How many tissues in a bone?

- Bone
- Red = haemopoietic marrow
- White/yellow = fatty marrow
- Blood vessels & nerves
- Cartilage
- Tendon = muscle to bone
- Ligament = bone to bone
- Periosteum and perichondrium
how we study bone structure

• Light microscopy  LM  Conventional & Confocal
• Polarised light microscopy  PLM
• Circularly polarised light  CPL
• Ground sections = undecalcified
• Decalcified sections, stained for cells
• TEM = undecalcified or decalcified
• SEM = undecalcified: shape or composition
• X-rays and microtomography  µCT
How many bone tissue types in a bone? Naked eye structure level

• ‘Compact’ = say more than 80% bone =
• ‘Cortical’ = position, but term often used to mean solid bone
• ‘Cancellous’ or ‘Spongy’ or ‘Trabecular’ = say less than 50% bone =
• ‘Medullary’ = position, but term sometimes used to mean open, porous bone
• What about the grey region between 50% and 80% BVF?
Compact vs cancellous, femoral neck, one becomes the other
What is cancellous?
Compact vs cancellous which is which?
Bone tissue types in a bone?
By origin - what was there first?

- Cartilage - majority of bones form in a cartilage model
- Membrane - *de novo* bone: clavicle, mandible, vault of skull
- Tendon - calcified tendon and sesamoid bones
How many types of cartilage in a bone?

- Calcified growth plate cartilage acts as temporary mould or scaffold for bone and is mostly replaced
- Calcified fibrocartilage where a tendon or ligament meets bone at a changing angle
- Articular Calcified Cartilage: also a growth site in the young, but always there to attach
- Hyaline articular cartilage
Growth plate cartilage, proliferative zone
Micro-structure level

- **Woven bone** - all start-up bone without a scaffold is ‘woven’, is itself a scaffold
- Calcified cartilage - scaffold for bone
- Calcified fibrocartilage
- Articular Calcified Cartilage
- **Lamellar bone** - appositional = it forms on a scaffold, mostly lamellar bone
- Sharpey fibre bone = calcified tendon or ligament
How many lamellar bone types?

Micro-structure level

- Lamellar means layered, actually sheets of branching bundles of collagen
- Primary parallel fibred
- Crossed lamellar - alternating light & dark in a polarising microscope
- Anything in between
- VIP differences in statistics!
How many lamellar bone types?
Micro-structure level

- **Circumferential lamellar** @ periosteal really indistinguishable from same type @ endosteal surfaces
- **Primary osteon(e)s** = scaffold of woven bone sprouted out from a surface: intervascular ridges
- **Secondary osteones or Haversian systems** = laid down within a tunnel dug into bone or any other calcified tissue in a bone
- ‘**Packets’** or Bone Modelling Units [BMU] in cancellous bone
3y non-pregnant non-lactating ewe rib TS

- Rapid annual growth mainly on one cortex
- Last year’s mostly 1° osteons, several cutting cones
- First year’s now mostly 2° osteons
LPL: slice examined between crossed linearly polarising filters

- ‘Immature’ [foetal, fracture callus, de novo] bone matrix contains some collagen fibre bundles large enough to be seen by LM. These are randomly oriented, but only those at 45° to the polarising filter are seen.

- Look = ‘woven’ [warp and woof] but
- fabric structure is a feltwork.
LPL: slice examined between crossed linearly polarising filters

- Collagen parallel to section plane and in 45° sectors appears bright
- Collagen perpendicular to section plane or parallel to filter planes [in 90° sectors] appears dark
- Maltese cross appearance of cross sectioned osteones: alternate light dark lamellae in 45° sectors
crossed linear polarisers
CPL = slice examined between crossed circularly polarising filters

- polar / 45° ¼λ plate/ bone section / 135° ¼λ plate / analysor
- All collagen parallel to section plane appears bright
- Collagen perpendicular to section plane appears dark
- No Maltese cross: alternate light dark lamellae in any azimuth
LPL vs CPL
Midshaft of horse radius

CPL for mapping collagen orientation
darker colours = more longitudinal
‘Ground’ or sawn sections

