WED twice a week for up to 56 days. Results: SEM (Scanning Electron Microscopy) demonstrated that compared to placebo, WED disrupted biofilm aggregates attached to the wound surface. WED significantly decreased (p<0.05, n=5) PAO1 burden on d35 post-infection. Closure of placebo treated biofilm infected wounds featured disrupted barrier function as indicated by high transepidermal water loss (TEWL). WED corrected (p<0.05, n=7) leakiness of such repaired skin. Next, we tested the efficacy of WED in eradicating already established biofilm infections in porcine burn wounds, resembling the clinically presented chronic wounds with biofilm infections. Wounds were infected for 7d to establish multispecies biofilm. Treatment of biofilm infected wounds with WED showed improved healing outcomes. WED significantly (p<0.05, n=5) improved wound re-epithelialization in biofilm-infected wounds. Conclusions: In summary, this work presents the first in vivo evidence demonstrating that WED is effective in preventing biofilm infection as well as in disrupting established biofilm from cutaneous wounds. (Supported by DoD W81XWH-11-2-0142, NIH NR013898 and unrestricted research gifts).
MW CHTN MANAGES CLINICAL TRIAL AND INVESTIGATOR CONSENTED STUDY BIOSPECIMEN REQUESTS.

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MW Collaborative Human Tissue Network (CHTN/NIH) works with funded, IRB approved investigator subject consented studies to access their human subject’s biospecimens for study. Biospecimens from individually consented subjects are procured according to investigator protocols, stored under controlled conditions and delivered to the investigator for subsequent study. Investigator applications are reviewed and approved (CHTN PI). Protocol biosample requests are posted on a secured server for procurement by Pathology Tissue Procurement Service (TPS). Eighty-three MW CHTN Division vetted/approved investigators currently have 134 active and on hold funded, IRB approved/reviewed research protocols posted for biospecimen service. In 2015, frozen tissue was the most common preparation type requested and distributed at 52% (4,552) of samples with snap frozen tissue comprising 43% (3,784) and frozen tissue in OCT 9% (768). Paraffin-embedded tissue blocks were 26% and fresh tissue 23% of total sample requests. Fresh tissues were preserved in CHTN provided media for 12% (1,040) and in investigator custom media for 11% (955). Fresh samples were shipped overnight or delivered the same day; others remained in transient storage until investigator arranged shipment. A variety of disease tissue types were requested and served including 4,156 malignant tissues, 2,127 adjacent unaffected tissues, 430 benign tissues and 1,419 normal tissues. Fluids including blood, plasma and urine were requested as components of Clinical Trials and accounted separately for 590 samples. MW CHTN has ongoing agreements with academic investigators to manage individually consented biospecimen collections for funded, IRB approved studies. Samples of specified preservation types are collected prospectively from consented subjects, processed or stored frozen for a single low processing fee and held until investigators are ready to begin their studies. MW CHTN facilitates basic, translational and clinical trial studies by managing approved investigators with subject consented studies for custom biosample procurement, controlled storage and shipping. National Cancer Institute (NCI/NIH), SUM1CA183713-03.
CHRONIC VENOUS INSUFFICIENCY

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Background:
Valve incompetence and muscle pump insufficiency cause microcirculatory changes and localized tissue ischaemia. Damaged valves may cause high pressure in veins below it leading to varicosities. Varicose veins exhibit proliferation of the collagen matrix with disruption and distortion of the muscle fibers. In severe cases, the muscle layer is completely disrupted, leaving only elastic tissue and collagen in the vein wall. Venous stasis ulcers may form in the lower extremities as tissue damage occurs in response to a complex inflammatory reaction that may be related to venous hypertension. Mesenchymal stem cells when implanted in the area surrounding the vein, through the process of transdifferentiation, differentiate into vascular endothelial cells and smooth muscle cells. These cells thicken the layer of collagen and also improve the elastin in the walls of the vein. Mesenchymal stem cells also have the ability to migrate to the site of injury or inflammation and participate in regeneration of damaged tissues. These cells stimulate proliferation and differentiation of resident progenitor cell and promote recovery of injured cells through growth factor secretion and matrix remodeling. Additionally, extracorporeal shock wave therapy (ESWT) has been shown to be effective in stimulating growth factors, angiogenesis and accelerating the inflammatory and early proliferative stages of repair. Thus, a combination of cellular therapy, platelet concentrate and ESWT was hypothesized to be effective in management of patients with chronic venous insufficiency.

The case of a 41 year old female patient with long standing venous ulcer successfully managed with cellular therapy and ESWT has been described.

Keywords: Varicose veins, Mesenchymal stem cells, ESWT
DIABETIC ULCER

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Background:
Diabetic ulcers develop as a result of the progressive and cumulative effects of long standing diabetes. Foot ulcers in diabetic patients are frequently a cause for leg amputation. Tissues can become ischaemic because of macrovascular disease (atherosclerosis), notably in the calf with relative sparing of proximal vessels and those in the foot. Ischaemia also results from microvascular disease such as thickened basement membrane, capillary wall fragility, thrombosis, vasomotor neuropathy etc.

Wound healing is a complex process which involves three sequential, yet overlapping phases: inflammation, proliferation and remodeling. Diabetes impairs the functions of cells involved in inflammation and wound healing namely neutrophils, macrophages keratinocytes, fibroblasts etc. This results in impaired cell adherence, chemotaxis, phagocytosis, and cytokine production. Additionally there is diminished response of these cells to growth factors and there may be increased apoptosis.

