Studying bone cells using electron microscopy

Aidan Seeley,
School of Medical Science, University of Aberdeen

What is the problem?

There are three types of cells in bone tissue: the osteoblast, which is responsible for formation of bone matrix; the osteoclast, responsible for bone-resorption; and the osteocyte which has a role in mechanical sensing and in the maintenance of mineral homeostasis. Osteocytes are found encased in cavities within the bone called lacuna. Osteocytes have many projections protruding from the cell body which travel through the bone in tunnels called canaliculi (see Figure 1). However, osteocytes are very difficult to observe because they are hidden within mineralized bone.

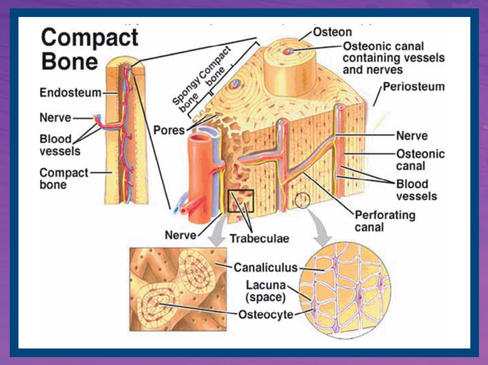


Figure 1: Structure of compact bone. (*Taken from:* Sheir, Butler & Lewis Hole's *Human Anatomy.* 10th Edition, McGraw Hill, Boston 2004.)

How are we trying to solve this problem?

In this project two methods were used to study osteocytes in their environment using the electron microscope. First, a method to freeze tissue under high pressure and then study very thin sections of that tissue to look inside the bone cells and, second, a method to study the lacuna and canaliculi by embedding bone with resin, cutting the block and etching the surface with acid to create casts.

What did we do?

Transmission Electron Microscopy (TEM) of Osteocytes

Tibia and femur from a three-month old rat were frozen using liquid nitrogen at high pressure. Samples were dehydrated by replacing any water within them with ethanol, a process known as automatic freeze substitution, and then embedded in epoxy resin. Very thin sections of the bone samples were cut using a diamond knife, stained with heavy metals and viewed using a TEM. This method allowed us to study the space between the osteocyte cell body and the lacuna.

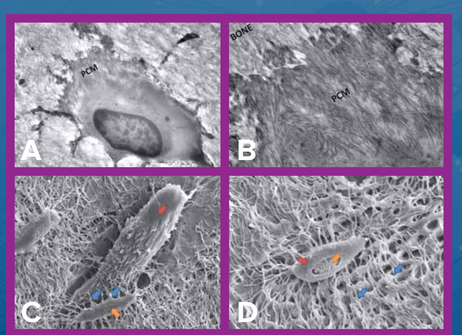
Scanning Electron Microscopy (SEM) of Osteocytes

Tibia and fibula taken from a three-month old rat were dehydrated and infiltrated with methymethacrylate (MMA) resin in a tissue processor. A very strong acid was applied to the bone surface to dissolve the main mineral in bone, hydroxyapatite, leaving the resin-filled spaces. Samples were then coated with a layer of gold and viewed using a SEM.

What did we see?

Images A and B show TEM micrographs of an osteocyte. The pericellular matrix (PCM) is filled with type I collagen fibres, which separate the osteocyte cell body from the cavity in which it resides.

Image C shows an overview of bone illustrating the interaction between the osteocyte cell body (orange arrows), the osteocyte process (blue arrows) and a blood vessel (red arrow). Image D shows the pericellular space (red arrow) which we know contains collagen fibres (see images A and B) in which an osteocyte resides (orange arrow) with canaliculi emanating from this space. These canaliculi contain the osteocyte processes and can be clearly seen by the blue arrow.



So what does that all mean?

Our studies illustrate that osteocytes reside within lacunae surrounded by a pericellular matrix containing unmineralized type I collagen, which suggests that osteocytes may have the capacity to synthesize and mineralize collagen fibres. Our images also show that osteocytes make contact with blood vessels. This study illustrates the complexity of the osteocyte network *in vivo* and why it is more beneficial to conduct anatomical studies in this way, rather than the major simplification of studying individual osteocytes *in vitro*. We conclude that the methods used here provide a useful tool to obtain detailed anatomical knowledge about the osteocyte network.

Who am I?

I am now a 4rd-year Biomedical Sciences student at the University of Aberdeen and I am hoping to pursue a career in research.

Acknowledgements

I wish to thank Medical Research Scotland for awarding me the Vacation Scholarship and Professor Helfrich, Lucy Wight, Gillian Milne, John Greenwood and Kevin Mackenzie for their support with the project.

Aidan was supervised during his Vacation Research Scholarship, "To study bone using electron microscopy methods – new insights with new methods?", by Professor Miep Helfrich.