Biology of Bone

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• Background
• Modelling / Remodelling of Bone
• Controlling bone cell activity
• The role of mechanical load
• Coordinating bone cell activity
• Biology in Orthopaedics
To meet mechanical need
Bone formation

- Bone formation is termed “ossification” or “osteogenesis”
- The “early” skeleton in an embryo is composed of fibrous membranes and hyaline cartilage
- During the process of ossification (around the 6-7th week of embryonic development):
  - Chondroblasts form cartilage
  - Osteoblasts form bone (mineralization)
Intramembranous Ossification

- Bone formation of the surface skull bones and clavicles
- Osteoblasts cluster around the centre of ossification
  - Here, osteoblasts secrete a collagenous matrix to form a framework for mineralization
  - The collagenous matrix is then calcified by the deposition of hydroxyapatite
  - The osteoblasts and their surrounding calcified matrix are now referred to as a trabecula
- Most of the trabeculae will be eventually destroyed and reformed to give a bone its final adult size and shape
Endochondral Ossification

- Replacement of cartilage with bone
- Primary ossification process for most bones of the body
  - Best exemplified in long bones
- During embryonic development, a cartilage model, or perichondrium, is laid down
  - Compact bone then forms around this area and is called the periosteum
    - Periosteal collar
- Cartilage grows outward from its center and is gradually calcified into bone tissue
  - Primary ossification centre: diaphysis
  - Secondary ossification centre: epiphysis
  - Two areas remain uncalcified cartilage: articulations, growth plate
Endochondral Ossification H&E

- reserve cartilage
- zones of cartilage
- proliferation
- hypertrophy
- calcification

bone marrow
newly formed bone
HCO₃⁻ - Cl⁻ - H⁺

ATPase + H⁺

Lysosomes

Cathepsin K

MMP9
Modelling / Remodelling of Bone
Growth
Bone grows in length because:
1. Cartilage grows here
2. Cartilage replaced by bone here
3. Cartilage grows here
4. Cartilage replaced by bone here

Remodeling
Growing shaft is remodeled by:
1. Bone resorbed here
2. Bone added by appositional growth here
3. Bone resorbed here

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Controlling bone cell activity
- Osteoclast
Osteoclastogenesis

SYSTEMIC HORMONES
PTH
1,25(OH)₂D₃

HAEMOPOIETIC CELLS

STEM CELL

OSTEOCLAST PRECURSORS

IL-1
IL-6
TNFα
TGFα
M-CSF
PTHrP
RANKL

IL-4
γ-IFN
TGFβ
OPG

calcitonin

CYTOKINES & GROWTH FACTORS

STROMAL CELLS

OSTEOBLASTS

MECHANICAL EFFECTS
RANKL / RANKL and OPG

Extracellular domain

Receptor activator of NF-κB ligand (RANKL)

Osteoclast differentiation factor (ODF)
Osteoprotegerin ligand (OPGL)
TNF-related activation-induced cytokine (TRANCE)

Cytoplasmic domain

Receptor activator of NF-κB (RANK)

Cysteine-rich domains

Osteoprotegerin (OPG)

Osteoclastogenesis inhibitory factor (OCIF)
TNF receptor-like molecule 1 (TR1)
RANKL / OPG levels regulate bone resorption

Differentiation

Fusion and activation

Apoptosis
RANKL intracellular signalling cascades
Crosstalk between immune and skeletal cells
Controlling bone cell activity

- Osteoblast
mesenchymal stem cell (MSC)

proliferation

commitment

lineage progression

osteoblast
chondrocyte
myoblast fusion
stromal fibroblast
tenoblast
preadipocyte

differentiation and maturation

osteocyte
chondrocyte
myocyte
stromal cells
tenocyte
adipocyte

bone
cartilage
cardiac
stroma
tendon
adipose tissue
Regulation of cell fate
Role of Wnt in skeletal patterning
Intracellular signalling in response to Wnt
## Regulating Bone formation

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<th>BMP Subfamily</th>
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Signal transduction events mediate the action of BMPs leading to regulation of osteogenic genes.
Control of osteoblast differentiation by transcription factors
Mechanical load regulates bone cell activity and bone mass
Nuclear architecture

- Ribosome
- ER lumen
- LBR
- MAN1
- HP1
- LAP2
- LAP1
- BAF
- SUN1/2
- ONM
- PNS
- INM
- Nuclear lamina
- NPC
- Chromatin
- Microfilament
- Nesprin 1/2
Levels of nuclear organisation
Propagation of signals

(A) Two connexons in register forming open channel between adjacent cells. Interacting plasma membranes. Connexon composed of six subunits. Gap of 2-4 nm.

(B) Connexins and connexons. Homomeric, heteromeric, homotypic, and heterotypic intercellular channels.
Intercellular calcium wave propagation during osteoblast differentiation

Coordinating bone cell behaviour
Mechanostat theory

Disuse Range
Resorption > Formation
modeling remodeling

Physiological Range
Resorption = Formation
homeostasis

Overload Range
Formation > Resorption
modeling remodeling

Pathological Overload Range
Formation > Resorption
modeling remodeling

Remodelling Rate

Bone Microstrain

50 1500 3000
Quiescence

Resorption

Mineralisation

Formation

Reversal
Time & Space

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Initiation of bone remodelling..
Osteocyte-mediated initiation of bone resorption

- Osteocytes prevent osteoclastogenesis – TGFβ / OPG

- Osteocytes produce RANKL / M-CSF

- The effects of osteocyte apoptosis are mediated by changes in the behaviour of bone-lining cells
Osteoclast recruitment

- Chemokines
  - Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2)
  - Stromal cell-derived factor (SDF-1, also known as CXCL12)
Reversal / Transition

- Factors released from the bone matrix
  - IGF-1, BMP-2, TGFβ, PDGF

- Factors released by osteoclasts
  - Cardiotrophin-1 (CT-1)
Cell:cell interactions
Termination of bone formation

- Osteocytes produce Sclerostin
  - Ligand for LRP5 & therefore prevents Wnt activated bone formation
  - PTH treatment and mechanical load reduce sclerostin expression by osteocytes
  - Lack of sclerostin leads to high bone mass diseases, Van Buchem disease and sclerosteosis
Biology in Orthopaedics

- Biologics
- Biomaterial engineering
- Cell therapies
Osteogenesis on 10-30µm grooved surfaces

Cell:cell interactions on grooved substrates

[Bar graph and histogram showing pixel counts and frequency of Connexin-43 gap junction size across different substrate widths (Flat, 10µm, 15µm, 30µm).]
Nanoscale disorder regulates osteoblast differentiation.

Autologous Chondrocyte Implantation

1. Healthy biopsy taken from non-load bearing region
2. Chondrocytes released by enzymatic digestion
3. Chondrocytes expanded in culture
4. Suspension of cultured autologous chondrocytes prepared
5. Defect debrided back to healthy cartilage
6. Periosteal graft sutured over defect
7. Cultured autologous chondrocytes injected under periosteal flap
8. Periosteal graft removed from medial tibia
Any questions?