BONE STRUCTURE AND BONE REMODELLING

Tim Arnett
Department of Anatomy and Developmental Biology
University College London
Gower Street
London WC1E 6BT, UK

Address correspondence to:

Timothy R Arnett
Department of Anatomy and Developmental Biology,
University College London, London WC1E 6BT, UK
tel: +44 (0)20 - 7679 3309
fax: +44 (0)20 - 7679 7349
e-mail: t.arnett@ucl.ac.uk

"The skeleton, out of sight and often out of mind, is a formidable mass of tissue occupying about 9% of the body by bulk and no less than 17% by weight. The stability and immutability of dry bones and their persistence over the centuries, and even millions of years after the soft tissues have turned to dust, gives us a false idea of bone during life. Its fixity after death is in sharp contrast to its ceaseless activity during life" (Cooke, 1955).

The aim of this brief review is to provide a basic overview of the formation, composition, structure and pathophysiology of bone.

Bone composition

Bone is a connective tissue that consists principally of a mineralised extracellular matrix plus the specialised cells, osteoblasts, osteocytes and osteoclasts. The structural component of the organic phase is type I (fibrous) collagen, which comprises about 90% of bone protein; the remaining 10% consists of a complex assortment of smaller, non-structural proteins, including osteonectin, osteocalcin, phosphoproteins, sialoprotein, growth factors and blood proteins. The inorganic phase is mainly tiny crystals of the alkaline mineral hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. These crystals enclose the collagen fibrils to form a composite material with the required properties of stiffness, flexibility and strength.

The three principal types of bone cell, osteoblasts, osteocytes and osteoclasts are considered below.

Osteoblasts and osteocytes

Osteoblasts, the bone forming cells work in groups to secrete and then mineralise patches of new bone matrix. Histologically, active osteoblasts appear as plump, cuboidal cells on the bone surface (Fig. 1), with the prominent rough endoplasmic reticulum characteristic of protein-secreting cells. Active osteoblasts express high levels of alkaline phosphatase, which probably aids mineralisation by liberating inorganic phosphate. Quiescent bone surfaces are covered by a near-continuous single layer of flattened, inactive osteoblasts, often referred to as 'bone-lining cells'. Osteoblasts are derived from mesenchymal

progenitor cells that in the bone marrow, at least, are also capable of differentiating into adipocytes and fibroblasts. Osteoblasts, or their progenitors, express receptors for many hormones, including parathyroid hormone, 1,25-dihydroxyvitamin D, sex steroids and corticosteroids; they are also responsive to, and may produce, a wide range of growth factors and cytokines (Table 1). During bone formation, some osteoblasts become engulfed by the accumulating matrix around them and differentiate into osteocytes, the interconnected, low-density network of cells that ramify throughout all living bone (Fig. 1). Osteocytes are thought to mediate the remarkable mechanical responsiveness of bone by functioning as strain sensors that communicate with cells (osteoblasts and osteoclasts) on bone surfaces (Ehrlich & Lanyon, 2002).

Osteoclasts

The destruction of bone is accomplished by osteoclasts, large motile, multinucleate cells that resorb characteristic 'scalloped' pits and trails in bone surfaces (Figs. 1, 2 & 3). Osteoclasts are derived by the fusion of mononuclear promonocytic precursors present in the marrow and circulation. They form resorption pits by attaching tightly to the bone surface, secreting protons to dissolve the mineral phase and proteolytic enzymes (chiefly cathepsin K) to degrade to collagenous matrix. Osteoclasts achieve a high surface area of interaction with the bone by means of a convoluted membranous organ, the 'ruffled border'; the adjacent resorption space can be considered as a specialised extracellular lysosome. Osteoclasts express high levels of tartrate-resistant acid phosphatase (TRAP), the function of which is uncertain. Mature osteoclasts express receptors for calcitonin, a potent inhibitory hormone and prostaglandins but appear not to be directly responsive to many other hormones or growth factors.