Stem cells have been shown to mobilize and home to ischemic and wounded tissue. These cells secrete chemokines and growth factors that promote angiogenesis and ECM remodeling, creating a local environment conducive to wound healing. In addition, vacuum assisted closure dressing delivers negative pressure to the wound. This dressing enables wound healing by induction of an increased local wound perfusion, micro-deformations at the wound surface and the removal of exudate thereby stimulating granulation tissue formation.

Thus, a combination of autologous cellular therapy and V.A.C dressing was hypothesized to be beneficial in management of diabetic ulcer.

We present a case of long-standing non healing diabetic leg ulcer in a 77 year old female patient.

Keywords: Diabetes, Wound healing, Mesenchymal stem cells, Vacuum dressing
Lipid Nanoparticles Composed of Quaternary Amine–Tertiary Amine Cationic Lipid Combination (QTsome) for Therapeutic Delivery of AntimiR-21 for Lung Cancer

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Supporting Information

ABSTRACT: microRNA-21 (miR-21) is an oncomiR that is frequently upregulated in human cancers. AntimiR-21 (AM-21) is an oligonucleotide complementary to miR-21 that is designed to inhibit its gene silencing activities. To facilitate efficient delivery of AM-21, a novel lipid nanoparticle formulation called QTsome, based on a combination of quaternary amine and tertiary amine cationic lipids, with a distinctive pH-responsive profile, was developed. QTsome/AM-21 comprising DODMA/DOTAP/DOPC/CHO/ MPEG-DPPE and AM-21 oligonucleotide exhibited a mean particle diameter of below 150 nm, moderate zeta potential (41±3.2 mV), excellent colloidal stability, and high drug loading efficiency (above 80%). In vitro study showed QTsome/AM-21 induced upregulation of miR-21 targets, including PTEN and DDAH1, in A549 cells while increasing their sensitivity toward paclitaxel (PTX). Finally, tumor regression, prolonged survival, and miR-21 target upregulation were demonstrated in an A549 xenograft mouse model. These data suggest that QTsome/AM-21 warrants further evaluation as an anticancer agent.

KEYWORDS: nanoparticles, microRNA, cancer, drug delivery, RNA interference, noncoding RNA gene regulation

1. INTRODUCTION

Lung cancer accounts for over 160,000 deaths in the United States each year. Non-small-cell lung cancer (NSCLC) accounts for over 80% of lung cancers and primarily to small-cell lung cancer (SCLC) is less responsive to surgery, radiation therapy, and chemotherapy. The destructive nature of NSCLC and the lack of effective means of treatment outline the critical need for new modes of therapy.

microRNAs are noncoding RNAs that regulate gene expression via RNA interference. Progression of NSCLC has been linked to overexpression of microRNA-21 (miR-21). miR-21 is an oncomiR that operates at the epigenetic level, directly and indirectly impacting pathways associated with cellular apoptosis, DNA repair, proliferation, invasion, metastasis, and drug resistance. Inhibition of miR-21 should result in upregulation of tumor suppressor genes that are miR-21 targets. Indeed, antimiR-21 (AM-21) has been shown to upregulate miR-21 targets (i.e., ANKR46, DDAH1, PTEN, RECK, PDCD4, TIMP3) while decreasing relative cell migratory and invasion potential. Moreover, miR-21 in ovarian cancer has been linked to resistance to chemotherapeutic paclitaxel (PTX).

As potential therapeutic agents, antimiRs are sensitive to nucleases, are rapidly cleared from blood circulation, and cannot penetrate the cellular membrane due to their high molecular weight and charge density. Incorporation of oligonucleotides into lipid nanoparticles (LNPs) combined with chemical modification of their backbone is a commonly used strategy to address these issues. A common chemical modification scheme is to introduce 2′-O-methylation on the ribose throughout, with phosphorothiate (PS) linkages at the two ends of an antimiR, which improves target affinity and nuclease stability relative to unmodified RNA. In designing LNPs, a cationic lipid is typically used to enable electrostatic complexation with the oligonucleotide, which is a polyanion due to the negative charges contributed by phosphates and/or PS in its structure.

Quaternary amine based cationic lipids, such as DOTAP and DOTMA, are permanently charged. They are used extensively

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in cationic LNPs for plasmid delivery for gene therapy. In contrast, tertiary amine based cationic lipids, such as DODAP and DODMA, are mostly uncharged at neutral pH and become fully ionized only at acidic pH, such as that found in endosomes. It has been shown that high zeta potential for a LNP is detrimental to its in vivo activity while introducing toxicity due to undesired interaction with blood components. Weakly charged LNPs can be prepared by combining an oligonucleotide with a tertiary amine based cationic lipid at low pH and in 40% ethanol to facilitate electrostatic interaction and oligo loading, followed by raising the pH and ethanol removal. At the cellular level tertiary amine based cationic lipids facilitate endosomal escape of the oligonucleotide by going back to positive charge in response to the low pH environment in the endosome following internalization of the LNPs.

QTsome is a novel formulation of LNP based on a combination of a quaternary amine based cationic lipid and a tertiary amine based cationic lipid. This design leverages the advantage of high charge density provided by the quaternary amine cationic lipid and the pH responsiveness of tertiary amine cationic lipid to achieve a balanced charge-vs-pH profile optimal for delivery of oligonucleotides. In addition to superior activity, an advantage of QTsome is that it can be produced using lipoid excipients (DOTAP, DODMA) that have previously been used in clinical trials and are readily available, providing a straightforward pathway toward rapid clinical translation.

