Identification and characterisation of vesicles in resorbing osteoclasts using electron tomography

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Background

- Osteoclasts are large multinucleated cells responsible for bone resorption. Upon activation, they polarise and adhere to the bone surface.
- They form 3 distinct membrane domains; the functional secretory domain (FSD), the basolateral domain (BL) and the ruffled border (RB) (Figure 1).
- Vesicular trafficking is indispensable for osteoclast function and for the formation of these domains, in particular at the ruffled border, where osteolytic enzymes are released onto the bone surface via extensive vesicular fusion with the plasma membrane.
- Few studies have tried to classify the vesicles near the RB into secretory or uptake pathways and given their size (50-200 nm) such studies are not possible by light microscopy alone.
- The RB is a highly convoluted and complex structure which is subject to erroneous interpretation when using 2D TEM imaging.

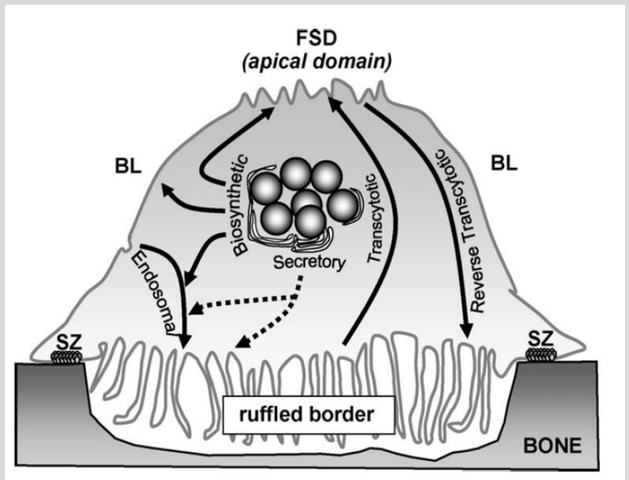


Figure 1. Illustration of a resorbing osteoclast on bone, demonstrating the multiple vesicular trafficking pathways involved in osteoclast function and the 3 membrane domains. (Coxon & Taylor, 2008)

Aims

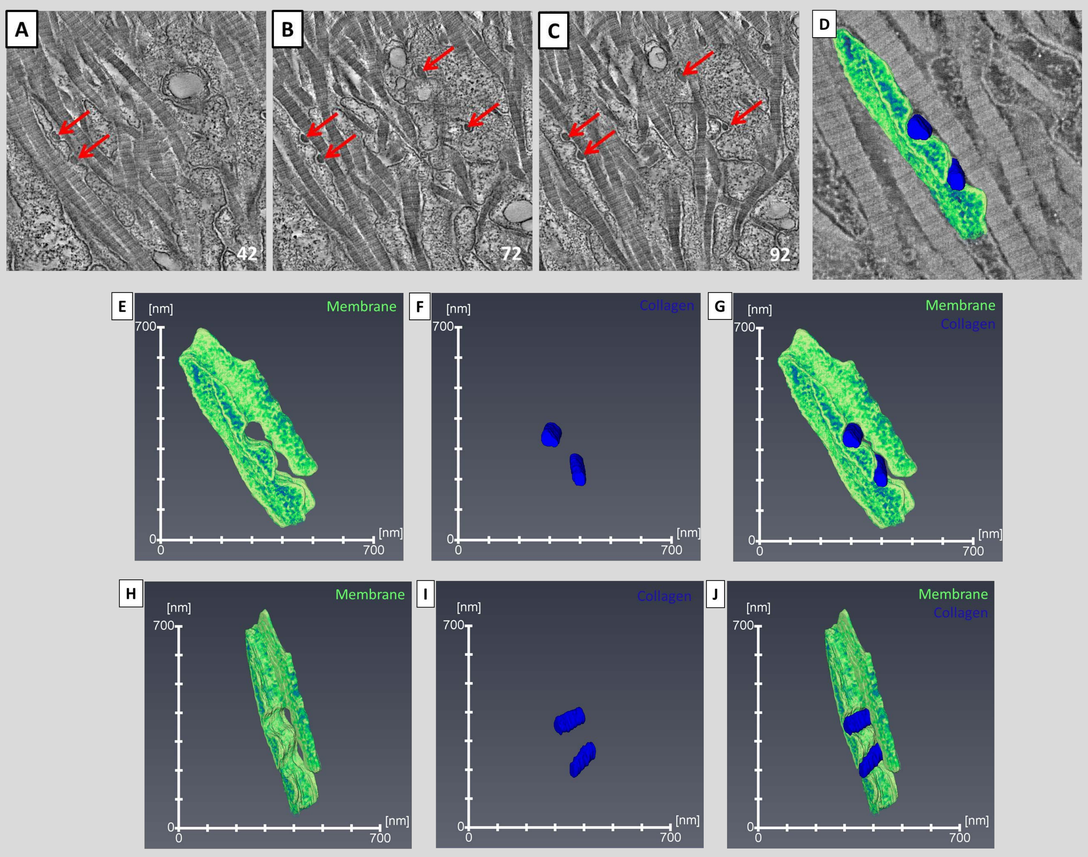
- To generate 3D reconstructions of vesicular structures at the RB using transmission electron tomography.
- To characterise these structures in an effort to better understand their function, content and relationship with the RB.

