Bone physiology

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Bone physiology

Aims

- Osseointegration
- Wound healing response
- Recap bone formation and remodelling

Osseointegration

- Characterised as a direct structural and functional connection between ordered living bone and the surface of a load carrying implant - ankylotic relationship (Branemerk et al, 1985)
- Compare to connective tissue encapsulated implants within bone described as "fibro-osteal integration."
- Former stable and desirable; latter unstable

Hard Tissue Interface

Fibro-osseo integration - least successful

- Distance between bone and gold implants - 40-60nm; fibrous capsule
- Distance between bone zirconium implants - 30-50nm - despite zirconium oxide layer
- Biotolerant

Bio-osseous intergation
- good result

Steel implant - fibrous capsule formation around a liquid filled void

Hard Tissue Interface

Osseointegration - best result

Bone comes into intimate contact with titanium (20nm)

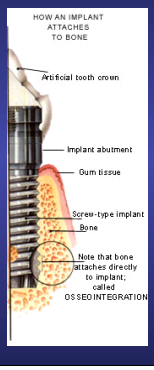
Similar response with tantalum and aluminium oxide

Bioinert

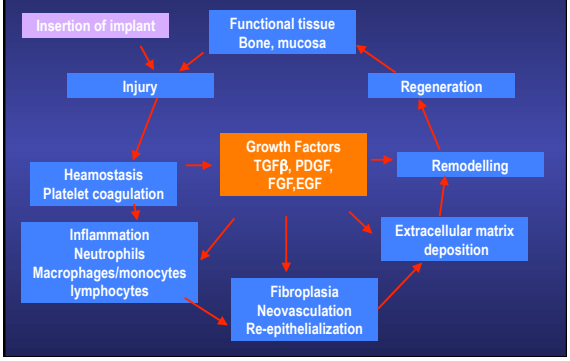
Titanium implant - connective tissue layer tightly adhering

Mechanism of Osseointegration

- Forcible insertion of implant material into bone results in a progression of events initiated by the injury.
- TRAUMA !!!!
- Directed towards the re-establishment of normal bone structure and function around the implant.
- Osseointegration occurs via multiple indistinct / overlapping phases.



Wound repair



Inflammatory Phase

Implant insertion - trauma (injury to blood vessels increases blood release).

- Protein adsorption - blood / other tissue fluid proteins.
- Blood coagulates - forms fibrin clot.
- Consists of aggregating platelets in a fibrin network (reduce blood flow).

Clot rich in - fibronectin, hyaluronan, vitronectin, thrombospondin.

Functions

- Reservoir of proinflammatory cytokines / growth factors.
- Provisional matrix for cell migration / activation.

Granulation Tissue Phase

Neutrophil / bacterial / ECM debris phagocytosed.

Fibrin clot ⇒ granulation tissue (fibrinolysis).

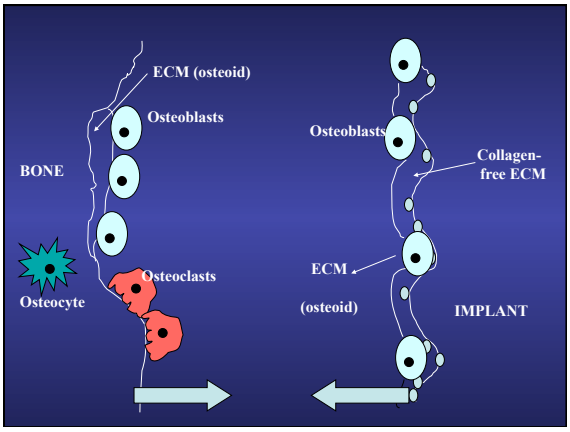
- Granulation tissue - rich in hyaluronan.
- Acts as a provisional matrix for osteoprogenitor cell differentiation (osteoinduction). Activated by BMPs.

Monocyte recruitment ⇒ macrophages.

- Macrophages release cytokines / growth factors.

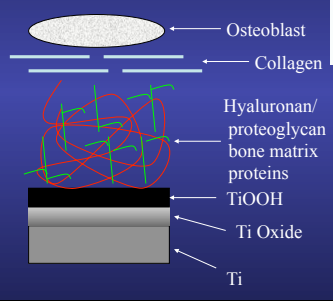
Hyaluronan degraded (hyaluronidase / ROS).