Application of the QTsome formulation toward the delivery of AM-21 is expected to increase its efficacy in vivo. The present study investigates the therapeutic potential of QTsome/AM-21 (QT/AM-21) in NSCLC using a cell line and a xenograft model. In vitro studies in A549 cells were carried out to evaluate the ability of QT/AM-21 to upregulate tumor suppressor targets of miR-21 and to reduce metastatic potential of tumor cells. Additional studies were completed to evaluate whether QT/AM-21 was able to increase sensitivity of A549 cells toward PTX. Finally, a xenograft mouse model was used to evaluate the in vivo efficacy of QT/AM-21 for inhibiting tumor progression and modulating targets of miR-21.

2. EXPERIMENTAL SECTION

2.1. Materials. 1,2-Dioleoyl-2,N,N-dimethyl-3-aminopropanoic acid (DODMA) was obtained from Corden Pharma (Boulder, CO, USA). 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol (CHOL) and Cremophor EL were obtained from Sigma-Aldrich (St. Louis, MO, USA). N-(Carboxymethyl)-polyethylene glycol 2000)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG) was purchased from NOF America Corp. (White Plains, NY, USA). A fully 2'-O-modified AM-21 oligonucleotide, u*c*a*acacucucuguaas*c*a*a, where asterisks represent phosphorothioate linkages, was obtained from Alpha DNA (Montreal, Quebec, CA, USA). PrimaTime qPCR assay primer probes and kits for DDAH1, PTEN, RECK, PDCD4, and TIMP3 target genes, with GUSB as a nontarget control and GAPDH as a housekeeping gene control, were purchased from Integrated DNA Technologies (Coralville, IA, USA). Goat IgG directed against DDAH1 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit IgG directed against GAPDH was purchased from Cell Signaling Technology (Danvers, MA, USA). Secondary antibody directed against goat or rat IgG conjugated with HRP was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Ammonium chloride was purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Synthesis of QTsome. QTsome were prepared by a serial ethanol dilution method. Initial dissolution of lipids under low pH and high ethanol resulted in a disperse lipid phase that was able to form electrostatic complexes with oligonucleotides. By increasing pH and reducing ethanol, LNPs became stabilized. Briefly, all lipids (X/Y/36/20/4 mol/mol, DODMA/DOTAP/DOPC/CHOL/DPPE-PEG) were dissolved in ethanol and then combined with an equal volume of AM-21 dissolved in citric acid buffer (20 mM, pH 4.5), maintaining a 10.1 lipid:AM-21 weight ratio. DODMA and DOTAP contents were varied with the sum of molar percentages of tertiary and quaternary amine maintained at 40 (X + Y = 40). This mixture was further diluted by equivalent volumes (1:1) of citric acid buffer, 300 mM NaCl, and then PBS (10 mM phosphate, 135 mM NaCl, pH 7.4). The resultant QTsome was concentrated by tangential filtration to remove unencapsulated AM-21 and ethanol, and to reach the appropriate final concentration. Samples were stored at 4°C prior to characterization. For long-term stability, 10% sucrose was added as a cryoprotectant and the QTsome formulation was either frozen or lyophilized.

2.3. Mean Particle Diameter and Surface Charge. Aliquots of QT/AM-21 were diluted in PBS. Particle size was measured by dynamic light scattering (DLS) on a NICOMP 370 submicron particle size analyzer (NICOMP, Santa Barbara, CA, USA). Aliquots of QT/AM-21 or LNPs containing only tertiary or quaternary amine cationic lipid were diluted in either citric acid buffer or PBS to determine the pH dependency of surface charge. Zeta potential measurement was conducted on a Zetasizer Nano ZS Analyzer (Brookhaven Instruments Corp., Westbury, NY, USA).

2.4. Drug Loading and Stability. Encapsulation efficiency for AM-21 was determined by Quant-IT RiboGreen RNA assay kit (Life Technologies, Carlsbad, CA, USA). Briefly, QT/AM-21 complexes were lysed with Triton X-100, and mean fluorescent intensity was compared with intact QT/AM-21 at 480 nm λex, 520 nm λem. The encapsulation efficiency was determined with the formula:

\[ \text{encapsulation efficiency} = \left(1 - \frac{\text{fluorescence without Triton X - 100}}{\text{fluorescence with Triton X - 100}} \right) \times 100\% \]

Formulation stability was evaluated at -20, 4, and 25°C over a period of 30 days. The particle size was periodically monitored by DLS; 10% sucrose was added as a cryoprotectant prior to storage.

2.5. Cell Culture. A549 cells were purchased from the American Type Culture Collection (Rockville, MD, USA) and grown in RPMI 1640 (Corning, Tewksbury, MA, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) and 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were maintained at 37°C and grown under a humidified atmosphere containing 5% CO2.

2.6. In Vitro Gene Regulation by QTsome-Encapsulated AM-21. A549 cells were grown in 24-well plates at a density of 7.0 × 10^4 cells/well 24 h prior to transfection. QT/AM-21 or QT/negative control (NC) of varying lipid composition was added at 50 nM oligonucleotide concentration.
in the presence of 20% serum containing medium to determine the optimal QTSome lipid composition. QT/AM-21 was also tested at concentrations of 1.56, 6.25, 25, and 100 nM to determine dose dependency of treatment. Cells were incubated at 37 °C with transfection medium for 4 h and then washed three times with PBS. Fresh complete cell culture medium was added, and the cells were incubated at 37 °C for an additional 44 h. RNA was isolated from cells by RNeasy 96 kit (Qiagen, Valencia, CA, USA). qRT-PCR was conducted with Taqman MicroRNA Assay (Life Technologies) or EXPRESS OneStep SuperScript qRT-PCR kit (Life Technologies) on an Applied Biosystems StepOnePlus RT-PCR system (Life Technologies). The relative amount of mRNA product was calculated and compared according to the 2^{-ΔΔCt} method. Western blot was completed to assess changes in protein expression following QT/AM-21 treatment. Cell lysates were denatured in sample buffer. Equal amounts of proteins were loaded and electrophoresed on 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The transferred blots were blocked with 5% nonfat milk in Tris-buffered saline (TBS, 150 mM NaCl, 20 mM Tris–HCl, pH 7.4) and incubated for 2 h at room temperature or overnight at 4 °C with primary antibodies in TBS, 0.05% Tween 20. After washing, the blots were reacted with HRP-conjugated secondary antibodies for 45 min and developed using an enhanced chemiluminescence (ECL) detection system. Quantitative analysis of Western blot bands was performed with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

