SUMMER SCHOOL

CERAMIC & GLASS SCIENCE & TECHNOLOGY, APPLICATION TO BIOCERAMICS & BIOGLASSES

Instituto de Cerámica y Vidrio, CSIC
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Biocompatibility and *in vitro* tests

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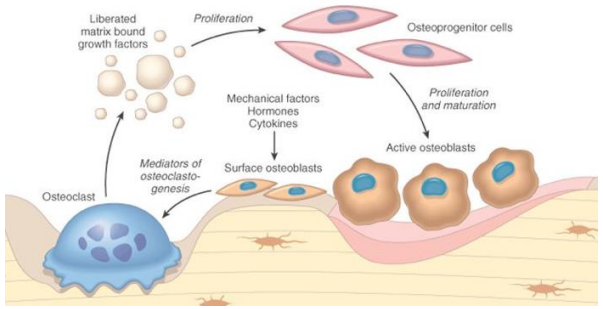
The Bone tissue

Complex mineralized living tissue, having the property of marked rigidity and strength while still maintaining some degree of elasticity.

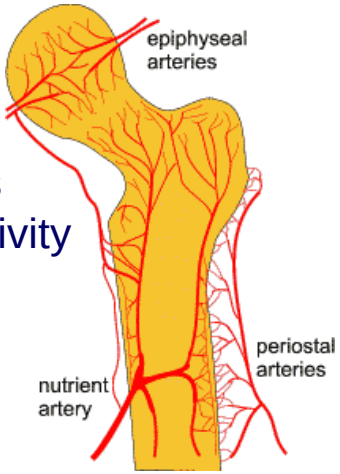
Trabecular bone
15 – 25% volume calcified.
Filled with bone marrow.
Metabolic function

Cortical bone
80 – 90% volume calcified.
Mechanical and protective functions

Bone is continuously in a **remodeling process**:
(resorption of old bone and formation of new bone)
Maintenance of bone tissue integrity and mineral homeostasis

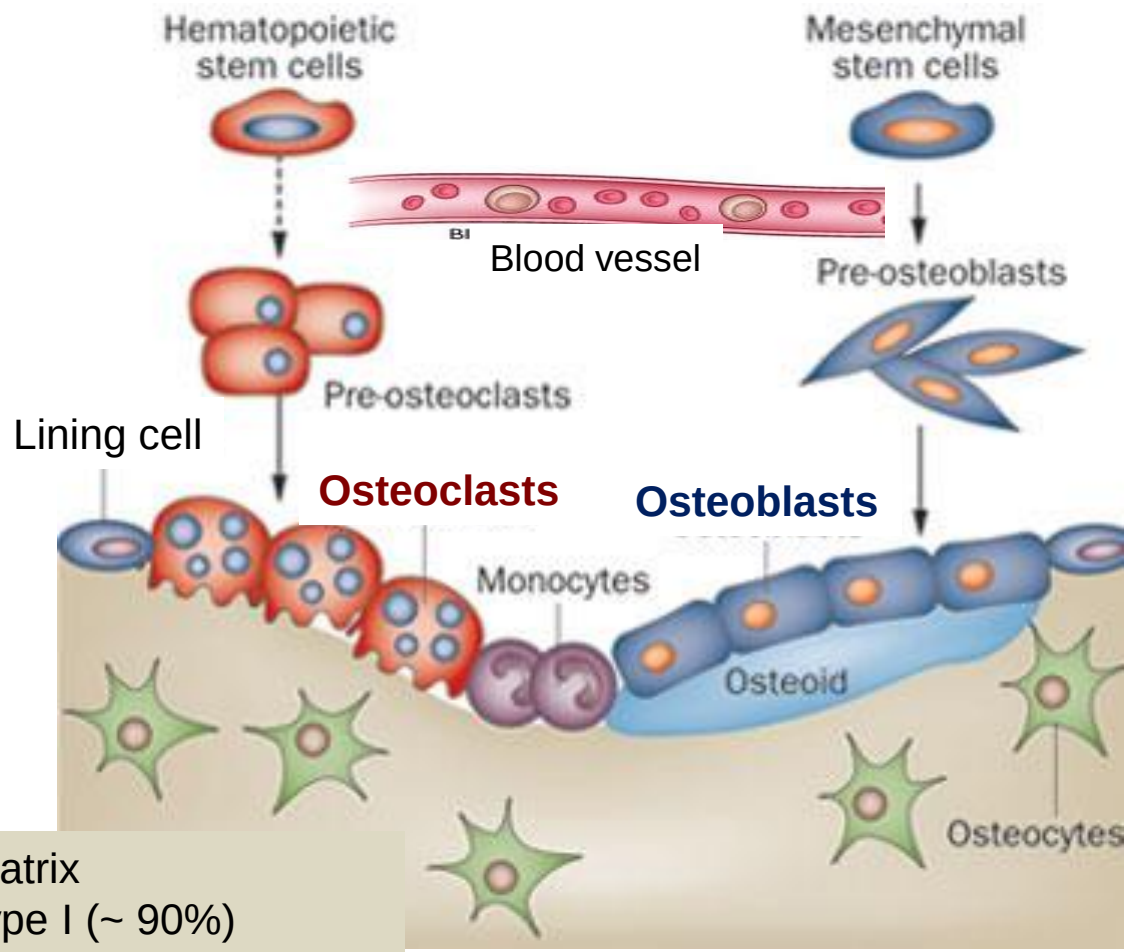


To accomplish this high metabolic activity …



Bone is **highly vascularized**
Supriment of oxygen, nutrients, progenitor cells, growth factors
Removal of metabolism products

The bone microenvironment: The bone cells



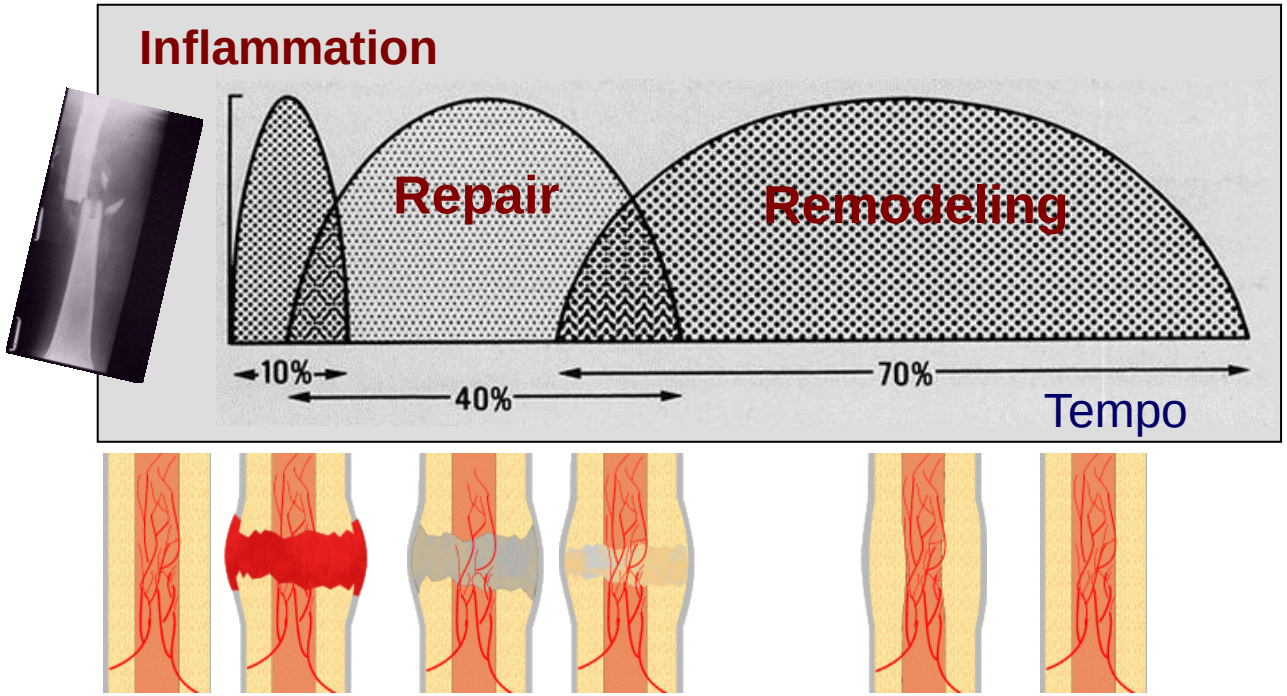
Calcified matrix
Collagen type I (~ 90%)
Glycoproteins, Proteoglycans,
Growth factors, cytokines, …
…Hydroxyapatite

- Osteoclasts
- Osteoblasts
- Bone surfaces undergoing remodeling

Osteocytes
Inside the matrix

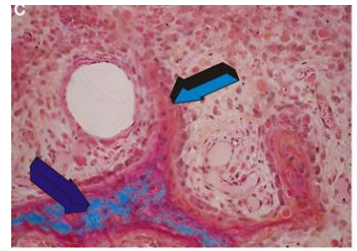
Bone lining cells
Resting bone surfaces

The Bone tissue: **Bone regeneration** (time line)



- Hematoma/inflammatory response/Angiogenesis
- Migration of bone precursor cells
- **Repair**:
  formation of an immature bone (primary stabilization)
- **Remodeling**:
  replacement of the immature bone by the mature bone
  (Occurs slowly over months to years and is strongly influenced by local mechanical stress placed on bone)

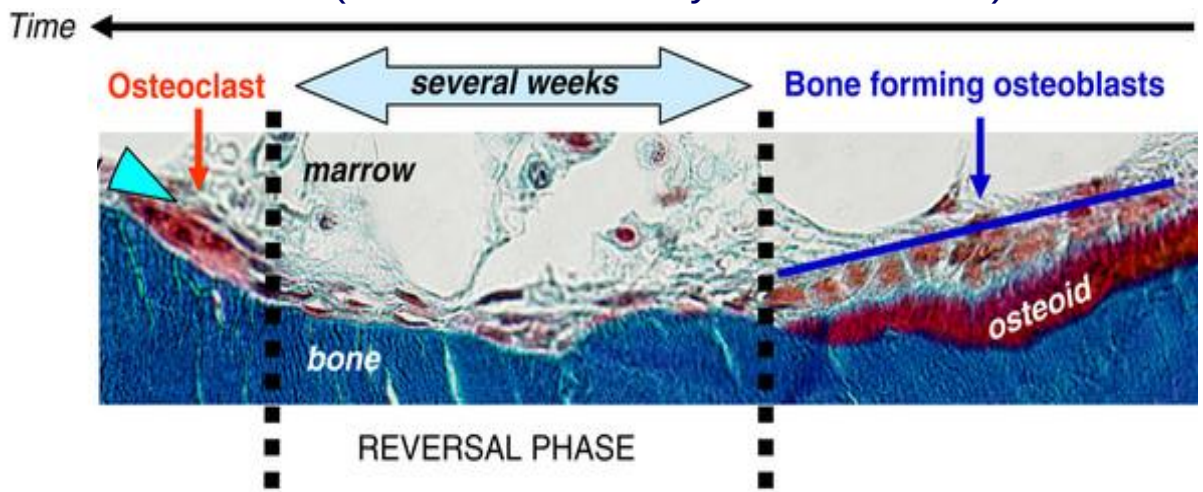
Bone formation



- Bone formation is always preceded by vascular invasion
- Osteogenesis occurs in the vicinity of newly formed blood vessels

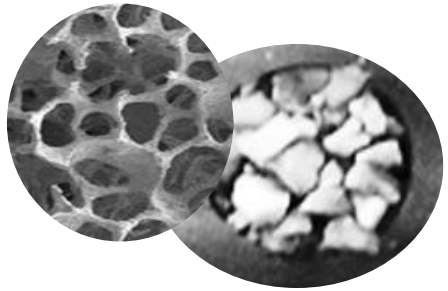
Remodeling

(coordinated activity of OC and OB)

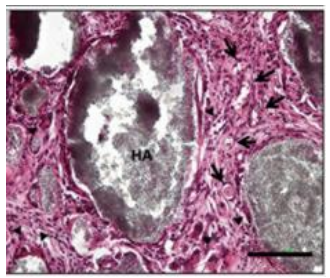


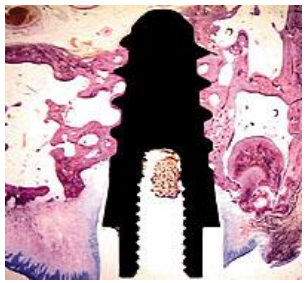
Regeneration of the form, structure and function

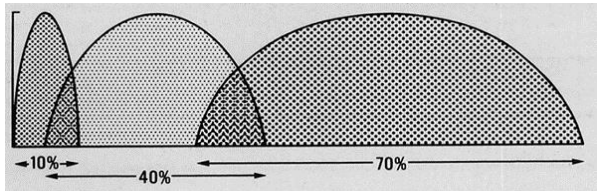
Incorporation / replacement of a bone graft



Similar to the regeneration process that occurs with a bone fracture





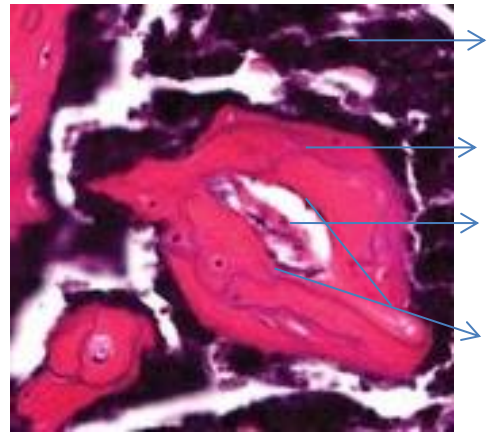


Following implantation

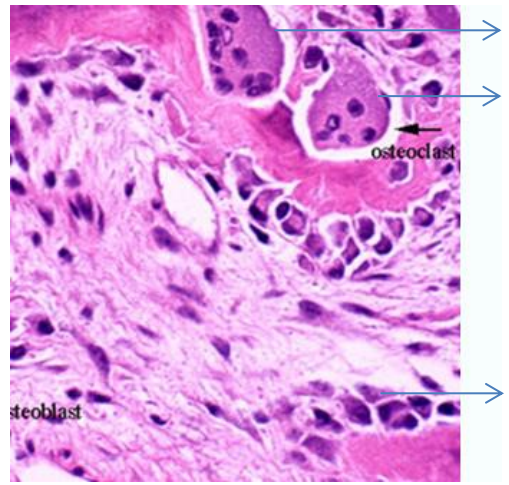
Inflammatory response/Angiogenesis
Protein adhesion to the material surface
Adhesion of osteoprogenitor cells to the material surface (via protein layer)
Formation of an immature bone
Remodeling phase: OC/OB activity

Bone formation

intimate interaction
angiogenesis/osteogenesis



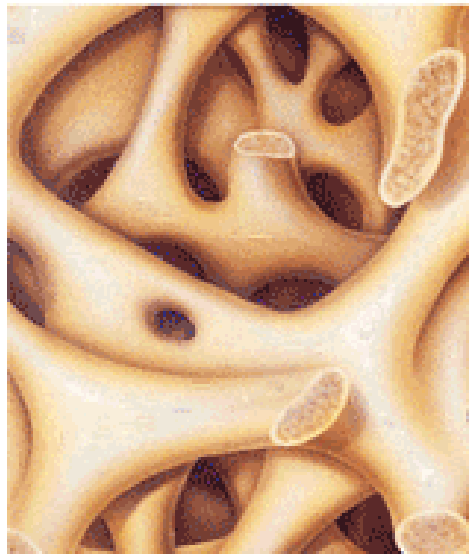
Remodeling



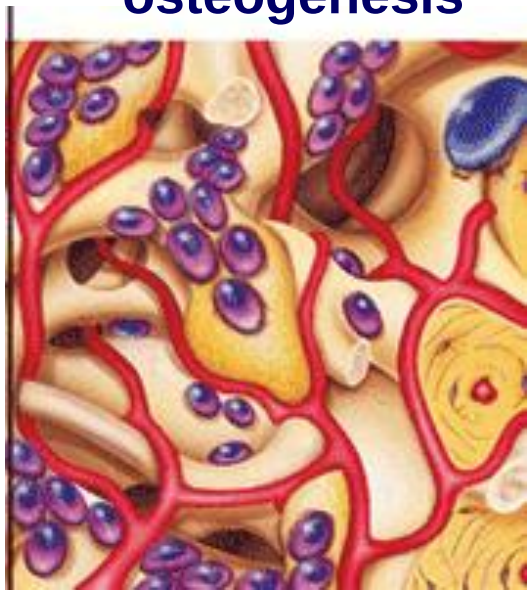
Cell culture models to address bone/biomaterial interaction

