Recent advances have contributed to our understanding of the factors contributing to the progression of osteoarthritis (OA) and how biological processes are related to clinical outcomes. Work has focused on cartilage, including how it is formed during skeletal development and how disrupted remodeling occurs during OA through activation of its cellular component, the chondrocyte. OA is a “whole joint” disease, in which cartilage erosion marked by loss of the collagen network is a critical determinant in OA progression. The disruption of cartilage homeostasis due to multiple potential causes, related to aging, genetic predisposition, trauma, or metabolic disorder, induces profound phenotypic modifications of chondrocytes.

A major challenge is the inability of the resident chondrocytes in cartilage is to lay down a new matrix with the same properties as it had when it was formed during development. Thus, researchers in the field are looking for strategies to prevent cartilage damage in the first place or to repair the damaged cartilage with cell-based tissue-engineering approaches. My laboratory studies the mechanisms of gene regulation by which stress- and inflammatory cytokine-induced signals suppress cartilage-specific matrix synthesis (type II collagen) in chondrocytes, and also induce expression of matrix metalloproteinase 13 (MMP-13), the pivotal collagen-degrading proteinase that marks OA progression. Recent studies have uncovered new roles of genes not previously known to act in cartilage in chondrocyte differentiation and OA. We have employed cell culture models, including high density monolayer and 3D pellet culture systems, using primary chondrocytes isolated from human or mouse cartilage or chondrocyte cell lines that facilitate mechanistic analysis of marker gene expression and pathway interactions. In vivo strategies employ mice to analyze the consequences of knockout or transgenic overexpression of critical genes and to model post-traumatic and genetic forms of OA. We have found that there are common mediators of these processes in human OA cartilage and are profiling temporal and spatial changes in gene expression and microRNAs during early through late stages of OA in mouse models.

Since the chondrocytes in adult human cartilage are normally quiescent and maintain the matrix in a low turnover state, understanding how they change their gene expression to promote matrix destruction and abnormal repair in OA may lead to identification of critical targets for therapy to block initiation of cartilage damage or promote effective cartilage repair. Early changes in the initiation of OA involve disruption of the pericellular matrix through signaling events mediated by chondrocyte receptors such as discoidin domain receptor-2 (DDR-2) and syndecan-4 and stress- and inflammation-induced kinase cascades that are also involved in mechanotransduction, including IKKs and MAPKs. These pathways converge on transcriptional regulation by NF-κB (canonical and non-canonical), ELF3, C/EBPβ, Runx2, and hypoxia-inducible factor (HIF) 2α of the cartilage-degrading enzymes, aggreganases and collagenases, especially MMP-13. A recent focus on epigenetic regulation of gene expression, has led to a new understanding of the role of DNA methylation status on MMP13 and IL1B expression in response to transcription factors and upstream signals. Epigenetic regulation by CpG methylation and microRNAs and elucidation of mechanisms over the time course of the disease initiation and progression in mouse models of OA will inform us about new approaches for designing therapies.

Bibliography


Relevant Review Articles:


MAPK in Innate Immune Inflammation of Osteoprogenitor Cells

Francis Y. Lee, M.D.
Professor with Tenure
Robert Carroll and Jane Chance Carroll Professor of Orthopaedic Surgery
Columbia University, New York, New York

Abstract
We are presenting data on innate immune function of osteoblastic cells. In a murine calvarial model of LPS-induced bone inflammation and resorption, locally delivered MAPK inhibitor remarkably prevented osteoclast formation and bone resorption. We further confirmed that ERK1/2 activation has a capability of modulating secretion of IL-6, RANKL, MCSF, and COX-2 in osteogenic lineage cells in response to LPS, TNF or metal particles, common stimulants of bone inflammation. Furthermore, we verified that blockade of phosphorylation of ERK1/2 in osteogenic lineage cells was sufficient to prevent production of aforementioned pro-inflammatory cytokines. Such disruption of ERK1/2 phosphorylation alone was sufficient to suppress LPS-, TNF- or titanium particle-induced calvarial osteolysis in αsTR-DN-MAPK1 mice. Our results unravel robust innate immune inflammatory function of cells of osteogenic lineage. A concept of using MAPK inhibitors as a new way of managing bone inflammation.
Hereditary Multiple Exostoses: Pathogenesis and Possible Treatments

