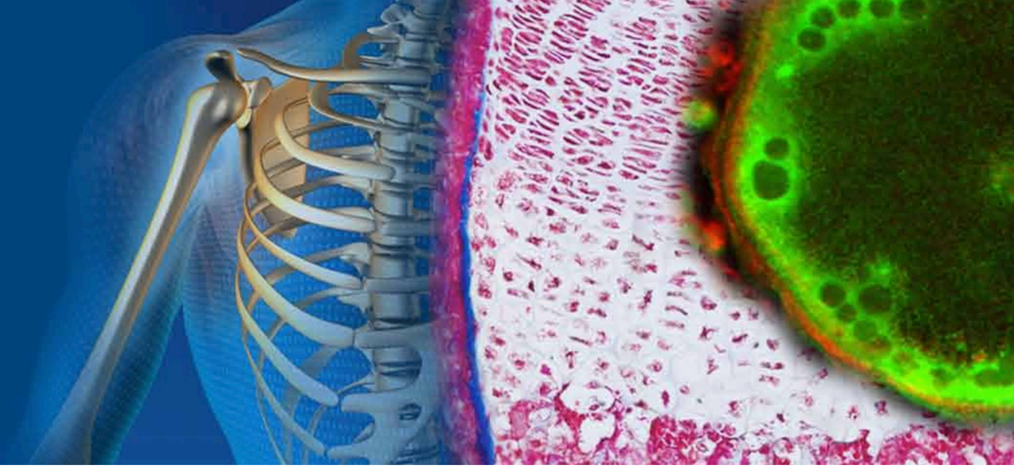
CENTER *for* MUSCULOSKELETAL RESEARCH



NID 464: Orthopaedic Basic Science Course

Bone Regeneration

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Fall 2019

Lecture Objectives

1. Introduction of clinical importance of bone regeneration/ healing and current limitations, and the goals of treatments
2. Introduction to basic biology of bone, clinical relevance of key factors in bone regeneration
3. Introduction of key molecules and cells involved in bone repair
4. Introduction to bone regeneration animal models and the cell delivery options

Definition: Bone Regeneration

Bone regeneration is a complex interplay well-orchestrated **physiological process** of anatomical, biomechanical and biochemical bone formation.

It is required for **recovery bone** tissue that lost by trauma, fractures, or surgical removal of locally invasive pathologies due to tumor or infection.

Rozalia Dimitriou, et al. BMC Medicine 2011, 66(9)
Bates P, et al. Basic Orthopaedic Sciences. 2007, 123-134.
Cho TJ, et al. J Bone Miner Res. 2002, 17: 513-520.
Einhorn TA, et al. Clin Orthop Relat Res. 1998, 355 (Suppl): S7-21.

Clinical Importance of Bone Regeneration

- Fracture repair
- Fracture nonunion
- Critical bone defect
- Bone infection
- Primary tumor lesion
- Secondary tumor lesion

Multiple Fragments Fracture:
Case example - Tibia

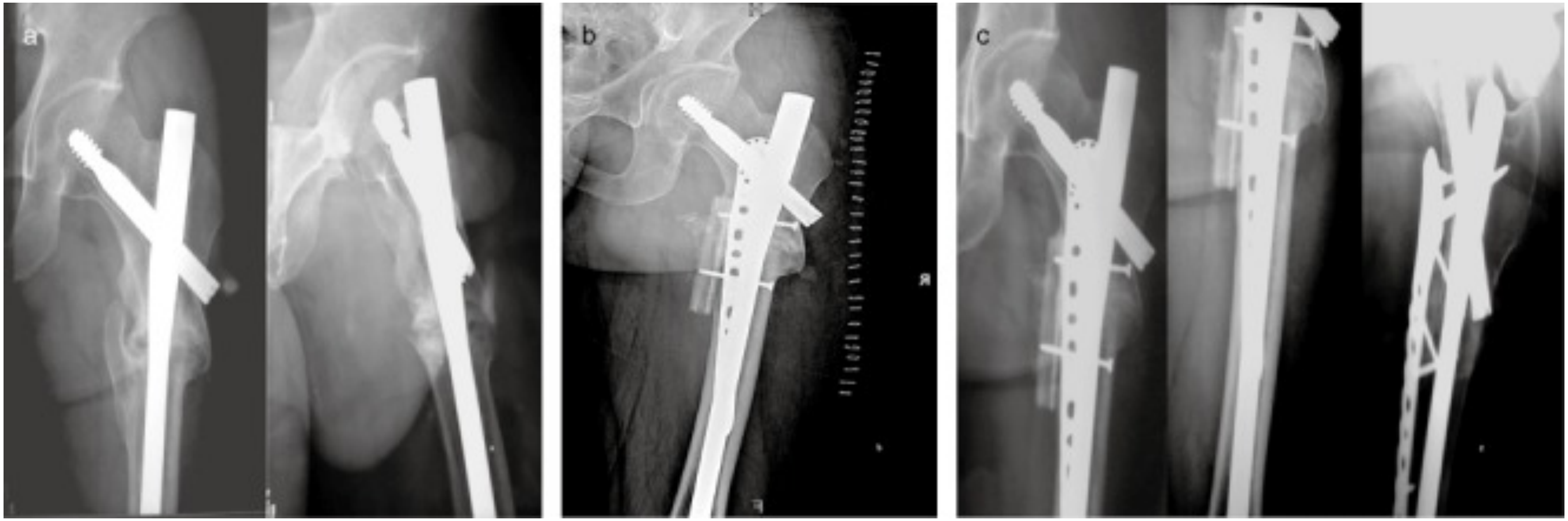
A 27-year-old healthy male was involved in a motorcycle collision, sustaining an isolated injury to the left lower extremity

Barei D. et al. J. Orthopaedic Trauma. 2007,21(8):574-578

Fracture nonunion:

Case example - Subtrochanteric fracture

A 36-year-old male patient with high subtrochanteric fracture treated with long Gamma nail. Ten months after the initial surgery, dynamization was used because of deficient callus formation.

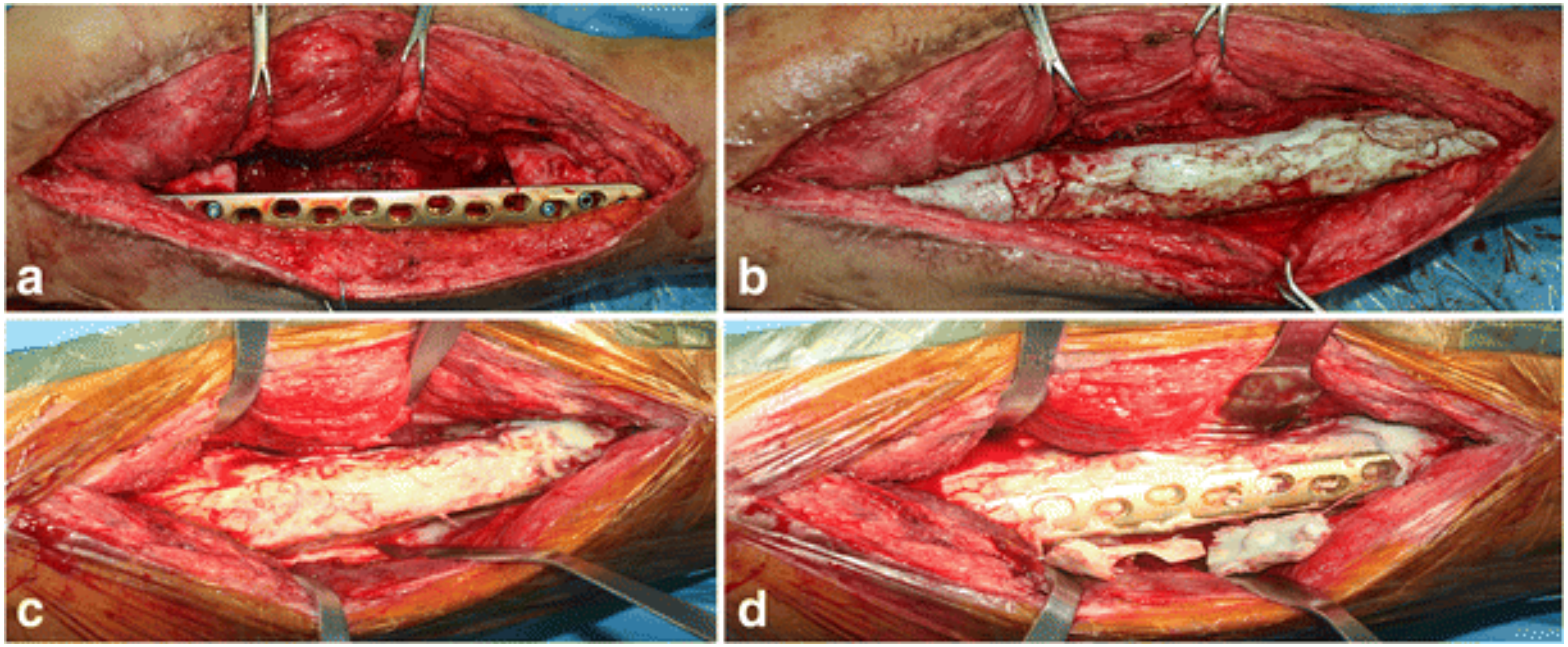


Gao et al. Orthop Surg. 2011;,3(2): 83–87

Critical Bone Defect:

Case example – Femoral fracture

Masquelet Technique

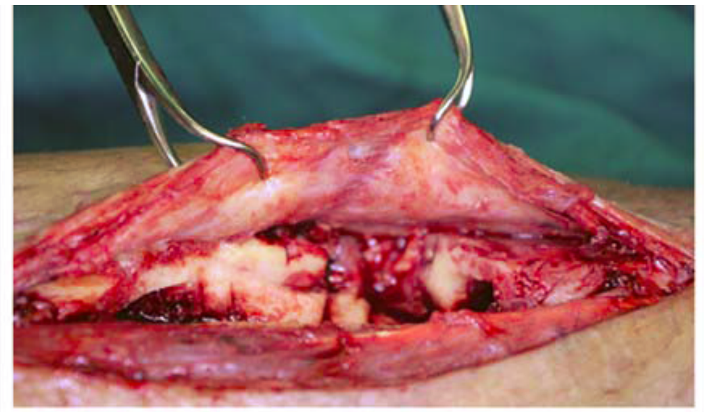


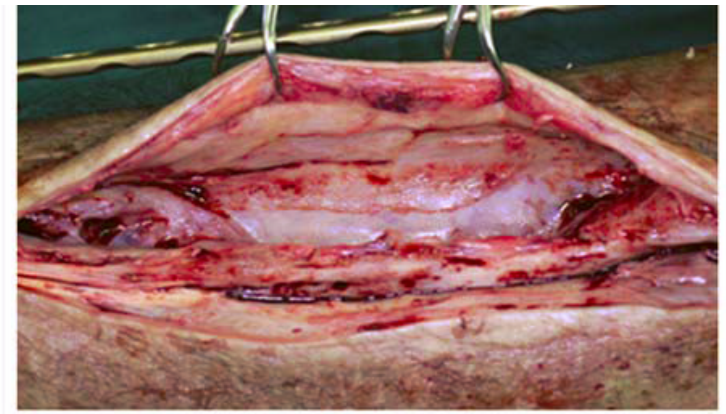
Xin Yu et al.. *International Orthopaedics, 2017.41:1851-57*

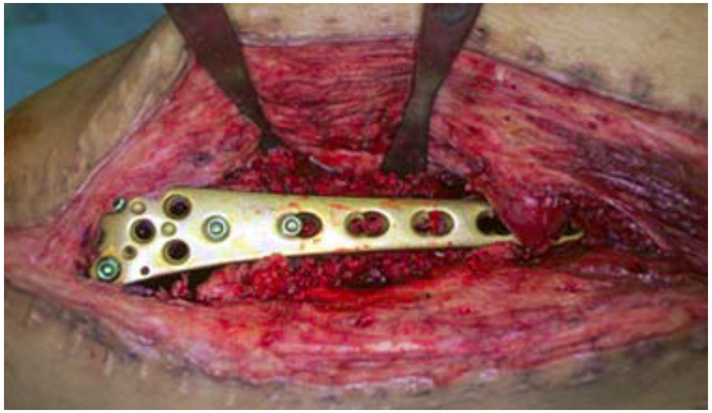
Critical Bone Defect:

Case example – Femoral fracture

Masquelet Technique







Xin Yu et al.. International Orthopaedics, 2017.41:1851-57

Critical Bone Defect:

Case example – Femoral fracture

Masquelet Technique

Xin Yu et al.. International Orthopaedics, 2017.41:1851-57

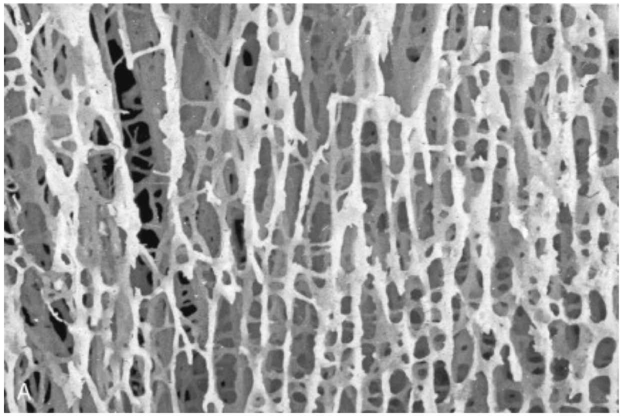
The goals of treatments and current limitations of bone regeneration

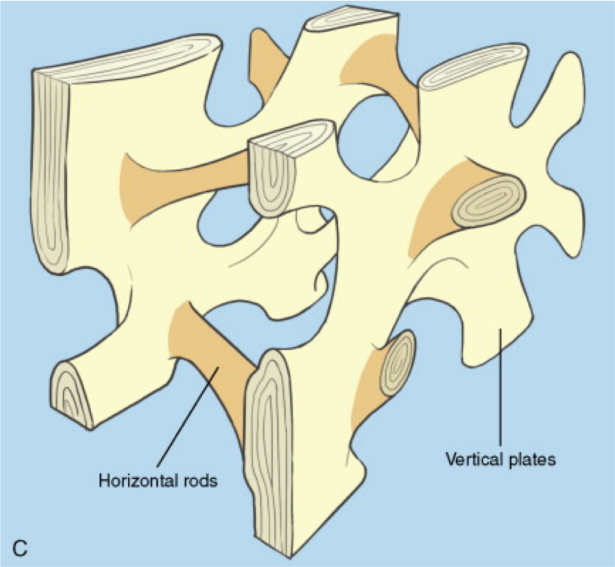
- The goals of fracture treatments are to restore the skeletal function
- Standard approaches widely used in clinical practice to stimulate or augment bone regeneration include:
  - Distraction osteogenesis and bone transport
  - Bone-grafting
    - autologous bone grafts,
    - allografts,
    - bone-graft substitutes or growth factors.
  - Two-stage procedure
    - Masquelet technique.
    - It is based on the concept of a "biological" membrane, which is induced after application of a cement spacer at the first stage and acts as a 'chamber' for the insertion of non-vascularised autograft at the second stage.
  - Non-invasive biophysical stimulation
    - Low-intensity pulsed ultrasound (LIPUS)
    - Pulsed electromagnetic fields (PEMF)

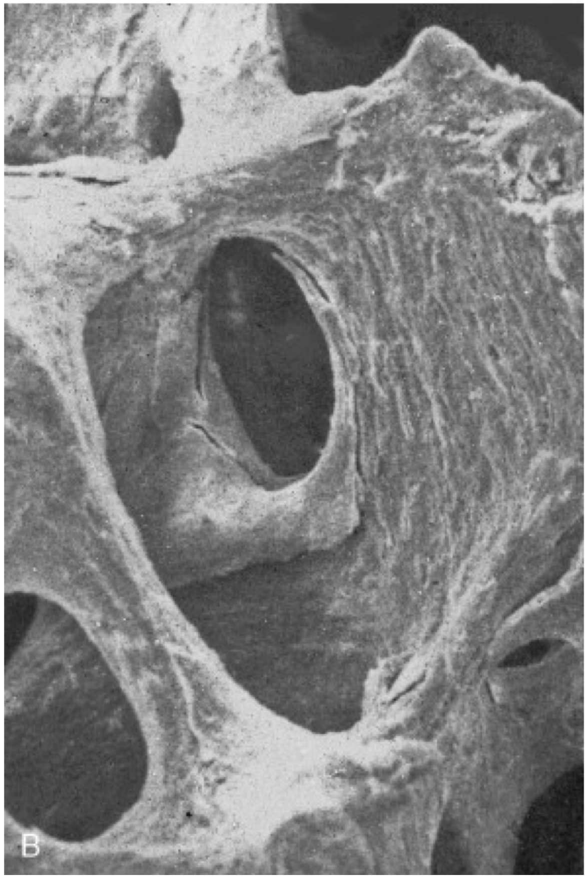
Aronson J, et al. J Bone Joint Surg Am. 1997, 79 (8): 1243
Green SA, et al. Clin Orthop Relat Re. 1992, 280: 136-142.
Giannoudis PV, et al. Injury. 2005, 36: S20-27.
Giannoudis PV, et al. Injury. 2009, 40: S1-3.