Relevant cell types and cell types interactions

Scaffold



Angiogenesis/ osteogenesis



Endothelial cells

Osteoblasts

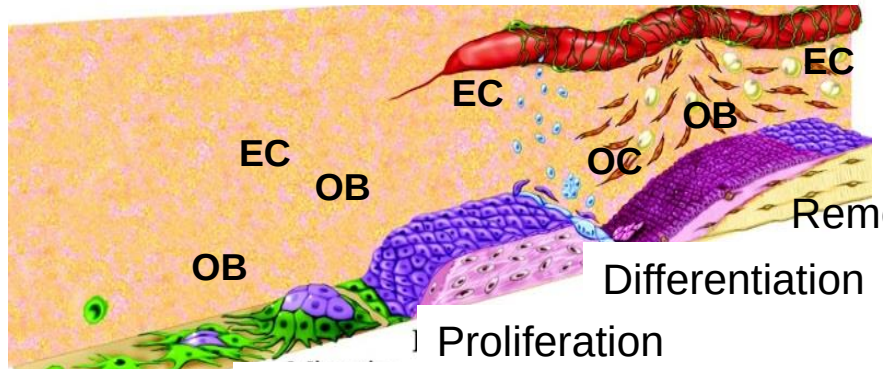
Remodeling

Osteoclasts

Osteoblasts

Cell culture models of the bone/biomaterial interface

Vascularization/angiogenesis



Osteoblast (OB) cell cultures
Bone formation

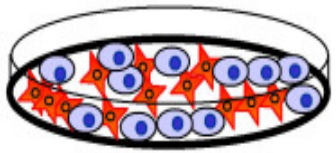
Mesenchymal stem cells

Pre-Osteoblasts

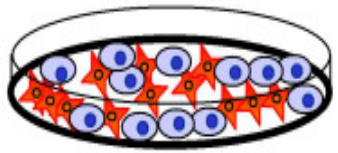
Osteoblasts

Endothelial cell (EC) cultures
Angiogenesis

EC/OB Co-cultures
Angiogenesis/ osteogenesis



OC/OB Co-cultures
Resorption/ formation

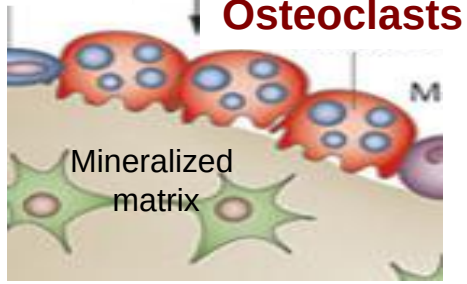


Osteoclast (OC) cell cultures
Bone resorption

Hematopoietic stem cells

Pre-Osteoclasts

Osteoclasts

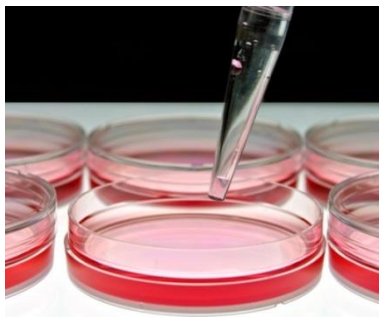


Cell cultures: equipment

Safety Cabinet

Incubator
37 ºC; humidified atmosphere
5% CO_2 / ar

Appropriate culture medium

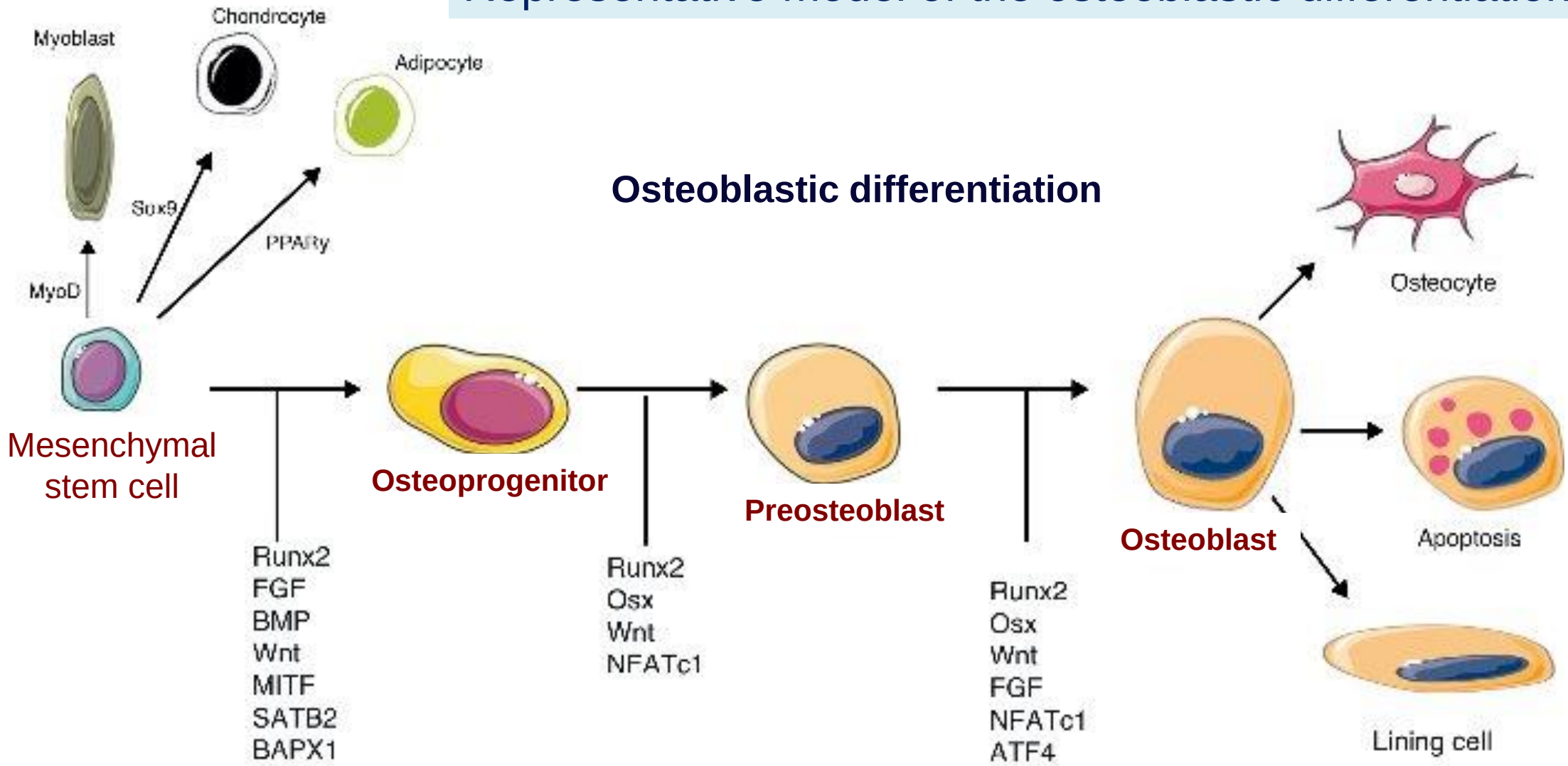


Culture flasks and plates

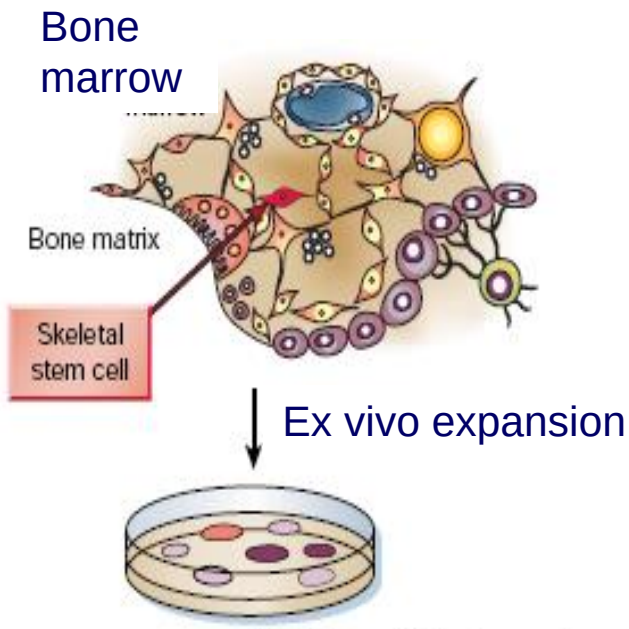
Osteoblastic cell cultures

Osteoblastic cell cultures

Representative model of the osteoblastic differentiation



Osteoblastic cell cultures



→ Primary culture

- Culture of a bone marrow suspension
- Outgrowth from bone explants
- Comercial MSC

37 ºC; 5% CO_2/air
1 – 2 weeks

(70 – 80% confluency)

→ Subculture
(to expand the cells)

Standard culture conditions:
Culture medium: alfa-MEM; DMEM
10% fetal bovine serum
Penicillin / Streptomycin;
Anphotericin B

50 mg/ml ascorbic acid
Dexamethasone (10 nM)
b-glycerophosphate (10 mM)

Osteoblastic differentiation

Characterization of the cell behaviour:

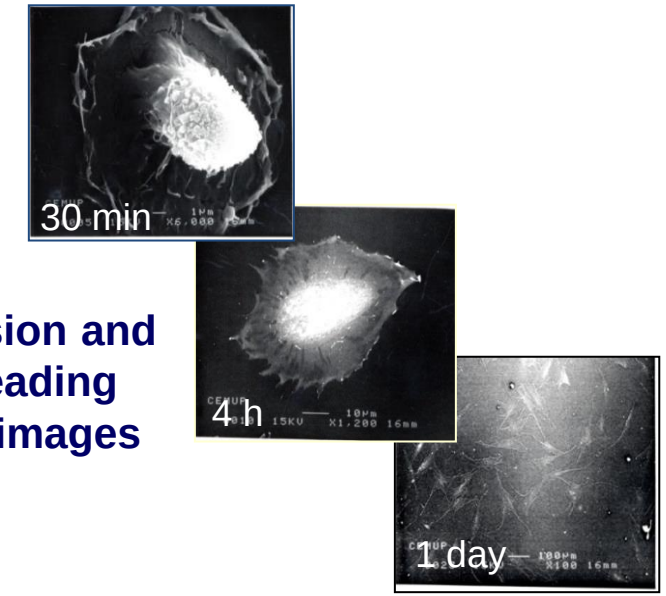
- Cell adhesion to the material substrate
- Cell viability/Proliferation (MTT, ADN, Protein)
- Apoptosis
- Cell cycle
- Morphology/F-actin cytoskeleton
- Focal adhesion points
- Expression of osteoblastic genes
  (Runx-2; Col-1; ALP; OC; RUNKL; OPG; …)
- Functional activity
  Alkaline phosphatase activity
  Formation of a mineralized matrix
- Intracellular signalling pathways

Biochemical, histochemical, immunohistochemical and molecular methodologies; SEM, CMSM

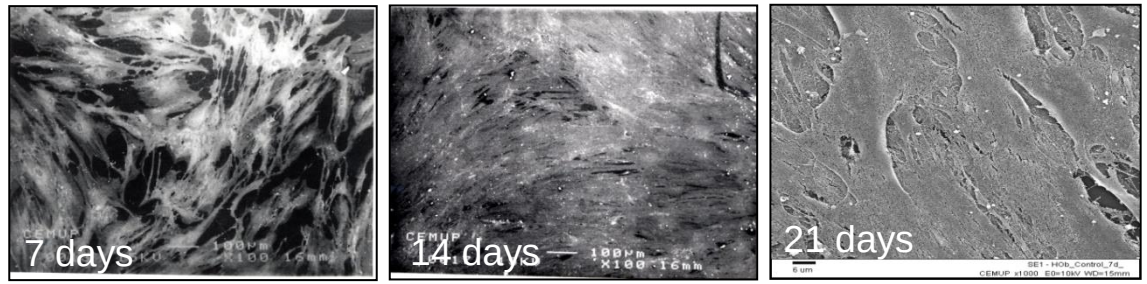
Osteoblastic cell cultures: Proliferation / differentiation pathway

Human bone marrow-derived osteoblastic cell cultures

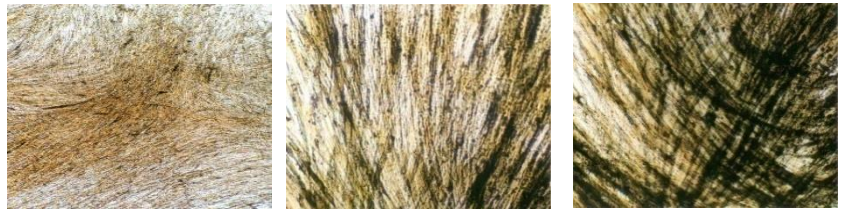
Characterization of the cell behaviour



Adhesion and spreading SEM images

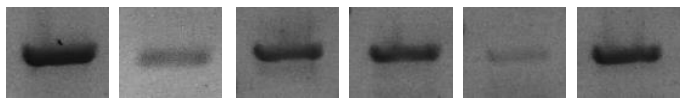


Cell proliferation (SEM images

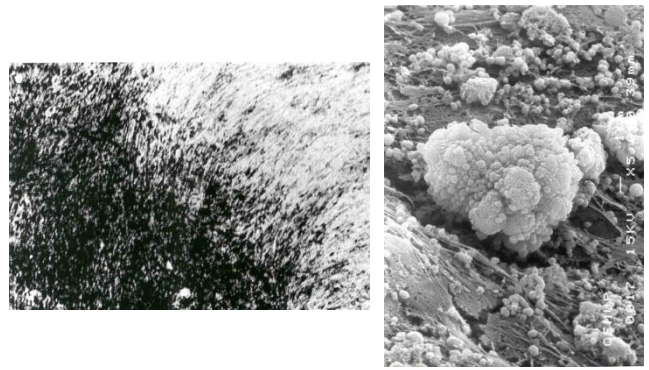


Alkaline phosphatase staining

Gene expression profile



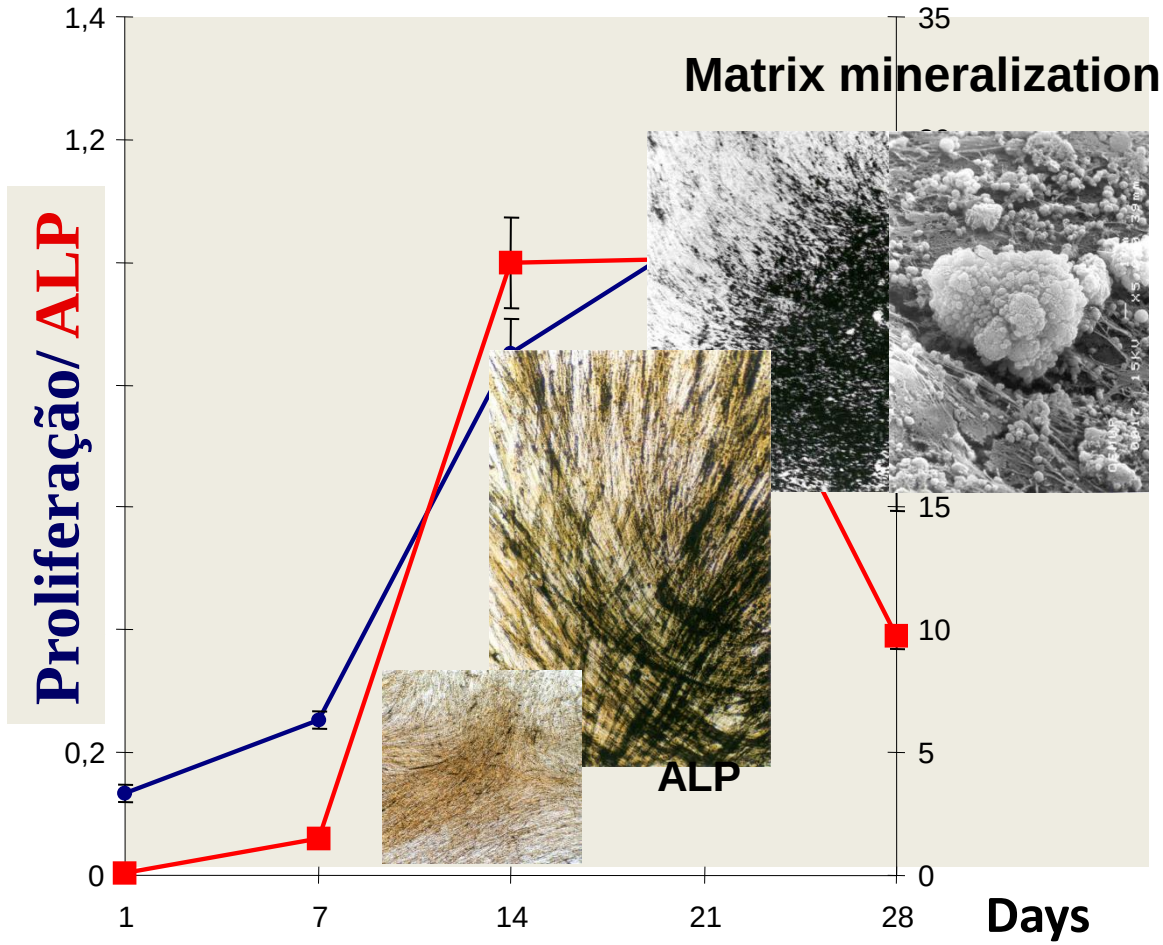
Runx-2; Col-1; ALP; OC; OPG; RANKL; …..



