THE BIOLOGY OF BONE REGENERATION

Theoretical basis of bone replacement

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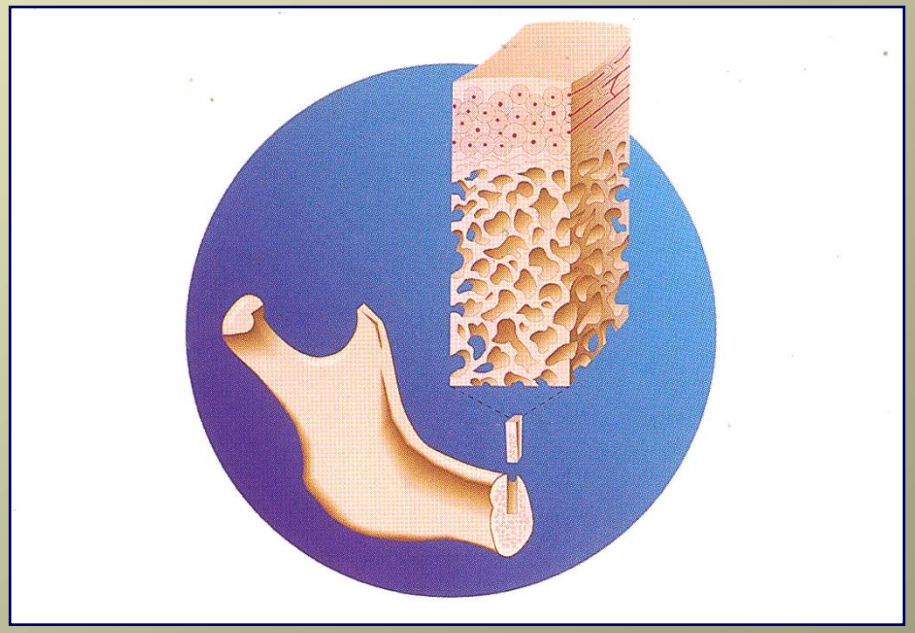
Role of bone tissue

- Maintance of calcium and phosphate homeostasis
- Sceleton
    - Mechanical stability
    - Moving
- Haemopoesis in medulla

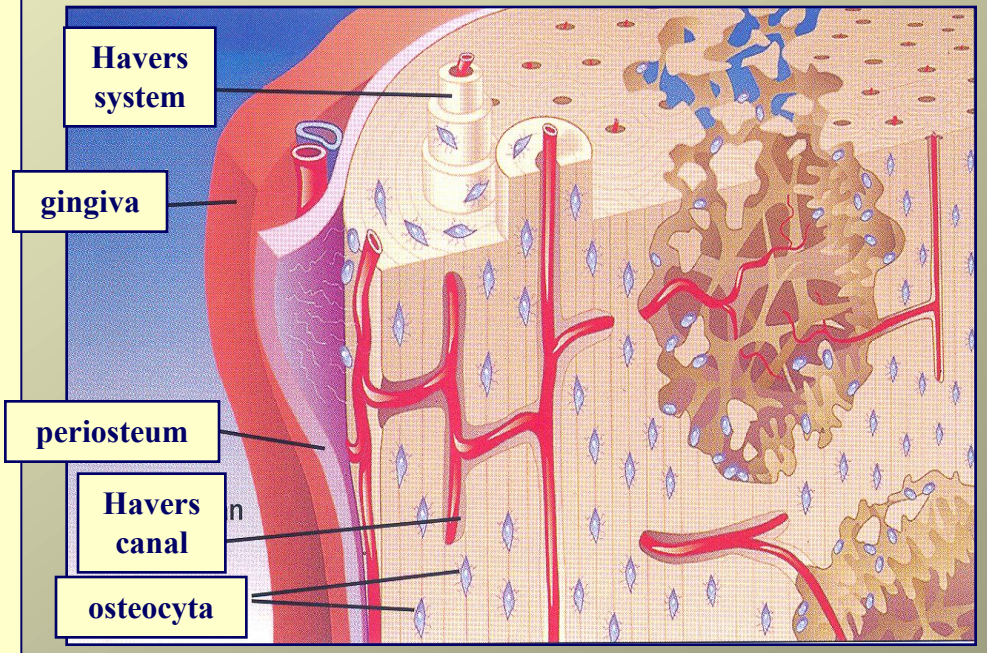
Macroscopic structure of bone tissue

- Cortical part
  - Compact structure
- Spongiosal part
  - Trabecular structure
  - Frame of medulla
- Periosteum
  - Outher fibrotic layer
  - Inner cell containing layer (cambium)
- Endosteum
  - Covers the inner surface of bone
  - Contains osteoblasts and osteoclasts



Microscopic structure of bone

- **Elements of bone:**
  - **Cells:**
    - **Osteoblast**
      - **Periosteal**
      - **endosteal**
    - **osteocyta**
    - **osteoclast**
  - **Extracellular matrix:**
    - **collagen**
    - **hydoxylapatit**
    - **proteoglikanes**
    - **osteocalcin**
    - **osteonectin**



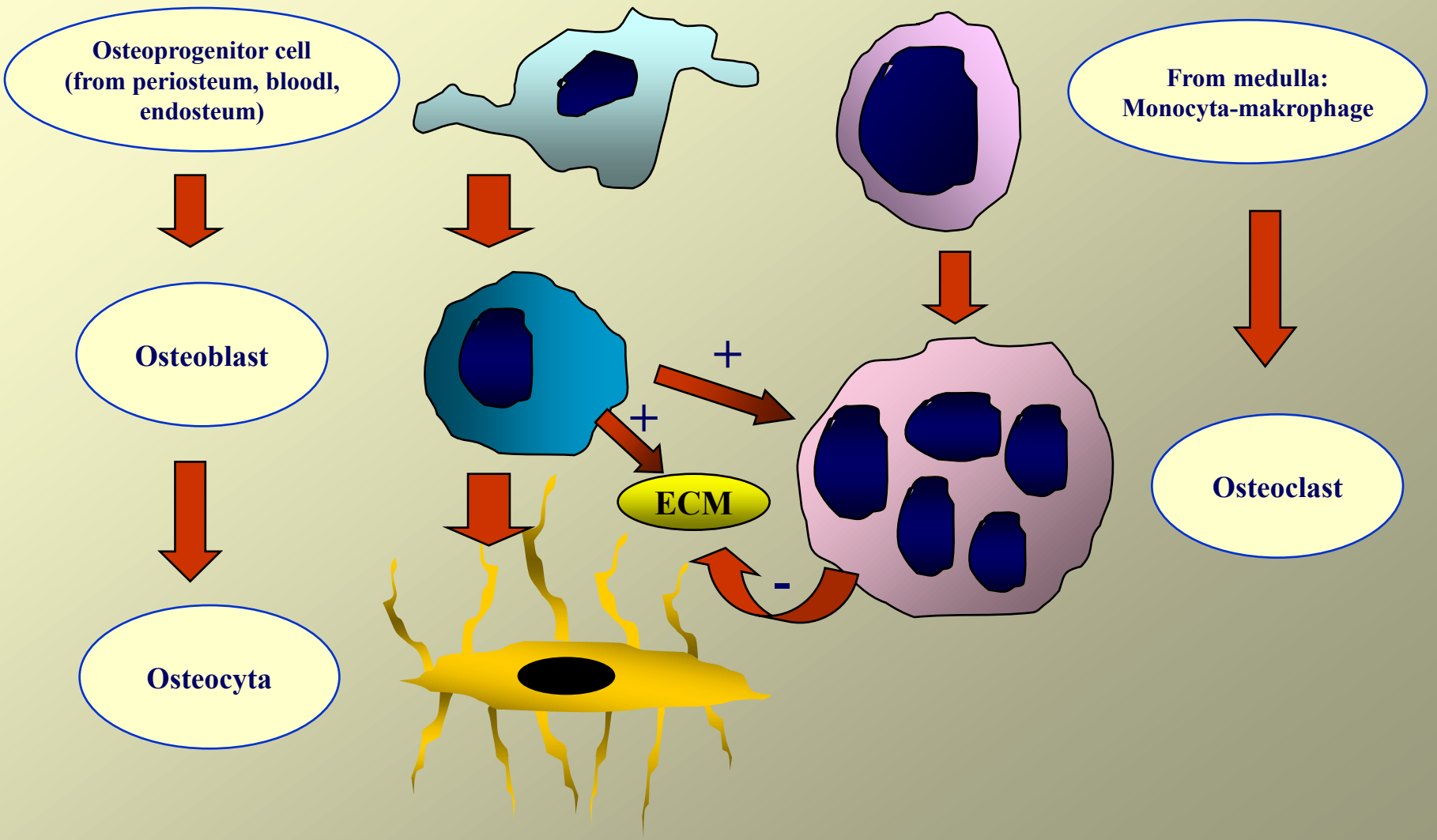
Role of bone cells

- **Osteoblast**
  - **ECM formation (collagen, osteocalcin, osteonectin, proteoglicane secretion)**
  - **ECM mineralization (hydroxylapatit formation)**
  - **Growth factor secretion (TGFβ, BMP, PDGF, TNF, IL-1, IGF)**
  - **osteoclast aktivation**
- **Osteocyta**
  - **Differentiate from osteoblast**
  - **They are copnnected to each other and to periosteal cells with dentritic extensions**
  - **Located concentrically around the Havers canals in the mineralized ECM**
- **Osteoclast**
  - **H^+ ion secretion → pH↓ →hydroxylapatit solution → ECM degradation**
  - **Hydrolitic enzym secretion → ECM degradation**

Types of bone formation

- **Primary (primer angiogen; Krompecher)**
- **Secondary**
  - **Desmogen (bone formation on connecting tissue base, e.g. scull bones)**
  - **Chondrogen (from chondrocytes, e.g. tubular bones)**
  - **Perichondral or periosteal (from inner cellular layer of periosteum)**

Steps of bone neoformation I.



Steps of bone neoformation II.

- I. phase bone
  - Non organized structure
  - Low biomechanical force
  - Low ECM mineralization
- Composite bone
  - Lamellar structure
- II. phase bone
  - High stability
  - Lamellar structure

Mechanism of bone formation

- **REMODELING**

  Based on the connected, sequencial activity of osteoclasts and osteoblasts leading to paralel bone formation and degradation and maintaining the stady stae condition of the bone. Ther is no change in the bone shape and mass normally.

  Regulated by autoregulative effects (paracrin, autocrin faktors)

- **MODELING**

  The process leading to continous change in bone shape and size after stop growing.

  There is independent activity of osteoblasts and osteoclasts from each other leading to separated bone absorption and new bone formation depending on the exogen effects (e.g. orthodontia).

  The process is activated during endosseal implant integration or after bone replacement.

  Regulated by growth factors.

Effect of physical forces on bone formation

- **Observation:**
  Exogen physical forces can influence the shape and size of forming bone.

- **Answer:**
  Exogen force → osteoblast aktivity↑ → calcification↑
  Bone compression → negatív elektric potencial↑ → osteoblast aktivity↑

Hormonal regulation of bone formation (Systemic regulation)

- **PARATHORMON (PTH)** — **serum Ca^{2+} level increases**
  formed in parathyroid gland
  bone absorption↑
  osteoclast activation through osteoblast aktivation
  Ca^{2+} reabsortion from bowels and urine ↑
- **KALCITROL (D_3 vitamin, 1,25 dihydroxy-cholecalciferol)** — **serum Ca^{2+} level increases**
  formed in liver and kidney
  bone absorption↑
  osteoclast activation through osteoblast aktivation
  bone mineralization↑
- **CALCITONIN** — **serum Ca^{2+} level decreases**
  formed in thyroid gland
  bone formation↑
  osteoblast aktivation↓

- **ANDROGENES, ESTROGENEs** — **bone formation↑**
- **GLUCOCORTICOIDS** — **Bone formation↓**

Definition of growth factors (citokines)

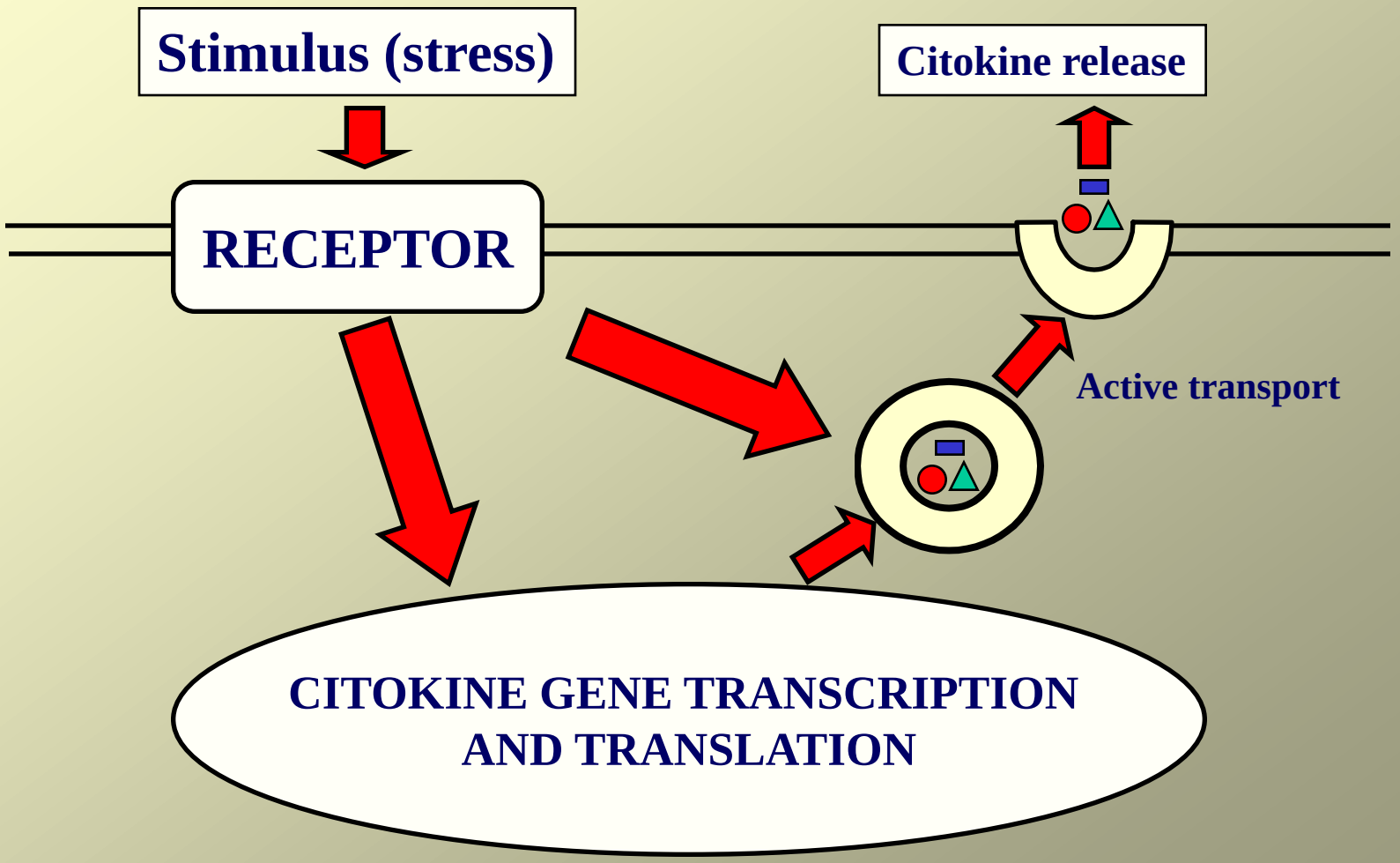
- **Low molecular weight proteins wich play role in the regulation of cellular proliferation-, differentiation and extracellular matrix formation.**

Origin of growth factors

- **Vascular endothel cells**
- **Fibroblasts**
- **Osteoblasts**
- **Macrophages**
- **Cellular elements of blood: <u>thrombocytes</u>**

  red blood cells

Regulation of growth factor formation



Growth factors playing role in bone formation

- Platelet Derived Growth Factor (PDGF-A és B)
- Transforming Growth Factor-beta (TGF-β_1, β_2, β_3)
- Bone Morphogenic Proteins (BMPs)
- Interleukinok
- Fibroblast Growth Factor (FGF)
- Epidermal Growth Factor (EGF)
- Insulin-like Growth Factor (IGF)
- Growth Hormon (GH)
- Angiotensin

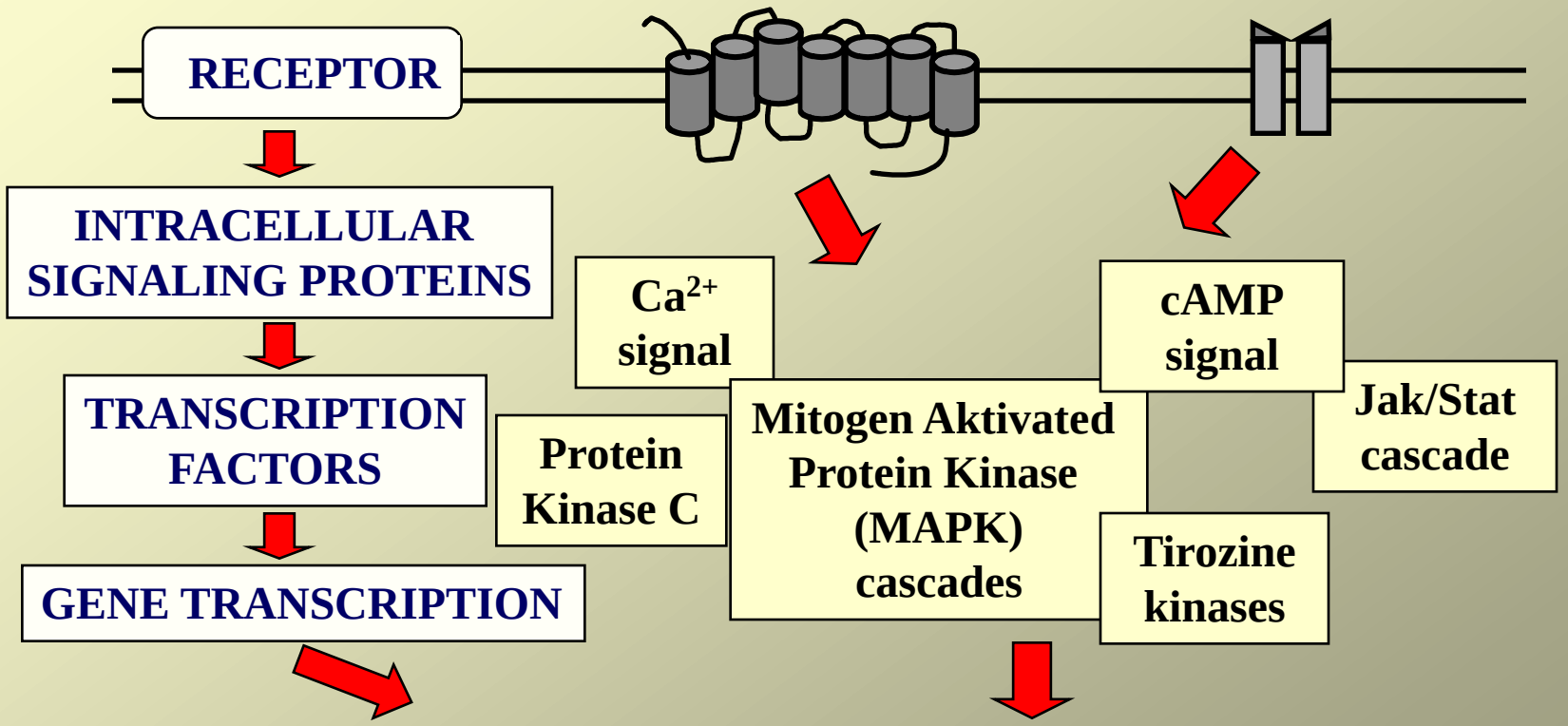
Other factors playing role in bone formation

- **Fibrin**
- **Extracellularis matrix proteins (ECM)**
- **Cell adhesion molecules (e.g. fibronectin, vitronectin)**
- **Integrines**

Effects of growth factors

- **Angiogenesis ↑**
- **Osteoblast activity ↑**
- **Chondroblast activity ↑**
- **Fibroblast activity ↑**
- **Mesenchymal stem cell activity ↑**
- **Extracellular matrix formation ↑**
- **Chemotaxis**
- **Haemopoetic cell formation↑**

Intracellular effects of growth factors



Clinical application of growth factors

- **Oral surgery**
  - Bone replacement (sinus elevation, onlay plasty, cyst loading, activation of skin-, mucosa wound healing)
  - To increase the efficacy of distraction bone prolongation
- **Paradontology**
  - Periodontal surgery
- **Orthopedia, traumatology**
  - To increase the bone fracture healing activity
  - To increase the efficacy of distraction bone prolongation

POSSIBILITIES OF GROWTH FACTOR „FABRICATION”

- **Platelet Rich Plasma (PRP)**
- **Platelet Rich Fibrin (PRF)**
  - Advantage: -cheap
    -autologous
    -includes every factor playing role in wound healing -
  - Disadvantage: -difficult to control the effect of it
    -technical difficulties
- **Gene technology (recombinant technic)**
  - Advantage: -separated „clean” factor production
  - Disadvantage: -expensive
    -there is no interaction between different factors
- **Stem cell therapy**
  - **Autologous**
  - **Heterolog**

ABOUT „PRP”

- **PRP = PLATELET RICH PLASMA**
- **Aim of application:**
  - **Thrombocyta concentratum formation (hematologic application)**
  - **Activation of soft- and bone tissue regeneration**
  - **Activation of tissue sealents (hemostasis)**
  - **Help of graft material internalisation**

THEORETICAL BACKGROUND OF A „PRP” APPLICATION

- **There is local growth factor release from thrombocyta α and dens granuls after activation of them**
- **PRP activates the regeneration of tissues in the first 48 hours through the concentrated growth factors released from thrombocytes**

METHODS OF „PRP” PREPARATION

- **PLASMAPHERESIS**
- **VERSATIL CENTRIFUGATION METHOD**
- **DOUBLE CENTRIFUGATION METHOD**

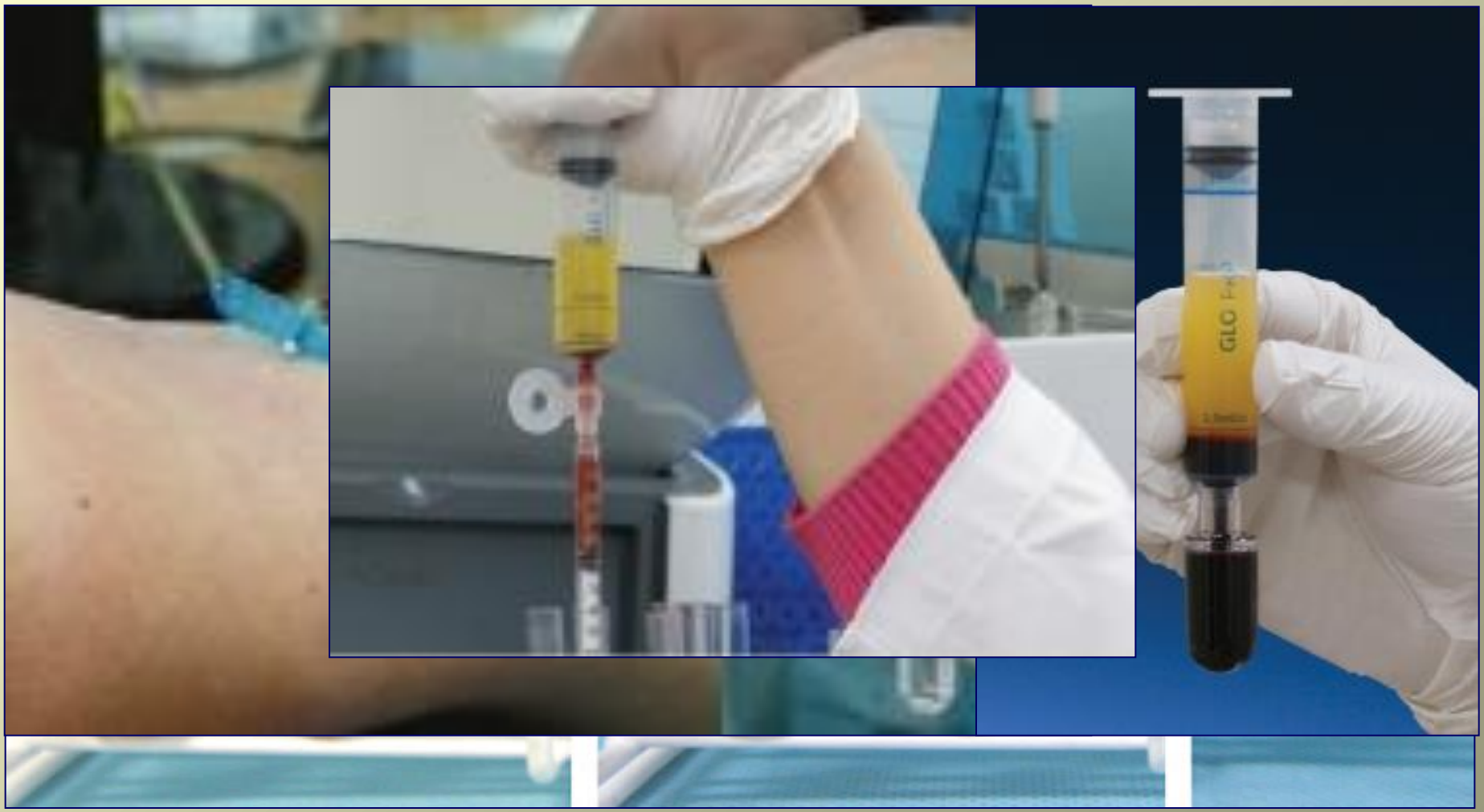
FORMS OF „PRP” APPLICATION

- **Gel (membrane) after addition of thrombin + Ca^{2+} or fibrin net**
  (=PRP-gel)

  PRF appli9cation is in this form
- **Plasma concentrate**

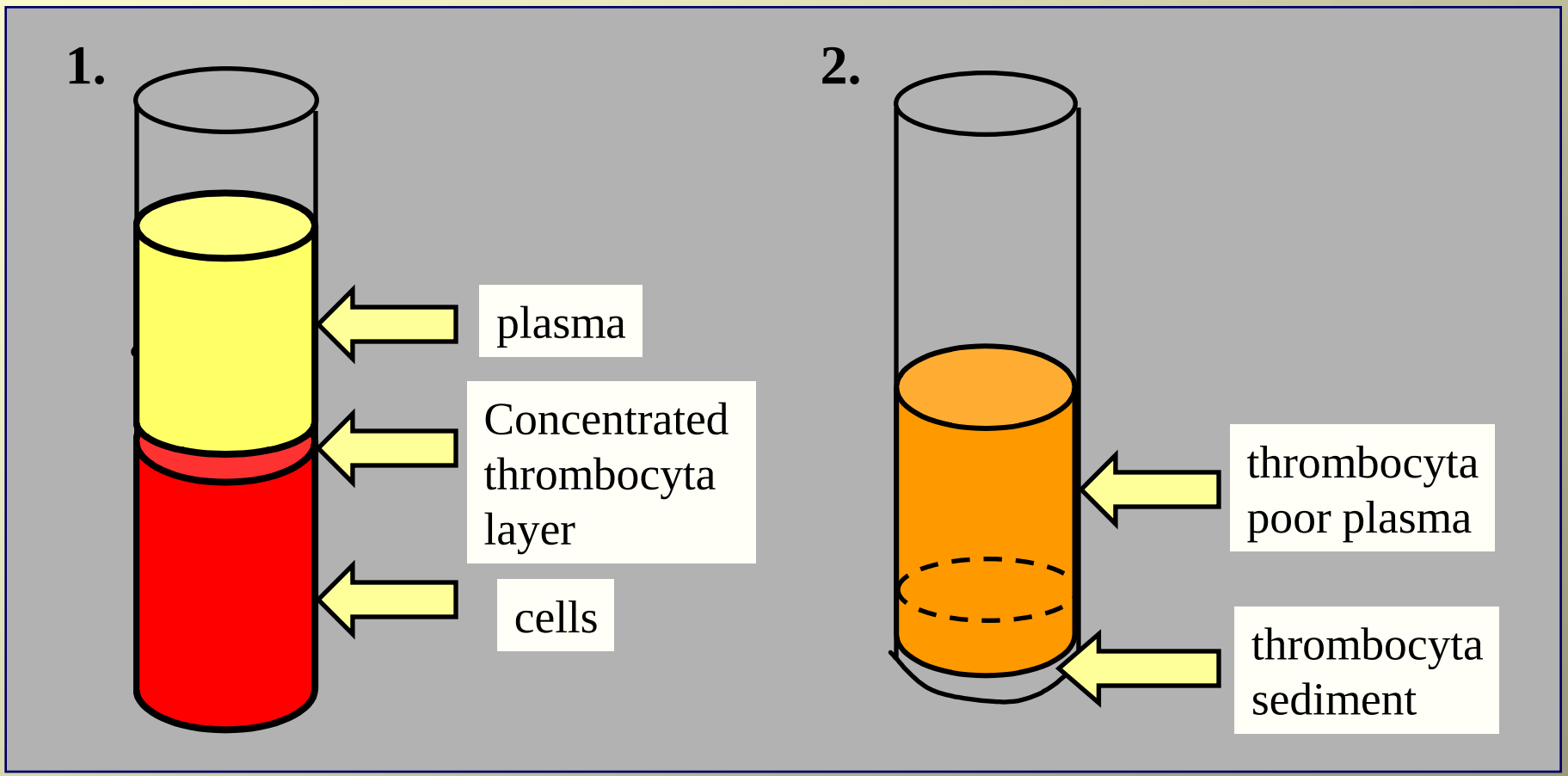
  (=PRP)

A „PRP” PREPARATION WITH DOUBLE CENTRIFUGATION



EQUIPMENTS

DOUBLE CENTRIFUGATION METHOD



INSTRUMENTS

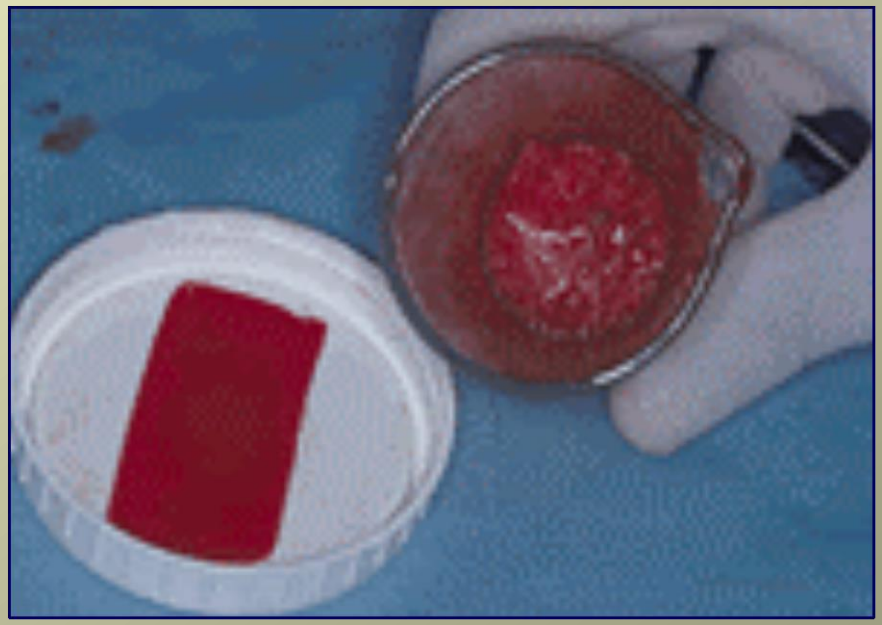
VERSATIL CENTRIFUGATION METHOD

thrombocyta poor plasma

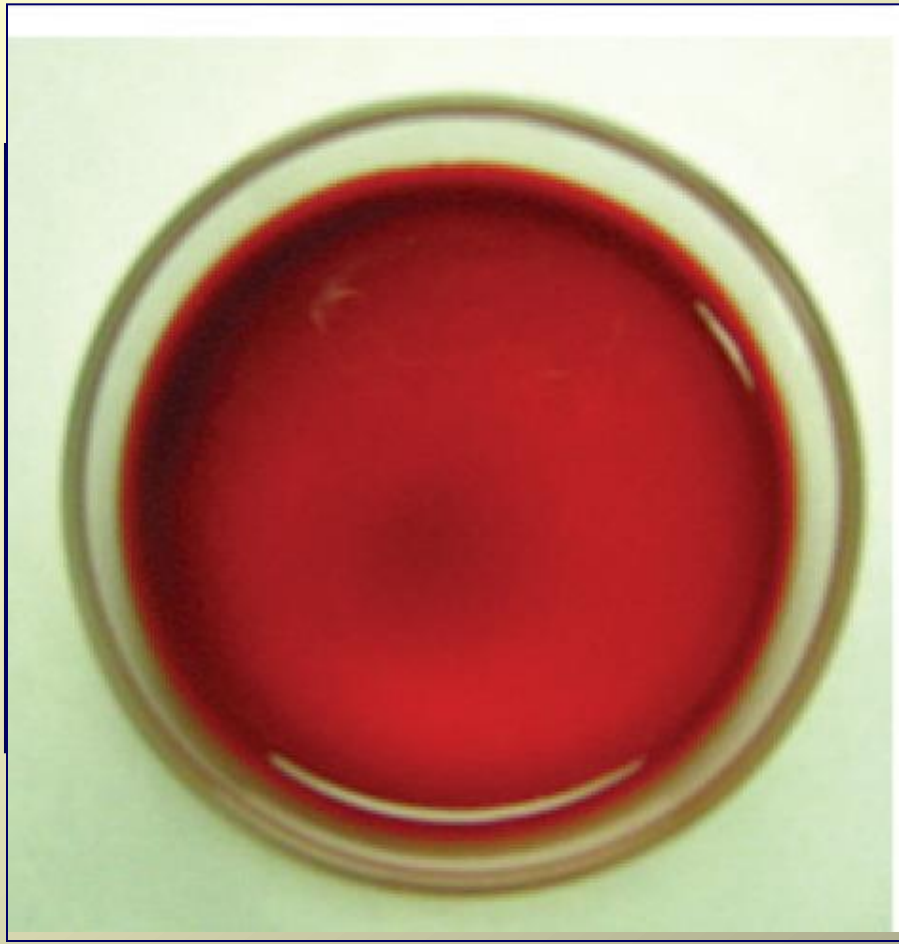
thrombocyta pellet

cells

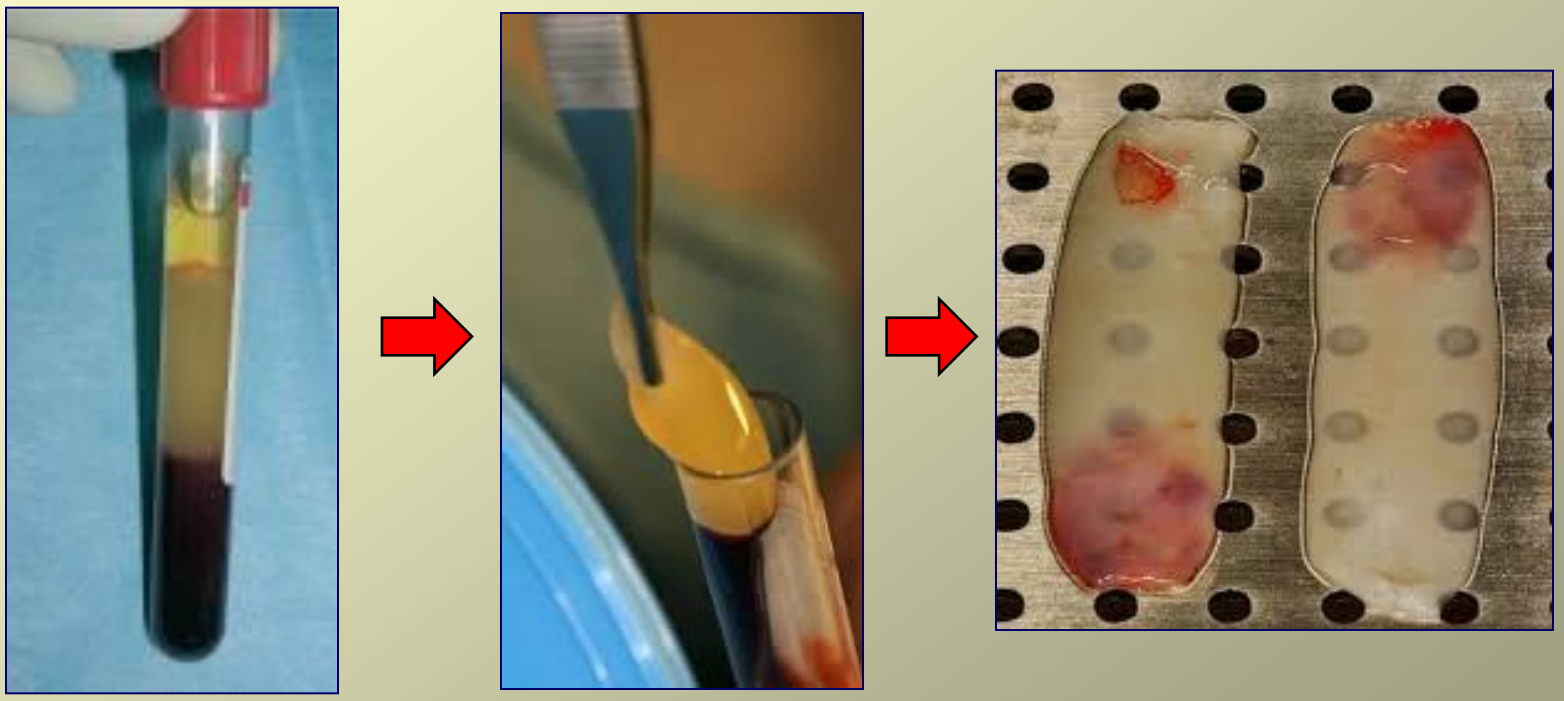
PRP GEL



„PRP-GEL”



PRF PREPARATION



SUMMARY

- **PRP contains 4-12x more thrombocyte then the normal plasma (Goal:>1,000,000/ìl)**
- **Histologic, histomorphometric tests prove that rate of new bone formation in case of bone replacement with PRP is higher with 50-70% then without PRP in the first 6 months.**

„TISSUE ENGINEERING”

- **Definition:**

  Methods using biologic mediator or biomaterial to regenerate the tissues of the body. The tissue regeneration can occur in vitro (in laboratory) or in vivo (e.g. absorbable biomaterials).

- **Aim:**

  The reconstruction of original biological conditions instead of secondary healing. The regenerated tissues should have the same biological and physiological quality like the original tissues.

THE FUTURE

- **Formation of new complex matrix materials (composite grafts) with optimal properties for bone replacement:**
- **There are osteogenic cells on their surface**
- **There is bioactive material release after activation from the matrix**
- **3 dimensional structure of the matrix should be ideal for osteoconduction a**
- **Mechanical properties of the matrix should be compatible to the surrounding tissues**
- **Biocompatibility**
- **The matrix should be absorbed during the new bone formation**

ROLE OF GENE THERAPY IN BONE REGENERATION

- Definition

  Insertion (transfection) of functional gene into the host organism instead of abnormal or missing genes or to produce proteins helping the regeneration process.

- Classification of gene therapies

  1) somatic cell gene therapy

  2) germinative cell gene therapy

ROLE OF GENE THERAPY IN BONE REPLACEMENT

- **Implantation of modified autologous cells producing growth factors playing role in bone neoformation (pl. BMP, PDGF, IGF, FGF)**
- **Administration of gene bound to vector in to the bone defect**
- **<u>GAM = gene activated matrix method:</u> Application of matrix material containing gene modified cell or gene bound to vector on the surface. The matrix can be collagen, autologous bone, allogen bone, polylactate etc.**
- **The safe and effective application of gene therapy will revolutionase bone replacement therapy of diseases with bone loss.**