Maurizio Pacifici

Division of Orthopaedic Surgery, The Children’s Hospital of Philadelphia

Hereditary Multiple Exostoses (HME) is a pediatric skeletal disorder characterized by benign cartilaginous tumors called exostoses that form next to the growth plates of long bones, ribs, vertebrae and other skeletal elements in both children and young adults. Because of their location, size and large number, the exostoses can cause a number of health problems that include growth retardation, skeletal deformities, chronic pain, blood vessel and tendon compression, and early onset osteoarthritis. In about 2 to 5% of HME patients, the exostoses can progress to malignancy, turn into osteosarcomas and thus become life threatening. The majority of HME cases are linked to heterozygous loss-of-function mutations in \textit{EXT1} or \textit{EXT2} that encode Golgi-associated glycosyl-polymerases responsible for heparan sulfate (HS) synthesis, leading to systemic HS deficiency in the patients. Recent studies by our group using mouse models of HME have clarified how the HS deficiency leads to exostosis formation, what progenitor cells are involved, what mechanisms are altered to instigate and sustain exostosis growth, and what skeletal sites are more prone to exostosis formation. I will also summarize data from in vitro models that have allowed us to clarify aspects of the cellular and molecular pathogenesis of HME and in particular the roles of signaling pathways. The latter studies have pointed to possible therapeutic possibilities and options that are being explored in animal models at the moment. In sum, our studies have provided novel insights into HME pathogenesis and are paving the way toward a better understanding of its intricacies and testing of possible treatments.
Molecular Pathogenesis of Osteosarcoma

Richard Gorlick

Richard Gorlick, MD, Professor of Pediatrics and Molecular Pharmacology, The Albert Einstein College of Medicine of Yeshiva University, Vice Chairman, Division Chief of Hematology/Oncology, Department of Pediatrics, The Children's Hospital at Montefiore, Bronx, New York 10467

OBJECTIVES: Osteosarcoma is the most common primary bone cancer that affects adolescents and young adults. Although it is a well-defined clinical entity easily recognized by its radiographic and pathologic appearance, numerous questions about its pathogenesis exist including the cell of origin and the specific genetic events leading to osteosarcoma formation. A series of experiments have been performed to address these questions.

METHODS: In an attempt to deduce the cell of origin of osteosarcoma, mesenchymal stem cells, pre-osteoblasts and osteoblasts have been serially transformed with SV40 large T antigen, hTERT and activated RAS. The mesenchymal stem cells have been serially transformed with SV40 large T antigen, hTERT and beta-catenin as well. Numerous functional assays have been performed on the resulting cells.

RESULTS: Introduction of SV40, hTERT and activated RAS into mesenchymal stem cells results in an undifferentiated sarcoma. Replacement of activated RAS with beta-catenin results in cells with enhanced osteogenic differentiation and inability to differentiate into the adipogenic lineage but these cells are not fully malignant. Introduction of SV40, hTERT and activated RAS into pre-osteoblasts results in cells capable of producing a tumor with the histologic appearance of an osteosarcoma but without the capacity for pluripotent differentiation which is usually present.

CONCLUSIONS: These results considered together suggest the cell of origin of osteosarcoma is a cell that is less mature than a pre-osteoblast. Studies are being performed to identify markers of intermediates in mesenchymal stem cell differentiation to pre-osteoblasts in order to more precisely define the cell of origin of osteosarcoma.
Gaucher’s disease (GD), the rare genetic disease affecting multiple organs, including bone and joints, is caused by the mutations of lysosomal enzyme glucocerebrosidase (GBA). Herein we report that deficiency of progranulin (PGRN), a growth factor with a unique structure and multiple functions, also causes GD unexpectedly, even if the level and activity of GBA are not decreased. Both ovalbumin-challenged and aged PGRN-deficient mice exhibit signs of GD, and relevant tissues are infiltrated with “Gaucher cells”, i.e., macrophages that show a characteristic “crinkled paper” cytoplasmic appearance resulting from the accumulation of glucosylceramide, the substrate of GBA. In addition, aged PGRN deficient mice display features of osteopenia in long bone and vertebrae, a well-documented symptom of GD. Recombinant PGRN promotes glucosylceramide clearance in PGRN-deficient macrophages and prevents the GD development in PGRN-deficient mice. PGRN binds directly to GBA and is required for the delivery of GBA to lysosome. Unbiased Mass Spectrometry approaches identify heat shock protein 70 (HSP70), an evolutionarily conserved molecular chaperone, as a GBA-associated protein that mediates trafficking of GBA through PGRN as an indispensable adaptor. Collectively, these findings not only demonstrate that PGRN is a novel co-chaperone of HSP70-mediated trafficking and plays an essential role in the GBA lysosomal delivery, but they also provide a new paradigm to guide therapeutic interventions for various HSP70-mediated pathologies and rare musculoskeletal diseases, including GD.
Biochemical MRI of Articular Cartilage: T1rho and T2 Mapping as a Noninvasive Biomarker of Early Cartilage Degeneration

Karen Sperling, MD, John Hardin, MD, Chris Peng, PhD, Everett Lai, MS, William Walter, MD, Erin Fitzgerald, MD, Ajay Lall, MD MS, Martin Levy, MD, Tony Wanich MD, Daniel Leong, PhD, Herb Sun, PhD, Neil Cobelli, MD

Objective: The development of DMOADs (disease modifying osteoarthritis drugs) has been hampered not only by the complex pathogenesis of osteoarthritis (OA), but by the relative lack of noninvasive biomarkers which can be used to detect and monitor early signs of cartilage degeneration. The purpose of this study was to determine whether knee pain or internal derangement including meniscal tears, ligamentous injuries or synovitis correlates with quantifiable changes in articular cartilage detected by biochemical MR imaging.

