PILOT AWARDS – FISCAL YEAR 2015

Institute on Aging Pilots

1. Effects of Autologus Jevenile, Adult, and Aged Tenocyte-Seeded Nanofibrous Scaffolds in Rotator Cuff Repair

PI: Andrew F. Kuntz, MD

Objective: Rotator cuff tears are a very common pathology, affecting millions of individuals each year, with a significantly increased prevalence in the elderly population. Despite extensive research aimed at improving surgical outcomes, repairs failure remains a common problem. The specific aim of this study is to determine the effects and mechanisms of action of juvenile, adult, and aged tenocyte-seeded nanofibrous scaffolds on tissue properties and joint mechanisms in a novel model of augmented rotator cuff repair.

Research Design: A total of fifty-four Sprague-Dawley rats will be divided into three groups based on age (juvenile, adult, and aged). Animals will be subjected to a validated four week overuse protocol to create a degenerate tendon prior to surgically-created tendon detachments to model an acute on chronic condition. Animals will undergo surgical detachment of bilateral supraspinatus tendons and harvest of the intra-articular biceps tendons. Biceps tendon tissue will be seeded into an electrospun poly(ε-caprolactone) (PCL) nanofibrous scaffold. Augmented supraspinatus repair will be performed during a second surgery in which one shoulder will receive a tenocyte-seeded scaffold while the other should receives an acellular scaffold to serve as an internal control. Animals will be sacrificed at 8 weeks after the second surgery and tissues will be harvested for biomedical and histologic analysis. Our hypotheses are two-fold: 1) Tenocyte-seeded scaffolds will decrease inflammation and increase collagen production at the repair site compared to scaffold only controls, resulting in enhanced tendon-bone healing with improved shoulder kinetics and mechanical properties. 2) While tenocytes will be successfully derived from rats of all ages, those from juvenile rats will result in greater improvement of functional outcomes in cuff healing.

Innovation and Application: The proposed research specifically addresses regenerative medicine and musculoskeletal repair by using bioengineered scaffolds in vivo to improve tendon repair after rupture in a clinically relevant and well-established animal model. Results will provide the critical preliminary data to allow for NIH or Veterans Affairs grant applications for funding of al full-scale study. As this approach uses FDA approved materials and minimally manipulated autologous cell populations, there exists direct precedence for rapid translation to clinical practice to address this important clinical problem. We expect to demonstrate the potential for autologous cell-seeded scaffolds to improve repairs in the aged population.

2. Rescue of Telomere Dysfunction Using Genome Editing and Pharmacological Approaches

PI: Christopher J. Lengner, PhD & F. Brad Johnson, MD, PhD

Abstract: Telomeres are the structures that cap the ends of chromosomes, and they comprise repeated DNA sequences bound by a protective protein complex called shelterin. Telomeres shorten with age, and several lines of evidence indicate shortening and consequent telomere dysfunction ("uncapping") contribute to the development of several age-related diseases. We have discovered a novel mechanism that helps maintain telomeres in a capped state, which may provide therapeutic avenues to treatment of age-related diseases. We have found that a positive feedback loop exists between the capped state of telomeres and expression of the components of a system that enables cells to communicate with one another, called the Wnt pathway. In particular, when telomeres are capped, the Wnt pathway is active, and the shelterin proteins that protect telomeres are expressed at optimal levels. In contrast, when telomeres begin to uncap, the loop is interrupted, leading to downregulation of the Wnt pathway and thus of shelterin protein levels, in turn driving further telomere uncapping. We discovered this mechanism using mice in which an enzyme called telomerase (which can normally lengthen telomeres and thus prevent their uncapping) has been inactivated genetically. These telomerase deficient mice develop prematurely shortened telomeres with age in tissues where telomerase is normally most active: most prominently the epithelium of the intestinal tract. Wnt signaling normally supports

