PHOSPHO1-mNeonGreen reporter cells are a robust model to study matrix vesicle biogenesis during osteoblast-driven mineralisation

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Background and aims:
Bio-mineralisation is an essential process in which bone cells initiate the laying down of mineral within the extracellular matrix (ECM) to form an organic-inorganic composite material that is both tough and stiff to support the physiological demands of bone. The delicate balance of inorganic phosphate and pyrophosphate concentrations is a major determinant of the rate of ECM mineralisation. This process is under the control of tissue non-specific alkaline phosphatase (TNAP) and PHOSPHO1, key phosphatases found in a specific set of extracellular vesicles termed “matrix vesicles” which are postulated to drive mineralisation. The biogenesis of matrix vesicles remains unclear, in part due to lack of imaging options available for such small vesicles, and the delicate nature of calcium phosphate mineral. This study aims to develop a robust imaging system that will allow us to study the mechanisms underpinning matrix vesicle biogenesis, and the process of mineral deposition within the ECM.

Methods:
Mineralisation was studied in the osteoblast cell line MC3T3. The calcium-binding compound, Alizarin red was used to confirm mineral deposition, and gene and protein expression assessed via qPCR and protein across the time course. Key matrix vesicle phosphatase PHOSPHO1 was tagged with mNeonGreen and live imaging performed with pseudo-super resolution (LSM880) and super resolution (ELYRA) microscopy in live and fixed cells. Reporter cell lines were generated using Fluorescence-activated Cell Sorting (FACS) with a Bigfoot Spectral Cell Sorter, and validated for matrix mineralisation capability and fusion protein expression. Organelle markers and immunofluorescence allowed the localisation of PHOSPHO1-mNeonGreen to be followed throughout the cell.

Results:
PHOSPHO1-mNeonGreen expressing cells are a robust model for imaging the intracellular stages of matrix vesicle production and biogenesis by osteoblasts. Using fluorescent PHOSPHO1 as a marker, we have followed the trafficking pathway of MV precursors in the endoplasmic reticulum to the trans-Golgi network; a process involving RAB8 vesicles and driven by microtubules. This suggests a RAB GTPase-dependant method of intracellular vesicle packaging and subsequent MV secretion by the osteoblast. Furthermore, confocal imaging shows PHOSPHO1 positive matrix vesicles appear to be released at actin-rich sites at the osteoblast cell membrane, possibly linked to focal adhesion sites. Time-lapse confocal imaging of cells surrounding mineralised nodules produced in vitro shows small PHOSPHO1-mNeonGreen objects present and interacting at the mineral-cell membrane boundary, suggesting evidence of matrix vesicle activity. Preliminary evidence with a TNAP-mKate2+PHOSPHO1-mNeonGreen double reporter line shows co-localisation of both matrix vesicle markers on intracellular objects, with object size and localisation as supporting evidence for potentially an MVB driven method of MV release.

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Conclusion:

PHOSPHO1-mNeonGreen expressing osteoblasts are a valuable and robust model for studying the process of bone mineralisation in vitro and allow our first insights into the intracellular steps of matrix vesicle biogenesis.

Keywords:

Super-resolution microscopy, cell matrix mineralisation