Basic Biology of Bone:
Bone structure

cancellous bone structure

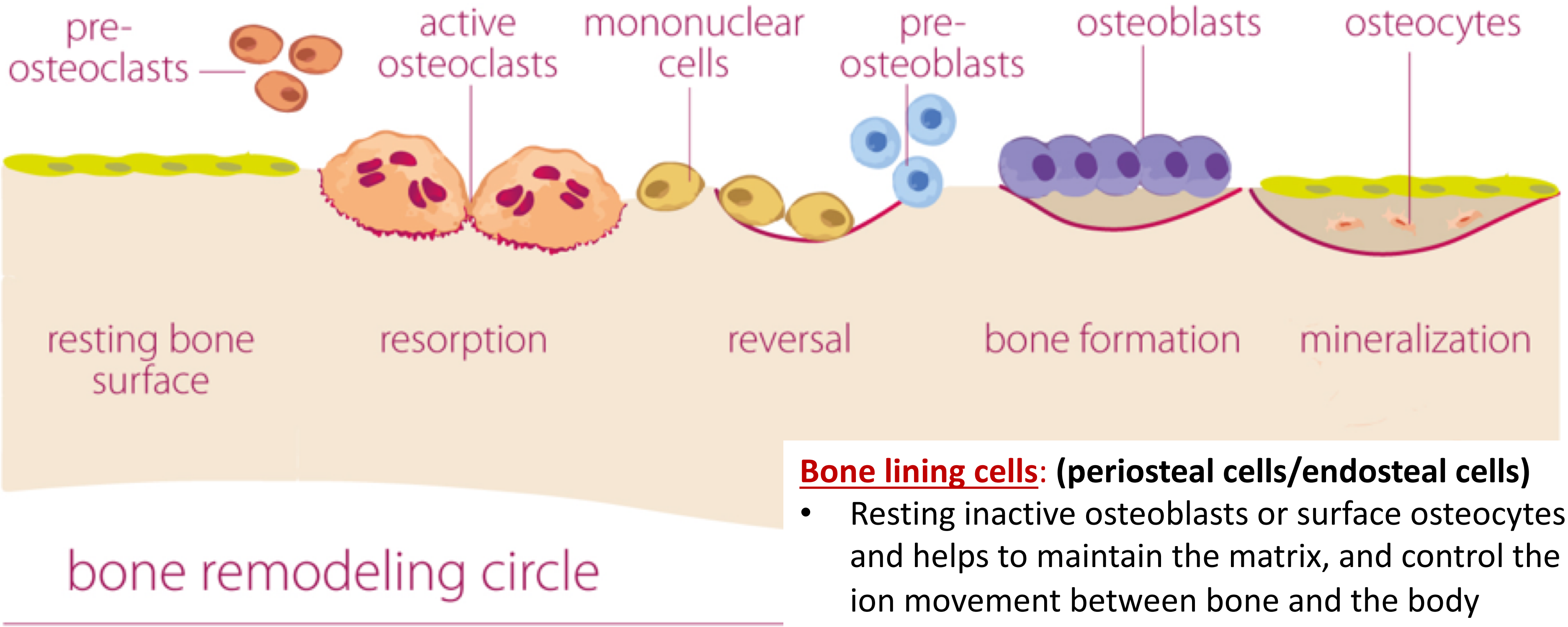






SEM

Basic Biology of Bone:
Bone cells

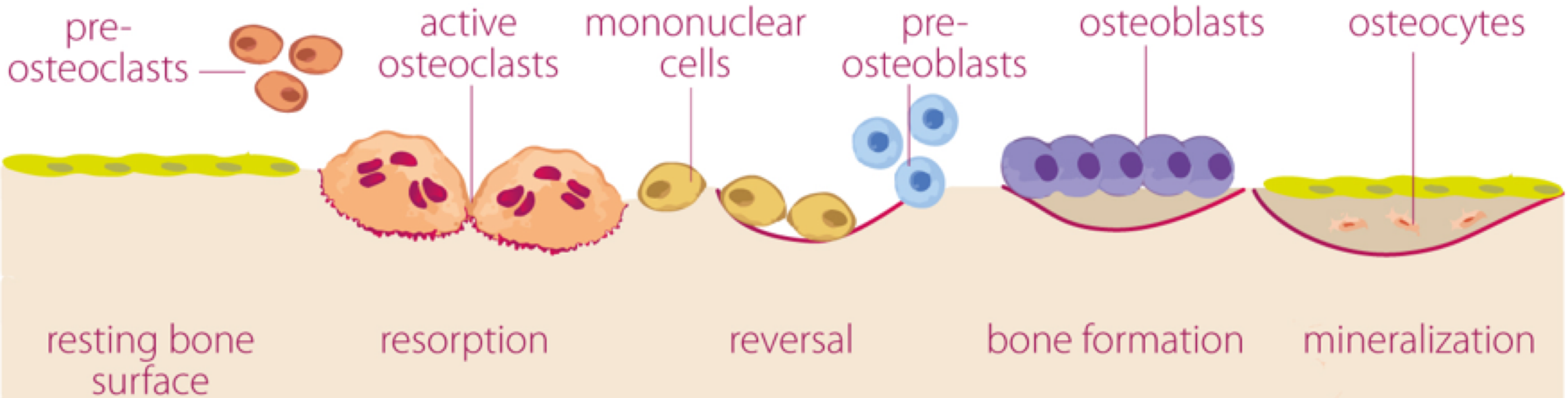


Bone lining cells: (periosteal cells/endosteal cells)
- Resting inactive osteoblasts or surface osteocytes and helps to maintain the matrix, and control the ion movement between bone and the body

Osteoblast (4-6%, secretion of bone matrix)
- Secretion collagen and calcium binding proteins of unmineralized bone, osteoid
- Regulate the activity of osteoclasts
- Maintain calcium homeostasis
- Quiescent bone lining cell or osteocyte

Clarke B. Clin. J. Am. Soc. Nephrol. CJASN. 2008, 3: S131–S139
S. C. Miller, et al. Scanning Microsc., 1989, 3: 953-960
J. D. Currey, et al. Bones: Structure and Mechanics. 2002.
R. Florencio-Silva, et al. BioMed Res. Int., 2015.

Basic Biology of Bone:
Bone cells



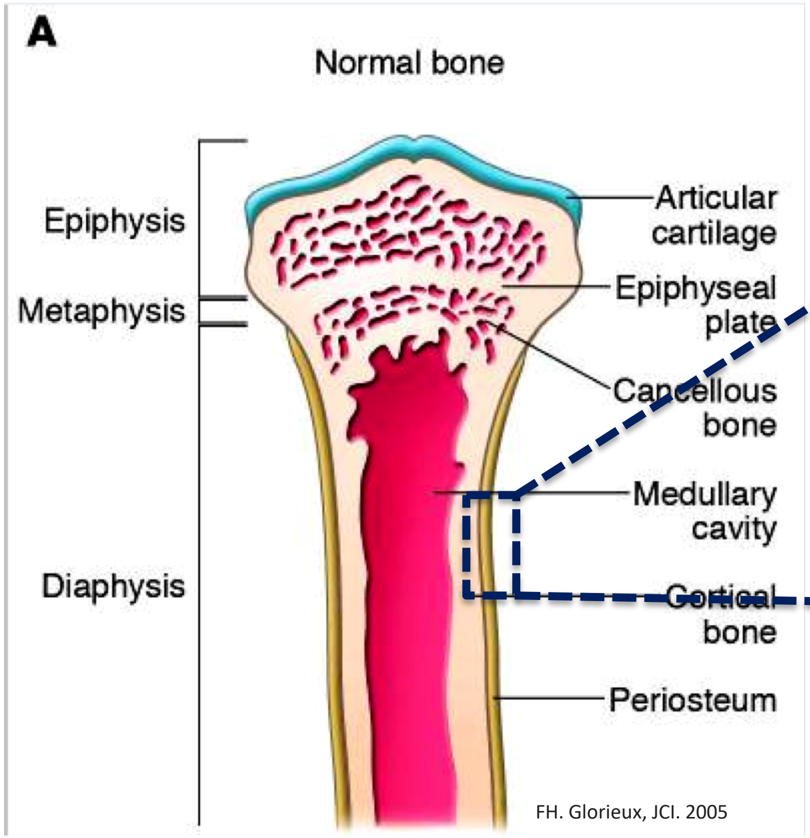
Osteocytes (90-95%)
- Embedded in the bone matrix with lacunae
- Canalicular network connect them to osteoblasts, other osteocytes and bone surface lining cells
- Mechanosensors transform mechanical load into chemical signals, which cause bone resorption by osteoclasts or bone formation by osteoblasts.

Osteoclasts (Multinucleated cell)
- Derived from mononuclear cells
- Resorb damaged bone tissue & matrix
- Dissolution of crystalline hydroxyapatite.

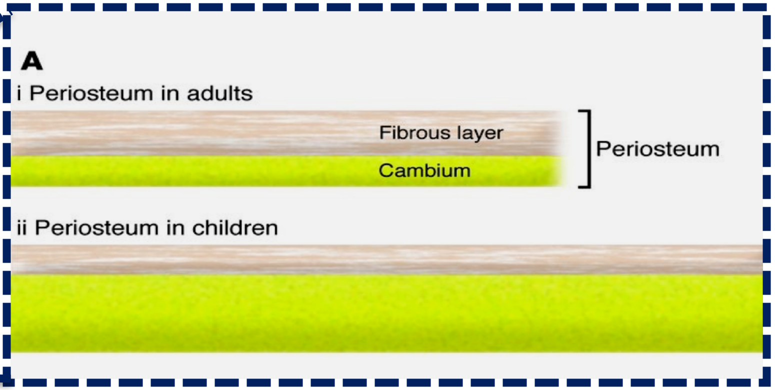
Osteoprogenitor cells (Endosteum+periosteum)
- Mitotically active stem cells
- differentiate into bone lining cells or osteoblasts

R. Florencio-Silva, et al. BioMed Res. Int., 2015.
E. J. Mackie, Int. J. Biochem. Cell Biol. 2003, 35: 1301
J. Klein-Nulend, et al. Bone. 2013, 54: 182
H. K. Väänänen, et al. J. Cell Sci., 2000, 113:377