the survival and division of stem cells that give rise to the intestinal epithelium, and when telomeres begin to uncap in these cells of the telomerase deficient mice, the resulting loss of Wnt signaling and additional telomere uncapping leads to stem cell dysfunction, apopotosis of epithelial cells, and intestinal atrophy. We found that treating these mice with any of several drugs that activate the Wnt pathway can restore the positive feedback loop and foster telomere capping an intestinal integrity. People with a rare disease called dyskeratosis congenita (DC) suffer from a deficiency in telomerase, and present with intestinal defects analogous to those in the mouse model, and thus provide an opportunity to ask if the lessons we have learned in mice apply to humans.
Here, we propose to 1) use state-of-the art genome editing to correct the underlying genetic defect in DC in human induced pluripotent stem (iPS) cells from these patients, 2) use the mutant and corrected iPS cells to generate intestinal tissue ("organoids") in culture and compare their health, 3) test the capacity of Wnt pathway activators to rescue telomere capping and tissue defects in the DC patient mutant and corrected intestinal organoids.
Thus we will determine if, as in mice, Wnt activators can restore capping to shortened telomeres in a model human tissue. The findings will demonstrate in proof of principal that Wnt agonists can be therapeutically effective for the treatment of intestinal pathologies in dyskeratosis congenita patients, and thus potentially for other pathologies in these patients as well as in a wider array of age-related diseases.

3. The Stimulation of Modeling-based Bone Formation in Estrogen-deficient Bone

PI: X. Sherry Liu, PhD

Abstract: Current postmenopausal osteoporosis treatments are categorized as either anti-catabolic, to reduce bone resorption, or osteo-anabolic, to increase bone formation. However, due to the coupling of bone resorption and formation, drugs that inhibit resorption often times also inhibit formation, and those that increase formation also increase resorption, thereby limiting their potential benefits. Recent data indicates a possible osteo-anabolic mechanism of intermittent parathyroid hormone (iPTH) through *modeling-based bone formation* (osteoblast formation without prior activation of osteoclast resorption). Through this pathway, combination therapy of iPTH and anticatabolic agents could activate new bone formation while blocking the accelerated bone resorption, leading to an additive effect above the available mono-therapies. However, the mechanisms of iPTH's activation of modeling-based bone formation are not clear. Recent studies suggest that mechanical stimulation and iPTH act independently, as well as synergistically, to stimulate new bone formation via the Wnt/β-catenin pathway by modulating the Wnt-signaling antagonist, sclerostin. Furthermore, both iPTH and Sclerostin antibody rescue disuse-induced bone loss, however, the rescue occurs with attenuated anabolic effects.
This may indicate a mechanically sensitive component of iPTH's anabolic effect on bone, which leads to our central hypothesis that iPTH substantially increases modeling-based bone formation which is locally regulated by mechanical stimulation via a Wnt/β-catenin signaling pathway. Accordingly, Aim1 is to identify modeling-based bone formation in response to iPTH. Bone remodeling and modeling processes will be monitored and quantified by a novel 3D in vivo bone dynamic imaging system for ovariectomized mouse tibiae treated with vehicle or iPTH with and without co-treatments with anticatabolic agents to minimize osteoclast activity. Aim 2 is to determine the impact of mechanical stimulation and the mechanistic roles of localized Wnt/β-catenin signals on iPTH-induced, modeling-based bone formation. A hindlimb suspension protocol will be used to reduce local mechanical stimulation while compressive dynamic loading will be applied to induce a controlled mechanical stimulation on TOPGAL mouse tibia, which contains reporters for Wnt/β-catenin signals. Local mechanical signals will be derived by finite element analysis and then registered with the in vivo bone dynamic image, as well as with β-gal stained images, to co-localize the occurrences of modeling-based formation, mechanical signals, and Wnt/β-catenin signals. The proposed studies are expected to advance our fundamental understanding of local signals activating modeling-based formation, a highly efficient but overlooked regenerative mechanism.

4. Tau Positron Emission Tomography Imaging in Young Onset Focal Dementia

PI: Ilya Nasrallah, MD, PhD & David Wolk

Abstract: Alzheimer's disease is a progressive dementia that is typically characterized by a prominent impairment in memory, however there are some uncommon subtypes with the most striking deficit is in another cognitive domain. While still less common than typical, amnestic Alzheimer disease, these subtypes are relatively more common in younger patients, where the onset of disease is under age 65. Patients with the logopenic variant of primary progressive aphasia (lvPPA) demonstrate a primary language impairment and those with posterior cortical atrophy (PCA) demonstrate a primary visuospatial impairment. Both of these conditions are

associated with the pathologic hallmarks of Alzheimer disease: neurofibrillary tangles containing tau protein and amyloid plaques. Corresponding to the symptomatic differences, pathologic and neuroimaging studies have identified unique regions of the brain that are affected in these syndromes - the left temporoparietal region in lvPPA and the occipital and posterior parietal lobes in PCA - differing from the more significant medial temporal lobe involvement of typical Alzheimer's disease.

