Osteocytes in Health and Disease

Nigel Loveridge
Bone Research Group Cambridge

With the very grateful help of:
Andy Pitsillides, Ken Poole, Brendon Noble, Jonathan Reeve, Mitch Schaffler
Osteocytes

Entombed osteoblasts

Canalicular system acts as a syncitium with connections to the bone surface

Important as mechano-sensors

Detectors of matrix damage
Bone Remodelling Cycle

- Osteoclast
- Osteoblasts
- Newly embedded osteocyte
- Mature osteocyte
- Bone lining cells
Osteocytes

Lynda Bonewald Sun Valley symposium 2005
Osteocytogenesis

Diagrammatic representation of the possible stages on osteoblast differentiation
1. Preosteoblast,
2. Preosteoblastic osteoblast,
3. Osteoblast,
4. Type 1 preosteocyte (osteoblastic osteocyte)
5. Type II preosteocyte (osteoid-osteocyte)
6. Type III preosteocyte,
7. Young osteocyte,
8. Old osteocyte.

Franz-Odenaal et al 2006
Possible Mechanisms for Osteocytogenesis

A: Osteoblasts secrete matrix in all directions.

B: Each osteoblast is polarized in a different direction but still secretes matrix in one direction only.

C: One generation of osteoblasts buries the next generation.

D: The osteoblast to be embedded slows down its matrix production compared to neighbouring osteoblasts.

E: Matrix secretion does not embed cells.
Possible Mediators of Osteocytogenesis

- TGFβ and the Smad Pathway
- Dental Matrix Protein I
- Sclerostin
TGFβ and osteocytogenesis

- Note the lack of staining in a mature osteocyte (arrow) and an osteoblast in the process of being embedded (arrowhead)
DMP-1

- DMP-1 exclusively expressed in osteocytes
- Seems to be involved in maintenance of the lacuno-canalicular system.
- Is reportedly responsive to loading (up-regulated)
Sclerostin

- Sclerostin - secreted protein product of the SOST gene
- Osteocyte specific and a powerful inhibitor of bone formation (BMP antagonist)
- Demonstrated when it’s absence results in the high bone mass disorder sclerosteosis
Sclerostin & Bone Formation

- Sclerostin/ Tol blue (G)
- Control (H)
- Alkaline phosphatase (I)
- Double demeclocycline labels (J)
Distance from Closest Bone Surface

Sclerostin +ve

Sclerostin -ve

Wilcoxon p<0.0001
Newly embedded osteocytes are sclerostin -ve

96.4 % of new osteocytes sclerostin negative within 16 days
Osteocytes and Bone Health

- Osteocytes required for bone health. In some cases osteocytes survive for many decades. In others osteocyte death is a cue for bone remodelling.

- Osteocytes are considered to be the main mechano-sensors in bone.

- Disruption of the canalicular system may result in cues to remodel damaged bone.
Micropetrosis

Mineralised Osteocyte Lacunae

Mineralised Haversian Canals

Courtesy Alan Boyde
Model: Rat Ulna Loading (Riggs/Lanyon Model)

Physiological Loading:
- Short daily period of dynamic loading:
  - 1200 cycles at 2 Hz;
  - peak strain = 4000µε
For 10 days

Supra-Physiological Loading:
- Short daily period of dynamic loading:
  - <1000 cycles at 2-4 Hz;
  - peak strain = 8000µε
  - Until shortened by 1.5mm or 30% loss of stiffness
Physiological Loading

A: - Loaded bone
B: - Contralateral control

- Note the increased double calcein labelling seen in the loaded bone

NO and Bone Turnover

- NO inhibits bone resorption and stimulates bone formation
- NO released in response to load
- eNOS predominant isoform in normal bone, especially osteocytes
Repeated episodes of bone loading in long-term culture: NO release increased during, but not after, loading.

Andy Pitsillides & colleagues
eNOS expression

mRNA

Protein

Andy Pitsillides & colleagues
Osteocytes release more NO than osteoblasts in response to mechanical strain in vitro.

**Summary:**
- Osteocytes release significantly more NO than osteoblasts.
- Strain-induced NO release is higher in osteocytes compared to osteoblasts.

**Graph:**
- The bar graph shows the nitrite concentration (µm) for Osteocytes and Osteoblasts under control and strain conditions.
- Osteocytes show a higher nitrite concentration under strain conditions compared to control.
- Osteoblasts show a lower nitrite concentration under strain conditions.

**Statistical Significance:**
- * indicates a statistically significant difference between control and strain conditions.
- ** indicates a very significant difference between control and strain conditions.
Relationship between loading-induced increases in G6PD activity and NO release

\[ R^2 = 0.984 \]
\[ P < 0.05 \]

% increase in NO release

% increase in G6PD activity

Egg type
Wild type
Meat type
Types of Cell Death

**Necrosis:** occurs in large areas of tissue and often provokes an inflammatory response. An example of this is avascular necrosis (osteonecrosis) where the blood supply fails.