- New blood vessel formation (angiogenesis).



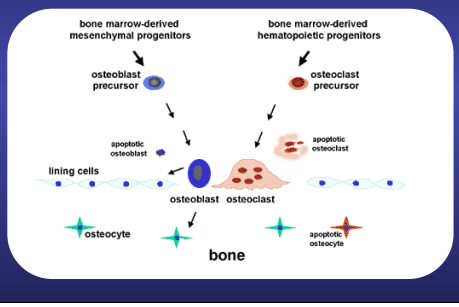
Proposed Model of Tissue-Titanium Interface

Does the Collagen free zone (lamina limitans function to provide substrata for cell adhesion?



Origins of bone cells

Osteoblasts, Osteocytes, Osteoclasts



Origin of Osteoblast

- Origin mesenchymal stem cells (stromal cells cells)
- Evidence *(Friedenstein 1976 and Beresford 1989)*
  - Bone marrow stroma, connective tissue cells which provide haematopoietic marrow structural support
  - Differentiate into many mesenchymal cells e.g osteoblasts as well as chondroblasts, fibroblasts, adipocytes and myoblasts.

Osteoprogenitor cells

- Stem cell population
- Osteoprogenitor cells
- Periosteum, PL, marrow spaces
- *C-myc, c-fos, cbfa 1*

Regulation of osteoblast differentiation

- Key regulator is *core-binding factor alpha-1* (Cbfa-1)
- Member of core –binding family of transcription factors
- Similar to the Drosophila (invertebrate) *runt gene* product *(Ogawa 1993)*
  - *Segmentation of body*
  - *Sex determination*
  - *Development of nervous system*

Regulation of osteoblast differentiation

- Cbfa-1 binds to and regulates the expression of a number of genes in cultured osteoblasts
  - *osteocalcin*
  - *type 1 collagen*
  - *bone sialoprotein*
  - *osteopontin*

Regulation of osteoblast differentiation

- Mice *(Ducy 1997)*
  - Cbfa-1 expression upregulated in osteoblast lineage
  - Upregulated by BMP-7
  - Down regulated by 1,25 di-OH Vit D3
- Cbfa deficient mice
  - Homozygous die at birth, resp failure, normal cartilage, no bone (membrane or endochonrial ossifcation)
  - Heterozygous skeletal abnormalities similar to cleidocranial dysostosis *(Komori 1997)*

Regulation of osteoblast differentiation

Regulation of osteoblast differentiation

- Bone morphogenic proteins (BMPs) determine osteoblast phenotype
- Members of TGF-beta superfamily
- Discovered 1965 *(Urist)*, purified from bone 7 types BMP-1 to BMP-7 *(Wang 1988)*

Regulation of osteoblast differentiation

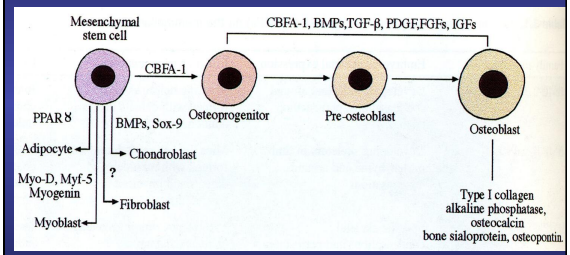
- BMPs
- Present at sites of skeletogenesis, axial vertebrate development, ectodermal ridge in developing limb bud
- Induce formation bone, cartilage and connective tissues