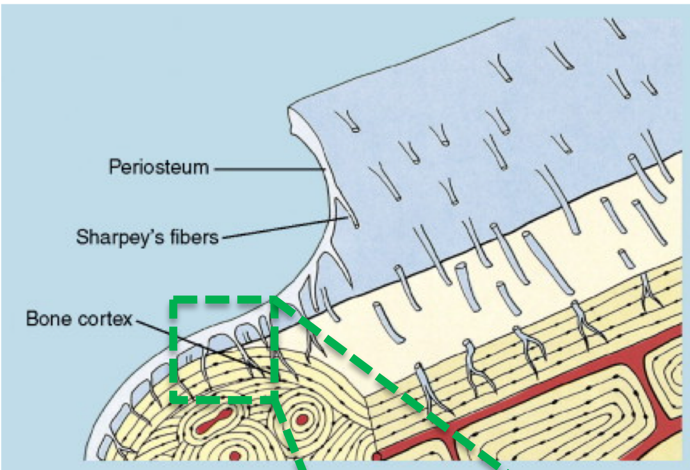
Basic Biology of Bone:
Periosteum

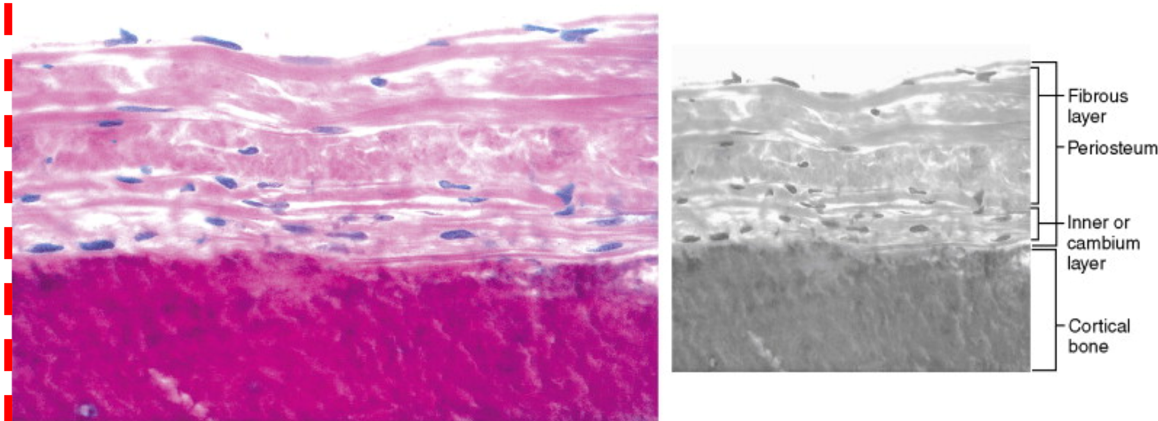


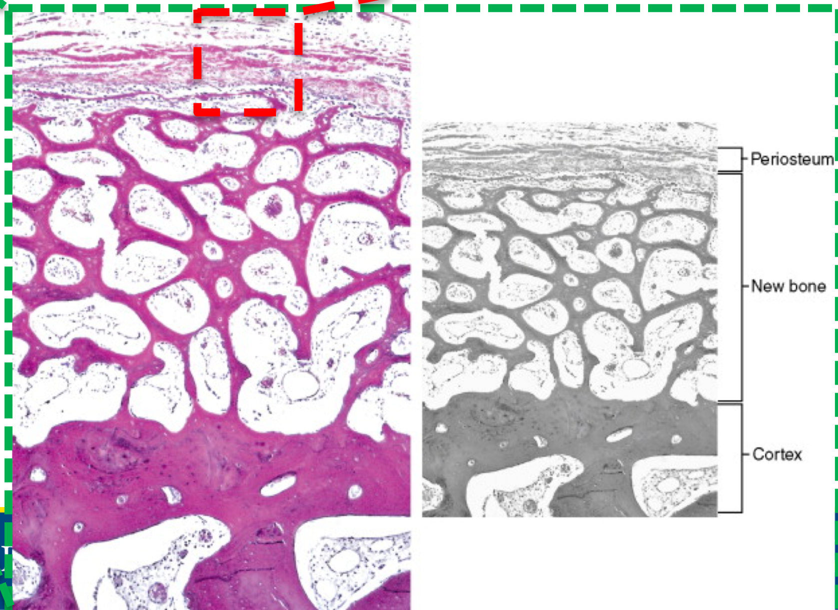
FH. Glorieux, JCI. 2005



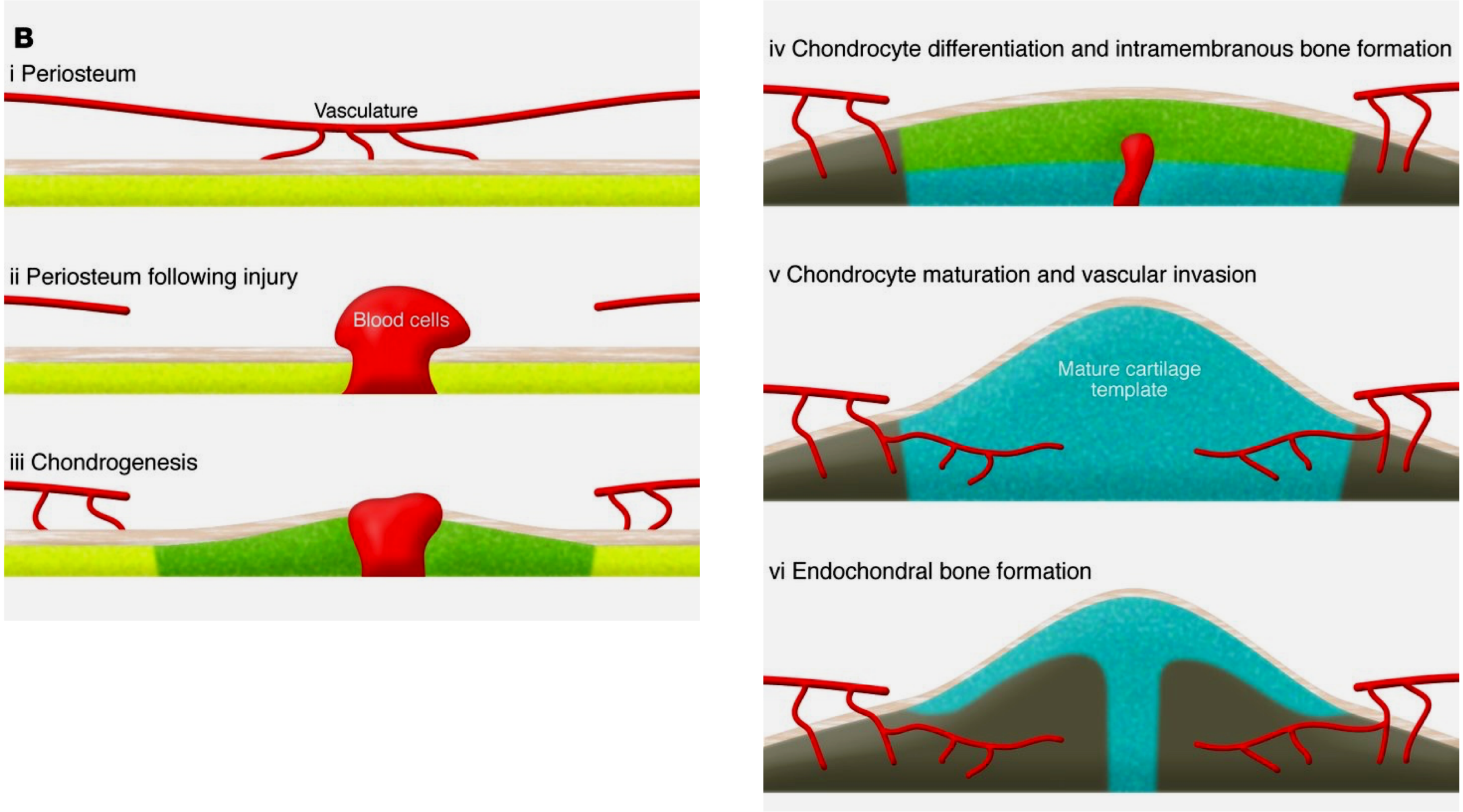
Basic Biology of Bone:
Periosteum





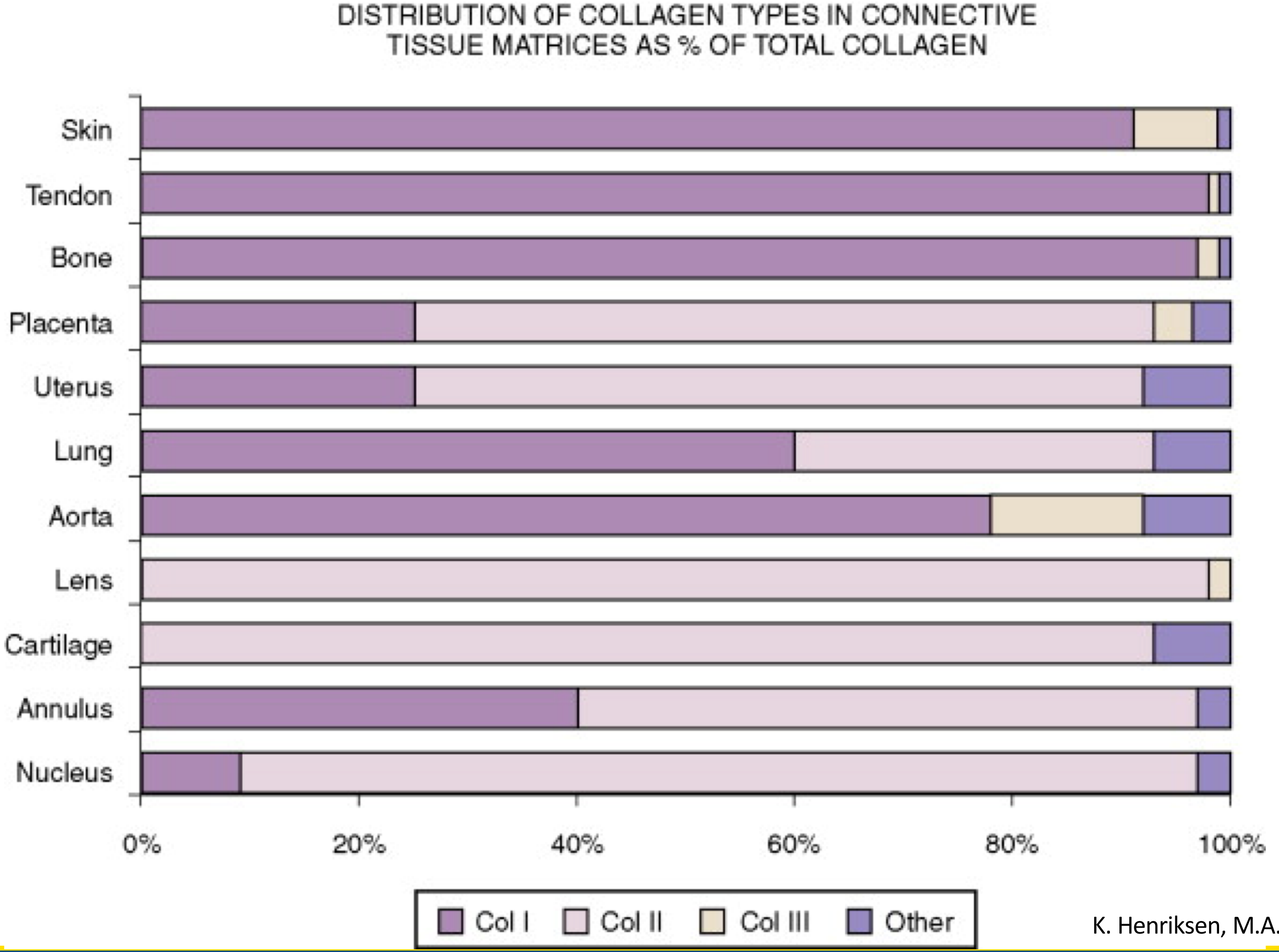


Basic Biology of Bone:
Periosteum-during fracture repair



Zuscik MJ , O'Keefe RJ, JCI 2008

Basic Biology of Bone:
Extracellular matrix- collagens in bone

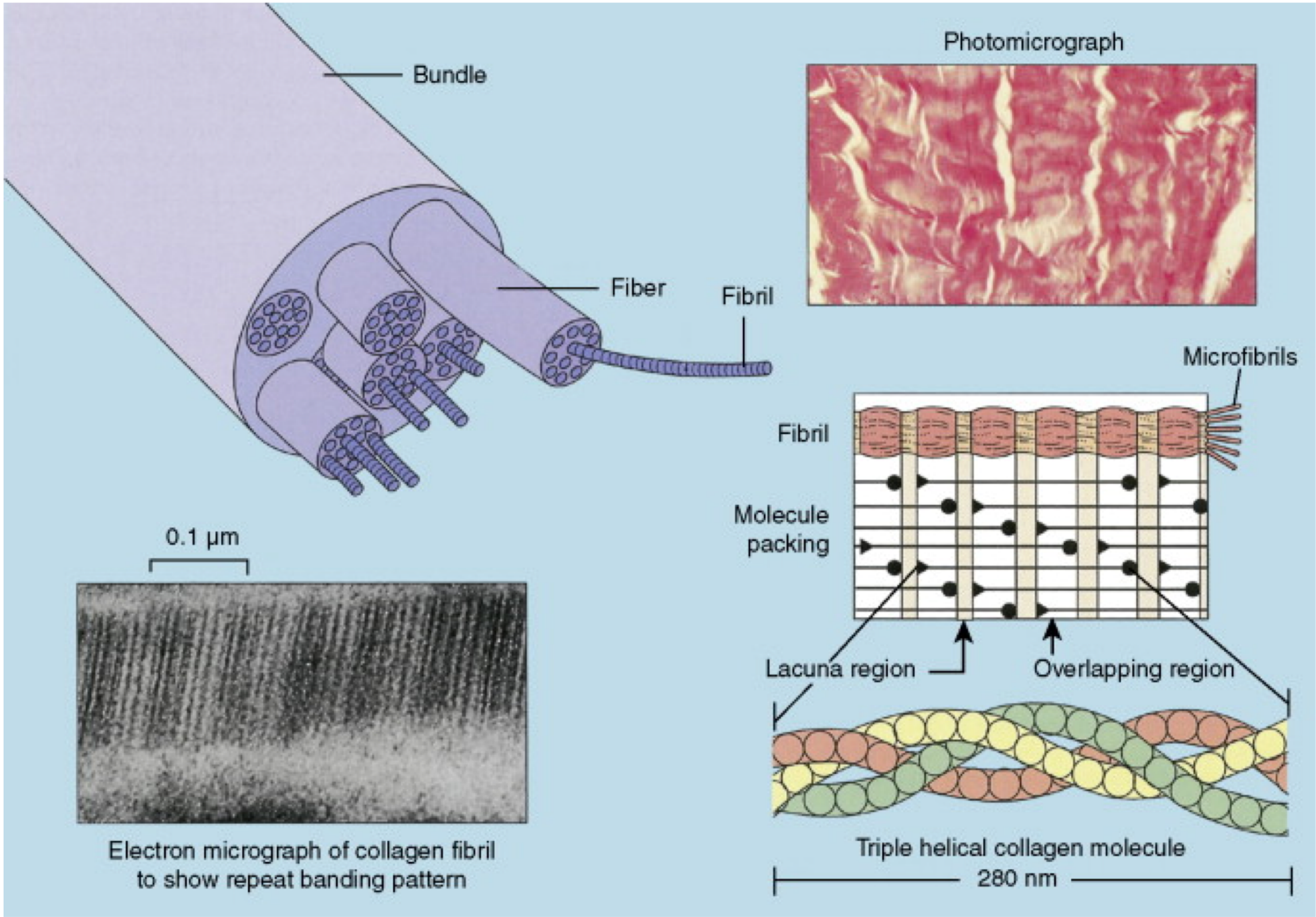


K. Henriksen, M.A. Karsdal. 2019

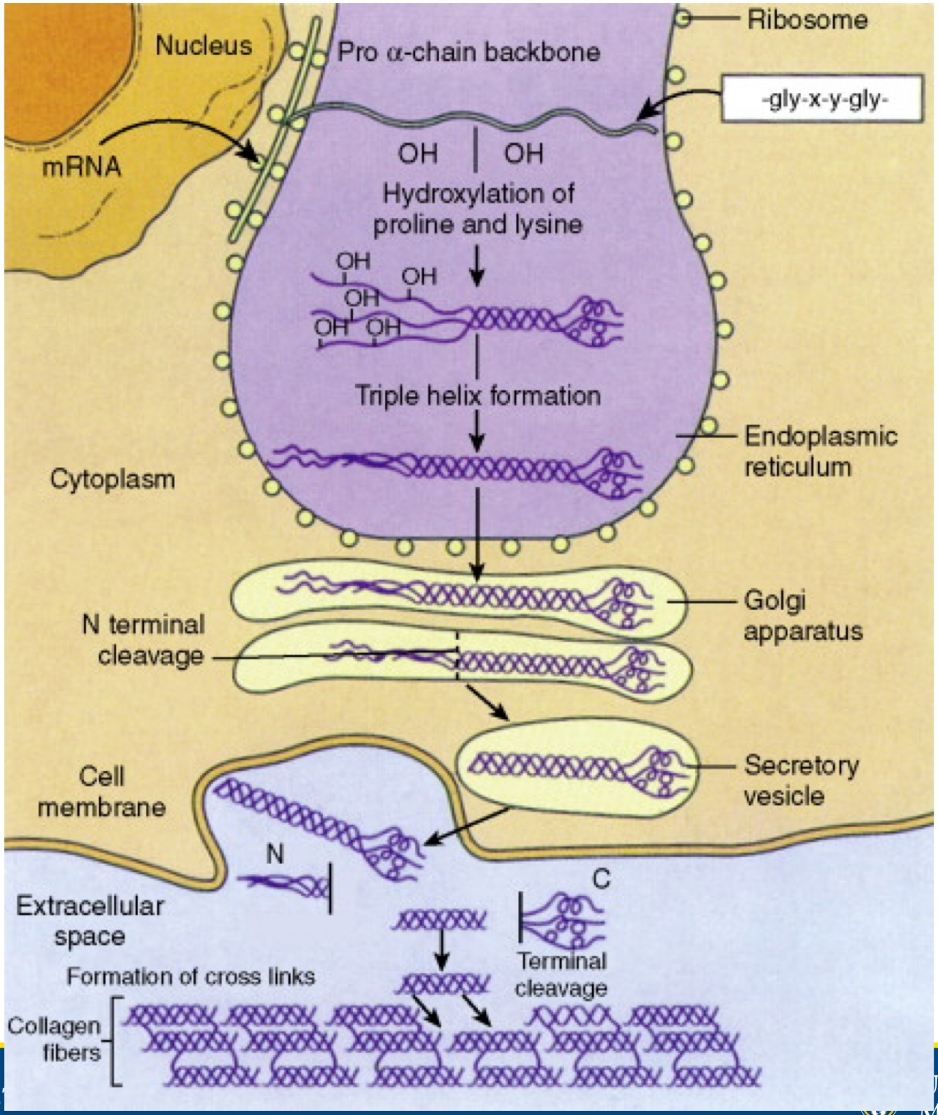
Basic Biology of Bone:
Bone matrix & collagens

Type	Genes	Structure	Representative Tissues	Disorders
I	COL1A1, A2	Fibrils	Skin, bone, tendon, dentin, etc.	Osteogenesis imperfecta, Ehlers-Danlos syndrome
II	COL2A1	Fibrils	Hyaline cartilage, vitreous body	Collagenopathy, types II and XI, spondyloepiphyseal dysplasia (SED)
III	COL3A1	Fibrils	Skin, vessels	Ehlers-Danlos syndrome (EDS)
IV	COL4A1, A2, A3, A4, A5, A6	Meshwork	Basement membranes	Alport's syndrome, porencephaly, Goodpasture's syndrome
V	COL5A1, A2, A3	Fibrils	Hamster lung cell cultures, fetal membranes, skin, bone, placenta, synovial membranes	Ehlers-Danlos syndrome (classic type)
VI	COL6A1, A2, A3	Short chain	Vessels, skin, intervertebral disc, placenta, heart	Ulrich myopathy, Bethlem myopathy
VII	COL7A1	Long chain	Dermo-epidermal junction	EDS, epidermolysis bullosa
VIII	COL8A1, A2	Short chain	Descemet membrane, endothelial cells	Corneal dystrophies
IX	COL9A1, A2, A3	Short chain	Cartilage specific hyaline cartilage, vitreous humor	Multiple epiphyseal dysplasia, Stickler syndrome
X	COL10A1	Short chain	Cartilage specific growth plate (hypertrophic cartilage)	Schmidt's metaphyseal dysplasia
XI	COL11A1, A2	Fibrils	Hyaline cartilage	Collagenopathy, types II and XI, Stickler syndrome
XII	COL12A1	?	Embryonic skin and tendon, periodontal ligament	?
XIII	COL13A1	Short chain	Endothelial cells, fibroblast, blood vessels	?
XIV	COL14A1	Glycoprotein	Fetal skin and tendon	?
XV	COL15A1	Interrupted collagen	Embryonic organs	?
XVI	COL16A1	FACIT	?	?
XVII	COL17A1	Transmembrane	Basement membrane	Epidermolysis bullosa
XVIII	COL18A1	Multiplexin	Vasculature	Knobloch's syndrome
XIX	COL19A1	FACIT		
XX	COL20A1	Short chain	Cornea	
XXI	COL21A1	FACIT	Skeletal muscle and heart	
XXII	COL22A1	FACIT	Tissue junctions	
XXIII	COL23A1	Transmembrane	?	?
XXIV	COL24A1	?	Bone, retina	(Marks embryonic bone formation)
XXV	COL25A1	Fibril	Brain specific	Alzheimer amyloid plaque component (senile Alzheimer's disease)
XXVI	COL26A1	Multiplexin	Uterus	?
XXVII	COL27A1	Fibril	Brain, lung	
XXVIII	COL28A1	FACIT	Lung	
XXIX	COL29A1	FACIT	Skin, lung	

