Regenerative Medicine and Bone Tissue Engineering

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Regenerative Medicine

- Regenerative medicine is the branch of medicine that develops methods to regrow, repair or replace damaged or diseased cells, organs or tissues.

- *Regenerative medicine* is a broad field that includes tissue engineering but also incorporates research on self-healing – where the body uses its own systems, sometimes with help foreign biological material to recreate cells and rebuild tissues and organs.

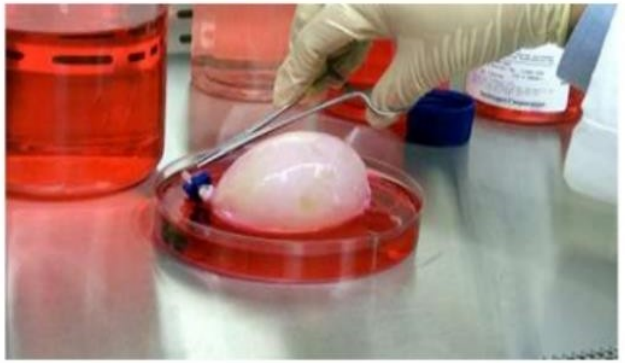
- Regenerative medicine includes the generation and use of

1. stem cell therapy,
2. tissue engineering,
3. the production of artificial organs
4. gene therapy

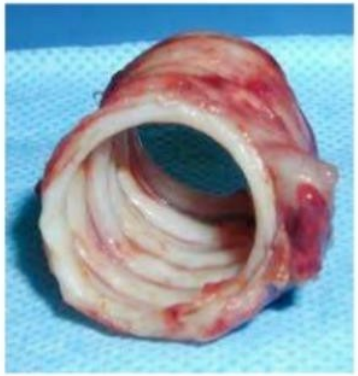
Areas of Regenerative Medicine

1. ***Artificial Organs: Medical Devices***

(Lab Grown Bladder)



2.Tissue Engineering & Biomaterials

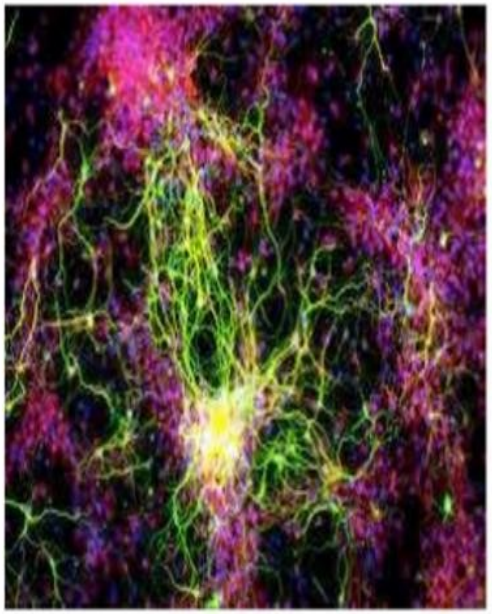


Scaffolds

Areas of Regenerative Medicine

3. *Cellular Therapies*

- Use of Stem Cells (From Patient)
- Development of Regenerative Medicine Treatments.
- Enhance Regeneration of Tissues and Organs.



4. *Clinical Trials*

- Many Currently in Progress.
- NIH and Private Organizations.

Artificial Organs

- The use of artificial organs has made remarkable progress in the last 10 years
- Biological compatibility is still not fully achieved and
- There are problems with preserving their functionalities after transplantation.

Organ Transplantation

The problems in organ transplantation

- The number of donors is low
- immunologic rejection despite recent improvements in immunosuppressive therapy.

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Tissue Engineering Approaches

- The most accepted definition for tissue engineering is the application of biology, chemistry and engineering principles to repair or reconstruct living tissues using biomaterials, cells and biosynthetic molecules alone or in combination.
- According to this definition, there are 4 approaches for tissue engineering:

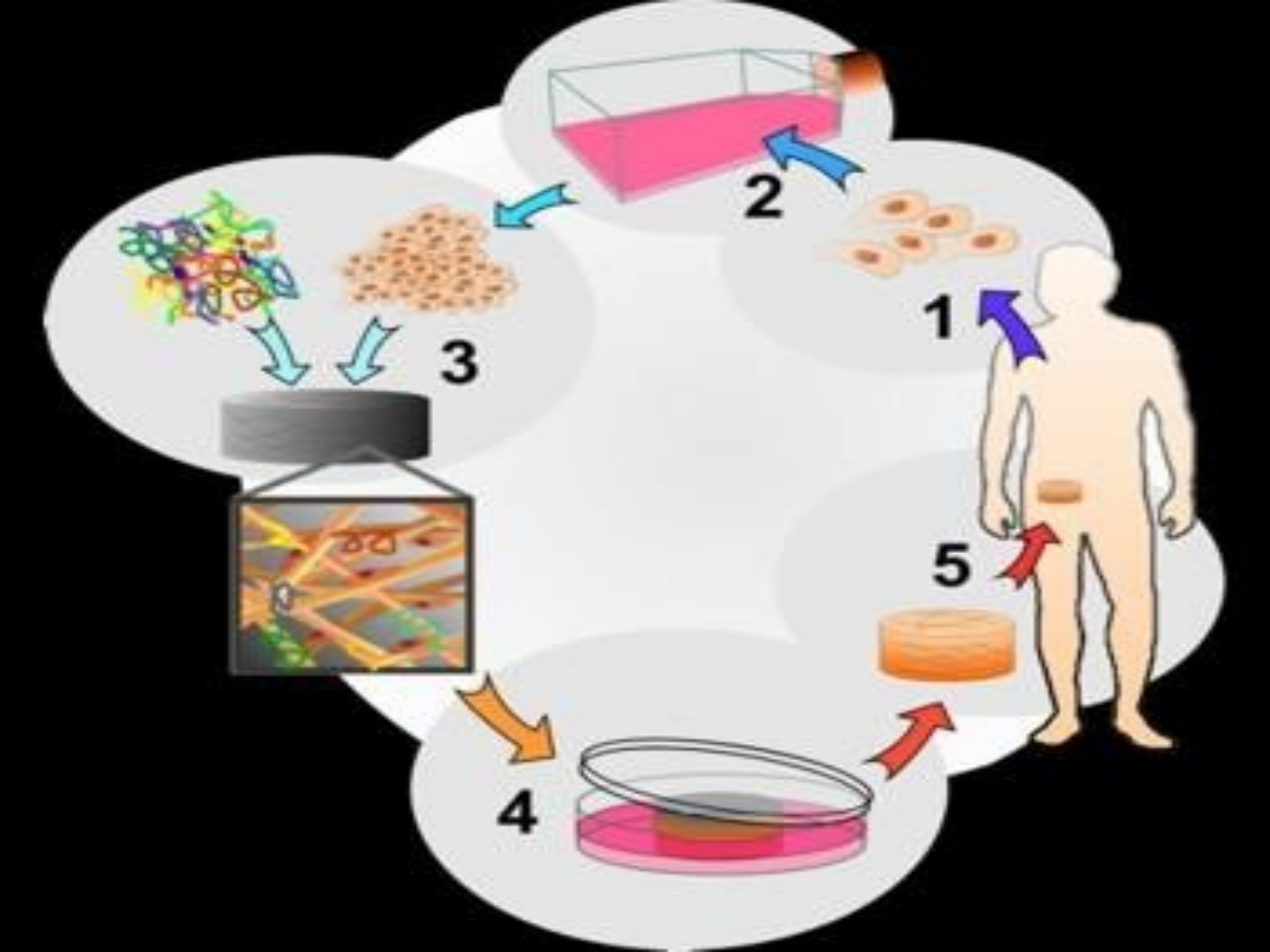
1 Use of biomaterials only

2 Use of cells only (cell transfer)

3 Biomaterials + biosynthetic molecules

4- Biomaterials + cell + biosynthetic

- The most studied approach is the 4th one.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Bone Tissue Engineering

Bone

- It is one of the roughest tissues of the body.
- It occurs when connective tissue turns into bone tissue.
- Bone is mechanically rigid and at the same time plastic, so it can renew itself in the face of mechanical interventions
- The substance between the cells in the bone consists of two parts, organic and inorganic molecules

Bones

- Functions
  - Support
  - Movement: muscles attach by tendons and use bones as levers to move body
  - Protection
    - Skull – brain
    - Vertebrae – spinal cord
    - Rib cage – thoracic organs
  - Mineral storage
    - Calcium and phosphorus
    - Released as ions into blood as needed
  - Blood cell formation and energy storage
    - Bone marrow: red makes blood, yellow stores fat

COMPOSITION OF BONE

Bone

Inorganic 65%

(Primarily calcium phosphate which is present in form of Highly insoluble crystals of Hydroxy apatite)

Organic 35%

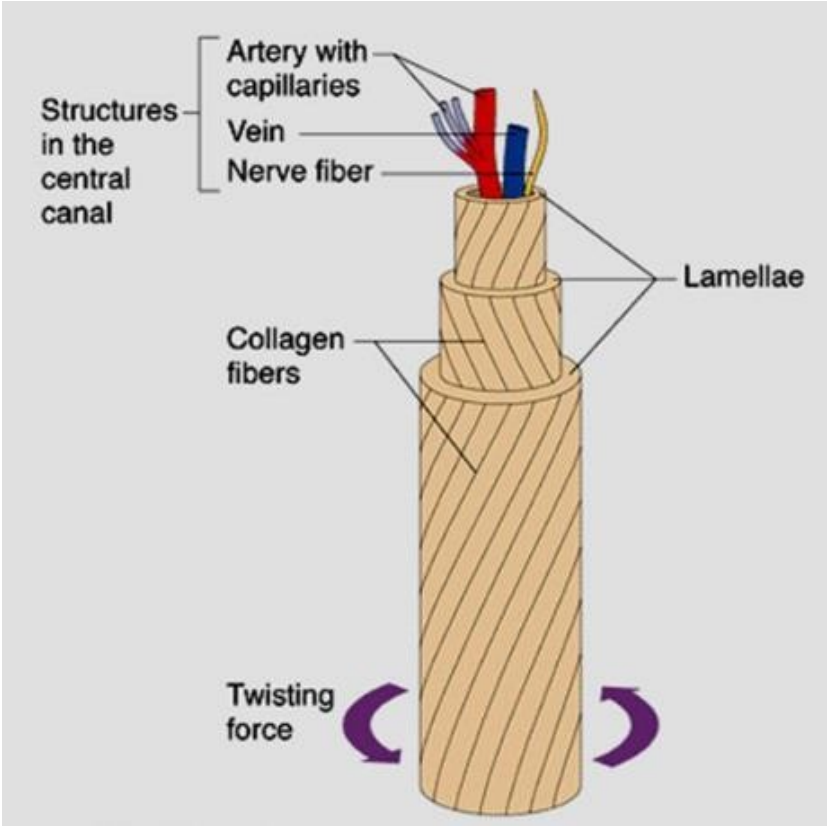
Collagen 88-89%

Non collagen 11-12%

Glycoprotein
Proteoglycan
Sialoproteins
Lipids

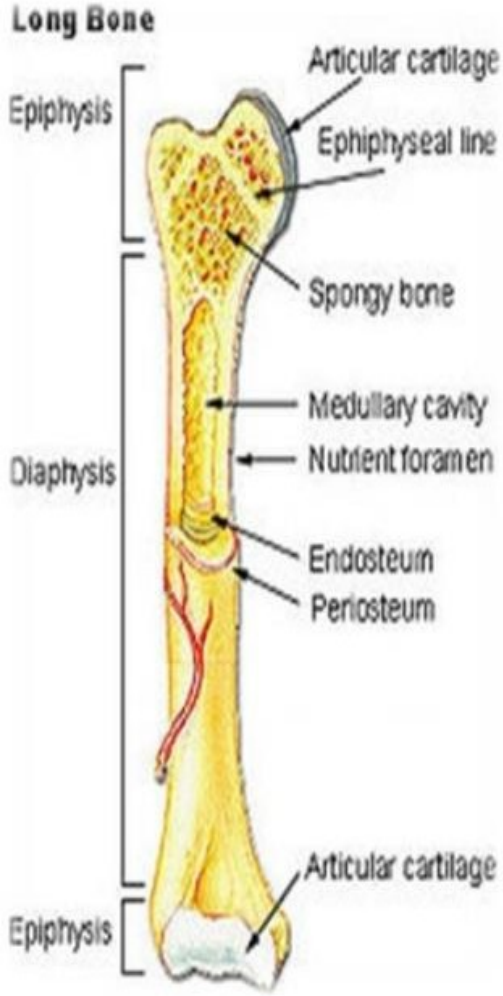
Collagen fibers provide bone with great tensile strength while Inorganic salts allow bone to withstand compression.