Family member	Embryonic skeletal expression	Effect of gene mutation	Effect of protein overexpression
BMP-2	Developing skeleton, around condensations, periosteum, teeth	Mice die before skeleton formed with defects in amnion, chorion and heart	In embryos, overexpression changes skeletal pattern; in adults, induces bone
BMP-4 (DPP)	Developing skelton, in limb mesenchyme and around condensations	Mice die before skeleton formed with mesoderm defects and impaired gastrulation	In embryos, overexpression changes skeletal pattern; in adults, induces bone
BMP-5	Specific skeletal condensations and periosteum	Viable mice missing skeletal elements and having impaired fracture repair: naturally ocfcurring muytation, short-ear (se/se)	In embryos, not known; in adults, induces bone
BMP-6 (Vgr-1)	Hypertrophic cartilage	Not yet reported	In embryos, changes skeletal pattern; in adults, induces bone
BMP-7 (OP-1)	Craniofacial condensations, perichondrium, hypertrophic chondrocytes	Mice die at birth; kidney and eye defects; also some polydactyly	In embryos, not known; in adults, induces bone
GDF-5 (CDMP-1; BMP-14)	Most developing joints	Viable mice and humans with short limbs and joint defects; natuarally occuring mutation, brachyhypodism (bp/bp)	In embryos, not known; in adults, induces cartilage, tendon/ligament
GDF-6 (CDMP-2; BMP-13)	Elbow and wrist joints	Not yet reported	In embryos, not known; in adults induces cartilage, tendon/ligament
GDF-7 (CDMP-3; BMP12)	Shoulder and digit joints	Not yet reported	In embryos, not known; in adults induces cartilage, tendon/ligament

Regulation of osteoblast differentiation

- In cell culture BMPs stimulate phenotypic markers of osteoblasts *(Asahina 1993)*
  - Alkaline phosphatase
  - Osteocalcin
  - cAMP response to PTH
  - Type 1 collagen synthesis
- Also direct entry into chondrogenic but inhibit the myogenic, adipogenic developmental pathways *(Gimble 1995)*

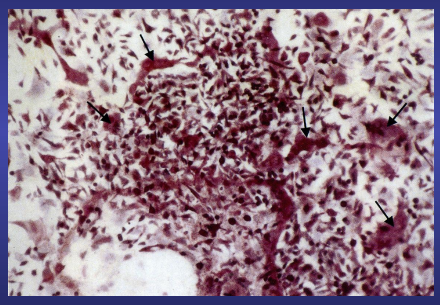
Regulation of osteoblast differentiation



Origin of osteoclasts

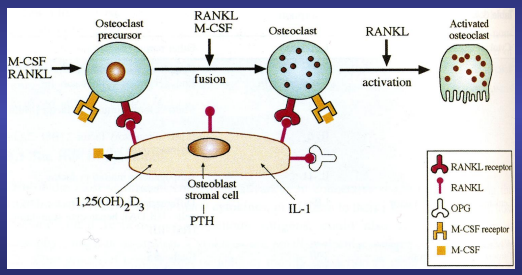
- Mononuclear precursor sells, migrate from bone marrow to skeleton via blood stream
- Mononuclear cells arrive and fuse together or remain single to form a pool for future osteoclast formation
- Control comes from
  - osteoblast derived signals and
  - growth factors from the bone matrix

Origin of osteoclasts

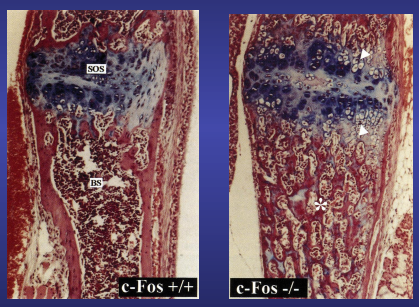


Bone remodelling;controlling mechanisms 0steoprotegerin/osteoprotegerin ligand

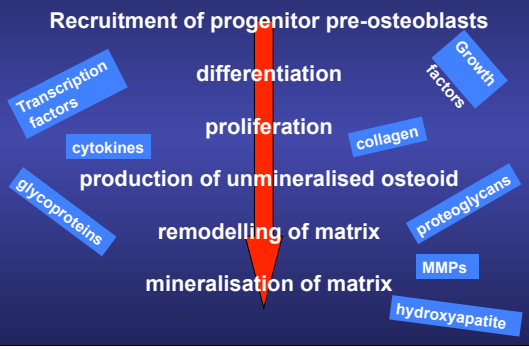
(Simonet 1997)