Materials and Methods: T1rho and T2 mapping, as well as routine MR sequences were performed on 52 (patients referred for pain and internal derangement of the knee. Pain, stiffness and functional limitation was assessed using KOOS questionnaires. Semiquantitative analysis of structural abnormalities using WORMS (Whole Organ MR Scoring) was performed and correlated with Quantitative measurements of T1 rho and T2 mapping of articular cartilage. Quantitative analysis was performed using dedicated in house developed software, with manual segmentation of discrete compartments on the medial and lateral femoral and tibial joint surfaces.

Results: Preliminary data demonstrates elevations of T2 and T1 rho in the setting of acute ACL injuries, particularly in segment at the posterior lateral tibia. Degree of synovitis did not show statistically significant relationship with T2 values. Full data and analysis will be presented.

Conclusion: T1 rho and T2 mapping show quantifiable changes in some but not all articular cartilage segments in the setting of various knee pathologies. Further longitudinal studies are necessary to assess the significance of these findings.
INTRODUCTION: Osteoarthritis (OA) is the most common form of musculoskeletal disease in the United States, affecting approximately 30 million people [1]. Symptoms of OA often only present after years of relatively slow progression. However, OA also occurs subsequent to traumatic joint injury, such as Anterior Cruciate Ligament (ACL) rupture in the knee. This specific circumstance leads to Post Traumatic Osteoarthritis (PTOA), which is a sub-set of the broader condition and makes up 12% of the overall disease burden in the US with associated costs of up to $3 billion annually [2]. The current paradigm for PTOA is that while acute injury eventually causes cartilage degradation, multiple joint tissues are involved from the outset and contribute to overall eventual ‘joint failure’. However, relatively little is known about the concomitant damage of other joint tissues. Since the knee is supported by stiff bone and calcified cartilage, it seems reasonable to propose that those tissues are also damaged by the acute injury, for example by generation of microdamage. Bone remodeling is also known to be involved in the early initiation and progression of OA. Recent intriguing data relates these two phenomena by showing that microdamage induces production of pro-resorptive factors in osteocytes following fatigue loading [3]. The subchondral region has a unique microarchitecture with three distinct regions: Calcified Cartilage (CC), Cortical Plate (CP) and Trabecular Bone (TB). It is not always recognized that these compartments differ not only morphologically but also mechanically and physiologically. Thus, if microdamage does occur following acute knee injury, and if it stimulates remodeling, then characterizing its spatial distribution among the subchondral tissues will be crucial to understanding this system. Here we characterize an in vivo model for ACL rupture, and resultant subchondral microdamage and quantify microdamage according to subchondral mineralized tissue-type as well as increased bone turnover as measured by dynamic histomorphometry.

METHODS: We modified the standard in vivo tibial axial loading configuration to include rotational and valgus components (Figure 1), both of which are known to contribute significantly to ACL rupture in humans. Under anesthesia, the knees of female Sprague Dawley rats (n=16, IACUC approved) were fixed at 80° flexion, using a novel custom-made fixture, and subjected to a compressive loading ramp (rate 0.1 mm/s) until rupture of the ACL was detected. Outright rupture of the ACL was the test endpoint and was defined by a rapid drop in load which was recorded in the system control software. Animals were sacrificed at days 0, 14, 28 and 56 days post injury. Fluorochrome labels were administered at the time of injury and then again 2 days prior to sacrifice. In each animal the contralateral limb served as the control. At necropsy, samples were dissected free of soft tissue, fixed in 10% NBF and either stained en bloc with basic fuchsin, using standard protocols for microdamage analysis, or processed directly for dynamic histomorphometry.

RESULTS: Animal weight was 278.94±18.4[g] and ACL failure load was 65.8±11.4 [N] with coefficients of variation at 6.6 and 17.3[%], respectively. Gross observation confirmed the rupture of ACL in all tested joints, with no evidence of ligament damage in the control group. In the day 0 group, microdamage was found in all three of the subchondral compartments of tested joints and microcrack density was calculated as 6.69±3.64, 2.65±0.65 and 2.84±0.80 [#/mm²], in CC, CP and TB regions, respectively (Figure 2). These data suggest that microdamage does indeed occur in the subchondral mineralized tissues following acute joint injury, at least in our model system. Furthermore, this suggests that each subchondral tissue is differentially affected – importantly, if the CC and CP regions prove to have osteoclastic activity co-localizing with microdamage this may have important consequences for disease progression in terms of porosity, vascular invasion and bone-cartilage cross talk. Initial data indicate that increased uptake of fluorochrome labels was present in injured joints after 4 and 8 weeks (Figure 3). Furthermore, preliminary data from standard OA scoring on histological sections stained with Safranin O also suggest that cartilage degradation is present after 2, 4 and 8 weeks in these animals.