Bone formation and bone types

Some parts of the skeleton, such as the long bones, are formed when mesenchymal cells in the limb buds of embryos differentiate into avascular cartilage rudiments (Olsen, 1999). These rudiments are then invaded by blood vessels; erosion of cartilage occurs in the centre of the rudiment; osteoblasts appear and begin depositing bone on internal and external cartilage surfaces. As this process continues, a zone of proliferating cartilage, the growth plate, becomes distinct near each end of the bone. The cartilage

ribbons produced by the growth plates serve as temporary template for further bone deposition, and provide a means by which rapid, controlled elongation of the long bones can occur. This process, known as "endochondral" ossification, is particularly impressive in large birds, where limb bones many centimeters long are formed in ~3 weeks between fertilisation and hatching of the egg. A similar process takes place during deer antler growth, where elongation can exceed 1 cm per day. The flat bones of the jaw and skull are formed directly by osteoblasts that differentiate from embryonic mesenchyme, without any intermediate cartilage stage, a process termed, rather misleadingly, 'intramembranous' ossification. Other bones are formed by a combination of endochondral and intramembranous ossification.

Bone that is formed rapidly during growth, or at fracture repair sites (termed 'woven' bone) is characterised by disorganised collagen fibre orientation, rapid matrix mineralisation and large, irregularly placed osteocytes. This is a temporary form of bone that is soon resorbed. It is generally replaced by the mature, lamellar form of bone which is deposited in discrete layers, each with a differing collagen fibre alignment; this arrangement confers strength and fracture resistance. Lamellar bone is characterised by relatively slow formation and turnover rates rates, delayed mineralisation of the organic matrix and regularly distributed osteocytes in small, ovoid lacunae.

Bone remodelling

During adult life, the skeleton undergoes a continual process of repair and renewal. Bone remodelling is a surface phenomenon: the turnover rate in trabecular bone may be up to ten times greater than in cortical bone, reflecting the large surface area presented by the former tissue (Fig. 2). Mineralised bone matrix is resorbed by OC and replaced in plywood-like layers, or lamellae, by groups of OB. This sequence of events is tightly coordinated both temporally and spatially. Under normal circumstances in young adults, remodelling activity keeps overall bone mass relatively constant. However ageing, the menopause and many other pathophysiological states can alter the balance of the turnover process, such that resorption begins to outstrip formation, leading to net bone loss and ultimately osteoporosis (Fig. 3; also: http://courses.washington.edu/bonephys/). This could be due not only to enhanced osteoclastic resorption but also to reduced

osteoblastic function. Trabecular bone sites, for example, in the vertebral bodies (Fig. 3) or in the ends of the long bones are particularly susceptible to remodelling imbalances, due to the relatively high turnover rate (~25% / year).

Bone growth, turnover and repair involves high levels of cellular activity, and requires an effective blood supply. This is in contrast with adult cartilage, an essentially primitive, avascular tissue with low cellularity and turnover rates. The importance of the vasular supply of bone, with its attendant network of fine nerve fibres is perhaps insufficiently recognised. Disruption of the blood supply to bone, for example because of inflammation, infection, tumours or fractures will result in hypoxia and acidosis, and may have profound negative consequences (see beow).

Regulation of bone cell function and bone turnover

Some of the main regulators of bone cell function are summarised in Table 1. This partial list gives an indication of the enormous complexity involved. Some of the classical systemic actions of hormones on bone may be mediated at tissue level via local production of growth factors and cytokines, and these effects may be mediated in turn by agents such as prostaglandins. The systemic and local actions on bone cells of simple inorganic moieties such as hydrogen ions and molecular oxygen (Arnett et al, 2003), phosphate (Yates et al, 1991) or nitric oxide (Ehrlich & Lanyon, 2001) also appear to be of considerable importance.

The strain resulting from mechanical loading is a key regulator of remodelling in some parts of the skeleton. The long bones and the vertebral bodies appear to require modest but regular loading cycles in order to maintain their mass. The mass and strength of bones in normal individuals is ultimately determined by the need to resist the loads and deformations resulting from the most extreme normal activities (for example, jumping off a wall 1-2 metres high on to a hard surface); this is the so-called ‘error strain hypothesis’ (Ehrlich & Lanyon 2001).

References

1. Cooke AM (1955) Osteoporosis *Lancet* i: 878-882 & 929-937.
2. Olsen BR 1999 Bone morphogenesis and embryologic development. In: American Society for Bone and Mineral Research Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, Edition 4 (Favus MJ *et al*, Editors). Lippincott, Williams & Wilkins, Philadephia, pp11-14. *Note that this volume contains many other useful, brief reviews*.
3. Arnett TR, Gibbons DC, Utting JC, Orriss IR, Hoebertz A, Rosendaal M, Meghji S 2003 Hypoxia is a major stimulator of osteoclast formation and bone resorption. Journal of Cellular Physiology 196: 2-8.
4. Ehrlich PJ, Lanyon LE 2002 Mechanical strain and bone cell function: a review. Osteoporosis International 13: 688-700.
5. Yates AJ, Oreffo RO, Mayor K, Mundy GR 1991 Inhibition of bone resorption by inorganic phosphate is mediated by both reduced osteoclast formation and decreased activity of mature osteoclasts. Journal of Bone and Mineral Research 6: 473-478.
6. http://courses.washington.edu/bonephys/ - *animations of the bone remodelling sequence in health and disease.*

