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Question for mathematics in biology workshop

Identifying the mediators of mechanotransduction between bone cells

Background:
Sensitivity to mechanical forces is one of the critical properties of many tissues in the body, including bone. Bone mass and architecture are regulated by mechanical forces, resulting in increase in bone mass in response to excessive loading and decrease in bone mass in the absence of physical activity (for example in immobilized patient or in astronaut)[1]. Bone in adult organism is constantly remodelled by the cooperative action of two cell types – cells of hematopoietic origin responsible for bone resorption, osteoclasts, and cells of mesenchymal origin responsible for bone formation, osteoblasts[2]. Different types of mechanical forces lead to cell membrane deformation, which is thought to be the triggering event for significant downstream cellular response to mechanical stimulation[3]. In addition, some evidence suggests that cells experiencing mechanical forces may communicate their status to remote cells which are not under direct influence of these forces.

The **objective** of our study is to assess the means by which mechanical stimulation is communicated among bone cells.

Experiments:
Osteoclasts and osteoblasts are cells of different developmental origin. In our experiments we either generated a co-culture of osteoclasts and osteoblasts from mouse bone marrow, or used the populations of only one cell type, osteoclasts formed from murine monocytic RAW 264.7 cells, or osteoblasts fromed from mouse mesenchymal C2C12 cells. Single cell was mechanically stimulated by a gentl touch of a micropipette and changes in cytosolic free calcium concentration ($[Ca^{2+}]_i$) were recorded and analyzed. Mechanical stimulation of an osteoclast or an osteoblast induced a transient increase in its $[Ca^{2+}]_i$. Stimulated bone marrow osteoblasts had significantly higher amplitude and rate of rise of $[Ca^{2+}]_i$. Mechanical stimulation of a single osteoclast or osteoblast also induced delayed transient elevations of $[Ca^{2+}]_i$ in neighboring non-connected cells, consistent with a release of a soluble mediator by mechanically stimulated cell. In cultures containing only osteoclasts, inhibitors studies suggest that the mediator is likely ATP or ADP, acting through suramin-sensitive P2 receptor. We have found that in cultures containing both osteoclasts and osteoblasts, when osteoclast was touched, the signal propagated significantly slower compared to the experiments when osteoblast was touched.

Questions:
Is it possible to estimate the molecular weight of a second mediator? Can we be certain that there is only 1 mediator released? If not, is it possible to assess if 2 (or 3?) mediators act concurrently (both released from the primary, stimulated cells) or sequentially (first signal is released from the stimulated cell, acts on the next neighbour, which in turn releases the second signal which acts on the more distant cells)?

References:
1. Turner CH (2006) Bone strength: current concepts. Ann N Y Acad Sci. 1068:429-46.
2. Taylor D, Hazenberg JG, Lee TC. (2007) Living with cracks: damage and repair in human bone. Nature Materials. 6(4): 263-8.
3. Liedert A, Kaspar D, Blakytny R, Claes L, Ignatius A. (2006) Signal transduction pathways involved in mechanotransduction in bone cells. Biochem Biophys Res Commun. 349(1):1-5.