To investigate the role of endosomal pH in QTSome-mediated delivery, the above studies were repeated with addition of 100 mM NH₄Cl, a lysosomotropic agent, into the medium during QT/AM-21 treatment to prevent endosome acidification.

2.7. Cell Viability Assay. A549 cells were grown in 96-well plates at a density of 2.0 × 10⁴ cells/well. Cells were treated with controls or QT/AM-21 at 30, 100, or 200 nM, with and without PTX (4.1 nM) dissolved in a small volume of 1:1 Cremerophor EL:ethanol solution. Relative cell viability was quantified by CellTitre 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) according to the manufacturer's protocol 72 h following the start of treatment. Briefly, 20 μL of 96-well assay solution was added to each well, and the plates were incubated for 1 h. The absorbance at 490 nm was recorded to determine cell viability.

2.8. In Vitro Tumor Cell Migration Assay. A scratch wound healing model was conducted to examine the migratory ability of A549 cells following treatment. A549 cells were plated at a density of 6.0 × 10⁴ cells/well in a 35-mm cell dish 24 h prior to transfection. A scratch wound across the dish was made using a 10 μL pipet tip immediately before treatment. Culture medium was removed and replaced with transfection medium containing QT/AM-21 or appropriate controls diluted in complete medium. Cells were allowed to proliferate at 37 °C for 48 h. Distances between the edges of the wound were measured on a Nikon E800 microscope (Nikon, Tokyo, Japan) with SPOT Advanced Imaging Software (version 5.0, Diagnostic Instruments Inc., Sterling Heights, MI, USA).

2.9. In Vitro Tumor Cell Invasion Assay. Matrigel (BD Biosciences, San Jose, CA, USA) was combined with serum-free RPMI 1640 culture medium in a 1:1 ratio; 70 μL of gel was added to each well insert of a 24-well plate. The gel was allowed to set for 1 h at 37 °C. A549 cells were seeded at 7.5 × 10⁴ cells/well in a volume of 100 μL/well on top of the gel in the insert. Transfection medium containing various formulations or controls at 2X concentration in a 100 μL volume were added to the top of the well inserts. Then, 500 μL of 10% fetal bovine serum supplemented medium was added as a chemotactrant below the Transwell insert. The plate was incubated at 37 °C for 48 h. Following the incubation period, cells remaining on the top of the well inserts were removed with a cotton swab. Well inserts were rinsed with PBS and placed in 500 μL of 0.25% trypsin solution for 1 h at 37 °C. Detached cells were counted on a hemocytometer.

2.10. In Vivo Therapeutic Activity of QT/AM-21. An A549 mouse xenograft model was generated by inoculating female athymic nude mice with 1.0 × 10⁶ cells/mouse. Tumors were allowed to reach a size of ~80 mm³ before treatment initiation (~2 weeks after inoculation). Mice (n = 10 per group) were dosed by tail vein injection with saline control, 0.5, or 1 mg/kg QT/AM-21. Tumor progression was monitored through the course of the study. Tumor volume was calculated according to the formula \( V = \frac{1}{2}L \times W^2 \). Mice were dosed every 3 days for the first three treatments and then once a week following the first dose for a total of seven doses. All mice were treated according to the guidelines deemed appropriate by the Institutional Animal Care and Use Committee (IACUC) of the Ohio State University (OSU).

2.11. Combination Therapy Analysis. Female athymic nude mice (n = 5 per group) were implanted with 1.0 × 10⁶ A549 cells/mouse, and treatment began when tumors reached a size of ≥100 mm³. Mice were treated by tail vein injection with saline control or 1 mg/kg QT/AM-21. Mice receiving PTX treatment as monotherapy or combination therapy received PTX dissolved in 1:1 Cremerophor EL:ethanol solution at a dose of 3 mg/kg via intraperitoneal injection. Mice were dosed on days 1, 3, 5, 8, 15, 22, 29, and were monitored over a 4-week period. At 48 h following the last dose, mice were euthanized, and tumors were collected and analyzed for miR-21 target gene expression.

2.12. In Vivo Gene Regulation by QTSome-Encapsulated AM-21. Tumors were harvested and placed in TRIzol reagent (Life Technologies) following treatment and were homogenized. mRNA was isolated per the manufacturer's protocol. qRT-PCR was then completed according to the same procedure as outlined above in section 2.6.

2.13. Statistical Analysis. All studies were done in triplicate unless otherwise indicated. Statistical analysis was conducted using Microsoft Excel Software (2013, Redmond, WA, USA). Student's t test was used to determine statistically significant difference(s) between two or more groups. A p value of 0.05 was selected as the cutoff for statistical significance.