Methodology

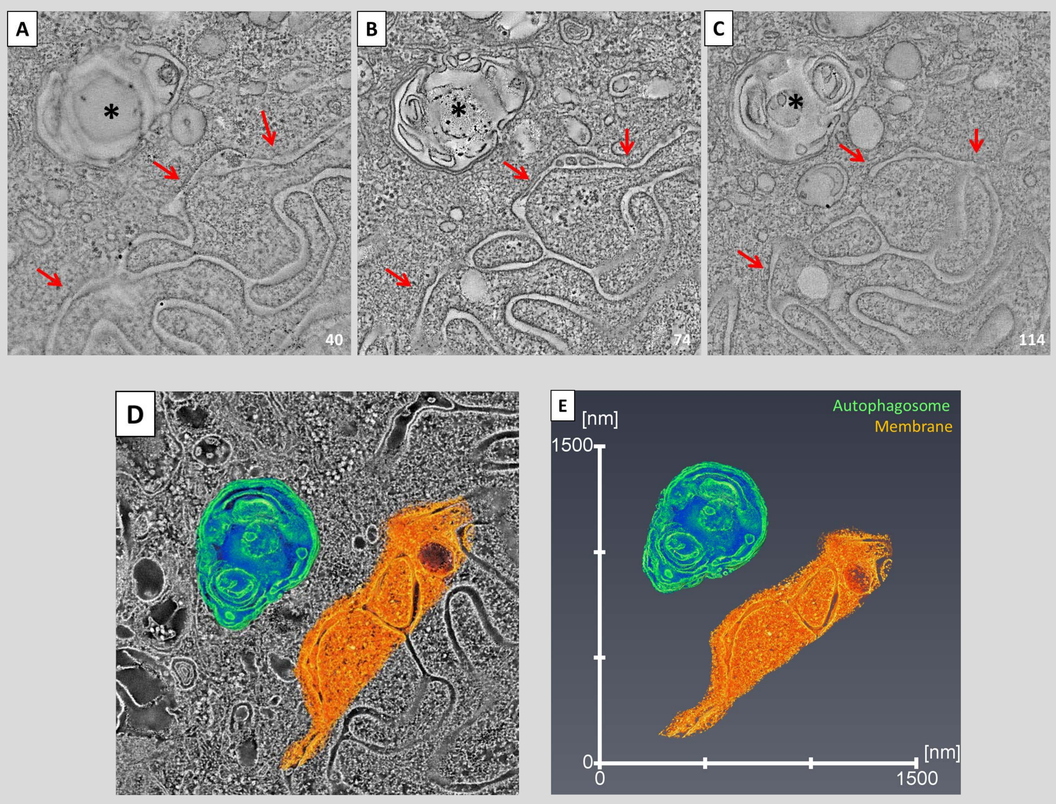
1. Osteoclasts were isolated from rabbit limbs and cultured on dentine discs.
2. The discs were processed for routine TEM and embedded in resin.
3. 200-300 nm thick sections were placed onto coated Cu grids.
4. Grids were placed in TEM for tilt series (±65) image acquisition.
5. IMOD software- tomogram generated.
6. Amira software – 3D renderings generated.