Table 1 - Regulation of bone cell function

(↑ = increases; ↓ = decreases; → = results in; OB = osteoblast; OC = osteoclast)

1. Hormones

Parathyroid hormone (PTH): ↑ plasma Ca^{2+} (critical regulator); ↑ OC formation, activity; ↑ OB proliferation, activity → ↑ bone turnover; intermittent PTH ↑ bone formation *in vivo*; continuous high-dose PTH ↑ bone resorption → bone loss.

1,25(OH)$_2$-vitamin D: ↑ gut Ca^{2+} uptake, plasma Ca^{2+}; ↑ OC formation, activity; ↓ OB proliferation, ↑ OB (and skin cell) differentiation; required for normal matrix mineralisation; deficiency → osteomalacia, rickets.

Calcitonin: ↓ plasma Ca^{2+} in young/hypercalcaemic animals; ↓ OC formation, activity; "emergency" hormone, not much effect in normal adults.

Glucocorticoids: necessary for normal bone development / function; excess → bone loss / osteoporosis; required for bone formation *in vitro*.

Growth hormone: required for normal bone growth.

Sex steroids (oestrogens & androgens): critical long-term, beneficial effects on bone maintenance; ↓ OC formation, activity; ?↑ OB activity; deficiency → ↑ bone turnover, osteoporosis.

2. Local (paracrine / autocrine) factors

Growth factors: mitogens; many produced by OB and other connective tissue cells, *eg* transforming growth factor ß, bone morphogenetic proteins, insulin-like growth factors I,II, platelet-derived growth factor, fibroblast growth factors; generally ↑ OC recruitment & activity, ↑ proliferation of OB & other cells.

Cytokines: products of immune & other cells; interleukins- 1, 2, 4, 6, 11, 13 & 17, tumour necrosis factor-α, RANK ligand, macrophage colony-stimulating factor ↑ OC formation/activity; interleukins 4, 10, 12, 13 & 18, interferon-γ & granulocyte macrophage colony-stimulating factor ↓ OC formation; interleukins may mediate some actions of PTH, 1,25$(OH)_2$-vitamin D & sex steroids.

Other molecules: Prostaglandins (normal OB product) ↑ OC recruitment, ↑↓ OC activity; mediate some actions of growth factors, cytokines, responses to mechanical stimuli & hypoxia. Leukotrienes ↑ OC formation/activity. Extracellular ATP ↑ OC formation/activity, ↓ OB activity; possible mediator of inflammatory bone loss. Bradykinin ↑ OC formation / activity; CGRP ↓ OC formation/activity

3. Inorganic agents (local / systemic)

Ions: extracellular H^+ (pH <~7.2) ↑ OC (required for OC activation), ↓ OB function. PO_4^{3-} ↓ OC formation/activity. Ca^{2+} ↓ OC formation/activity (small effect). Sr^{2+} ↑ bone formation. F^- ↑ bone formation.

Oxygen & free radicals: hypoxia ($PO_2 \leq 5\%$) ↑ OC formation, ↓ OB function. Nitric oxide ↑↓ OC formation/activity, ↑↓ OB activity; required for normal bone remodelling, mediates responses to loading.

4. Mechanical effects

cyclical loading ↑ OB activity, bone formation, adaptive remodelling; unloading (bed rest, micro-gravity) ↑ OC formation/activity; large changes in hydrostatic pressure ↑ osteocyte death.