3. RESULTS

3.1. Particle Size and Surface Charge. Previous studies have demonstrated the need for small particle size (<150 nm) and moderate zeta potential (+10–30 mV) to potentiate delivery of oligonucleotides. QTSome particle size measurement by DLS indicated particles of approximately 80–170 nm in diameter (Figure 1A). Particles with greater amounts of quaternary amine (15–40 mol%) achieved particles of smaller size (<120 nm). The miminization of particle size was obtained at equal molar percentages of quaternary and tertiary amine, which may signify the point at which the QTSomes adopted a condensed structure. Zea potential measurement (Figure 1B) revealed formation of QTSome particles of 12.49 ± 1.45 mV in PBS (pH 7.4) and 29.89 ± 8.16 mV in citric acid buffer.
Figure 1. Particle size and surface charge of QTSomes and LNPs. QTSome of varying mol % quaternary and tertiary amine content were prepared and analyzed by DLS for the effect on nanoparticle size (A). QTSome and LNP composed of only quaternary or tertiary amine cationic lipids were placed in buffer at physiological pH (7.4) and acidic pH (4.0) to determine the pH responsive effect on surface charge (B). Data is presented as the mean ± SD of three independent samples (n = 3).

(pH 4.0), thus demonstrating the pH responsive behavior of a conditionally ionizable LNP formulation. As expected, the pH responsiveness of QTSomes fell between that of those LNPs containing only tertiary amine cationic lipid and those with only quaternary amine cationic lipid. The charge on tertiary amine only LNPs changed from 3 to 15 mV with increasingly acidic pH. Conversely, the charge on quaternary amine only LNPs did not vary much with changes in buffer pH (Figure 1B).

3.2. Drug Loading and Colloidal Stability. Encapsulation efficiency obtained during QTSome synthesis was 83.3 ± 4.17%. Further studies by size exclusion chromatography analysis on a Sepharose CL-4B column (Figure 2A) showed approximately 90% of oligonucleotide within the encapsulated drug fractions,18–19 with very little oligonucleotide remaining in the free drug fractions.18–19 The formulation (with 10% sucrose as cryoprotectant) further demonstrated high stability under storage at −20, 4, and 25 °C over a period of 30 days, maintaining a size of ~110 nm (Figure 2B).

3.3. Determination of Optimal Lipid Combination. A series of QTSome formulations were evaluated to determine the best ratio of quaternary and tertiary cationic lipid for transfection. Formulations were identified as QT(mol % quaternary amine)-(mol % tertiary amine) in Figure 3. Treatment with 50 nM AM-21 revealed QTSome with 15 mol % DOTAP and 25 mol % DODMA to perform the best in upregulation of DDAH1 (1.33-fold), and this combination of lipids was therefore chosen for further in vitro and in vivo investigation. Formulations QT10-30 and QT5-35 also performed well, with 1.32and 1.27-fold upregulation of the target genes, respectively. Formulations containing only quaternary or tertiary cationic lipid did not perform as well relative to the quaternary–tertiary combinations.

3.4. Regulation of miR-21 and miR-21 Targets. Treatment with 100 nM QTSome AM-21 resulted in significant downregulation of miR-21 and upregulation of its downstream targets (Figure 4A,B). Relative to the untreated control, miR-21 level was decreased by 50.3 ± 2.1% following administration of
Figure 4. Upregulation of miR-21 and its target genes by QT/AM-21 in A549 cells. (A) Expression of miR-21 by qRT-PCR, relative to RNU44. (B) Upregulation of miR-21 target genes, relative to GAPDH. A549 cells were treated for 4 h with 1.56, 6.25, 25, or 100 nM QT/AM-21 to demonstrate the effects of dose dependency on target (C) mRNA and (D) protein expression levels. Relative gene expression to GAPDH was evaluated 24 h following the start of treatment. (E) Relative gene expression of DDAAH1 following QT/AM-21 treatment in the presence of 100 mM NH4Cl. Results are reported as the mean ± SE of three independent plates.

QT/AM-21. In contrast, little to no effect on target gene regulation was observed for the scrambled negative control (NC) group. Tumor suppressors PTEN and PDCD4 were upregulated 2.7- and 1.3-fold, respectively. Matrix metalloprotease inhibitors RECK and TIMP3 were both upregulated by approximately 1.5-fold. Migration inhibitors ANKRD46 and DDAAH1 were upregulated 1.2- and 3.0-fold, respectively. Meanwhile, little to no change in expression was seen for GUSB control across treatment groups.

3.5. Dose and pH Dependency. Varying dosages of QT/AM-21 between 1.56 and 100 nM were administered and qRT-PCR was conducted to evaluate the relationship between AM-21 concentration and miR-21 target gene upregulation (Figure 4C). miR-21 targets DDAAH1, PTEN, and RECK all demonstrated a direct correlation between AM-21 dose and mRNA levels. DDAAH1 and PTEN were relatively more sensitive to changes in concentration relative to RECK. In addition, concentration-dependent upregulation of DDAAH1 in response to QT/AM-21 was shown at the protein level by Western blot (Figure 4D). Furthermore, QT/AM-21 treatment of A549 cells in the presence of 100 mM NH4Cl demonstrated a significant reduction in DDAAH1 upregulation. This might be due to the inhibition of endosomal release of the oligonucleotide (Figure 4E). By preventing the acidification of the endosome, overall cationic charge of QTsome would have been diminished as the tertiary amines remained neutral and thus unable to interact with negatively charged lipids of the endosomal membrane. The results of this finding suggest a pH-dependent mechanism of QTsome delivery.
3.6. Cell Viability. Treatment with free AM-21 or empty QTosome did not result in significant cytotoxicity as analyzed by MTS assay (Figure 5). Likewise, the combination of QT/AM-21 at 50 nM did not demonstrate much cytotoxicity. However, increases in cytotoxicity were observed at increased concentrations of QT/AM-21 (100, 200 nM). Addition of PTX alone diminished cell viability by 40%. Addition of free AM-21 or QTsome lipids to PTX did not result in significant decreases in cell viability. However, substantial gains in cytotoxicity were observed with QT/AM-21 treatment in a dose-dependent manner. Cell viability was reduced to 51.2%, 41.0%, and 31.8% at 50, 100, and 200 nM doses, respectively. The differences between the QT/AM-21 and PTX monotherapies and the combination therapy were both significant at p < 0.05. Changes in cell morphology were also noted by microscopy. Minor changes in cellular morphology were observed for control treatment groups and for low doses of QT/AM-21. Major alterations in morphology were observed corresponding to increasing cytotoxicity for increasing doses of QT/AM-21 and especially QT/AM-21 with PTX (Supplemental Figure 1).