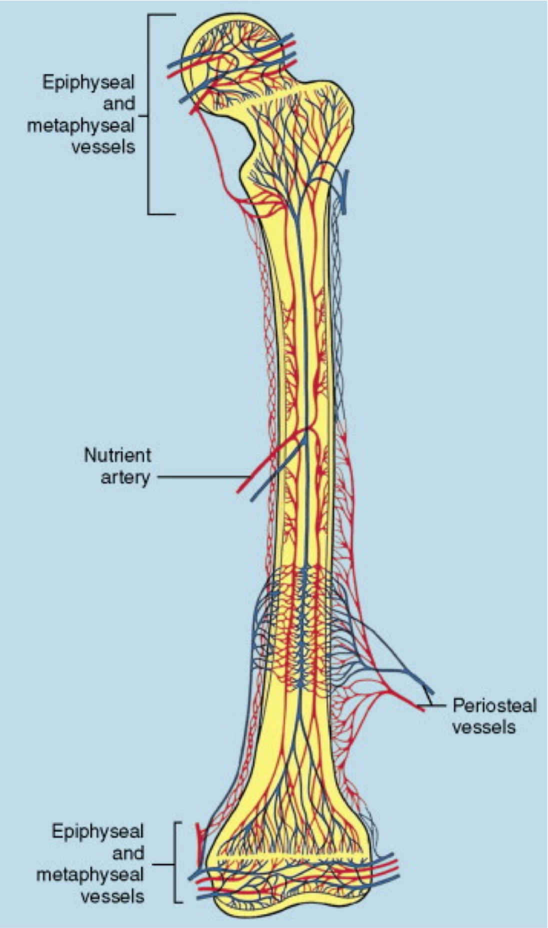
Basic Biology of Bone:
Normal Collagen structure

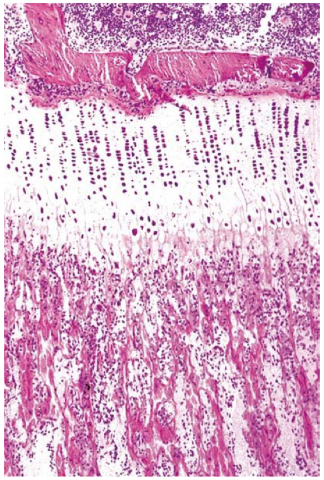


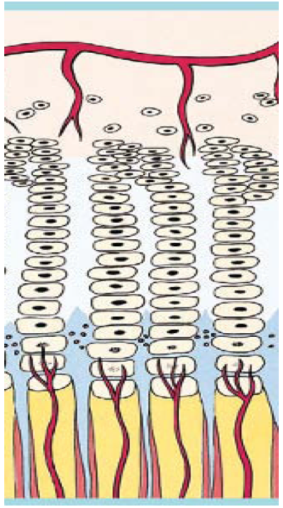
Basic Biology of Bone:
Intra- and extracellular collagen synthesis

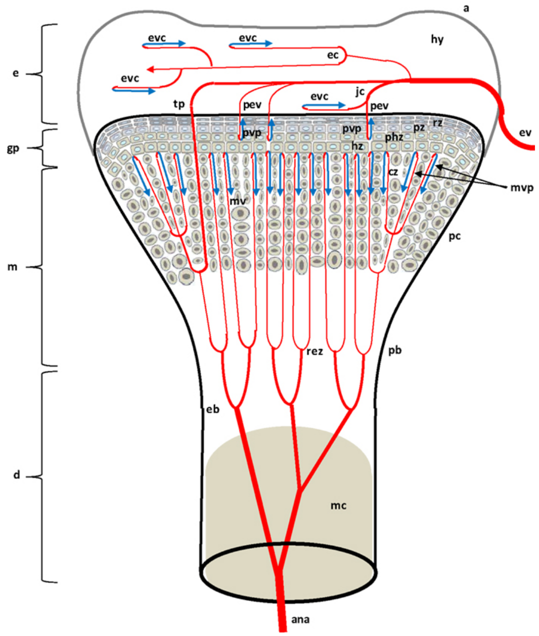


Basic Biology of Bone:
Blood supply



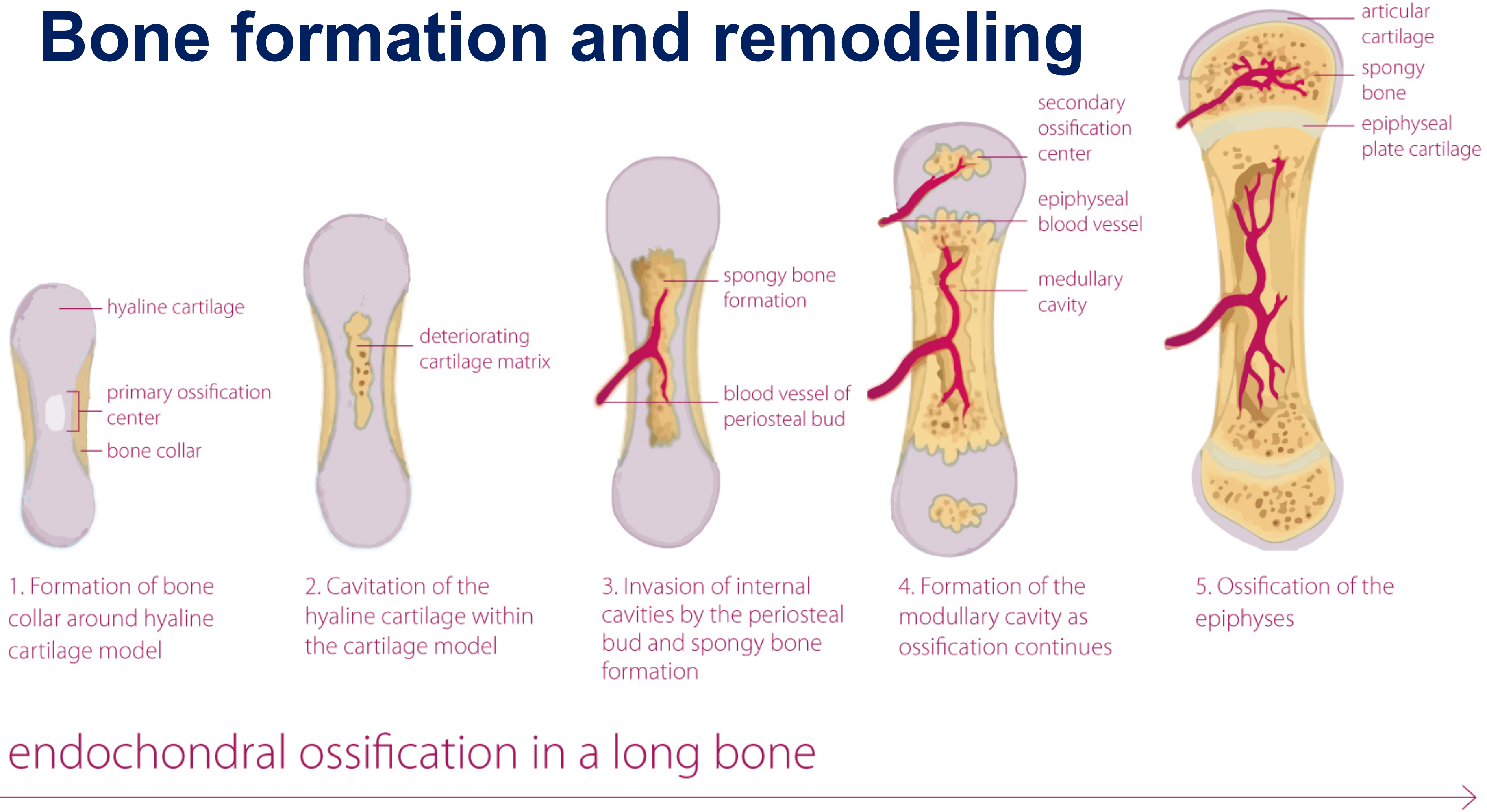






Robert F. Wideman, et al.
Front. Endocrinol., 2013

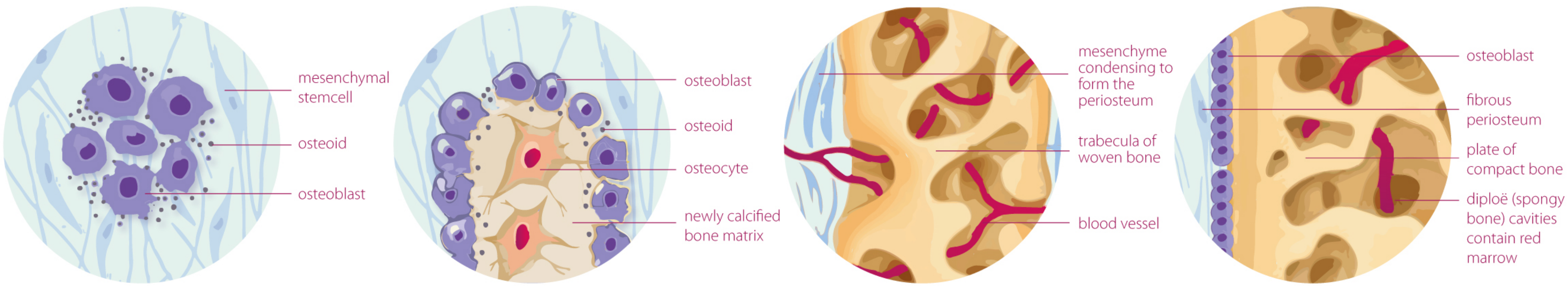
Basic Biology of Bone:
Bone formation and remodeling



Translational Orthopaedic **Biomaterials Interest Group**

Basic Biology of Bone: Intramembranous bone formation

- Ossification center formation
- Calcification
- Trabeculae formation
- Periosteum development



1. An ossification center appears in the fibrous connective tissue membrane

2. Bone matrix (osteoid) is secreted within the fibrous membrane

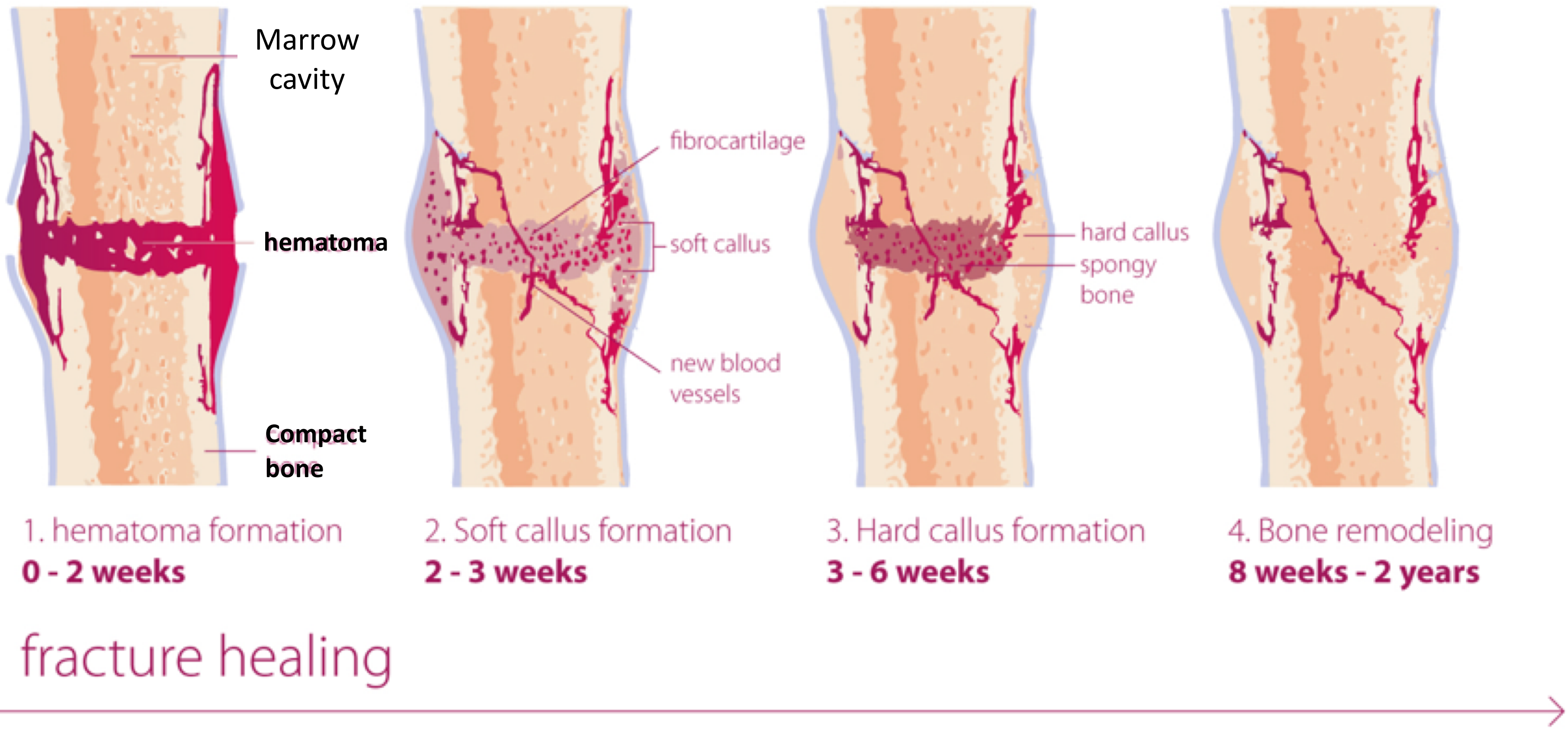
3. Woven bone and periosteum form

4. Bone collar of compact bone forms and red marrow appears

intramembranous bone formation

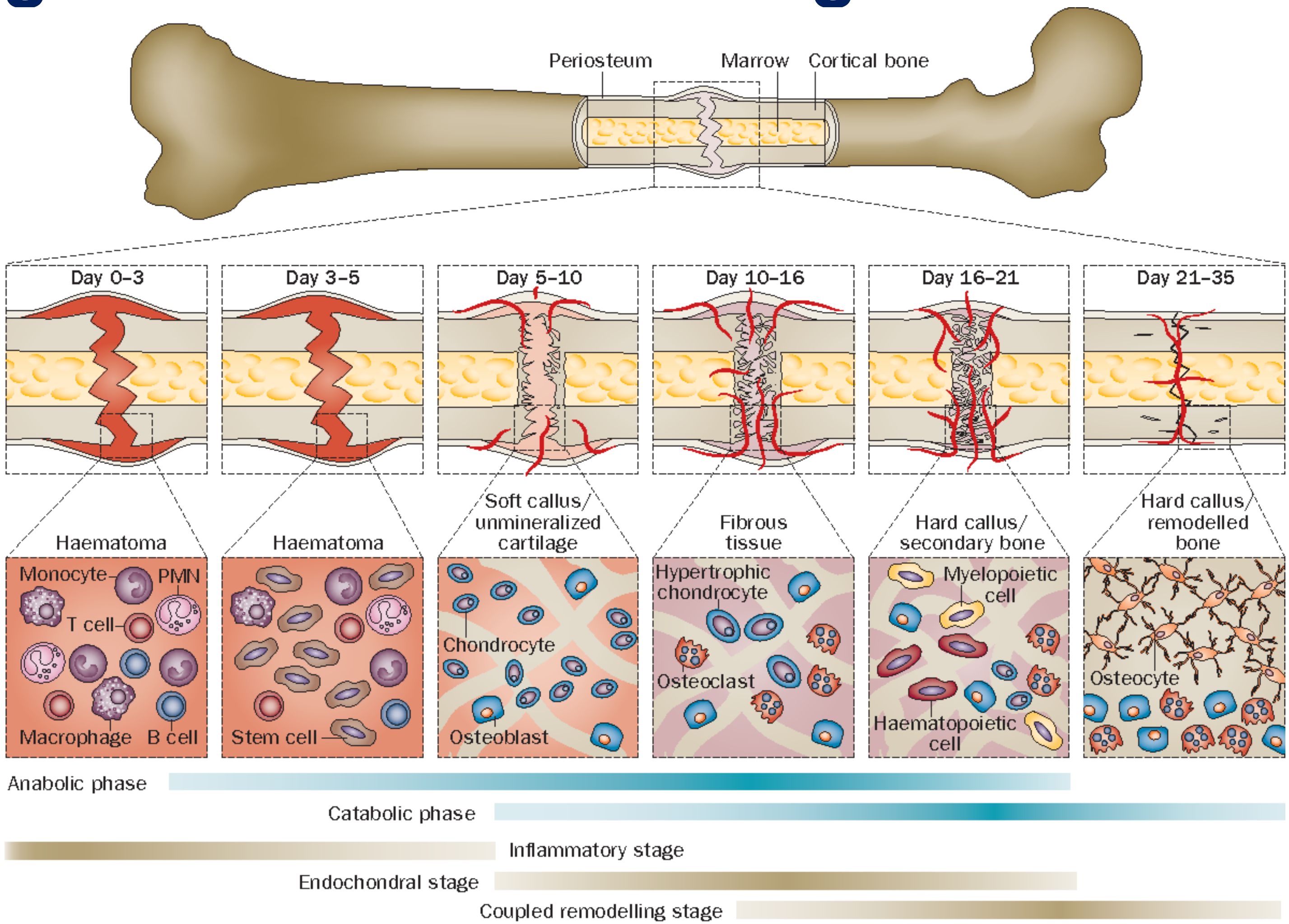
Translational Orthopaedic
Biomaterials Interest Group