DISCUSSION: Here we characterize a novel animal model for ACL rupture, which generates subchondral bone microdamage differentially among the subchondral bone structural units as well as subsequent bone resorption. Many of the current animals models of PTOA induce the disease via a surgical procedure or and injection of catabolic factors into the joint capsule. These approaches do not replicate the mechanical environment of injury faithfully since the mechanical overload, which must pass through the joint at the time of injury, is missing. Therefore, by incorporating those aspects, our model allows us to study the contribution of microdamage in the subchondral regions to disease initiation and progression in the overlying cartilage and other surrounding tissues. The magnitude of the failure loads in our study were carefully monitored, while different positioning of the joint during testing would allow lower failure loads, 65N is approximately equivalent to 20X BW in this model. This compares well with the joint reaction forces experienced in the human injury condition. Microdamage appears to be differentially distributed among the three subchondral mineralized tissue regions following this testing protocol. This appears to be related to the remodeling response to injury in this model. Figure 3 shows evidence of increased uptake of fluorochrome labels in the subchondral bone of these animals 4 weeks after injury. We also report that evidence of degradation of the overlying cartilage is also present at this time-point (data not shown). Taken together, these data suggest that there may be a direct link between microdamage in subchondral bone, the subsequent remodeling response and the eventual cartilage break down that characterizes PTOA.

SIGNIFICANCE: These data are particularly significant at the translational level. Although the role of subchondral bone in OA has been investigated before, there has never been a clear link between acute microdamage, the subsequent biological responses and eventual cartilage degradation. From a translational perspective, this work may lead to development of novel early intervention bone-targeted treatment strategies, once the underlying cell and molecular mechanisms of this injury-induced disease process are fully characterized and understood.

ACKNOWLEDGEMENTS: New York School of Medicine Department of Orthopaedic Surgery. Austin Ramme for technical assistance.

Title: Mechanisms of mineral metabolism during pregnancy and lactation in mice with X-Linked Hypophosphatemia (XLH)

Steven M. Tommasini, Ph.D.

Abstract:
In postmenopausal women, estrogen loss and vitamin D deficiency are associated with a decline in intestinal calcium absorption. The negative calcium balance leads to increased serum PTH levels and, consequently, age-related bone loss. Further, continuous administration of PTH also has catabolic effects on the skeleton and hyperparathyroidism is associated with increased cortical porosity, similar to that described in post-menopausal osteoporotic bone. A better understanding of cortical bone loss would provide new targeted therapies for osteoporotic fractures, which affect nearly 200 million women worldwide, accounting for more days hospitalized than many other diseases, including diabetes, myocardial infarction, and breast cancer. Given the lack of an *in vivo* model, the etiology and mechanisms underlying increased cortical porosity are poorly understood. Our goal is to develop an evidence-based rationale for therapies to treat osteoporosis characterized by a negative calcium balance and elevated PTH. We investigated bone loss using a murine model that phenocopies increased cortical porosity in the face of increased mineral demand. During pregnancy, maternal adaptations to high mineral demand include more than doubling intestinal calcium absorption by increasing vitamin D production. However, in patients with X-linked hypophosphatemia (XLH), vitamin D production and intestinal calcium absorption are impaired. Therefore, the pregnant Hyp mouse, a murine model of XLH, is an ideal model for studying the skeletal effects of impaired mineral homeostasis. Further, to accommodate the increased mineral demands of lactation, Hyp mice increase intracortical remodeling similar to that described in post-menopausal osteoporotic bone. We hypothesized that, with impaired vitamin D production and intestinal absorption of phosphate and calcium, pregnancy in the Hyp mouse will lead to amplified cortical bone resorption, as cortical bone is the largest reservoir of mineral in the Hyp mouse. The increased resorption resulted in a bony phenotype similar to that seen in secondary hyperparathyroidism including increased cortical porosity. The data revealed dramatic increases in intracortical porosity in Hyp mice characterized by elevated serum PTH, increased vascularity, and elevated type-I collagen matrix-degrading enzyme MMP-13. By studying a novel model of cortical remodeling, we aim to better our understanding of the interactions of vitamin D, mineral homeostasis, and PTH on bone strength.
Stem cell activation during neonatal healing
Kristen Howell, Ronen Schweitzer, Alice H. Huang

In adults, tendon healing is thought to be regulated by extrinsic cells residing within the epitenon or tendon sheath that migrate into the injury and produce scar tissue [1, 2]. In contrast, embryonic tendons heal without scar, via a regenerative pathway that is intrinsic to fetal cells [3, 4]. This regenerative potential may be retained even after birth, as tendons injured during a neonatal stage (postnatal day 7, P7) recover matrix and mechanical properties faster compared to older juvenile stages [5]. However, the full potential for regeneration in neonatal tendon remains unknown and the biological mediators have yet to be identified. The purpose of the current study was therefore to evaluate neonatal tendon healing using a full transection model of acute tendon injury.