3.7. Invasion and Migration. Wound healing assay was completed to monitor the relative mobility of AS49 following treatment with AM-21. QTsome lipids or AM-21 alone did not confer significant decreases in cell migration in the wound region. With 100 and 200 nM AM-21 treatment, mobility was reduced to 43.0 and 22.2% relative to the untreated control (Figure 6A). Matrigel was used to simulate biological conditions of the basement membrane. The ability of cancer cells to migrate plays a role in determining metastatic potential and increases with cancer progression. Treatments with QT/AM-21 at 50, 100, and 200 nM were able to reduce migration to 87.7, 76.7, and 62.6%, respectively, while QTsome lipids alone or free AM-21 did not significantly retard cell invasion (Figure 6B).

3.8. In Vivo Dose Response. Treatment with QT/AM-21 demonstrated strong antitumor activity (Figure 7) at 1 mg/kg, but diminished activity at 0.5 mg/kg, suggesting a strong dose-dependence in therapeutic response. Treatment of tumors initiated at ~80 mm³ and ended at 816, 618, and 172 mm³ for the untreated, 0.5 mg/kg, and 1 mg/kg groups, respectively. Moderate differences in terms of body weight were observed between the treated and untreated groups. Liver and spleen weights remained fairly consistent between the two groups, suggesting little to no significant toxicity for these organs. Tumor weight was over 16-fold lower for the treated group compared to the untreated group (Figure 8). In terms of median survival time, the untreated group was 21 days, the 0.5 mg/kg treated group was 24 days, and the 1 mg/kg treated group was significantly prolonged, at 33 days (Figure 9).

3.9. In Vivo Combination Therapy. PTX and QT/AM-21 combination therapy was evaluated for therapeutic efficacy. Treatment began when tumors reached ~80 mm³ in volume. Tumors progressed to 380, 246, 201, and 138 mm³ for the untreated, PTX, QT/AM-21, and combination treatment groups, respectively (Figure 10). Furthermore, qPCR conducted on tumor sections revealed moderate to strong upregulation of DDAH1 and PTEN (Figure 11). DDAH1 and PTEN were only modulated slightly by PTX, by 1.7- and 1.5-fold, respectively. DDAH1 was upregulated 3.4-fold while PTEN was upregulated 2.5-fold with QT/AM-21. DDAH1 was strongly upregulated by 5-fold and PTEN was upregulated 4.1-fold with the QT/AM-21/PTX combination therapy.

4. DISCUSSION

miR-21 is involved in a number of pathways regulating tumor progression and resistance to chemotherapy. Therefore, it can serve as a prognostic and diagnostic biomarker for NSCLC. miR-21 is also found to have similar roles in other cancers as
well, including ovarian, breast, and prostate cancer. AM-21 therapy to inhibit miR-21 is a potential strategy for the treatment of NSCLC and other cancers. The in vivo application of AM-21 and related oligonucleotides for therapy is hindered by several physical and biological barriers to their in vivo delivery. LNPs are employed to improve delivery of oligonucleotides. Quaternary amines are the most commonly used class of lipids for gene therapy delivery and form strong electrostatic interactions with negatively charged oligonucleotides. Meanwhile, tertiary amines are used for delivery of oligonucleotides, such as siRNA. For example, the tertiary amine lipid 1,2-dilinoleoylxy-3-dimethylaminopropane (DLinDMA) is a major component of a version of the stable nucleic acid lipid particles (SNALP) delivery system developed by Telomira Pharmaceuticals. Tertiary amine-based lipids are pH responsive, which has been shown to be critical to oligonucleotide delivery.

In the present study, a pH-sensitive carrier, QTsome, was evaluated for the delivery of AM-21 to NSCLC. Tertiary amine cationic lipids form the pH-sensitive component of QTsome. Upon exposure to acidic conditions as found in the endosome, the tertiary amine cationic lipid becomes cationized, enabling...
interaction with negatively charged lipids in the endosomal membrane and facilitating endosomal release. Release of drug from the endosomal compartment is a critical step in determining antimiR efficacy. 6,12,13 Meanwhile, inclusion of quaternary amine lipids can contribute to structural stability of LNPs under physiological pH. A positively charged quaternary amine can better interact with the negative charge of the oligonucleotides, thereby resulting in particles of smaller average particle size relative to particles containing only tertiary amine lipids. In terms of pH-dependence of surface charge, QT15-25 displayed an intermediary response to pH relative to QT40-0 or QT0-40. QT/AM-21 furthermore demonstrated excellent colloidal stability and efficient drug loading, which are important factors for clinical translation. It is worth noting that LNP formulations also based on a combination of two pH-sensitive lipids, one cationic and one anionic, called Smarticles, have recently been used in clinical studies for therapeutic delivery of miR-based oligonucleotides with promising initial data. QT'somes may be further stabilized by the addition of cryoprotectant and by lyophilization for long-term stability. 12,13