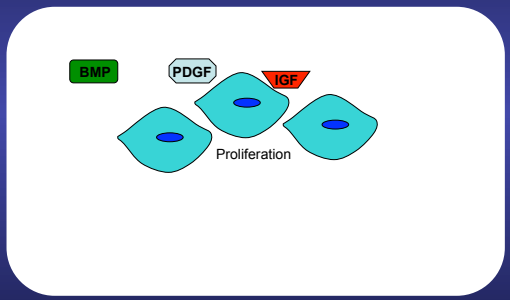
Origin of osteoclasts



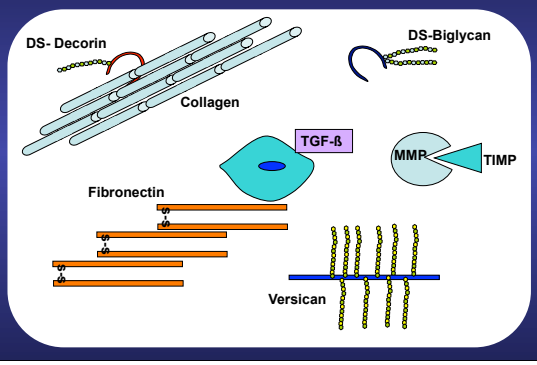
Formation of bone



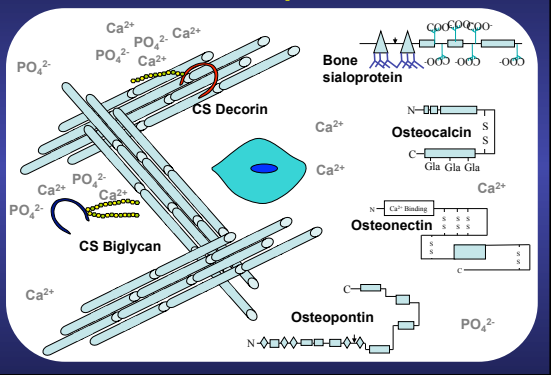
Cell differentiation and proliferation



Osteoid formation



Mineral deposition

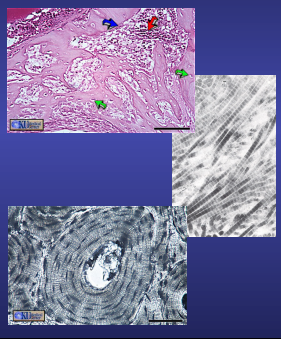


Initial Bone Formation

Woven bone - immature

- Deposition of woven bone 4-6 weeks after surgery
- Rapidly formed; 1mm within 2 days
- Characterised by random felt-like orientation of fibres
- High vasculature
- Numerous irregularly shaped osteocytes
- Low mineral density

Remodelled bone

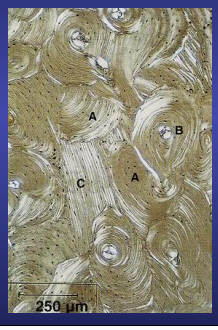


- Subsequent remodelling of woven bone to form lamella bone
- Collagen packed as parallel fibres with alternating courses in several planes
- Slowly formed, 1-1.5µm/ day
- Parallel-fibered bone may also result where fibres run parallel but without a preferential plane

Coupling of bone resorption and formation

- Connective tissue undergoes continual synthesis and degradation
- Old bone resorbed by osteoclasts and new bone made by osteoblasts
- Past activity marked by reversal lines

histology



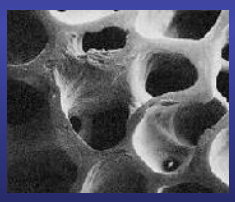
remodelling

remodelling

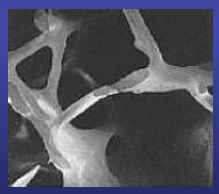
Reversal lines

Bone remodelling

normal



osteoporotic



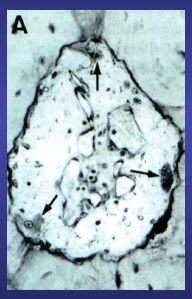
Bone structure and remodelling

- Why remodel?
  - Calcium homeostasis
  - Removal old bone
  - Adaptation to strain and exercise
  - Repair of microfractures
- 25% cancellous bone yearly replaced *cf* 3% of cortical bone
- Whole skeleton replaced every 10 years

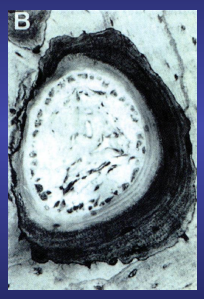
Bone structure and remodelling

- Remodelling takes place in discrete packets
- Basic multicelluar units (BMU)
- BMU life span of 6-9months
- Each location is resorbed every 2-5 years
- Osteoclasts dig out osteonal tunnels
- 9 osteoclasts at 50 micrometers/day *(Jaworski 1992)*
- Osteoblasts fill in the tunnel in reversal phase
- secondary osteons, 200micron wide and upto 10mm long!

