CDMI
CENTER FOR DISRUPTIVE MUSCULOSKELETAL INNOVATIONS

Integrated in vivo and in vitro high-throughput analyses of osteocyte-mediated bone remodeling

University: University of California San Francisco	
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Need and Industrial Relevance:

NEED: Understanding the control of bone mass has yielded essential therapies to treat bone fragility, including those that target parathyroid hormone or sclerostin ***(Figure 1)***. However, *at least half of people with fragility fractures do not have low bone mass, pointing to the importance of bone quality*.

A key aspect of bone quality is the material quality of bone extracellular matrix (ECM). Defects in the material quality of bone contribute to bone fragility in aging, diabetes, and other conditions. The deterioration of bone quality has significant orthopaedic and dental implications, including the integration of implants and osteonecrosis. Currently, *therapies for bone quality simply do not exist*.

Agents that control bone quality have great and untapped therapeutic potential for treating skeletal disease or for improving orthopaedic implant fixation. Because the protection and improvement of bone quality could be clinically transformative, *our long-term goal is to discover new diagnostic markers for bone quality and new therapies to treat it.*

INDUSTRIAL RELEVANCE: Toward the long-term goal of identifying novel therapies to improve bone quality and prevent bone fragility, this project aims to develop a novel, integrated panel of in vivo and in vitro assays to monitor the cellular control of bone quality by osteocytes, called OMBRE.

When implemented, OMBRE will support industry and basic science in the implementation of high-throughput screens (HTS) and performance of pre-clinical analyses to identify compounds that improve bone quality systemically or locally near orthopaedic devices.

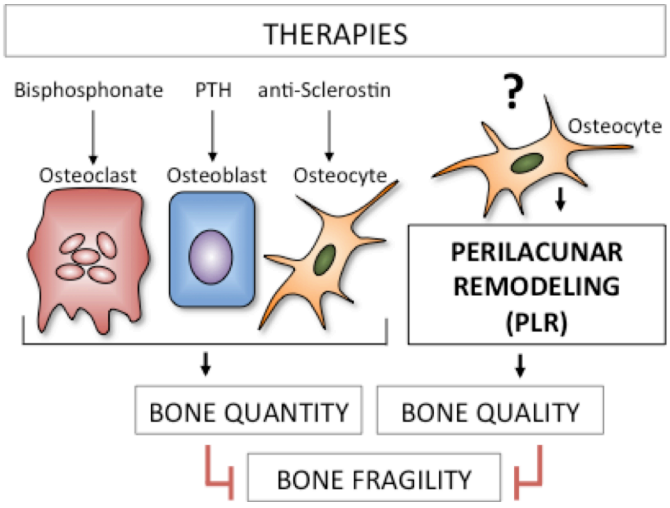

Figure 1: *Schematic of therapies targeting cellular participants in bone remodeling to improve skeletal health.*

Project Aims:

This project promotes the development of new therapies to improve bone quality and prevent bone fragility by streamlining the analysis of perilacunar remodeling by osteocytes ***(Figure 1)***. **Perilacunar remodeling (PLR)** is a dynamic process by which osteocytes dynamically resorb and then replace the local bone matrix. Recent studies by our group and others reveal that PLR maintains bone quality, systemic mineral homeostasis, and the lacunocanalicular network. Our ongoing studies strengthen the causal link between PLR, bone quality, and diseases ranging from osteoarthritis and diabetes to osteonecrosis and aging. Nonetheless, many questions remain about the regulation and role of PLR in skeletal health and disease, in part, because reliable outcome measures to effectively study PLR are still needed.

With partial support of our **2014 CDMI Project**, we developed in vivo outcome measures to investigate the effect of physiologic, pathologic, or pharmacologic interventions on PLR. Here we extend this effort by developing a comprehensive approach to evaluate PLR in vivo and in vitro, ultimately to advance the development of therapies to improve bone quality. Specifically, we aim to:

Aim 1: Develop in vitro measures of PLR function for high throughput screening.

Based on PLR-dependent changes in osteocyte pH and bone matrix resorption, we will develop and validate in vitro fluorescent PLR assays that can be used in high-throughput screens to identify novel PLR-regulatory compounds.

Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.

The OMBRE Core will provide novel, streamlined in vitro and in vivo analysis of PLR for basic, pre-clinical, or clinical studies.

When implemented, the OMBRE Core will support scientists, including those affiliated with the CDMI, by providing PLR analyses as they seek to identify new therapies to improve bone quality systemically or locally near orthopaedic devices.

Methods:

Aim 1: Develop in vitro measures of PLR function for high throughput screening

We propose to develop a novel high-throughput screen to identify compounds that regulate osteocyte-mediated PLR. We will work in partnership with the UCSF Small Molecule Development Center (SMDC), led by Dr. Michelle Arkin, to design, validate, and ultimately, perform this screen.

Based on their utility in our current studies, we plan to use MLO-Y4 osteocyte-like cells for our primary high-throughput screen (HTS). Our multiplexed screen will use multichannel fluorescence to detect cell viability, in addition to two functional PLR outcomes, namely intracellular pH and the expression of a promoter/reporter construct for a prototypical PLR gene, such as MMP13. Intracellular pH will be measured using a fluorescent SNARF dye, which will show a drop in pH as osteocytes acidify their microenvironment to induce PLR. Key milestones include development and validation of the pH assay, identification of a prototypical PLR gene, cloning and validation of a PLR gene promoter-reporter construct, and adaptation of these assays into a multiplex assay.

Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.

The OMBRE Core will develop and perform a series of protocols that provide a comprehensive approach to evaluate the role and regulation of osteocyte-mediated perilacunar remodeling in bone health and disease. This research is needed to advance the development of therapies to target

perilacunar remodeling to improve bone quality.

This Core will integrate and streamline a suite of rigorous histologic, cellular and molecular analyses for in vitro studies and for in vivo analysis of specimens from mouse, rat, and human bone. In vitro analyses will derive from the studies proposed in Aim 1.

In vivo protocols include 1) analysis of collagen organization using picrosirius red staining, polarized light microscopy, and ImageJ, 2) analysis of lacunocanalicular networks using Ploton silver stain and Image J, and 3) analysis of PLR gene expression using a series of validated PLR gene primer sets. We have observed surprising levels of variability in PLR and the ability to detect it across this range of conditions. Therefore, protocol optimization will be done in an iterative fashion until the methods can be reproducibly performed on mouse and human femora and mandible. Validated protocols and service will be made available to CDMI members as Core services.

Milestones:

- Identify prototypical PLR-inducible gene for 1st *in vitro* PLR functional outcome – Dec 1, 2016
- Final protocol for OMBRE I: Collagen Organization – January 15, 2017
- Finish development of 2nd *in vitro* PLR functional outcome: intracellular pH assay – February 1, 2017
- Final protocol for OMBRE II: Canalicular Stain – March 1, 2017
- Finish cloning and sequence validation of novel PLR-reporter construct – April 1, 2017
- Final protocol for OMBRE III: PLR Gene Expression – May 1, 2017
- Finish validation of both *in vitro* PLR outcomes for high-throughput screen (HTS) – July 1, 2017
- Final protocol for OMBRE IV: In vitro intracellular pH Assay – August 1, 2017
- Complete pilot HTS using *in vitro* PLR outcomes – September 1, 2017
- Final protocol for OMBRE V: In vitro PLR Reporter Assay – September 30, 2017

Deliverables:

- *December 2016 – conference call*
- *Spring 2017 – Spring Symposium @ UT (conference call option for non-UT teams)*
- *June 2017 - OMBRE Protocols I-III*
- *June 2017 – conference call*
- *September 2017 – Fall Symposium @ UCSF (conference call option for non-UCSF teams)*
- *October 31, 2017 - Final written report including results and OMBRE Protocols I-V*

General Budget Outline:

Personnel	$	24,000
Tissue Culture Supplies	$	6,000
Chemicals and Supplies	$	6,364
Total Direct	$	36,364
Indirects (10%)	$	3,636
Total	$	40,000

Start Date: October 2, 2016 **End Date**: September 30, 2017