Matrix mineralization (SEM)

Osteoblastic cell cultures: Proliferation / differentiation pathway

Human bone marrow-derived osteoblastic cell cultures

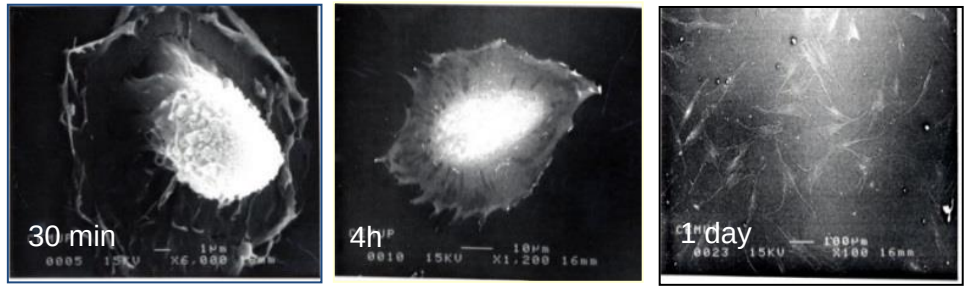


Inverse relationship between proliferation and differentiation

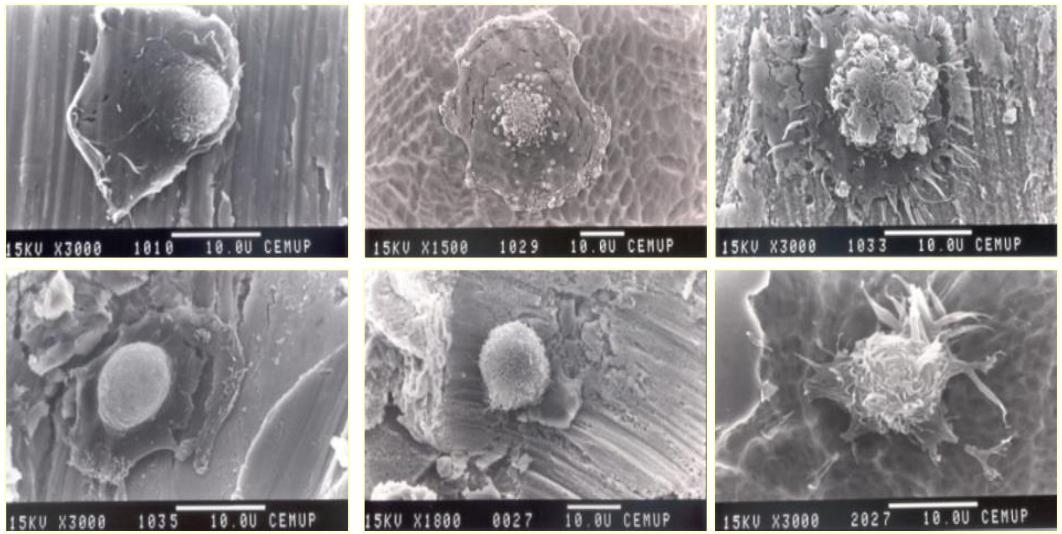
Osteoblastic cell cultures: cells / biomaterials interactions

Human bone marrow-derived osteoblastic cell cultures

Cell ahesion: standard tissue culture plates

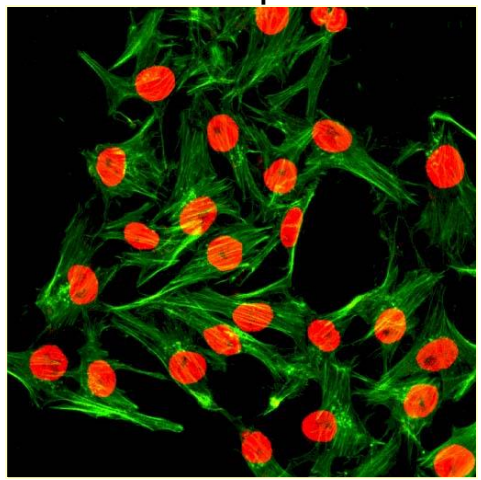


Ahesion to HA substrates with different topographies; 30 min

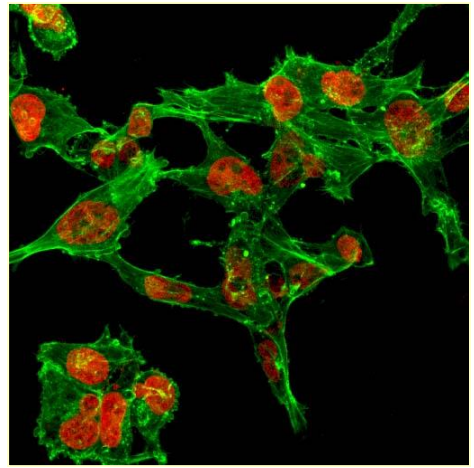


Cell morphology

Culture plates

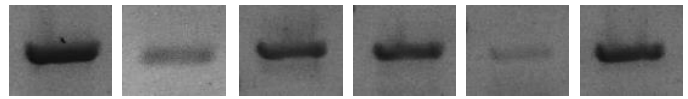


Degradable ceramic



CLSM of cells stained for F-actin cytoskeleton (green) and nucleus (red)

Gene expression profile

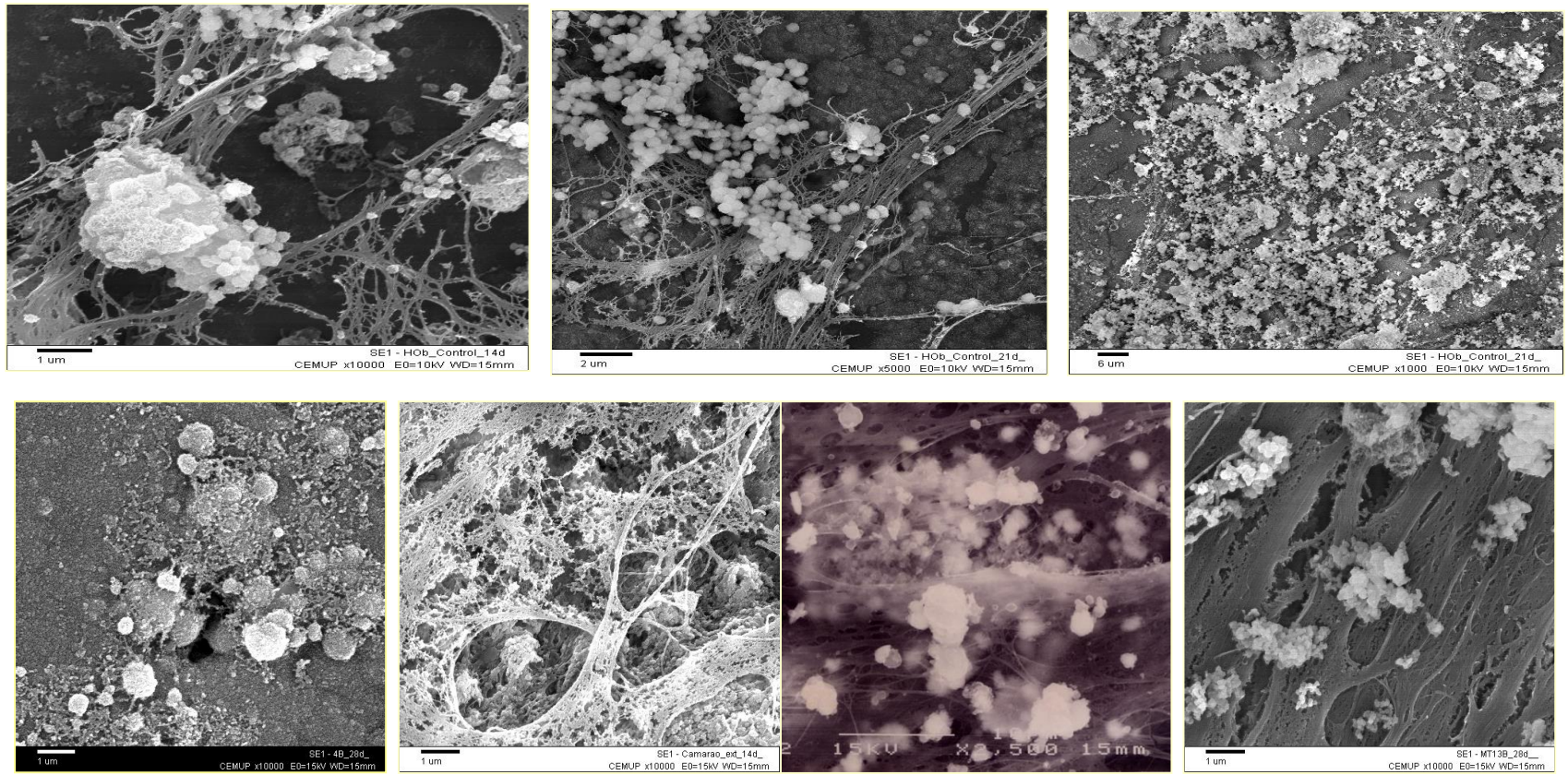


Runx-2; Col-1; ALP; OC; OPG; RANKL; …..

Osteoblastic cell cultures

Human bone marrow-derived osteoblastic cell cultures

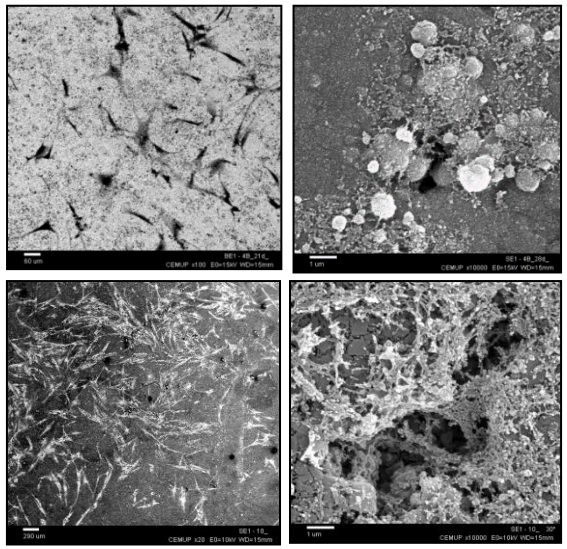
Formation of a cellular mediated mineralized matrix in several biomaterials



Osteoblastic cell cultures

Illustrative studies

Ceramic composites

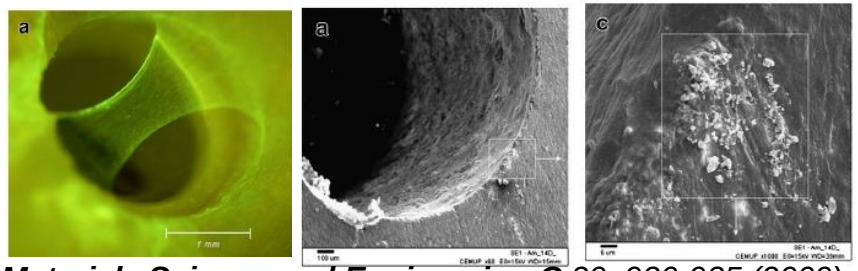


Bonelike®

Glass/Si3N4 composites

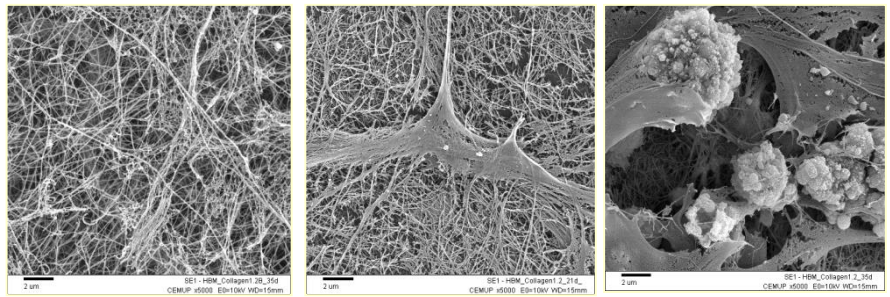
Biomaterials, *26: 485-493 (2005)*

Macroporous ceramics



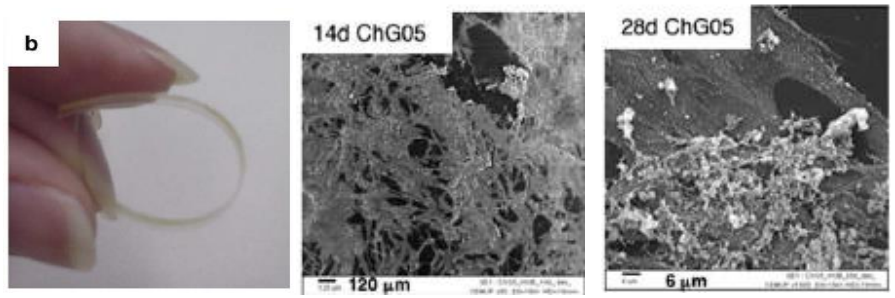
Materials Science and Engineering C *29: 930-935 (2009)*

