Removing or Truncating Cx-43 in Osteocytes Alters Nanoscale Composition and Microscale Mechanics

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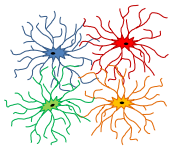
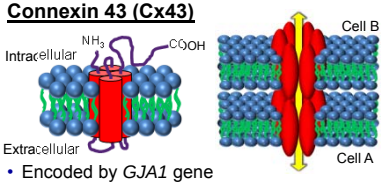
INTRODUCTION

Osteocytes (Ot)

- Many embedded throughout bone
  - Dendritic processes interconnected
  - Exchange nutrients/waste via gap junctions (GJs)
- May participate in mineral homeostasis
- Produce sclerostin, block Wnt signalling
- Sense load/transmit signal to other cells
  - Cx43 modulates response to loading



Connexin 43 (Cx43)



- Encoded by *GJA1* gene
- 6 Cx43s → hemichannel, 2 adjacent docked hemichannels → GJ
- GJ closed if C-terminus phosphorylated
- Small molecule transport, interactions with structural/signaling molecules

Study Contribution

- Mechanical and compositional implications of tissue specific deletion of Cx43 and the removal of the C-terminal domain are investigated at the micro and nanoscales
- Understanding the domain and tissue specific functions of Cx43 may lead to new interventions exploiting Cx43 to increase bone mass/strength in diseased patients

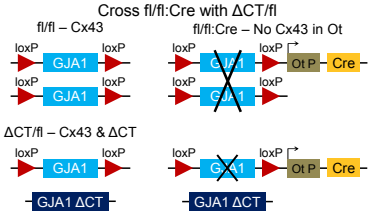
HYPOTHESIS

Removing/truncating Cx43 alters microscale mechanics of bone via compositional and morphological changes at the nanoscale.

MATERIALS AND METHODS

Animals

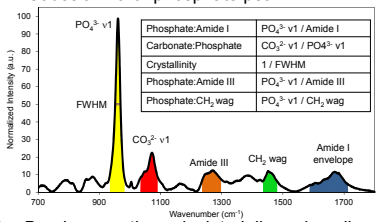
- Cre under control of 8 kb DMP-1 promoter (Ot P) deletes floxed Cx43 (fl) in Ots only (fl:Cre)
- Truncated Cx43 lacks C-terminus (ΔCT)
- All mice on C57BL/6 backgrounds



- Sacrificed at 18 weeks (n=6-12 per group)

Raman Spectroscopy

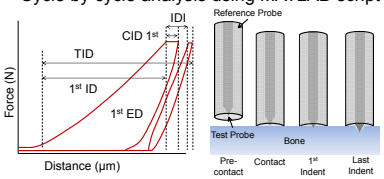
- Right tibiae, 5 locations per bone
- Distal to tibia-fibula junction, medial surface
- Unprocessed hydrated surface
- Gaussian fit for phosphate peak FWHM



- Band area ratios calculated, linear baseline

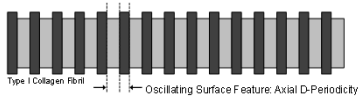
Reference Point Indentation (RPI)

- Distal tibia after Raman, medial surface
- Sample hydrated in PBS bath
- 2 N indents for 10 cycles, BP3 probe
- Cycle by cycle analysis using MATLAB script



AFM Imaging and Analysis

- Distal right tibia mounted lateral side up, EDTA demineralization (n=4 per group)
- 3.5 μm x 3.5 μm images in air
- Collagen D-periodic spacing from 2D Fast Fourier Transform (2D FFT) power spectrum
- 10-15 fibrils/location, ~55 total/bone



Statistical Analysis

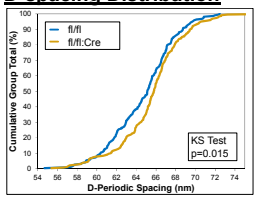
- Mean comparisons using two-way ANOVA
  - Cre and ΔCT main effects
  - Transformation if assumptions violated
- D-spacing distribution differences tested with Kolmogorov-Smirnov (KS) tests
  - Bonferroni correction for multiple comparisons, p<0.0167 significant

