

## **RANKL and OPG Activity is Regulated by Injury Size in Networks of Osteocyte-like Cells**

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Osteocytes have been shown to detect microdamage and initiate a remodelling response. However, the mechanism of detection and initiating of the response is yet to be elucidated. Bone remodelling involves the RANKL-RANK-OPG system, which is responsible for the functioning of the Basic Multi-cellular Unit (BMU)[1]. Following microdamage, RANKL binds to RANK, initiating osteoclastogenesis. This stimulates the release of OPG, which sequesters RANKL, stimulating osteoblast activity [2]. Additionally, the frequency of BMUs has been shown to correlate with increased numbers of microcracks in bone [3]. This study aims to characterise the relationship between RANKL and OPG release and varying microdamage size.

MLO-Y4 cells were transfected with a plasmid containing the reporter gene luciferase under the control of the RANKL promoter. These cells, in addition to non-transfected cells, were cultured in 3D matrices. Planar defects were applied with 160-800 µm needles. Production of RANKL and OPG were quantified by ELISA. RANKL promoter activity was assessed by luciferase activity.

RANKL release increased significantly at 400µm injury size compared to other injuries. OPG release showed an inverse relationship to this, with 400µm damage being significantly lower. 800µm samples followed a different trend with both RANKL and OPG release being lowest in this group. RANKL promoter activity showed a doubling in activity with increase in injury size up to 400µm where it was significantly highest. Again, RANKL production was seen to decrease at 800µm. A theoretical model was developed allowing these results to be extrapolated to actual defect sizes. Based on previous theoretical analysis, our model showed a reduced frequency of ruptured processes, which was normalized by the control level. Thus, theoretical data showed increase in cell process rupture correlates with cytokine activity.

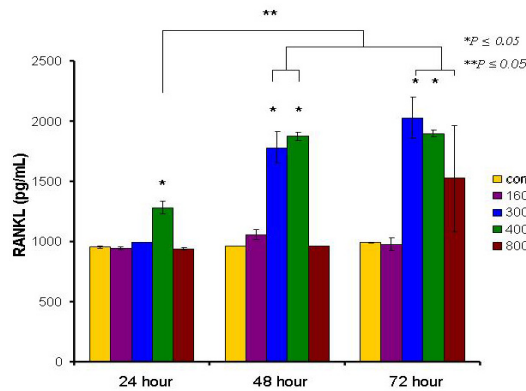


Fig 1: RANKL release over 72 hours

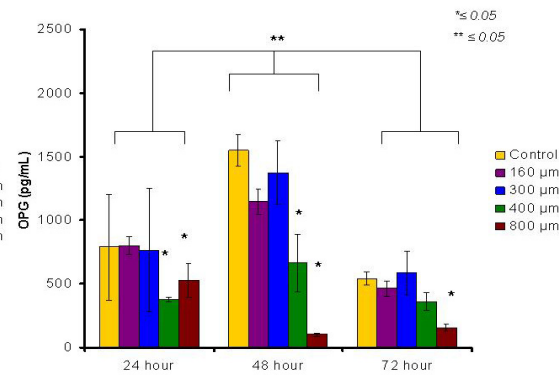


Fig 2: OPG release over 72 hours

Our results demonstrate that RANKL and OPG release is controlled by injury size. The data is complemented by the use of theoretical models which can further allow the study of the release of bone-repairing cytokines, and may allow us to predict what will happen at critical injury sizes.

### References

- [1] Bekker, P., et al, Journal of Bone and Mineral research. 16:348-360; 2001
- [2] Robling, J., et al. Annual Review of Biomedical Engineering 8:455-498; 2006
- [3] Verborgt, O., et al. Bone. 10:201-214; 1989

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