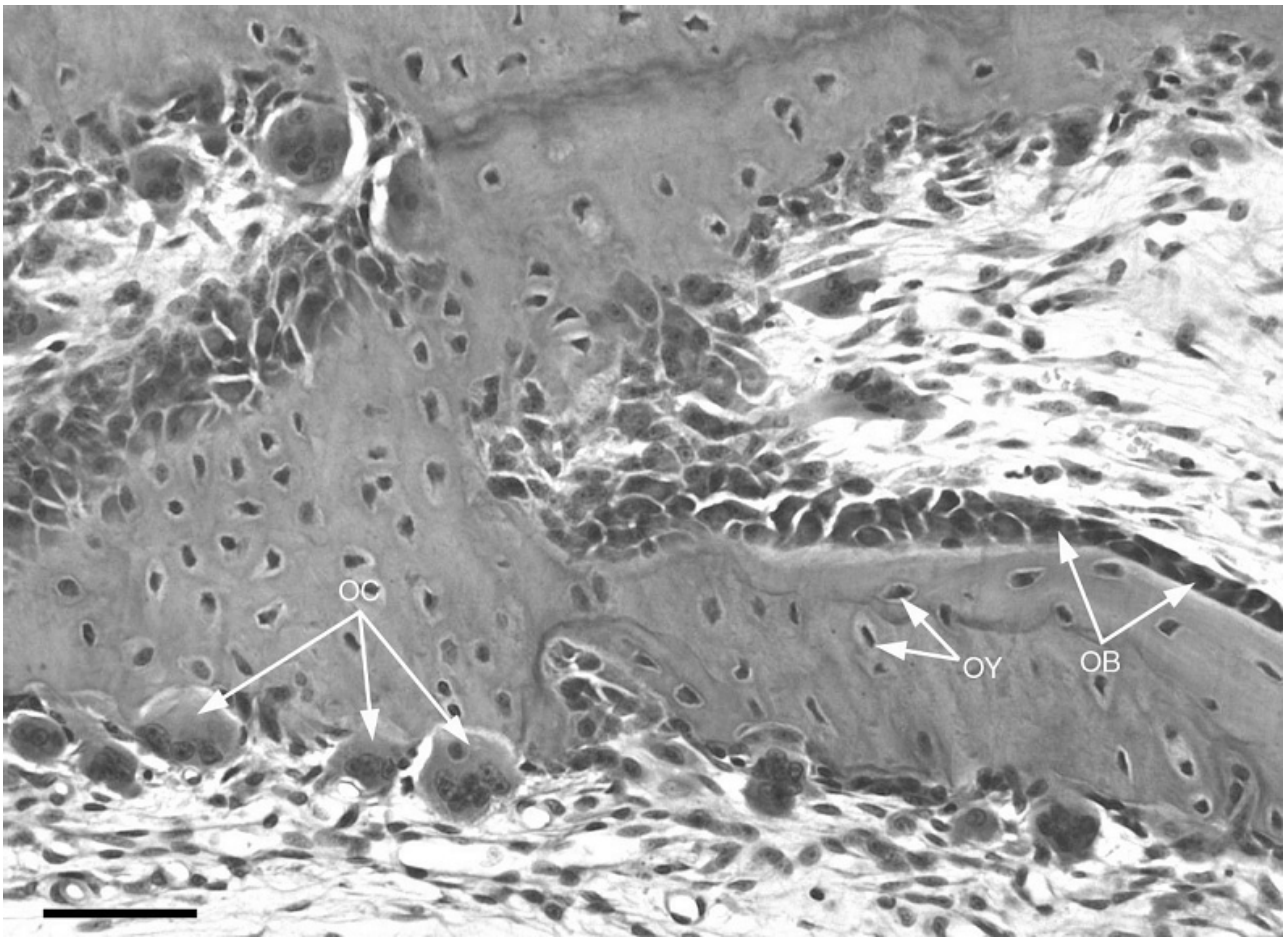

Figure 1. Section of rapidly remodelling bone from the jaw of a young animal, showing: active osteoblasts (OB) forming bone; osteocytes (OY) entombed in bone matrix; multinucleate osteoclasts (OC) forming resorption pits in the bone surface. Scale bar = 50 μm.