- Organic substances constitute 30-35 % of bone tissue.
- Most of these materials contain Type 1 collagen fibrils
- The collagen fibers are arranged parallel to each other.
- Hydroxyapatite crystals are found between collagen fibers
- Hydroxyapatite is a substance that gives texture to the tissue



A typical long bone consist of following

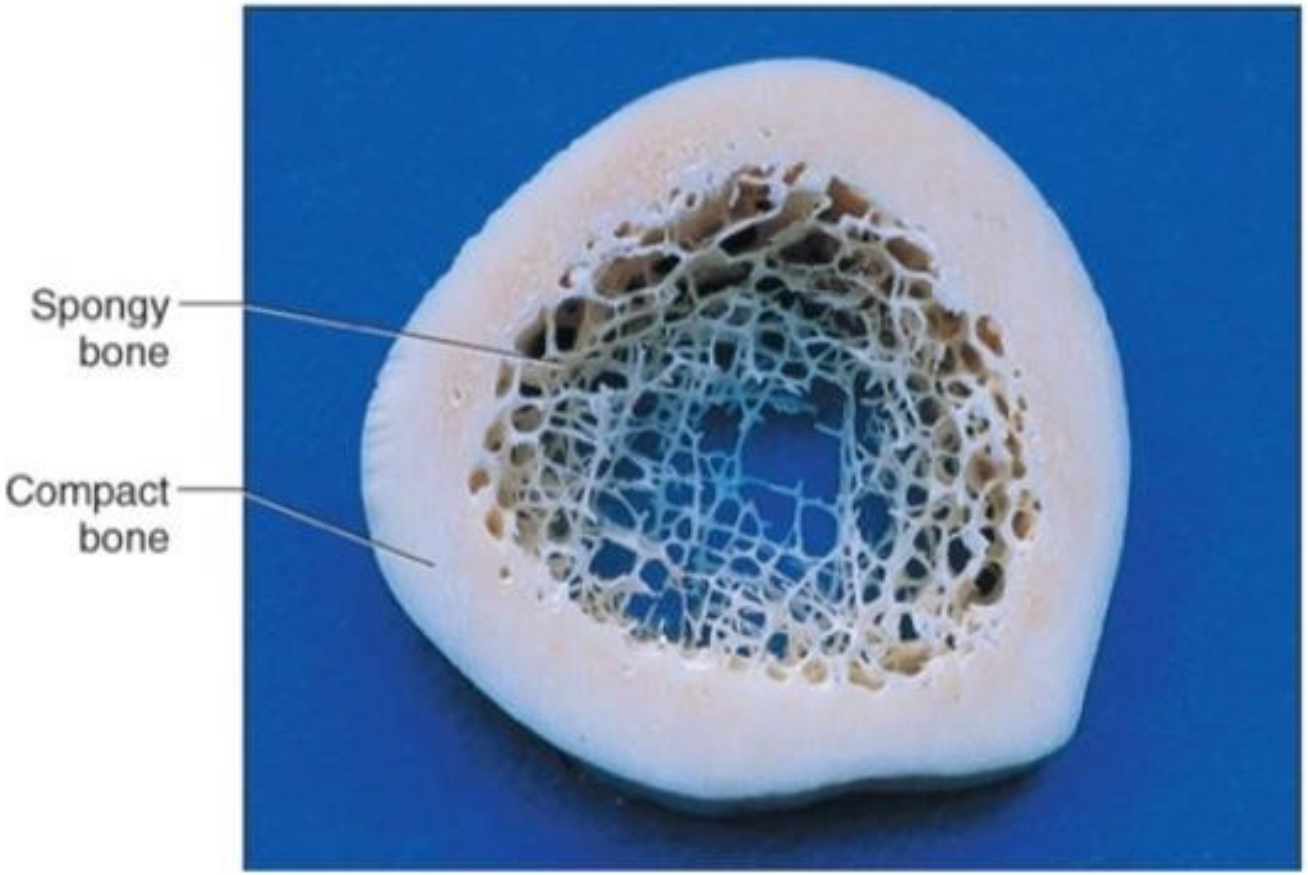
- Diaphysis
- Epiphysis
- Metaphysis
- Articular cartilage
- Periosteum
- Medullary or marrow cavity
- Endosteum



Gross structure of bone:

- Epiphysis: Ends of a bone
- Diaphysis: Shaft of a bone
- Metaphysis: Joining of Epiphysis and Diaphysis. This is the most vascular part of a bone. Because most of the supplying arteries and veins enters through this area.
- Periosteum: Outer covering of bone. This area is pain sensitive.
- Endosteum: thin layer lining the medulary cavity.

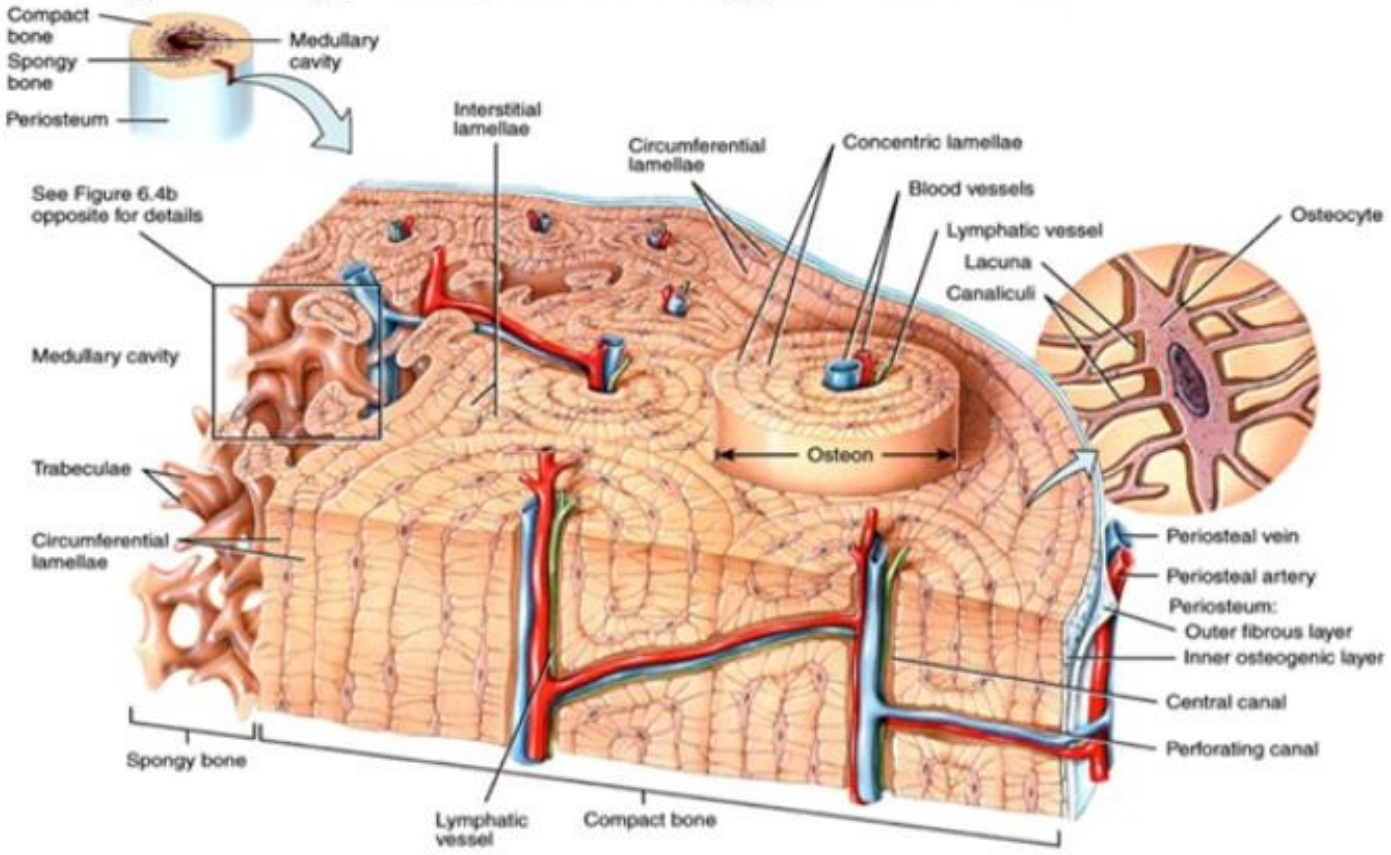
Microscopic Structure of Bone



STRUCTURE

A. COMPACT BONE – strong, weight-bearing, stress-resisting bone with few spaces

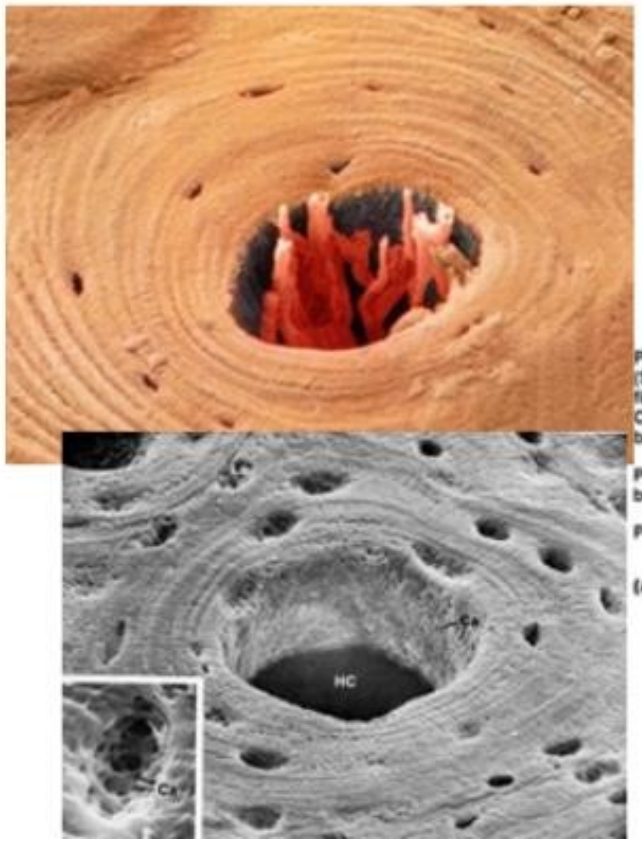
1. OSTEON "HAVERSIAN SYSTEM" – repeating unit, run length of shaft