Basic Biology of Bone:
Fracture healing stages



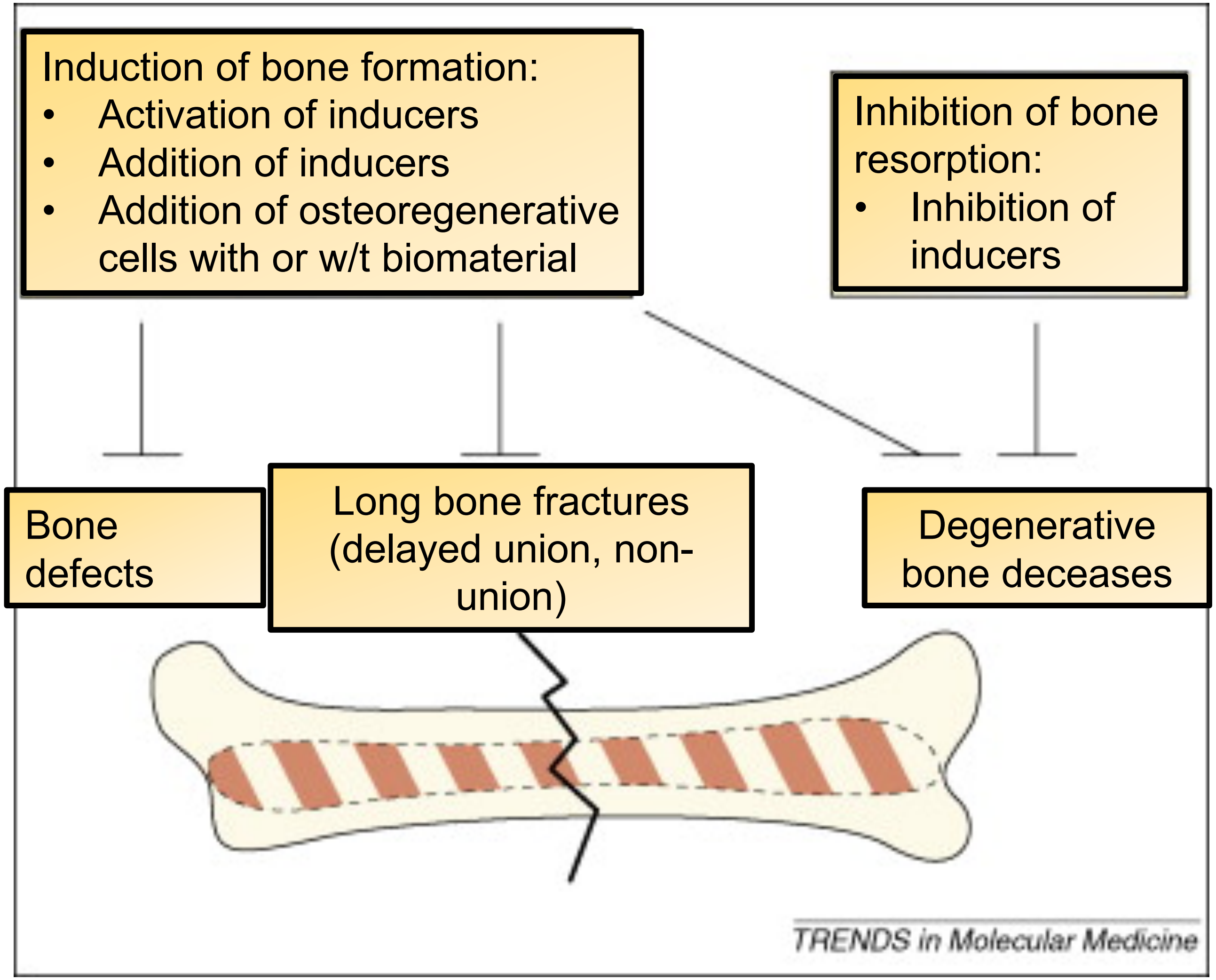
Translational Orthopaedic
Biomaterials Interest Group

Basic Biology of Bone:
Stages of Fracture Healing



Einhorn T A, Gerstenfeld L C. Nature Reviews Rheumatology, 2015, 11(1): 45.

Key Molecules & Cells involved in Bone Regeneration



Clinical relevance of
Key factors in bone repair

Key factors tested	Observations	References
PTHrP/PTH	Used for osteoporosis; efficient for increasing bone mass when intermittently administered	[85]
BMP2	Used for spine fusion, bone nonunion and bone defects; clinically efficient for bone repair and regeneration; some adverse effects observed (osteolysis and ectopic bone formation)	[34]
BMP7	Used for spine fusion and bone nonunion; clinically efficient for bone repair	[35]
Wnt–β-catenin	LiCl used as a specific inhibitor of GSK3β to increase bone mass post-fracture and to diminish fracture risk Bortezomib, proteasome inhibitor used in treatment of multiple myeloma (MM); also increases bone mass Anti-DKK1 monoclonal antibody (BHQ880) used to inhibit osteolysis in MM or to increase BMD Anti-sclerostin antibody used to increase bone mass GSK3b inhibitor (603281-31-8)	[68,69,86–90]
RANKL/OPG	Targeting RANKL to treat osteoporosis; e.g. denosumab (anti-RANKL antibody), which can be used with biphosphonates	[91]
Biphosphonates	Widely used for osteoporosis, bone necrosis, osteogenesis imperfecta and some osteolytic tumors (MM) (zoledronate, alendronate, risedronate); some adverse effects noted (osteonecrosis, inhibition of osteogenesis)	[92]
TGFβ	Used as a bone nonunion marker	[93]
Platelet-rich plasma (growth factor substitute)	Used in maxillofacial surgery and for bone defects with or without biomaterials with or without osteoregenerative cells (randomized controlled trials required)	[94]
MSCs or osteoblasts	*In vitro*-expanded MSCs (or osteoblasts) used for bone defects, osteonecrosis, immune rejection; randomized controlled clinical trials are required	[83]

Key molecules and cells
Involved in bone repair

Key factors	Function	*In vivo* and *in vitro* effects	References
Extracellular messengers			
IL-1, IL6, TNFα	Elicit inflammation and migration	*In vitro* inhibit osteoblastic differentiation, but *in vivo* TNFα is crucial for bone repair; role of IL-6 is controversial (anti-or pro-osteogenic probably, depending on soluble IL-6 receptor)	[3,4]
SDF1	Chemotactic factor	Allows MSCs homing both *in vitro* and *in vivo*	[71]
TGFβ	Mitogenic factor, osteogenic factor	Can induce osteoblast differentiation at the early stage of immature cells but can also inhibit osteogenesis in committed cells	[17,23]
BMP2	Osteogenic factor	Osteochondrogenic factor; might initiate bone formation and bone healing and can induce expression of other BMPs	[18,20,30]
BMP4	Osteogenic factor	Osteochondrogenic factor *in vivo* and *in vitro*	[18,21]
BMP7	Osteogenic factor	Osteogenic factor *in vivo* and *in vitro*; active on more mature osteoblasts	[18]
Noggin	BMP2, 4 and 7 specific inhibitor	Suppresses osteoblastic differentiation	[18]
FGFb	Angiogenic and mitogenic factor, osteogenic factor (controversial)	Mutations induce chondrodysplasia and craniosynostosis; can stimulate Sox9; might be a negative regulator of postnatal bone growth and remodeling	[72]
IGF-I, II	Mitogenic factors, osteogenic factors	Stimulates growth plate formation, endochondrate ossification and bone formation by osteoblasts	[73]
VEGF	Angiogenic and vasculogenic factor	Most potent angiogenic and vasculogenic factor; crucial at the onset of bone formation	[74]

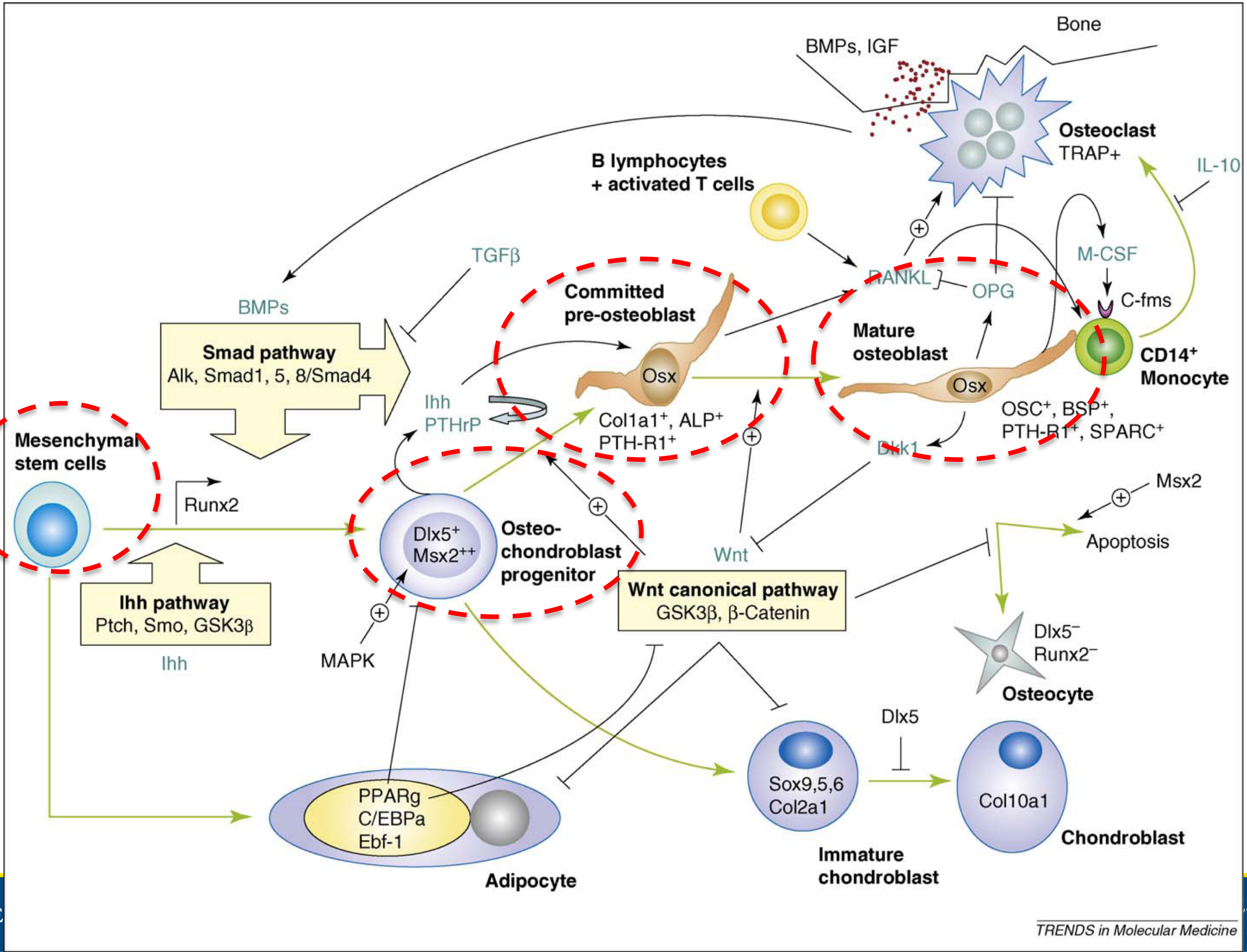
Key molecules and cells
Involved in bone repair

Key factors	Function	*In vivo* and *in vitro* effects	References
Extracellular messengers			
PIGF	Angiogenic and vasculogenic factor	Induces proliferation and osteogenic differentiation of MSCs; crucial for vascularization	[75]
PDGF	Mitogenic and chemotactic factor	Highly mitogenic factor for MSCs and chemotactic for MSCs, osteoblasts and perivascular cells	[76]
Wnts	Mitogenic and osteogenic factors	Depending on Wnt type, crucial for osteoprogenitor proliferation; can also inhibit final osteoblast maturation	[43]
DKK1	Inhibitor of Wnt signaling	Strongly inhibits osteogenesis of MSC and osteoprogenitor cells; can stimulate terminal maturation	[36]
Ihh	Osteochondrogenic factor	Pivotal role for growth plate and endochondral formation; can inhibit osteoblast differentiation; might induce PTHrP expression	[13,14]
PTHrP	Osteochondrogenic factor	Pivotal role for growth plate and endochondral formation; can induce or inhibit osteogenesis	[14,65]
OPG	Decoy receptor of RANKL, inhibition of RANKL	Strongly inhibits bone resorption and has a pivotal role in bone remodeling	[77]
RANKL	Induces osteoclastogenesis	Strongly stimulates bone resorption and has a pivotal role in bone remodeling	[77]
M-CSF	Induces osteoclastogenesis	Crucial for osteoclastogenesis.	[2]
Gastrointestinal serotonin	Neurotransmitter inhibiting osteogenesis	Expressed by enterochromatin cells, inhibits bone formation and repressed by Lrp5	[54]

Key molecules and cells
Involved in bone repair

Key factors	Function	*In vivo* and *in vitro* effects	References
Intracellular messengers			
MAPKs	Transduce osteogenic signaling by phosphorylation	Crucial for regulation of intracellular signaling induced by osteogenic factors (still controversial)	[56]
PKA/CREB	Transduce osteogenic signaling	Can transduce osteogenic signaling (still controversial); possible indirect effect	[66]
β-Catenin	Osteogenic transducer factor	Pivotal role in transducing osteogenic signal from Wnt and is negatively regulated by GSK3β	[68]
Runx2	Early osteogenic transcription factor	Master regulator of early osteogenesis; *runx2*$^{-/-}$ mice died, with no bone formation	[9]
Osterix	Late osteogenic transcription factor	Master regulator of late osteogenesis, inhibiting chondrogenesis	[78]
Dlx5	Osteogenic homeobox protein	Induces osteoblast maturation but inhibits osteocyte formation	[24]
Msx2	Osteogenic homeobox protein	Induces proliferation of immature cells; responses depend on Dlx5 quantity	[24]
NF-κB	Inflammation transducer factor, inhibits osteogenesis	Inhibits the differentiation of MSCs and committed osteoblastic cells	[79]
Cells			
MSCs	Origin of osteoblasts	Can form bone *in vivo* and osteoblasts *in vitro*.	[80–82]
Osteoblasts	Osteogenic professional cells	Generate bone formation	[83]
Adipose tissue-derived multipotential cells	Multipotential cells	Can give rise to bone *in vivo* and *in vitro* but are less effective than bone marrow MSCs	[84]