**Apoptosis:** Is focal and does not provoke an inflammatory response. Can be either active through death receptors or passive through lack of cell survival agents.
Physiological Loading

Number of apoptotic osteocytes with fragmented DNA

Peak strain magnitude

Supra-Physiological Loading

Rat ulna: 10 days after fatigue

Rat ulna: Control

Supra-physiological

A: Control bone
B: Overloaded
C: High power

Supra-physiological loading

% osteocytes with fragmented DNA

7 Days

14 Days

CONTROL LOADED

A apoptotic osteocytes associated with microcracks (Mdx)

Verborgt et al, 2000

(-) = No TUNEL Staining
(+)= TUNEL positive cell
E.lac = Empty lacunae/
TUNEL positive debris
Apoptotic osteocytes at resorption spaces

(-) = No TUNEL Staining
(+) = TUNEL positive cell
E.lac = Empty lacunae/
TUNEL positive debris
Osteocytes and Bone Disease

- Post-menopausal Osteoporosis
- Hip Fracture
- Sclerosteosis
- Osteoarthritis

Dunstan et al. (1993) CTI 53:S113-S117

AGE (years)
Iliac trabecular bone loss after 6 months of GnRH analogue therapy can be marked.

PRE-treatment

POST-treatment
Oestradiol changes during GnRH therapy

Tomkinson et al J Clin Endocrinol Metab 1997
Percentage of apoptotic osteocytes before and after treatment with GnRH analogue

Tomkinson et al. J Clin Endocrinol Metab 1997
Ovariectomy in Rats

% Apoptosis

Morphology
Nick Translation

Sham
Ovx
Ovx + E₂

Hip Fracture Incidence Forecast in European Community
Density of eNOS+ve Osteocytes

![Bar chart showing the density of eNOS+ve osteocytes in control and case groups, comparing inferior and superior regions. The chart indicates significant differences (p=0.0004) between control and case groups in the inferior region, with no significant difference (p=0.17) in the superior region.](image)
Location of eNOS+ve Osteocytes (Minimum)

Inferior Region

Superior Region

Distance from canal surface (µm)

Control
Case

Control
Case

NOS -ve
NOS +ve
Superior aspect

Inferior aspect

Formation of giant canals

Do not resorb

eNOS positive

eNOS negative

Case

Control

Formation of giant canals
Sclerosteosis

- Rare autosomal-recessive disease
- Systemic skeletal syndrome, bone mass ↑↑
- Markedly increased bone formation
- Afrikaner population of South Africa
- Clinically:
  - hyperostosis
  - narrowing of skull
  - cranial nerve compression
  - headaches
  - facial palsy
  - hearing loss
  - ↑↑BMD
  - syndactyly
  - tall stature
  - nail dysplasia
  - strong teeth
  - ICP
- ↑ cortical thickness and cancellous bone volume
- ↑↑ bone formation rate
- Stylomastoid foramen
Sclerosteosis Radiology: Hands

Normal Hand

Sclerosteosis
Bone Histology in Sclerosteosis
Hip OA and Femoral Neck Fracture

- Femoral neck fracture is uncommon in hip OA and it is has been suggested that hip OA offers protection in the form of increased cancellous bone strength.

- In osteoporosis (OP) there is decreased bone mass whereas in hip OA bone mass is increased.

- In hip OA there is increased bone formation at more sites than in controls.

- Is this related to a decreased sclerostin expression?
Comparison of Percentage Ct.Ar. and Cn.Ar. for all Biopsies

* P < 0.01

Jordan et al ASBMR 1999
Sclerostin in active and inactive osteons

- Used adjacent ALP sections to mark forming (red) and quiescent (yellow) for analysis on the polarized light image.

- For each subject, 10 forming and 10 quiescent osteons were measured. (where possible)
Bone surface undergoing active formation

No difference in osteocyte density between OA and control groups.

- In osteons undergoing bone formation density of sclerostin +ve osteocytes was higher:
  
  OA: 229 mm² ± 58  Control: 373 mm² ± 56, p=0.02

No difference in density of sclerostin +ve osteocytes within quiescent osteons

- % sclerostin +ve osteocytes within osteons undergoing bone formation higher in OA:
  
  OA: 49.1% ± 7.0  Control: 74.2% ± 7.1 p= 0.01

- Mean distance of sclerostin +ve osteocytes from the bone surface was higher in OA:
  
  OA: 64 µm ±5  Control: 48 µm ±5 p=0.024
Does the decreased sclerostin allow greater bone formation?

Control osteon

Sclerostin -ve

OA osteon

Sclerostin +ve
Summary

- Osteocytes are embedded osteoblasts. Changing the rate of osteocytogenesis will have effects on bone formation.

- Osteocytes are important regulators of bone turnover especially in relation to mechanical loading & damage repair. It is possible that all bone turnover is regulated by the osteocyte network.

- Failures in osteocyte activity may be responsible for some musculo-skeletal diseases.
And now for a break to allow the brain to recover

QuickTime™ and a YUV420 codec decompressor are needed to see this picture.