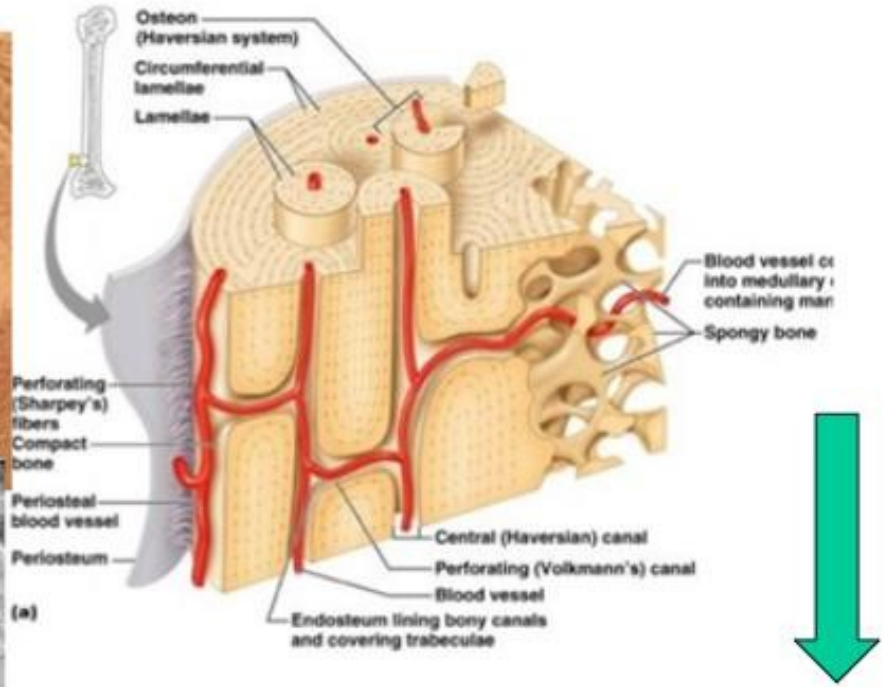


STRUCTURE

A. COMPACT BONE – strong, weight-bearing, stress-resisting bone with few spaces

2. HAVERSIAN (CENTRAL) CANALS – present in each osteon; lengthwise canals supplying nerves & vascular tissue

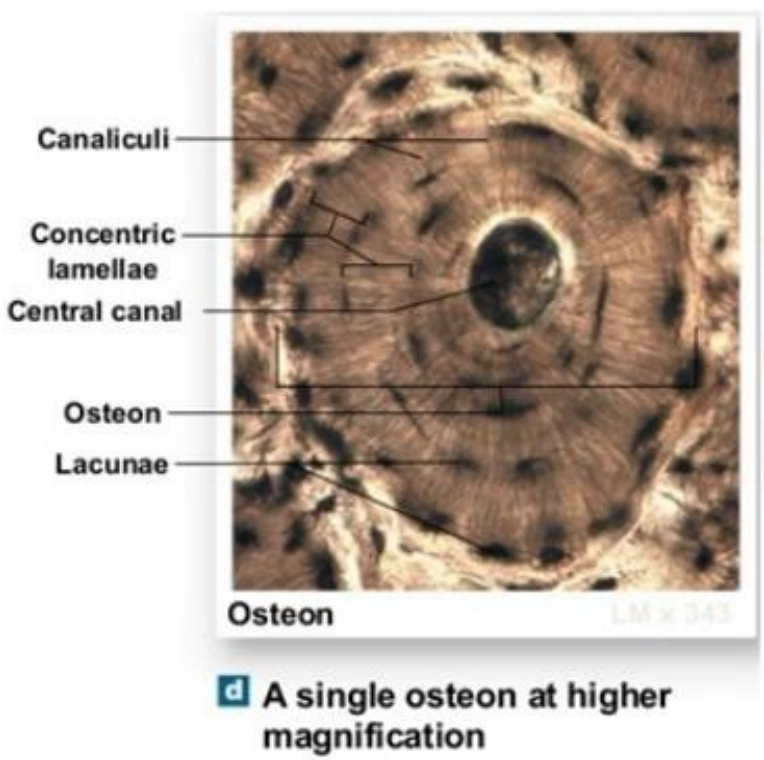


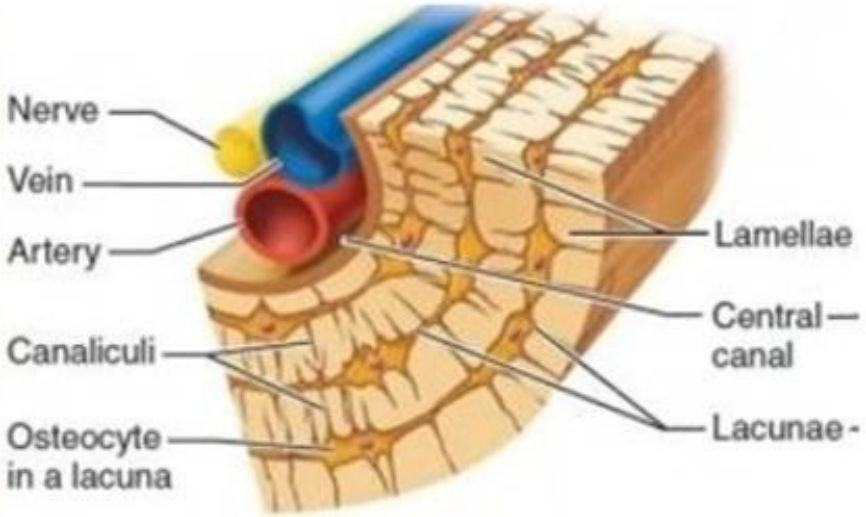


STRUCTURE

COMPACT BONE – strong, weight-bearing, stress-resisting bone with few spaces

3. LAMELLAE – rings of hard calcified matrix surrounding *haversion canals*

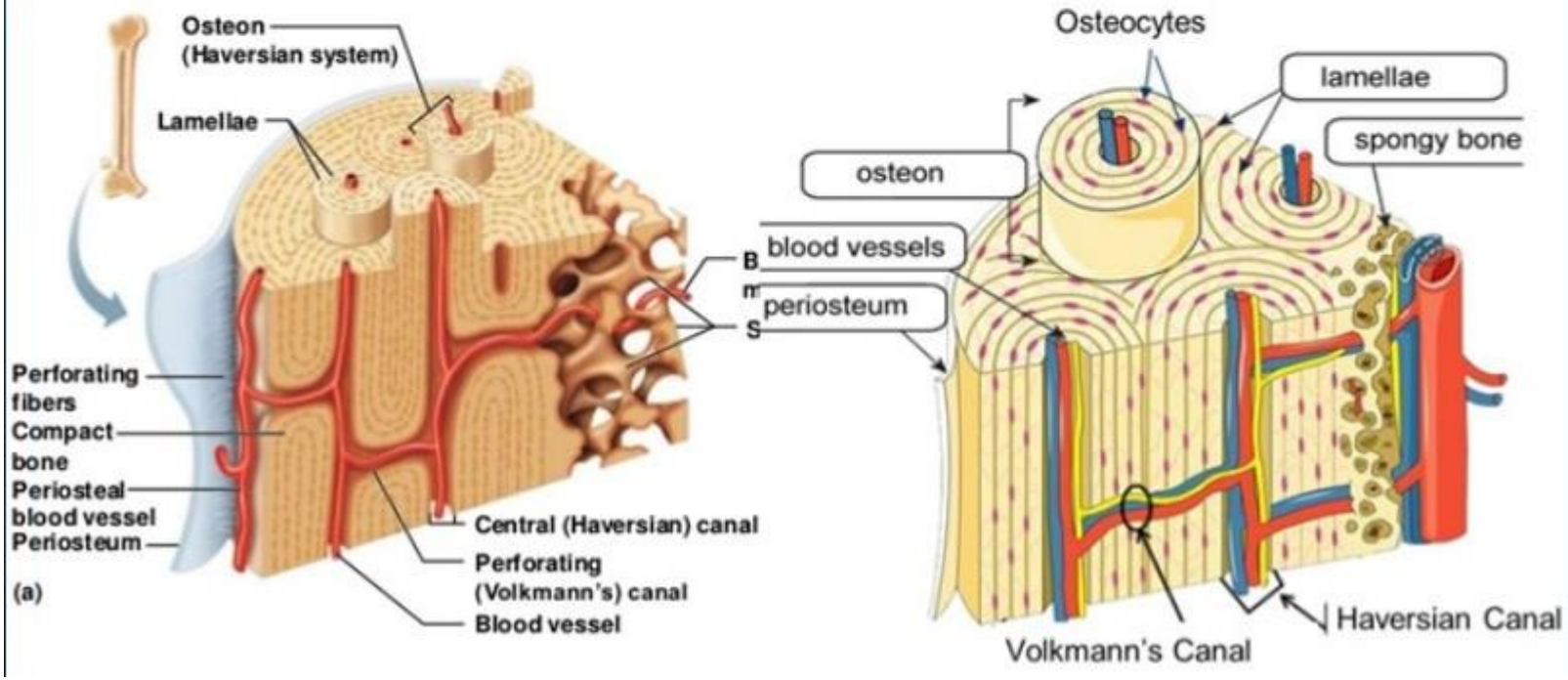




STRUCTURE

COMPACT BONE – strong, weight-bearing, stress-resisting bone with few spaces

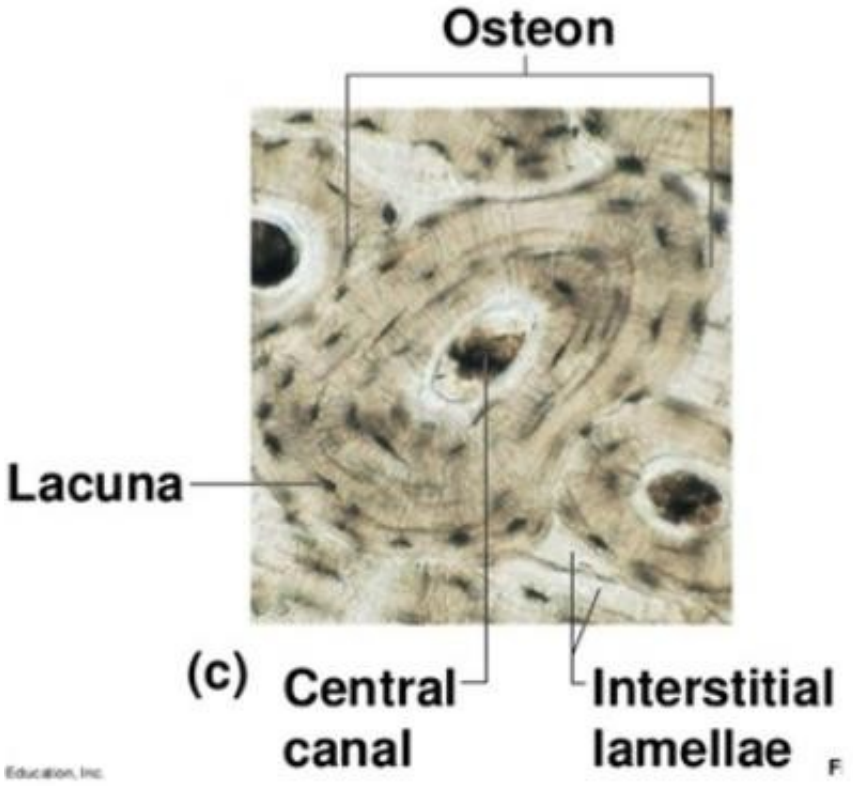
4. PERFORATING (VOLKMANN'S) CANALS – cross-sectional canals supplying nerves & blood vessels; connect w/ blood vessels of periosteum, central canal & marrow cavity

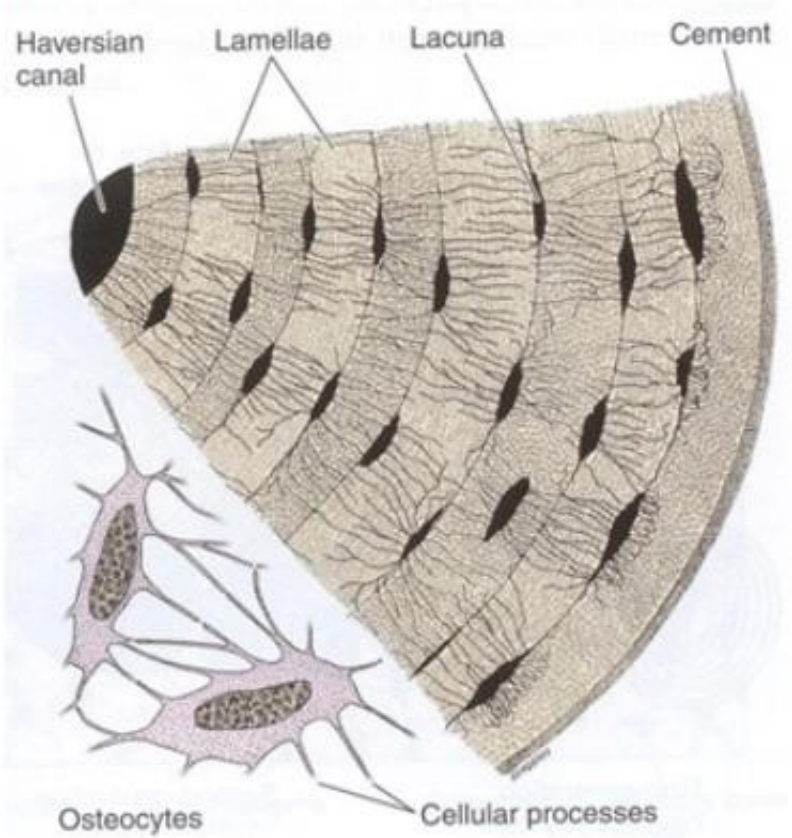


STRUCTURE

A. COMPACT BONE – strong, weight-bearing, stress-resisting bone with few spaces

5. LACUNAE – small openings containing osteocytes -between lamellae

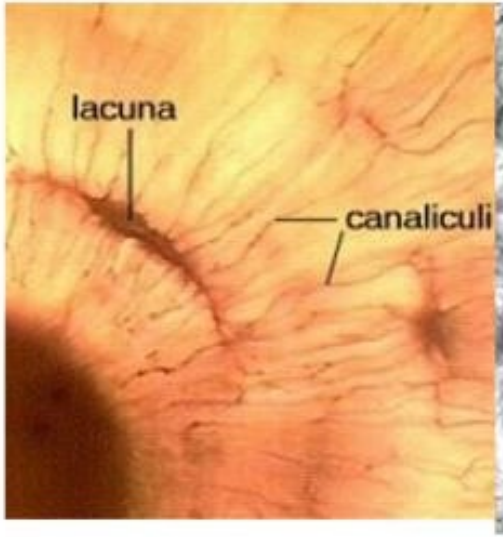


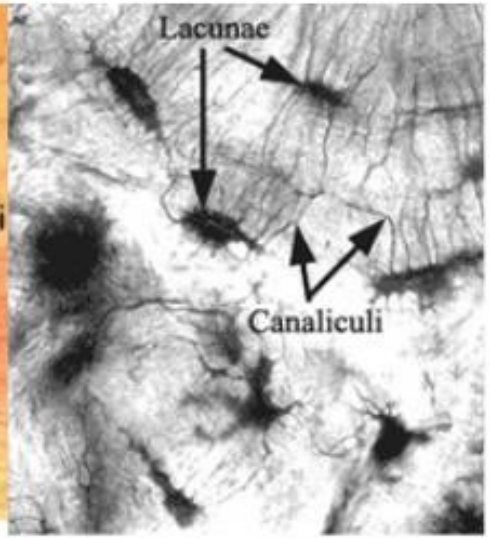


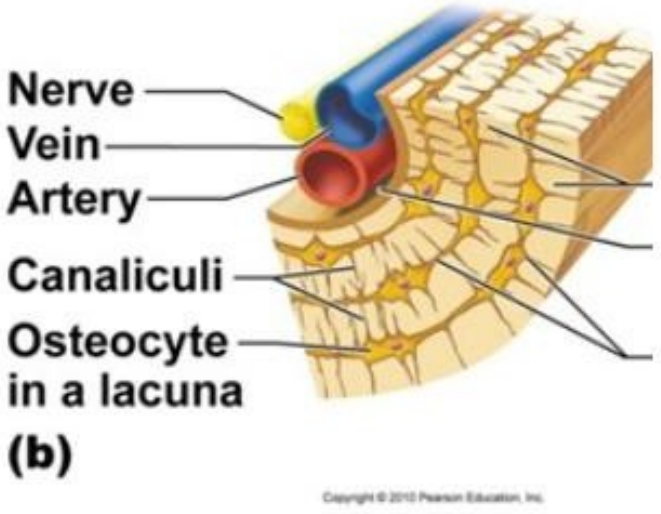
STRUCTURE

A. COMPACT BONE – strong, weight-bearing, stress-resisting bone with few spaces

6. CANALICULI – ("tiny canals") containing osteocyte extensions and fluids connecting both lacunae and *haversion canals* together (creates branching network for transportation of nutrients and wastes) osteocytes communicate with one another







Blood and nerve supply of Bone:

Blood: Bone has a rich vascular supply, receiving 10-20% of total CO. major contributing arteries are-

* Diaphyseal Nutrient artery
* Metaphyseal and Epiphysial arteries
* Periosteal arterioles

Nerves: Rich in articular extremities, vertabrae, and flat bones. Most of the cases nerves accompany with arteries.