Bone resorption

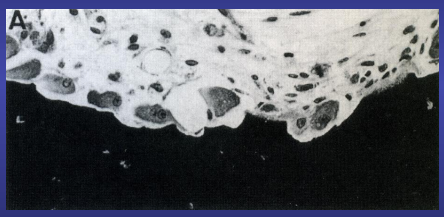


Bone resorption

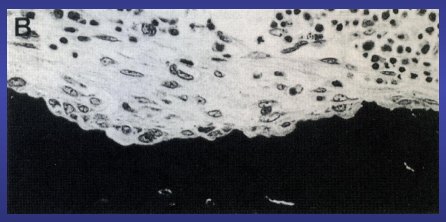


Bone resorption
secondary osteon

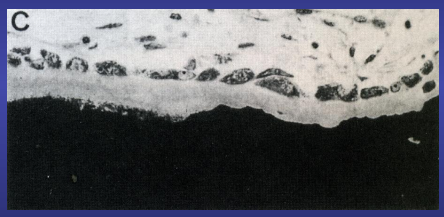
Resorption phase
(trabecular bone)
osteoclasts resorb to 70 micrometers



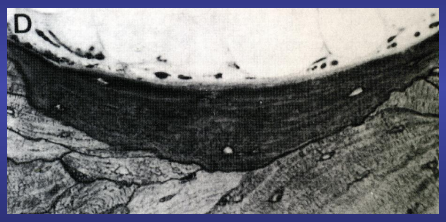
Reversal phase
osteoblast prescursors migrate



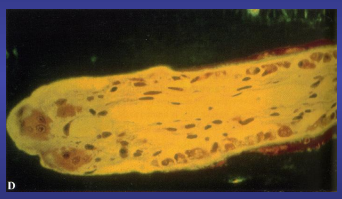
Formative phase
osteoblasts secrete osteoid



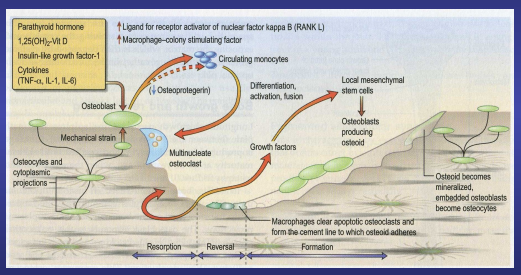
BMU reversal line



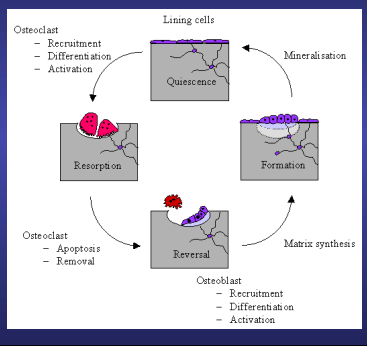
Bone resorption
secondary osteon
200 micrometeres wide and 10mm long



Bone remodelling



Bone remodelling



Bone formation/implant surfaces

- Surgical implant placement
  - Interface implant and bone
  - Bone, marrow tissue, haematoma, bone fragments
  - Inflammatory and mesenchymal cell migration from adjacent vessels and marrow stroma towards interface surface
  - Haematoma replaced by BV and connective tissue
  - MNGC cover surfaces but decrease with time

Bone formation/implant surfaces

- Early phase healing (4-16 weeks)
  - Osteoblast form woven bone from trabecullae and endosteal surface
  - Resorption
  - Bone formation secondary osteons
  - Further woven bone in voids between cut bone and implant
  - Increased bone condensation towards implant and into threads

Bone formation/implant surfaces

- Late phase healing (4-12 months)
  - Amount of bone in threads increases with time
  - Bone implant contact increases
  - Lamellar bone replaces woven bone

Bone formation/implant surfaces

- Complete healing appears to take longer than conventional 3-6 months (Branemark 1977)
- Implant stability
  - Soft bone and dense bone issue
  - Primary stability
- Early loading *(Ko et al J Dent Res 2003)*
  - Enriched osteoid, indistinct osteoblasts with denser staining nucleii, increased mineral matrix production, increased morphogenic protein production
  - Gene expression