In Study 1, the Achilles tendon in the right hindlimbs of P5 ScxGFP pups was transected at the mid-substance and left unrepaired, in accordance with IACUC protocol, while the left hindlimbs were used as non-injured contralateral controls. Mice were sacrificed 3, 10, and 14 days after injury (n=3) and transverse cryo-sections were obtained and imaged via fluorescence microscopy. Since the tendon-muscle unit is ordinarily under tension, transection of the tendon resulted in immediate retraction of the muscle, leaving an empty ‘gap’ space between the original Achilles tendon stubs. Transverse sections of hindlimbs 3 days after tenotomy showed intense proliferation within the original tendon stubs, compared to the contralateral control limb, and more limited proliferation within the gap space (not shown). At this time, the gap space was completely devoid of ScxGFP+ cells. By day 14, a continuous and aligned ScxGFP+ ‘neo-tendon’ had formed within the gap space, connecting the original tendon stubs, suggesting that rapid regeneration of tendon may be possible in neonates. In Study 2, we investigated the cellular mediators of neonatal tendon healing, which we hypothesized may be tendon stem cells. Although multi-potent, colony-forming cells have been isolated from tendons [6], the source and identity of these cells and their in vivo activity remains unclear. One well-established method to identify potential stem cells is via pulse-chase labeling; a low dose of tamoxifen is given to sparsely label cells, and clonal expansion is determined at a later timepoint. As there are no definitive markers for tendon stem cells, we evaluated the expression of Sox9, which has been used to identify stem cells in a number of tissues, including hair follicles, skin and intestines [7-9]. Furthermore, very early tendon progenitors are also derived from a Sox9-expressing population (although Sox9 is not expressed at later embryonic stages) [10, 11]. Using the inducible Sox9CreERT2 line [10] combined with the AIt4 Rosa26-TdTomato Cre reporter (RosaT) [12], we labeled putative stem cells via tamoxifen administration at P0 and found that Sox9-lineage cells were largely localized within the tendon (ScxGFP+), although a few ScxGFP- cells near the epitenon were also labeled (not shown). Immunostaining for laminin to highlight the epitenon showed that the Sox9-lineage cells were laminin-negative, and thus were distinct from epitenon cells. To evaluate whether Sox9-expressing cells may represent a resident tendon stem cell population, tamoxifen was given at P2 and P3 to Sox9CreERT2;RosaT;ScxGFP pups to label Sox9+ cells prior to Achilles tenotomy at P5 (1.25 mg/pup). While control tendons showed sporadic labeling of tenocytes (ScxGFP+) at day 10 after tenotomy, the injured tendon showed dramatic expansion and localization of RosaT+, Sox9-lineage cells near both cut ends of the original Achilles stubs. Transverse sections taken through the gap region also revealed a high density of Sox9-lineage cells within the forming ‘neo-tendon’, co-expressing ScxGFP. Finally, in Study 3, we performed Achilles tenotomy on 1-year old adult ScxGFP mice (n=3) and examined the hindlimbs 14 days after injury. We found that at 1 year of age, ScxGFP expression was markedly reduced in all tendon. Interestingly, the injured Achilles tendon stubs showed dramatic re-activation of ScxGFP relative to the other tendons in the limb and in the contralateral control. In all 3 mice, we found formation of scar tissue bridging the tendon stubs; however, the neo-tissue remained ScxGFP-negative, suggesting there may be distinct mechanisms that regulate neonatal vs adult tendon healing. Although fibrosis is the default pathway in the healing of many adult tissues, a few recent studies suggest that neonatal tissues such as the heart and cochlear hair cells have the capacity to activate regenerative pathways, either through progenitor/stem cell types or through differentiated, lineage-restricted cells [13, 14]. In these studies, we showed that neonates re-established a ScxGFP+ neo-tendon after full transection injury, and that neo-tendon formation is mediated by Sox9-lineage cells. We further showed that adult tendons healed via formation of ScxGFP-negative tissue. Although these results are intriguing, additional analyses of tendon-specific markers and later timepoints are required to determine whether neonatal tendon healing is truly regenerative. While Sox9CreERT2 largely labeled tenocytes, a few ScxGFP-negative cells near the tendon were also labeled - future studies will therefore use the ScxCreERT2 line to distinguish the activity of these cell populations during neonatal healing. Collectively, this work will establish a foundation for future experiments to test gene function in cell recruitment and tendon differentiation, and to distinguish the mechanisms that regulate fibrosis vs tendon-specific differentiation, thus defining targets for future therapies.

Muscle and bone are known to act as a functional unit and communicate biochemically during tissue development and maintenance [1]. The muscle-bone crosstalk appears to be bi-directional. Muscle-derived factors (myokines) have been found to affect bone functions in vitro [2]. Bone marrow-derived mesenchymal stromal cells (MSCs) and mechanically stimulated MLO-Y4 osteocytes were found to be capable of modulating muscle functions [3,4]. The cross-talk could occur through vasculature and/or interstitial pathways [5]. In this talk, I will focus on our recent studies in quantifying the transport characteristics of the local interstitial pathway. This interstitial consists of the hierarchical network of muscle extracellular matrix (endomysium, perimysium, and endomysium) [6], the fibrous collagen-rich periosteum, as well as the mineralized bone tissues.