HISTOLOGY OF BONE TISSUE

- Like other connective tissues, bone tissue contains an abundant matrix surrounding the cells. The matrix is about 25% water, 25% protein fibres and 50% mineral salts.

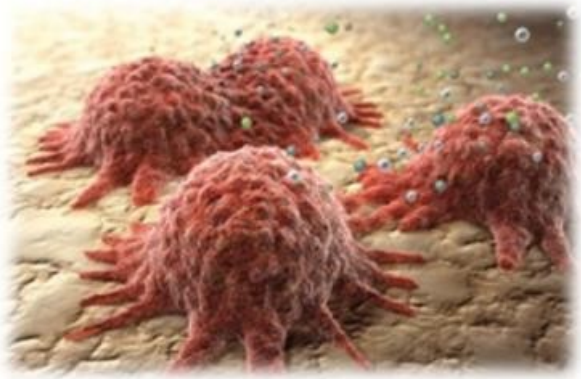
- There are **4 types of cells** in bone tissue.
  - **Osteoprogenitor cells** are unspecialized cells derived from mesenchyme, the tissue from which all connective tissues are derived. They undergo mitosis and develop into osteoblasts. They are found in the periosteum, endosteum and in canals that contains blood vessels.

- **Osteoblasts** are the cells that form bone, but they have lost the ability to divide by mitosis. They secrete collagen and other organic components needed to build bone tissue.

The differentiation of osteoproginitor cells into osteoblast is acclererated by some hormones and some bone proteins called Skeletal growth factors.

Function-

1) Role in formation of bone matrix
2) Role in calcification (through the alkaline phosphatase enzymes)
3) Synthesis of proteins.



- **Osteocytes-** these cells are concerned with maintenance of bone.

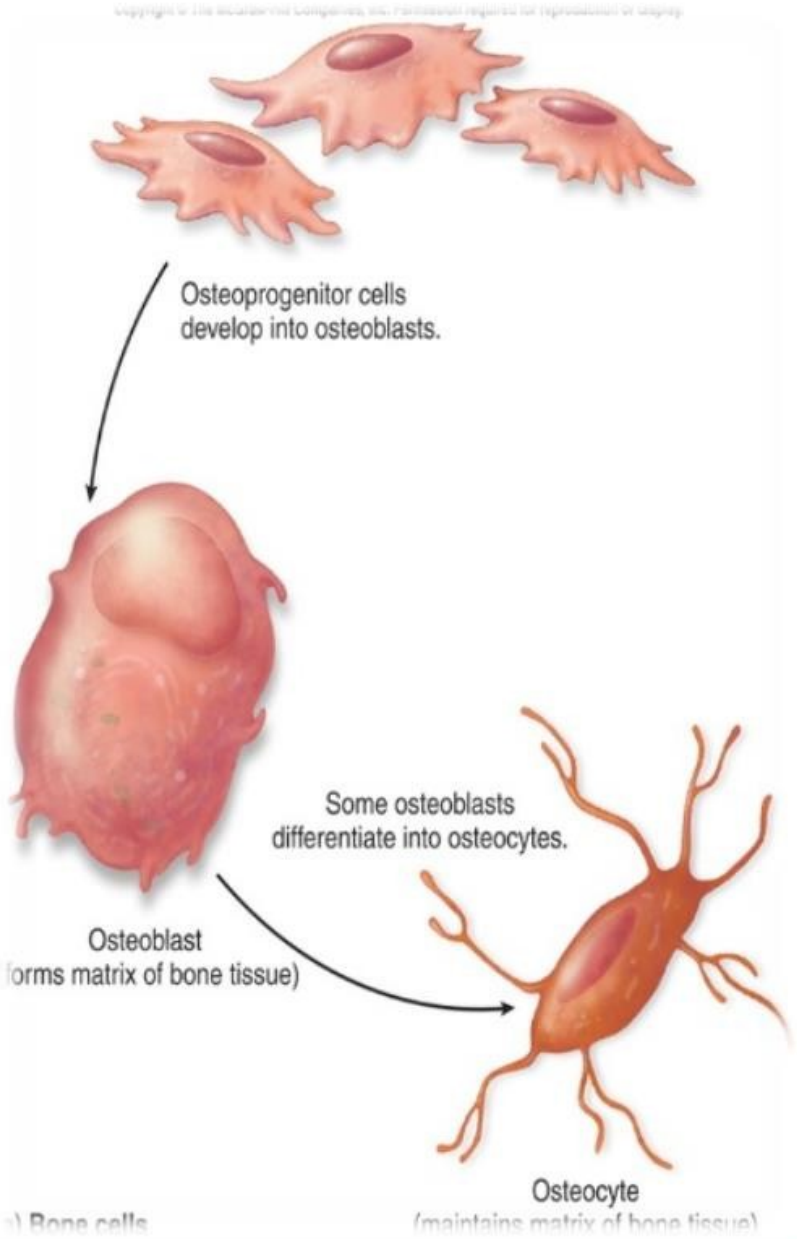
  Small flattened and rounded cells embedded in bone lacunae.

  Derived from mature Osteoblast.

  Function-

  1) Help to maintain the bone as living tissue because of there metabolic activity.

  2) Maintain the exchange of calcium between the bone and ECF.



- **Osteoclast-** Concerned with bone resorption.

Giant phagocytic mutinuecleated cells found in the lacunae of bone matrix.

Derived from hemopoietic stem cells via monocytes.(CFU-M)

Function-

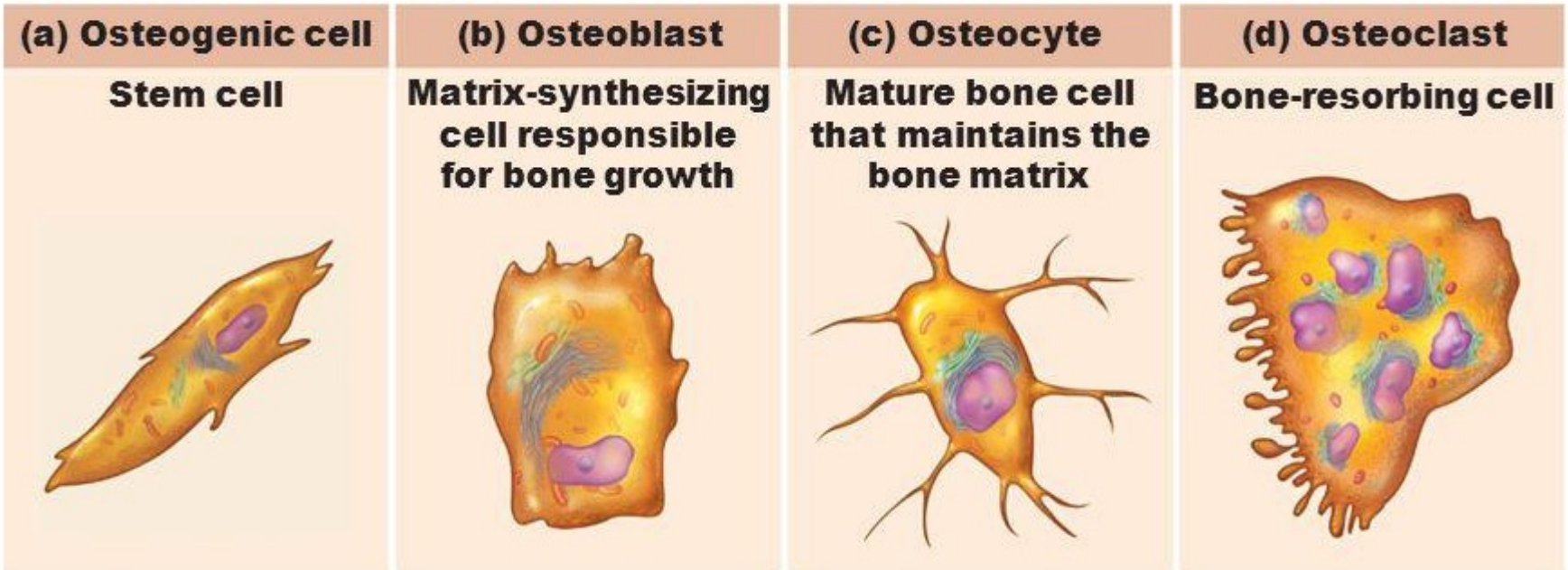
Responsible for bone resorption during bone remodelling.

Synthesis and release of lysosomal enzymes necessary for bone resorption in to bone resorbing compartment.

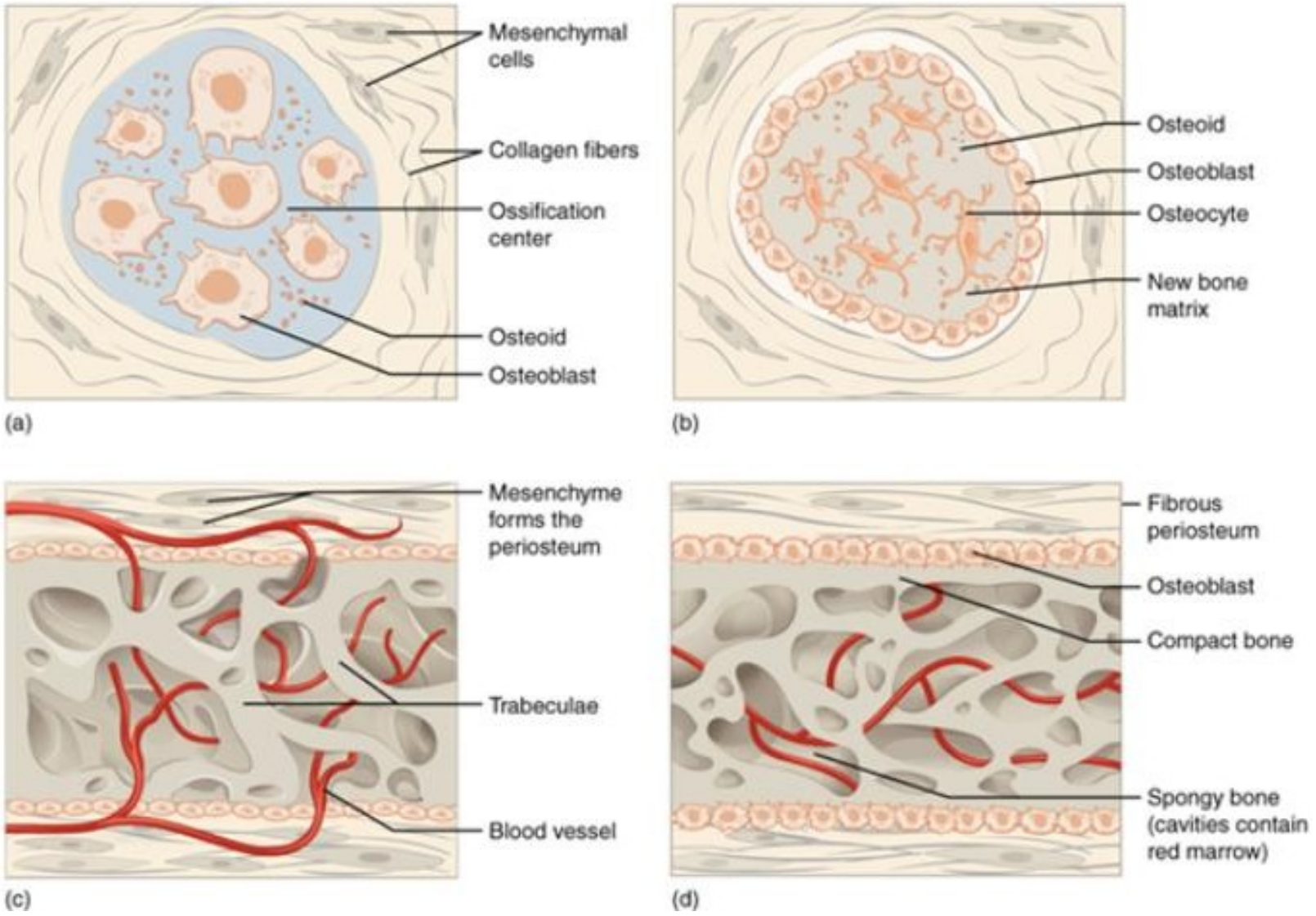
Types of Bone Cells

- Osteogenic Cell: stem cell in the inner layer of the periosteum; that gives rise to osteoblasts
- Osteoblasts: Bone-forming cells
- Osteocytes: Mature bone cells
- Osteoclasts: Bone-destroying cells; Break down bone matrix for remodeling and release of calcium

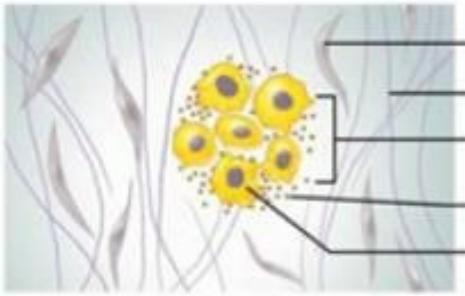
Bone remodeling is a process executed by both osteoblasts and osteoclasts



Intramembranous Ossification

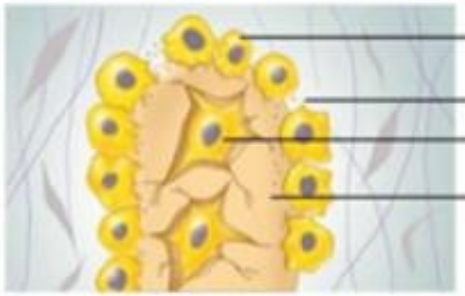


Intramembranous Bone Development



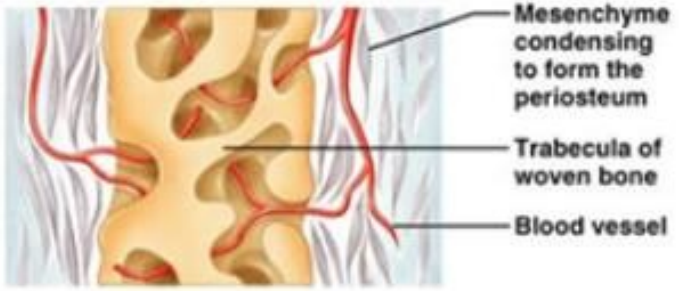
① **An ossification center appears in the fibrous connective tissue membrane.**

- Selected centrally located mesenchymal cells cluster and differentiate into osteoblasts, forming an ossification center.



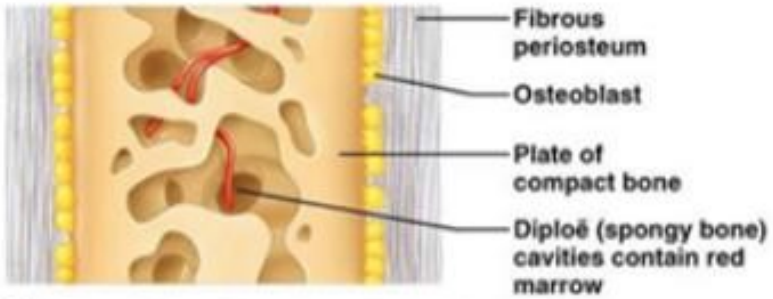
② **Bone matrix (osteoid) is secreted within the fibrous membrane.**

- Osteoblasts begin to secrete osteoid, which is mineralized within a few days.
- Trapped osteoblasts become osteocytes.



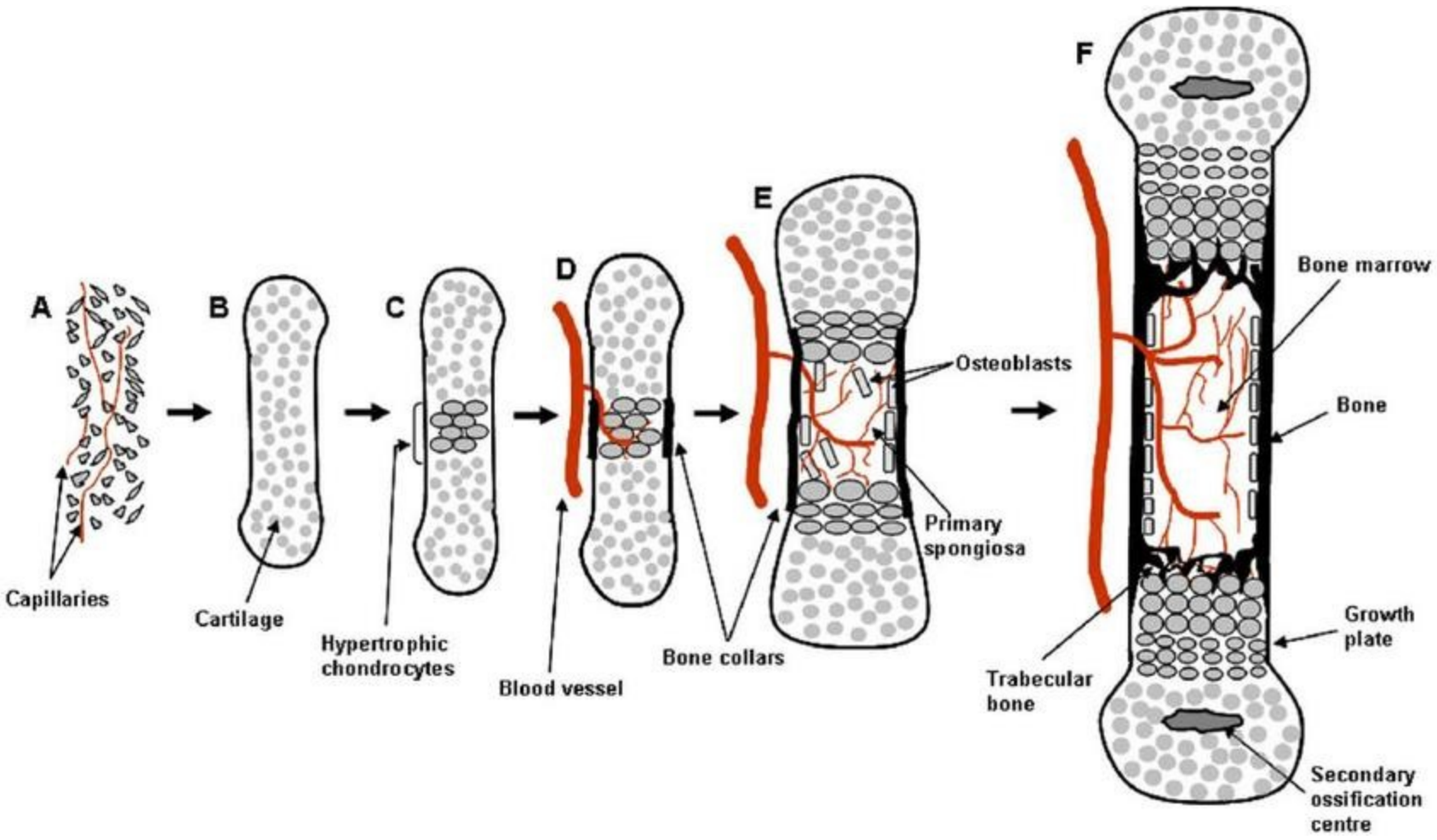
③ **Woven bone and periosteum form.**

- Accumulating osteoid is laid down between embryonic blood vessels, which form a random network. The result is a network (instead of lamellae) of trabeculae.
- Vascularized mesenchyme condenses on the external face of the woven bone and becomes the periosteum.

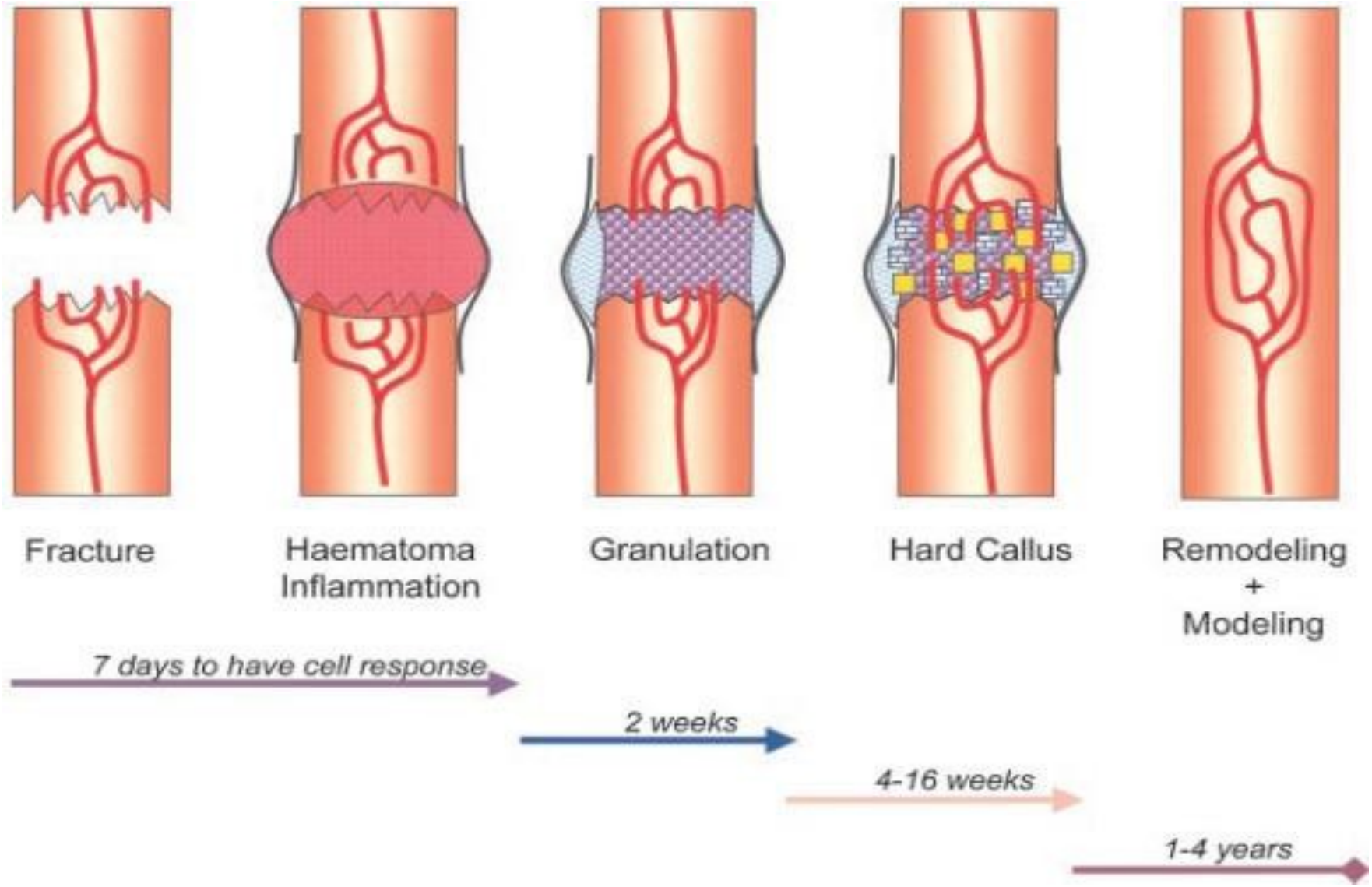


④ **Bone collar of compact bone forms and red marrow appears.**

- Trabeculae just deep to the periosteum thicken, forming a woven bone collar that is later replaced with mature lamellar bone.
- Spongy bone (diploë), consisting of distinct trabeculae, persists internally and its vascular tissue becomes red marrow.