- Embedded in a high refractive index mounting medium like DPX or balsam
- Nothing can be seen in ordinary transmitted light microscopy
- By nothing, I mean nothing but!!!
- Confocal reflection and fluorescence imaging is excellent
Lamellae seen by reflection confocal microscopy
osteones of different ages seen by confocal autofluorescence microscopy
‘Ground’ or sawn sections

- Mounted dry in air, light is strongly reflected by low refractive index, air=1, osteocyte lacuna spaces in high index [1.44] bone: lamellae may also show
- Mounted wet in water, light is reflected at low refractive index, H₂O=1.33, osteocyte lacuna spaces in high index [1.44] bone
- Microradiography
Decalcified or demineralised sections for light microscopy

- Bone is too hard to cut as continuous sections with a knife.
- It is hard because of its calcium phosphate [carbonate apatite] salt content.
- It can be softened by removing same:
  - either with any acid
  - or with a chelating agent e.g. EDTA
Decalcified or demineralised sections for light microscopy

• Tissue decalcified in bulk after fixation
• using formaldehyde or glutaraldehyde as protein cross-linking agents
• embedded in molten paraffin wax, cooled
• cut into 5-15µm sections
• dewaxed in xylene  
  contd
Decalcified or demineralised sections for light microscopy

- Xylene removed with ethanol
- Ethanol removed with water
- Tissue stained, e.g. haematoxylin and eosin, H&E
- Water removed with ethanol
- Ethanol removed with xylene
- Mounted in RI = 1.5 balsam or DPX in xylene, same RI as glass coverslip
Decalcified or demineralised sections for light microscopy

- Stained sections let you see the cells
- BUT all fat is gone - dissolves in ethanol & xylene etc
- AND all mineral is gone, and much of the magic in bone is the mineral
- AND you can see hardly anything of the matrix structure
Transmission electron microscopy

- TEM: fix in glutaraldehyde and OsO$_4$
- dehydrate and embed in plastic [epoxy]
- cut 0.05µm sections
- optional staining with U and Pb
- shoot through 80keV+ electrons
- capture transmitted electrons on photo film
Transmission electron microscopy

- Bone salt ‘crystals’ are so tiny that they can be deformed over the 60° knife edge
- Undemineralised sections can be cut, but very few people do it: diamonds are needed
- Interpretation confused between added electron density of Os, U and Pb and natural density of bone salt
- AND bone salt dissolves in OsO₄ etc etc contd
Transmission electron microscopy

- High magnification = small field of view = blinkered vision, poor sampling
- Useless for bone mineral, where unfixed unstained anhydrous handling is required
- Solution for TEM of mineral phase is to use ion beam or fast atom beam thinning of thin sections
- NOT a popular research field!
Scanning electron microscopy

- Look AT, not THROUGH, a sample, but in a vacuum, so it has to be dry.
- Great news for bone, which does not contain too much water [it is replaced by bone salt] and does not shrink too much.
- Q: But what do we see?
- A: Cells !! unless we get rid of most or all of them. How do they do that?
Scanning electron microscopy

- Find a flat bone & peel off layers of cells above the osteoblasts and osteoclasts, or
- Wash and digest off all the cells to expose the collagen rich bone matrix surface, or
- Dissolve all bone matrix that is not mineralised using proteases, alkalis, oxidising agents, when we have a mineral front preparation [(superficially) anorganic or deproteinised]
GA fixed CPD
intercellular gaps arise
during critical point
drying process
30kV SE image
EDAX analysis obtained
from bone beneath!

Rat parietal osteoblasts *ex vivo*
Osteoclasts liberate live osteocytes.
Rat calvarium, GA in TES, Leishman’s, Toluene AD, 10kV SE
Density of osteocytes and canaliculi

PMMA internal cast of sheep rib
Normal mature female

30y F L4
1kV SE
4mm slice
2.7mm grid background
bone packet distribution by BSE-SEM: note that they do not fit the prior resorption fields
Vital labelling

• Tetracycline
• calcein
• alizarin
• etc etc etc
Multiple tetracycline labels
human femur shaft