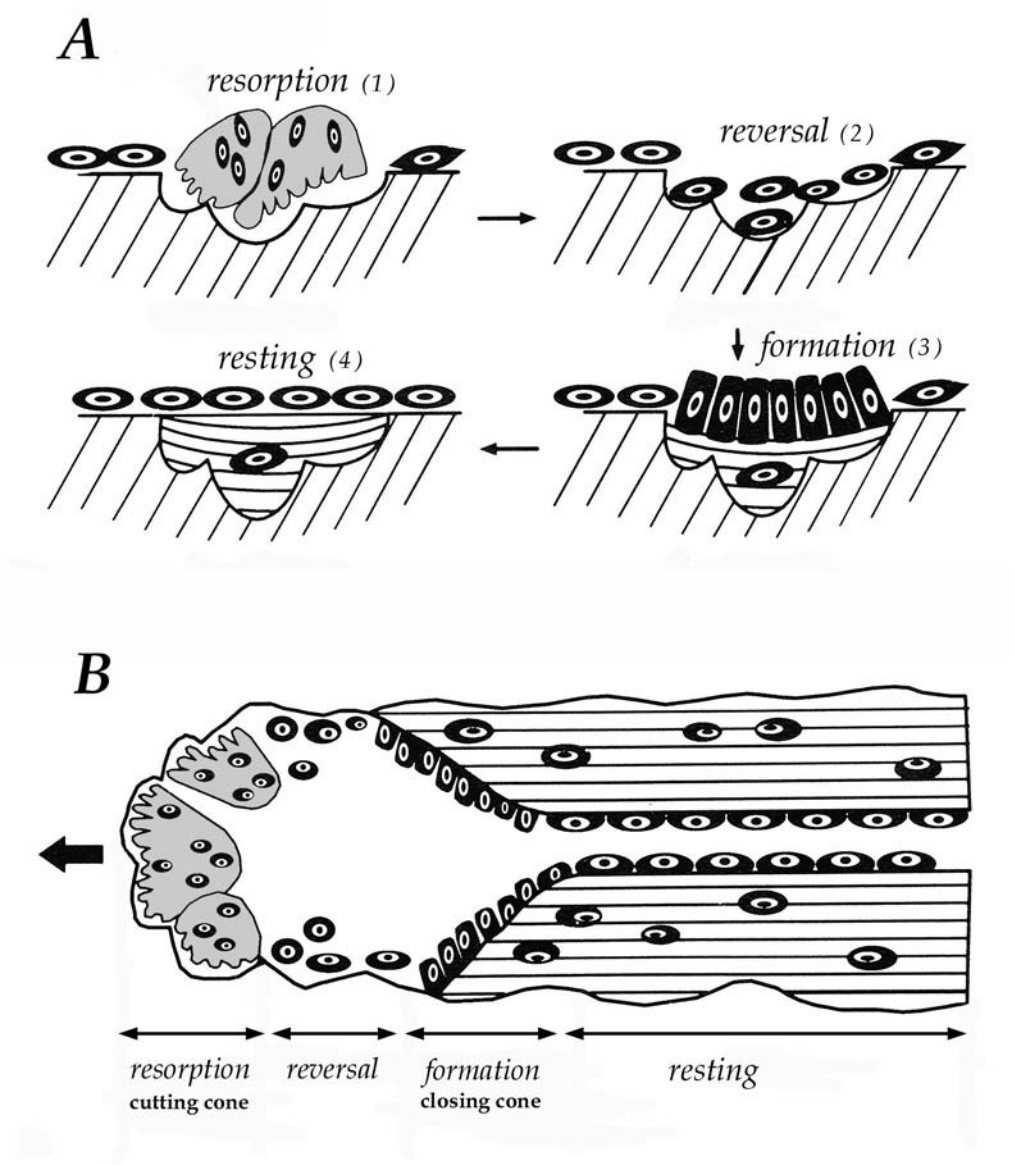

Figure 2 A: Schematic of remodelling sequence on a trabecular (cancellous) bone surface. Resorption *(1)* is initiated following recruitment and activation of osteoclasts (shaded grey); in the reversal phase *(2)*, osteoclasts either die by apoptosis or migrate to other sites, and the resorption cavity is temporarily invaded by mononuclear cells; active osteoblasts (shaded black) then appear at the site and begin forming new lamellae of bone matrix *(3)*, with differing collagen fibre orientation to pre-existing lamellae; when bone formation ceases *(4)*, osteoblasts assume a quiescent, flattened morphology and the bone surface returns to a resting state. Note that when changes in bone shape or size are required, cavities may be overfilled or underfilled. **B:** Remodelling sequence in cortical (compact) bone. Osteoclasts (formed from precursors that extravasate from an adjacent blood vessel) resorb tunnels ~0.1 mm in diameter and ~2 mm in length along the axis of the bone; following a reversal phase, osteoblasts begin depositing concentric layers of new bone matrix to gradually infill the tunnel; the central canal of the mature secondary (Haversian) osteon is lined by resting osteoblasts, and contains a blood vessel.