I will share two lines of our research, which help us to gain quantitative understanding of the muscle-bone cross-talk mechanisms. First, I will introduce our approach that combines fluorescence confocal imaging and mathematical modeling (Fig. 1) and our findings of the solute/fluid transport characteristics such as diffusivity and convection inside bone [7,8,9] and characteristic transport time and solute diffusivity across periosteum (Fig. 2) [10]. Second, the hydraulic permeability of skeletal muscles measured from large and small animal species through creeping tests and finite element analysis (Table 1) will be presented. These quantitative measures allow us to determine the overall permeability of the interstitial transport pathway and to identify the barriers for such muscle-bone cross-talk. In particular, these transport constrains will help biologists to identify potential agents for the cross-talk.


Transport Mechanisms for Muscle-Bone Cross-Talk
Liyun Wang, Ph.D.
Department of Mechanical Engineering, University of Delaware

Fig. 1. Confocal imaging of fluid and solute transport in bone. (A) setup. (B) Loading profile. (C-D) Fluorescence recovery after photobleaching (FRAP) technique. (E-F). data analysis and modeling to extract diffusivity and convection of solute in bone (ref.8).

Fig. 2. Penetration times for several myokines in periosteum were predicted based on the empirical relationship derived from experimental data (ref. 10).
<table>
<thead>
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<th>Mechanical Property</th>
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<td></td>
<td>Relatively Young (N = 18)</td>
<td>Relatively Old (N = 3)</td>
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<td>Age = 11.9±1.4 weeks</td>
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<td>Matrix Modulus (kPa)</td>
<td>4.1 ± 1.7</td>
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<td>3.2 ± 1.4</td>
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Note: Values are presented as mean ± SD. Pairwise comparisons were performed using one way ANOVA and Tukey tests. $p^1$: young murine vs. old murine. $p^2$: young murine vs. bovine. $p^3$: old murine vs. bovine.
Cartilage and bone cross-talk via mechanically sensitive microRNA, Qian Chen, Kun Yang, Dept of Orthopaedics, Alpert Medical School of Brown University/Rhode Island Hospital, Providence, RI

Background and Objective: Growth plate chondrocytes are critical for endochondral bone elongation during skeletal development. MicroRNAs play important roles in this process since cartilage specific suppression of miRNA expression in Dicer-null growth plates induces severe skeletal growth defects. MiR-365, isolated by microarray analysis for mechanical loading stimulated miRNAs, is upregulated by cyclic loading of growth plate chondrocytes. It is both necessary and sufficient for mechanical stimulation of chondrocyte proliferation and differentiation in vitro by directly targeting histone deacetylase 4 (HDAC4). The role of miR-365 in skeletal development in vivo, however, is unknown.

Methods: To study the effect of cartilage specific expression of miR-365 on skeletal development, we created conditional miR-365 transgenic mice with LoxP/Cre system and bred them with col2a1-Cre mice to drive miR-365 expression specifically in chondrocytes.

Results: MiR-365 expression level is upregulated for 6 fold in cartilage tissues, which is similar to the extent of miR-365 upregulation by mechanical loading. These miR-365 transgenic mice exhibited premature ossification, accelerated secondary ossification formation and increased bone mineral density.

Discussion and Conclusion: Cartilage-specific expression of miR-365 affects post-natal skeletal development in vivo. Enhancing bone mineral density by up-regulating miR-365, either by cyclic loading or by therapeutic administration may provide a potential means to counter bone loss and/or to accelerate fracture healing process.

Acknowledgement: This study is supported by P20GM104937.
Remodeling of Bone Tissue on Bisphosphonates

J. Christopher Fritton, Ph.D.

Department of Orthopaedics, NJ Medical School, Rutgers University, Newark, NJ

The detection of damage signals by osteocytes within bone tissue is critical to normal targeted remodeling, or turnover of bone tissue. This detection may be influenced by drugs, diet and exercise.

Bisphosphonate drugs were specifically designed to combat osteoporosis by reducing bone tissue remodeling. We have found in cortical bone tissue that bisphosphonates also appear to reduce the density of osteocyte lacunae, the production of normal-sized new microstructural elements (osteons) and the apparent-tissue elastic modulus. With long-term use these alterations may lead to a reduction in bone’s fracture resistance under cyclic fatigue loading and could offer a partial explanation for the association of bisphosphonate treatment with low-energy, atypical fractures of cortical bone.

While bisphosphonates are taken by millions of older individuals to reduce fracture risk at the hip and spine, they may also be of benefit for reducing bone loss in the relatively young astronaut during and after long-term space flight. Any possible side-effect in these otherwise very healthy individuals should be minimized. We have been examining the mechanical consequences of suppressed remodeling with a bisphosphonate under conditions of immobilization (6 months), followed by remobilization (12 months). Our results suggest that at the cortical-tissue level, recovery of mechanical toughness may take longer than the time (twice the immobilization period) thought to recover cortical structural properties, regardless of previous treatment with a bone-sparing bisphosphonate.

