SOST and WNT-11 are regulated by different stimuli in mechanically loaded bone

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Introduction: Osteocytes (OCYs) are mechanosensory cells in bone that are responsible for regulating bone adaptation to mechanical loads [1]. OCYs differentially express several genes in response to mechanical loading. The strain level at which OCYs respond to mechanical stimuli is unknown. In trabecular bone, formation and resorption are not limited to regions of high and low strain respectively [1-2]. Identifying OCYs with altered gene expression may provide insight into the physical stimulus required for a cellular response. We found that SOST is downregulated, and Wnt-11 is upregulated following loading using bulk RNA sequencing [3]. Expression levels of these genes may identify mechanically stimulated OCYs. Our objective was to investigate how gene expression is affected by local tissue strains in trabecular bone. We quantified differences in OCYs expressing SOST and Wnt-11 and used 2-pt correlation to determine whether SOST and Wnt-11 expression is correlated to the mechanical environment of the OCYs.

Methods: Cylindrical bone explants (N=8) 8 mm in diameter and 1 cm tall were prepared from cervical vertebra of 6 mo. old female pigs and cultured in a bioreactor. After 48h, the explants were subjected to two bouts of 1200 cycles of compressive loading to 196 N with 1 hour between bouts daily for three days. (Fig. 1a). Four control samples were cultured without loading. Samples were fixed 6 h after the final load, demineralized, embedded in paraffin, and sectioned at a thickness of 5 µm. The sections were stained using RNAScope Multiplex Kit for SOST and Wnt-11 using custom probes. The stained sections were imaged at 20x magnification using confocal microscopy. OCYs were identified and the intensity of the signal for each OCY was measured (Fig. 1b). Samples were µ-CT scanned immediately after being removed from the bioreactor and finite element models were created to determine the deviatoric tissue strain corresponding to the measured displacement during loading. Stained fluorescence *in-situ* hybridization (FISH) sections were aligned to the finite element model (Fig. 1c) Two-point correlation was used to determine whether the tissue strain magnitude was spatially correlated to the gene expression. The 2-point correlation function, g(r), measures the probability of a point with a specific feature being located at a distance, r, from a point with another feature of interest as a multiple of random probability. We correlated the location of high and low expression of WNT-11 and SOST to trabecular tissue locations with strain lower than 1500 or 1000 µε, respectively, and to regions subjected to greater than 2000 µε.

Results: More SOST+ OCYs were detected in the unloaded vs. loaded explants (Fig. 1d), while more WNT-11+ OCYs were found in loaded compared to unloaded explants. Tissue strained below 1000 µε was twice preferentially found near SOST+ OCYs while tissue strained over 2000 µε was less likely to be found near SOST+ cells than to all OCYs. SOST positive OCYs were less likely than SOST negative OCYs to be near regions subjected to more than 2000 µε. However, SOST- OCYs were similarly likely to be in high strain regions as all OCYs (Fig. 1e, f). More OCYs express high amounts of Wnt-11 in loaded bone compared to unloaded bone (Fig. 1g). Wnt-11 negative osteocytes were much more likely to be regions with strain below 1500 µε than Wnt-11 positive OCYs, and, conversely, Wnt-11 positive OCYs were more likely to be near regions subjected to more than 2000 µε than Wnt-11 negative OCYs (Fig. 1h, i).

Discussion: Loading in trabecular bone results in a heterogeneous strain field within the mineralized tissue, such that OCYs are located within tissue subjected to a range of strains. As such, OCY regulation of gene expression is also spatially heterogeneous based on the local tissue strain. Most OCYs express SOST in unloaded bone, while most do not in loaded bone. In contrast, WNT-11+ OCYs are uncommon in unloaded bone, and less than half of OCYs are WNT-11+ following loading. As such, SOST- OCYs are not as strongly associated with high tissue strain as WNT-11+ OCYs. SOST expression is regulated by strains within a 200 µm radius of the OCY, while WNT-11+ OCYs occur in highly strained regions of up to 500 µm. This suggests individual genes may be regulated by different magnitudes or measures of strain, which may be a mechanism by which trabecular architecture is maintained within the heterogeneous loading.

Significance: Understanding the strain levels that are associated with mechanobiological gene expression will help decipher the mechanisms of mechanotransduction and identify unique targets for treating or preventing bone loss.

REFERENCES: 1. Schaffler MB, et. Al 2014, 94(1):5-24; 2. Cresswell EN, et al. J Biomech. 2016; 3. Machireddy M, et al. In: ORS 2022 Annual Meeting,Feb 4, 2022 – Feb 8, 2022; Tampa, FL

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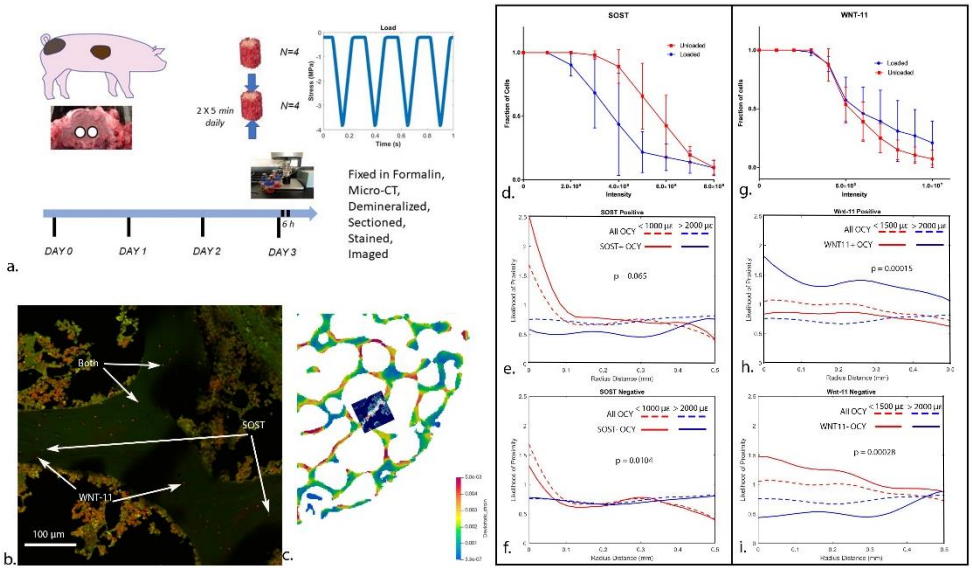

a) Experimental setup b) Wnt-11 and SOST RNA expression was labeled by FISH and imaged with confocal microscopy. c) Histological sections were aligned to the finite element results. d). More osteocytes expressed SOST at high levels in the unloaded vs. loaded explants. e.) Tissue with strain higher than 2000 µε was about half as likely as random to be found near SOST+ OCYs while strains lower than 1000 µε were more likely to be found near SOST+ OCYs. f) Regions of strain greater than 2000 µε were likely to be found near SOST- cells g) More osteocytes express high amounts of Wnt-11 in loaded bone compared to unloaded bone. h, i) Tissue strained beyond 2000 µε was over 1.5 times more likely than random to be found near WNT-11+ cells, while highly strained tissue was unlikely to be found near WNT-11- osteocytes. The opposite was true for tissue with strains below 1500 µε.