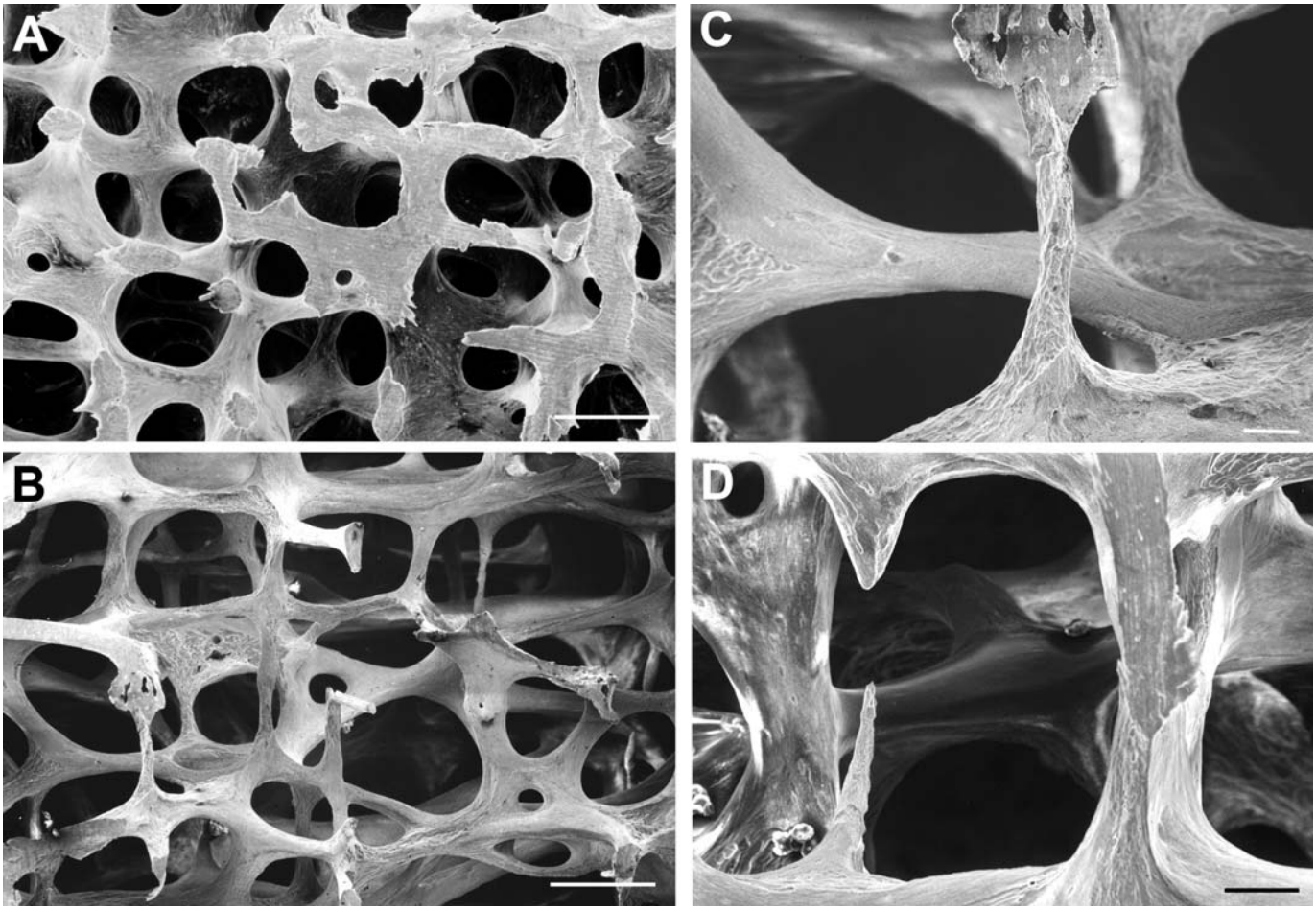

Figure 3 A, B: Scanning electron micrographs of vertical sections through the bodies of the third lumbar vertebrae (after removal of bone cells and soft tissues) of a normal 30 year old woman and a 71 year old woman, respectively. Severe osteoporotic changes are evident in **B**; the trabeculae are eroded to thin, mechanically insufficient rods; scale bars = 500 μm. **C:** Higher power detail of plate **B**, showing extensive osteoclastic resorption pits on the vertical trabecular element in the foreground; this element displays a possible microfracture callus at the top; scale bar = 100 μm. **D:** Trabecular element perforated by osteoclast action, with clearly visible profiles of resorption pits; scale bar = 100 μm.