Tissue Engineering

Part 2 - *Lecture 4*

Bioceramics & Bone Tissue Engineering

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TYPE OF BIOCERAMICS

In order to be classified as a bioceramic, the ceramic material must exceed such properties:

1. Should be nontoxic
2. Should be noncarcinogenic
3. Should be nonallergic
4. Should be non inflammatory
5. Should be biocompatible
6. Should be biofunctional for its lifetime in host

Multiple Configurations

- Powders
- Granules (Solid & Porous)
- Porous Shapes (Wedge, Cylinder)
- Settable Pastes
- Settable Putties

Settable Injectable

Fast Set Putties

Drillable Formulations

Porous Preforms

Polymer Ceramic Composites

TYPE OF BIOCERAMICS

1 Relatively Inert (Non-absorbable) Bioceramics
2 Non-inert Bioceramics (Resorbable) Bioceramics
3 Surface Reactive (Semi-inert) Bioceramics

Notes Absorbable : Capable of being absorbed or taken in through the pores of a surface

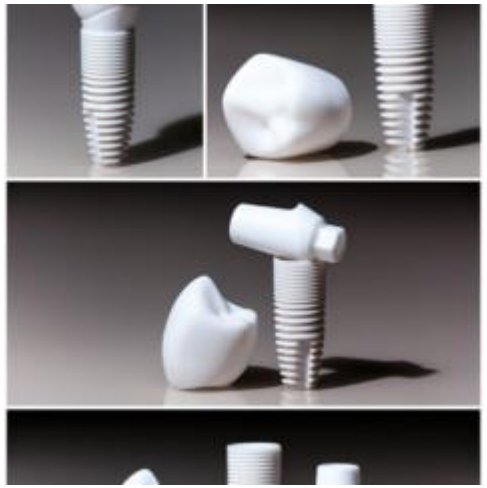
TYPE OF BIOCERAMICS

Types of Implant–Tissue Response
If the material is toxic, the surrounding tissue dies.
If the material is nontoxic and biologically inactive (nearly inert), a fibrous tissue of variable thickness forms.
If the material is nontoxic and biologically active (bioactive), an interfacial bond forms.
If the material is nontoxic and dissolves, the surrounding tissue replaces it.

Types of Bioceramic Tissue Attachment and Their Classification	
1. Dense, nonporous, nearly inert ceramics attach by bone growth into surface irregularities by cementing the device into the tissues or by press-fitting into a defect (termed "morphological fixation").	Al_2O_3 (single crystal and polycrystalline)
2. For porous inert implants, bone ingrowth occurs that mechanically attaches the bone to the material (termed "biological fixation").	Al_2O_3 (polycrystalline) Hydroxyapatite-coated porous metals
3. Dense, nonporous surface-reactive ceramics, glasses, and glass-ceramics attach directly by chemical bonding with the bone (termed "bioactive fixation").	Bioactive glasses Bioactive glass-ceramics Hydroxyapatite
4. Dense, nonporous (or porous) resorbable ceramics are designed to be slowly replaced by bone.	Calcium sulfate (Plaster of Paris) Tricalcium phosphate Calcium–phosphate salts

RELATIVELY INERT (NON-RESORBABLE) BIOCERAMICS

- Maintain their physical and mechanical properties while in host.
- Resist corrosion and wear
- Have all the six (6) desired properties of implantable bioceramics.
- Have a reasonable fracture toughness.
- Typically used as structural-support implant such as bone plates, bone screw and femoral heads.



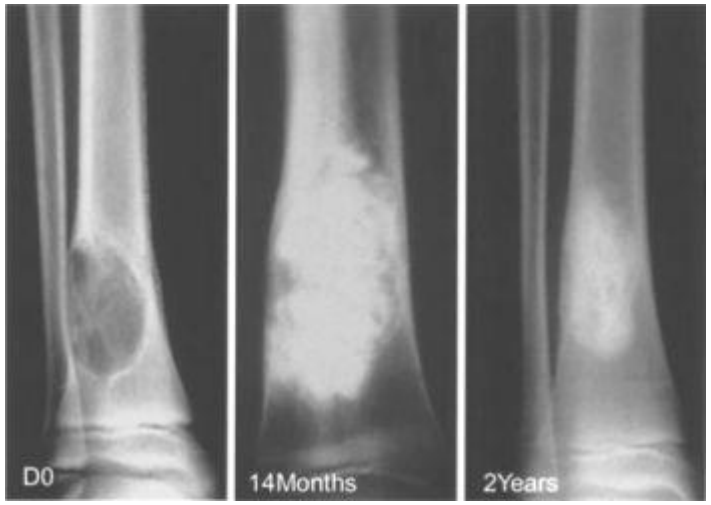
Properties of medical-grade alumina and zirconia[4,6,7]

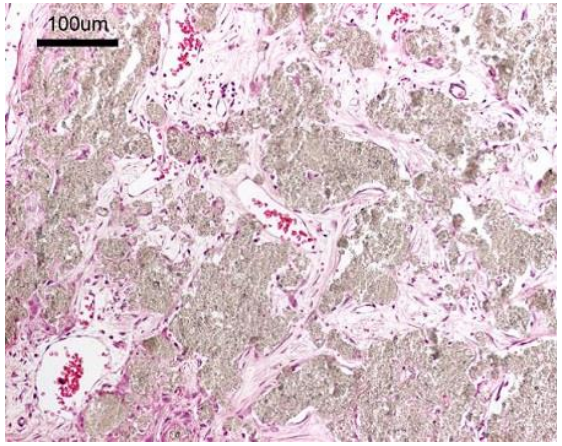
Property	Alumina	Y-TZP	Mg-PSZ
Chemical composition	Al_2O_3 + MgO	ZrO_2 + Y_2O_3	ZrO_2 + MgO
Type of material	Corundum polycrystalline	Polycrystalline tetragonal zirconia	Partially stabilized zirconia
Standards	ISO 6474	ISO/DIS 13356	None
Elastic modulus	380 GPa	210 GPa	210 GPa
Hardness	2000 HV 0.1	1250 HV 0.1	1250 HV 0.1
Bending strength	>500 MPa	>900 MPa	>500 MPa
Fracture toughness	4 MPa $m^{1/2}$	8 MPa $m^{1/2}$	10 MPa $m^{1/2}$
Grain size	<2 μm	<0.5 μm	30 μm

TYPE OF BIOCERAMICS

NON-INERT BIOCERAMICS (RESORBABLE) BIOCERAMICS

- Chemically broken down by the body and degrade
- The resorbed material is replaced by endogenous tissue
- Chemicals produced as the ceramic is resorbed must be able to be processed through the normal metabolic pathways of the body without evoking any deleterious effect.
- Synthesize from chemical (synthetic ceramic) or natural sources (natural ceramic)





NON-INERT BIOCERAMICS (RESORBABLE) BIOCERAMICS

Examples of Resorbable Bioceramics

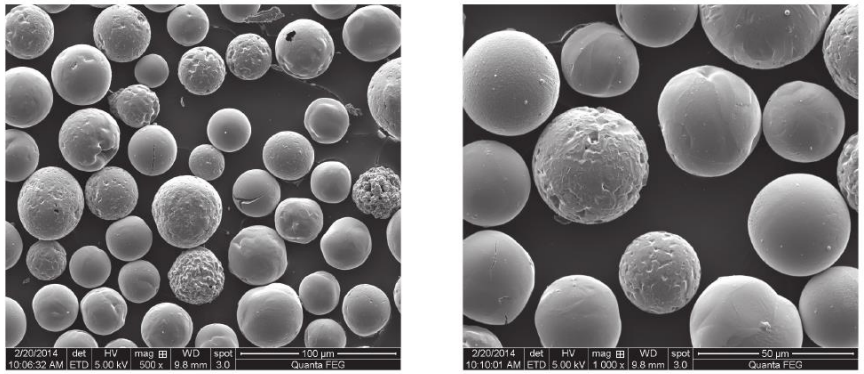
1. Calcium phosphate
2. Calcium sulfate, including plaster of Paris
3. Hydroxyapatite
4. Tricalcium phosphate
5. Ferric-calcium-phosphorous oxides
6. Corals

NON-INERT BIOCERAMICS (RESORBABLE) BIOCERAMICS

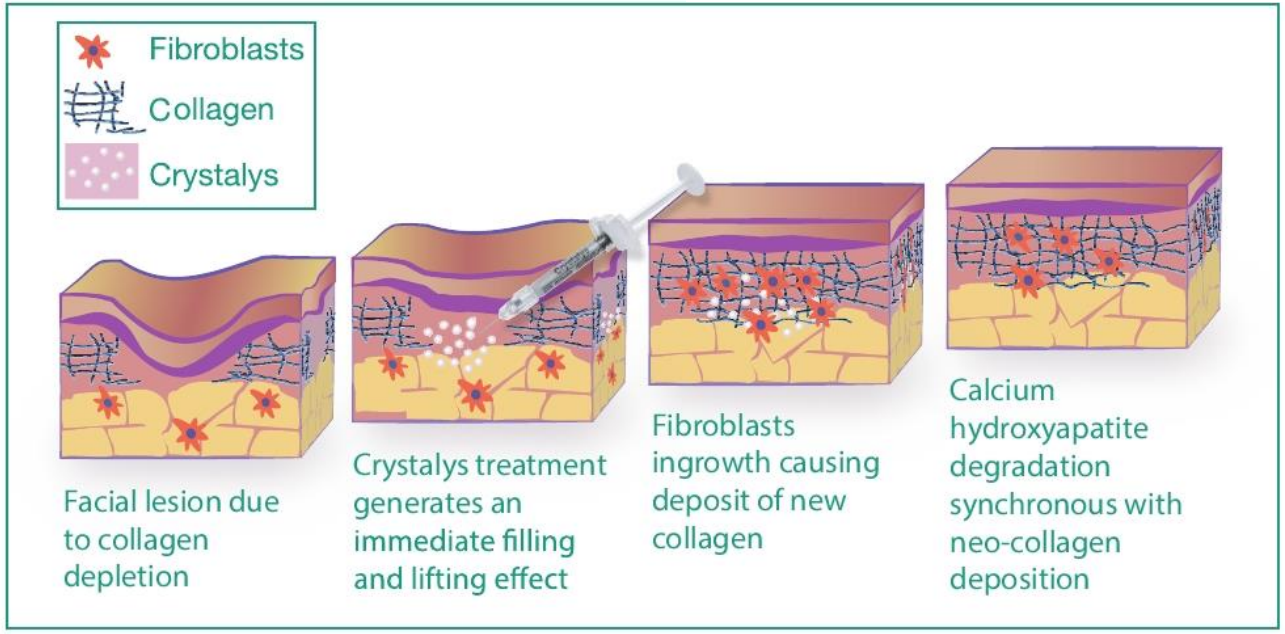
Synthetic ceramic

Calcium phosphate and Hydroxyapatite

- Can be crystallized into salts such as Hydroxyapatite.
- Hydroxyapatite (HAP) has a similar properties with mineral phase of bone and teeth.
- Important properties of HAP:
  - Excellent biocompatibility
  - Form a direct chemical bond with hard tissue