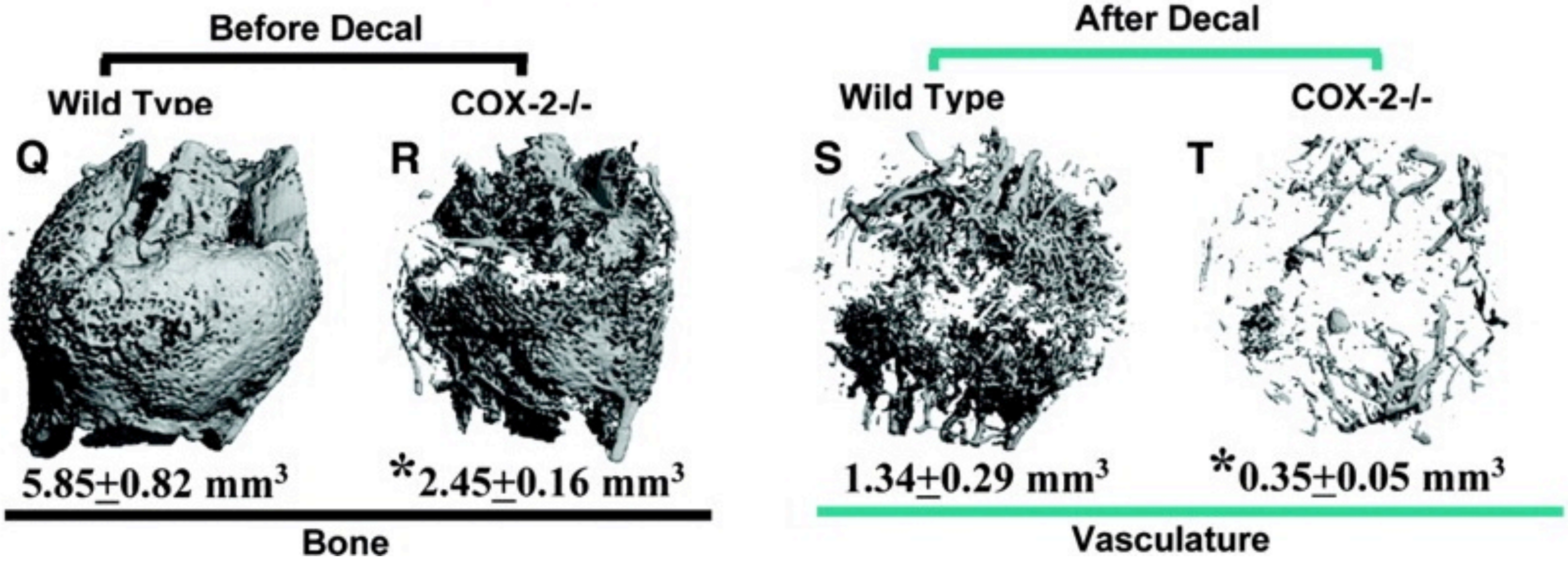
Bone remodeling after fracture



Bone Regeneration:
Animal models & the cell delivery options

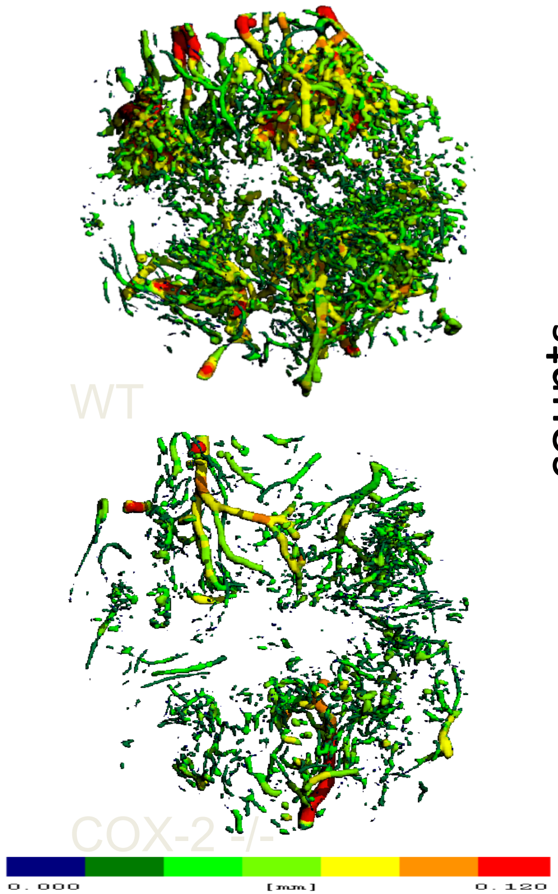
- Fracture
  - Tibial
  - Femoral
- Graft
  - Isograft
  - Autograft
  - Allograft

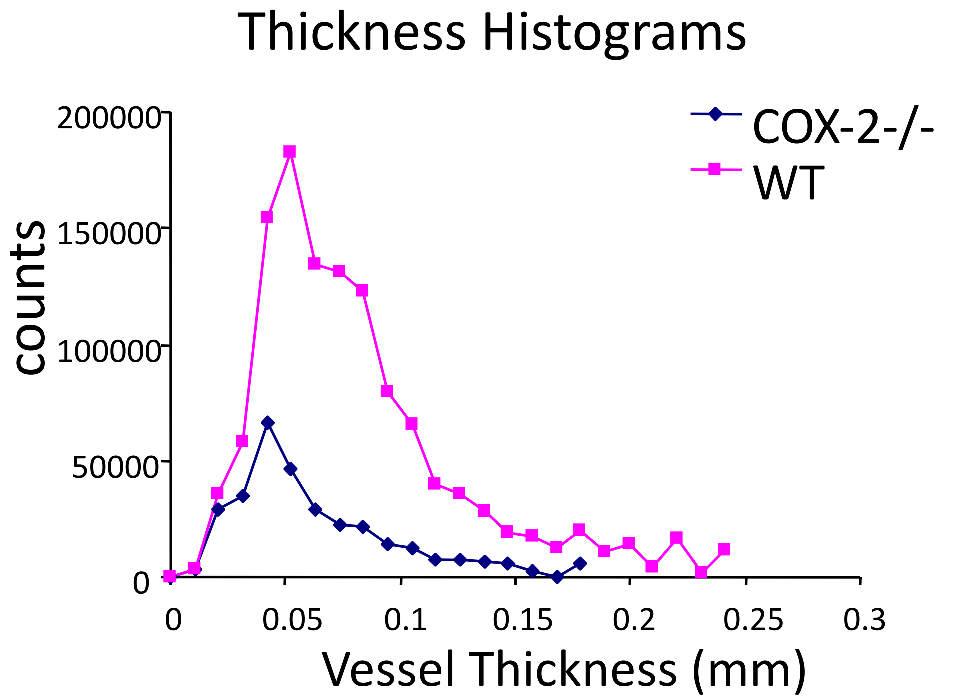
Animal models：
Fracture- New bone and vascularity