QT/AM-21 was able to downregulate miR-21 and upregulate several key targets of miR-21 including tumor suppressors, matrix metalloproteinase inhibitors, and migration inhibitors. PTX kills cancer cells by stabilization of microtubules, which prevents mitosis. Patients normally respond to PTX with a 40–80% response rate, but many of these patients develop resistance to PTX over time. 10,11 The combination of QT/AM-21 and PTX demonstrated greater ability to reduce cell proliferation than the combination of free AM-21 and PTX, suggesting greater uptake and/or efficacy of AM-21 when delivered via QT. In an ovarian cancer model, resistance against PTX is suggested to be regulated by miR-21's effect on hypoxia-inducible factor-1α (HIF-1α) and P glycoprotein (P-gp). 12,13 Decreased migratory and invasion potential was observed in a dose-dependent manner in response to QT/AM-21 treatment. Lung cancer is often diagnosed late in its development, when metastasis has already begun. Therefore, QT/AM-21 addresses a critical need that is currently unmet by chemotherapy administered in the late stage of the disease.

Treatment with QT/AM-21 in a xenograft mouse model was able to significantly suppress tumor growth at 1 mg/kg. Targets of miR-21 were also found to be upregulated at this dose. Relative increase in body weight indicated mice treated with QT/AM-21 as having better health and body condition. 14 Liver and spleen weight did not differ much between the two groups, suggesting that the formulation was not toxic to those organs. Liver and spleen are highly vascularized organs involved in the reticuloendothelial system with large local populations of macrophages, and LNPs may accumulate in these organs due to their similarities with tumor vasculature. 15,16 The combination of PTX and QT/AM-21 demonstrated greater therapeutic activity than either agent administered alone. Interestingly, PTX appeared to have effects on miR-21 targets. Actually, this is consistent with previous findings, showing effects of PTX on PTEN and DDAH1 expression levels. 17 Within certain cytotoxic thresholds, PTX has been found to increase the expression as well as the activity of PTEN. It is therefore plausible that upregulation of DDAH1 can occur by a similar mechanism.

Additional studies will be required to validate the safety and efficacy of QT/AM-21. Studies by Western blot would confirm whether the increase in mRNA for other target genes correlates with an increase in the associated protein levels. 17 It would also be interesting to see if miR-21 in NSCLC also regulates HIF-1α as in ovarian cancer to verify the mechanism by which miR-21 promotes resistance against PTX. Cellular uptake studies with pathway inhibitors would be likewise helpful to understand the mechanism behind uptake of QTs, specifically to determine if QT'somes are taken up in the same clathrin-mediated endocytosis pathway as in the case of quaternary- or tertiary-based LNPs. 17 Incorporation of antimiR oligonucleotides into lipid nanoparticles is generally based on their electrical charge rather than base composition. Therefore, application of QT'somes is not limited to delivery of AM-21. QT'somes may be used to deliver virtually any type of antimiRs, siRNAs, or miR mimics. 17 Addition of a targeting agent may improve the specificity of delivery of QT'somes to NSCLC cells. 17 Further toxicity, pharmacokinetic, and pharmacodynamics studies are needed to better characterize QT/AM-21 and aid its translation to the clinic.

5. CONCLUSION

QT'somes were prepared by a modified ethanol dilution method. QT'some nanoparticles exhibit small particle size, moderate zeta potential, high drug loading capacity, and long-term stability. In vitro analyses indicate a strong, dose-dependent upregulation of miR-21 targets with greater activity than formulations with either quaternary or tertiary amine cationic lipids alone. The combination of quaternary and tertiary amine cationic lipids forms a pH-sensitive system that is stable and possesses fusogenic activity in the endosome. Moreover, increased sensitivity to PTX and reduced migration
and invasion were demonstrated with QT/AM-21 treatment. In vivo analyses in tumor-bearing mice reveal QT/AM-21 induced tumor regression, upregulated miR-21 target genes, enhanced antitumor activity with combination therapy, and prolonged survival. Thus, these studies suggest that QT/AM-21 warrants further evaluation for therapeutic applications in NSCLC and other types of cancer.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.5b00878.

Image showing changes in cell morphology (PDF)

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Notes

The authors declare no competing financial interest.

**REFERENCES**


(9) Xie, Z.; Cao, L.; Zhang, J. miR-21 regulates p53-mediated sensitivity and hypoxia-inducible factor-1α expression in human ovarian cancer cells. Oncol. Lett. 2013, 6 (3), 795–800.