Collagen substrates



Connective Tissue Research, *50: 336-346; 2009*

Chitosan hybrid membranes

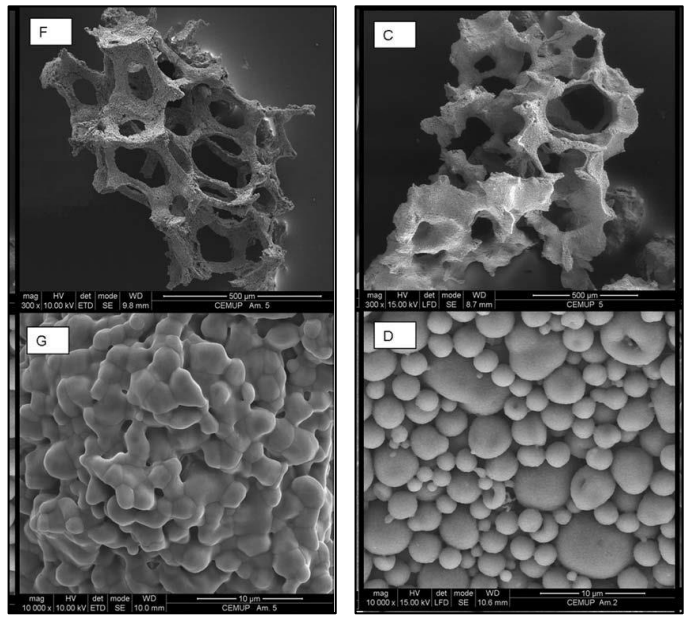


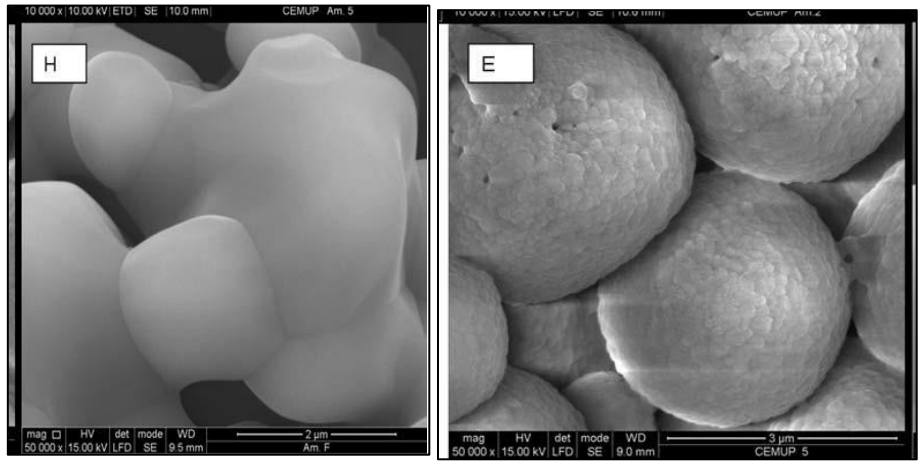
Biomaterials 26: 485-493 (2005) ***Acta Biomaterialia*** *5: 346-355 (2009)*

Osteoblastic cell cultures

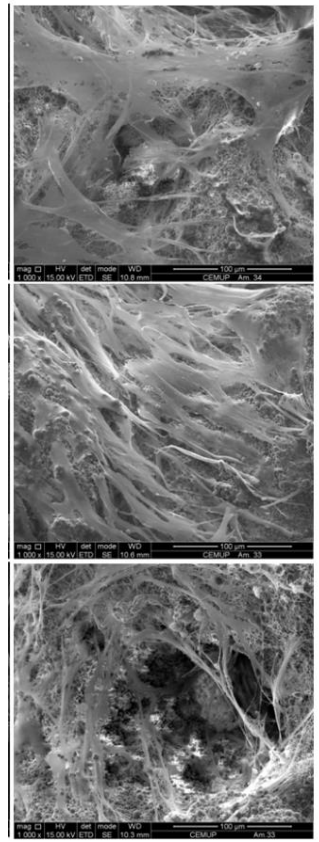
Illustrative study

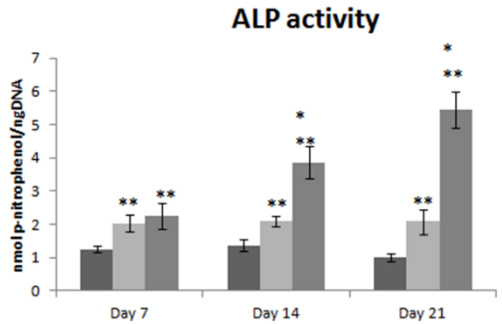
microHA **nanoHA**





Micro and Nanostructured macroporous ceramic scaffolds



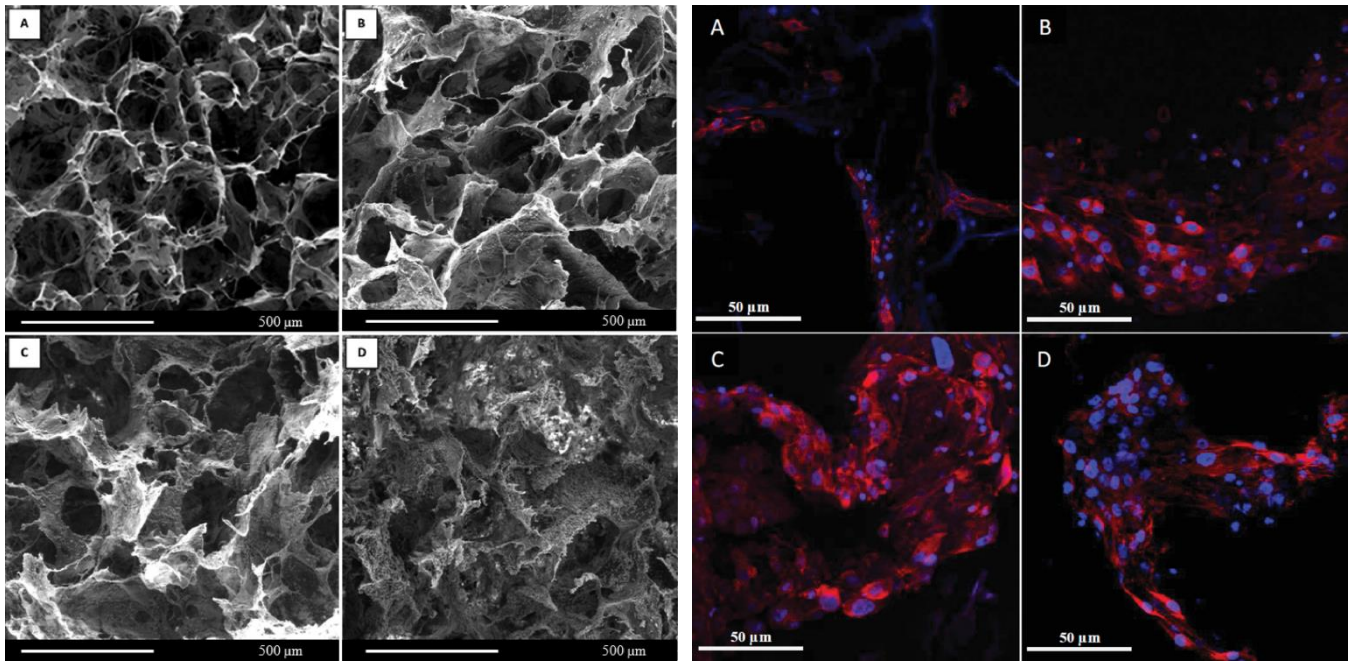


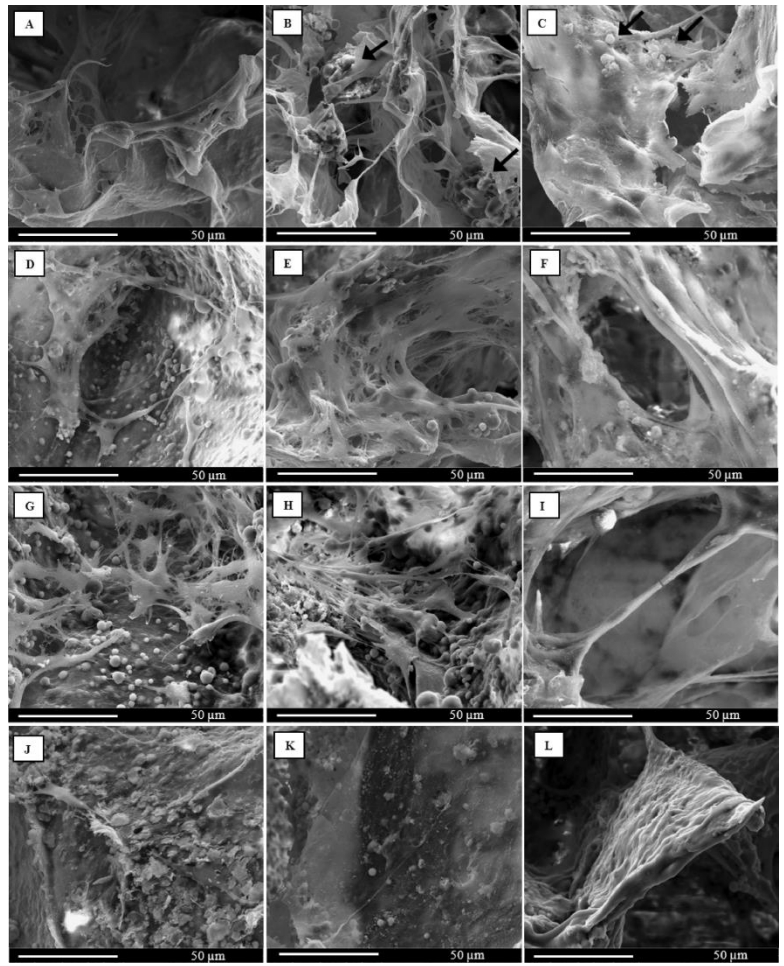
J Biomed Mater Res A 101: 1080-1094 (2013)

Osteoblastic cell cultures

Illustrative study

Collagen-nanohydroxyapatite biocomposite scaffolds



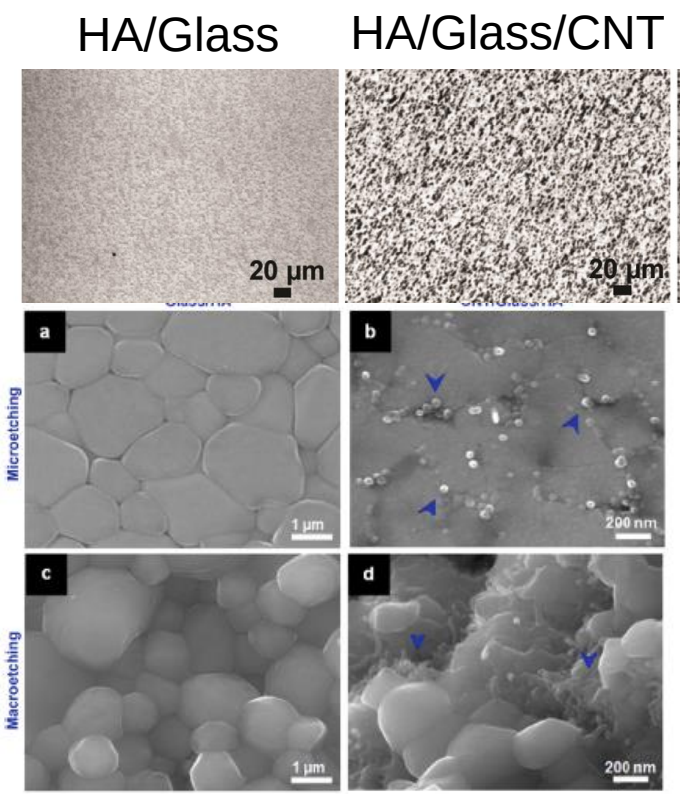


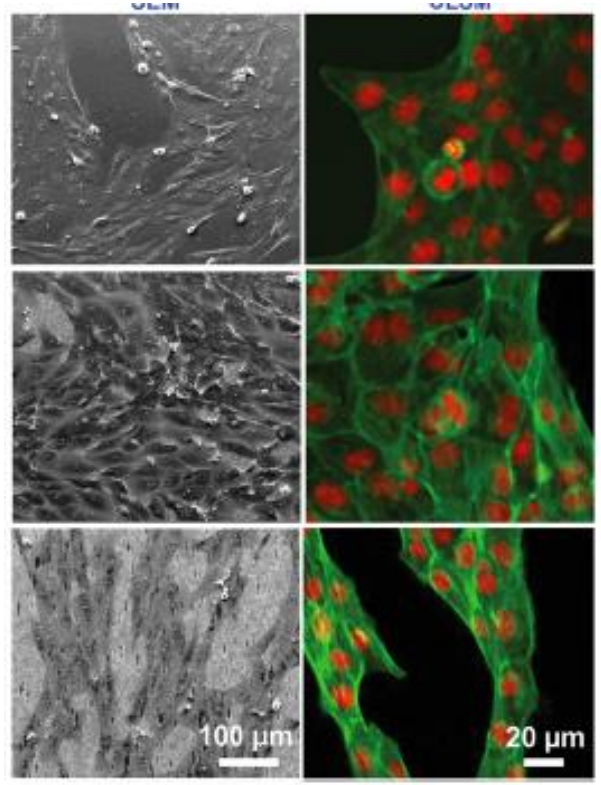
J Biomed Mater Res Part A: 101A: 10–1094 (2013)

Osteoblastic cell cultures

Illustrative study

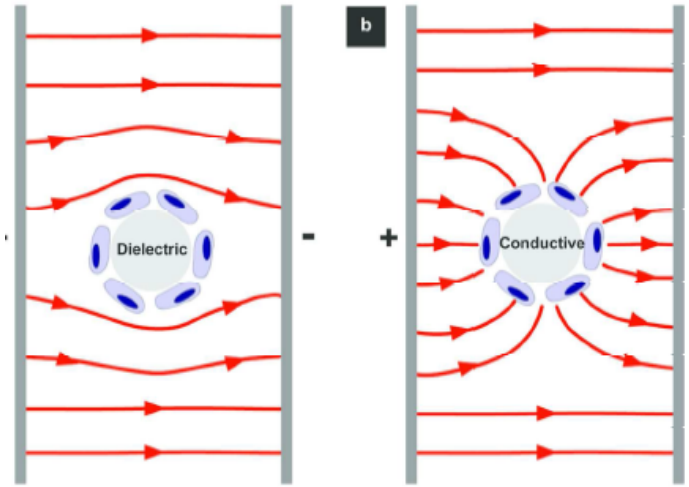
Bioceramics/carbon nanotubes (CNT) composites
(conductive substrates) Electrical stimulation





HA/Glass

HA/Glass/CNT



Nanoscale; 04 (2015)

J Materials Chemistry B; 3:1831-45 (2015)

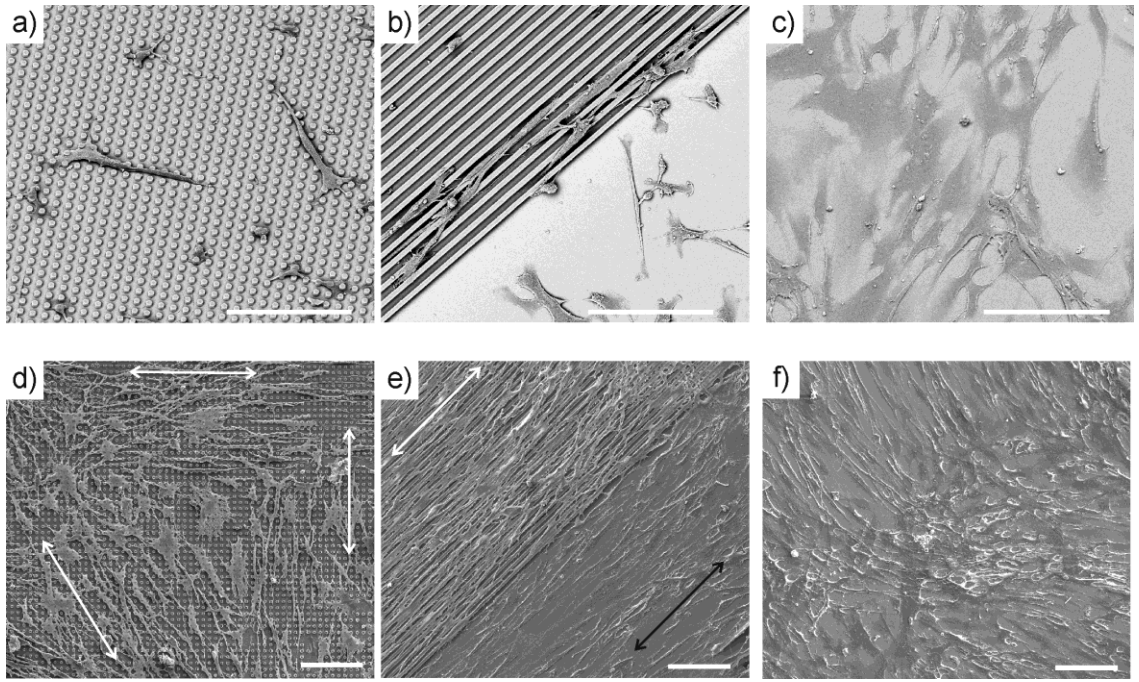
J Biomedical Nanotechnology; 10:725-743 (2014)