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Musculoskeletal pain arises from nocioceptor activation in muscle, joints, bone and other tissues due to inflammatory and other local injury processes. Neuropathic pain can also arise through excitation of sensory neurons by direct compression, chemical stimuli, infection or lesion of peripheral nerve. Cell bodies of the sensory neurons are located in dorsal root ganglia along the spinal cord and neck where they are outside the blood brain barrier and thus are potentially more accessible to pharmacological therapy than central targets. Although their excitability and other properties may be modulated by glia within the ganglia, sensory neurons make their first synapses in the dorsal horn of the spinal cord. From there, the ascending pathways separate, the slower nocioceptor C and Aδ fibers traveling along the spinothalamic tract to the thalamus and cortex and the faster Aα, Aβ and some Aδ fibers mediating touch, pressure and temperature traveling along the dorsal column pathway, synapsing first in the medulla and then in the thalamus. Slower conduction velocity of nociceptors than other modalities create opportunities to “gate” pain, and descending pathways from the amygdala can modulate pain activity at each site of synaptic integration. Numerous ions and receptor types have been proposed as the “pain channel”, and it seems likely that at least several are commonly activated by nociceptive stimuli, including transient receptor potential (Trp) and other types of potassium channels, certain type 2 purinergic (P2) receptors and certain types of sodium and calcium channels. A variety of receptors at relay sites is endogenously modulated by cytokines and growth factors and has been therapeutically targeted, and novel cell targets include central and peripheral glia and new molecular targets are gap junctions and pannexins. In this presentation we will describe use of various techniques to quantify behavioral phenotypes and mechanisms in mouse models of pain and how we are beginning to apply them to musculoskeletal disease.
Repair of Dense Connective Tissues via Biomaterial-Mediated Matrix Reprogramming of the Wound Interface

Robert L. Mauck, PhD

Mckay Orthopaedic Research Laboratory
University of Pennsylvania
Translational Musculoskeletal Research Center
Philadelphia VA Medical Center

The soft tissues of the musculoskeletal system are vital for the efficient and pain free execution of the activities of daily living. However, as a direct consequence of their central role in load bearing activities, these tissues are commonly injured, and present a frustrating scenario for the clinical repair. Namely, these tissues all have an intrinsic healing response that can best be described as ‘poor’, and at worst as ‘nonexistent’. Intrinsic repair of these tissues in adults is limited in part by the challenging mechanical environment in which healing takes place, but also by the dense matrix composition (which enables load bearing) and the low density of endogenous cells. These latter two issues limit the number of native tissue and endogenous progenitor cells that can successful migrate to the wound interface to participate in repair.

To address this deficit, we have developed a structurally motivated tissue engineering approach based on organized nanofibrous assemblies. These scaffolds are composed of ultra-fine biodegradable and biologic fibers that recreate the structural anisotropy typical of fiber-reinforced tissues, and can also serve as a 3D micro-pattern for directed cellular behavior and a template for new tissue formation. Expanding the functionality of such scaffolds, we have developed novel fabrication strategies to instill dynamic and multi-functional characteristics (including controlled drug delivery) to enhance scaffold colonization. In this talk, we will discuss new technology designed to address limitations in tissue repair that arise as a consequence of decreased cell mobility through the dense ECM. Specifically, we will describe a new biomaterial mediated approach that improves native tissue repair via local reprogramming adult tissue.

Using nanofibrous systems engineered to deliver a controlled level of degradative enzyme, we will illustrate reprogramming of the adult ECM to a ‘fetal-like’ tissue state in terms of matrix density and mechanics. Using these materials in a variety of in vitro and in vivo repair models (including a large animal meniscus repair model), we will show how this reprogramming of the wound interface leads to enhanced repair. This work has the translational potential to rapidly improve clinical treatment for a number of degenerative and traumatic injuries common to dense connective tissues for which there are no regenerative solutions.
Mechanotransduction in Musculoskeletal Tissue Regeneration Induced by Dynamic Acoustic Force and Fluid Flow

Yi-Xian Qin, L. Bi, M. Hu, M. Pritz, S. Uddin
Department of Biomedical Engineering
Stony Brook University, Stony Brook, NY
Email: Yi-Xian.Qin@stonybrook.edu

Introduction: Mechanotransduction has demonstrated potentials for tissue adaptation in vivo and in vitro. Although a wide range of studies have been done, mechanism for this mechanical effect on bone regeneration is unknown and still under active investigation. A potential mechanism, by which bone cells may sense mechanotransductive signals, is through deformation and streaming of bone cells and their surface structures, to trigger osteogenesis. The purpose of this study was to evaluate the role of acoustic radiation force (ARF) in bone remodeling and osteogenesis, and biological responses of stem cells to induced fluid flow and mitigation of osteopenia.

Method: In Vivo: Closed femoral fracture procedures were performed on thirty-six Sprague–Dawley rats after they were randomly divided into three groups. In group FSU, the rats were disuse-induced by HLS, and ARF treatment was administered daily to the fractured femur; in group FSC, the rats were HLS but without ARF treatment; in group NFC, no HLS and ARF treatments were applied after fracture. In vivo μCT was used to exam the fractured femur immediately and two weeks after fracture. Five weeks later, femurs of fracture were harvested and subjected to ex vivo μCT evaluation and four-point bending to assess morphometric and mechanical properties. Undecalcified samples of fracture healing areas were embedded by polymethylmethacrylate and sectioned and stained with Von Kossa and hematoxylin-eosin.