A
B
C
D
E
F
Capillaries
Cartilage
Hypertrophic chondrocytes
Blood vessel
Bone collars
Osteoblasts
Primary spongiosa
Bone marrow
Bone
Growth plate
Trabecular bone
Secondary ossification centre

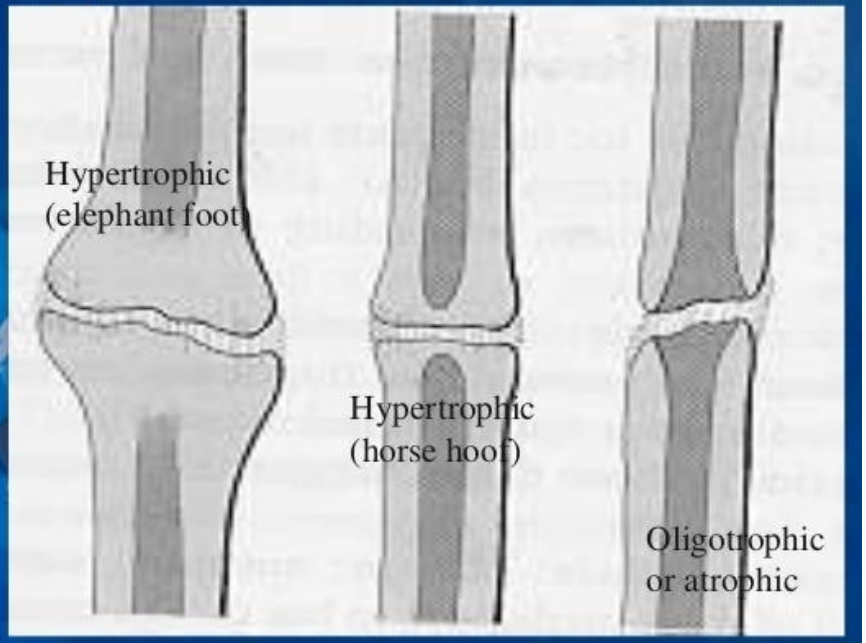
Bone can heal by itself



- Some bone diseases, tumors or bad fractures can cause major bone damage.
- Bone tissue has the ability to renew itself, but it does not fill the large gaps that occur.
- At this situation, **non-union bone formation** is occured and the bone can not heal by itself

Non union of Fractures

Types of Non-union



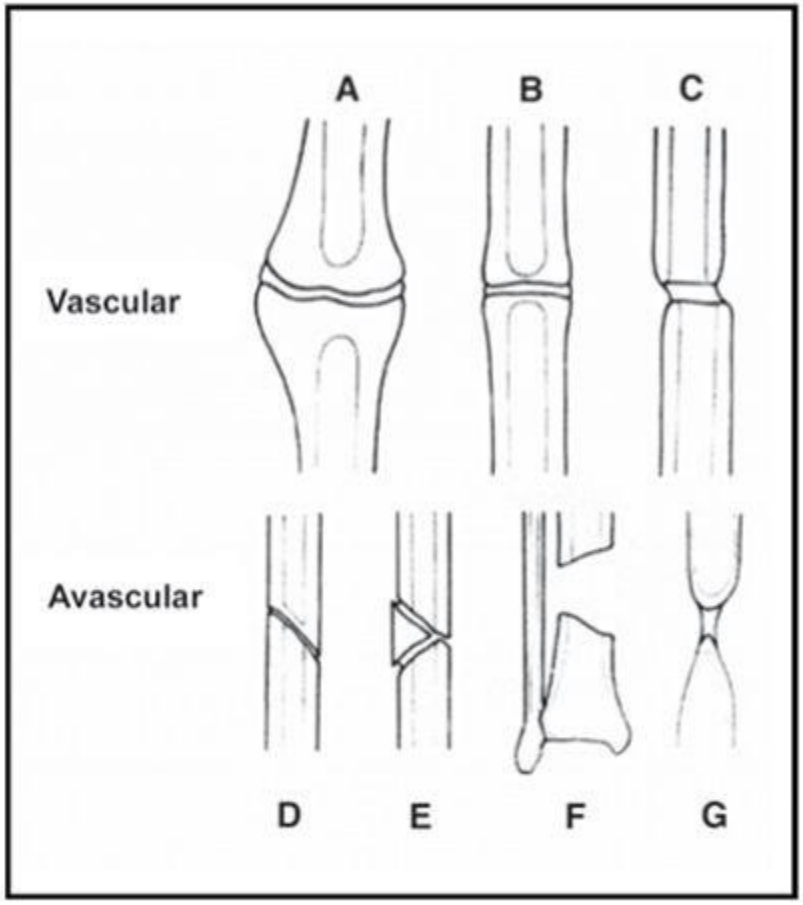
Malunion

Nonunion

Nonunion

Nonunited fractures form two types of pseudoarthrosis:

- **Hypervascular or hypertrophic**
  - With biological reaction capacity
- **Avascular or atrophic**
  - Without biological reaction capacity



Vascular: A = hypertrophic (elephant foot), B = normotrophic (horse foot), C= hypotrophic. Avascular: D = torsion wedge, E = multifragmented, F = bone gap, G= atrophic.

CAUSES OF NON-UNION

Injury

- Distraction at fracture site
- Soft tissue loss
- Bone loss
- Soft tissue interposition

Bone

- Poor blood supply
- Hematoma
- Infection
- Pathological lesion

Surgeon

- Poor splintage
- Poor fixation
- Impatience

Patient

- Age
- Poor medical condition
- Smoking and Alcohol

Drugs

- NSAIDs
- Fluoro-quinolone

Functions of Bone Graft

- osteoconduction
  - provides matrix for bone growth
- osteoinduction
  - growth factors encourage mesencymal cells to differentiate into osteoblastic lineages
- osteogenesis
  - transplanted osteoblasts and periosteal cells directly produce bone
- structural support

BONE GRAFTS

- Autografts
- Allografts

BONE GRAFTS
AUTOGRAFTS

- Enhance osteogenic response
- Have osteoinductive and osteoconductive potential
- Structural support to maintain fracture reduction
- Generally harvested from patient's iliac crest
- Finite quantity available
- Donor-site morbidity

Autografts

- Histocompatible
- Non-immunogenic
- possess the essential components to achieve osteoinduction (i.e., bone morphogenetic proteins (BMPs) and other growth factors), osteogenesis (i.e., osteoprogenitor cells) and osteoconduction (i.e., three-dimensional and porous matrix)

Disadvantages of Autografts

- Autologous bone transplants are very expensive procedures,
- They may result in significant donor site injury and morbidity, deformity, scarring
- They are associated with surgical risks as well: bleeding, inflammation, infection, and chronic pain.

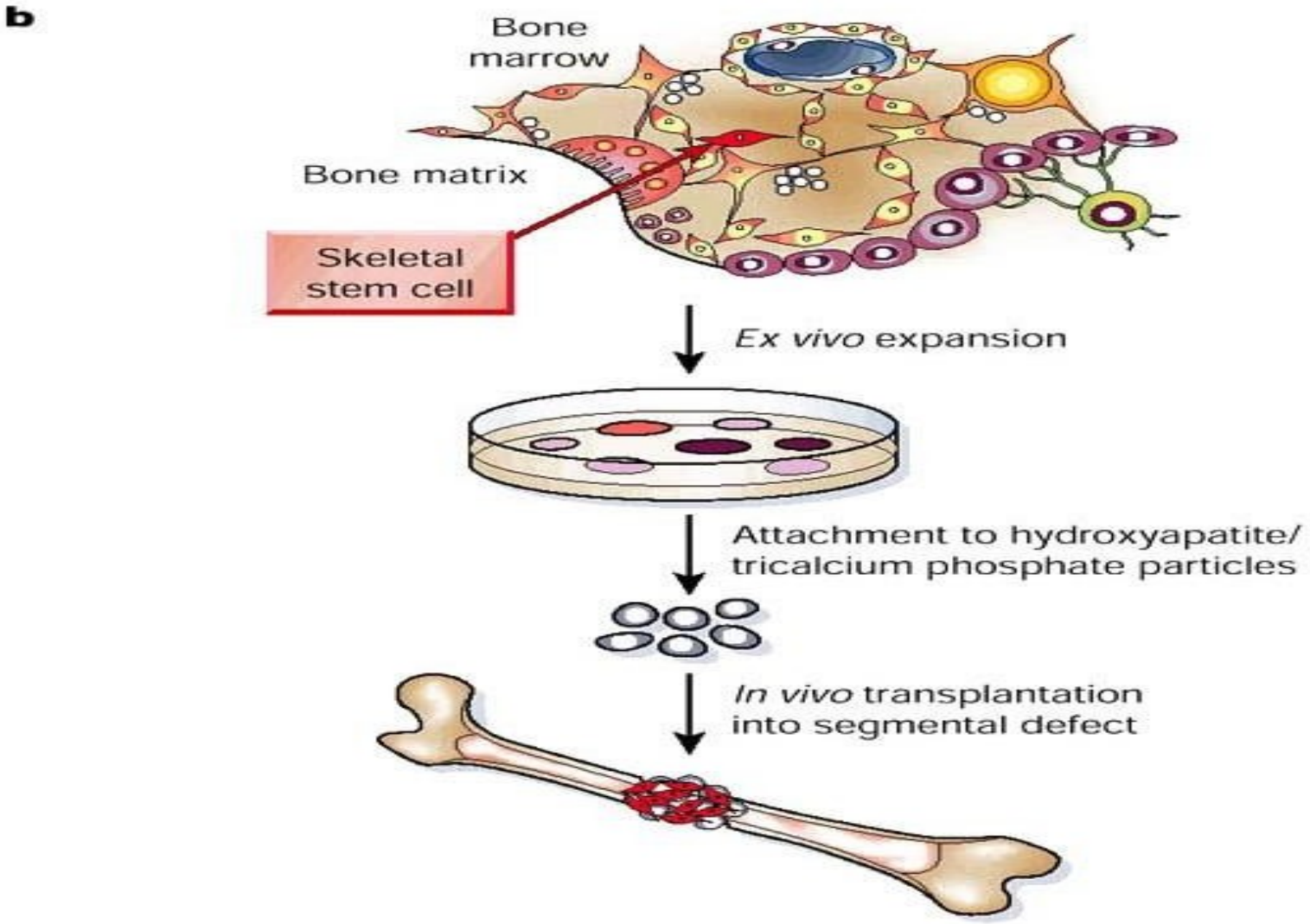
BONE GRAFTS
ALLOGRAFTS

- No osteogenic potential
- Mechanically improve fracture stability
- No donor-site morbidity
- Possible disease transmission

Disadvantages of Allografts

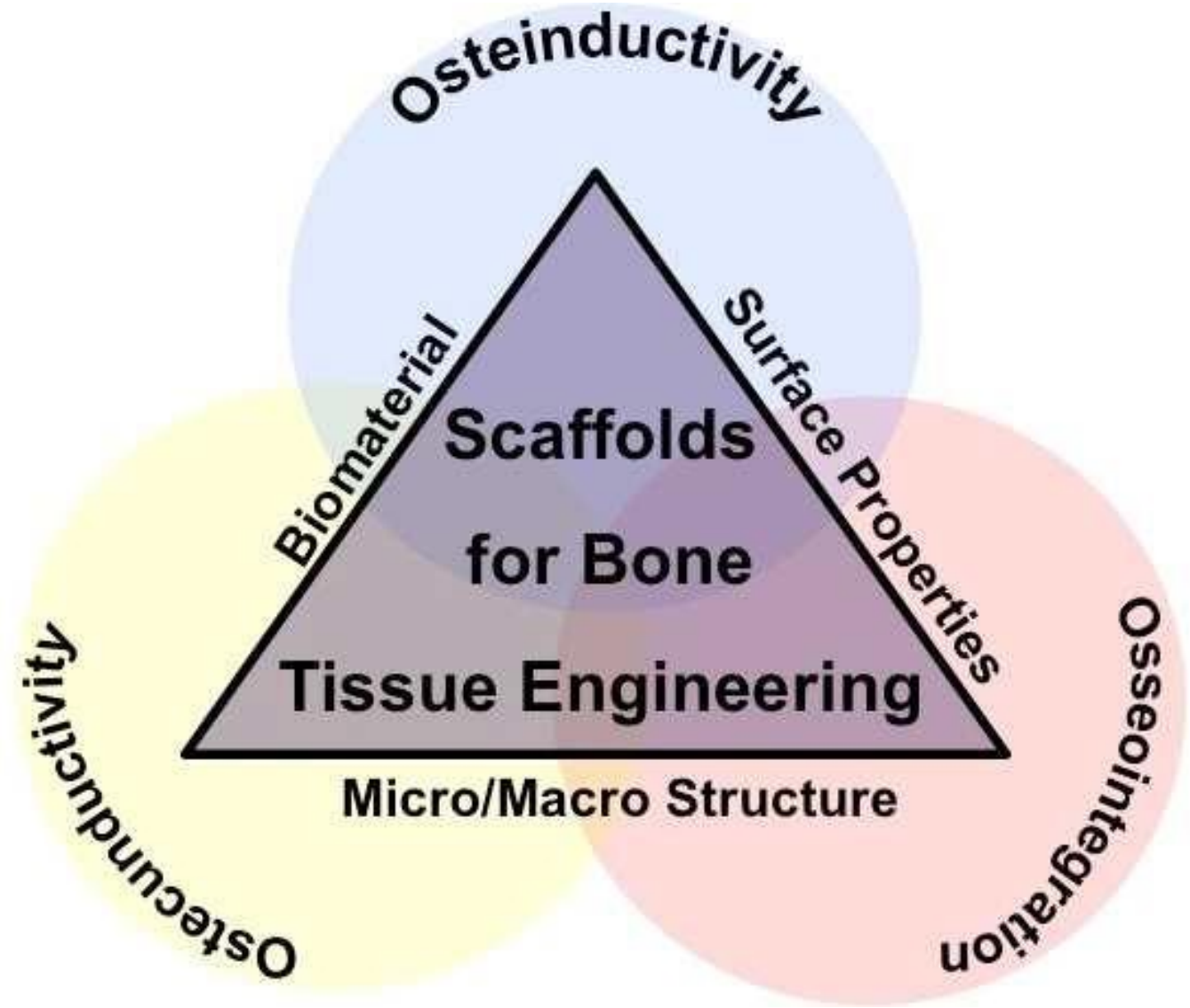
- In comparison to autografts, allografts are associated with risks of immunoreactions and transmission of infections.
- They have reduced osteoinductive properties and no cellular component, because donor grafts are devitalized via irradiation or freeze-drying processing.