Nanotechnology 25**;** 145602 (2014)

Osteoblastic cell cultures

Illustrative study

Guided proliferation of osteoblastic cells on patterned surfaces



J Biomed Mater Res B, 101: 762-9 *(2013)*

Dental Materials, 28:1250-1260 *(2012)*

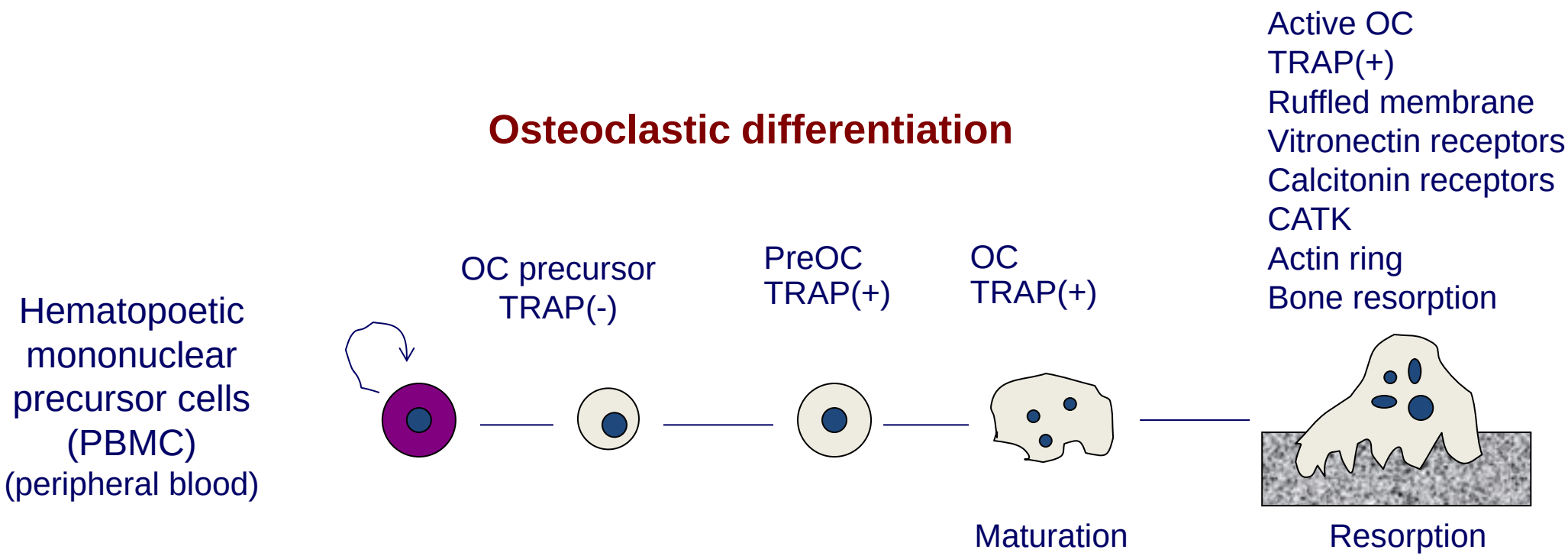
Dental Materials 27: 581-589 (2011)

Microsc Microanal 16:670-67 (2010)

Osteoclastic cell cultures

Osteoclastic cell cultures

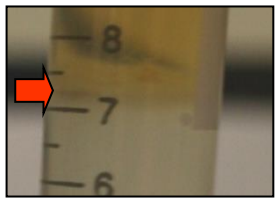
Representative model of the osteoclastic differentiation



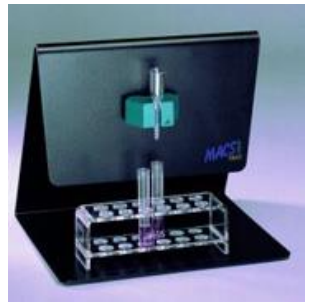
Osteoclastic cell cultures

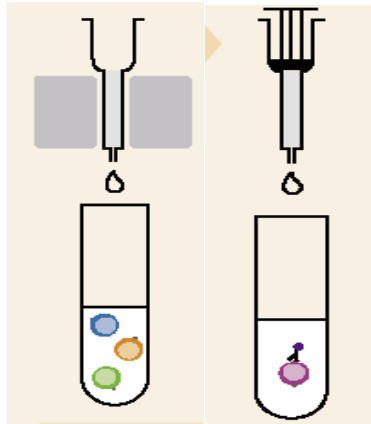
Peripheral blood

Buffy coat



Magnetic separation of CD14+ cells





Obtention:

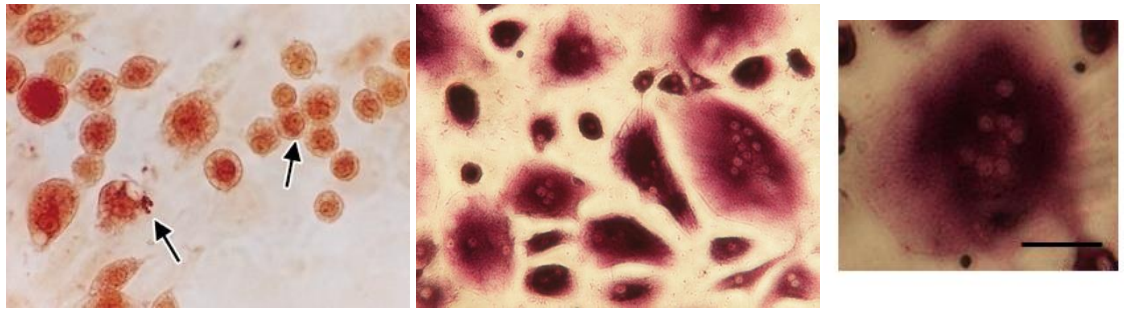
- Isolation of the peripheral mononuclear cells from a buffy coat
  (Buffy coat + PBS) + Histopaque:
  mixt of monocytes, platelets and lymphocytes
- Magnetic separation of CD14+ cells
- Cell culture (2 x 10^6 cell/ml); 21 days
  a-MEM; 10% human AB serum;
  1% glutamine; 30 mg/ml ascorbic acid
  25 ng/ml MCSF; 30 ng/ml RANKL

Characterization of the cell behaviour:

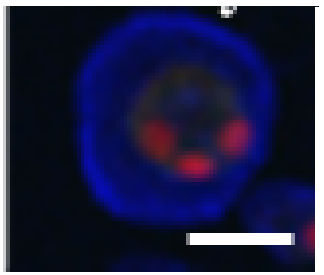
- Cell adhesion to the material substrate
- Total protein content
- Apoptosis
- Morphology
- Formation of actin rings
- Immunostaining of Calcitonin and Vitronectin receptors
- Expression of osteoclastic genes
  (c-myc; c-src; TRAP; CATK, CA; …)
- Functional activity
  TRAP activity
  Formation TRAP+ multinucleated cells
  Resorption activity
- Intracellular signaling pathways

Osteoclastic cell cultures

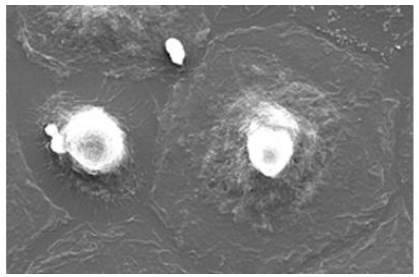
Formation of multinucleated cells (TRAP staining)