Morphology of Crystalys CaHA microspheres:
Scanning electron micrographs at x 500 (left) and x 1000 (right) magnification



TYPE OF BIOCERAMICS

NON-INERT BIOCERAMICS (RESORBABLE) BIOCERAMICS

- Application:
  - Bone substitute in a granular or a solid block.
  - Temporary scaffold which is gradually replaced by tissue
  - Orthopaedic and dental implant coating
  - Dental implant materials
- Drawback:
  - Complicated fabrication process and difficult to shape

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)

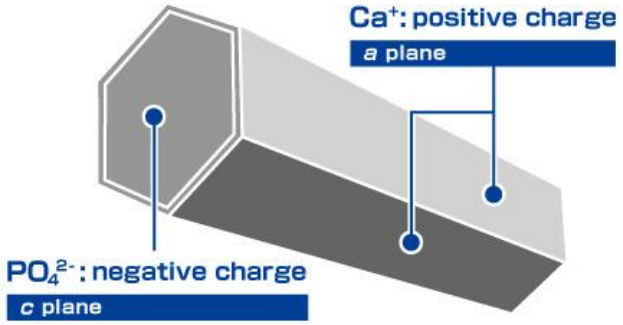
HAp as bone mineral detected in 1926 by x-ray diffraction studies (De Jong)

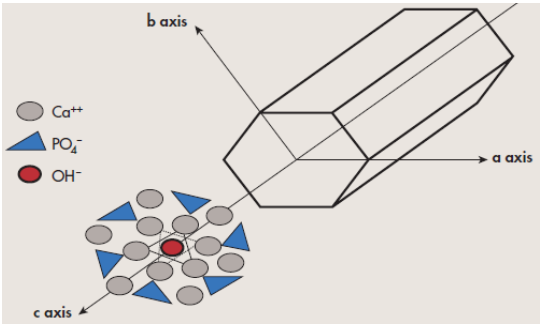
Broadening of diffraction lines → small crystal size

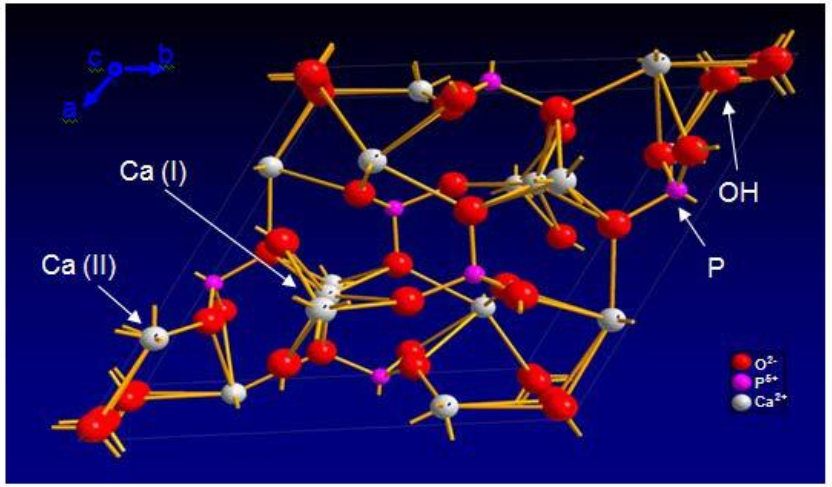
Since 1970's accepted as biomaterial

Used for
- Middle ear
- Alveolar ridge maintenance
- Maxillofacial surgery + dental
- Bone substitute material
- Vertebra substitution
- Orthopedic implants

Bone HA (SEM)







HA crystal structure

Calcibon®

Production routes

- ***Purely synthetic***

1. From concerning powder of CPs
2. Forming of structures by pore formation (easily soluble filler, foaming, ..)
3. Sintering

- ***By removement of organic components***

1. Starting form (animal) bone
2. Oxidative firing to remove all organic matter or chemical deproteinization

Example Endobone®

- Endobon® - for metaphyseal compression fractures

Features

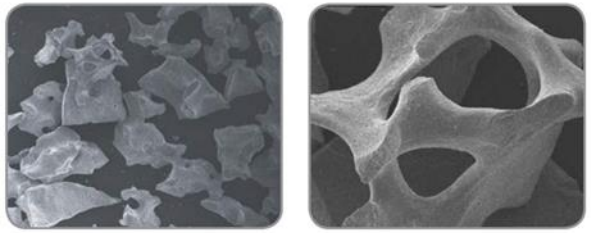
- interconnecting pore system
- pore size 100-1.500 µm
- fully deproteinated
- highly biocompatible
- augmentation with autologous bone graft, bone marrow aspirate, blood or PRP
- several forms (granules, blocks, cylinders) and different granules available
- Filling defects, Alveolar ridge augmentation including esthetic contouring defects, Extraction socket grafting

The Material

manufactured in a two-stage high-temperature process:
Bovine-derived hydroxyapatite that has been fully deproteinized by tw
temperature process for protection from bacteria, viruses and prions
1. pyrolysis at a temperature above 900 °C
2. sintering at a temperature above 1.200 °C

Small Granules
500–1000 µm particle size preferred for grafting smaller defects, such as extraction sockets.

Large Granules
1000–2000 µm particle size preferred for grafting large defects, such as sinus elevations.



SEM images of Endobon Xenograft Granules at 20x and 100x showing the micro and macro pores in the particles.

Two Year Histology

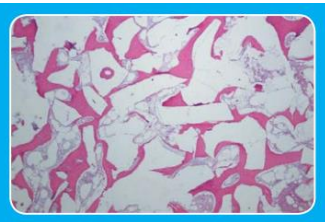


Fig. 1. Histologic core of the regenerated bone showing that the Endobon Xenograft Granules integrated with newly formed bone.

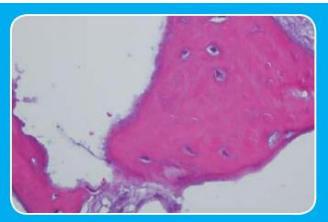


Fig. 2. At 40x magnification, note the newly formed bone (violet) and mature bone (pink) that surround the Endobon Xenograft Granules (white).

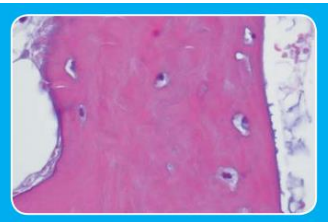


Fig. 3. At 60x magnification, lamellar bone is well formed.

TYPE OF BIOCERAMICS

Surface reactive (semi-inert) bioceramics

- Direct and strong chemical bond with tissue
- Fixation of implants in the skeletal system
- Low mechanical strength and fracture toughness
- Examples:
  - Glass ceramics
  - Hydroxyapatite
  - Dense nonporous glasses

Bioglasses and Bioactive glass ceramics

Mostly based on SiO_2 (CaO-P_2O_5-SiO_2-System)

Bioglass® - glass that binds to living tissue discovered by Hench and Wilson in 1971 – induces greater osteoblastic activity than (thermally treated) HAp

(45S5Bioglass® with 24.5 wt% CaO, 24.5 wt% Na_2O, 6 wt% P_2O_5,45 wt% SiO_2)

Glass phase transforms to HAP under physiological conditions

(initial layer forms within 1 h)

Different other materials on market

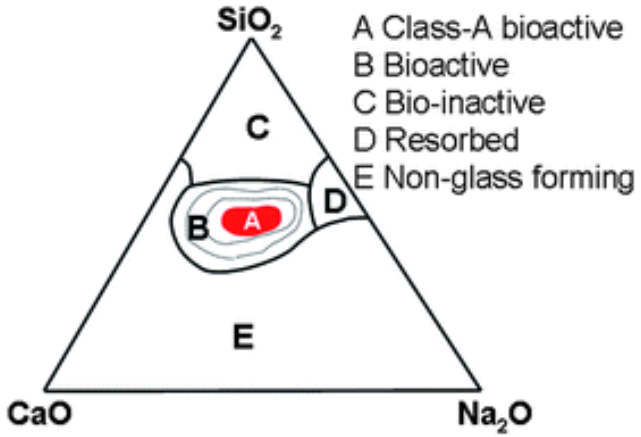
Bioverit®, Ceravital®

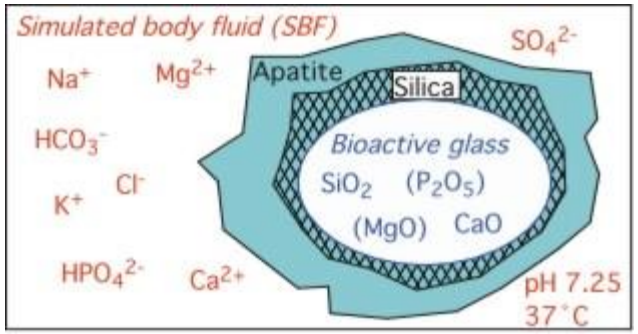
Non load-bearing applications

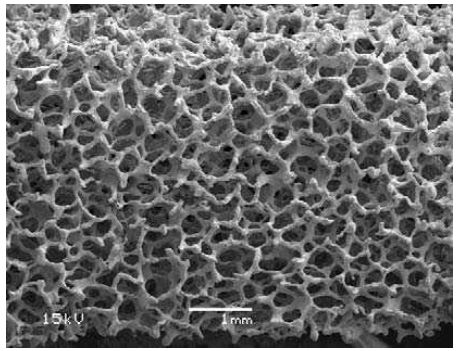
- *Middle ear*
- *Alveolar ridge maintenance*
- *Maxillofacial surgery + dental*
- *Bone substitute material*
- *Vertebra substitution*
- *Orthopedic implants*