RESULTS

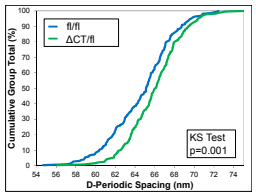
Group		ED (μJ) †	US (N/μm) *	1st US (N/μm) *	Crystallinity *	PO_4^{3-}/Amide I
		RPI			Raman	
fl/fl	n=9	4.16 ± 0.68	0.240 ± 0.020	0.232 ± 0.020	0.0493 ± 0.0027	1.06 ± 0.22
fl/fl:Cre	n=12	4.63 ± 0.72	0.215 ± 0.020	0.206 ± 0.021	0.0515 ± 0.0029	1.09 ± 0.18
ΔCT/fl	n=7	3.83 ± 0.60	0.259 ± 0.024	0.249 ± 0.025	0.0490 ± 0.0016	0.89 ± 0.08
ΔCT/fl:Cre	n=6	4.80 ± 1.32	0.250 ± 0.038	0.240 ± 0.037	0.0508 ± 0.0025	1.03 ± 0.13

* p<0.05 main effect of Cre; † p<0.05 main effect of ΔCT

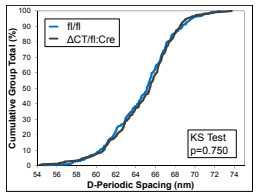
D-spacing Distribution



Removal of Cx43 from Ot alters distribution



ΔCT with floxed Cx43 alters distribution



ΔCT without floxed Cx43 in Ot normal distribution

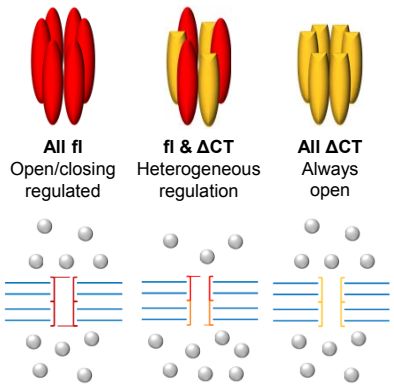
DISCUSSION

Animal Model

- fl/fl endogenous Cx43
- Cre only removes Cx43 in Ot, other cells retain Cx43
- ΔCT/fl phenotype heterogeneous
  - 12 Cx43/gap junction
  - Each Cx43 either fl or ΔCT

Uniform Cx43 for Normal Collagen

- Uniform Cx43 expression (either all ΔCT or all endogenous Cx43) necessary for distribution similar to fl/fl control
  - ΔCT with endogenous Cx43 alters D-spacing
  - No Cx43 in Ot alters collagen D-spacing
    - No gap junction-mediated signaling



Cx43 Removal Alters Crystallinity/US

- Crystallinity increased when floxed Cx43 removed
- Unclear if crystallinity cause or effect of altered collagen
  - Larger D-spacing → larger crystals
  - Less demineralization → less collagen collapse
- Endogenous Cx43 in Ot increases resistance to indentation

ΔCT May Alter Mineralization/US

- PO_4^{3-}/Amide I trended down with ΔCT (p=0.070), less mineralized matrix
- Both US and 1st US trended up with ΔCT (p=0.058 and p=0.062)
- Unclear why less mineralized matrix would increase resistance to indentation

Truncated and floxed Cx43 Interact

- Changes in ΔCT/fl rescued in ΔCT/fl:Cre by removing floxed Cx43 from Ot
  - D-spacing restored to fl/fl in ΔCT/fl:Cre
  - Reduced trend for PO_4^{3-}/Amide I restored in ΔCT/fl:Cre
- ED reduced in ΔCT/fl but increased in ΔCT/fl:Cre

Limitations and Future Directions

- Osteocytes not stained, effect may be stronger in perilacunar tissue
- Unclear if due to secondary mineralization or perilacunar remodeling
- Whole bone mechanical properties
- Cortical and trabecular growth

Removal or truncation of Cx43 in osteocytes resulted in altered collagen morphology and microscale mechanics in mice