LOCAL MECHANICAL ENVIRONMENT REGULATES SCLEROSTIN IN SINGLE OSTEOCYTES IN VIVO

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Introduction

Mechanical loading is one of the major factors controlling bone mass. Osteocytes, bone cells that are embedded in the matrix, are the main sensors of the mechanical microenvironment. It is believed that these cells regulate formation and resorption activity by a number of biochemical signals, among them sclerostin (SCL), an inhibitor of bone formation. However direct *in vivo* experimental data quantifying these signals at the single-cell level is still missing. Here, we present a method to quantify and directly compare SCL levels in single cells with the local remodelling activity and the mechanical microenvironment.

Methods

The sixth caudal vertebrae of 2 adult female C57BL/6 mice were dynamically loaded as described earlier [Webster *et al*, 2008]. *In vivo* micro computed tomography (µCT, 10.4µm, figure 1B) scans were performed at week 0, 2, 4 after which 3D dynamic morphometric parameters were assed using advanced registration techniques [Schulte *et al*, 2011] (figure 1D). Micro finite element (µFE) analysis was used to calculate the strain energy density (SED, figure 1C). Samples were harvested one day after the last loading cycle, decalcified, embedded in paraffin, sectioned (8µm) and stained for SCL using immunohistochemistry [Nakashima *et al*, 2011] (figure 1A). To quantify SCL levels, the number of positive stained pixels inside each osteocyte was counted. Osteocytes were mapped into the *in vivo* 3D µCT volumes and the corresponding µFE models [Truessel *et al 2012*]. They were grouped according to regions of formation, quiescence and resorption (figure 1E). SED was quantified around each osteocyte location (sphere with 40µm radius) prior to both loading and sacrifice. The change in SED (ΔSED) was compared to SCL level.

Results

We found that SCL levels were significantly decreased in osteocytes closer to regions of bone formation than bone resorption (figure 2A). Furthermore, SCL level was significantly decreased in osteocytes associated with positive ΔSED compared to osteocytes associated with negative ΔSED (figure 2B).

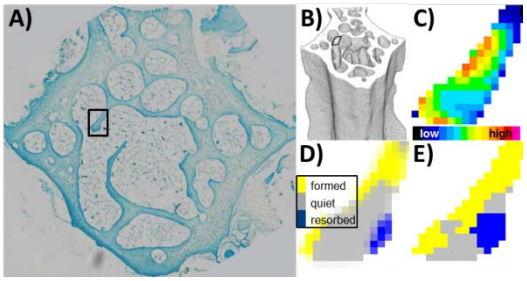

Figure 1: The different modalities. A) Histology section, B) µCT volume, C-E) 2D slices that corresponds to the rectangle in A&B). C) µFE-based SED map, D) dynamic morphometry map and E) bone grouped according to D).

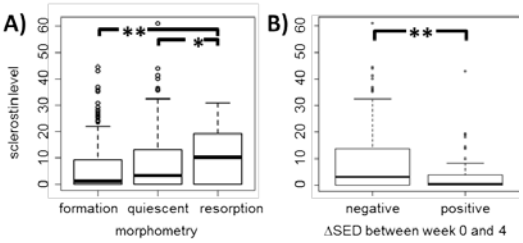

Figure 2: Sclerostin level of osteocytes. A) Grouped according to the dynamic morphometric parameters (figure 1E); B) according to the change in the mechanical microenvironment over time. ($p<0.05$, ** $p<0.01$, pairwise Wilcox test)*

Discussion & Conclusion

At the single-cell level SCL is correlated *in vivo* with mechanical and bone remodelling parameters. The methodology presented here will be extended to include other key molecules and will further our understanding of local remodelling processes in bone.

Acknowledgment

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References

Nakashima *et al*, Nat Med 17:1231-1234, 2011
Schulte *et al*, Bone, 48:433-442, 2011
Truessel *et al*, J Biomech 45(S1):S108, 2012
Webster *et al*, Comput Method Biomech 11:435-441, 2008