Bone physiology

StJohn Crean
BDS MBBS FDSRCS FFGDP(UK) FRCS
FRCS(OMFS) PhD FHEA
UCLan
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 (Branemark 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-osseous 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 integration
  - good result

Steel implant - fibrous capsule formation around a liquid filled void
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

Insertion of implant

Injury

Heamostasis
Platelet coagulation

Inflammation
Neutrophils
Macrophages/monocytes
lymphocytes

Functional tissue
Bone, mucosa

Growth Factors
TGFβ, PDGF, FGF, EGF

Remodelling

Regeneration

Extracellular matrix deposition

Fibroplasia
Neovascularization
Re-epithelialization
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 $\Rightarrow$ granulation tissue (fibrinolysis).
- Granulation tissue - rich in hyaluronan.
- Acts as a provisional matrix for osteoprogenitor cell differentiation (osteoinduction). Activated by BMPs.

Monocyte recruitment $\Rightarrow$ macrophages.
- Macrophages release cytokines / growth factors.

Hyaluronan degraded (hyaluronidase / ROS).
- New blood vessel formation (angiogenesis).
BONE

IMPLANT

ECM (osteoid)

Osteoblasts

Osteoclasts

Collagen-free ECM

Osteocyte

ECM (osteoid)
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 \textit{(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
- $C\text{-}myc$, $c\text{-}fos$, $cbfa\ 1$
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 endochondrial ossification)
  - 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
<table>
<thead>
<tr>
<th>Family member</th>
<th>Embryonic skeletal expression</th>
<th>Effect of gene mutation</th>
<th>Effect of protein overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP-2</td>
<td>Developing skeleton, around condensations, periosteum, teeth</td>
<td>Mice die before skeleton formed with defects in amnion, chorion and heart</td>
<td>In embryos, overexpression changes skeletal pattern; in adults, induces bone</td>
</tr>
<tr>
<td>BMP-4 (DPP)</td>
<td>Developing skeleton, in limb mesenchyme and around condensations</td>
<td>Mice die before skeleton formed with mesoderm defects and impaired gastrulation</td>
<td>In embryos, overexpression changes skeletal pattern; in adults, induces bone</td>
</tr>
<tr>
<td>BMP-5</td>
<td>Specific skeletal condensations and periosteum</td>
<td>Viable mice missing skeletal elements and having impaired fracture repair: naturally occurring mutation, short-ear (se/se)</td>
<td>In embryos, not known; in adults, induces bone</td>
</tr>
<tr>
<td>BMP-6 (Vgr-1)</td>
<td>Hypertrophic cartilage</td>
<td>Not yet reported</td>
<td>In embryos, changes skeletal pattern; in adults, induces bone</td>
</tr>
<tr>
<td>BMP-7 (OP-1)</td>
<td>Craniofacial condensations, perichondrium, hypertrophic chondrocytes</td>
<td>Mice die at birth; kidney and eye defects; also some polydactyly</td>
<td>In embryos, not known; in adults, induces bone</td>
</tr>
<tr>
<td>GDF-5 (CDMP-1; BMP-14)</td>
<td>Most developing joints</td>
<td>Viable mice and humans with short limbs and joint defects; naturally occurring mutation, brachy hypodism (bp/bp)</td>
<td>In embryos, not known; in adults, induces cartilage, tendon/ligament</td>
</tr>
<tr>
<td>GDF-6 (CDMP-2; BMP-13)</td>
<td>Elbow and wrist joints</td>
<td>Not yet reported</td>
<td>In embryos, not known; in adults induces cartilage, tendon/ligament</td>
</tr>
<tr>
<td>GDF-7 (CDMP-3; BMP12)</td>
<td>Shoulder and digit joints</td>
<td>Not yet reported</td>
<td>In embryos, not known; in adults induces cartilage, tendon/ligament</td>
</tr>
</tbody>
</table>
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

Mesenchymal stem cell

CBFA-1, BMPs, TGF-β, PDGF, FGFs, IGFs

CBFA-1

Osteoprogenitor

Pre-osteoblast

Osteoblast

Type I collagen, alkaline phosphatase, osteocalcin, bone sialoprotein, osteopontin.

PPARγ

Adipocyte

BMPs, Sox-9

Chondroblast

Myo-D, Myf-5

Myogenin

Myoblast

Fibroblast
Origin of osteoclasts

• Mononuclear precursor cells, 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
Osteoprotegerin/osteoprotegerin ligand
*(Simonet 1997)*
Origin of osteoclasts
Formation of bone

Recruitment of progenitor pre-osteoblasts

differentiation

proliferation

production of unmineralised osteoid

remodelling of matrix

mineralisation of matrix

Transcription factors
cytokines
glycoproteins
Growth factors
collagen
proteoglycans
MMPs
hydroxyapatite
Cell differentiation and proliferation

- BMP
- Cbfa-1
- Progenitor cell
- Osteoblast differentiation
- IGF
- PDGF
- Proliferation
Osteoid formation

DS - Decorin

Collagen

Fibronectin

Versican

TGF-β

MMP

TIMP

DS-Biglycan

Collagen
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, 1-1.5 \( \mu m \)/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
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 multicellular units (BMU)
- BMU life span of 6-9 months
- Each location is resorbed every 2-5 years
- Osteoclasts dig out osteonal tunnels
- 9 osteoclasts at 50 micrometers/day \textit{(Jaworski 1992)}
- Osteoblasts fill in the tunnel in reversal phase
- Secondary osteons, 200 micron wide and upto 10 mm long!
Bone resorption
Bone resorption
Bone resorption
secondary osteon
Resorption phase
(trabecular bone)
osteoclasts resorb to 70 micrometers
Reversal phase
osteoblast precursors migrate
Formative phase
osteoblasts secrete osteoid
BMU reversal line
Bone resorption
secondary osteon
200 micrometeres wide and 10mm long
Bone remodelling

- **Osteoclast**
  - Recruitment
  - Differentiation
  - Activation

- **Lining cells**

- **Quiescence**

- **Mineralisation**

- **Resorption**

- **Formation**

- **Matrix synthesis**

- **Reversal**

- **Osteoclast**
  - Apoptosis
  - Removal

- **Osteoblast**
  - Recruitment
  - Differentiation
  - Activation
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