Results

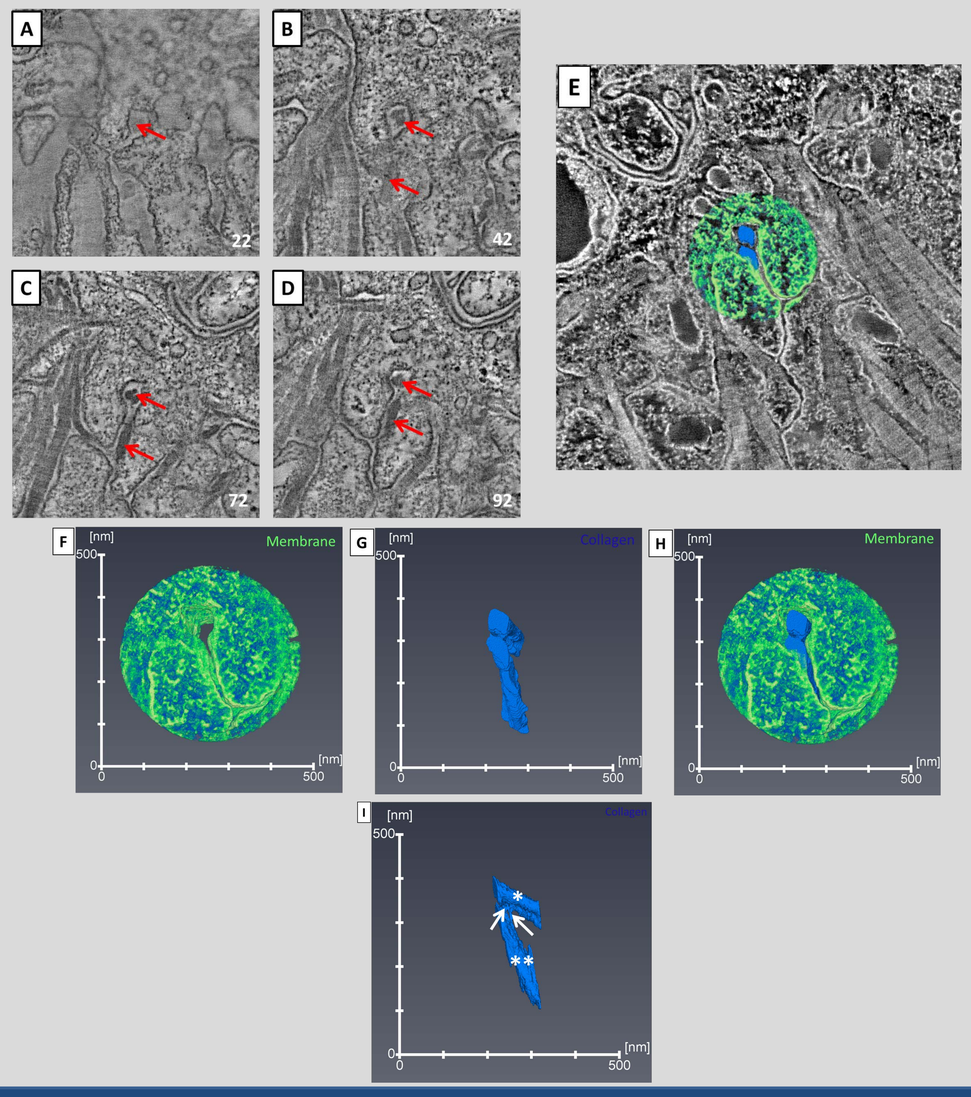
Single membrane-bound vesicles with electron dense content and distinct halos (A-C, red arrows), previously thought to be secretory lysosomes, were found to be tangential sections of collagen fibrils encased in RB membrane following 3D rendering (D-J).