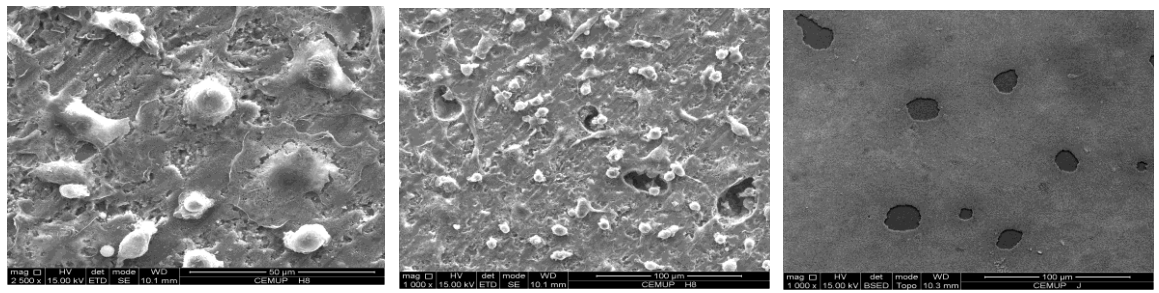
Actin ring



SEM

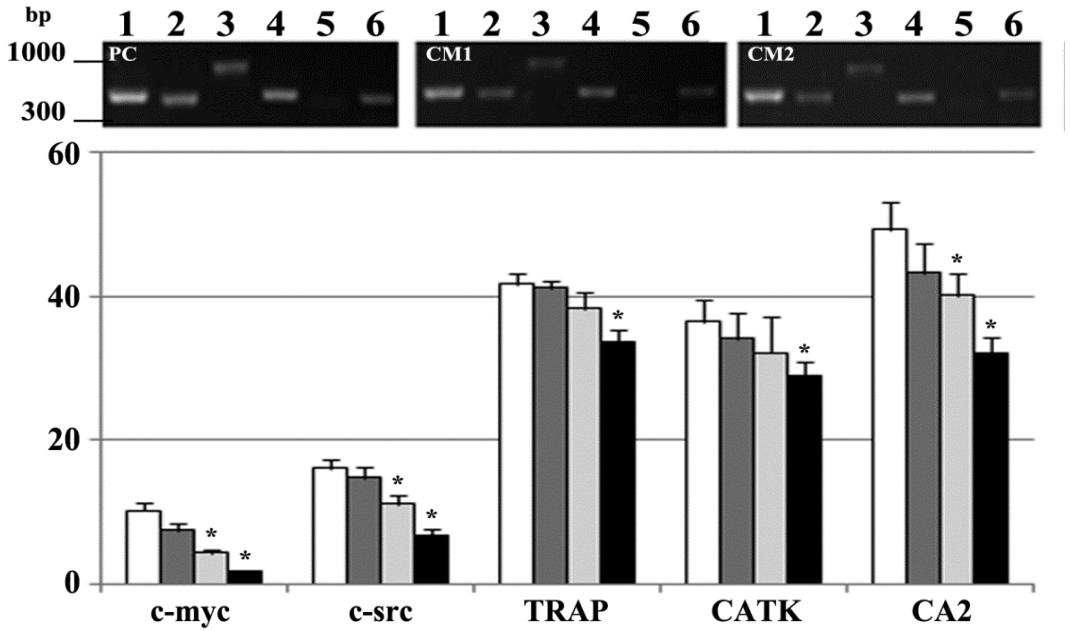


SEM: resorption activity



Characterization of the cell behaviour

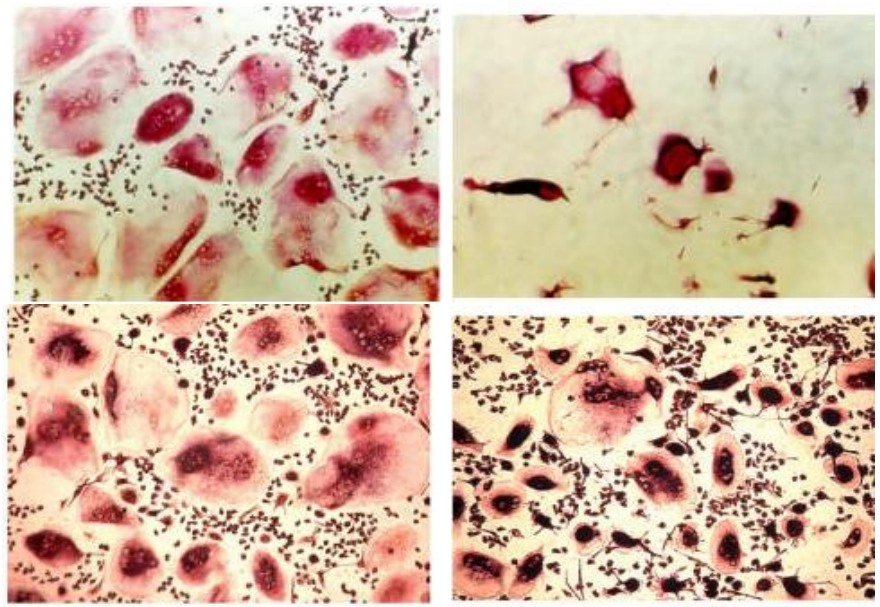
Expression of osteoclastogenic genes



Osteoclastic cell cultures

Illustrative studies

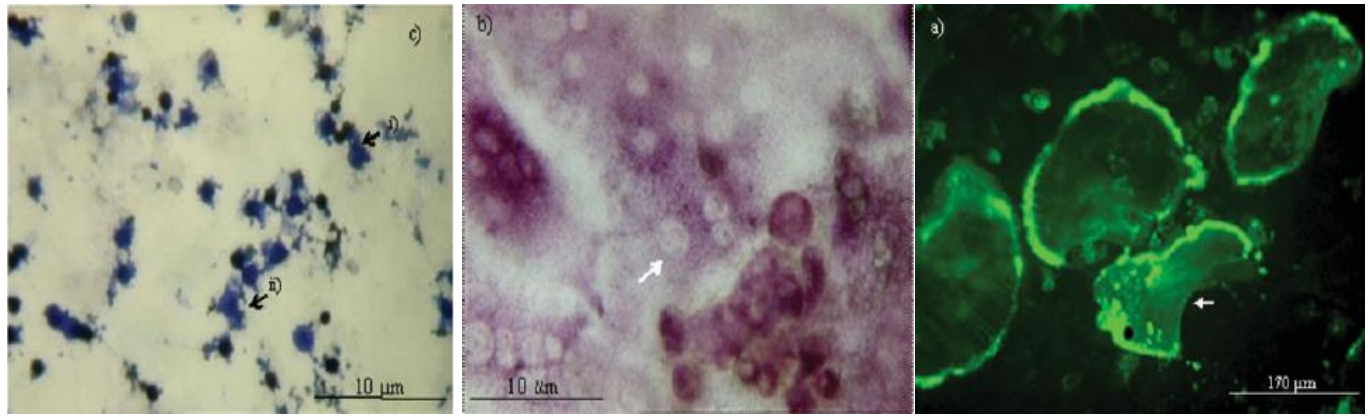
TRAP staining in different biomaterials



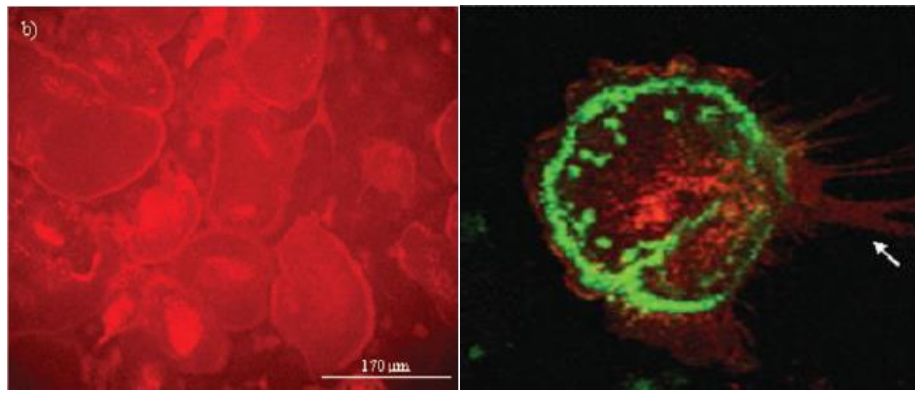
Hydroxyapatite seeded with OC cells

Multinucleated cells

Actin rings



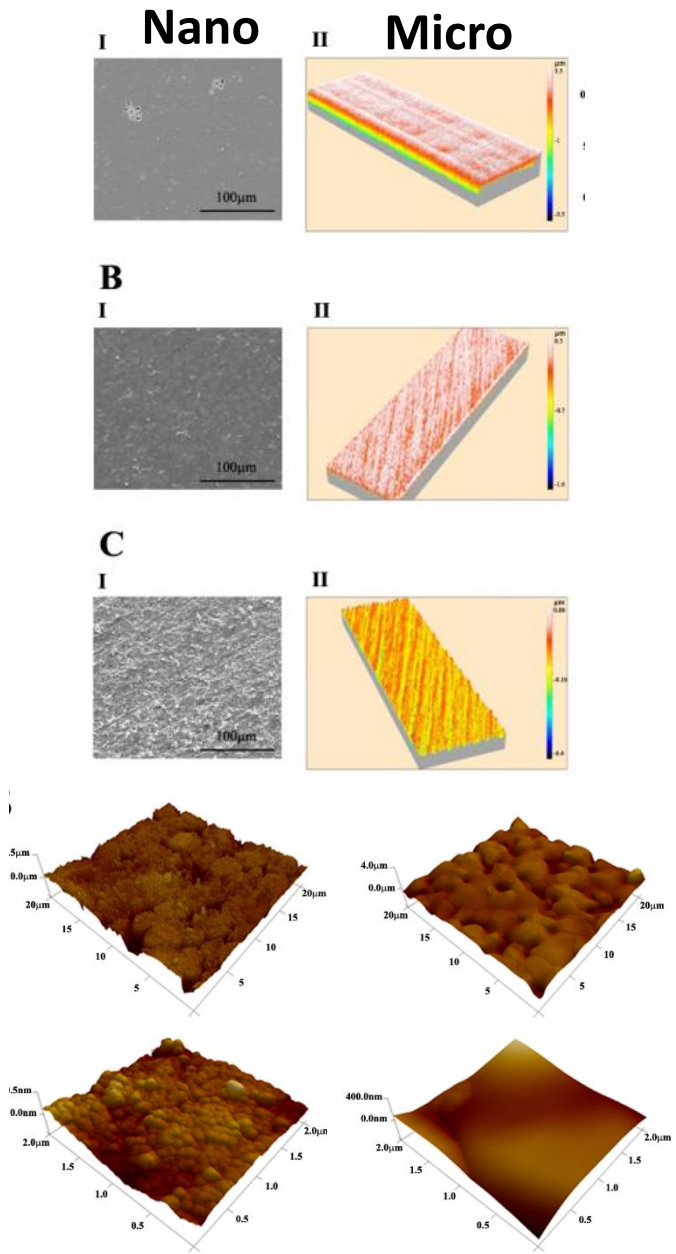
Vitronectin receptor



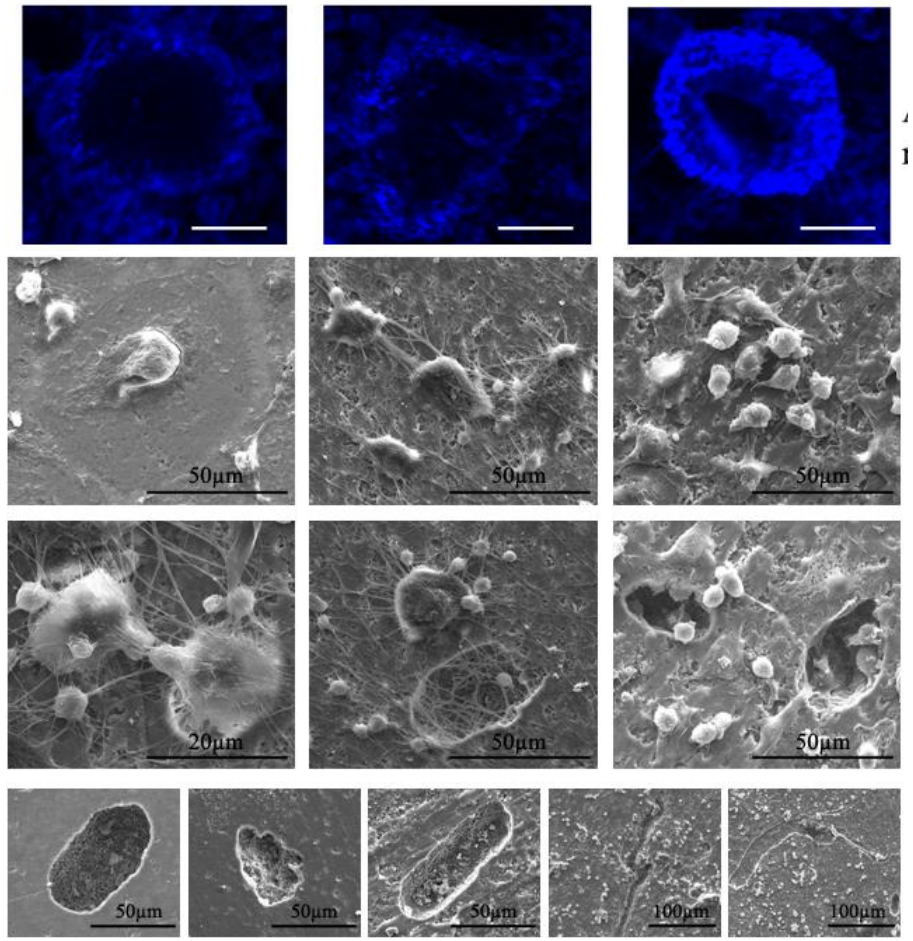
Osteoclastic cell cultures

Illustrative study

Modulation of bone cell behaviour by surface topography in **Hydroxyapatite substrates)**



Osteoclastic cell cultures

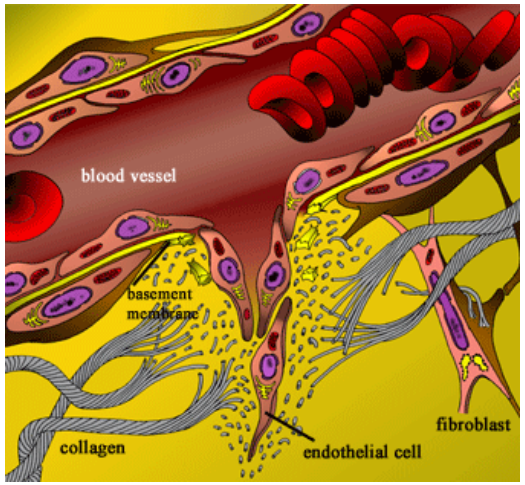


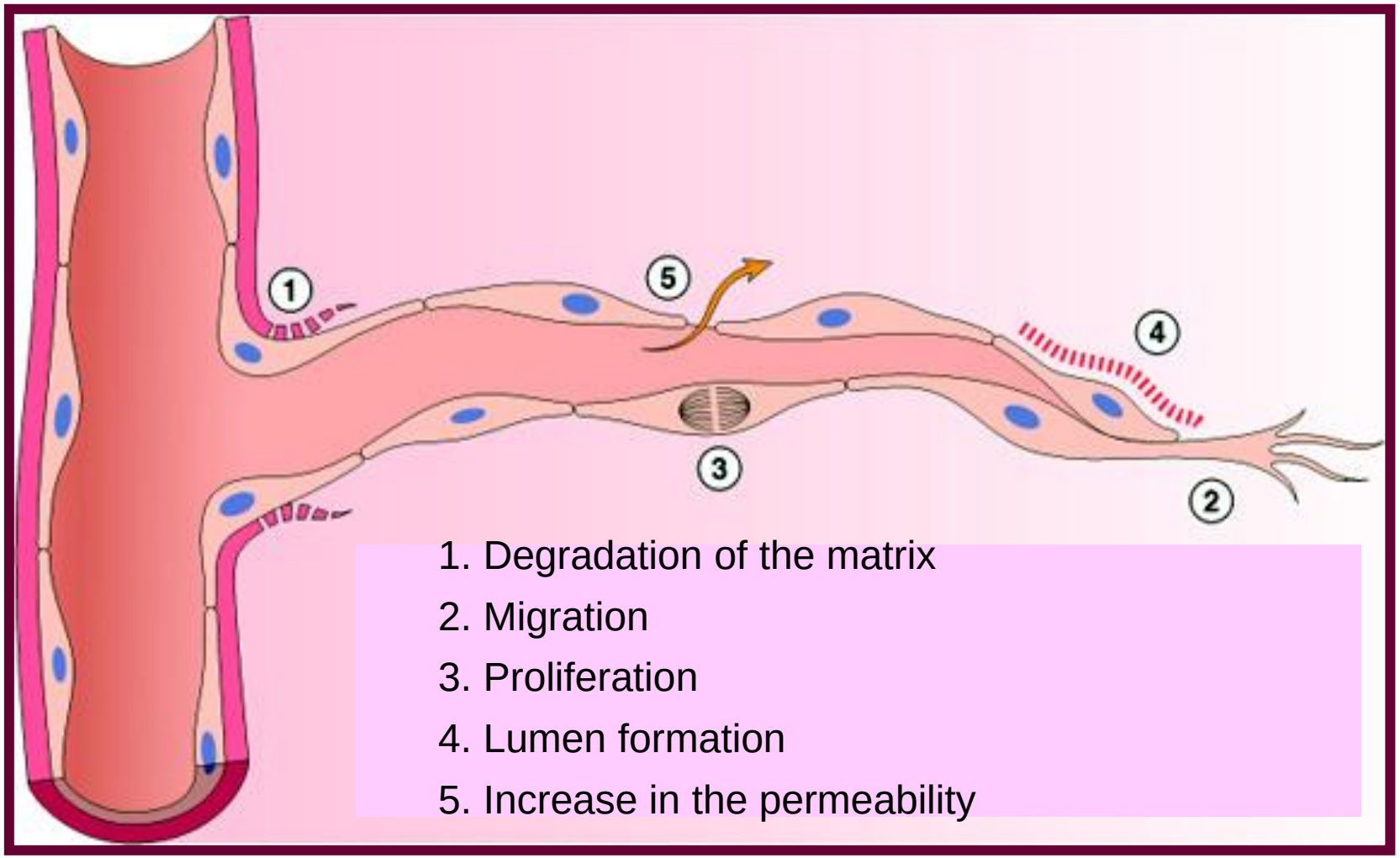
Acta Biomaterialia 8:1137-45 (2012)

Endothelial cell cultures

Endothelial cell cultures

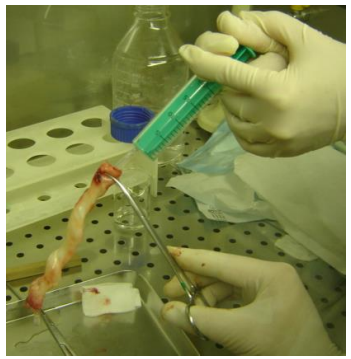
Representative model of angiogenesis

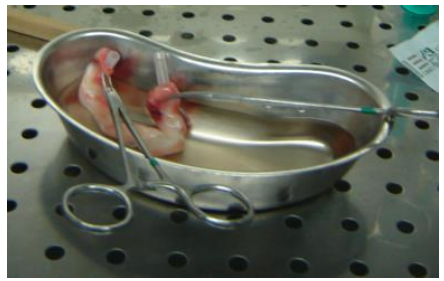


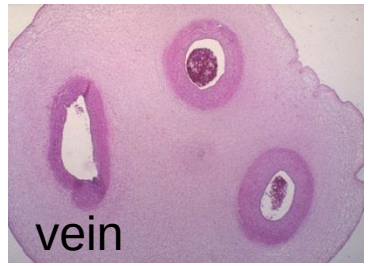


Endothelial cell cultures

Human umbilical vein endothelial cells (HUVECs)





Obtention:

Endothelial cells isolated from umbilical vein
Culture in 1% gelatine pre-coated substrates
Medium M199; 20% fetal bovine serum
1% glutamine; penicillin/ctreptomycin
Trypsin / EDTA solution (70 – 80% confluency)
• First subculture (2x 10^4 cell/ml)
1% heparin; 1 mg/ml EGFS; 7 days

Characterization of the cell behaviour:

- Cell adhesion to the material substrate
- Cell viability/proliferation. Pattern of cell growth
- Apoptosis
- Cell cycle
- Morphology / F-actin cytoskeleton
- Immunostaining of PECAM-1, VE-caderin, vWB
- Expression of endothelial genes
  (PECAM-1, VE-caderin, factor vWB)
- Functional activity
  Production of NO
  Formation of tubular-like structures

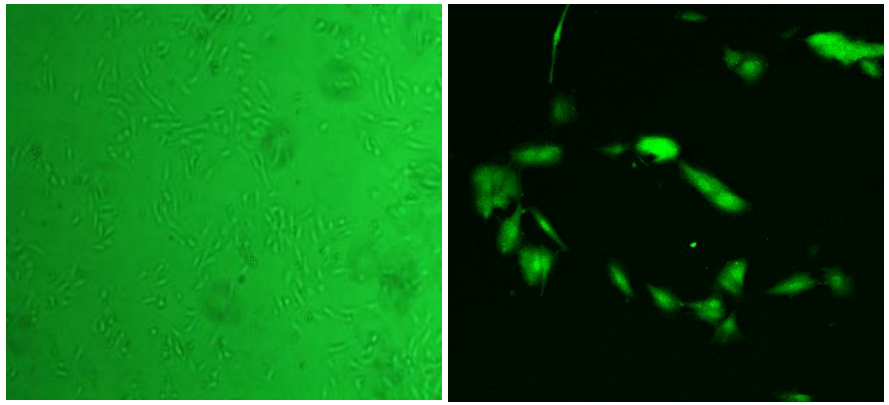
Commercial endothelial cells of different origins

Microvascular endothelial cells
Appropriate culture medium for angiogenic differentiation

