Single-cell Spatial Biology in Bone Adaption and Regeneration

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Motivation

- Age-related bone loss has also been attributed to the decrease in mechanical usage of the skeleton.
- Conversely, it has been demonstrated that mechanical loading results in enhanced bone formation.
- Thus, a detailed understanding of the mechanobiological processes governing load-regulated bone remodeling down to single cell level will help identify new molecular targets for prevention and treatment of bone fractures.
- Today, I would like to take you on a multiscale journey through bone from biomechanics to spatial mechanobiology.
Human vertebral bone
Failure mechanisms in trabecular bone

Human vertebral bone

Müller, Nature Rev Rheumatol, 2009
Failure mechanisms in trabecular bone

Human vertebral bone

Thurner et al., Bone, 2006
C3\textit{lit} femur posterior cortex

Voide et al, Bone, 2009
Introduction

• Maintenance and adaptation of bone morphology results from an orchestrated remodeling process

• Locally coordinated by osteocytes with biochemical signals resulting in increased or decreased bone formation or resorption activities, also in bone regeneration

• To better understand bone adaptation and regeneration, we have to better understand how osteocytes sense and process mechanical loading within their local microenvironment

Ultra-high-resolution micro-CT of osteocyte lacunae displaying spatial variability in lacunar sphericity (Goff et al. Bone, 2022)
Mouse model of bone adaptation (C57BL/6)

Cyclic mechanical loading of the 6th caudal vertebra (CV6, tail)
3000 cycles, 10 Hz, 8 N, 3x/week for 4 weeks [Webster et al, 2008]

Weekly *in vivo* micro-CT measurements

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Lambers et al, Bone, 2011
Local mechanical feedback loop

*In vivo* micro-CT: bone remodeling
*In vivo* micro-FE: mechanical environment

Bone formation
Bone resorption

500 µm

High
Low

Mouse model of bone regeneration (C57BL/6)

- Time-lapsed in vivo microCT to discriminate
  - different healing phases
  - physiological versus impaired healing patterns
  - effects of mechanical loading
In vivo imaging of regeneration and healing in cyclically loaded bone

![Diagram showing bone formation and resorption in loaded and control conditions.](image-url)
Associating mechanics with bone regeneration
Linking mechanics and molecular spatially (2D-3D registration) - Correlative Multimodal Imaging

- 2D-3D affine transformation
- Locate cell positions from histology to microCT
- Multiscale analysis
  - Protein expression (e.g. RANKL, Sclerostin, Piezo1, YAP, TAZ)
  - Remodeling
  - Mechanical strain
Linking mechanics and molecular analyses - Prematurely aged CRISPR/Cas9 based fluorescent reporter mice

GFP mCherry DAPI

Bone formation Bone resorption

CONTROL

LOADED

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8/29/2022
In silico prediction of bone regeneration using micro-MPA

**cells**
- vasculature
- hematopoietic stem cell
- immune cell
- (pre-)osteoclast
- skeletal stem cell
- osteoblast
- lining cell
- (pre-)osteocyte

**cellular processes**
- motility
- differentiation
- proliferation
- cell respiration
- mechanosensation
- receptor-ligand binding
- cytokine & matrix production

**physical properties**
- mineral
- oxygen
- $\varepsilon_{\text{eff}}$
- cytokines
- reaction-diffusion
- mineralization
- diffusion
- micro-FE

murine femoral defect with simulated vascularization
In silico prediction of bone regeneration using micro-MPA
Spatial mechanobiology in osteocytes
Local *in vivo* Environment - LivE Imaging

http://www.rna-seqblog.com
Spatial mechanomics in single osteocytes

control

loaded

expression relative to hprt

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expression relative to hprt

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Trüssel et al., WCB 2014
Results - hierarchical clustering

expression hierarchical clustering

A (n=6)  B (n=45)  C (n=23)

samples

gene z-score

-6  -4  -2  0  2  4  6

Trüssel et al, ASBMR 2015
Results - hierarchical clustering

SED (high/low) probability within cluster **

remodeling probability within cluster *

high SED
low SED
forming
quiescent
resorbing
Results - mechanical microenvironment

- **Wnt Signaling**
  - *Sfrp1*
  - ***Ctnnb***
  - **Cx43**

- **Growth Factors**
  - ***Bmp2***
  - *Bmp7***

Numbers of subpopulations:
- high n = 27
- low n = 47

Osteoblast builder

Osteocyte coordinator

Trüssel et al, ASBMR 2015
Results - remodeling microenvironment

- Sfrp1
  - Wnt related
  - Expression (log2)
  - for n = 28
  - qui n = 31
  - res n = 15

- Mmp14
  - Osteolysis
  - Expression (log2)

- Car2

- Mmp13
  - osteocyte coordinator

Trüssel et al, ASBMR 2015
LivE - from 2D to 3D

remodeling

SED

ΔSED

→ Temporal gradient: increase vs. decrease of SED over time

**sclerostin**

**sclerostin**

mechanical loading

Remodeling patterns over time:
- **Sclerostin+** and **Sclerostin−** patterns indicate different remodeling phases.
- **SED** (sclerostin expression density) variations show high and low regions indicating mechanical loading.
- **ΔSED** (change in SED) highlights regions of increase and decrease over time.

Statistical analysis:
- Temporal gradients show significant differences (**p<0.001**).
- Box plots depict the distribution of Sclerostin expression with **ΔSEDmax** indicating positive and negative changes over time.

Scheuren et al., SSBE 2017
LivE - spatial mechanomics
Cell-based micro-MPA in silico simulations of bone remodeling

Time: 0.00

Lining cell
Osteoblast
Preosteoclast
Osteoclast

Week 0-1
Week 2-3
Week 1-2
Week 3-4

8/29/2022
In silico profiling of spatial mechanomics protein expression

Hierarchical clustering

K-means clustering using PCA
Conclusions

• Time-lapsed in vivo imaging allows longitudinal quantification of bone adaptation and regeneration using a multiscale mechanobiology approach

• LiviE imaging allowed clarification of the role of a wide range of genes as key players in the mechanically induced bone adaptation and regeneration process

• Spatial mechanobiology allows coupling of biochemical information with the mechanical and remodeling microenvironment of osteocytes

• Spatial mechanobiology informs cell-based in silico models of bone remodeling to predict realistic outcomes for bone adaptation and regeneration

• In the future, this will facilitate better understanding of biochemical signaling pathways in bone adaptation and regeneration in vivo

Chiapparini, ..., Schneider, Müller, J Biomech, 2012