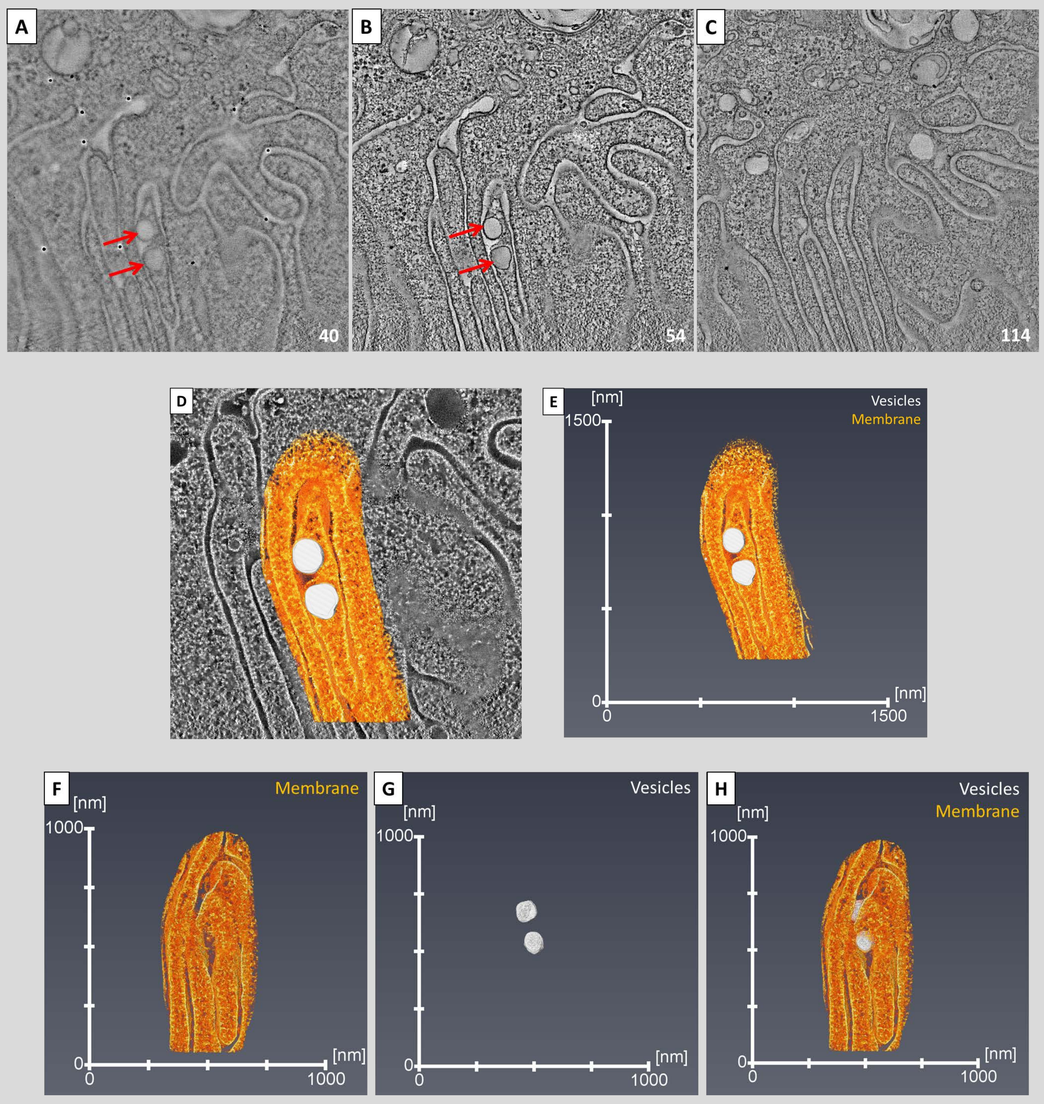
We regularly found double membrane-bound large vesicles (*= autophagosome) near the ruffled border (A-C, red arrows). 3D reconstruction showed that there was no contact between the autophagosome and RB membrane (D &E).



Single membrane-bound vesicles (A-D) with moderate electron dense content located near the RB were often associated and retained connections (I) with extracellular collagen (red arrows) fibre tips. 3D renderings (E-I) showed that these vesicles appeared to have taken up small amounts of degraded collagen and therefore may be part of the uptake pathway.



Vesicles (red arrows) without notable content were found near the RB (A-C). 3D rendering showed that the vesicles were extracellular (D-H).



Summary

- ***TEM tomography is necessary for accurate interpretation of vesicular structures at the ruffled border.***
- *We have identified new events at the RB that suggest mechanisms for uptake of collagen:. 1) Uptake of partially degraded collagen from a collagen tip. 2) Relationship between RB membrane and collagen fibres may indicate anchoring system between membrane and fibre.*
- *In this instance, we found that there was no interaction between an autophagosome and RB membrane.*
- *We have identified extracellular vesicles at the ruffled border. This may indicate that osteoclasts release extracellular vesicles during resorption.*