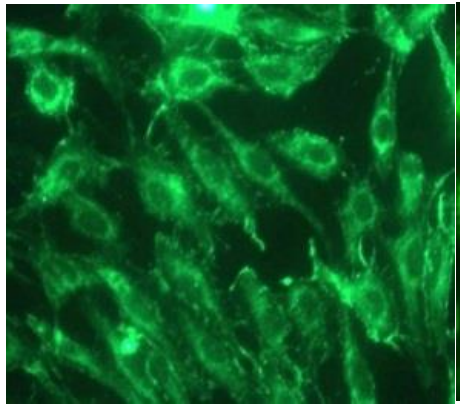
Endothelial cell cultures

Characterization of cell behaviour

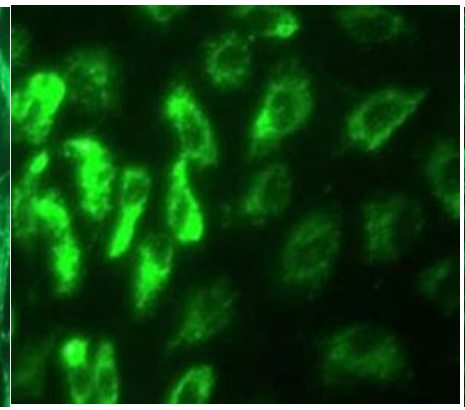
Circular pattern of cell proliferation



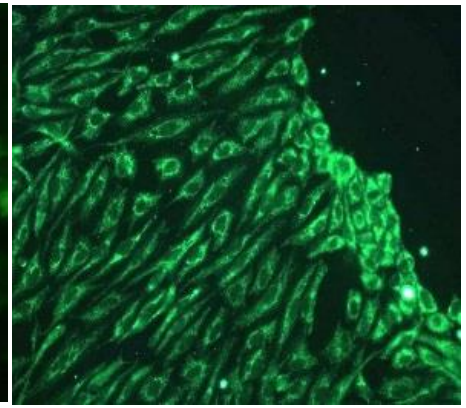
PECAM-1



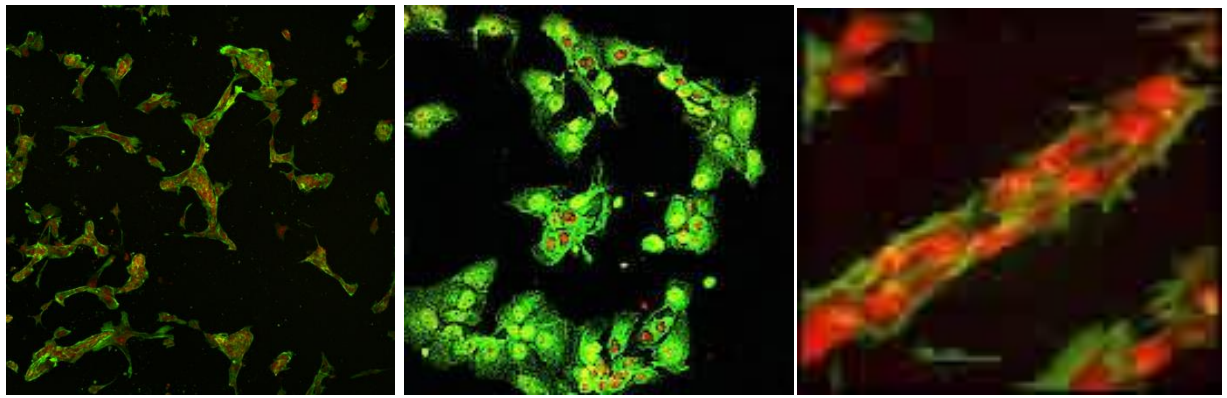
factor vWB



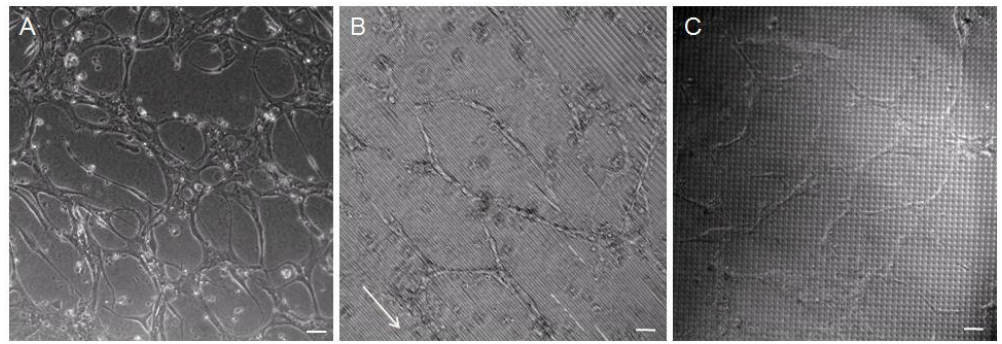
VE-cadherin



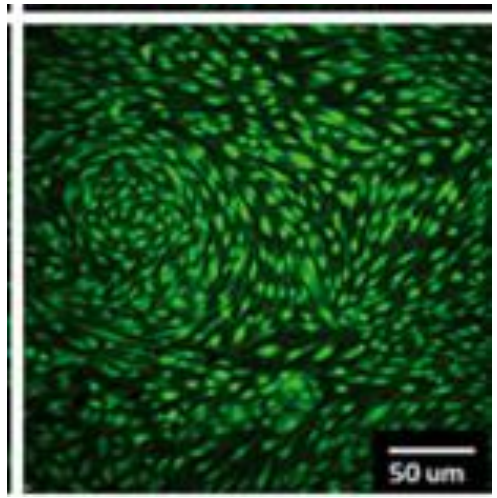
Capillary-like tube formation



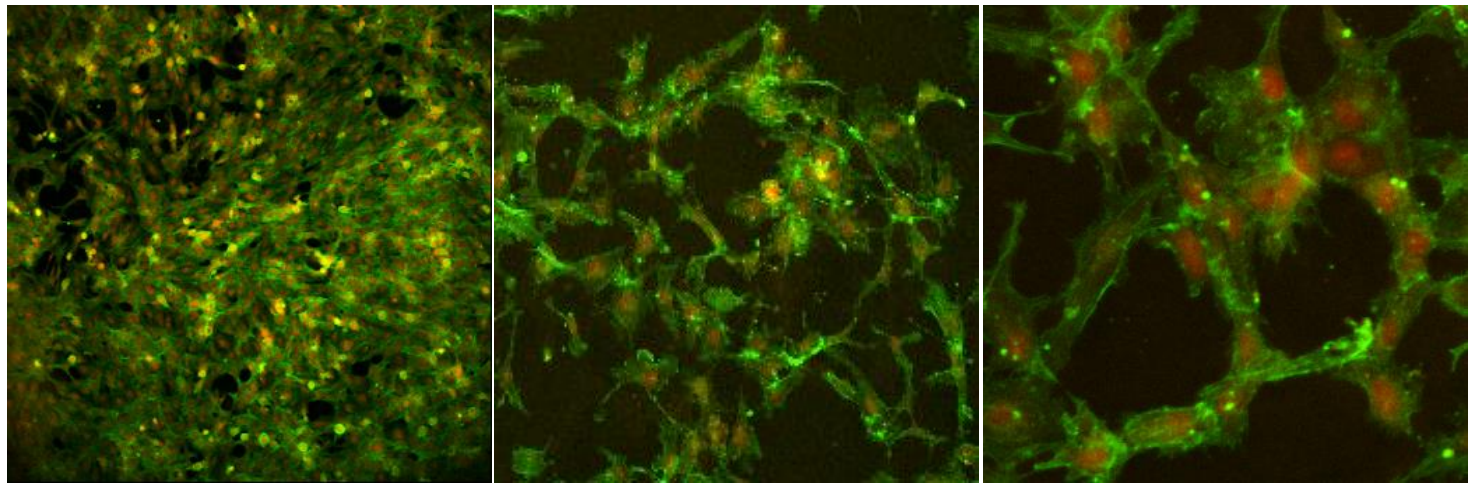
Capillary-like tube formation



Endothelial cell cultures



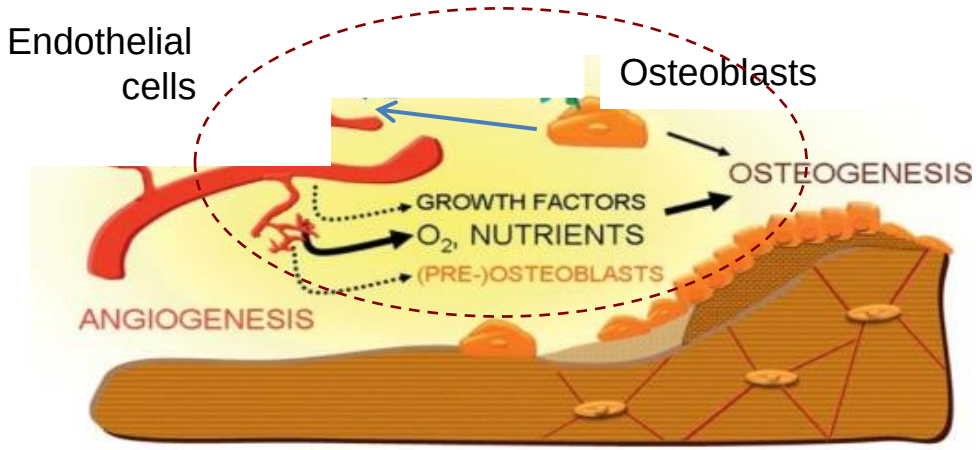
Formation of tube-like structures after the addition of a extracellular matrix (Matrigel)



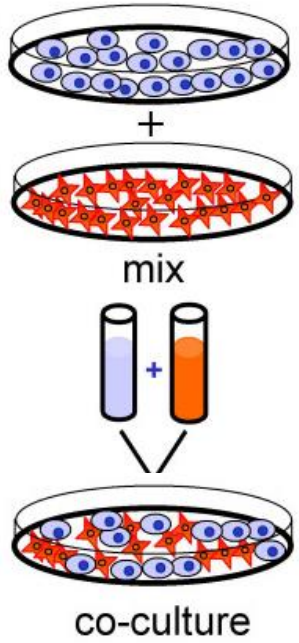
Co-cultures of endothelial and osteoblastic cells

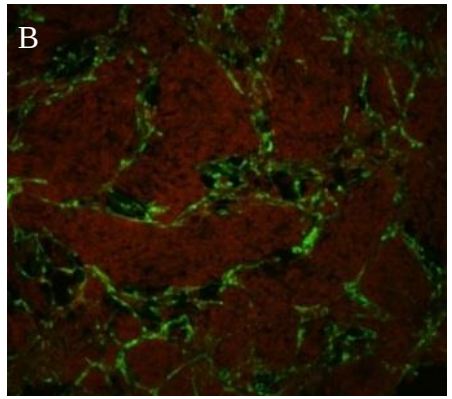
Co-cultures of endothelial and osteoblastic cells

Angiogenesis/Osteogenesis



OB/EC interaction





OB (red)

EC (green)

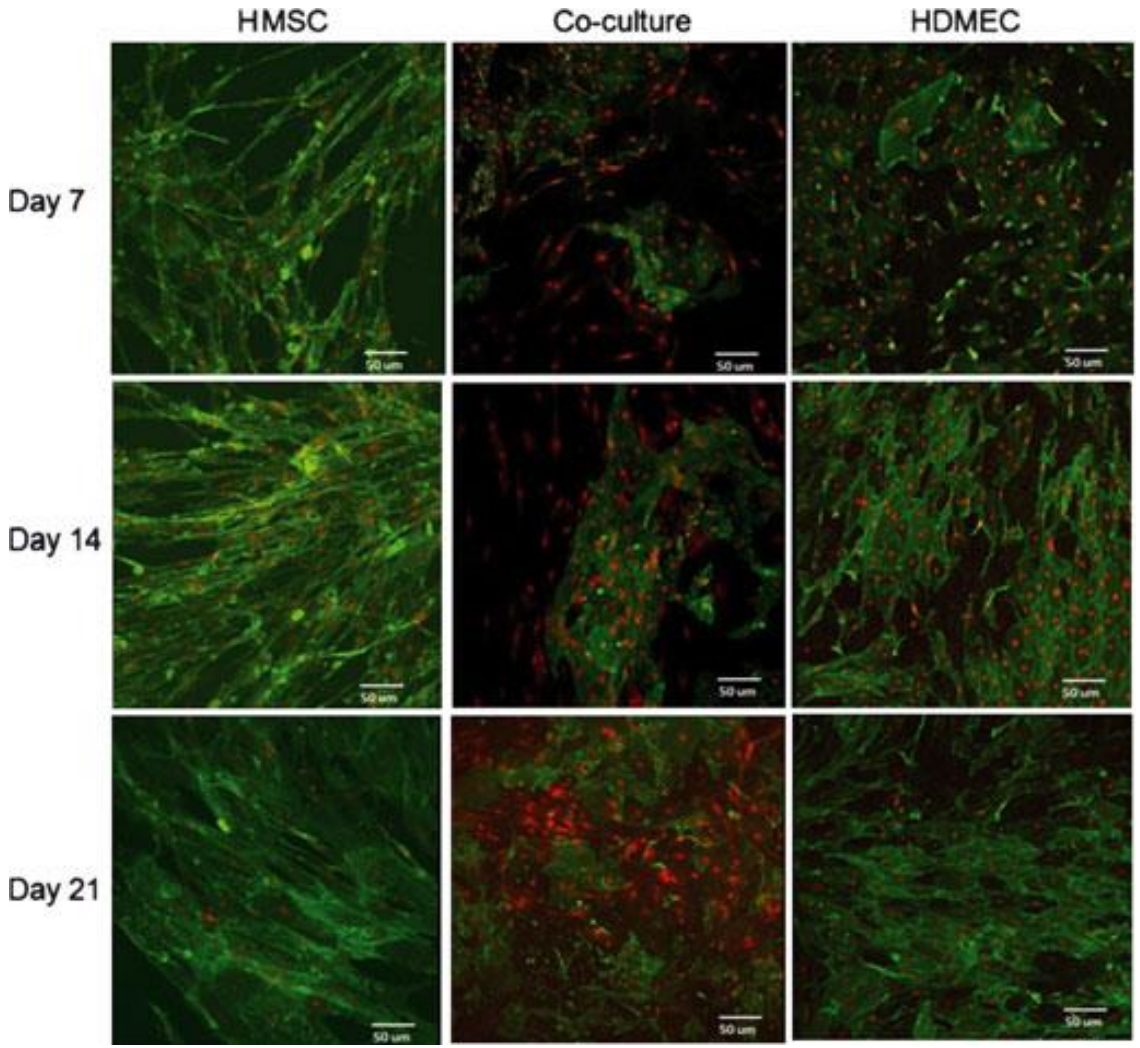
Separation of the two cell populations (flow cytometry)

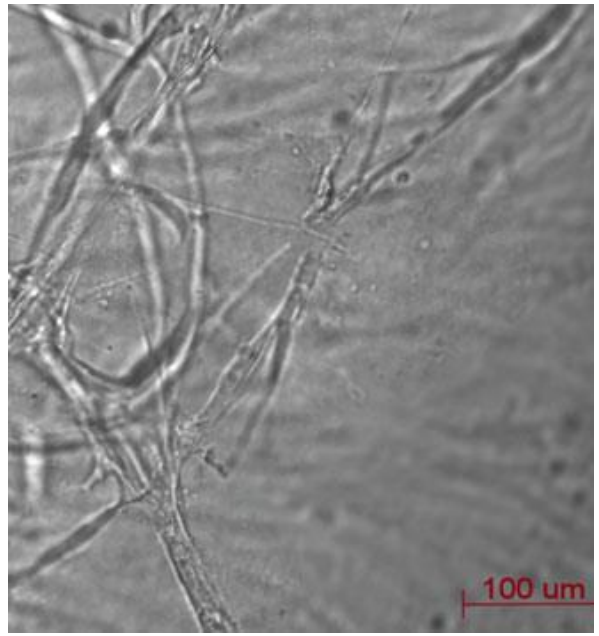
Characterization of each population for typical phenotype features

Co-cultures of endothelial and osteoblastic cells

Illustrative study

Immunostaining of osteoblast cells, endothelial cells and co-cultures





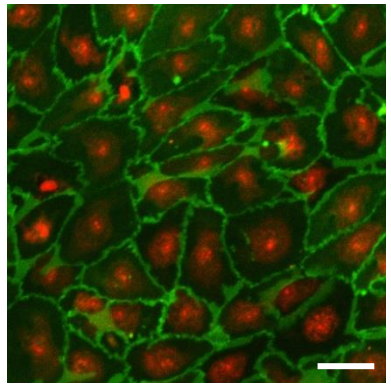
Formation of tubular-like structures

Cell proliferation; 45:320-334 (2012)

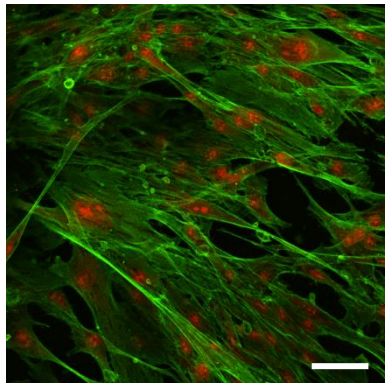
Co-cultures of endothelial and osteoblastic cells