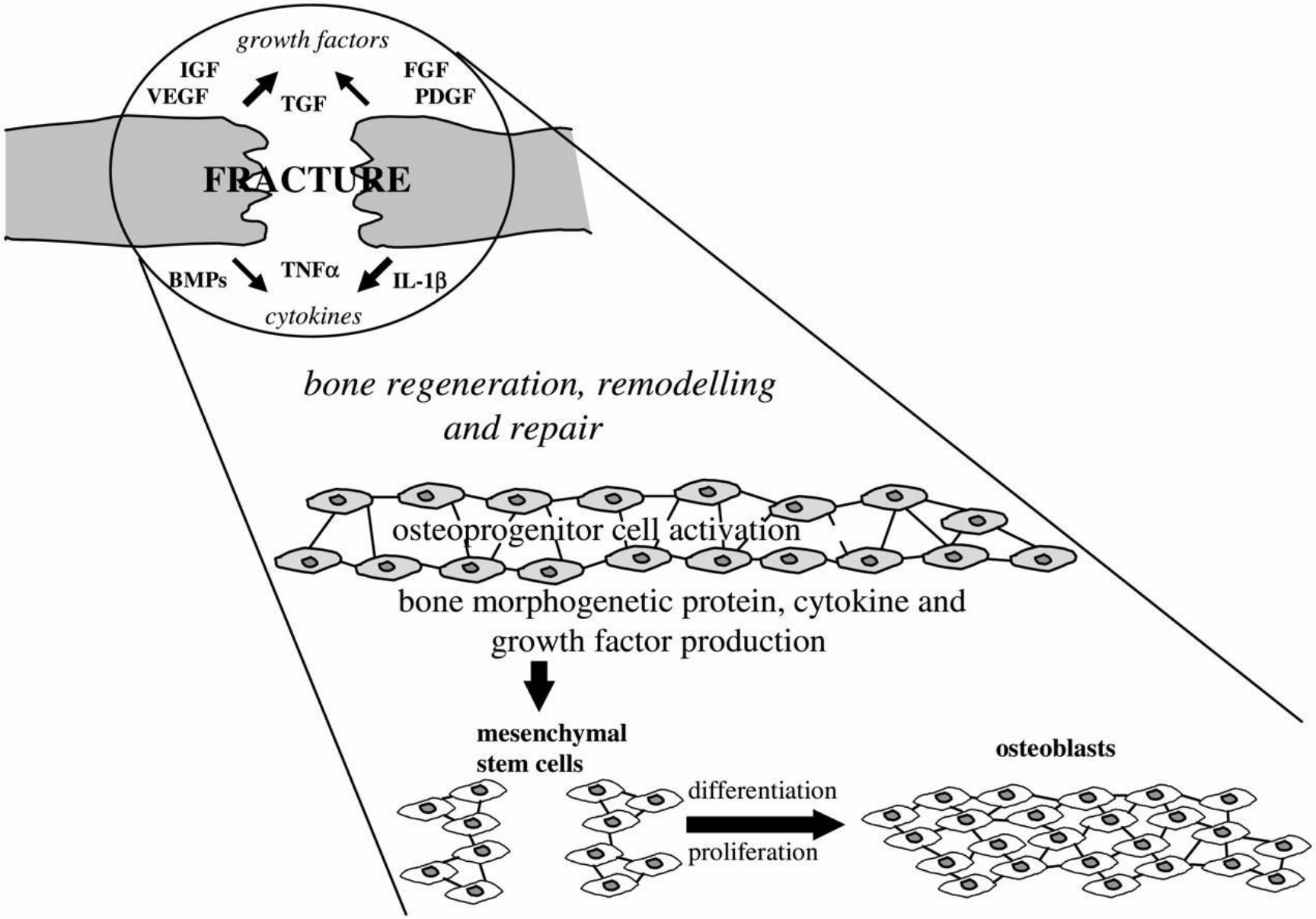
Bone tissue engineering



Bone tissue engineering

- The classic BTE paradigm highlights several key players:
- (1) a biocompatible scaffold that closely mimics the natural bone extracellular matrix niche,
- (2) osteogenic cells to lay down the bone tissue matrix,
- (3) morphogenic signals that help to direct the cells to the phenotypically desirable type, and
- (4) sufficient vascularization to meet the growing tissue nutrient supply and clearance needs.


Osteinductivity
Biomaterial
Surface Properties
Scaffolds
for Bone
Tissue Engineering
Micro/Macro Structure
Osteoconductivity
Osseointegration

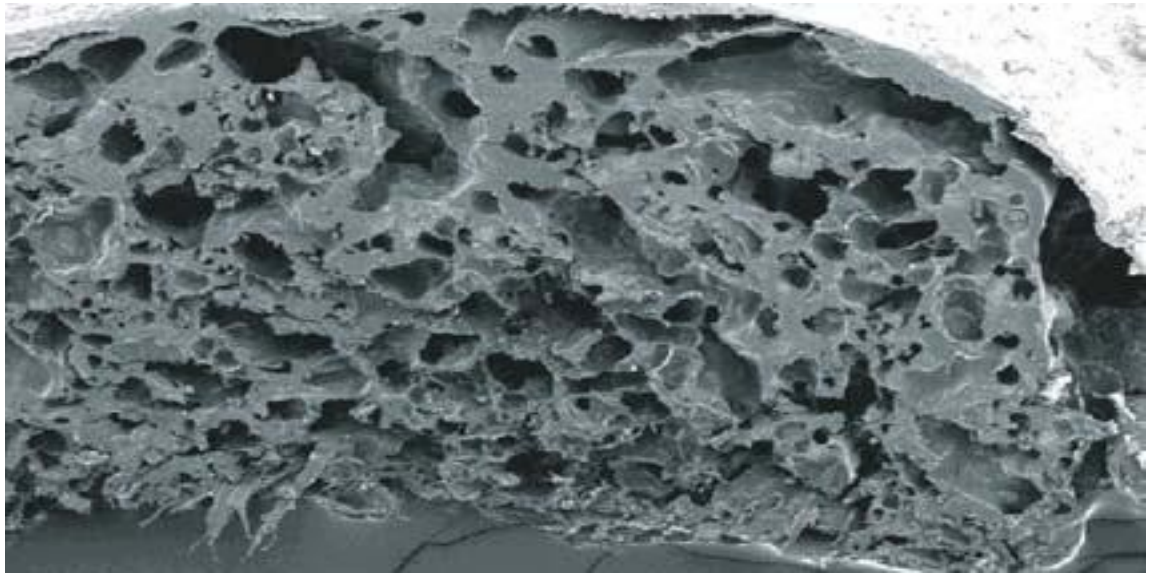

growth factors
IGF
VEGF
TGF
FGF
PDGF
FRACTURE
BMPs
TNFα
IL-1β
cytokines
bone regeneration, remodelling and repair
osteoprogenitor cell activation
bone morphogenetic protein, cytokine and growth factor production
mesenchymal stem cells
differentiation
proliferation
osteoblasts

Ideal Bone Scaffold

- Characteristics
  - Biodegradable
  - Interconnected porosity
  - Biocompatible
  - Handleablity
  - Osteoconductive or osteoinductive
  - Cheap
- Examples
  - Hydroxyapatite
  - PLGA
  - Gelatin
  - Chitosan

- There is roughness on the natural bone surface of about 100 nm in size.

- It is very important to include such nano details on the surface of bone implants commonly used in bone tissue damage



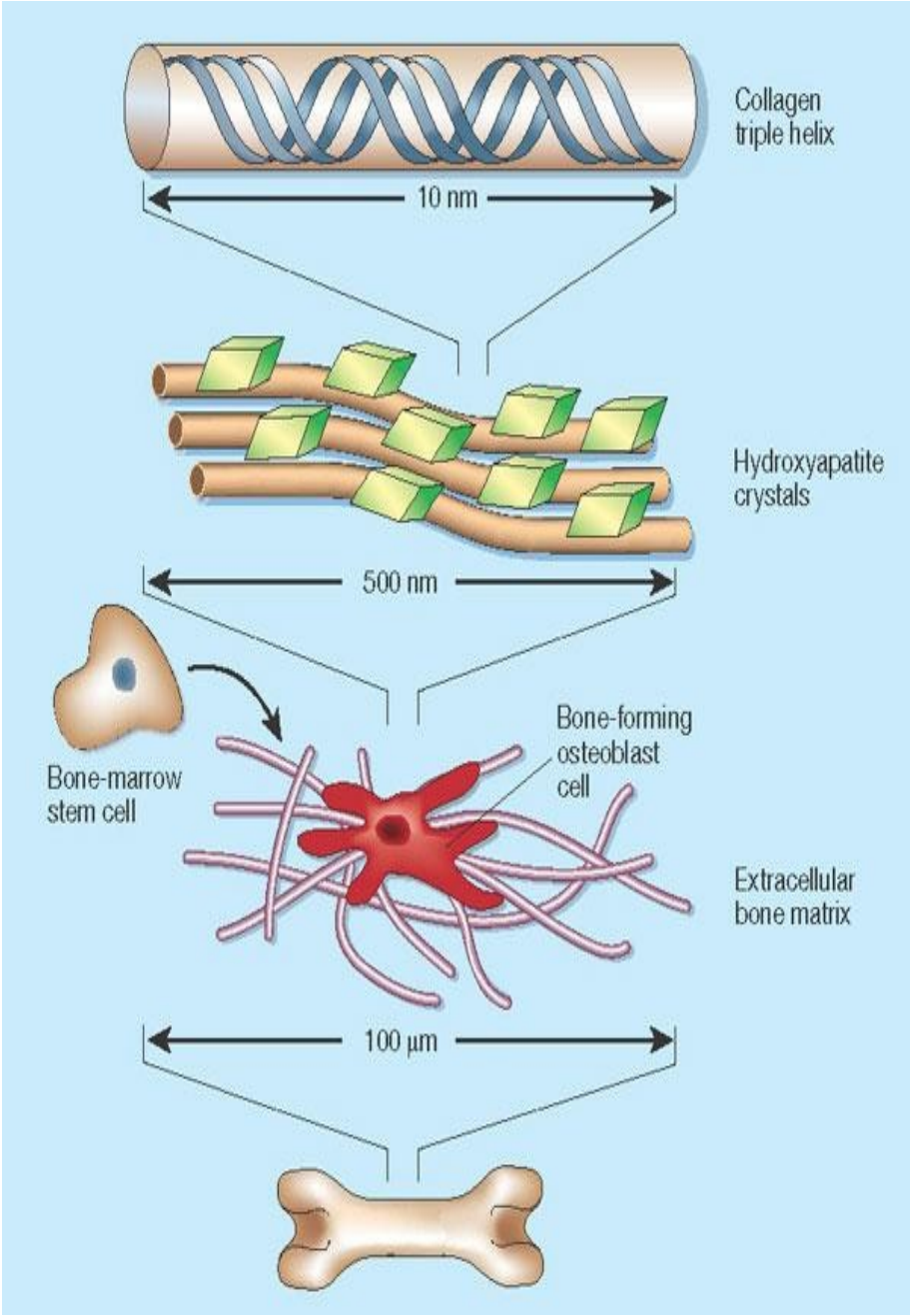
- If the implant surface is smooth, the body will try to reject the implants.
- The smooth surface will trigger the production of threadlike tissue covering the implant surface, which reduces bone implant interaction
- The strength of the implant diminishes and the infection occurs.
- **The nano-sized pieces reduce the risk of rejecting the body's implants and also promote osteoblast production.**

- The use of bioceramics such as **hydroxyapatite (HA)** and **tricalcium phosphate** in bone tissue engineering is the mostly applied technique.

- These structures are similar in chemical and structural terms to the minerals in the bone tissue.