TYPE OF BIOCERAMICS

- ***Type of glass ceramic***
  - Bioglass
  - Ceravital
- Both are SiO_2, CaO, Na_2O and P_2O_5 systems
- Bioglass composition manipulated to induce direct bonding with the bone
  - Must simultaneously form a calcium phosphate and SiO_2 – rich film layer on surface of ceramic for this to happen
  - With correct composition will bond with bone in approximately 30 days







TYPE OF BIOCERAMICS

Orthopedics products based upon 45S5 bioglass

Trauma	Long bone fracture (acute and/or comminuted); alone and with internal fixation
	Femoral non-union repair
	Tibial plateau fracture
Arthroplasty	Filler around implants (acetabular reconstruction)
	Impaction grafting
General	Filling of bone after cyst/tumor removal
Spine fusion	Interbody fusion (cervical, thoracolumbar, lumbar)
	Posterolateral fusion
	Adolescent idiopathic scoliosis

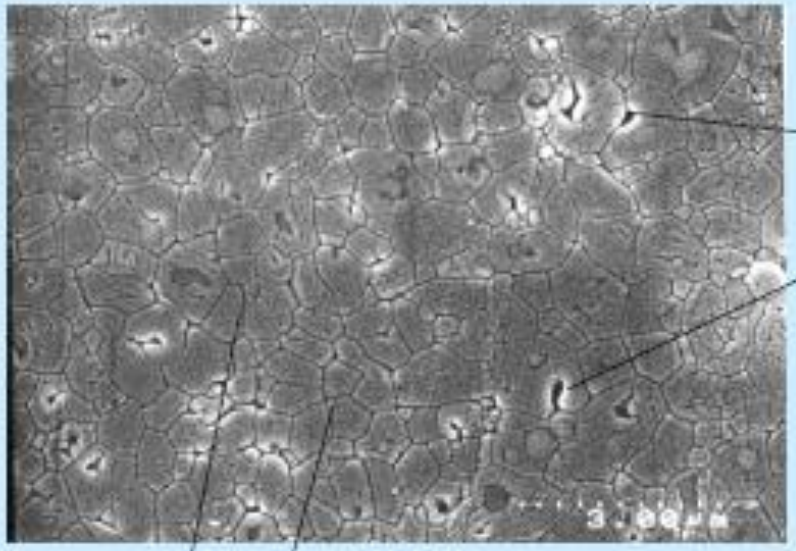
Cranial-facial products based upon 45S5 bioglass

Cranioplasty	Facial reconstruction
General oral/dental defects	Extraction sites
	Alveolar ridge augmentation
	Sinus elevation
	Cystectomies
	Osteotomies
Periodontal repair	

Dental-maxillofacial-ENT products based upon 45S5 bioglass

Toothpaste and treatments for dentinal hypersensitivity
Pulp capping
Sinus obliteration
Repair of orbital floor fracture
Endosseous ridge maintenance implants
Middle ear ossicular replacements (Douek MED)

- Glass ceramic properties



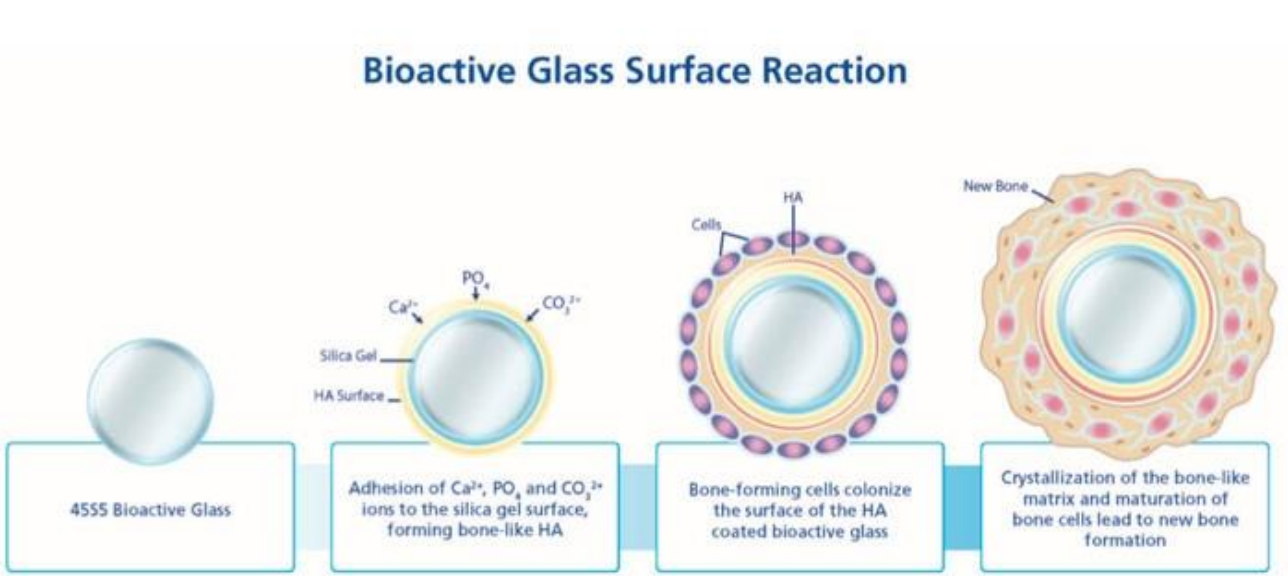
POROSITY

- Open or closed
- Decrease Young's modulus
- Decrease strength
- Decrease hardness
- Decrease thermal conductivity

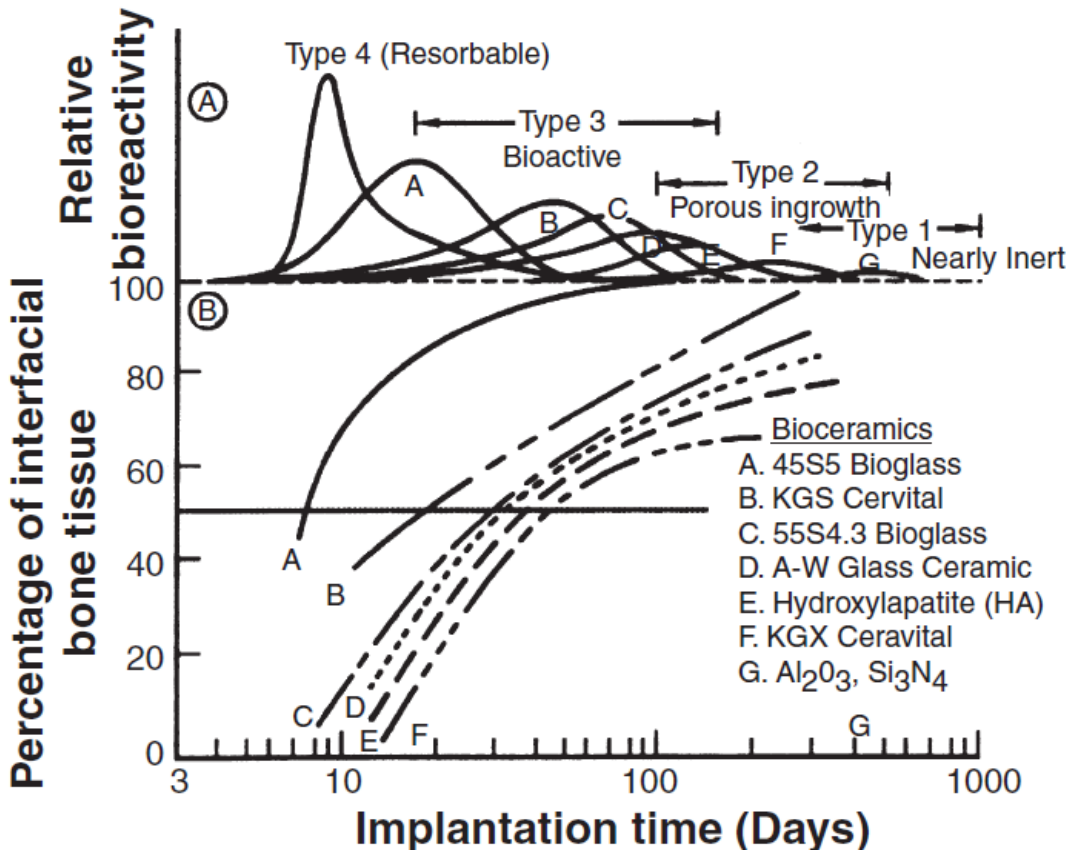
Fig. 3.20

- Collectors of impurities
- Glassy phase
- Stress concentration
- Microcracking source

GRAIN BOUNDARIES



TYPE OF BIOCERAMICS



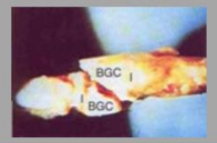
Bioceramic Material Characteristics and Properties
Composition
Microstructure
Number of phases
Percentage of phases
Distribution of phases
Size of phases
Connectivity of phases
Phase state
Crystal structure
Defect structure
Amorphous structure
Pore structure
Surface
Flatness
Finish
Composition
Second phase
Porosity
Shape

Bioactivity spectrum for various bioceramic implants:

(a) relative rate of bioreactivity

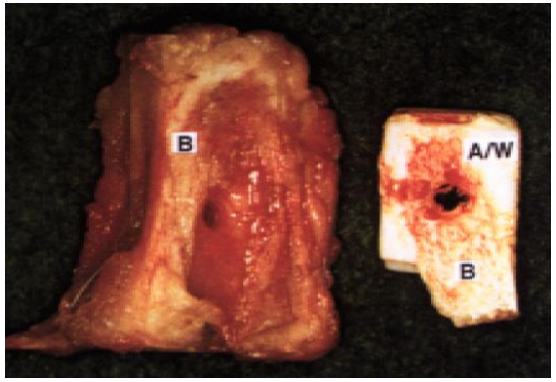
(b) time dependence of formation of bone bonding at an implant interface. Various products are studied (A – G), from bioactive resorbable to inert material

Failure Loads of Some Bioceramics at 8 Weeks after Implantation

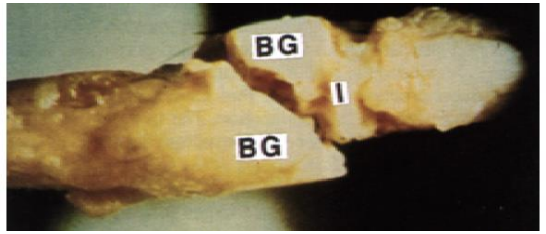


Material	Failure Load (kg|)	Location of Fracture
Dense alumina	0,13 ± 0,02	Interface
Bioglass 45S5	2.75 ± 1.80	Within material
Ceravital (GC)	3.52 ± 1.48	Within material
Cerabone A-W (GC)	7.43 ± 1.19	Within bone
Hydroxyapatite	6.28 ± 1.58	Within material

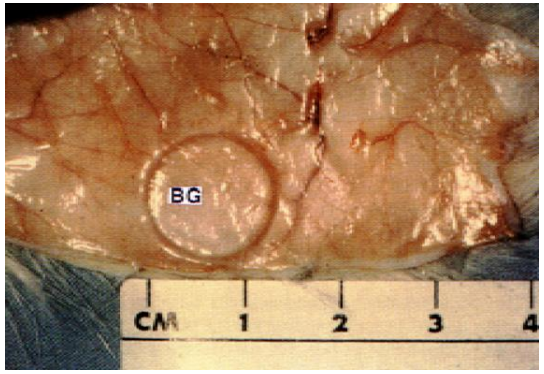
Conclusion:
Bioceramics with higher Bioactivity as Bioglass and Ceravital , have lower strength than bone.



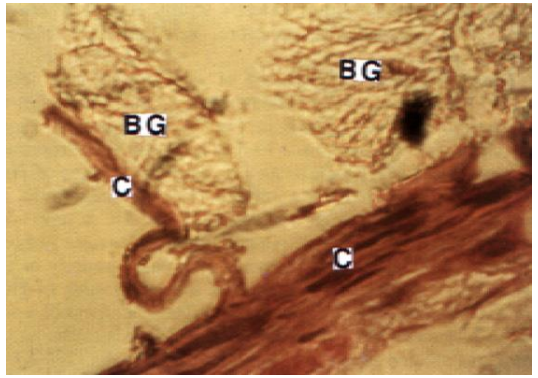
Interfacial adherence of A/W bioactive glass-ceramic is stronger than either bone [B] or implant [A/W]. (Photo courtesy Professors T. Yamamura and T. Kokubo, Kyoto University.)



Fracture of 45S5 bioactive glass [BG] segmental bone replacement in monkey due to torsional loading. Note the bonded interface [I] that survived the load to failure. (Photo courtesy of Professor George Piotrowski, University of Florida.)



Adherant soft connective tissue bonding immobilizes a 45S5 bioactive glass [BG] implant. (Photo courtesy of Dr. June Wilson.)



Adhesion of collagen fibers [C] to bioactive glass bonding gel layer [BG] is stronger than cohesive bonding of collagen to itself. (Photo courtesy of Dr. June Wilson, University of Florida.)

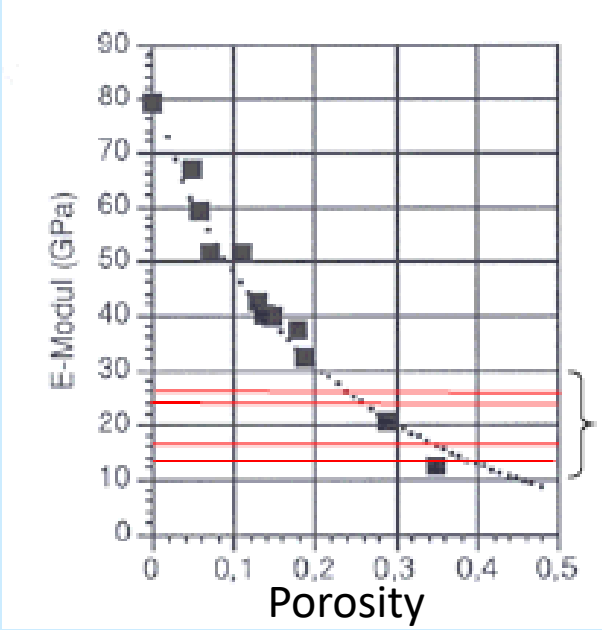
Composition and Mechanical Properties of Bioactive Ceramics Used Clinically

Property	Bioglass 45S5	S45PZ	Glass-ceramic Ceravital	Glass-ceramic Cerabone A/W	Glass-ceramic Ilmaplant L1	Glass-ceramic Bioverit	Sintered hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (>99.2%)	Sintered β-$3CaO \cdot P_2O_5$ (>99.7%)
Composition (wt%)								
Na_2O	24.5	24	5–10	0	4.6	3–8		
K_2O	0		0.5–3.0	0	0.2	3–8		
MgO	0		2.5–5.0	4.6	2.8	2–21		
CaO	24.5	22	30–35	44.7	31.9	10–34		
Al_2O_3	0		0	0	0	8–15		
SiO_2	45.0	45	40–50	34.0	44.3	19–54		
P_2O_5	6.0	7	10–50	16.2	11.2	2–10		
CaF_2	0			0.5	5.0	3–23		
B_2O_3	0	2						
Phase‡	Glass	Glass	Apatite Glass	Apatite β-Wollastonite Glass	Apatite β-Wollastonite Glass	Apatite Phlogopite Glass	Apatite	Whitlockite
Density (g/cm^3)	2.6572			3.07		2.8	3.16	3.07
Vickers hardness (HV)	458 ± 9.4			680		500	600	
Compressive strength (MPa)			500	1080		500	500–1000	460–687
Bending strength (MPa)	42§			215	160	100–160	115–200	140–154
Young's modulus (GPa)	35		100–150	218		70–88	80–110	33–90
Fracture toughness, K_{IC} ($MPa \cdot m^{1/2}$)				2.0	2.5	0.5–1.0	1.0	
Slow crack growth, n (unitless)				33			12–27	

†Reference 15. ‡Apatite is $Ca_{10}(PO_4)_6(O,F)$, β-wollastonite is $CaO \cdot SiO_2$, phlogopite is $(Na,K)Mg_3,(AlSiO_{10})F_2$, and whitlockite is β-$3CaO \cdot P_2O_5$. §Tensile.

Porosity Effects on Mechanical Properties

Porosity can be used to tailor the Young's modulus of a glass or ceramic to that of bone

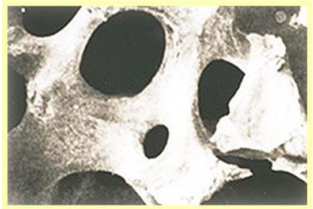


✓The Young's modulus of ceramics and glasses is much higher than that of bone

✓For implant applications, the Young's modulus of the implant must be matched to that of bone (avoid stress shielding)

✓Adding porosity to decrease the Young's modulus of the implant

Range of variation of the Young's modulus of cortical bone



Human Bone

Hydroxyapatite

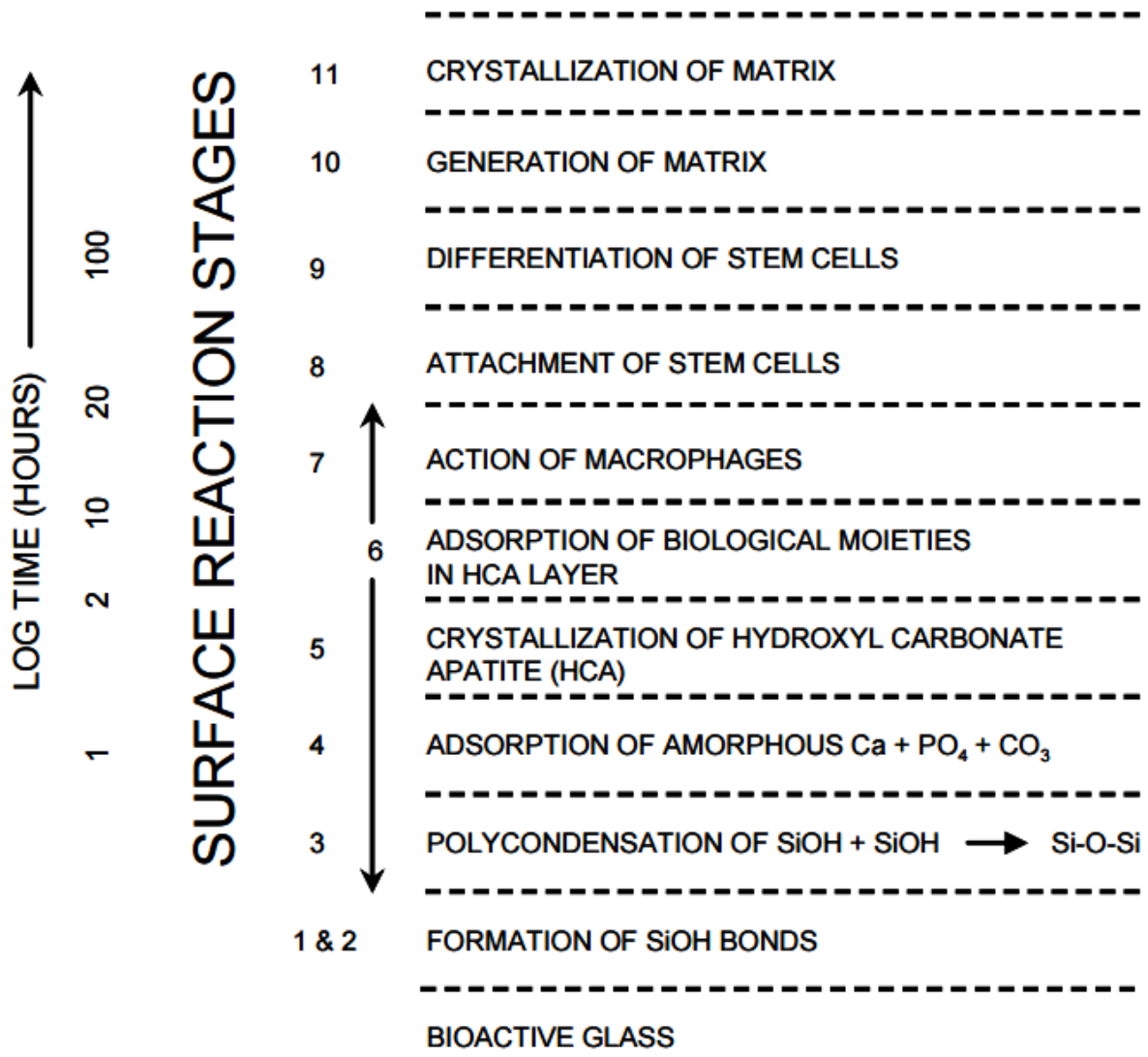
SURFACE REACTIVE (SEMI-INERT) BIOCERAMICS

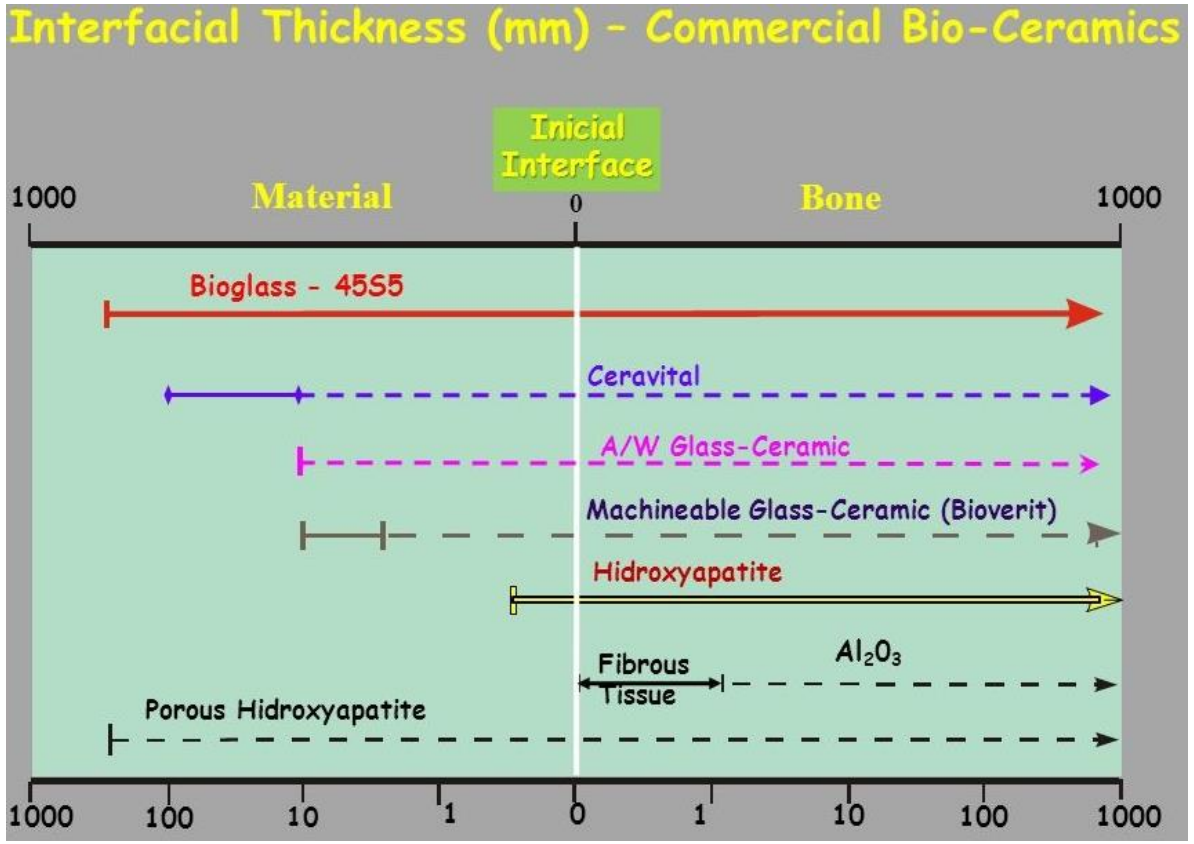
- Application of Glass Ceramic
  - Orthopaedic and dental implant coating
  - Dental implant
  - Facial reconstruction components
  - Bone graft substitute material
- Main limitation:
  - Brittleness
  - Cannot be used for making major load bearing implant such as joint implant

Bioceramics	Bending strength (MPa)	K_C MPa.m $^{1/2}$
Cerabone A/W	215	2,0
Bioverit I	140-180	1,2-2,1
Hydroxyapatite	40-70	not specified
Bioglass 45S5	**70**	**0,6**

45S5 Mechanical Properties are their "Achilles Heel"

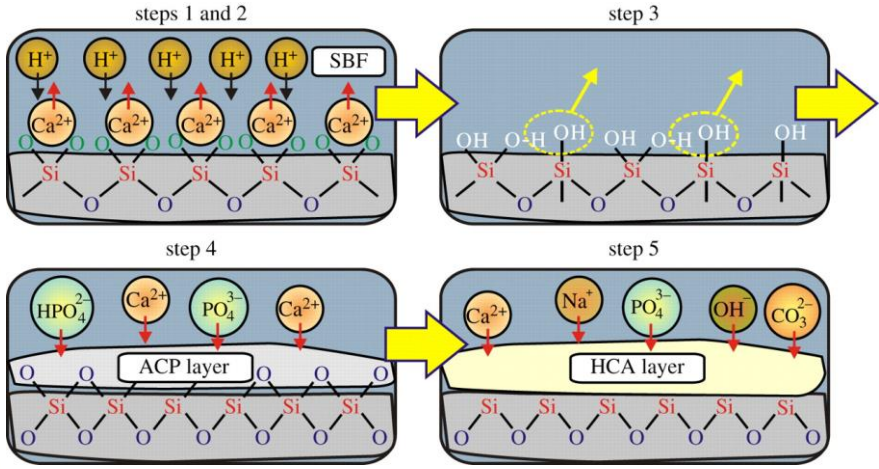
Sequence of interfacial reactions involved in forming a bond between tissue and bioactive ceramics

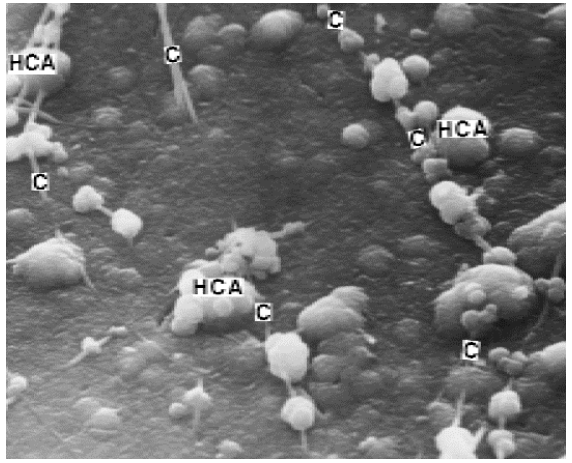




Sequence of interfacial reactions involved in forming a bond between tissue and bioactive ceramics

CHEMICAL/BIOLOGICAL PROCESS	STAGES	EFFECT PRODUCED
Interchange Na^+/H_3O^+	1	Formation of silanol groups (Si-OH)
Loss of soluble silica	2	Release of $Si(OH)_4$ and formation of Si-OH
SiO_2 condensation and polymerisation	3	Formation of siloxane bonds (Si-O-Si)
Migration of Ca^{2+}, PO_4^{3-} to the silica rich layer	4	Formation of amorphous calcium phosphate (a-CaP)
Crystallisation including OH^- and CO_3^{2-}	5	Crystallisation of hydroxycarbonate apatite (HCA)
Adsorption of biological moieties to the HCA layer	6	Adsorption and desorption of growth factors
Action of macrophages	7	Preparation the implant site for tissue repair
Adsorption of osteoblasts stem cells	8	Attachment of stem cells
Differentiation and proliferation of osteoblasts	9	Simultaneous proliferation and differentiation of the cells
Generation of matrix	10	Growth factors stimulating cell proliferation and formation extracellular matrix proteins
Crystallisation of matrix	11	Mature osteocytes in a collagen-HCA matrix





SEM micrograph of collagen fibrils [C] incorporated within the HCA layer [HCA] growing on a 45S5 bioactive glass substrate *in vitro.*

BIODEGRADATION OF CERAMIC

DEFINITION

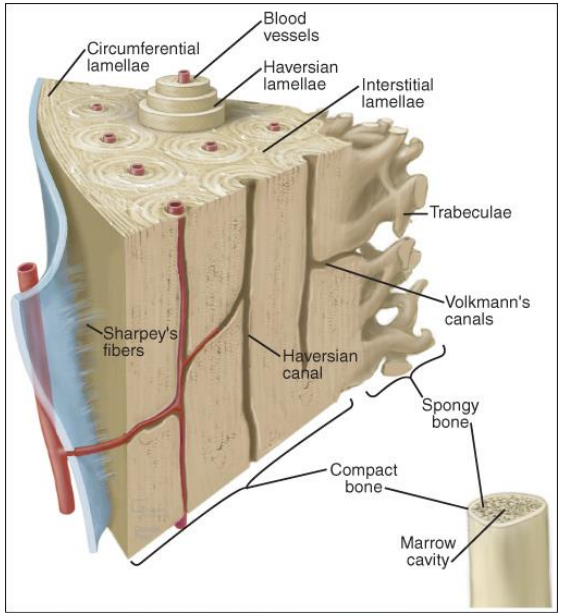
- Biodegradation: chemical breakdown of a material mediated by any component of the physiological environment (such as water, ions, cells, proteins, and bacteria).
- Bioerosion is breakdown including chemical degradation or other process in which bond cleavage is not required (e.g. physical dissolution), of a material mediated by any component of physiological environment.

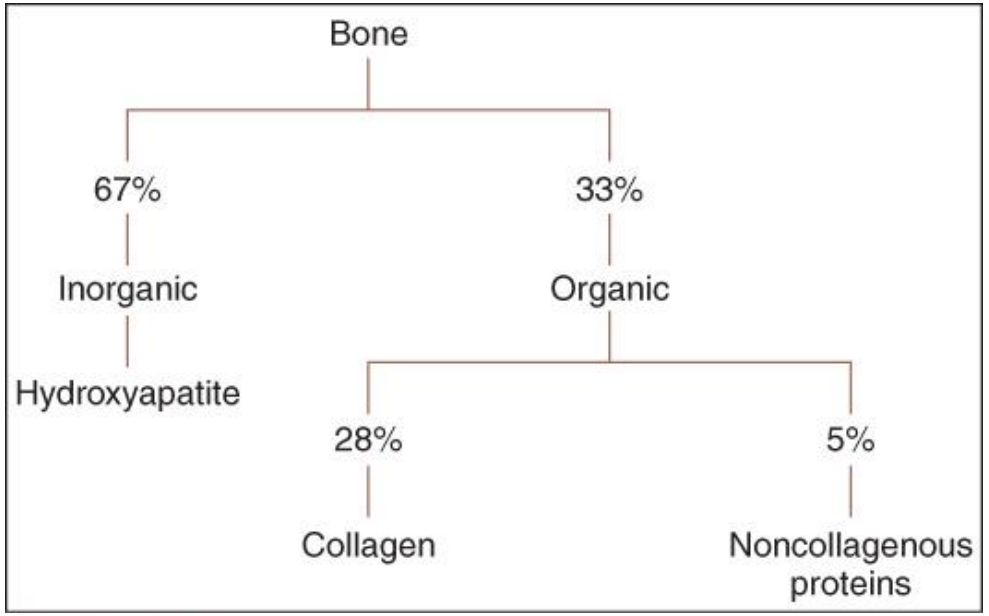
* Level of bioactivity I_B for several Bioceramics

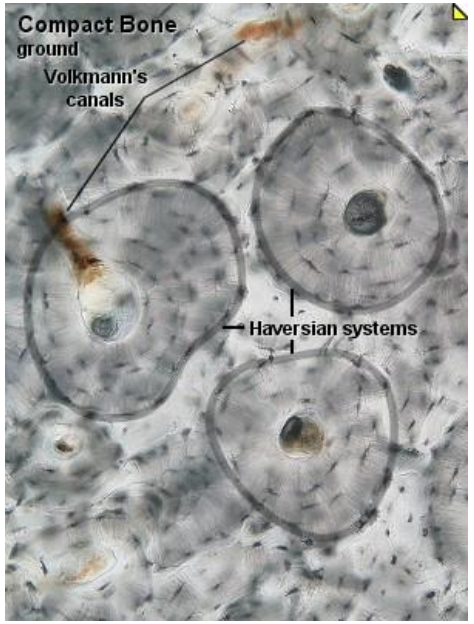
Bioceramics	$I_B = 100/t_{0,5}$
Bioglass 45S5	12,5
Ceravital	5,6
A/W	3,2
Hydroxyapatite	3,1
Ceravital KGX	2,3
Al_2O_3	0

Anatomy and function of bone

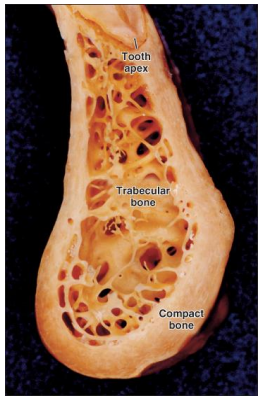
Beneath the hard outer shell of the periosteum there are tunnels and canals through which blood and lymphatic vessels run to carry nourishment for the bone.





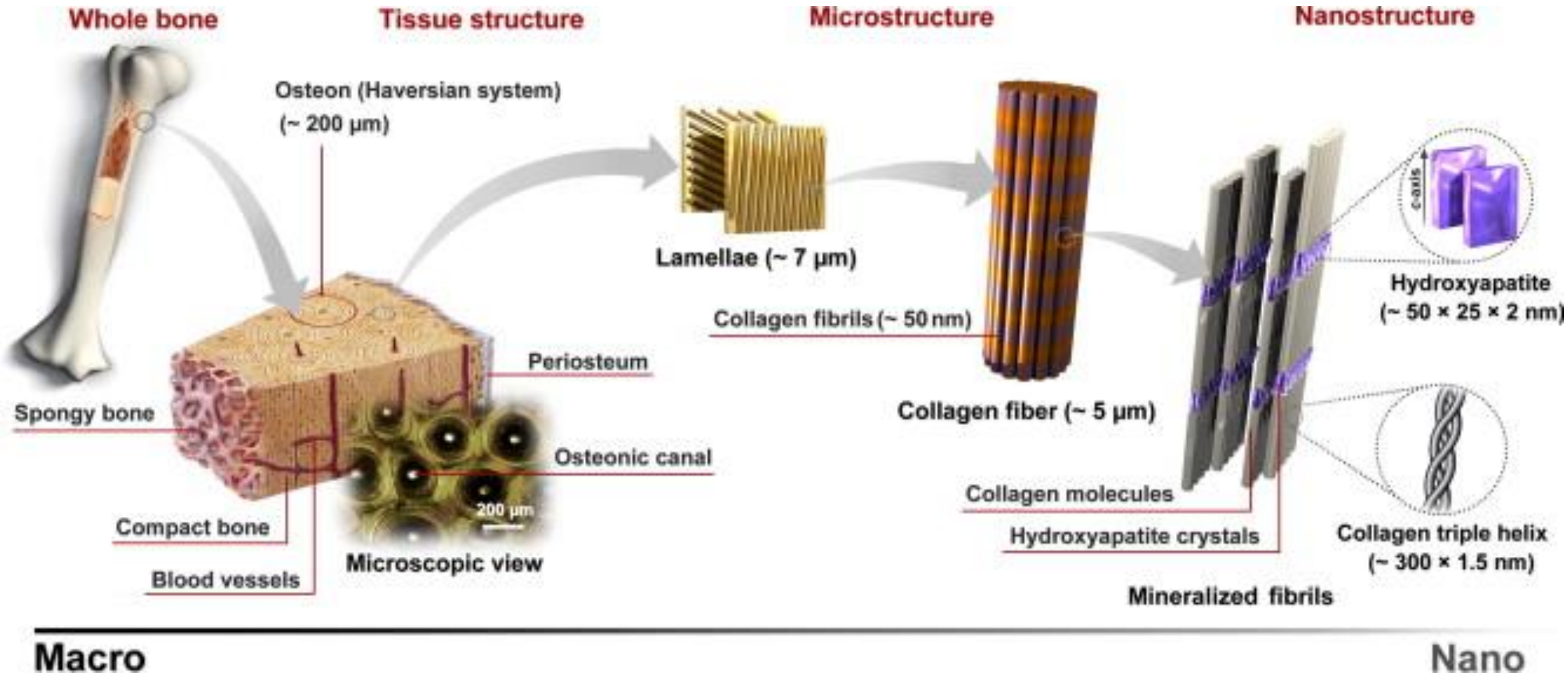


What are the functions of bone?



- Bone provides shape and support for the body, as well as protection for some organs.
- Bone also serves as a storage site for minerals and provides the medium (marrow) for the development and storage of blood cells.

Anatomy and function of bone



The different types of bone cells

Osteoprogenitor cells: Contribute to maintaining the osteoblast population and bone mass. Located in the periosteum and endosteum and differentiate into osteoblasts.

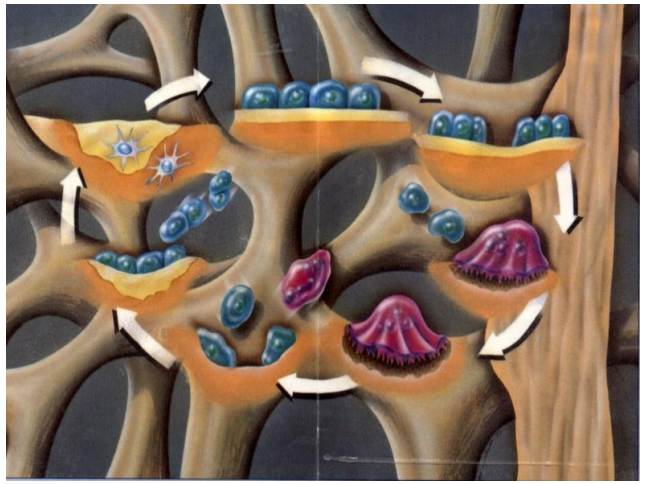
Osteoblasts: synthesize the bone matrix on the bone forming Surfaces that give bone its strength.

