Reviewer’s report

Title: Cyclic hydrostatic pressure: Effect on synthesis by human macrophages and osteoblasts of sRANKL, OPG and RANK, and possible effects on osteoclast recruitment.

Version: 2 Date: 20 September 2005

Reviewer: Gerald Atkins

Reviewer’s report:

General
This is an interesting study of the release of OPG and soluble RANKL by macrophages and cocultures of macrophages and osteoblasts in response to cyclical hydrostatic pressure, and the expression of RANK on macrophages. While generally well written, the discussion is too long, in parts highly speculative, and should be pruned to a more succinct form. The results are somewhat overinterpreted as they do not take into account OPG and sRANKL synthesis as opposed to their secretion from cells. Also transmembrane RANKL is not included as a variable. The observation that pressure upregulates RANK expression by macrophages is interesting and I believe novel, and might point to a biological role in this respect for hydrostatic pressure in instances of periprosthetic implant loosening.

-----------------------------------------------------------------------------------------------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) Methods, pages 5&6: No data on resorption resulting from cocultures of macrophages and osteoblasts are reported for this study, rather the authors reference their manuscript in press (reference 26). Based on the morphology and size of the TRAP-positive cells shown in figure 5, I would be surprised if these cells, generated even in monocultures of macrophages, could resorb dentine, as few of these in my view look like classical multinucleated osteoclasts. Either the authors should revise their results and provide an indication of the resorptive capacity of these cells in their experiments or reference to this aspect of the study should be removed from the paper.

2) The hypothesis that sRANKL is the only contributor to the RANKL pool ignores the expression of transmembrane RANKL expression, in particular by osteoblasts. What is the evidence that the expression of transmembrane RANKL is not a player in this system?

3) Methods page 7: Do the authors take into account the formation of sRANKL/OPG complexes in conditioned media, can these be detected by the respective ELISAs employed, and what impact does this have on the interpretation of the data?

4) Methods, page 7: ‘Effect of hydrostatic pressure on OPG, sRANKL and RANK synthesis’: ‘Synthesis’ is a misnomer and should be replaced by ‘secretion’. RANK expression is not described here.

5) Page 9 and Table 1: sRANKL and OPG are measured in terms of pg or ng/ml respectively. It is widely considered that the ratio of agonist to antagonist (RANKL:OPG) is the important determinant of the net biological effect. Therefore, to adequately interpret the changes in their relative levels, these should be expressed as a molar ratio, and this ratio should be included in Table 1.

6) Results, page 10: Immunostaining is a qualitative and non-linear measure, and notoriously
variable across fields. If the authors do not have a quantitative assessment of RANK expression, the language throughout this section should be modified to indicate this.

7) Table 2: This is difficult to interpret. Does ‘–/+’ indicate that in one experiment there were no positive cells and in another there were some, or that it was difficult to say if they were stained or not? Also, how can there be 1 and 4 cycles in the ‘no pressure’ group? The legend is misleading – how can the results be +/− SE for this type of analysis?

8) Results page 10: The pressure protocol would be better referred to in terms of cycles rather than hours, as ‘4 hours of pressure’ implies 4 consecutive hours rather than four consecutive days each with a one hour cycle.

9) Results page 11, Figure 5: It is difficult to see from these images that cells are mono- or multinuclear – I see no arrows as promised in the figure legend. Is there evidence that these cells resorb dentine?

Discussion:

10) Page 12, line 16: The results in fact show secretion of both sRANKL and OPG – not synthesis as stated, in response to pressure. There is certainly good evidence that OPG at least, is released from cytoplasmic stores by both osteoblasts and endothelial cells in response to cytokines such as TNF. Synthesis would be better analysed using a PCR approach. All subsequent occurrences of ‘synthesis’ should be changed to ‘secretion’.

11) Line 17: The authors state that “the ratio of sRANKL/OPG was not affected...”. In fact, the ratio is not presented in the figures/tables and should be formally represented. All subsequent references to this ratio should be altered in light of further analysis.

12) Also, see my question above, as to whether total or only uncomplexed sRANKL and OPG are detected by the respective ELISAs, as this will potentially profoundly influence the interpretation of these data.

13) Page 13, line 4: The issue of the sRANKL:OPG ratio is somewhat glossed over: Firstly, that the ratio of sRANKL:OPG remains unchanged remains unsubstantiated (see above).

14) Line 6: Secondly, the results show that levels of these potent factors are dynamic and it is likely that changes of these in the bone microenvironment do in fact influence osteoclast activity and net bone resorption.

15) Page 14 line 19: References should be included for MIP, MCP and M-CSF involvement in recruitment and differentiation.

16) Page 15: Reference and explain Wolff's law. The ensuing paragraph is highly speculative and should be omitted.

17) Page 15, bottom: References should be inserted for diffusion of OPG and sRANKL from osteoblasts, and for the requirement of cell cell contact.

-------------------------------------------------------------------------------

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Abstract Background: OPG/sRANKL/RANK triumvirate: soluble RANKL is not generally or necessarily considered to be the main controller of bone resorption, rather RANKL per se should be the second member of the triumvirate.
2) Table 1: ‘P’ should be formally defined in the figure legend.

3) Results page 10: ‘addition of pressure’ would read better as ‘application of pressure’.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests.