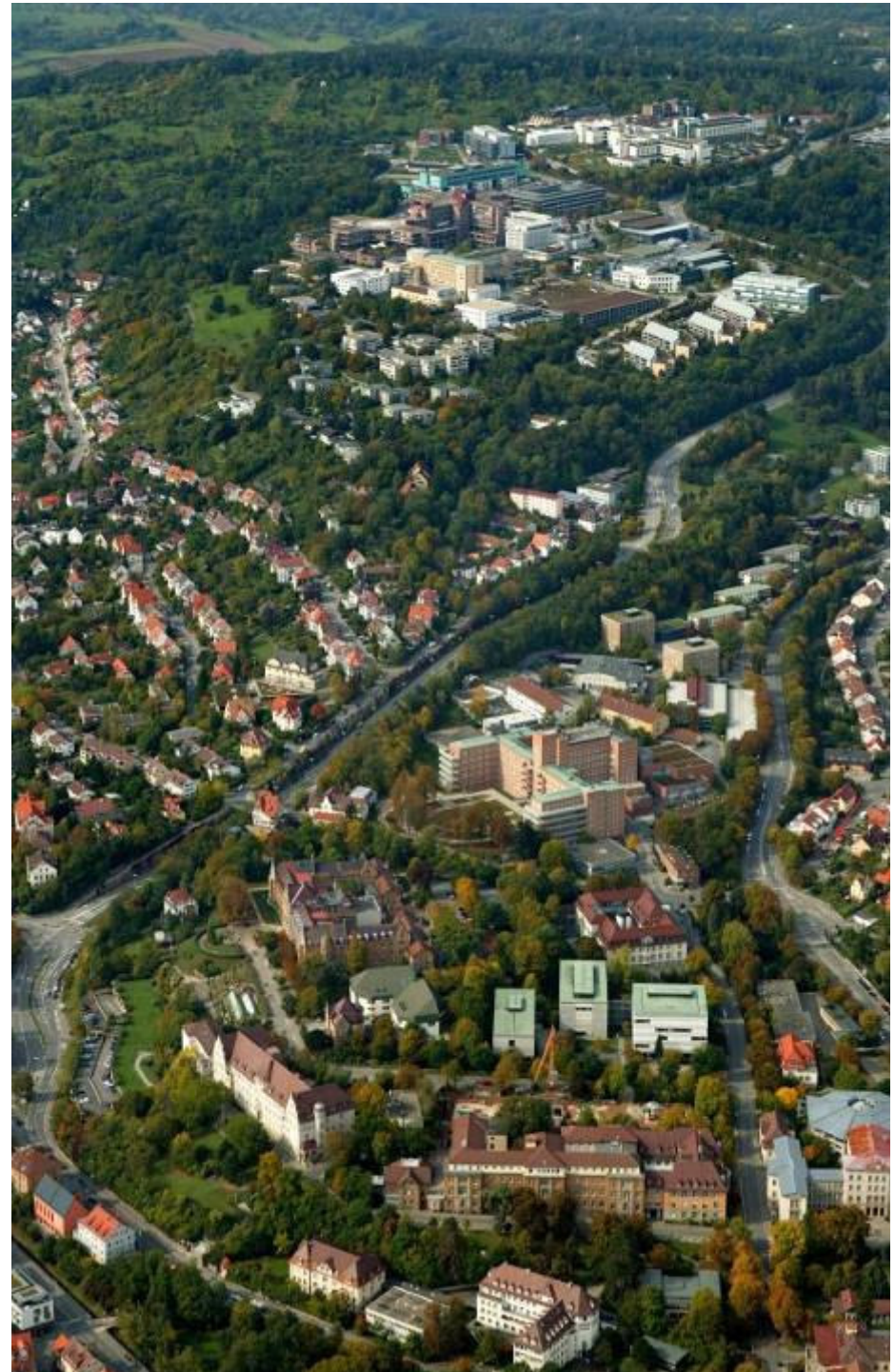


Bone tissue engineering / areas of application for mesenchymal stem cells

Prof. Dr. D. Alexander-Friedrich

Department of Oral and Maxillofacial Surgery
University Hospital Tübingen

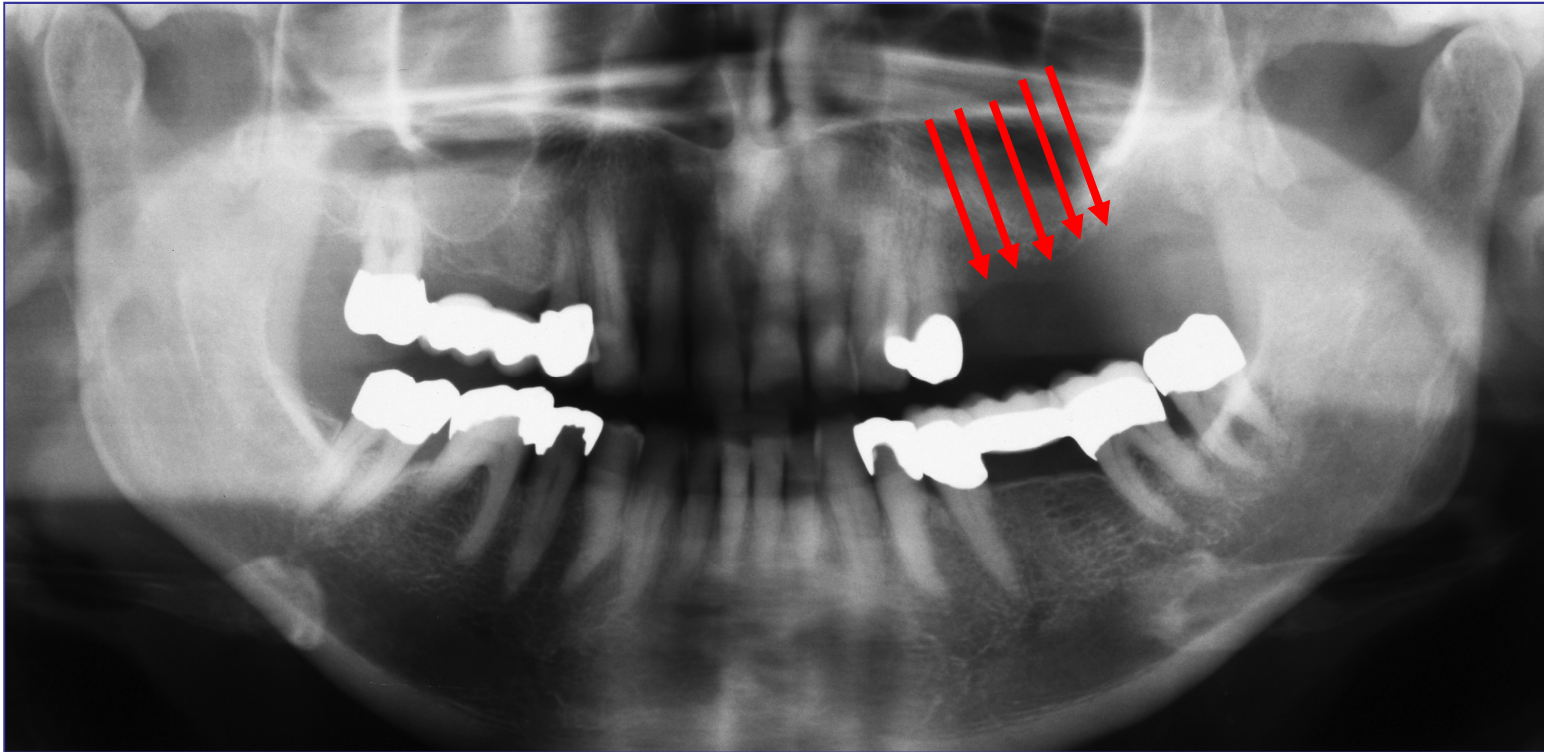


Indication I



Toothless jaws – edentulism

Indication I



Jaw bone atrophies

Indication II



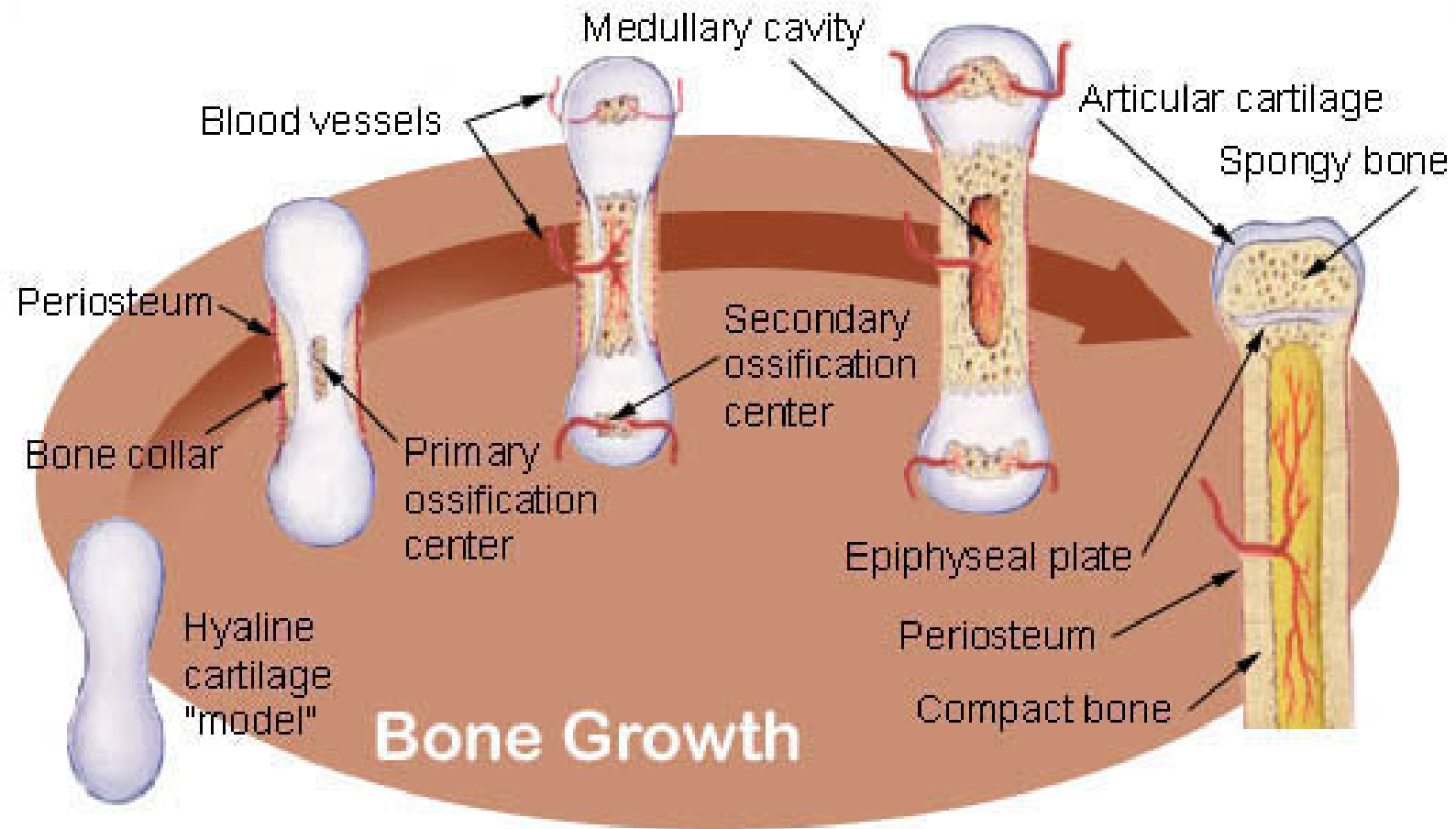
Carcinoma of the oral cavity

Indication III



Cleft lip and/or palate

Bone growth



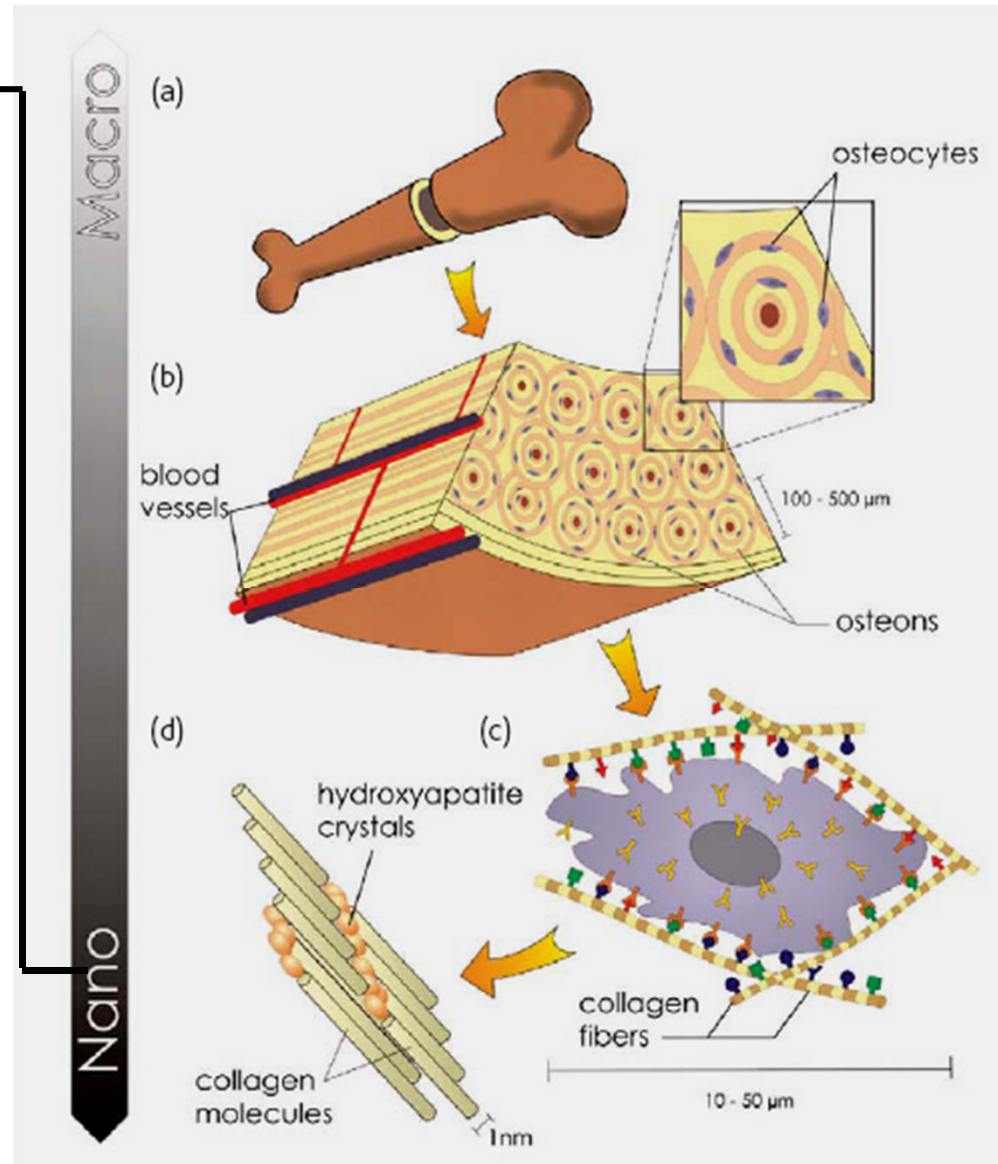
Hierarchical organization of bone

Bone ECM

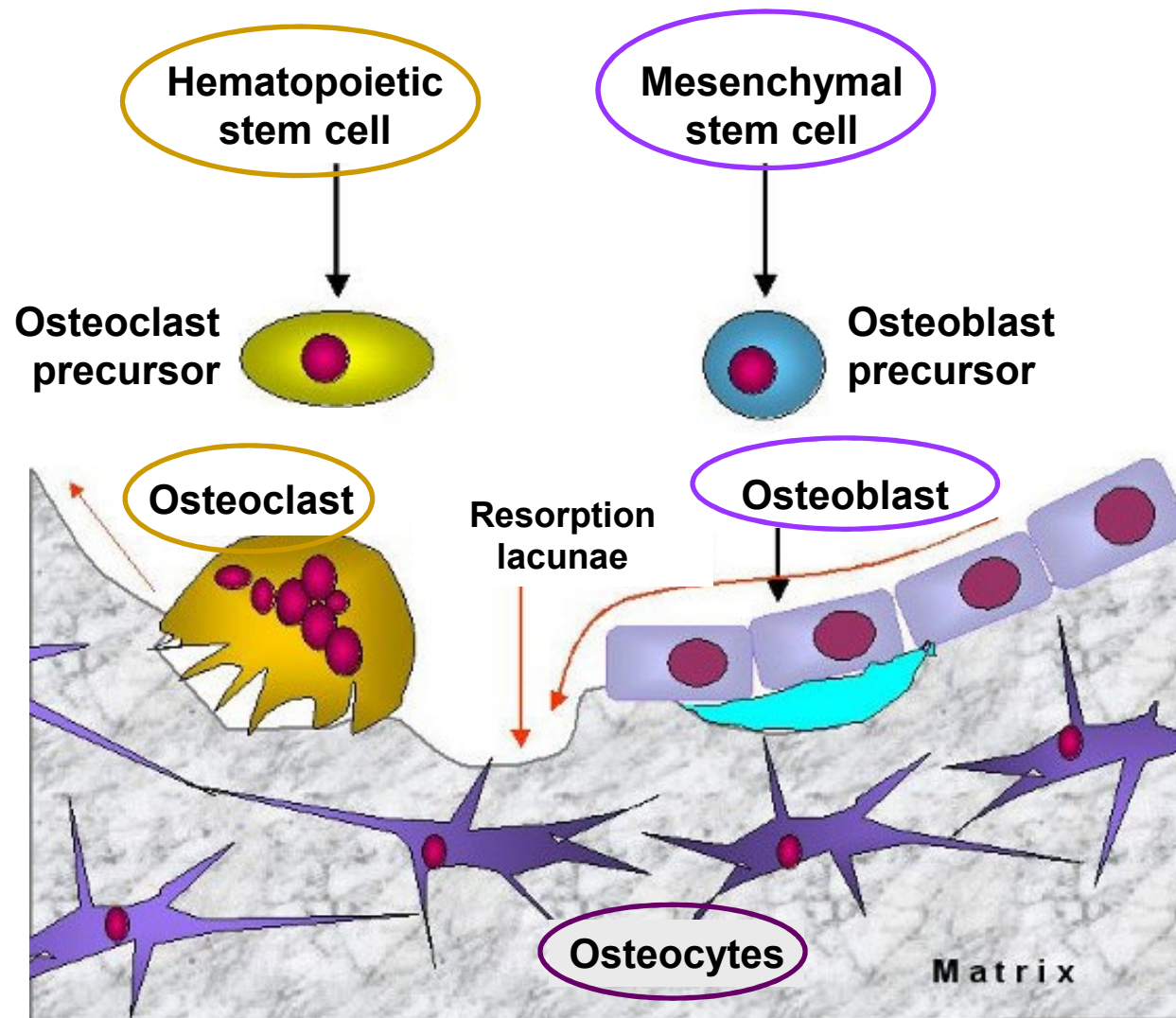
- mineralized inorganic components (hydroxyapatite)
– 60-70%

- non-mineralized organic components (collagen type I)
 - non-collagenous matrix proteins (glycoproteins, proteoglycans, bone sialoproteins)
- 20-30% + water

**MM Stevens et al.,
Science 2005**

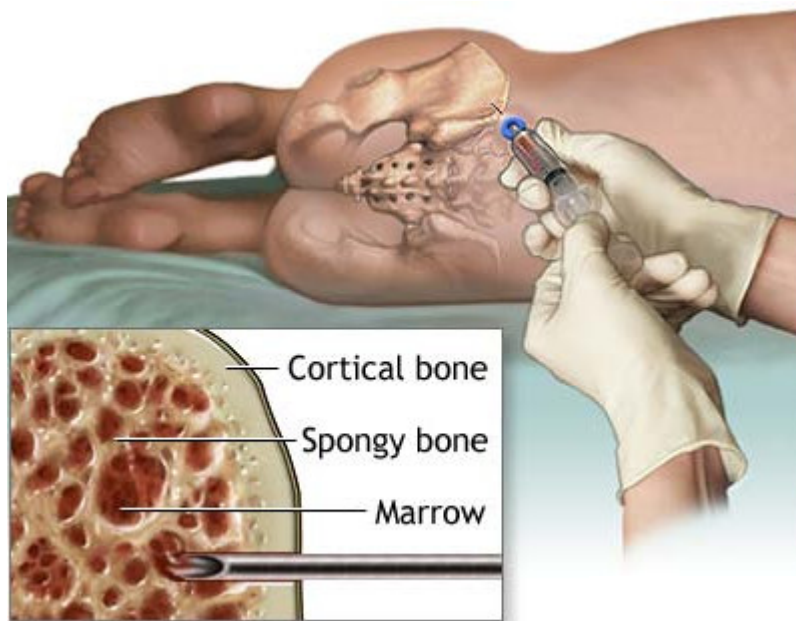


Cells of bone tissue: Osteoclasts / osteoblasts / osteocytes

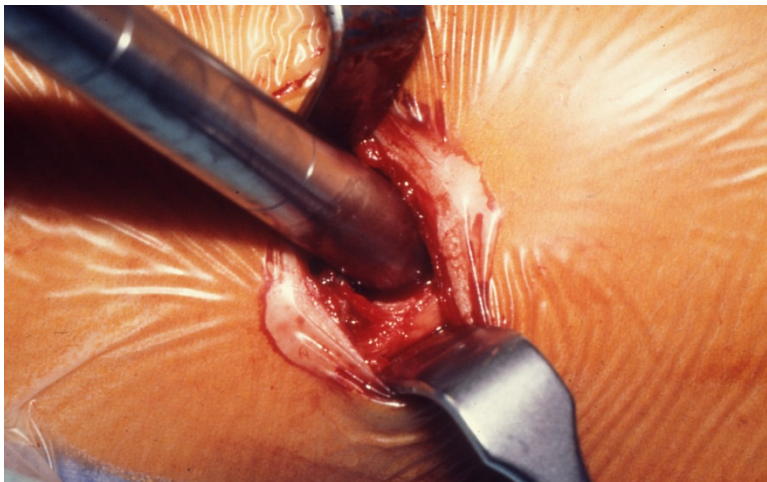


<http://www.andreas-unterberger.at/2012/07/chronische-entzuendungen-sind-ausloeser-von-knochenschwund/>

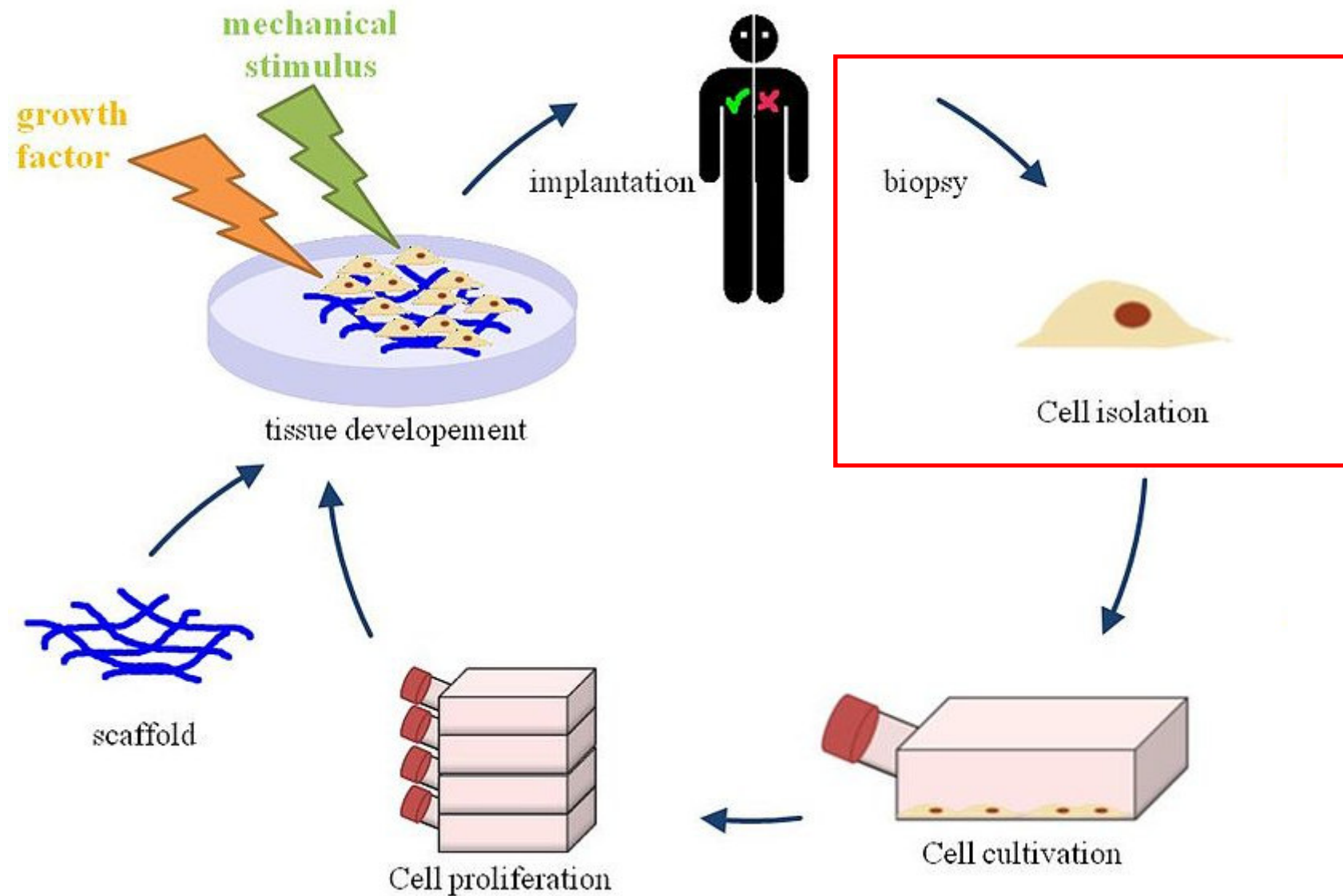
Gold standard for autologous bone regeneration



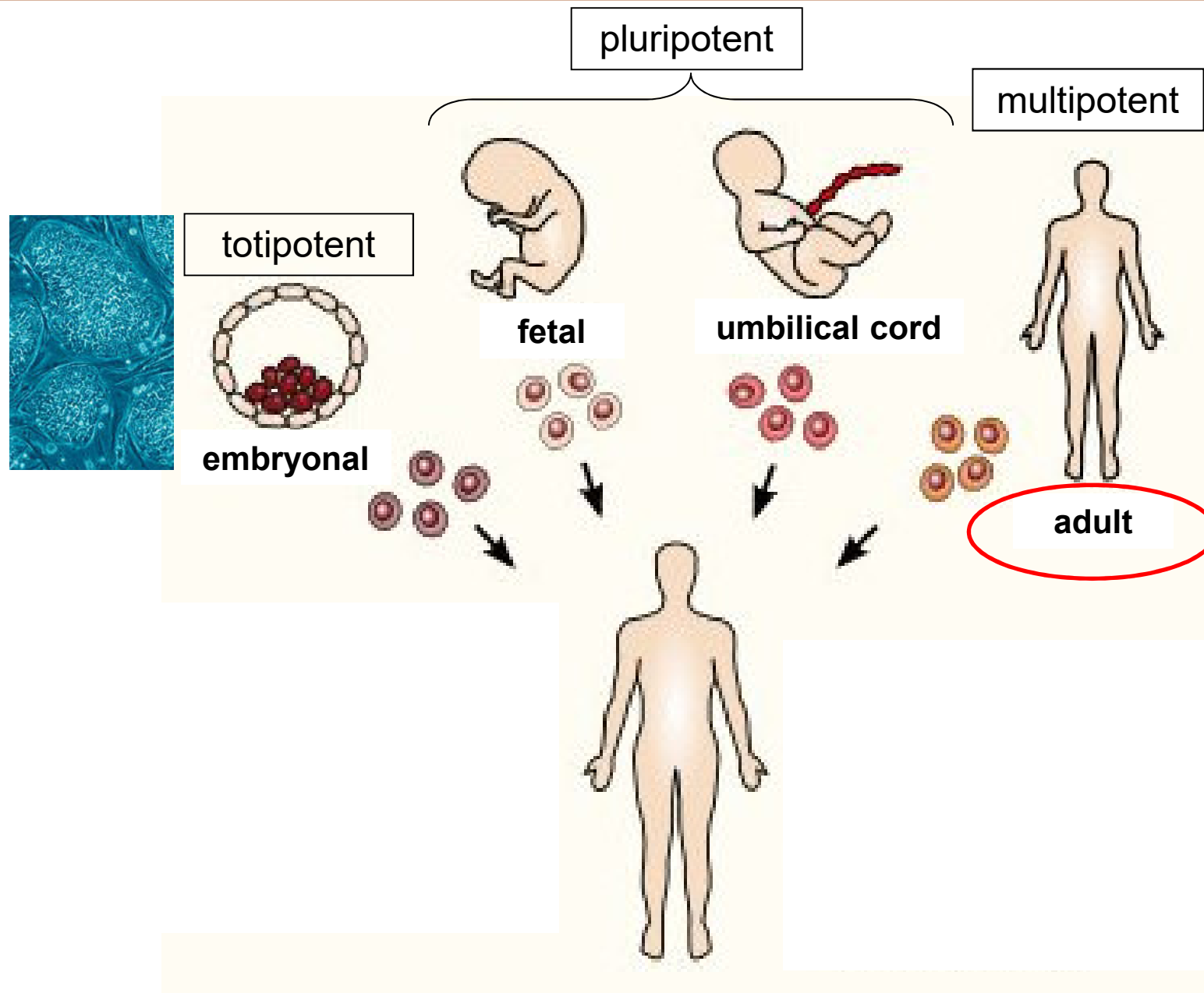
- spongy bone/marrow biopsies
- sufficient bone material is required
- morbidity of the donor site



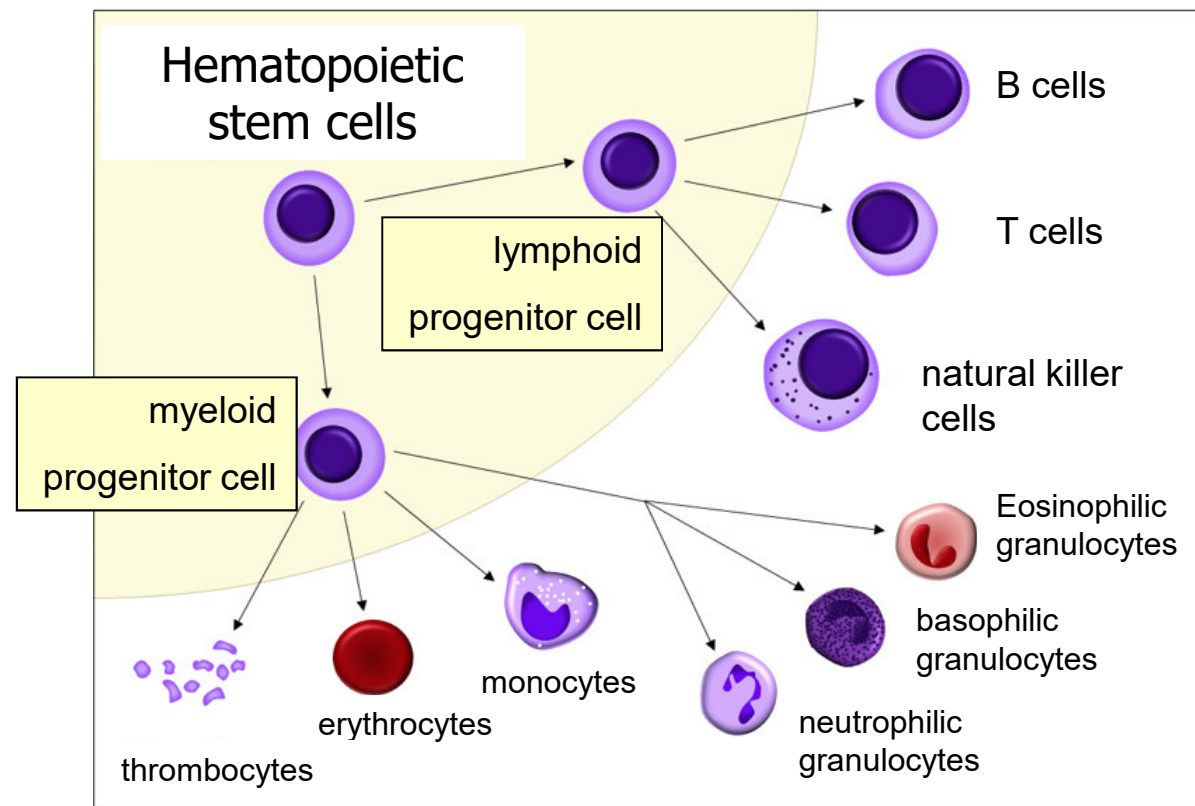
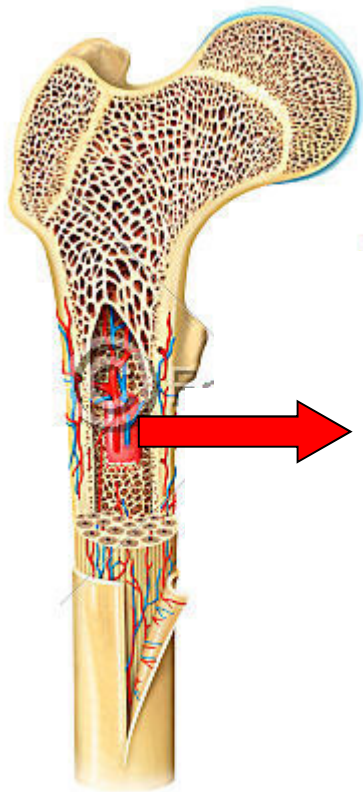
Tissue Engineering approach



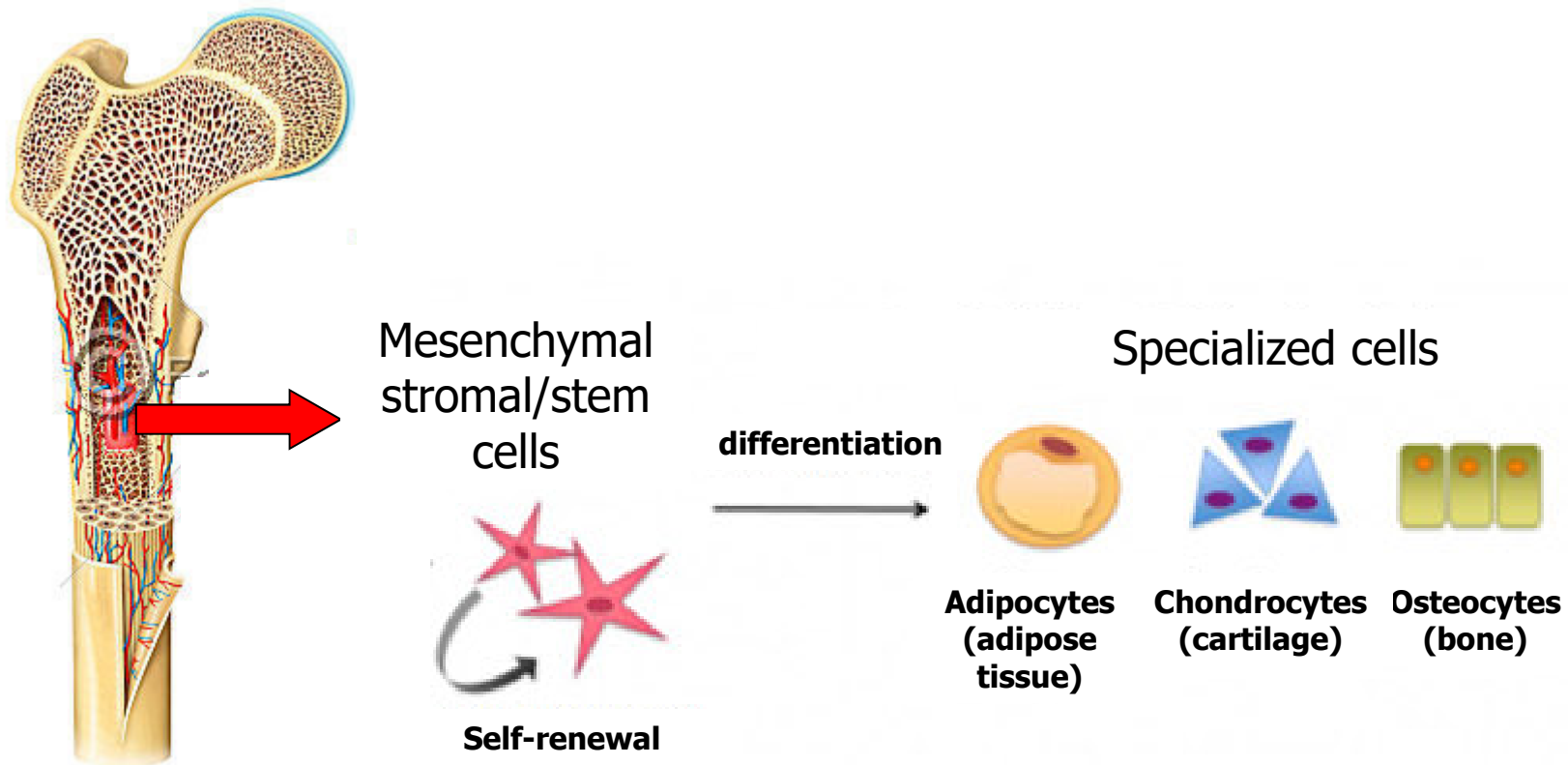
Stem cells



Bone marrow



Bone marrow

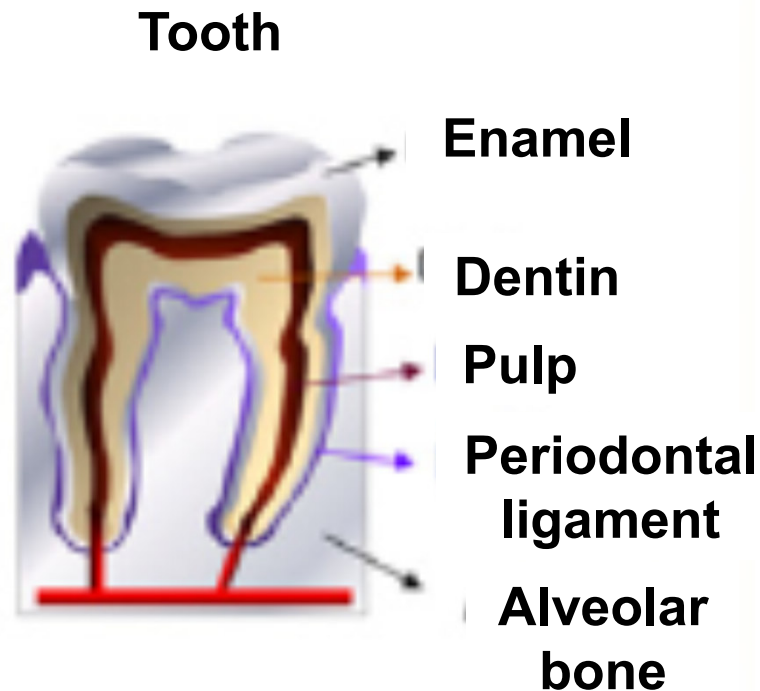


Mesenchymal stem cells (MSCs)

- **adherent** mesenchymal stem cells
 - Dominici criteria (*Cytotherapy 2006*):

1. adherence to plastic in standard culture conditions		
2. phenotype	positive ($\geq 95\%$)	negative ($\leq 2\%$)
	CD105	CD45
	CD90	CD34
	CD73	CD14 or CD11b
		CD79 α or CD19
		HLA-DR (MHC class II)

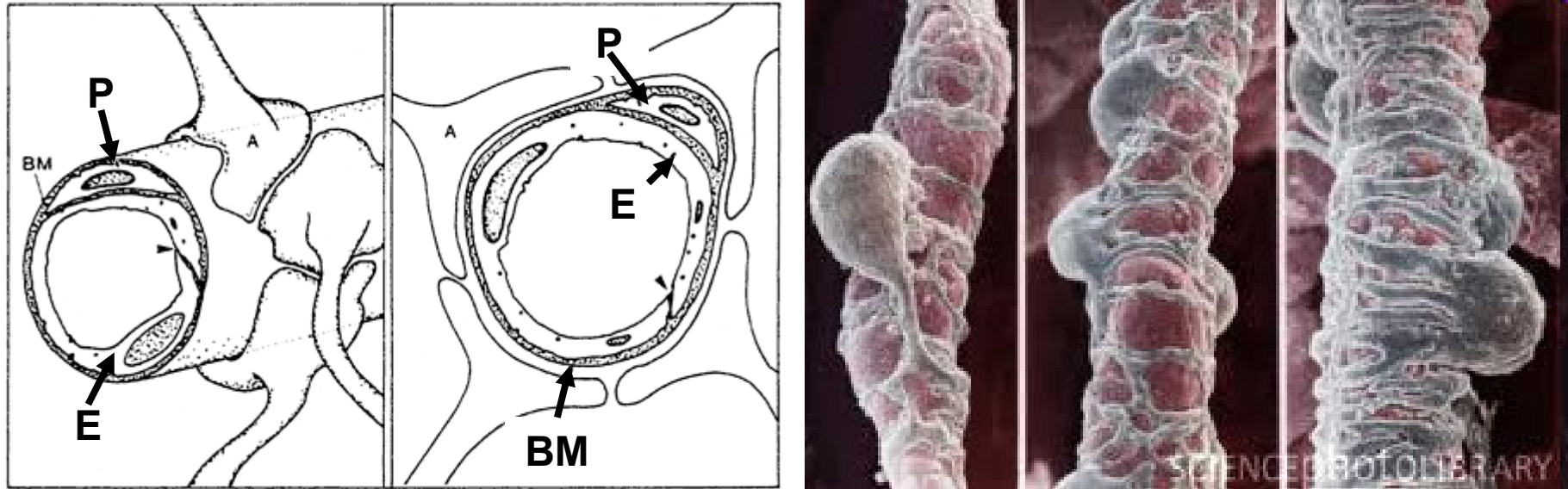
MSCs from other tissues



Dental Pulp Stem Cells

- dental pulp
- cord blood
- cord Wharton's jelly
- adipose tissue
- amniotic fluid
- compact bone
- periosteum
- synovial membrane
- articular cartilage
- fetal tissues

Are mesenchymal stem cells pericytes?

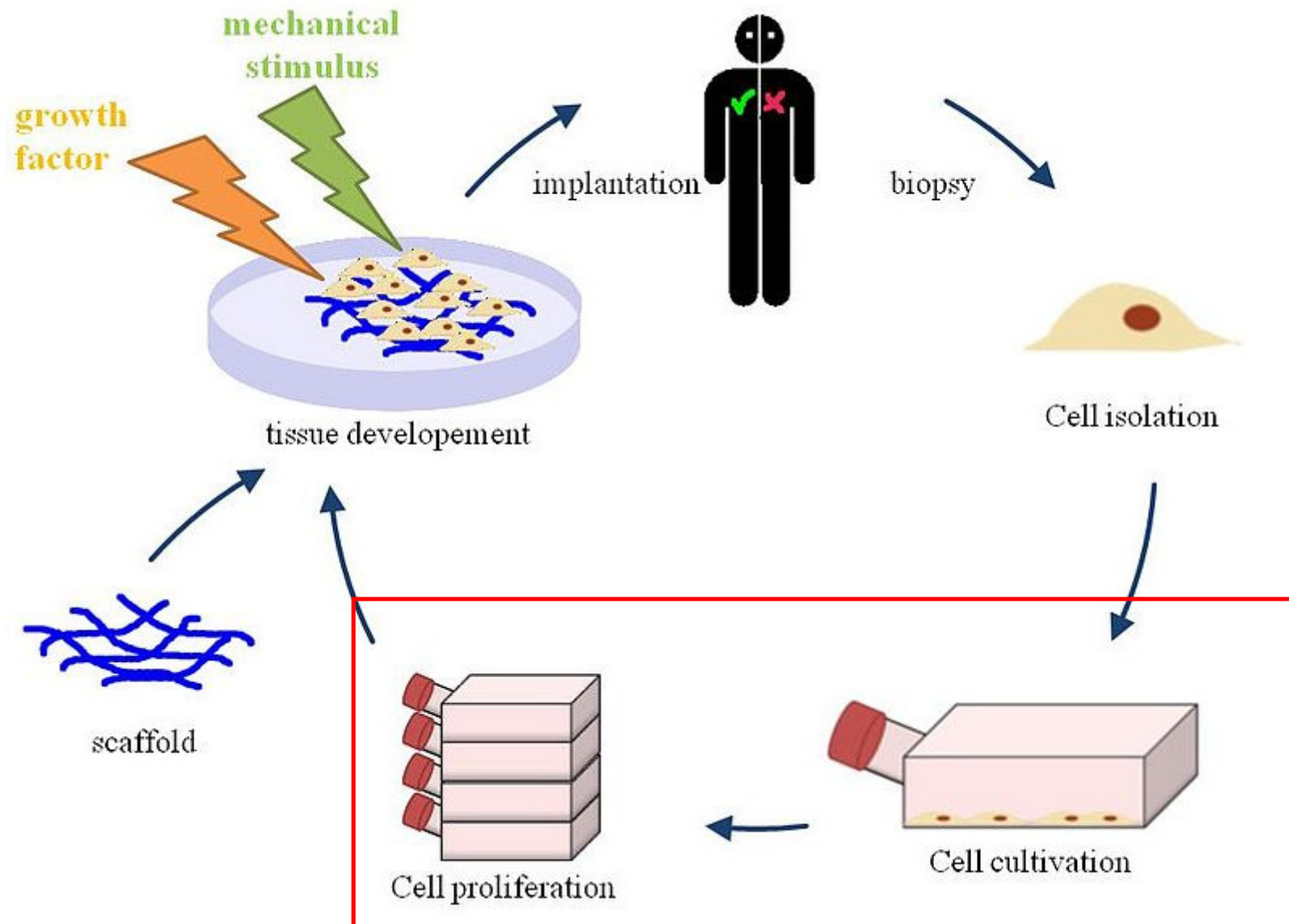


Pericytes (P) also known as mural or Rouget cells are closely associated with capillary endothelial cells (E) with which they share a common basal lamina (BM). Pericytes are able to differentiate into adipocytes, osteoblasts, fibroblasts, smooth muscle cells and macrophages.

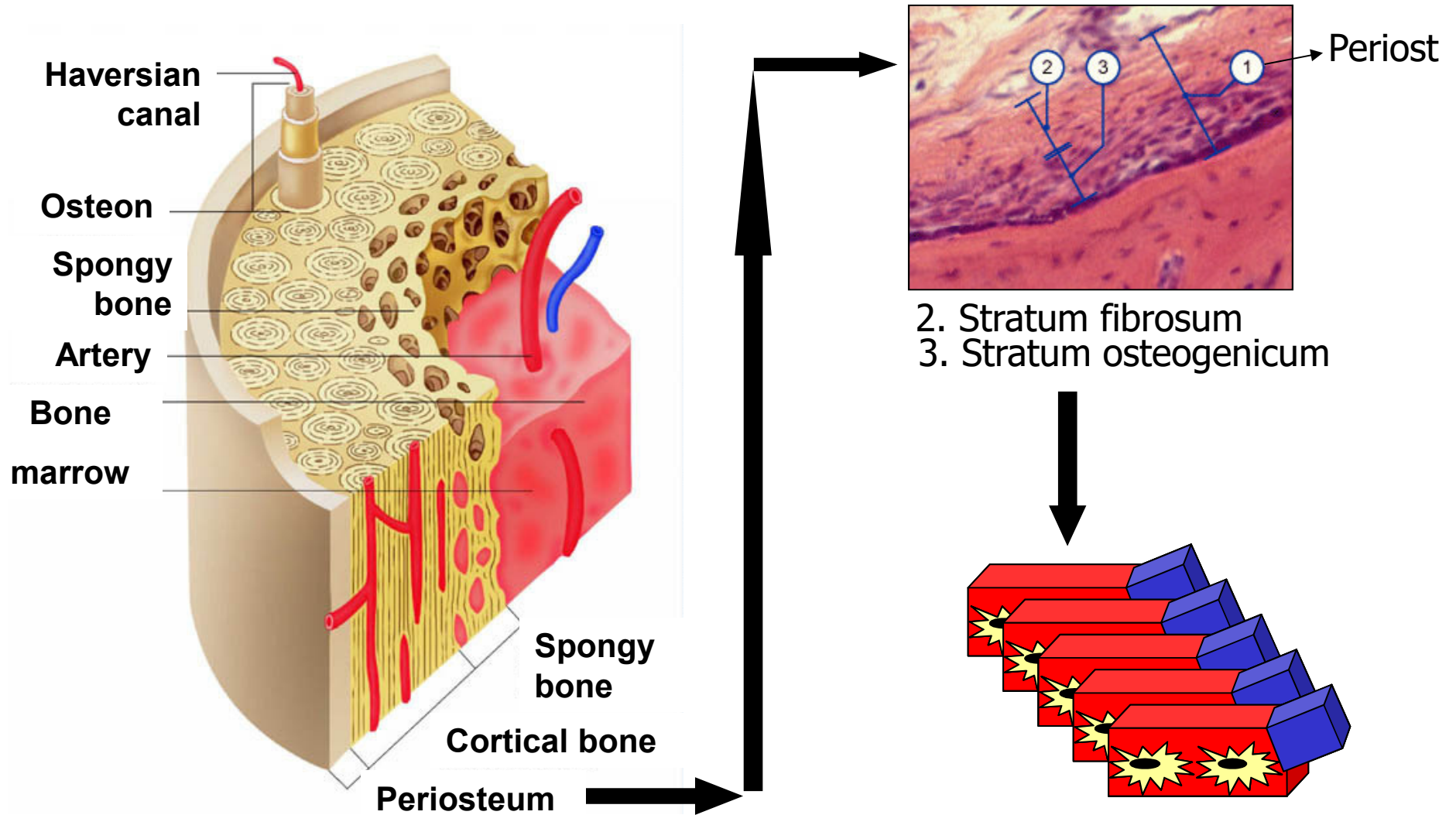
Pericytes were demonstrated to be CD105+, CD146+ and CD31-, CD34-

G Allta and J.G. Lawrensona, Cell Tissues Organs 2001

Tissue Engineering approach

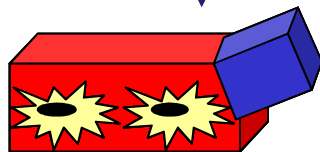
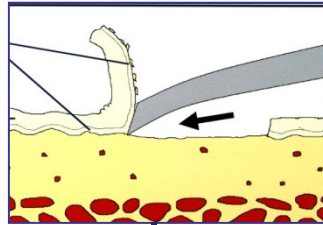


Periosteal tissue

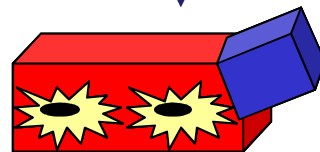


In vitro cultivation of stem cells

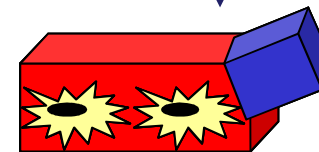
**Isolation and
expansion of
periosteal cells**



untreated



**osteogenic
supplement 1**



**osteogenic
supplement 2**

**proliferation
analysis**

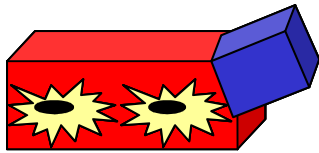
**Quantification of
calcium
precipitates**

gene expression analysis

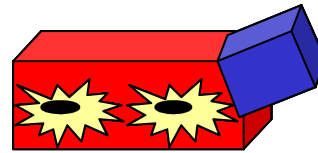
**protein-
expression
analysis**

stainings

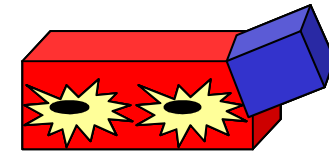
Gene expression analysis of differentiating cells



untreated



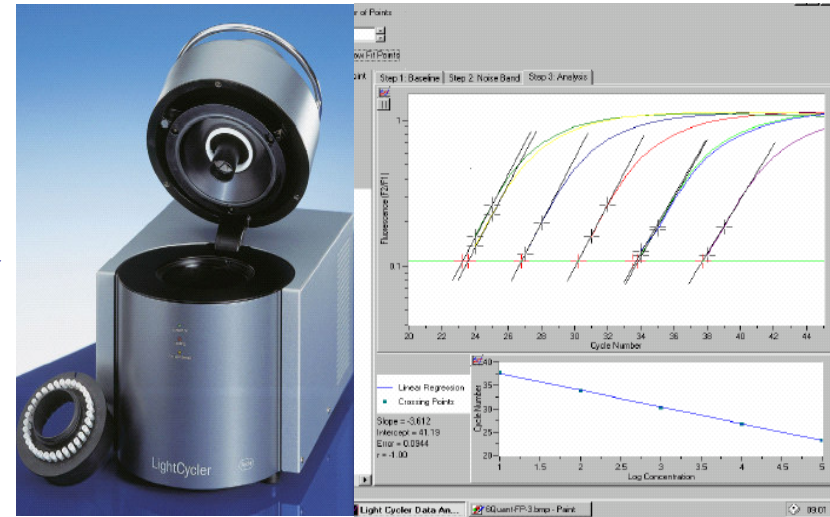
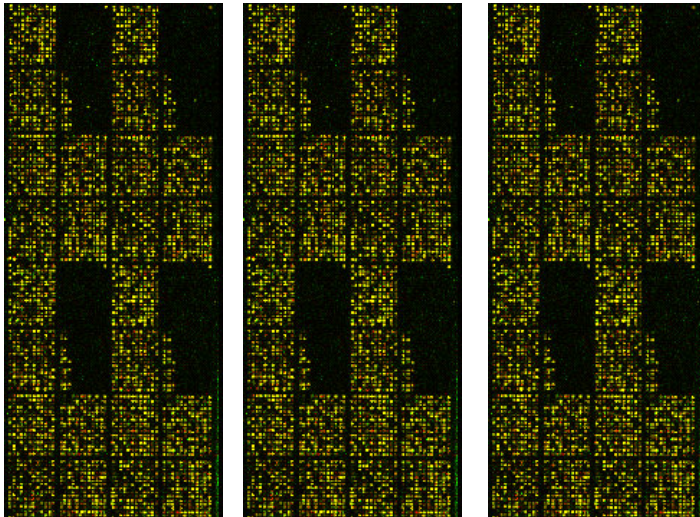
osteogenic
supplement 1



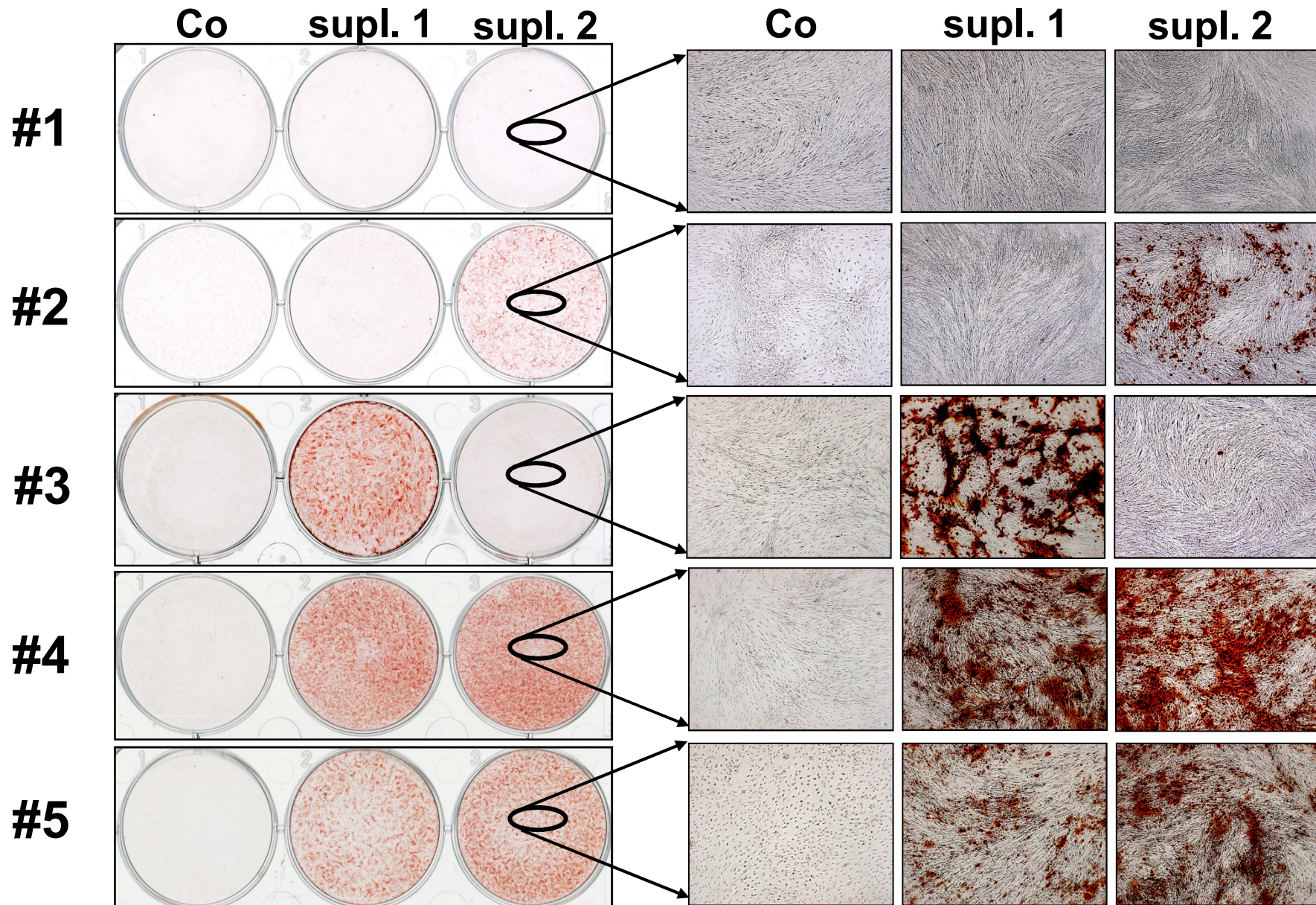
osteogenic
supplement 2

Microarrays

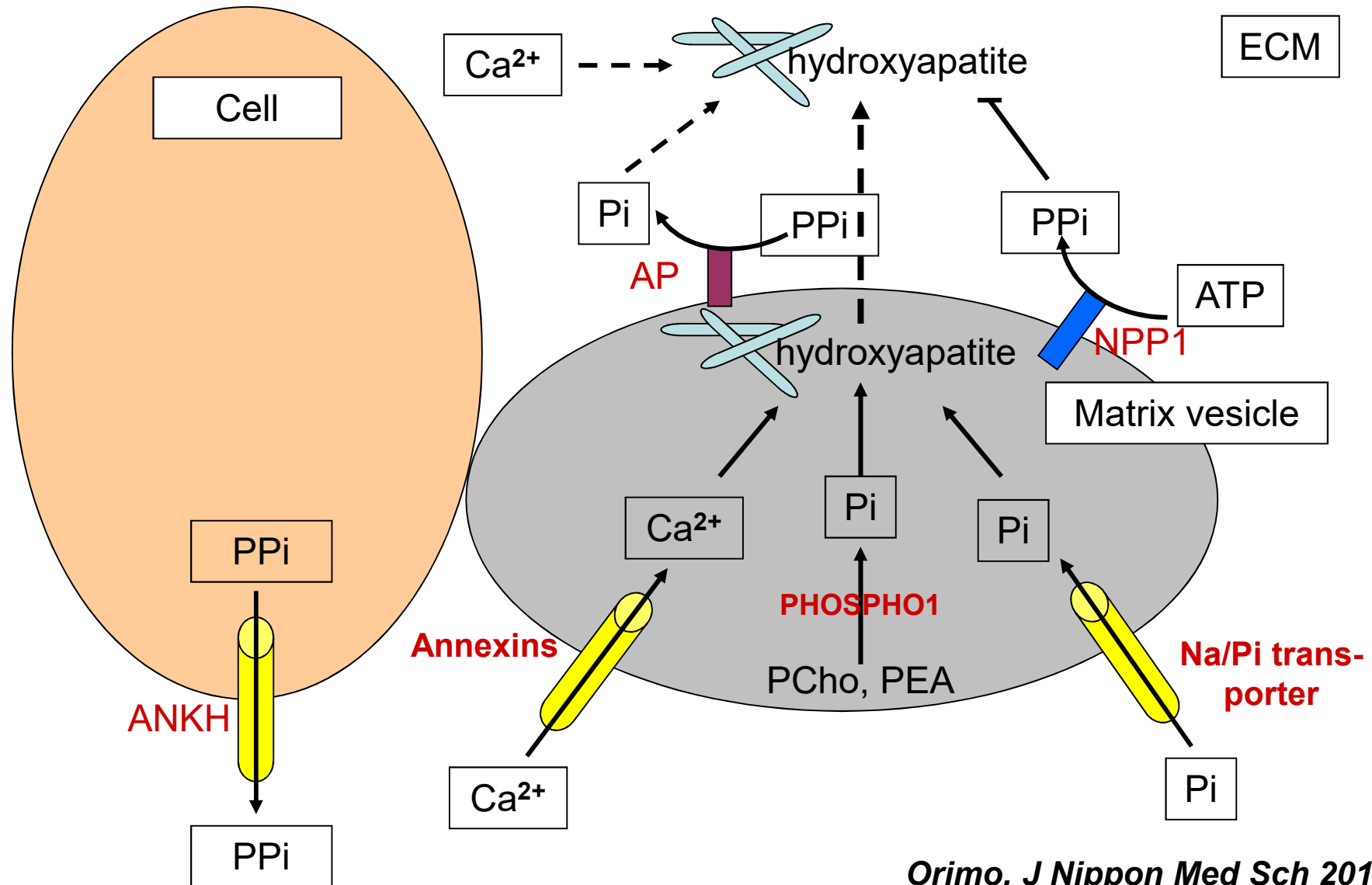
quantitative PCR



Different osteogenic potential of periosteal cells

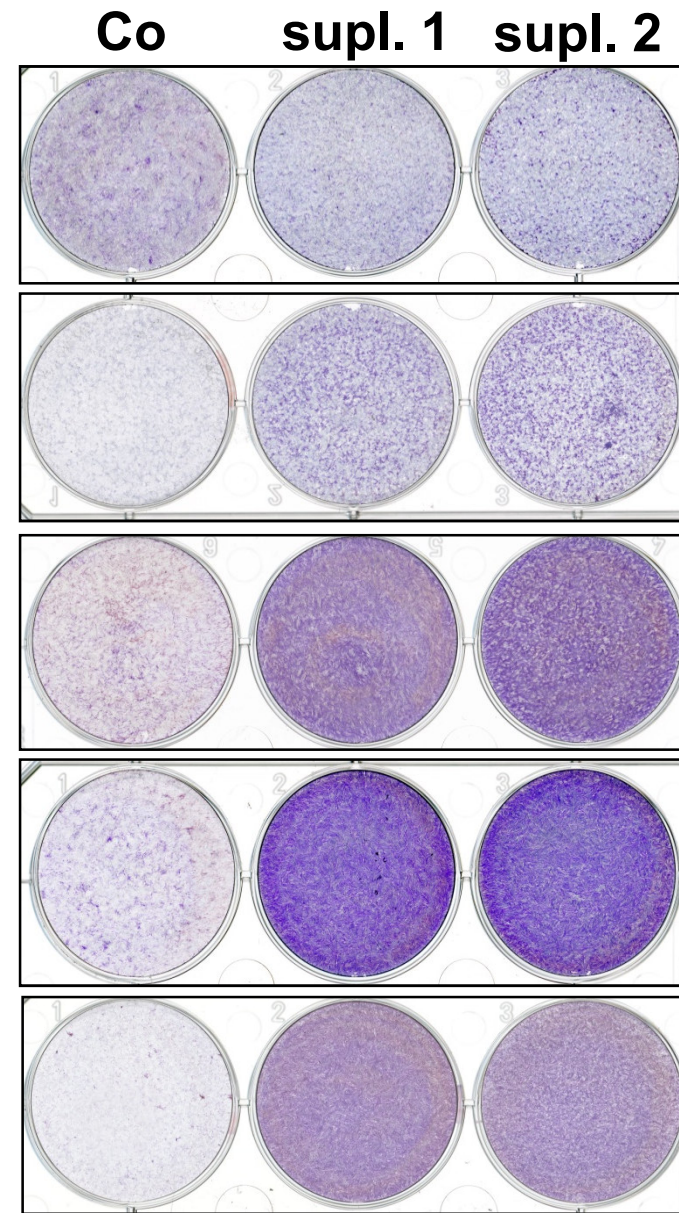
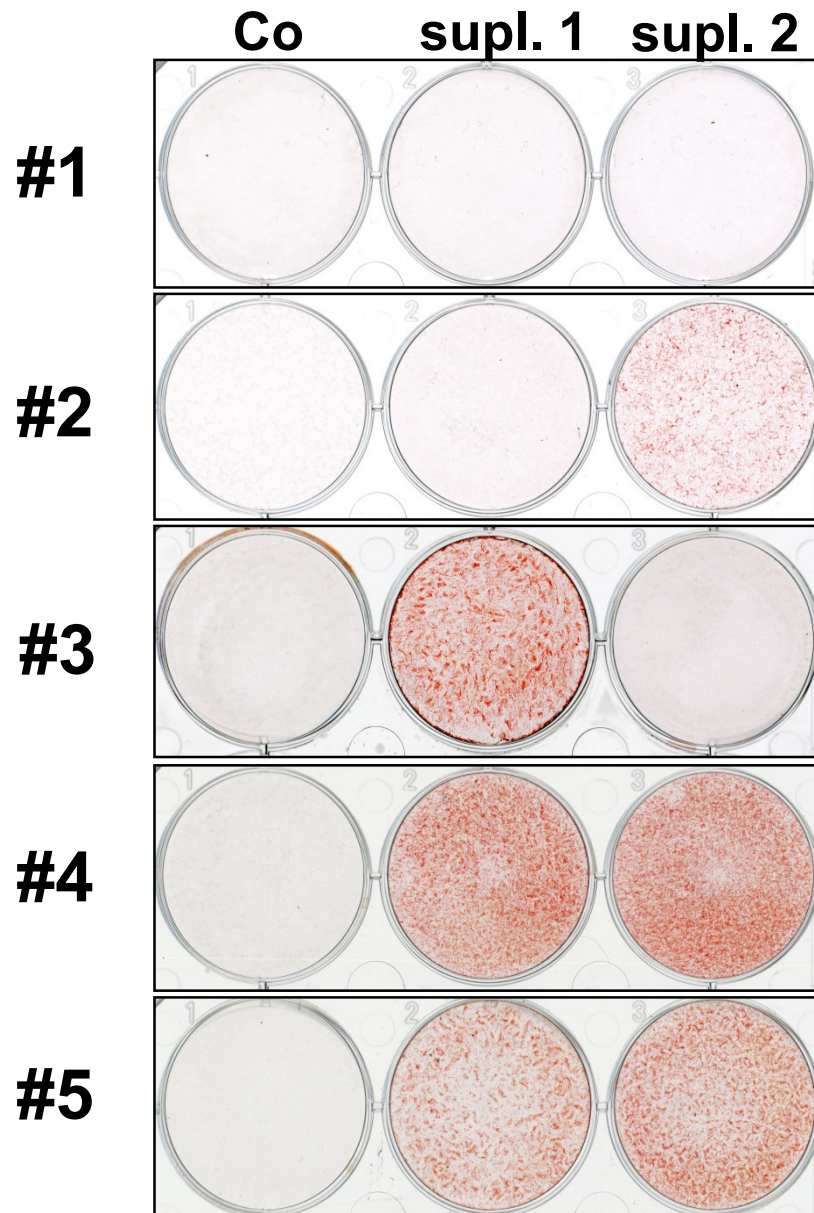


Schematic diagram of the mineralization process

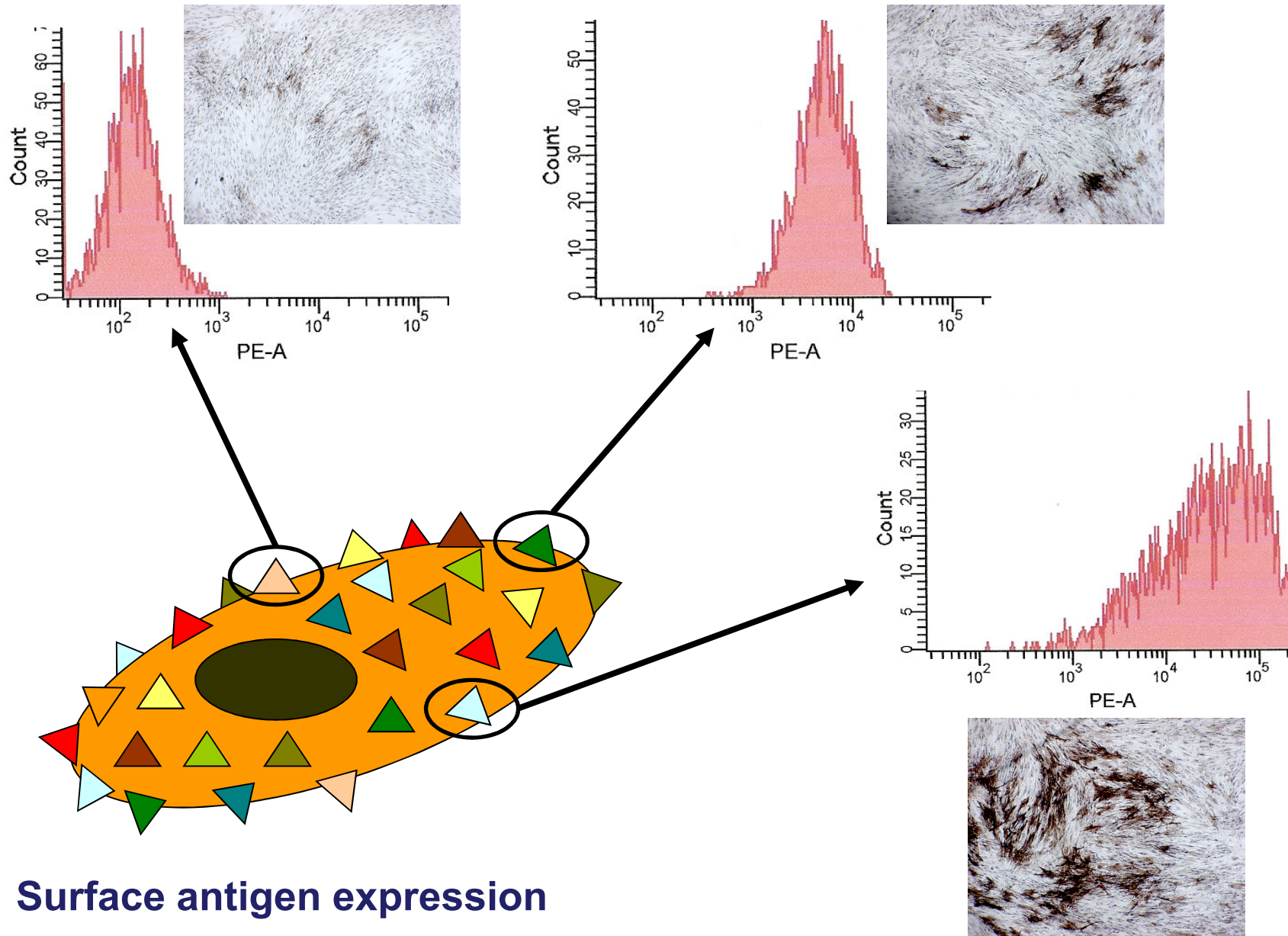


Orimo, J Nippon Med Sch 2010

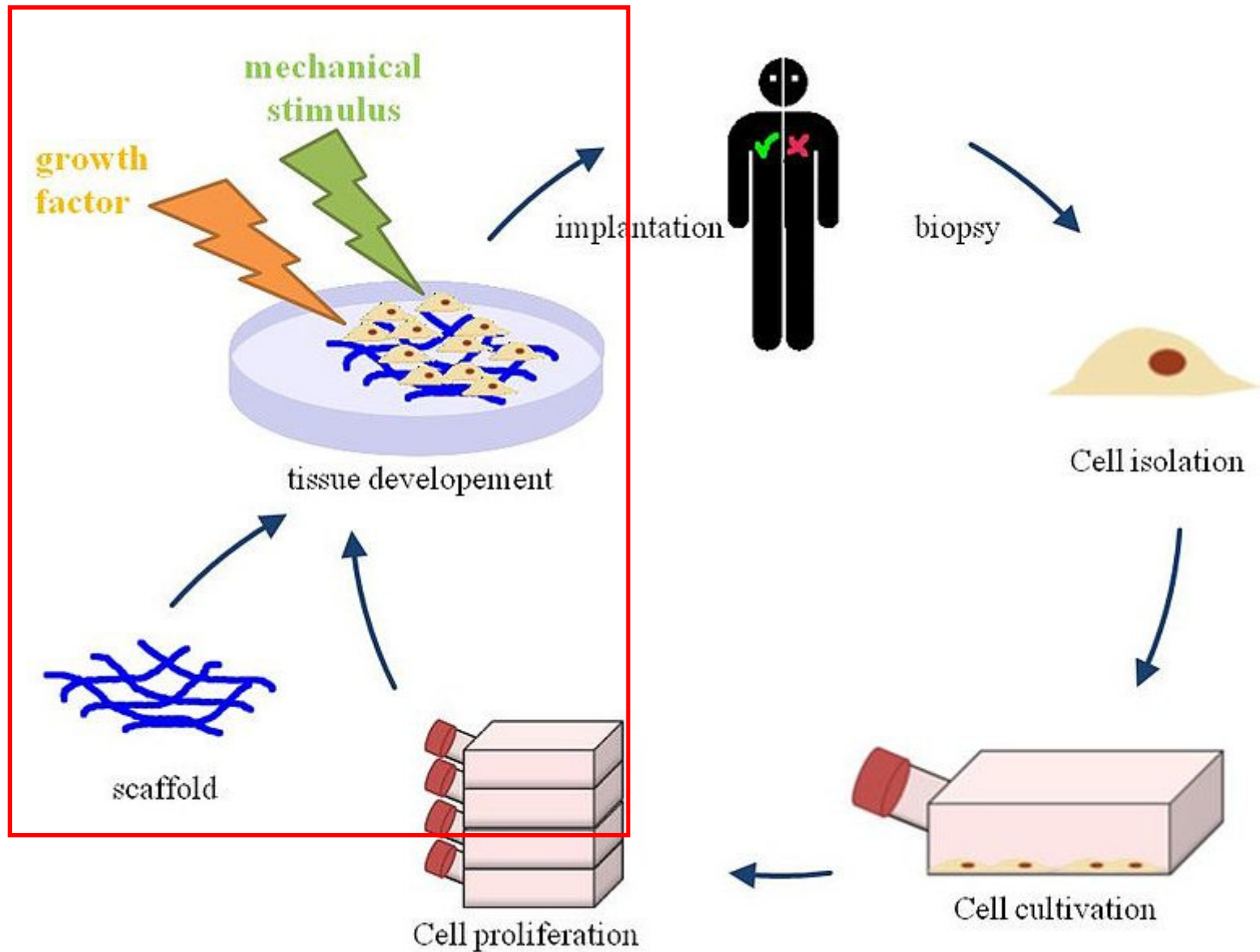
Correlation with the alkaline phosphatase activity



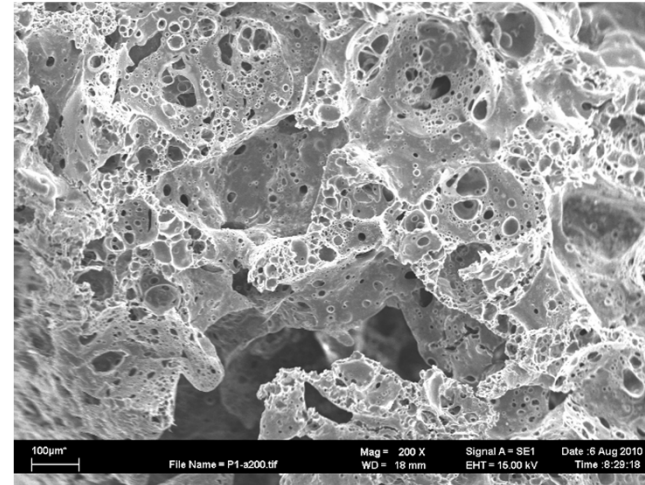
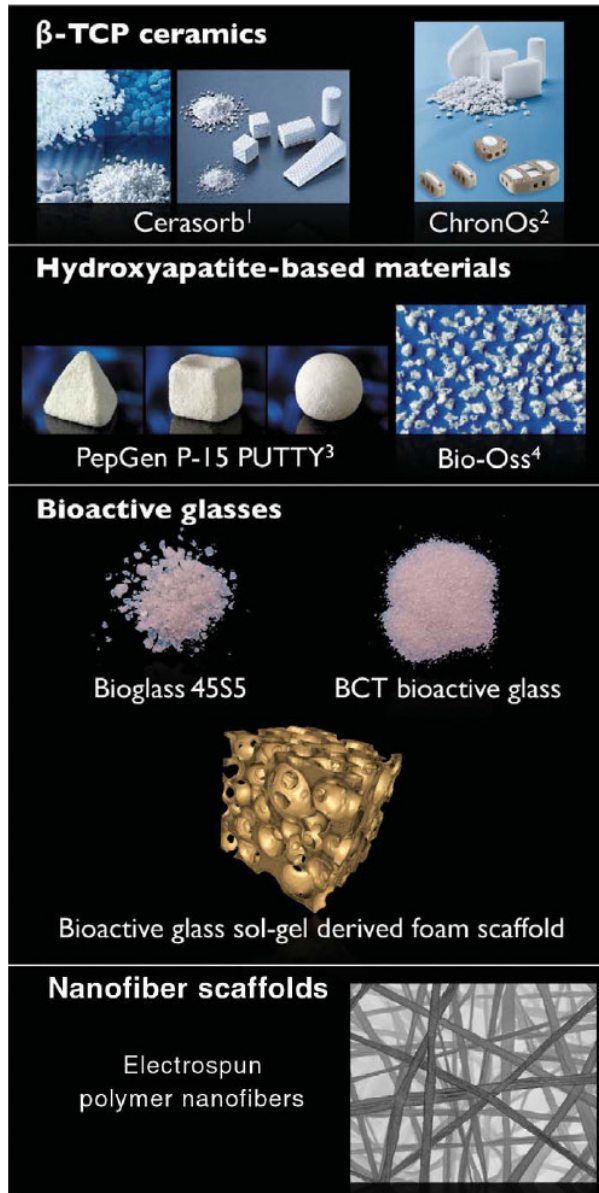
FACS (fluorescence activated cell sorting)



Tissue Engineering approach

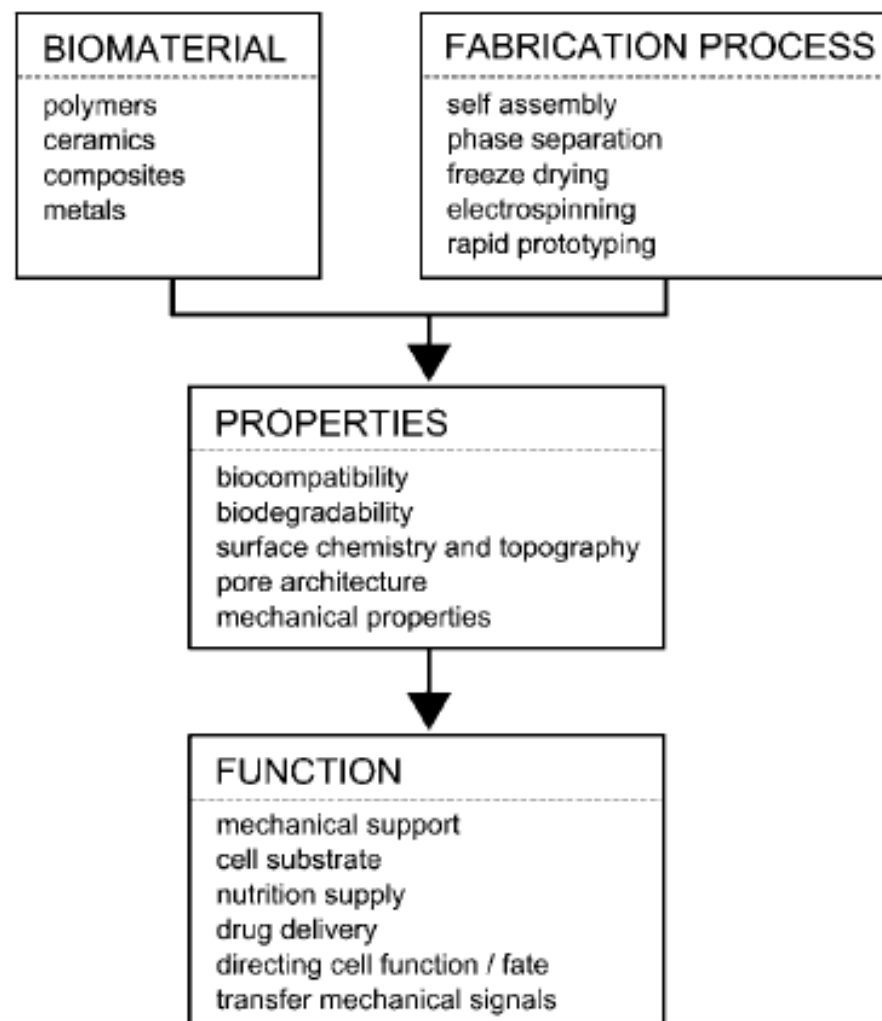


Biomaterials for bone TE



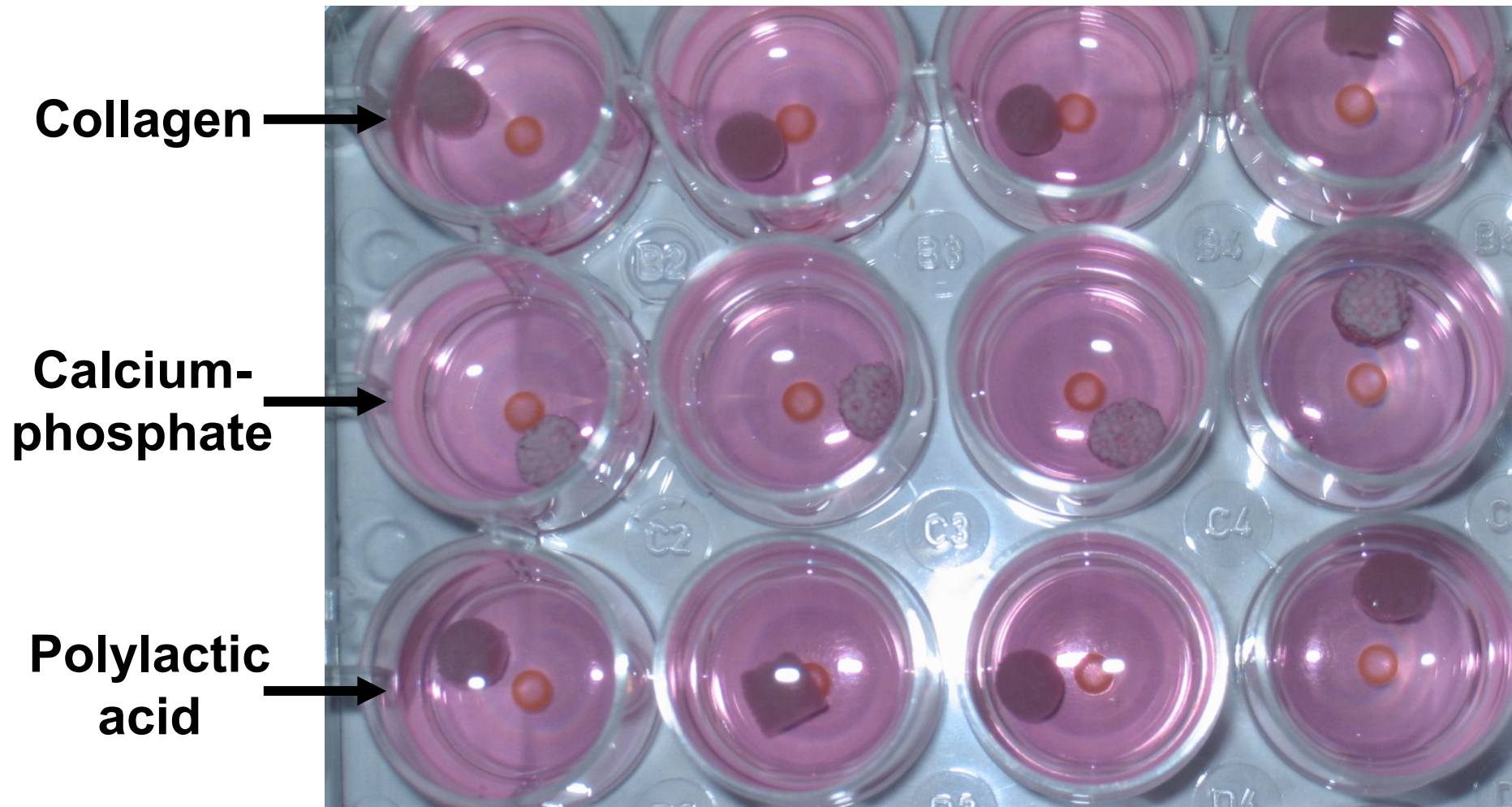
- bioactive inorganic materials
- biological polymers
- composite materials

Fundamental features of biomaterials for bone regeneration

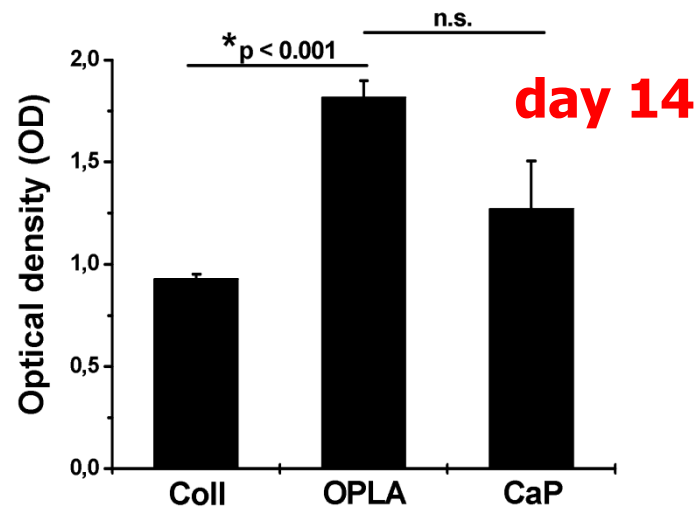
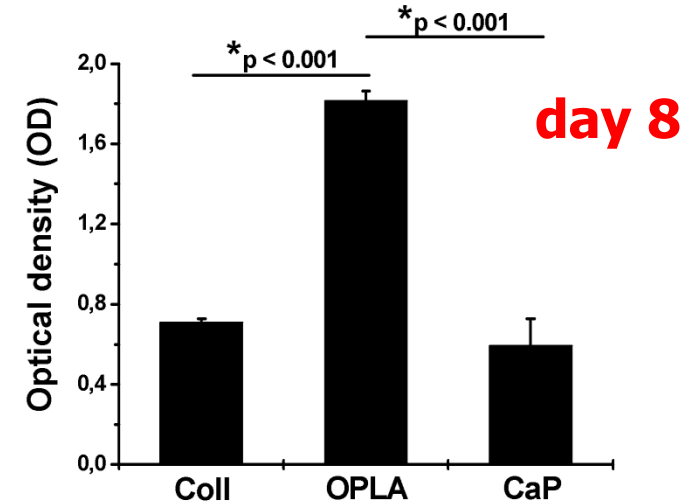
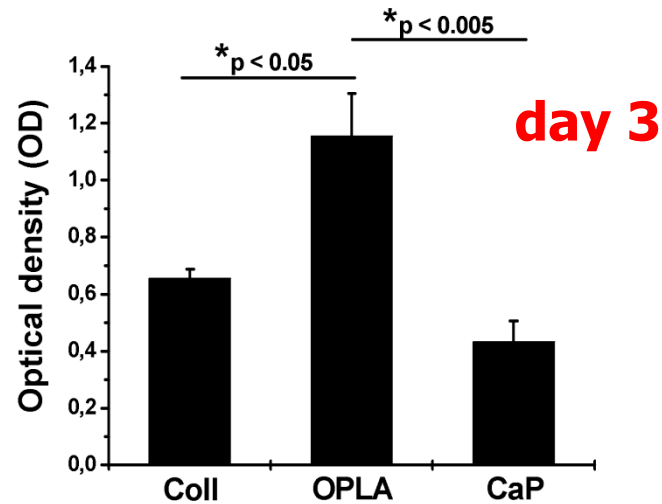


3D biomaterials for different stem cell types

Periosteal cell-seeded biomaterials

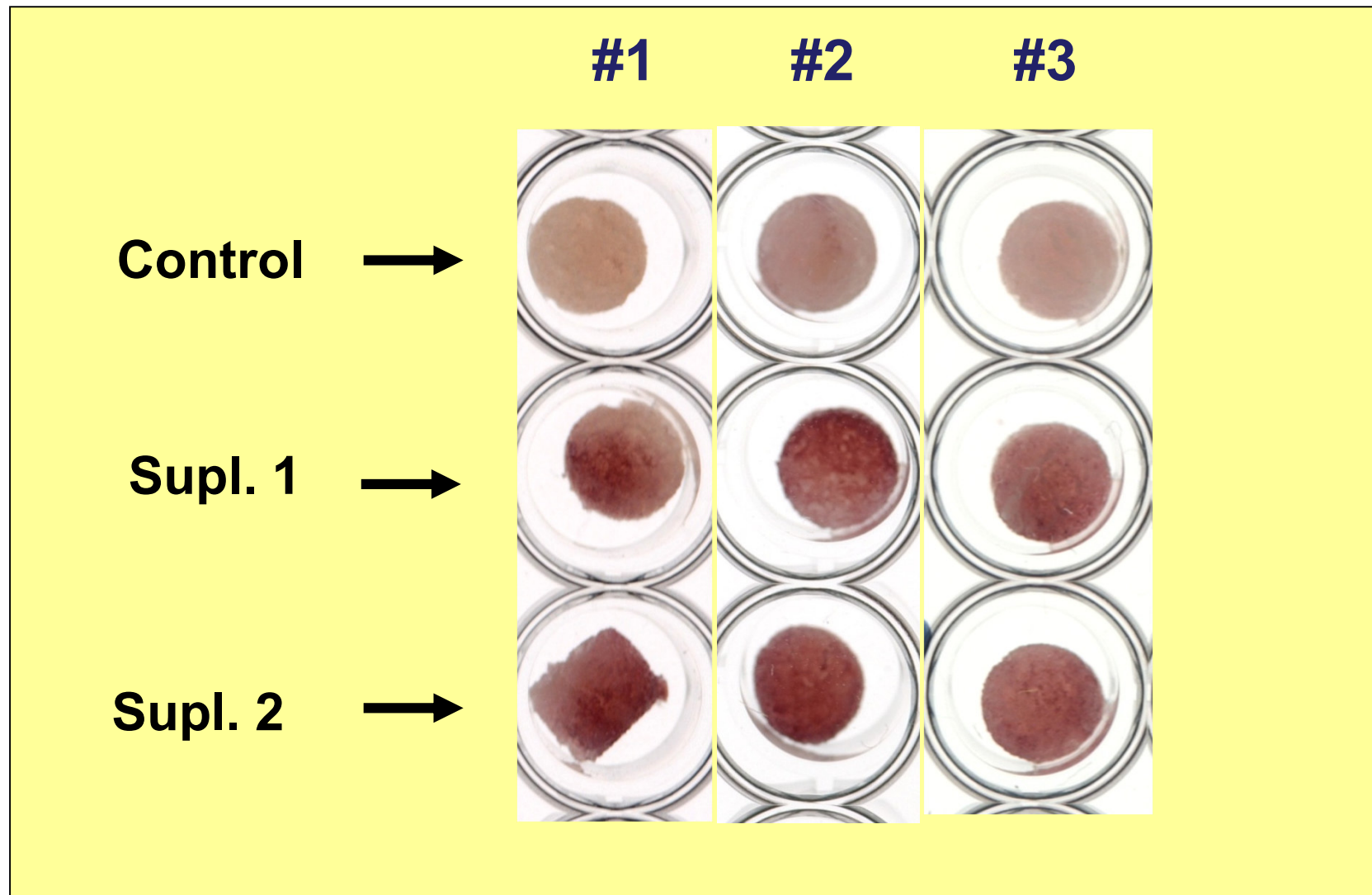


Proliferative behaviour of periosteal cells within different biomaterials



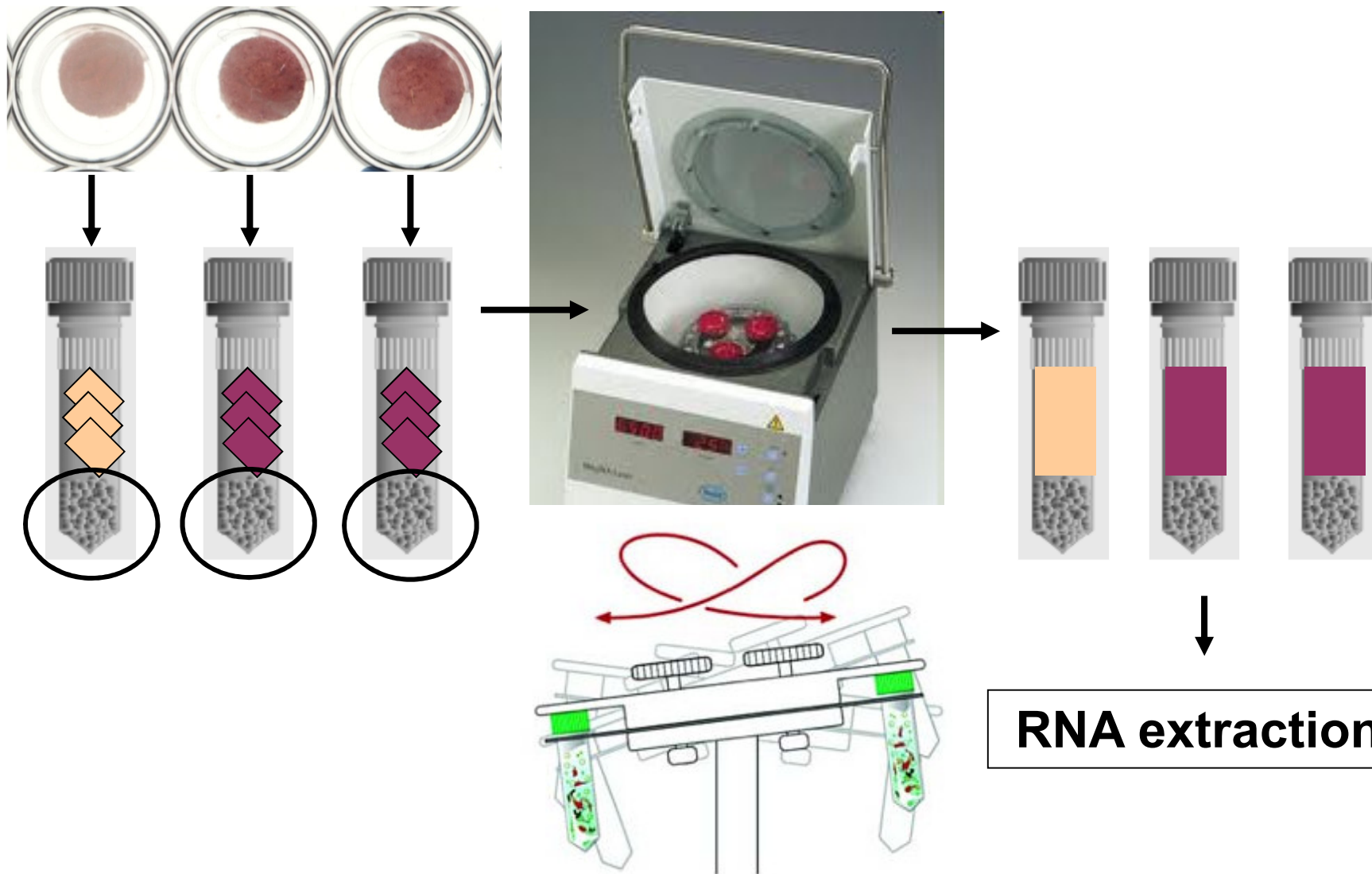
Osteogenic differentiation of cell-seeded polylactic acid 3D constructs

Detection of alkaline phosphatase



RNA extraction from cell-seeded 3D constructs

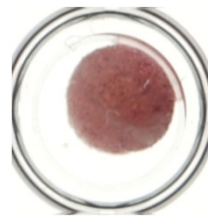
MagNA Lyser



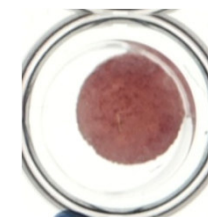
Gene expression analysis in cells growing within 3D scaffolds



untreated



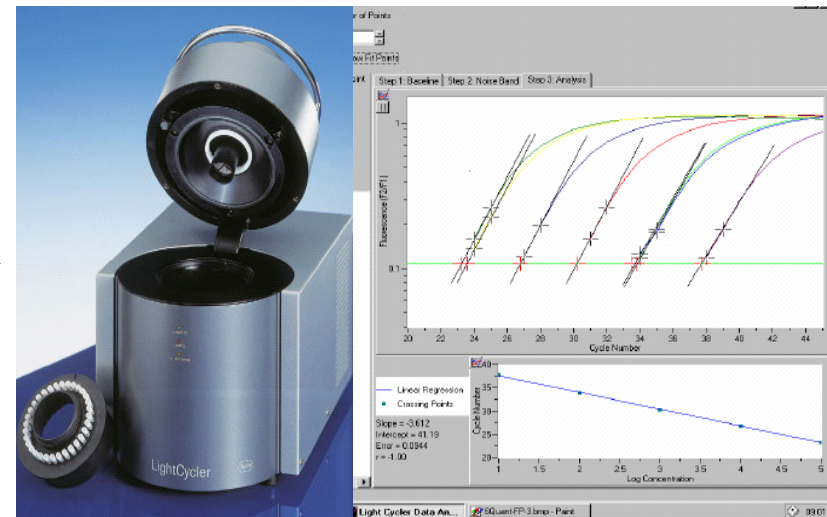
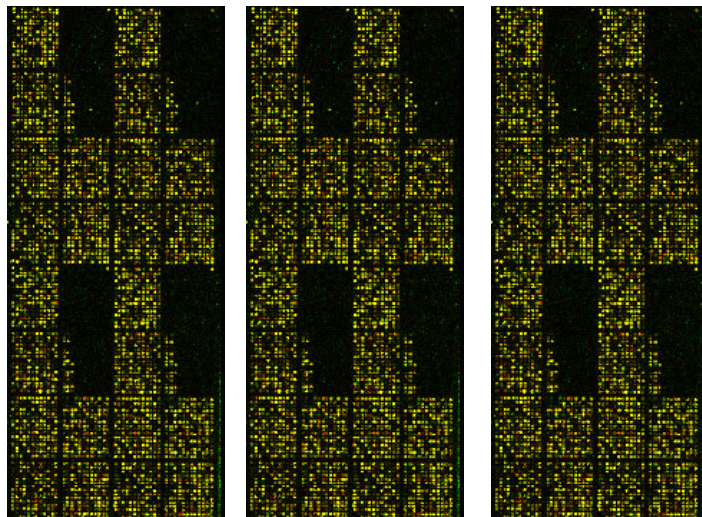
osteogenic
supplement 1



osteogenic
supplement 2

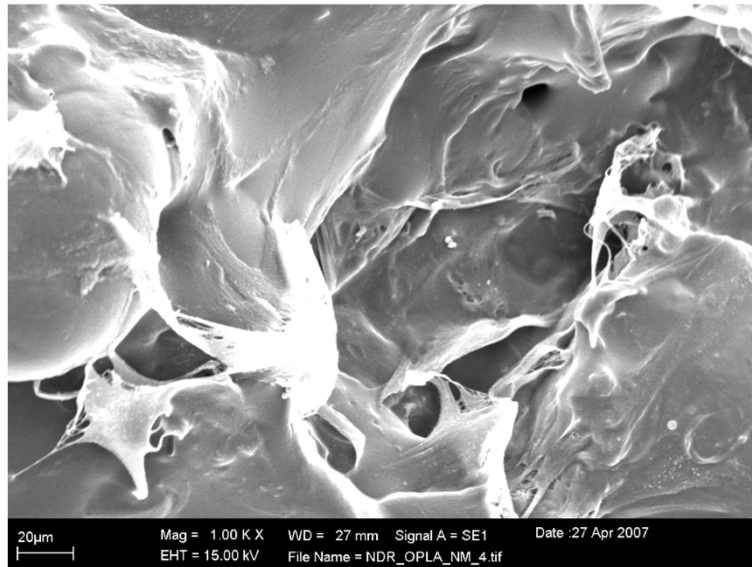
Microarrays

quantitative PCR



Electron microscopy of cell-seeded scaffolds

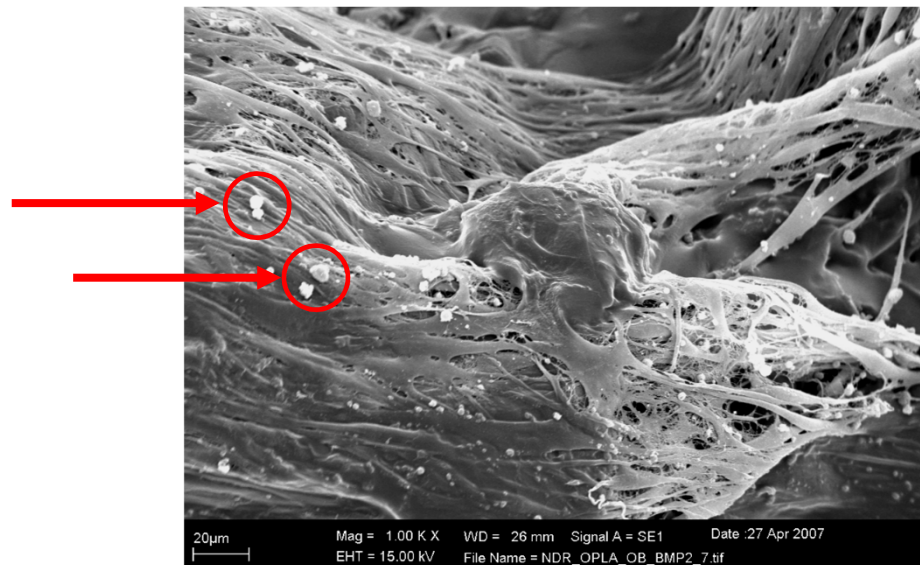
untreated control



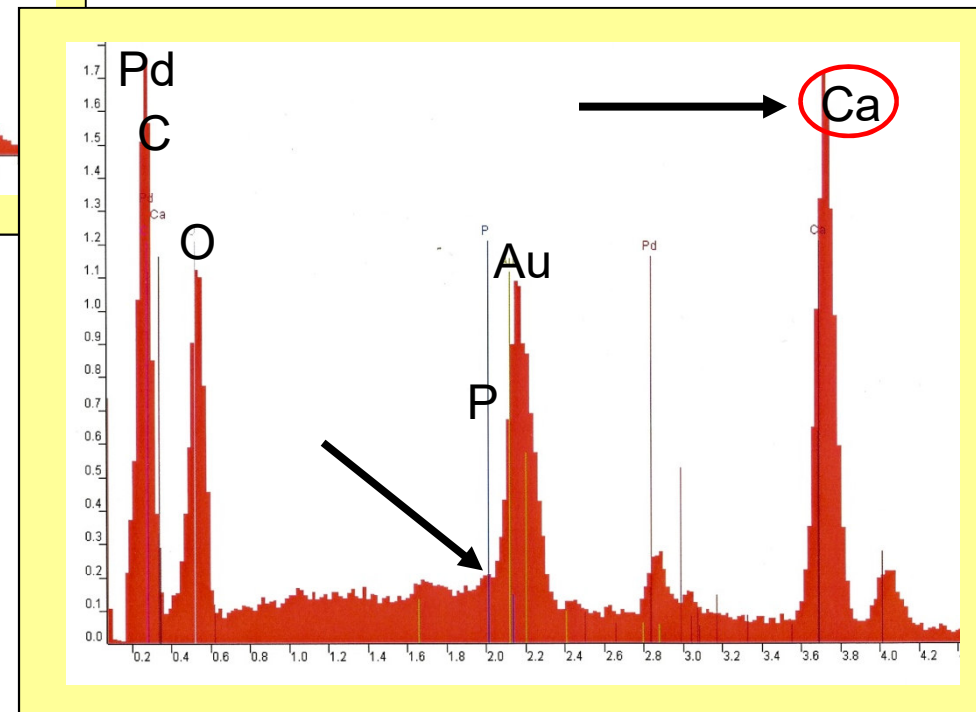
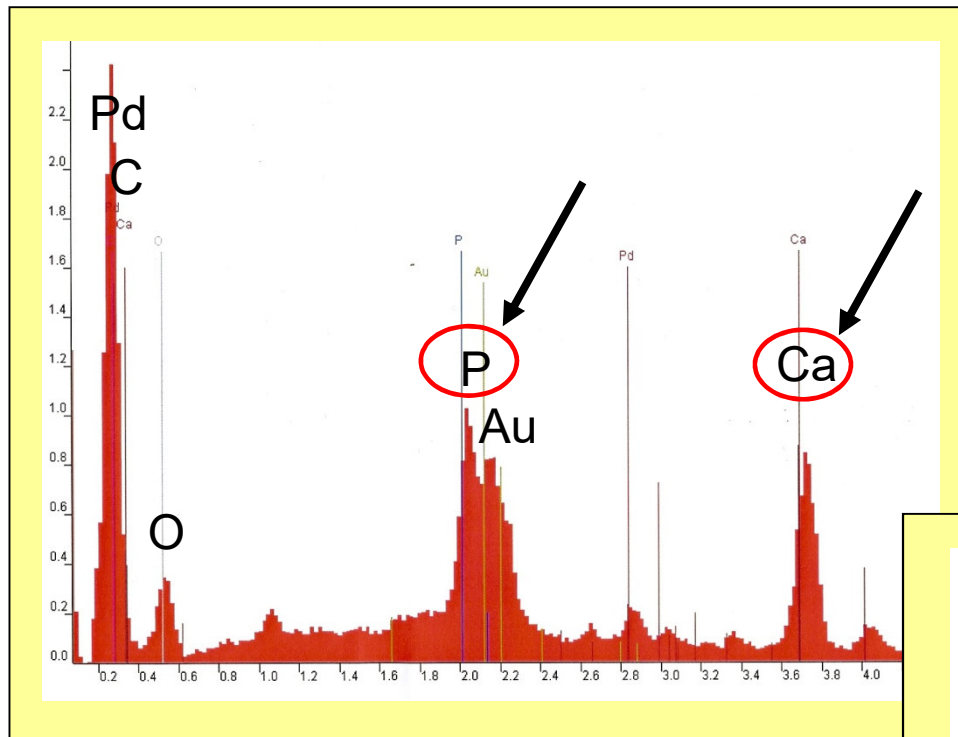
ost. suppl. 1



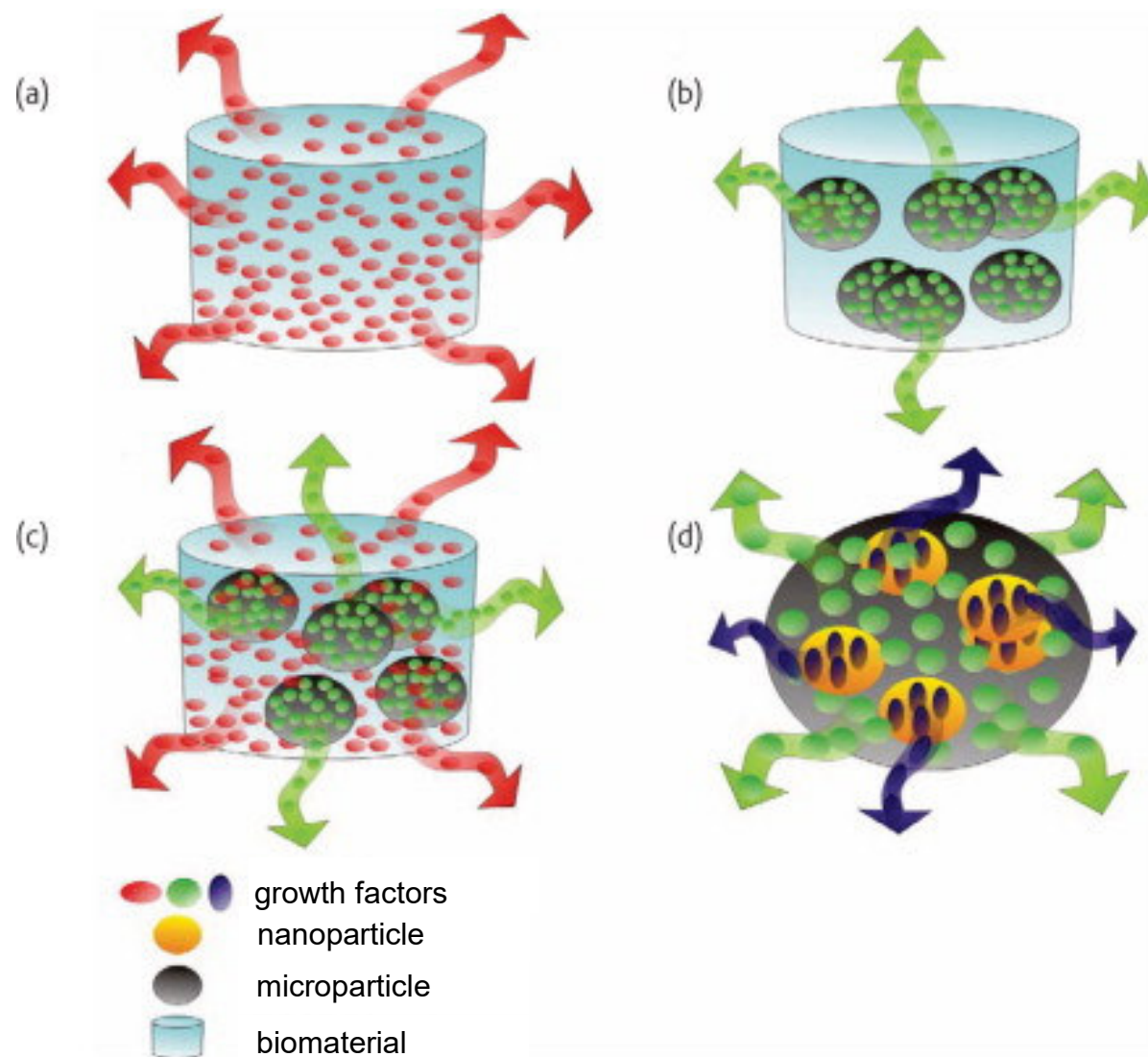
ost. suppl. 2



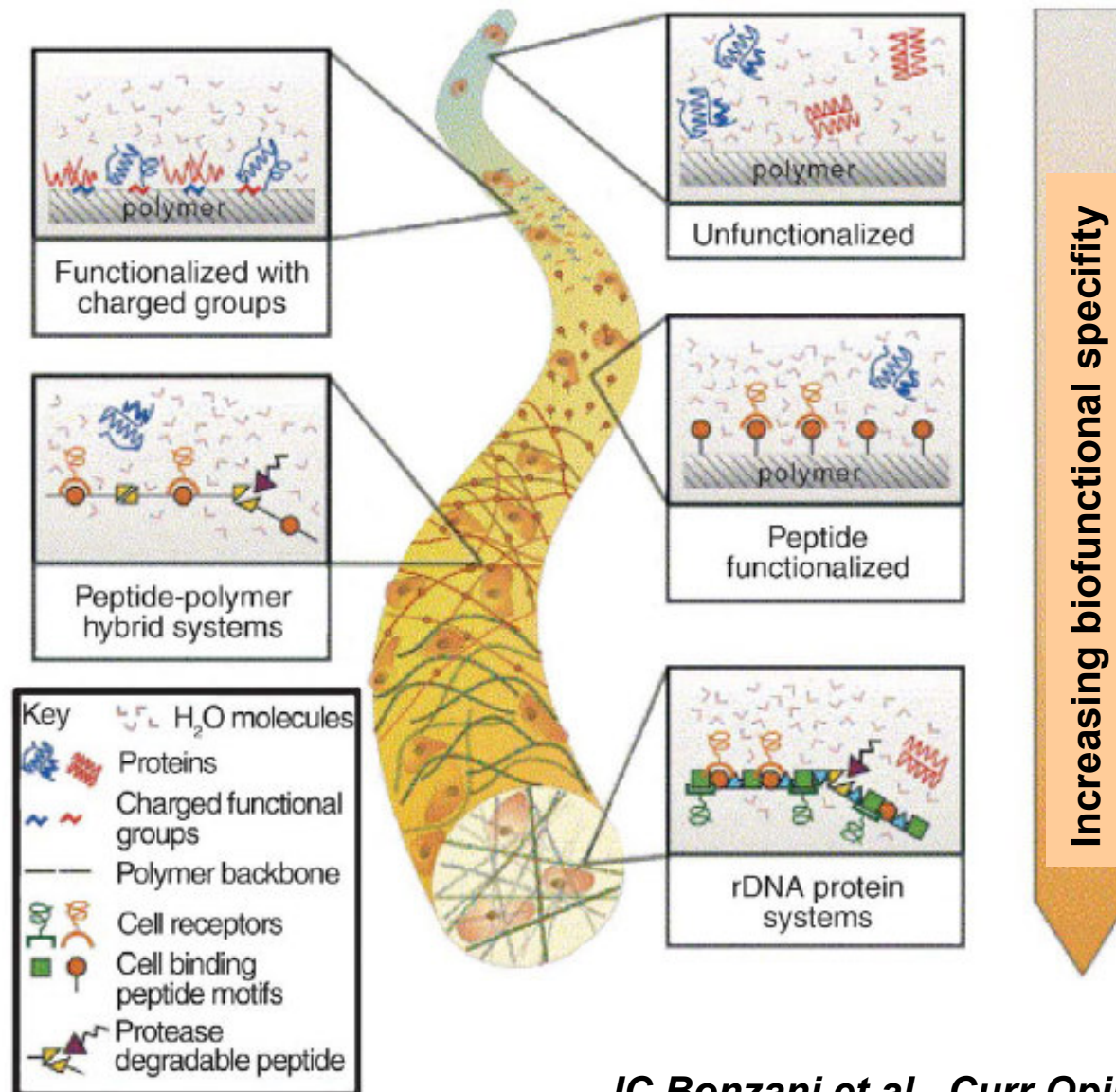
Element analysis by EDX spectrometry



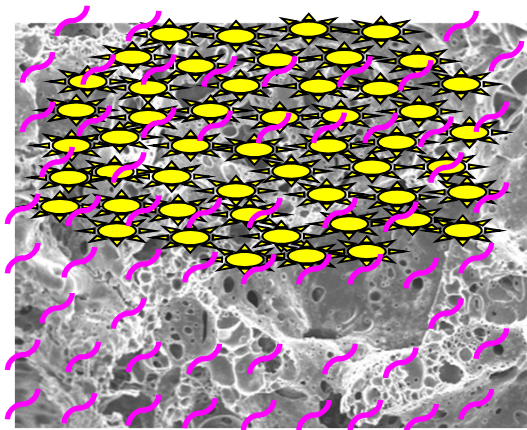
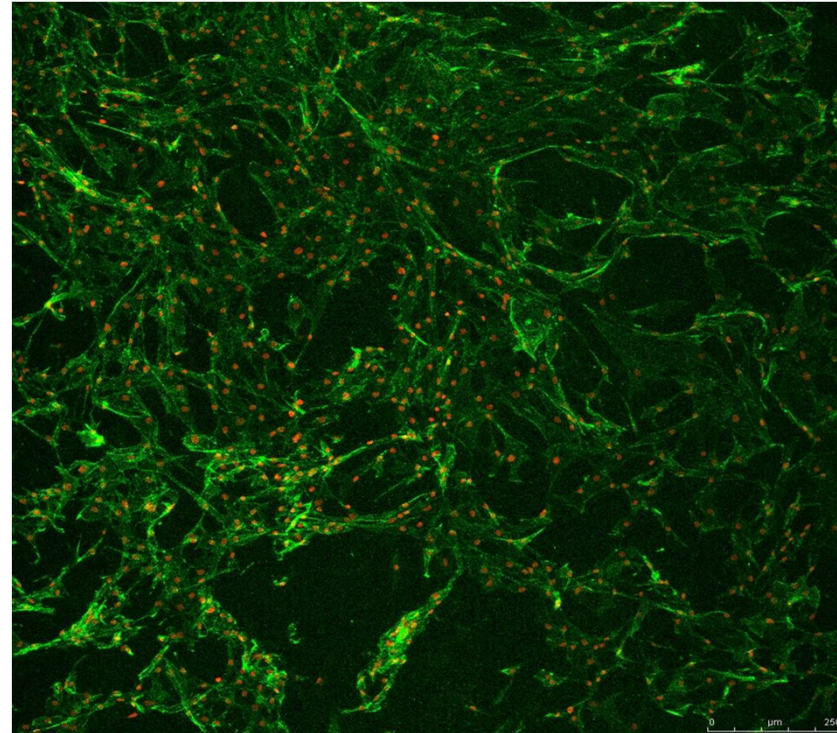
Potential biofunctionalization approaches



Enhancing material biofunctionality

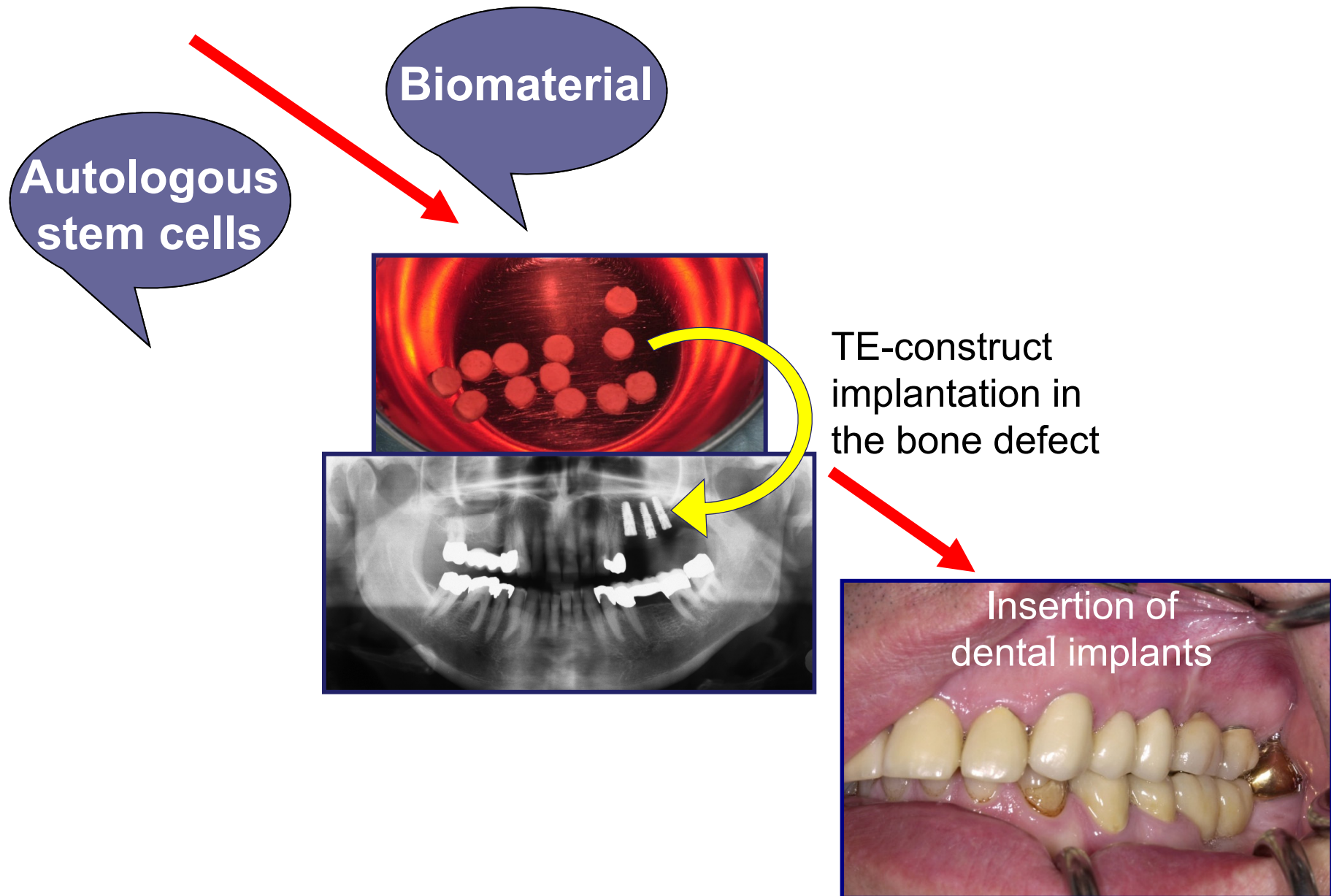


Enhancing material biofunctionality

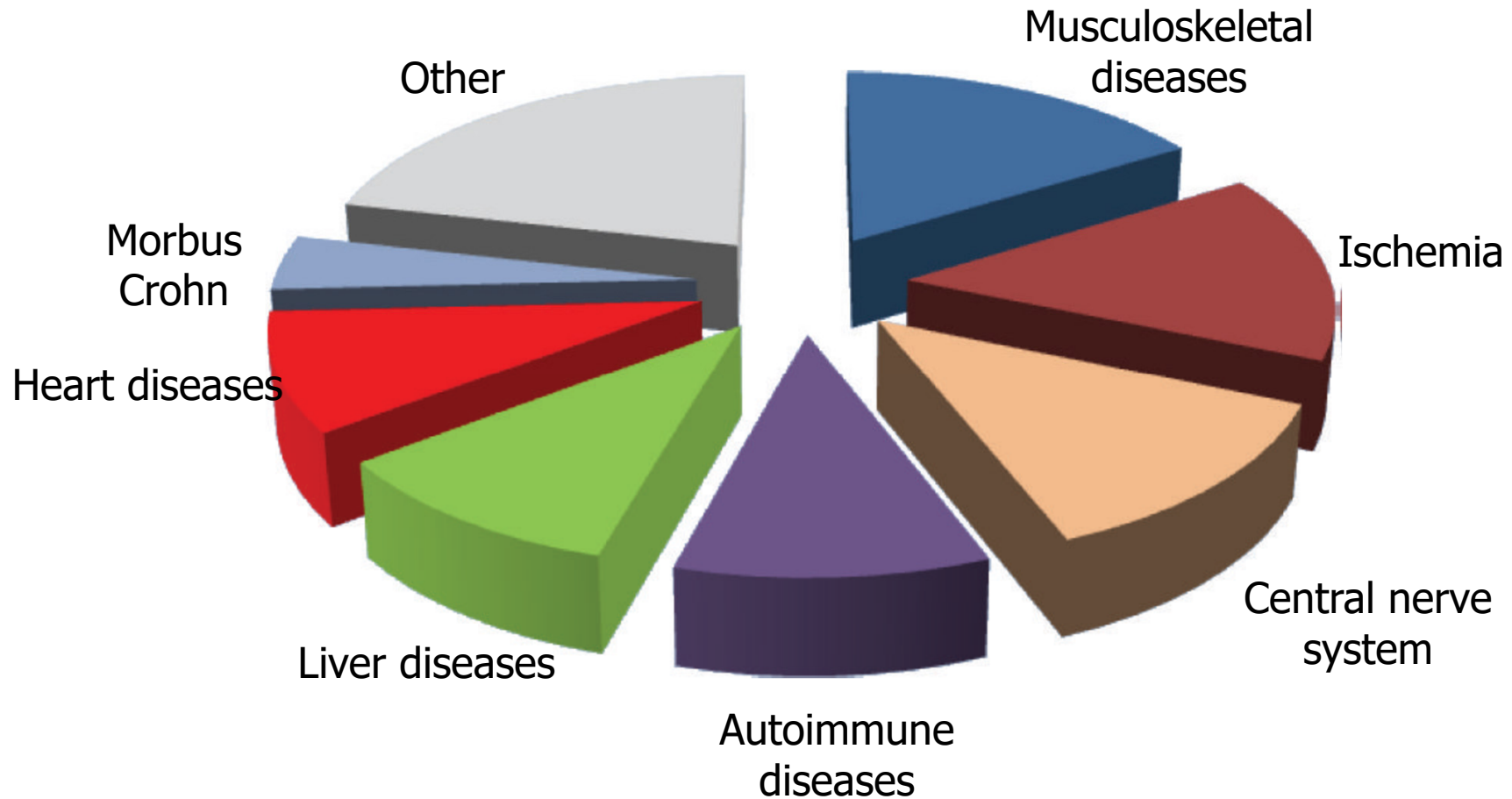


 activating molecules
 MSCs

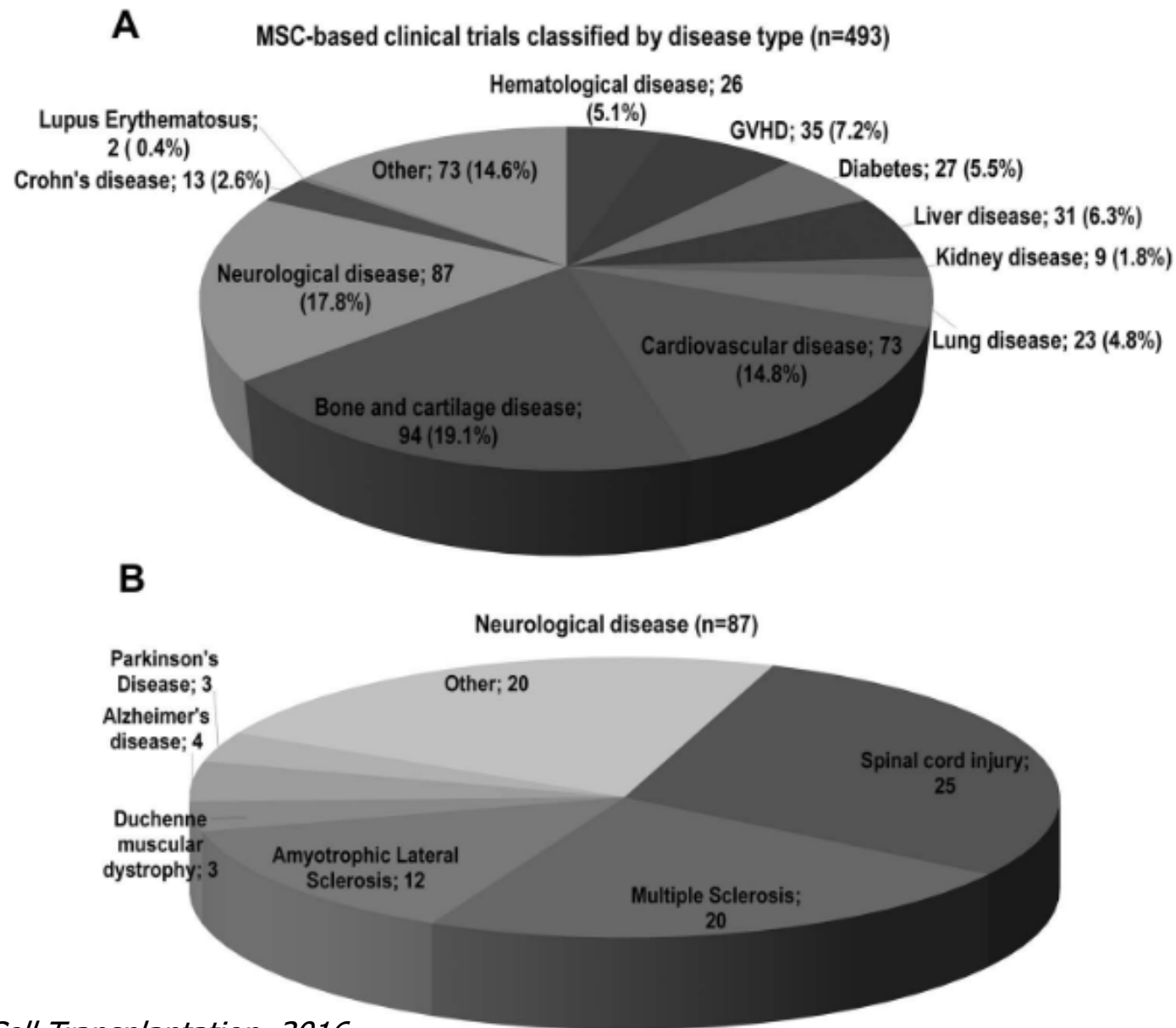
TE approach in the oral and maxillofacial surgery



Clinical trials with MSCs (n=252)



Clinical trials with MSCs (n=493)



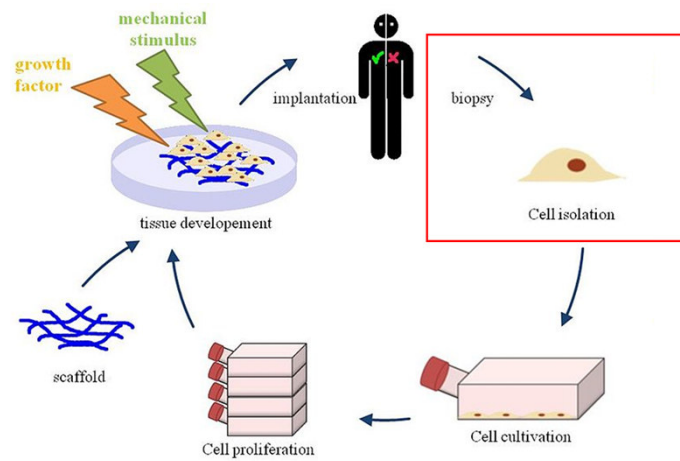
Clinical trials with MSCs (n=903)

Table 1. Distribution of clinical trials registered at *clinicaltrial.gov* by the medical field

Medical speciality	# of CT	%	Phase1	Phase2	Phase3	Phase4	Total
Traumatology	234	25.9	72	137	23	2	234
Pneumology	99	11.0	43	53	3	0	99
Neurology	97	10.7	31	61	4	1	97
Cardiology	83	9.2	21	50	12	0	83
Immunology	78	8.6	14	50	14	0	78
Hepatology	53	5.9	11	39	2	1	53
Endocrinology	47	5.2	13	30	4	0	47
Dermatology	31	3.4	9	22	0	0	31
Gastroenterology	30	3.3	9	20	1	0	30
Nephrology	27	3.0	12	15	0	0	27
Hematology	25	2.8	9	13	2	1	25
Oncology	24	2.7	10	13	1	0	24
Gynecology	16	1.8	5	11	0	0	16
Psychiatry	16	1.8	2	13	0	1	16
Urology	16	1.8	9	6	1	0	16
Ophthalmology	11	1.2	3	7	1	0	11
Odontology	10	1.1	2	8	0	0	10
Other	6	0.7	3	3	0	0	6
Total	903	100	278	551	68	6	903

Applications of MSCs

Tissue engineering



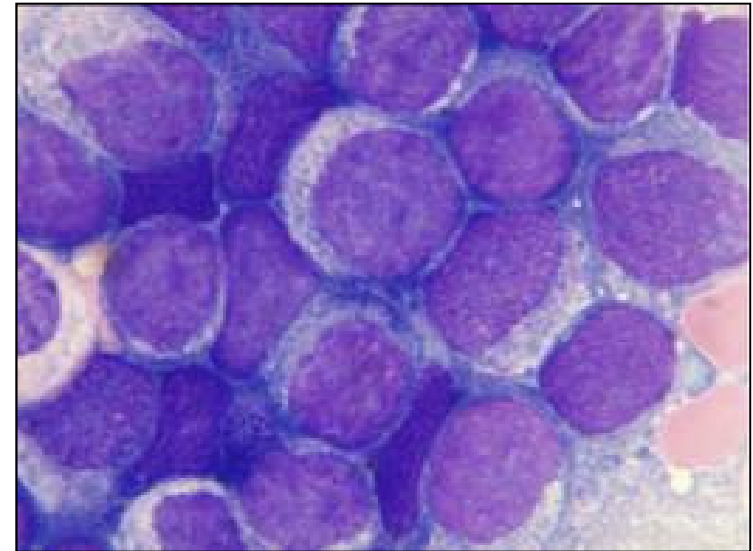
Regenerative potential
of MSCs

Additive therapy for organ transplantations



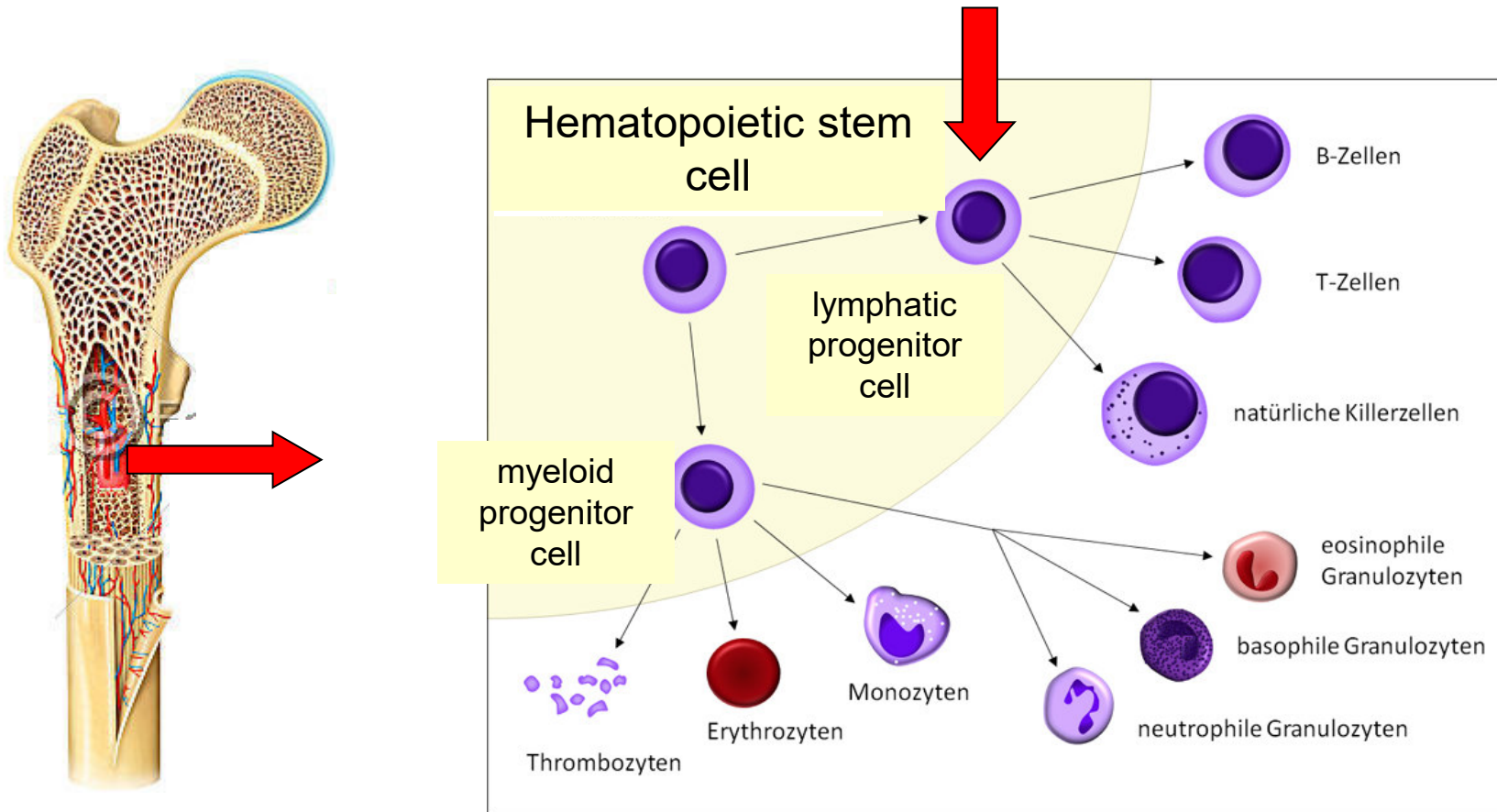
immunoregulatory
potential of MSCs

Acute lymphatic leukemia (ALL)



The most frequent tumor disease in childhood

Malignant degenerated cell



Therapeutic option – allogeneic hematopoietic stem cell transplantation

Chemo-,
radiotherapy



Leukapheresis

Isolation of hematopoietic stem
cells after **G-CSF** pre-
treatment

Complication after allogenic hematopoietic stem cell transplantation



<http://www.google.de/>



Graft-versus-host disease (GVHD) is the most frequent complication (30-40%) after an allogeneous bone marrow or hematopoietic stem cell transplantation. The transplanted cells recognize the host cells as foreign and attack them.

http://en.wikipedia.org/wiki/Graft-versus-host_disease

Treatment option for GvHD

- ➔ **MSC application**
- ➔ **50-60% of treated patients are responder!!!**

Treatment option for kidney transplantation

Autologous MSCs

	+ MSC	Ø MSC
acute rejection	7.5% 	21.6%
kidney-function	83.7% 	69.9%
infections	52.8% 	60.8%

MSCs inhibit the immune response