Osteocytes: organized throughout the mineralized bone matrix that support bone architecture, its function is to help maintain bone as living tissue.

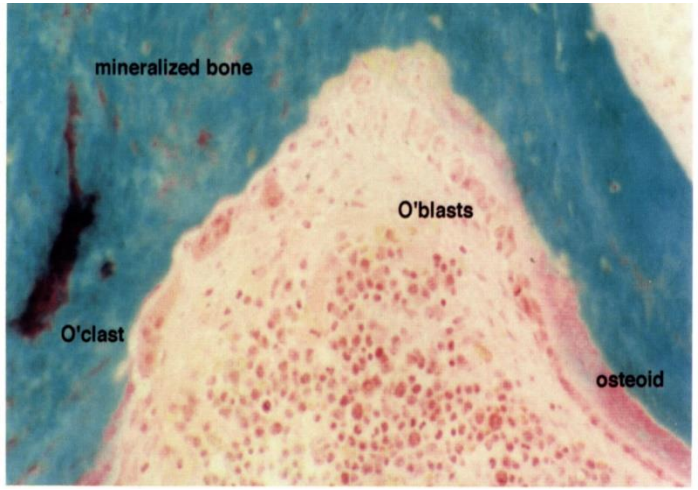
Osteoclasts: large multinucleated cells (20-100 µm) derived from fusion of monocytes/macrophages and resorbs bone. its function is to absorb and remove unwanted tissue.

Fat cells and hematopoietic cells: found within the bone marrow.

Ematopoietic cells: that produce blood cells.



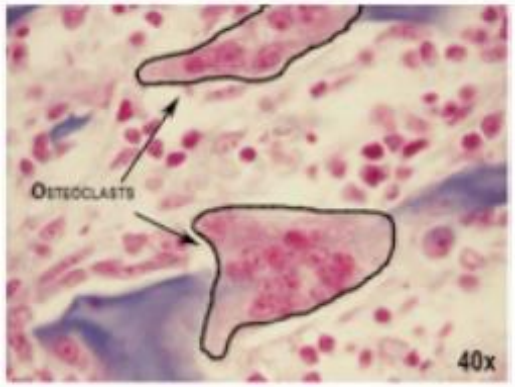
Bone Remodeling

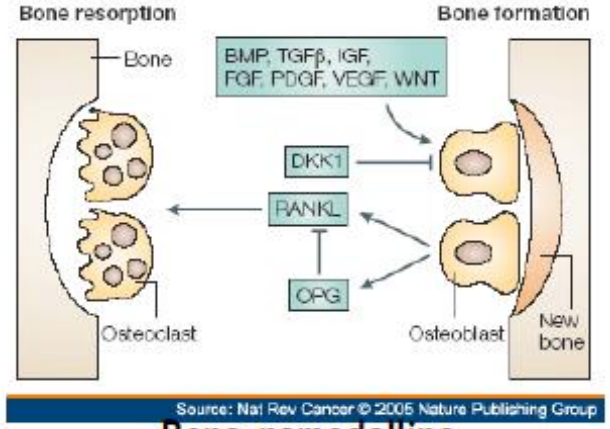


Five Phases of Remodeling

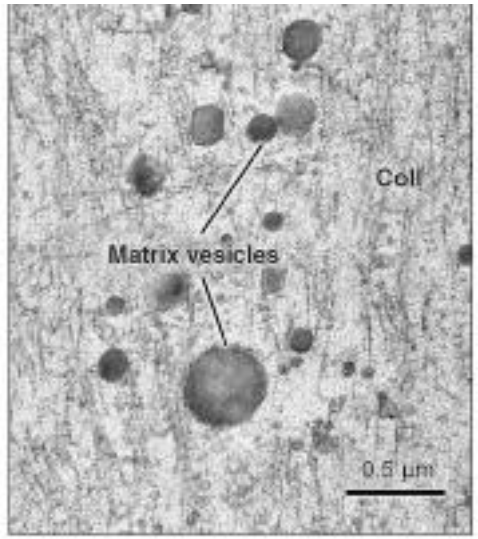
1. Activation of Osteoclasts
2. Resorption of Bone
3. Reversal Phase
4. Formation of Bone
   Activation of Osteoblasts
   Mineralization
5. Resting

The different types of bone cells



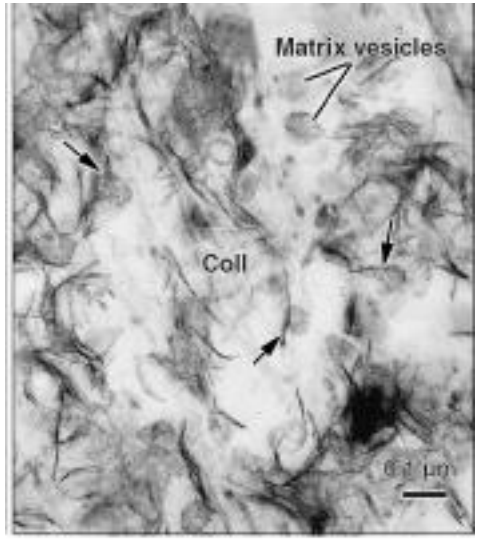


Bone remodelling



Initiation

Not dependent on alkaline phosphatase



Propagation

ECM mineralization

Dependent on alkaline phosphatase

- Physiologic bone mineralization in mammals refers to the ordered deposition of apatite on a type I collagen matrix
- The mineral is an analog of the geologic material, hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ - provides mechanical rigidity and load bearing strength to the bone composite

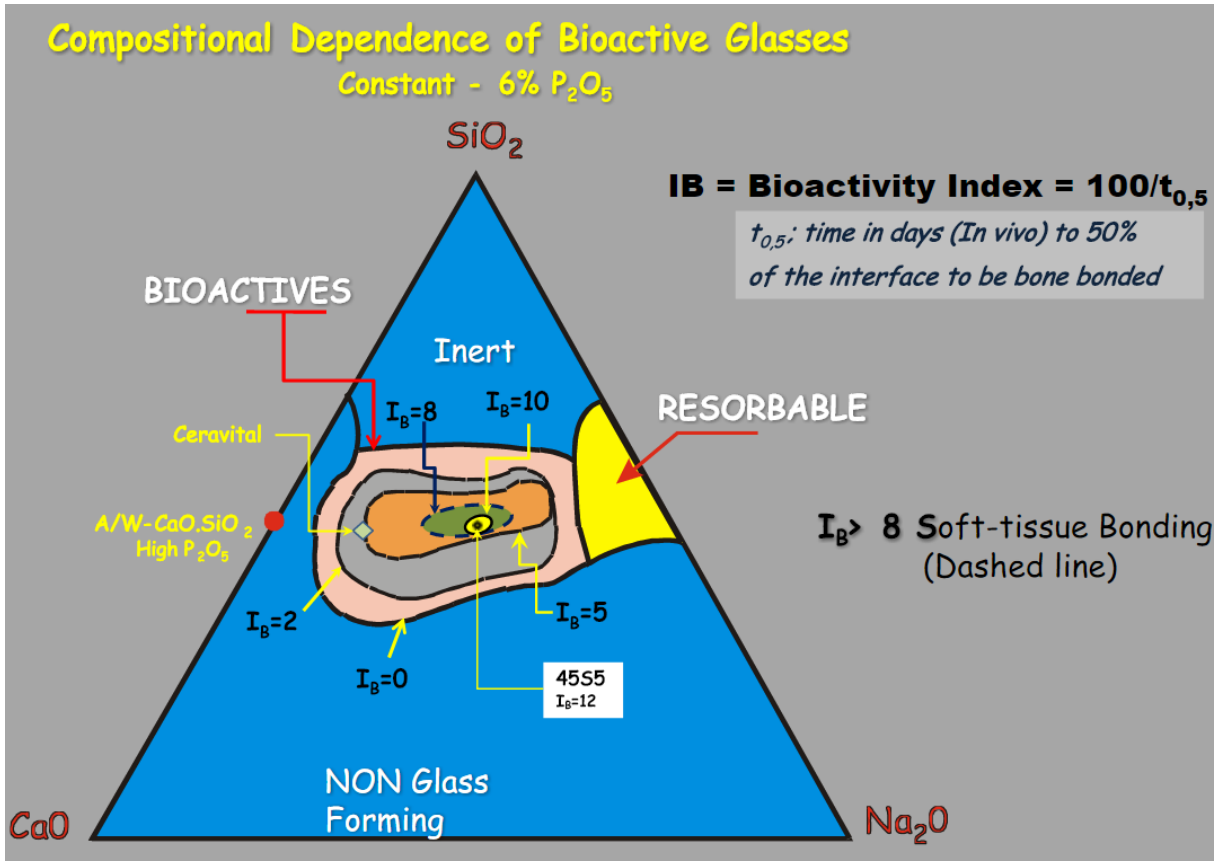
BIODEGRADATION OF CERAMIC

BIODEGRADATION

ceramic degrade primarily via dissolution, this because ceramic formulation are highly soluble in aqueous environment