Clinically, there are unmet needs to be able to objectively monitor progression of pathologic changes in the brain as well as to differentiate these syndromes from others with similar symptoms but associated with differing pathological changes. Unfortunately, neurodegeneration sufficient to cause atrophy or abnormal cerebral metabolism, which are currently measured by available conventional neuroimaging studies, occurs relatively late in the course of disease. Imaging agents for cerebral amyloid, using positron emission tomograohy (PET), confirm the presence of amyloid in these syndromes but do not distinguish between them, nor demonstrate significant change as the disease progresses. Newly developed PET radiotracers that target tau neurofibrillary tangles, including one named T807, may be able to do both, as cerebral neurofibrillary tangle distribution is thought to differ between these syndromes and also to match regions of neurodegeneration. The first aim of this proposal is to study the distribution of T807 uptake in these subtypes of Alzheimer disease, to determine whether it can detect regional differences in tracer uptake between them. The second aim is to determine whether the distribution of T807 correlates with brain atrophy detectable by MRI and with results of cognitive testing.

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5. Does Chronic Insomnia Lead to Accumulation of Beta Amyloid?

PI: Philip Gehrman, PhD

Abstract: Individuals with dementia frequently have sleep problems that negatively affect daily functioning and quality of life. It has been thought that the sleep problem occurs as a result of the dementia, but it is now recognized that chronic poor sleep may come first in some cases and increase the risk of developing dementia. This may occur because, during sleep, the brain is able to clear away waste products from normal metabolic processes. Poor sleep may interfere with this process. Some of these metabolites, such as beta amyloid, can be toxic to the brain if they build up over time.
The goal of this project is to measure beta amyloid, and other metabolites, in five individuals with chronic insomnia and compare them to five good sleepers. Participants will complete standard questionnaires and have their sleep monitored at home for one week. They will then come to the hospital for an overnight stay. During this time, blood samples will be taken every two hours for 24 hours, and spinal fluid will be collected once at night and once the next morning. Sleep will also be monitored overnight. Beta amyloid and other metabolites will be measured in both blood and spinal fluid. The results of this study will help us understand how chronic poor sleep may increase the risk for dementia and inform the development of interventions designed to reduce the likelihood of developing dementia.

6. Within-individual Variability as a Biomarker of Incipient Dementia in Mild Cognitive Impairment

PI: David R. Roalf, PhD

Abstract: Mild Cognitive Impairment is associated with an increased risk of dementia, and early and accurate detection of cognitive impairments that lead to dementia would benefit clinical management. To date, the preponderance of cognitive research in MCI has emphasized overall group differences in mean performance. Despite the fact that the clinical presentation and progression of dementia is varied, there has been little work in developing measures tapping these individual differences. Within-individual variability (WIV), or fluctuations, in behavioral performance is common in healthy aging and more pronounced in neurological and psychiatric disorders. Importantly, higher WIV is predictive of clinical outcome. While individuals with MCI show measurable deterioration in cognitive function that is greater than expected based upon an individual's age and education these early signs of cognitive impairment are subtle and may go undetected using traditional neuropsychological measures. Despite its being a major research focus in recent years, establishing the diagnosis of MCI and

monitoring disease progress using neuropsychological function over time remains challenging. WIV has been shown to confer unique predictive information about cognitive functioning beyond mean performance and is specific to an individual. Therefore, the identification of mechanisms underlying cognitive variability is crucial for advances in identification of MCI and eventual treatment of early dementia.

With funding from the University of Pennsylvania Institute on Aging and Alzheimer's Disease Core Center (ADCC), I propose to address the crucial need by measuring neurocognitive variability in individuals with Alzheimer's disease, MCI and healthy aging. The primary aim of the proposed research is to better understand the utility of WIV in neurocognitive ability as an indicator of neurological integrity and its viability as an early indicator of cognitive impairment in MCI. The proposed work will utilize the tremendous resources of the ADCC in two ways: 1) by completing the retrospective assessment of neuropsychological data collected at the ADCC to measure global neuropsychology WIV and associate it with biomarker data (e.g. MRI, CSF), and 2) prospective collection of computerized neuropsychological tests that will allow for the systematic domain-specific measurements of WIV.