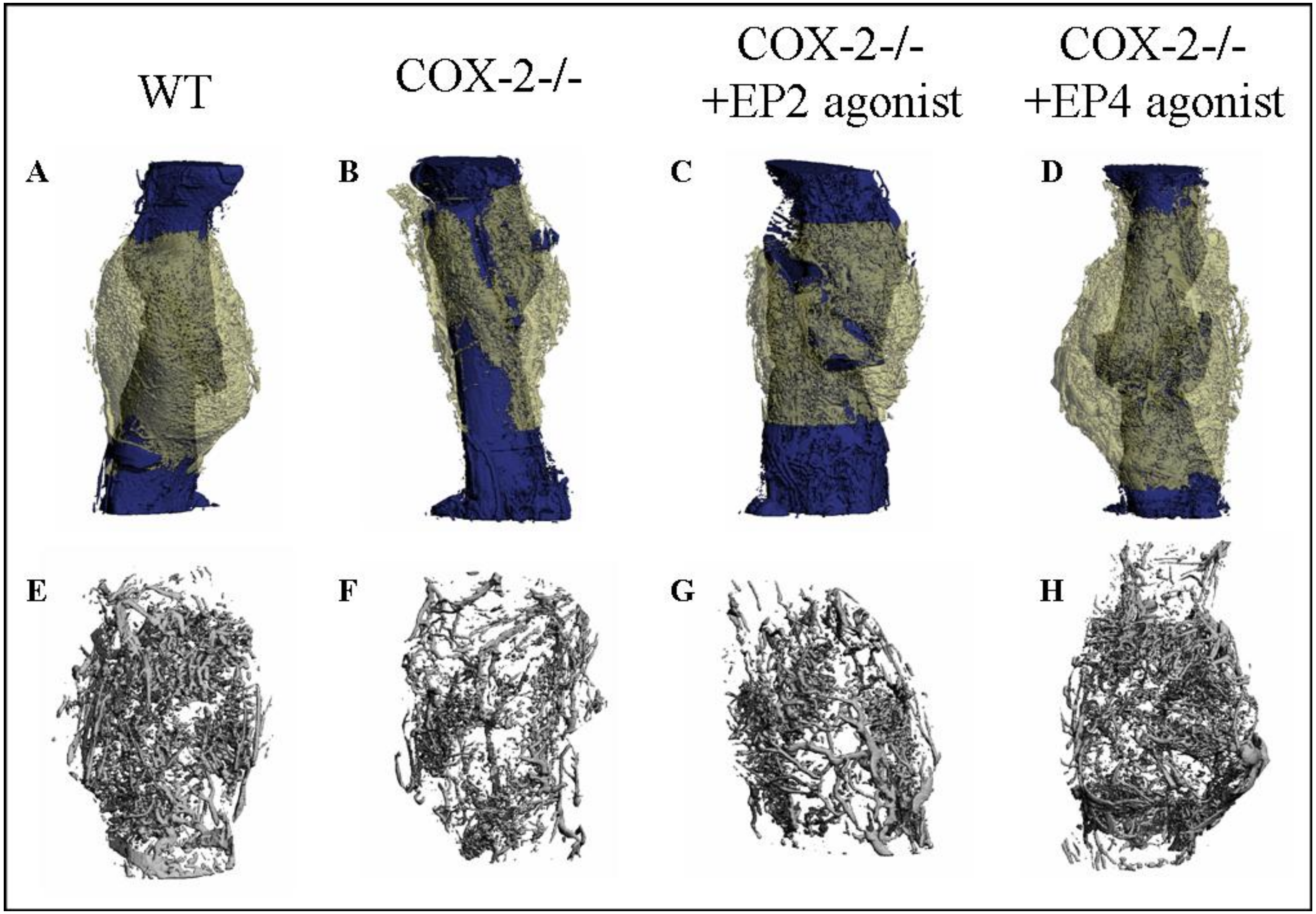
Xie C, et al. AJP 2009

Animal models：
Fracture- New bone and vascularity



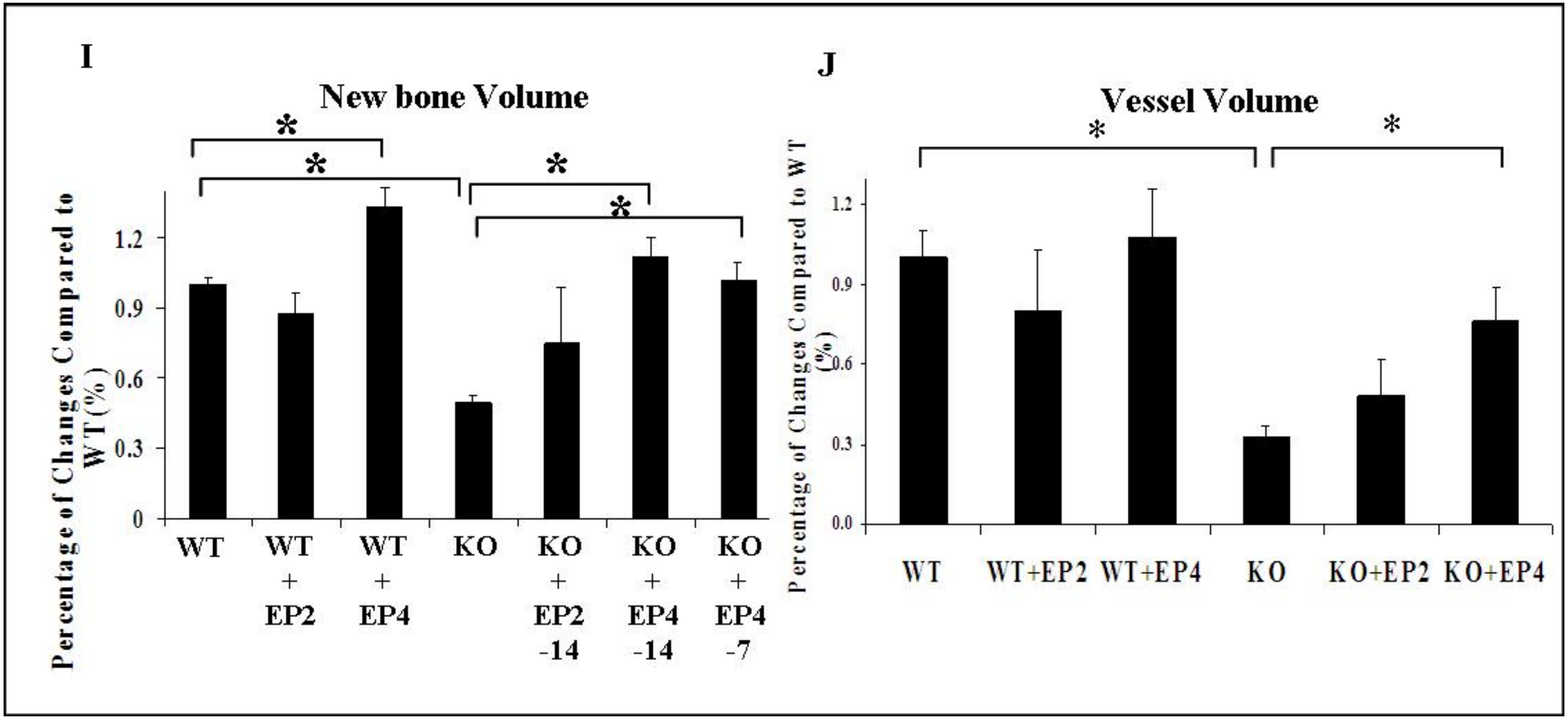


Animal models：
Fracture- New bone and vascularity



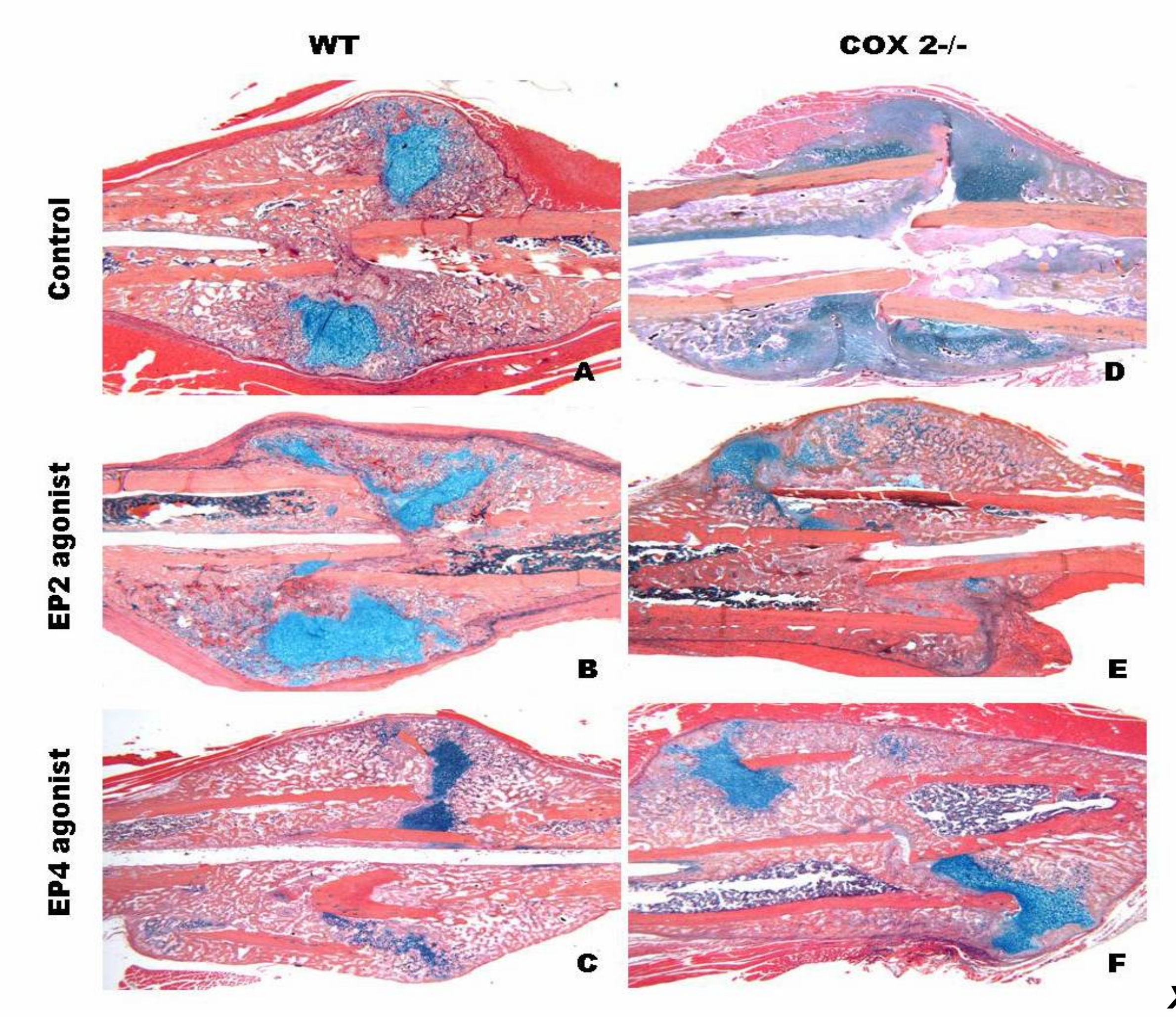
Xie C, et al. AJP 2009

Animal models：
Fracture- New bone and vascularity



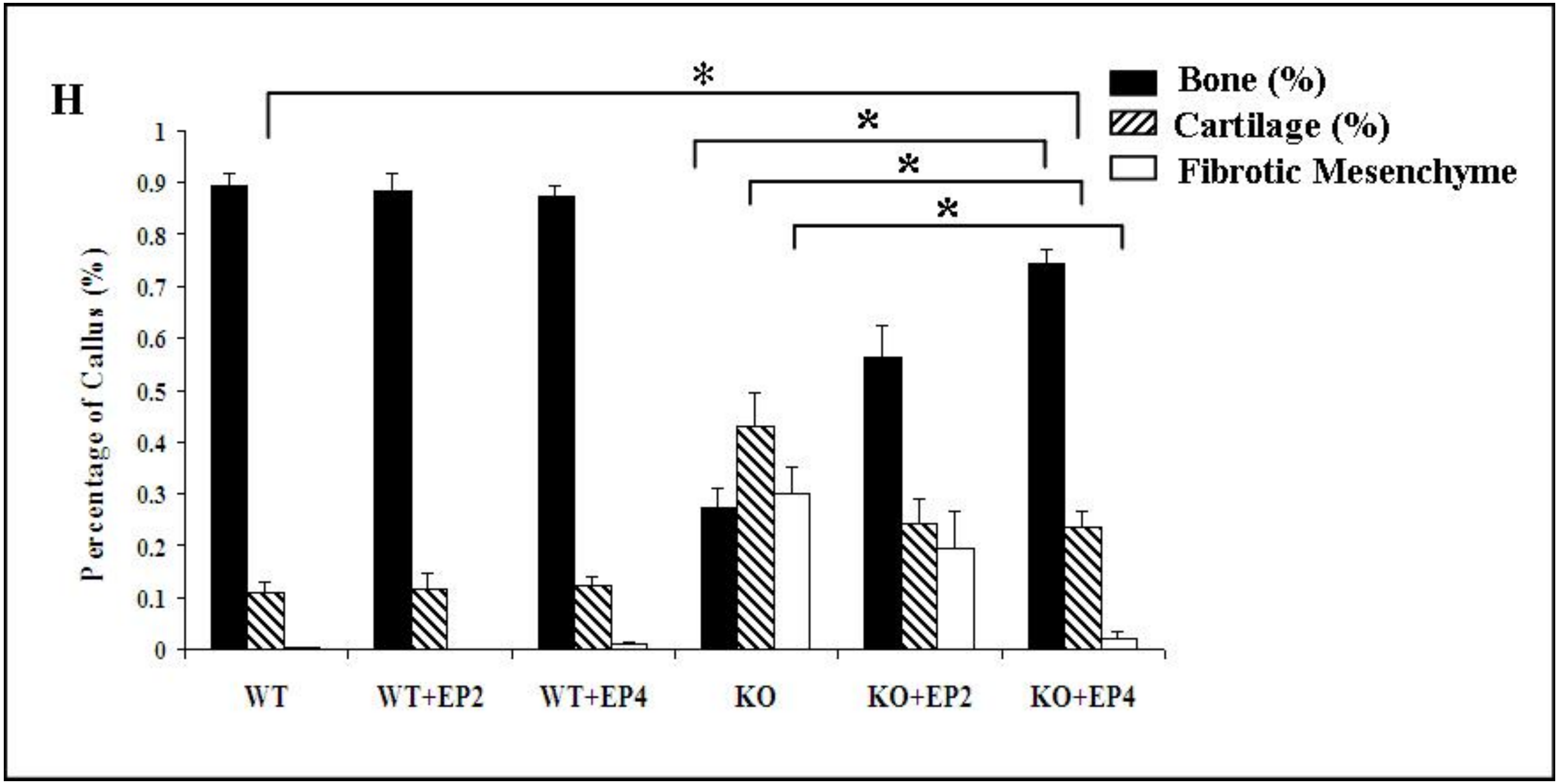
Xie C, et al. AJP 2009

Animal models：
Fracture- New bone and vascularity



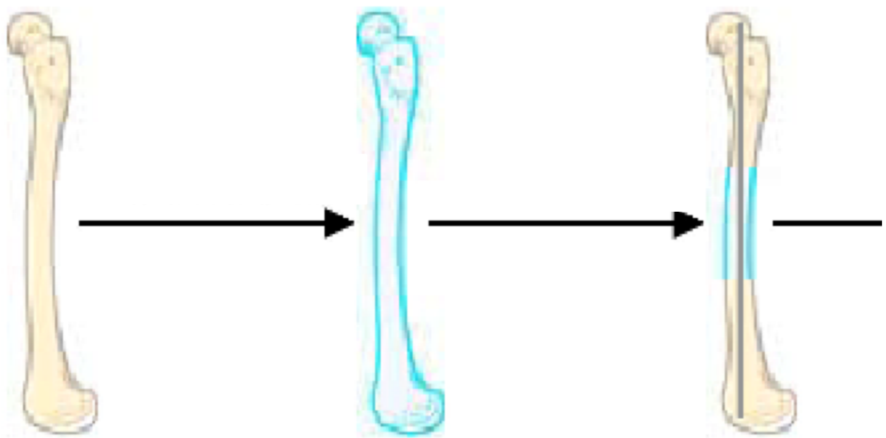
Xie C, et al. AJP 2009

Animal models：
Fracture- New bone and vascularity



Xie C, et al. AJP 2009

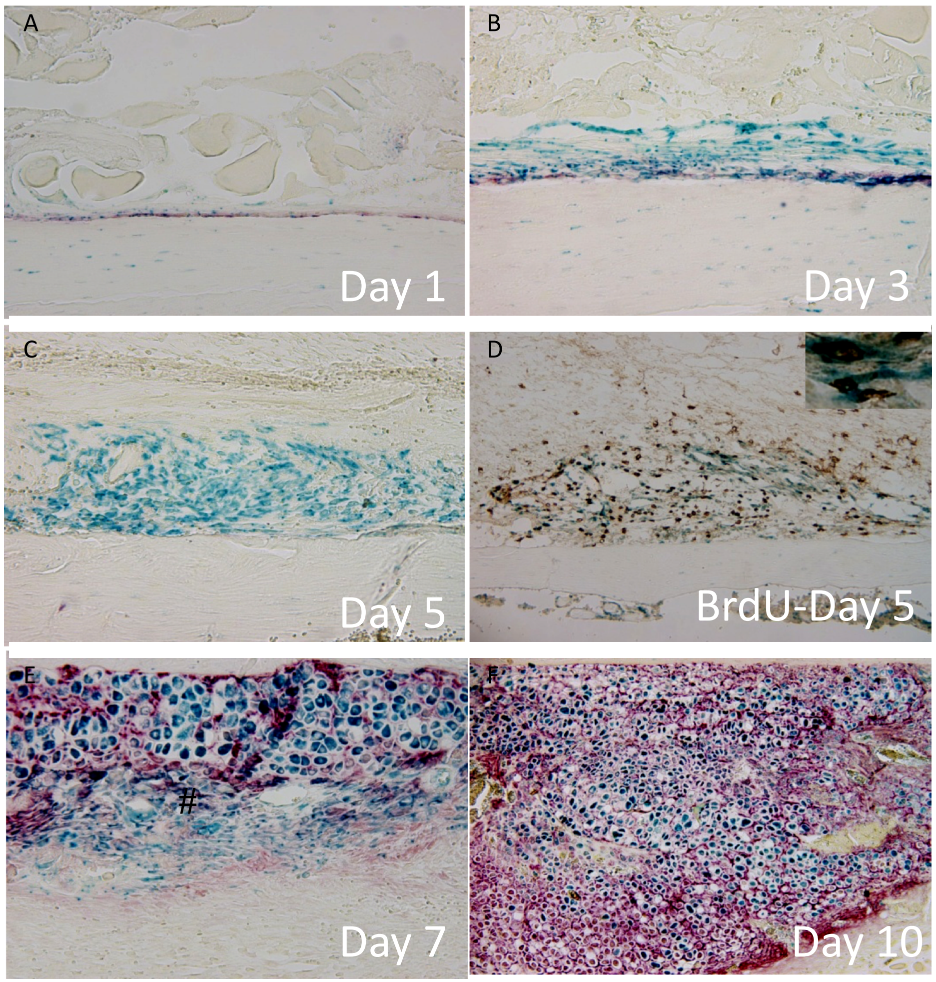
Animal models：
Bone grafting

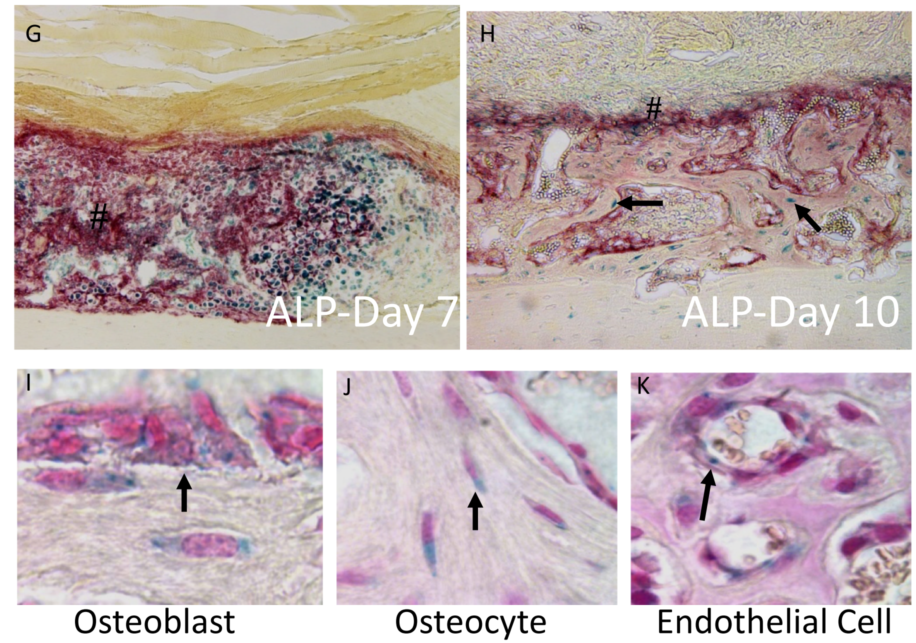


Analyzing donor periosteal stem cell fate and cell dependent healing

Recipient **Donor Rosa 26A** **Post-transplantation**

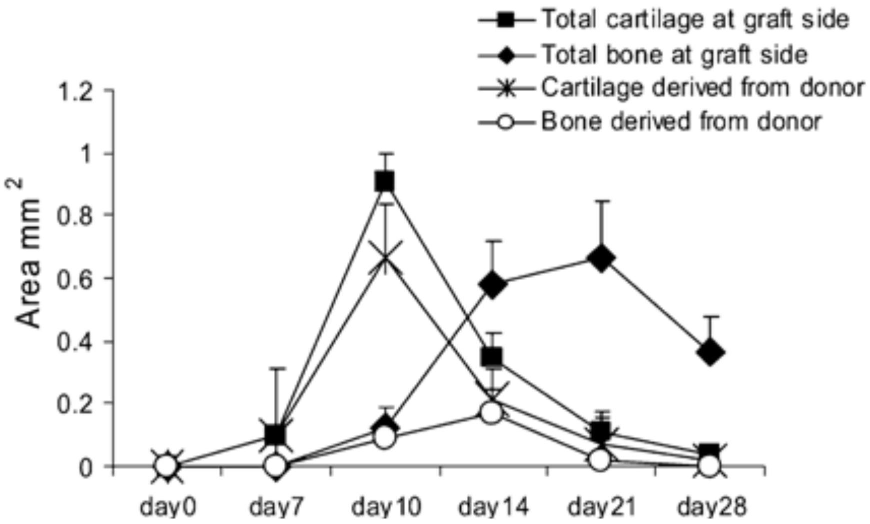
Characterization of Donor Periosteal Progenitor Cell Differentiation in Femoral Isograft Model



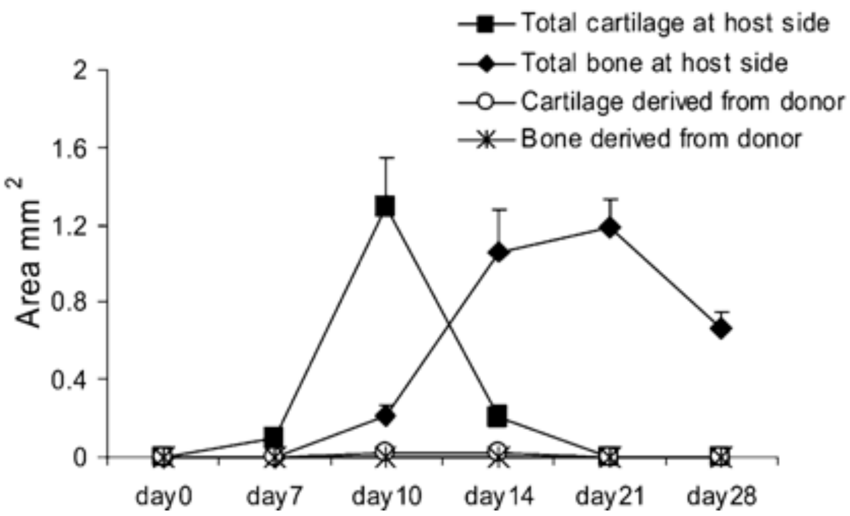


Zhang XP, Xie C. et al. JBMR 2005

Histomorphometric Quantification of Donor Cell Contribution to Osteogenesis in Live Isograft Healing