- For this reason, bioceramics stimulate osteoblasts proliferation and osteogenic differentiation

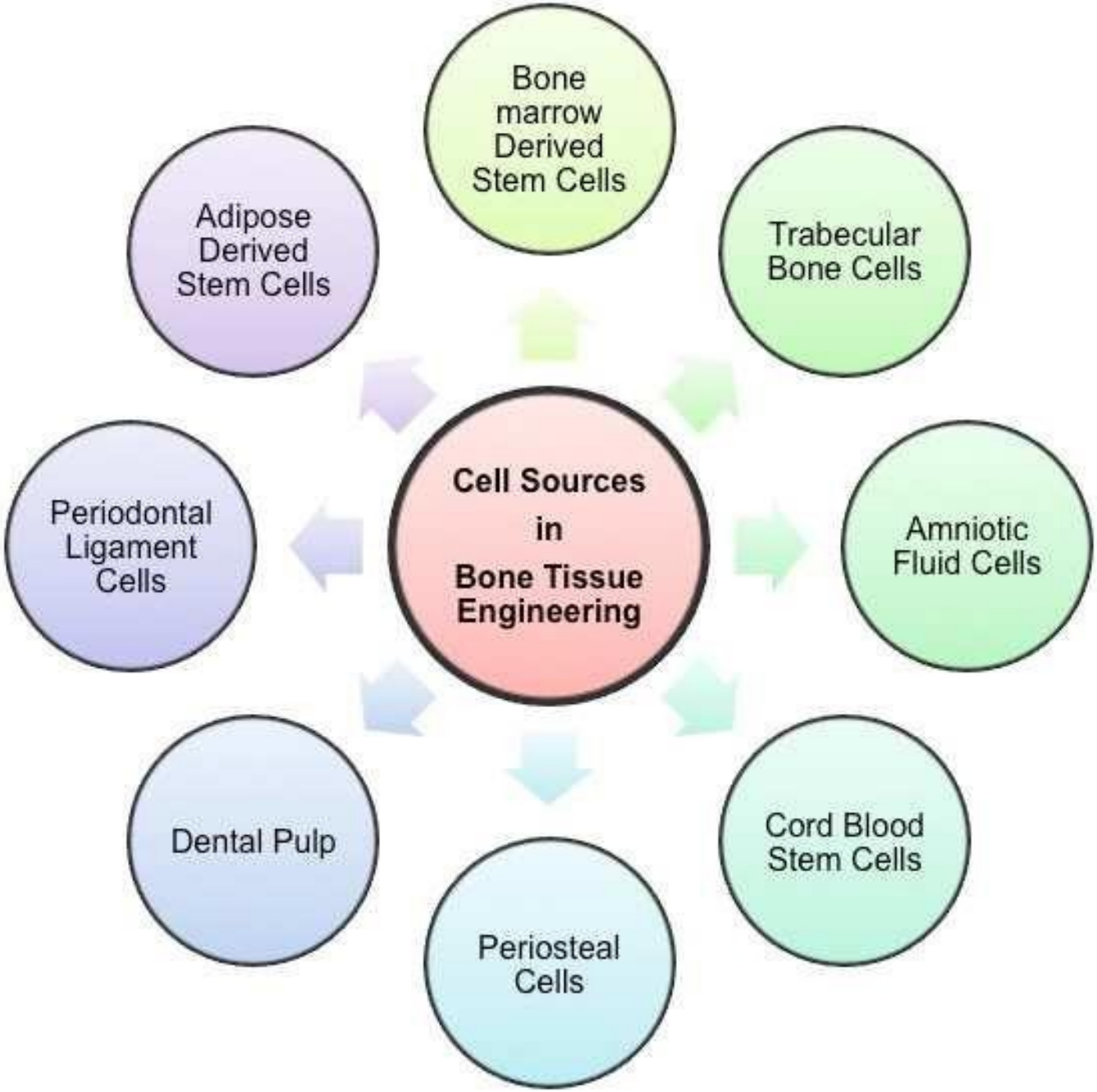
- Studies are underway to combine hydroxyapatite nanoparticles with polymers.
- The biodegradability of the polymer is combined with the osteoconductivity of hydroxyapatite.
- It was determined that the hydroxyapatite nanoparticles incorporated into the polymer scaffold changed the pore structure of the material and made protein absorption more suitable



- Composites of hydroxyapatite and various polymers, including poly(lactic acid) (PLA), PLGA, gelatin, chitosan, and collagen have been successfully fabricated and have demonstrated enhanced bone formation *in vitro* and/or *in vivo*.

Nanoparticles

- In recent years studies have used nanoparticles to increase the mechanical strength of these materials
- The use of nanoparticles allows for better imitation of the environment inside the body, as the organic and inorganic minerals present in the bone tissue are also nano-structured
- These structures, which provide a suitable surface geometry and mechanical strength, support the interaction between the material and the surrounding tissue


Bone marrow Derived Stem Cells
Trabecular Bone Cells
Amniotic Fluid Cells
Cord Blood Stem Cells
Periosteal Cells
Dental Pulp
Periodontal Ligament Cells
Adipose Derived Stem Cells
Cell Sources in Bone Tissue Engineering

Cell Source	Advantages	Disadvantages
Bone marrow-derived mesenchymal stem cells (BM-MSCs)	(i) High osteogenic potential (ii) Studied extensively	Low abundance; requires extensive *in vitro* expansion
Adipose-derived stem cells (ASCs)	(i) Similar osteogenic characteristics as BM-MCSs (ii) Highly abundant; easy to harvest surgically	More studies are needed to test their use in bone repair
Embryonic stem cells (ESCs)	(i) Pluripotency (ii) Capable of differentiating into all cell types in bone	(i) Ethical and regulatory constraints (ii) Produce teratomas when transplanted *in vivo*
Umbilical cord blood mesenchymal stem cells (CB-MSCs)	(i) High availability (ii) Broad differentiation and proliferation potential (iii) Higher *in vivo* safety than embryonic stem cells	(i) More difficult to be isolated than MSCs from the marrow (ii) More studies are needed to test their use in bone repair
Induced pluripotent stem cells (iPSCs)	(i) Pluripotency (ii) Capable of differentiating into all cell types in bone	(i) Reprogramming efficiency is low (ii) Require extensive expansion (iii) Safety concerns; limited clinical application

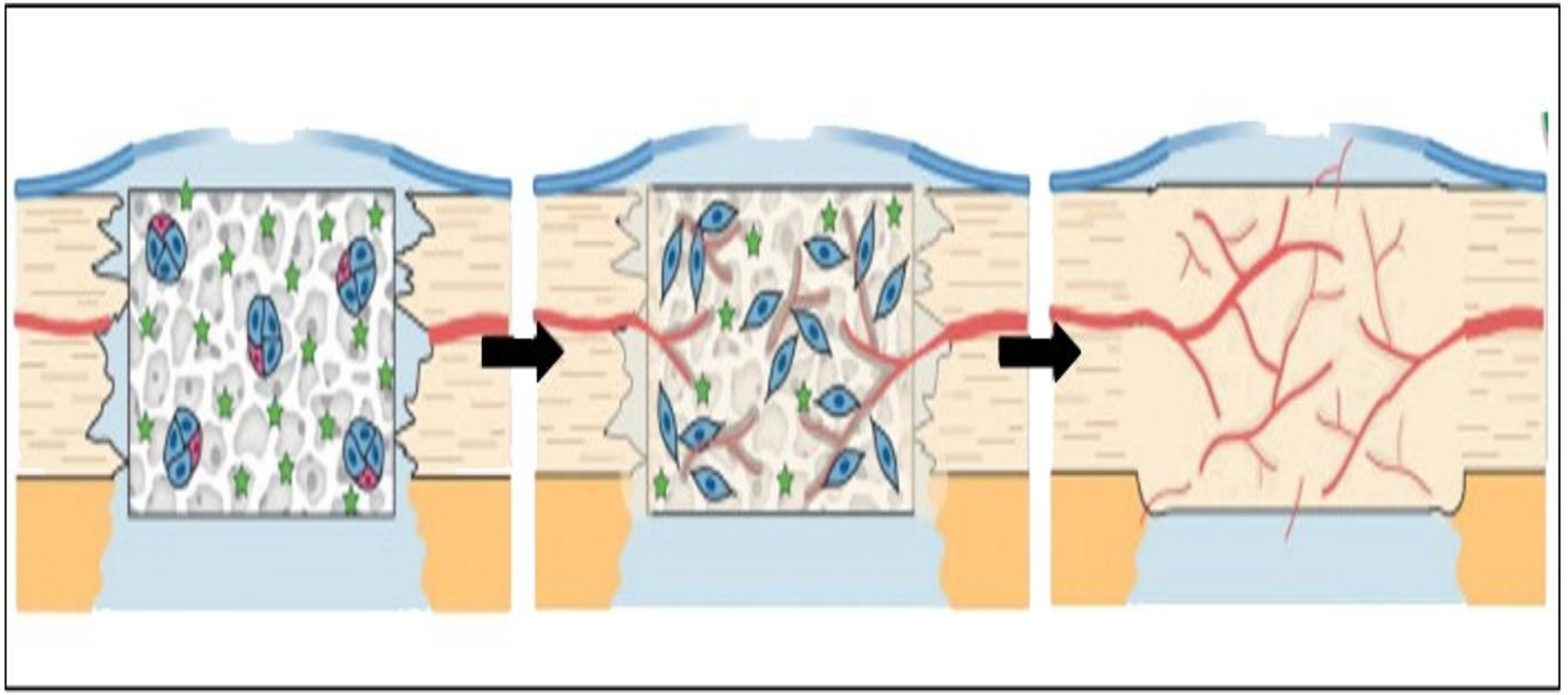
Growth Factors for Bone Tissue Engineering

- Growth factors that play a role in the restructuring of bone tissue

  - BMP (bone morphogenetic protein)
  - TGF (transforming growth factor)
  - PDFG (thrombocyte-derived growth factor)
  - VEGF (vascular endothelial growth factor)

- These growth factors affect osteogenic differentiation by acting alone and together

Orthotopic vascularization



Bone

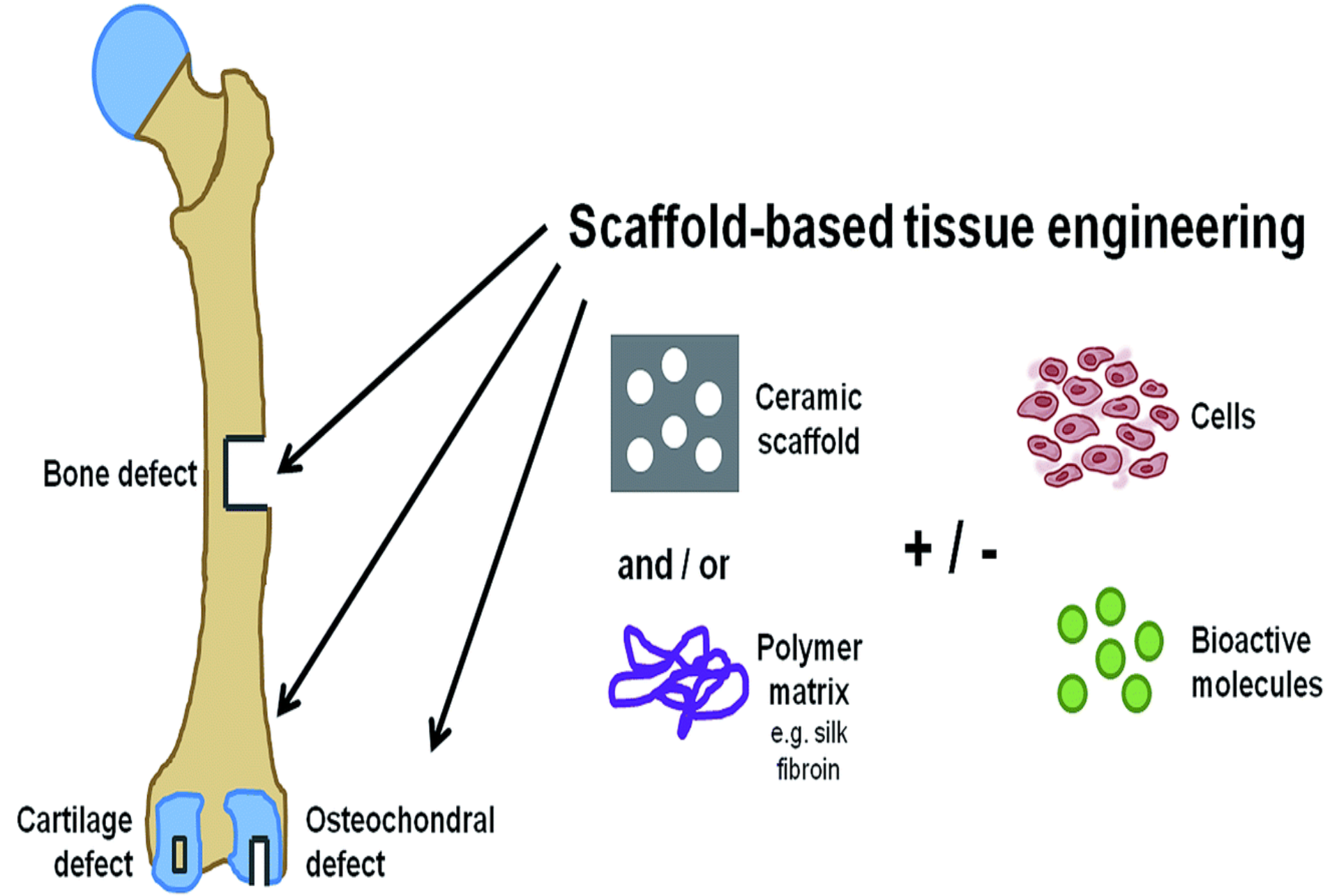
Scaffold

Stem cell

Endothelial cell

Growth factors

Vessel


Scaffold-based tissue engineering
Bone defect
Cartilage defect
Osteochondral defect
Ceramic scaffold
and / or
Polymer matrix
e.g. silk fibroin
+ / -
Cells
Bioactive molecules