ELEVATED VACUUM SUSPENSION PRESERVES RESIDUAL LIMB SKIN HEALTH IN LOWER LIMB AMPUTEES

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A growing number of clinical trials and case reports support qualitative claims that use of an elevated vacuum suspension (EVS) prosthesis improves residual limb health on the basis of self-reported questionnaires, clinical outcomes scales, and wound closure studies. Here, we report first efforts to quantitatively assess residual limb circulation in response to EVS. Residual limb skin health and perfusion of lower-limb amputees (N=10) were assessed during a randomized crossover study comparing EVS to non-elevated vacuum suspension (control) over a 32 week period using non-invasive probes (transdermal water loss, laser speckle imaging, transcutaneous oxygen measurement) and functional hyperspectral imaging approaches. Regardless of the suspension system, prosthesis donning decreased perfusion in the residual limb under resting conditions. After 16 weeks of use, EVS improved residual limb oxygenation during an activity of treadmill walking. Likewise, prosthesis-induced reactive hyperemia was attenuated with EVS following 16 weeks of use. Skin barrier function was preserved with EVS but disrupted after control socket use. Taken together, outcomes suggest chronic EVS use improves perfusion and preserves skin barrier function in lower limb amputees. This trial is registered on clinicaltrials.gov, identifier: NCT01839123.
Novel fluorescence microscopic imaging with greatly extended depth of field
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Wavefront-engineered microscope with greatly extended depth of field (EDoF) is designed and demonstrated for volumetric imaging with near-diffraction limited optical performance. The general polynomial phase mask has the capability for robustness and custom merit function, which could extend the depth of field. The phase mask is placed in between the objective lens and the tube lens for ease of use, and the test is with a dyed USAF resolution target. In Zemax simulation for a setup using 32X objective (NA = 0.6), the EDoF is 10μm whereas a conventional one has a DoF of 0.75μm, indicating a 13 times increase. In experiment, a 20X objective lens with NA = 0.4 was used and the corresponding phase plate was designed and fabricated and the defocus fluorescence images showed significant improvement in EDoF, while the focus image had the same resolution as conventional focused image. A simple and efficient architecture for extending the DoF of the microscope has been verified. The general polynomial phase function is very effective. The DoF has been extended by more than 10 times. This method of EDoF provides a new approach to volumetric biomedical imaging and industrial inspection. It may play significant role in observing dynamic changes in a 3D volume.
3D-Printing a Closed Disposable Seeding System for Rapid Preparation of Patient-Specific Tissue Engineered Vascular Grafts

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The first FDA approved tissue-engineered vascular graft (TEVG) for the palliation of congenital cardiac anomalies is prepared via an open technique in an ISO-Class 7 clean room. Autologous bone marrow mononuclear cells (BM-MNCs) are enriched by density-gradient centrifugation and subsequently vacuum seeded onto a biodegradable tubular scaffold. The time, labor, and resource-intensive nature of graft preparation along with the complexities of maintaining a GMP-compliant clean room limit the widespread adoption of this approach. Attempts to optimize scaffold seeding have resulted in the development of a filter-based system for BM-MNC isolation and a closed, disposable seeding device, which have previously been validated in small and large animal models. Furthermore, the integration of pre-operative imaging studies, computational hemodynamic modeling, computer-aided design, and 3D printing would allow for the creation of an optimal patient-specific TEVG scaffold and closed seeding system. In this study, we present an improved single-use TEVG seeding prototype fabricated by 3D printing that is suitable for preclinical evaluation. A commodity fused deposition modeler (Ultimaker 2) was used to print the components of the seeding system from poly-lactic acid filament over the course of 36 hrs. Next, we used pre-operative MRI imaging of a candidate TEVG recipient with dextrocardia, transposed great arteries, and total anomalous pulmonary venous return (TAPVR) status post TAPVR repair and right-sided bidirectional Glenn shunting to design a patient-specific seeding system. This complex case represents a situation in which the ideal vascular conduit would not be the currently utilized linear graft of constant diameter, and highlights the advantages and feasibility of our approach. Translation of the 3D-printed closed disposable seeding system to the clinic would allow for an off-the-shelf patient-specific TEVG for point-of-care treatment of congenital heart disease.
Oral Supplementation of Fermented Papaya Preparation to Type 2 Diabetes Mellitus Patients Improves the Respiratory Burst Function in Chronic Wound Macrophages

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Wound macrophages are responsible for the production of reactive oxygen species (ROS) during their characteristic respiratory burst (RB) utilized during phagocytosis and as an intracellular signaling molecule to recruit granulocytes to the wound site. Type 2 diabetes mellitus (T2D) patients have been reported to exhibit compromised RB compared to non-T2D counterparts. We have shown that wound macrophages from diabetic (db/db) mice orally supplemented with fermented papaya preparation (FPP, 0.2g/kg body weight) exhibited an increased production of ROS when stimulated by phorbol 12-myristate 13-acetate (PMA). Mice receiving FPP showed improved wound healing outcomes compared to placebo counterparts. Peripheral blood monocytes (PBM), the circulating precursor of wound macrophages, isolated from T2D patients orally supplemented with FPP (3g, 3 times/day) also demonstrated enhanced ROS production when stimulated with PMA (40nM, 30min) compared to non-T2D counterparts. We hypothesize that supplementation of FPP to T2D patients will improve wound macrophage RB ROS production, allowing for improve wound healing outcomes. T2D patients currently undergoing negative-pressure wound therapy (NPWT) are being recruited from the OSUWMC Comprehensive Wound Center. Thirty patients are being randomized into two groups; FPP supplemented and standard of care (SoC). NPWT dressings are being collected and lavaged to yield wound macrophages for assay. Oxidant production in isolated wound macrophages is being measured using 2',7’-dichlorofluorescin diacetate (DCFDA, 5μM). Patients are enrolled for a total of 12 weeks, during which time images of their wounds are collected at weeks 0, 3, and 12 to track wound closure. Wound macrophages isolated from T2D patients who received FPP supplementation exhibited increased RB ROS production compared to SoC counterparts. Compromised RB function in innate immune cells represents a serious threat for T2D patients with non-healing wounds. FPP is able to support RB function in wound macrophages. The current trial is still ongoing. As more patients complete the study, we hope to gain evidence that FPP supplementation will confer improved wound healing outcomes for T2D patients with chronic wounds.

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