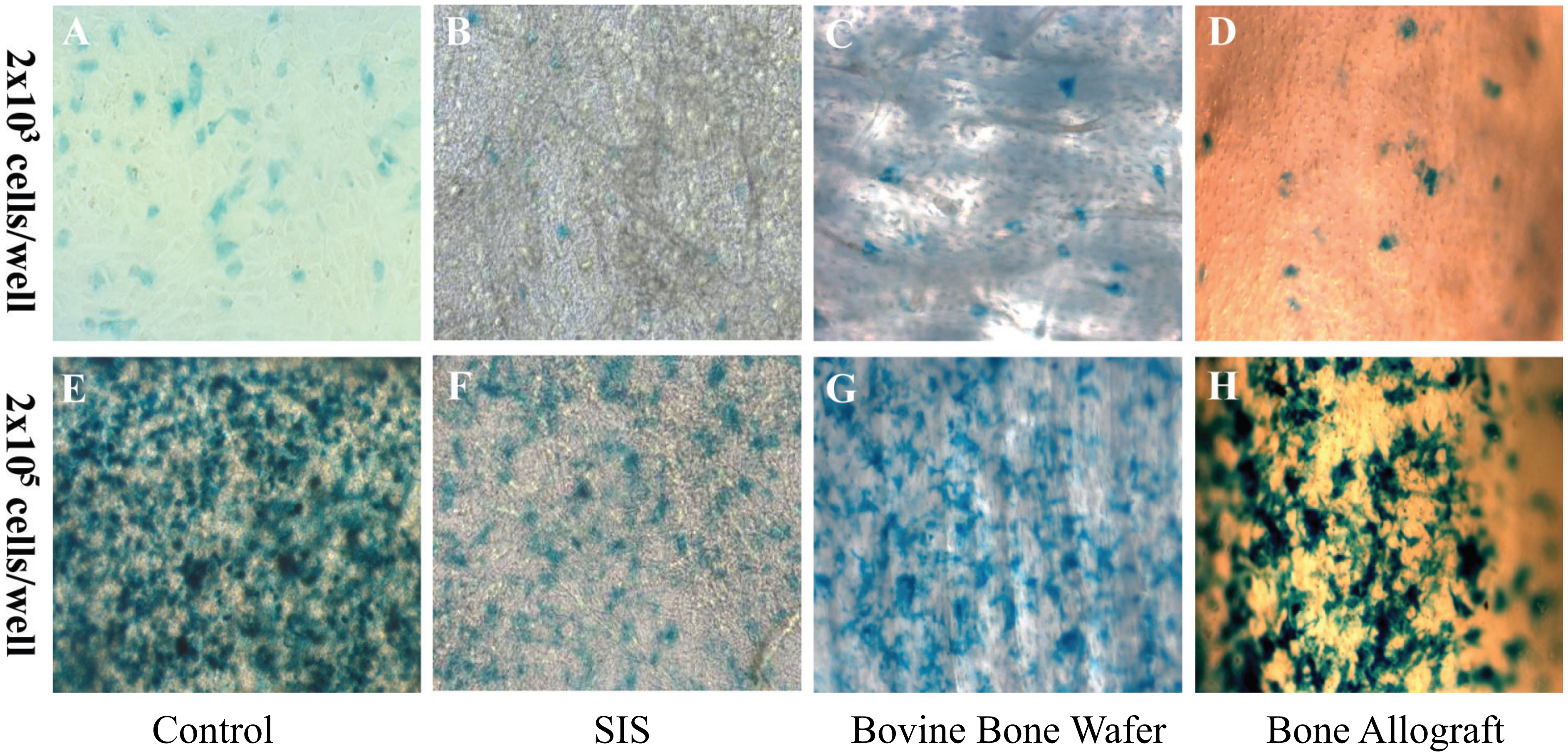


Graft side



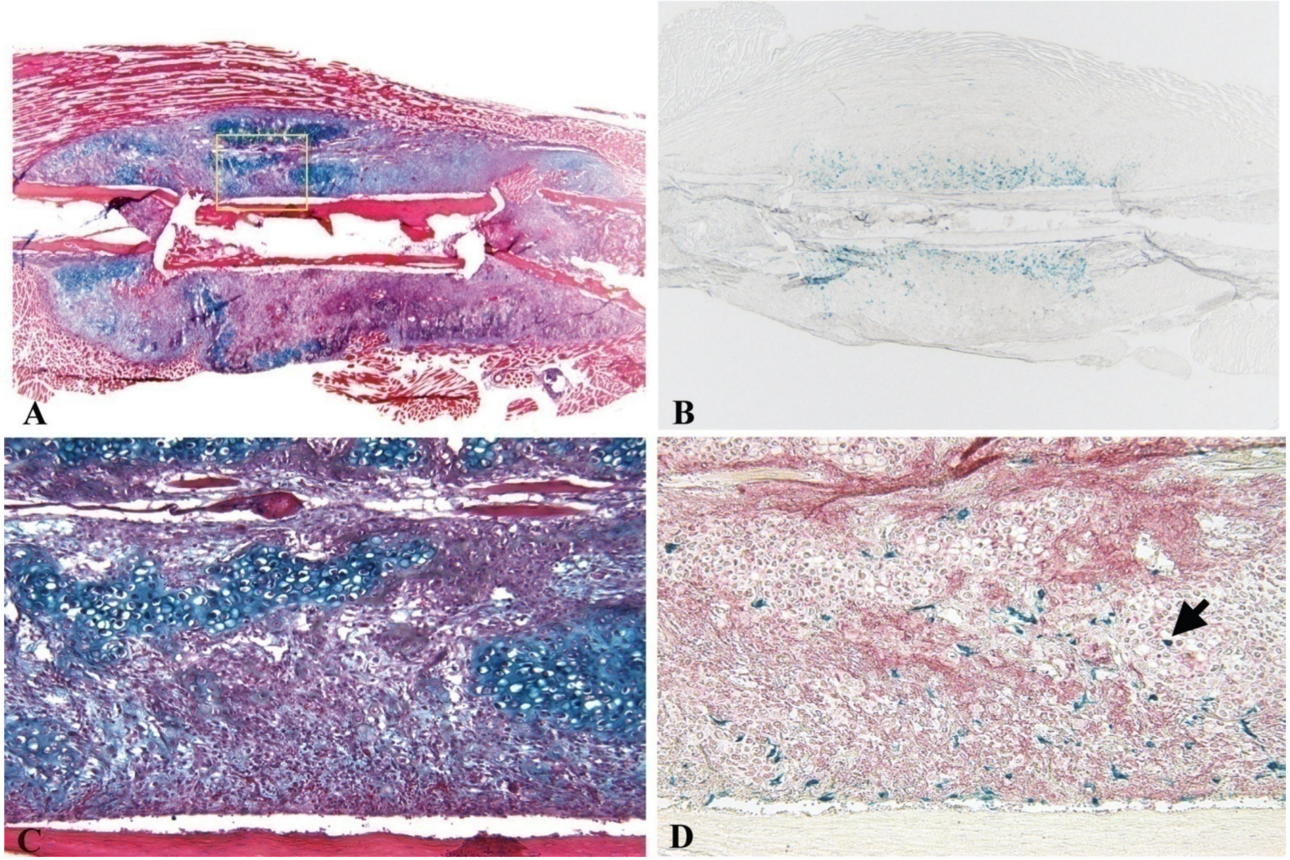
Host side

The Growth & Viability of C9 Cells on Scaffold or Bone



Xie C, et al. Tissue Engineering 2007

The Bio-integration & Survival of C9 Cells After Their Transplantation on Coated Allografts *In Vivo*

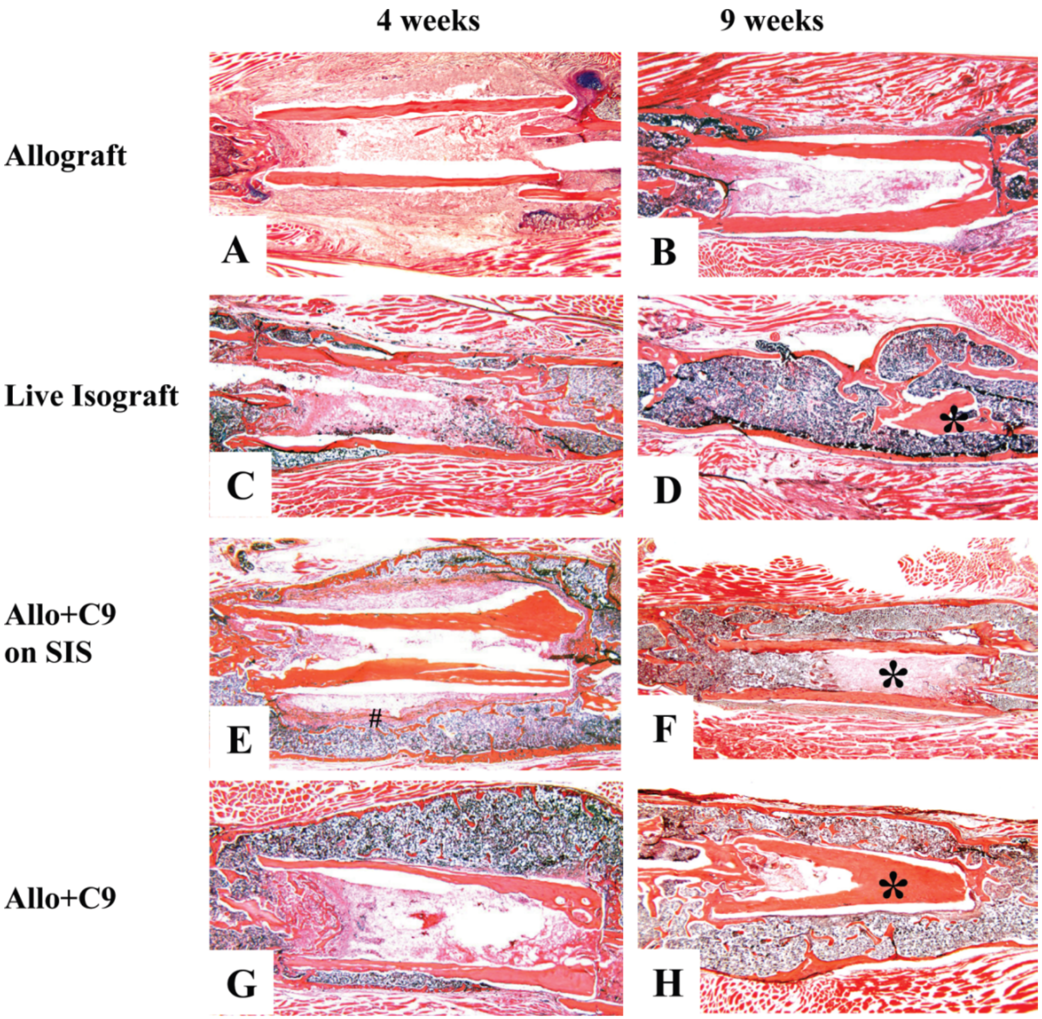


H&E

Beta-Gal/ALP

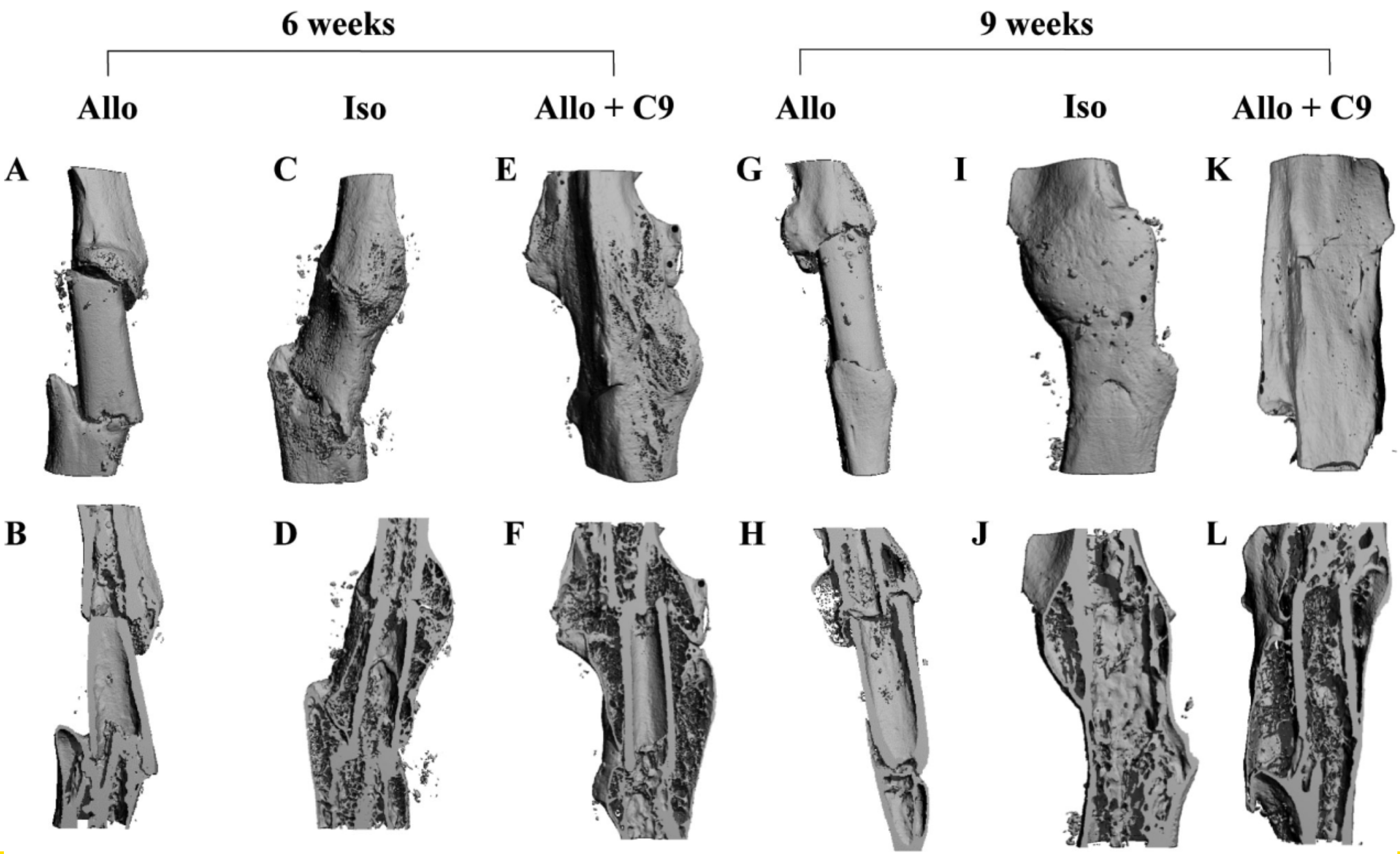
Xie C, et al. Tissue Engineering 2007

Induction of Periosteal-like New Bone Callus in C9-Coated Allografts



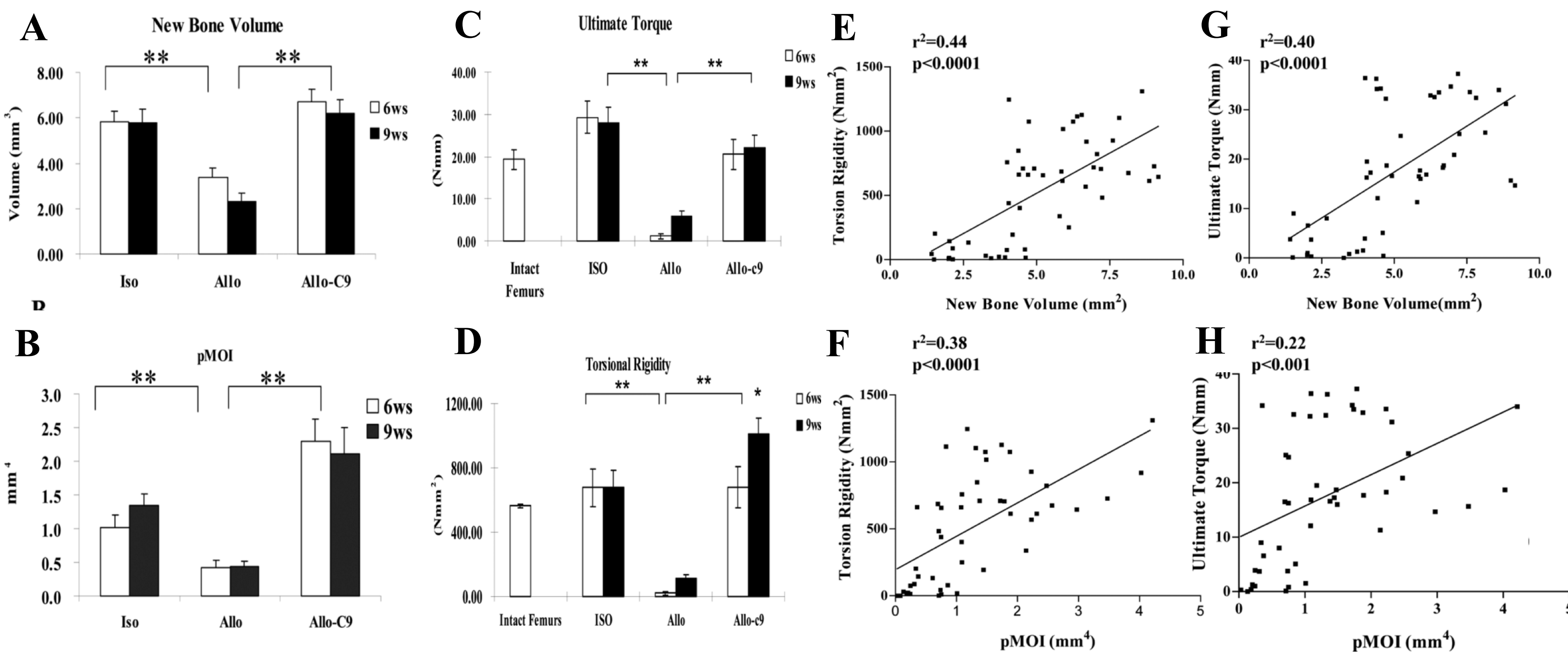
Xie C, et al.
Tissue Engineering 2007

3-D Micro-CT Imaging Confirmed Histological Findings of New Bone Formation on BMP2-Coated Allografts



Xie C, et al. Tissue Engineering 2007

A Significante Increase in Bone Formation & Biomechanics in BMP2-Allografted Femurs



Xie C, et al. Tissue Engineering 2007

Summary

1. A high demand for bone regeneration/ healing in clinical application
2. There are still vast limitations of bone regeneration to achieve the goals of treatments needed
3. Understand the basic biology of bone and its clinical relevance of critical factors are the foundation of translational research
4. The key molecules and cells that involved in bone repair, combined with animal models and the cell delivery options are the only way to transfer research outcomes from bench to bed.

Beipanjiang Bridge

World's Highest Bridge

(Duge Elevation)