In Situ: Female C57BL/6J mice of 3 months old were used to obtain fresh femur samples immediately after euthanasia. For dynamic fluid flow loading, the drilled hole of each femur sample was tightly sealed by a 24-gauge catheter that connects to a fluid flow pump loader with loading frequencies of 1 Hz, 10 Hz, and 20 Hz stimulation (n = 4 per group) for 10 sec of baseline, 30 sec of loading, and 10 sec of post loading. Real time confocal imaging with 40X objectives, 488-nm laser excitation, and 2 frames/sec was performed to capture the calcium signals of the osteocytes within the bone that was subjected to dynamic fluid flow stimulation. The fluorescent intensity of the cell body was extracted as a function of time using the microscope software. By normalization to the baseline intensity, the percentage of response cells, calcium spike magnitude, and the time to the first spike were quantified.

Results: In Vivo: Although there were no significant changes of morphometric properties proved by in vivo μCT among the three groups at the 0 and 2 weeks (P>0.05), ex vivo μCT showed 30% (p<0.05) bone volume increase in treated group than the control in 5 weeks. 4-pt mechanical bending test showed 5-week ultrasound treated group was 48% (p<0.05) higher than the sham control in Young's modulus. Histological staining showed a higher ratio of lamellar bone formed in the fracture areas of FSU (36%), compared to control groups (20%). In contrast, only cartilage-like tissues and wove bone formed in the fracture areas in the NFC and FSC groups.

In Situ: At baseline level, osteocytes were imaged at a focal plane ~40mm below the periosteal surface. Dynamic fluid flow stimulation at 1 Hz did not induce any calcium response within the osteocytes. However, 10 Hz and 20 Hz of loading lead to 95% and 68% of responsive cells, respectively. Results of peak magnitude normalized to the baseline value showed that loading of 10 Hz lead to 10% greater calcium spike magnitude compared to 20 Hz of loading. Moreover, loading of 10 Hz exhibited about 21% shorter calcium initiation time compared to 20 Hz of loading.

Discussion: In a functional disuse in vivo model, ARF has shown significance in promoting initial callus forming and mineralization, as well as enhanced mechanical strength in fracture. It is indicated that ARF induced fluid flow can regulate osteogenesis, and enhance fracture healing. Direct fluid flow stimulation indicated that that osteocytes displayed calcium spikes in response to in situ dynamic fluid flow stimulation into intact mouse femur. The number of responsive cells, calcium spike magnitude and initiation time were dependent on the loading frequencies. Real time observation of the calcium signals clearly indicated the instant in situ osteocytic response to the mechanical signals derived from dynamic fluid flow stimulation, providing better understanding of cellular mechanism of tissue regeneration via mechanotransduction.
Regeneration of tissues that function as native replacements remains to be broadly realized. A common approach for tissue regeneration is cell delivery, including stem cells that are transplanted directly or as committed tissue progenitors. However, cell-based therapy encounters several barriers in translation towards clinical therapeutics. Immune rejection, pathogen transmission, potential tumorigenesis, packaging/storage/shipping, and anticipated difficulties in clinical adoption, cost reimbursement and regulatory approval are among some of the roadblocks. Economic viability of cell delivery, especially if it requires substantial ex vivo cell manipulation, is far from trivial. I will present emerging data from my laboratory and others in several recent reports that chemotactic cell homing is responses for the regeneration of multiple and, in some cases, complex tissues, such as dermal, muscle, dental, cardiac, cartilage and bone. These data suggest an emerging concept that single or complex tissues can regenerate by the homing of endogenous cell lineages and potentially without cell transplantation. A multitude of approaches will be discussed to orchestrate cell homing including active recruitment of host endogenous cells by chemokines, cytokines, drugs, polymeric materials and bioengineering models. Information on the mechanisms of cell homing will be explored primarily by in vitro studies of cell migration, cell recruitment and cell motility in 2D and 3D models. Endogenous stem cells may accelerate clinical application of stem cell technology.
Low back pain is the second most frequent cause of doctor visits and is commonly associated with defects in the intervertebral disc (IVD) including herniation and annulus fibrosus defects. There are few minimally invasive treatments available to repair defects in the IVD, or to augment surgical microdiscectomy procedures with annulus fibrosus closure techniques. The objectives of this work are to analyze multiple strategies for annulus fibrosus repair using adhesive biomaterials and space filling scaffolds. Fibrin is a commonly used adhesive sealant that can be enhanced with genipin crosslinking (FibGen) in order to stabilize and stiffen the material. This presentation describes several formulations of FibGen for biomechanical repair, and as a carrier for delivery of drugs and cells. Experiments involve biomechanical performance, drug delivery kinetics, organ culture and in vivo evaluations. Result indicate that FibGen offers promise as an annulus fibrosus repair material and can also serve as a carrier for delivery of bioactive components.

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