Illustrative studies

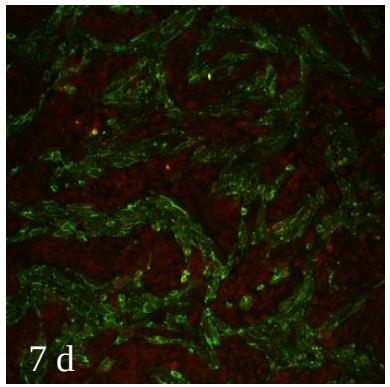
Endothelial cells

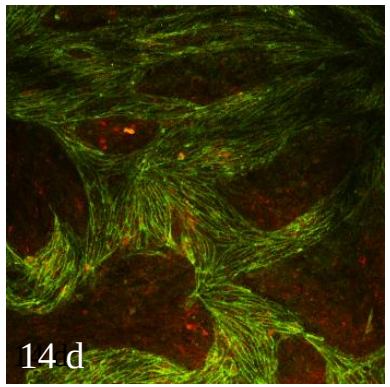


Osteoblasts



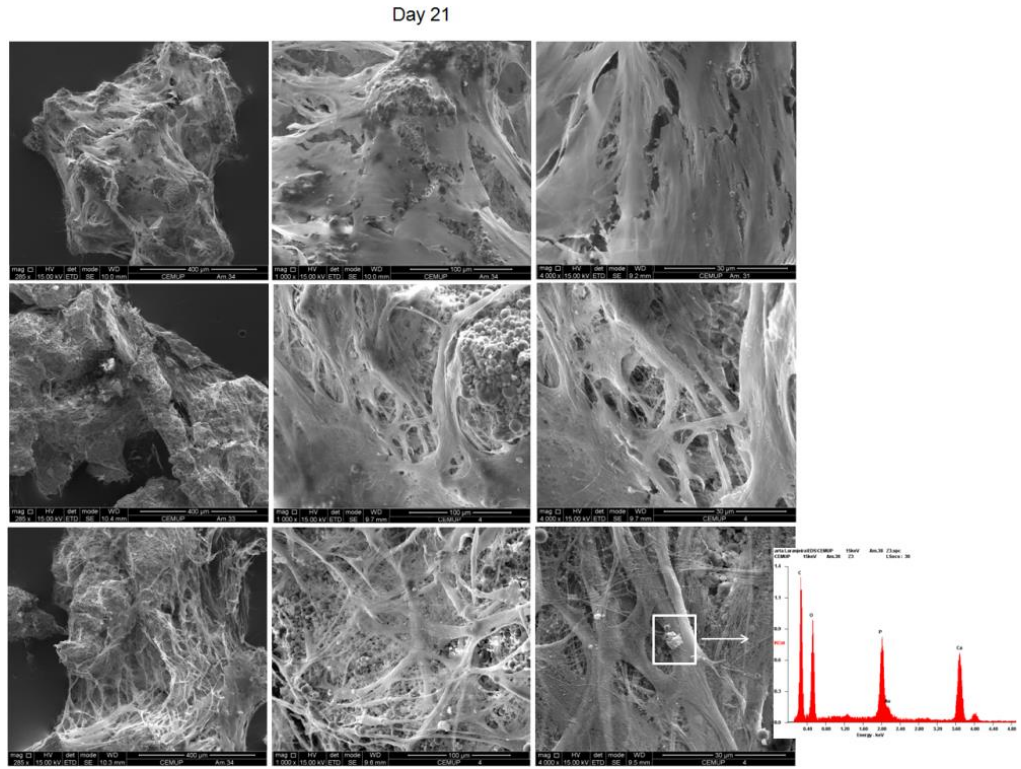
Co-cultures





Macroporous granules of nanostructured-hydroxyapatite agglomerates

Co-cultures of osteoblastic and endothelial cells

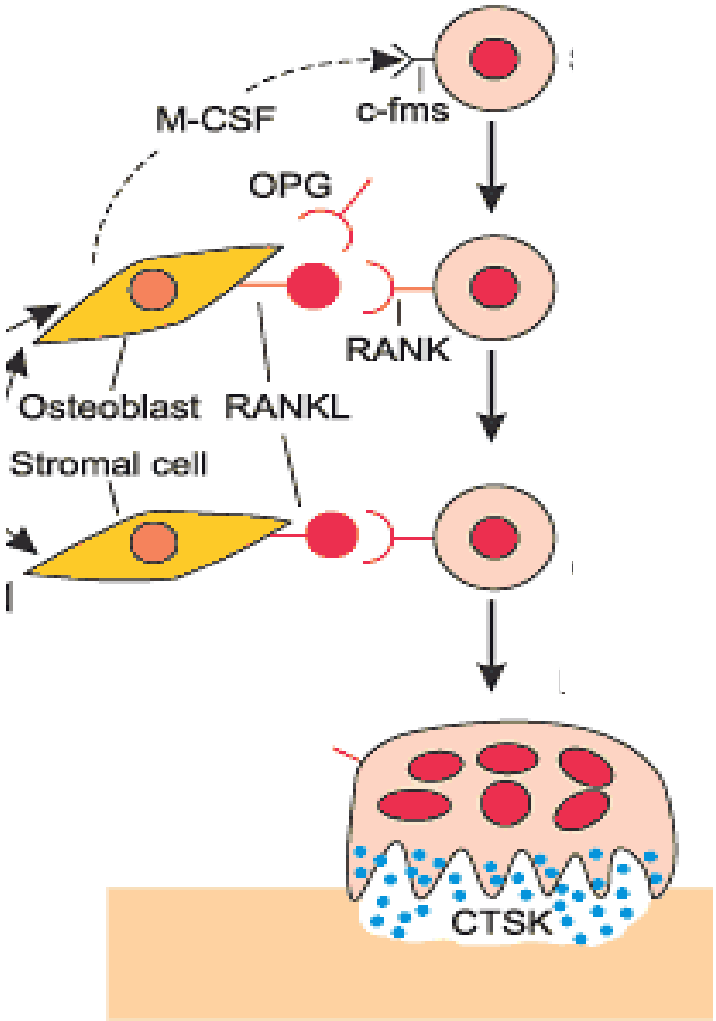


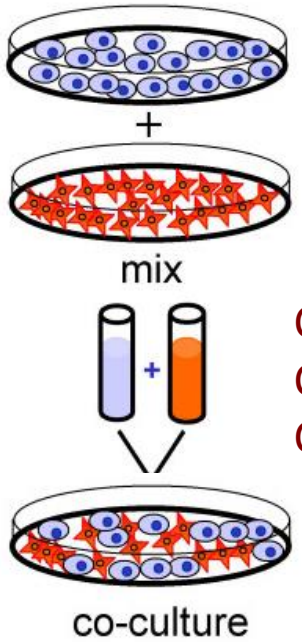
Co-cultures of osteoblastic and osteoclastic cells

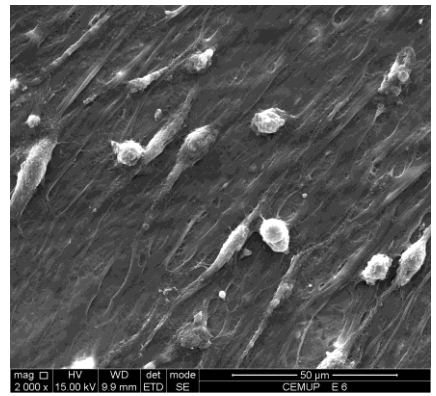
Co-cultures of osteoblastic and osteoclastic cells

OB/OC interaction

Hematopoetic mononuclear precursor cell (PBMC)







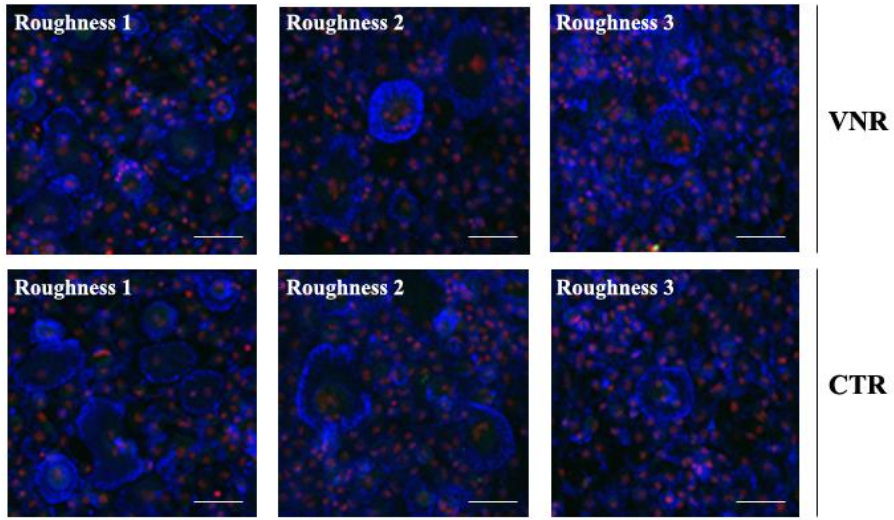
Separation of the two cell populations (flow cytometry)

Characterization of each population for typical phenotype features

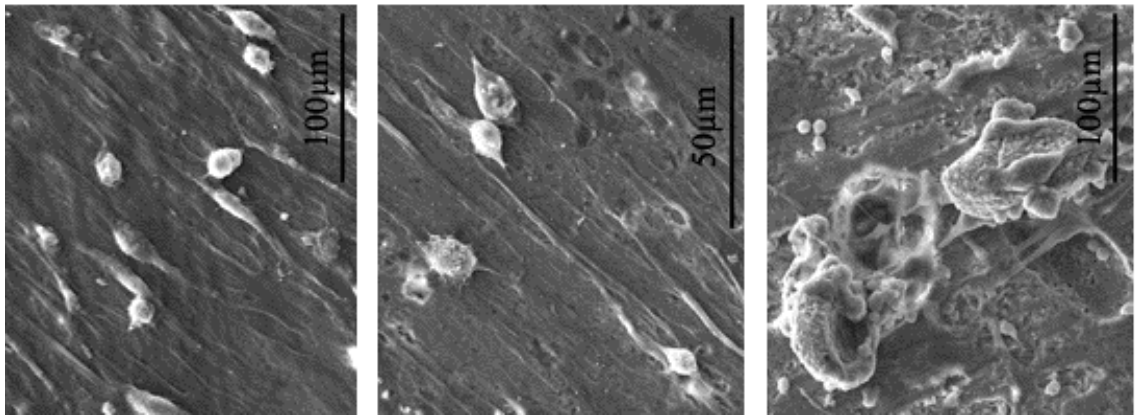
Co-cultures of osteoblastic and osteoclastic cells

Illustrative study

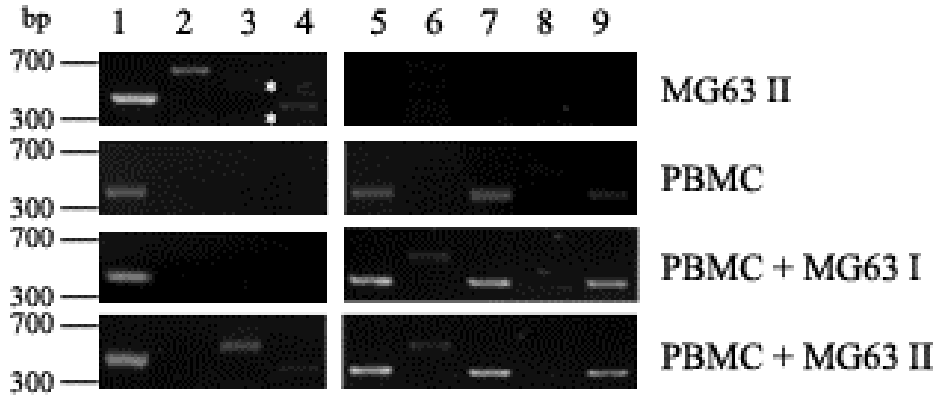
Vitronectin and Calcitonin receptors

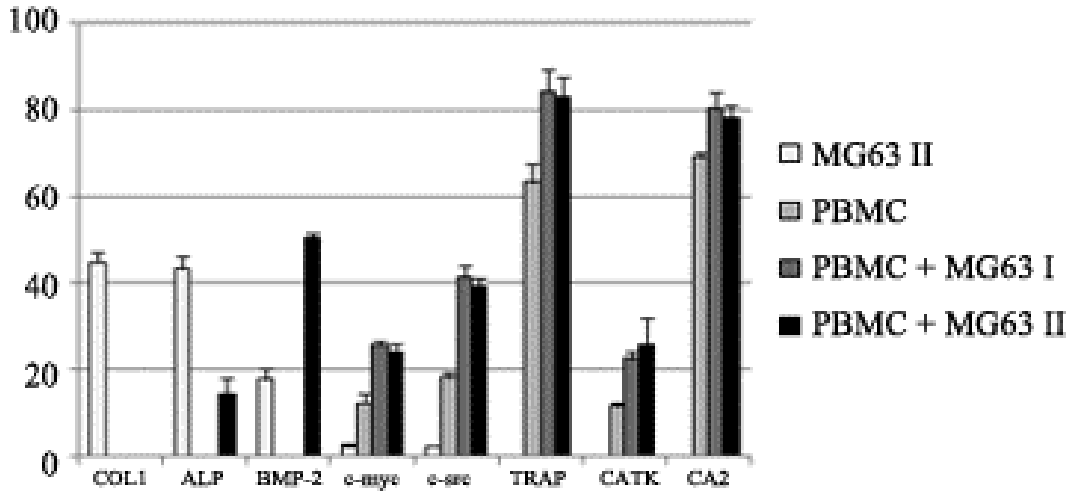


SEM



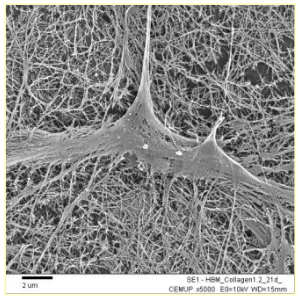
Gene expression



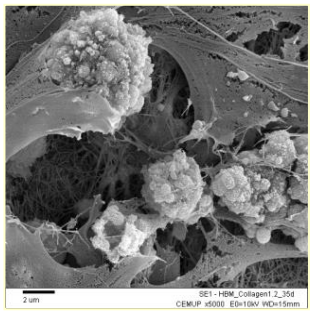


Representative Cell culture models:

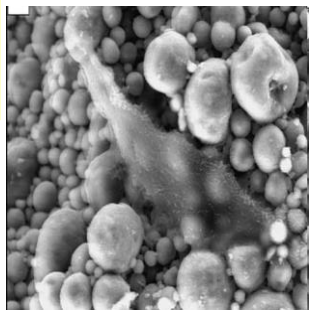
To address the cytocompatibility of biomaterials regarding cells involved in the bone regeneration events

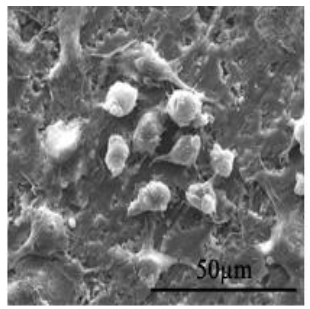


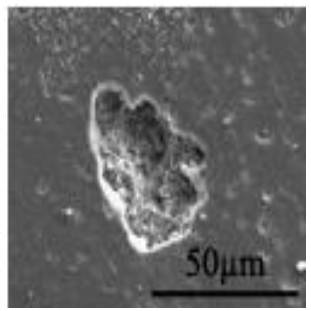
To perceive key Biomaterial/Cell interactions, to understand cellular recognition of material surfaces, and specific cellular events leading to efficient new bone growth



….. To exploit/optimise relevant cellular/material interactions to improve bone regeneration events







In vitro models

Advantages

Information on the molecular and cellular behaviour in controlled experimental conditions

To address specific aspects of the cellular behaviour in the absence of the in vivo complexicity

Limitations

- Alteration of the cell phenotype with the culture time
- Absence of the integrated molecular, cellular and tissue in vivo complexicity

In vitro observations can not be extrapolated to *in vivo*

First stage of biological response to biomaterials

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