Uncontrolled degradation

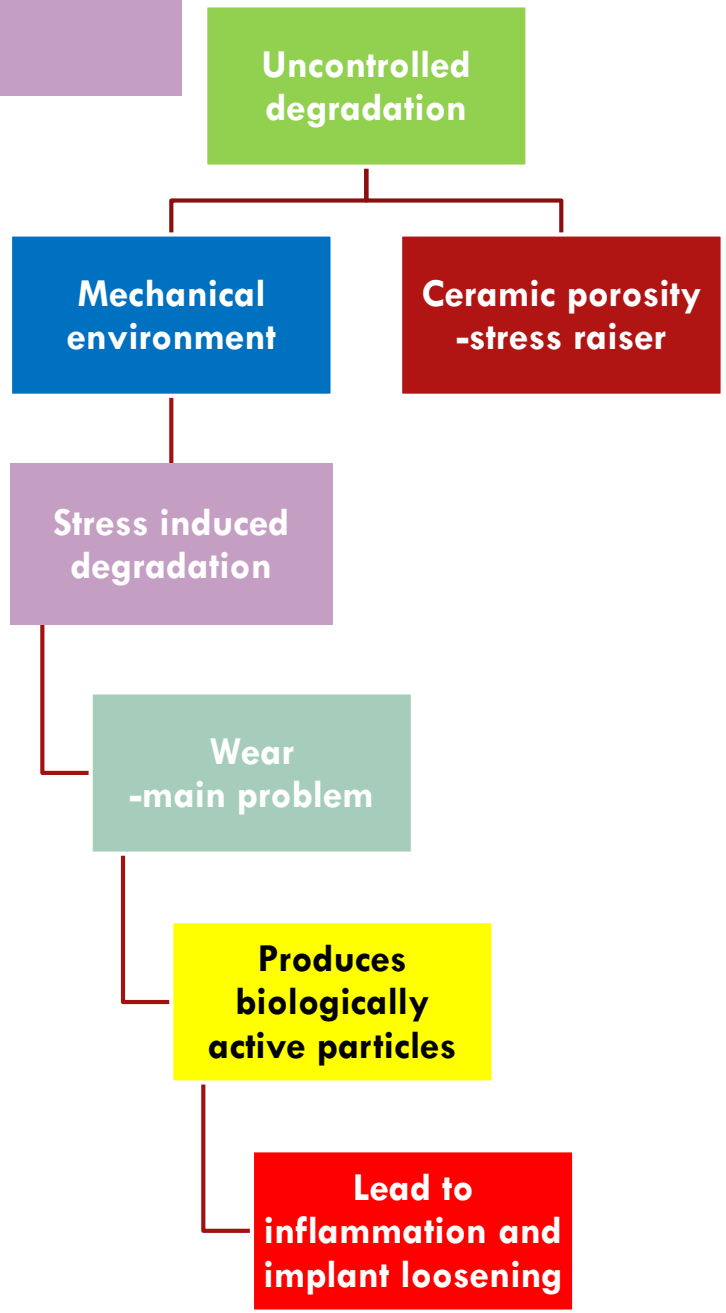
Controlled degradation



Uncontrolled Degradation

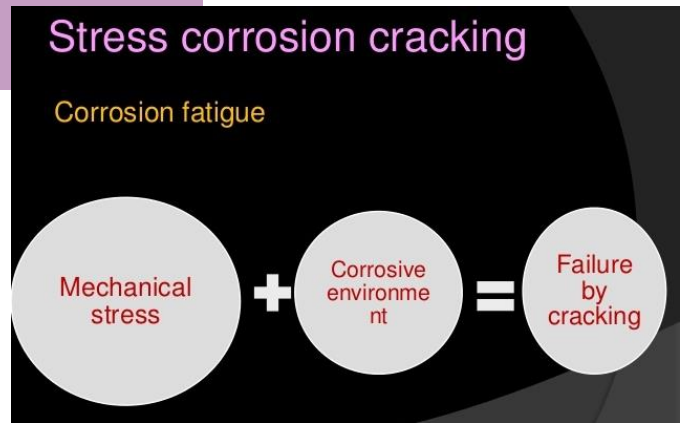
*DEPEND ON TWO FACTOR

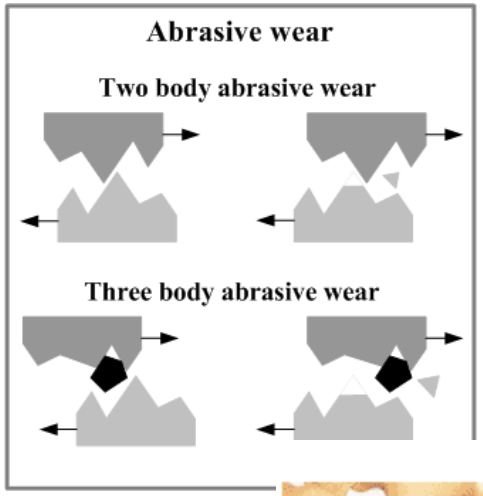
- Mechanical environment
  - Stress induced degradation can occur in ceramics under tension.
  - If crack is formed in these materials, the tensile stress may lead to further dissolution at the crack tip and material fracture.
- Ceramic porosity
  - Pores are stress raiser thus may increase the formation of cracks or the rate of their propagation.

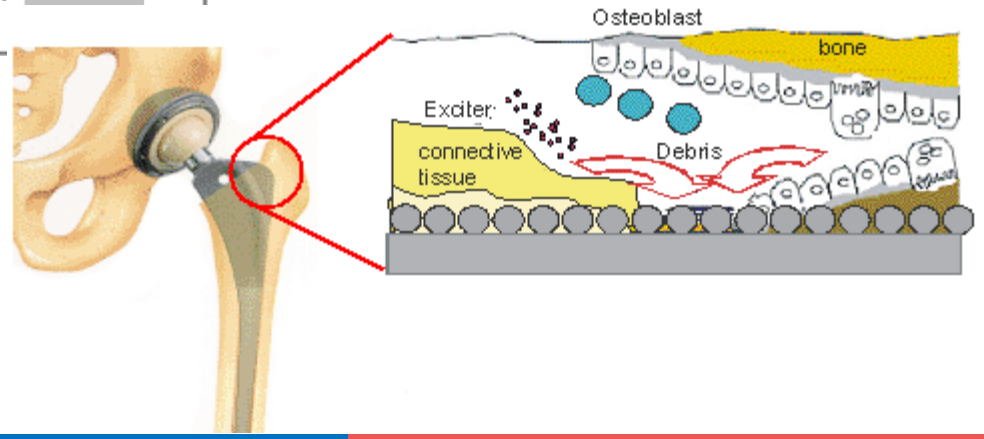


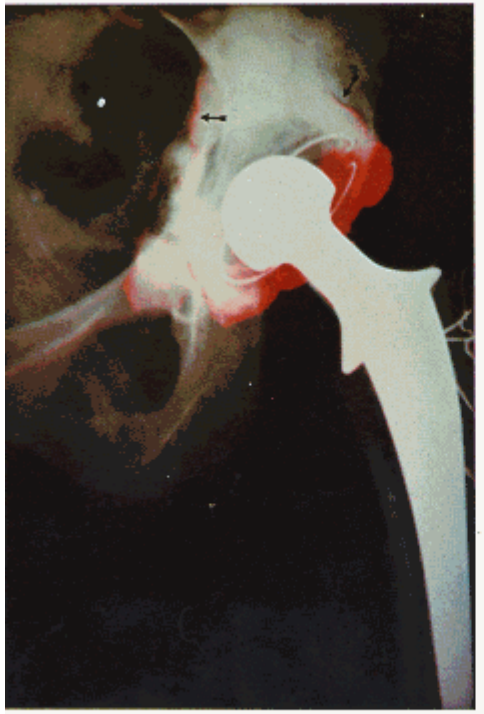
Uncontrolled Degradation

- Uncontrolled degradation will cause WEAR.
- WEAR → the generation of fine wear particles that can lead to inflammation and implant loosening.







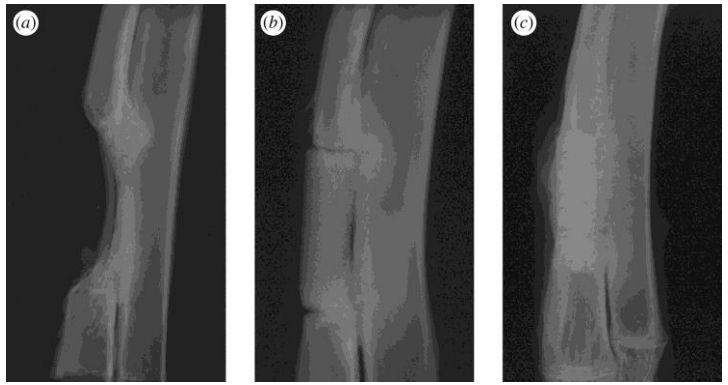


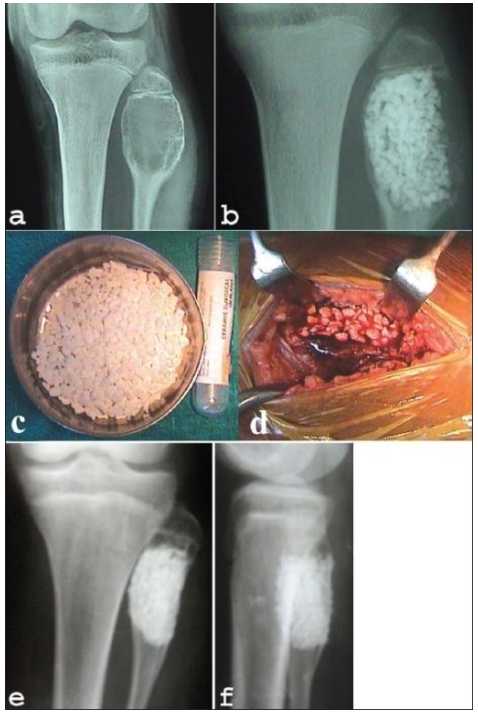
Color Plate I.1. Color subtraction technique applied to this film confirms definite loosening with obvious contrast around acetabular component (arrows).

Color Plate I.2. Color subtraction dramatically confirms femoral component loosening (arrow).

Controlled Degradation

- Degradation is desirable.
- Controlled biomaterial degradation can be used as an important part of tissue engineering and drug delivery therapies.
- For these application, the temporary nature of the material is ideal to promote localized tissue healing or release of a bioactive agent without the need for second surgery to remove implant.





Controlled Degradation

- Biodegradable ceramics are usually type of calcium phosphate, such as
  - Hydroxyapatite, HA ($Ca_{10}(PO_4)_6(OH)_2$)
  - Tricalcium phosphate, TCP ($Ca_3(PO_4)_2$)
- Biodegradable ceramic generally degrade by dissolution (influenced by the solubility of the ceramic formulation in media and the pH of the media) coupled with physical disintegration.

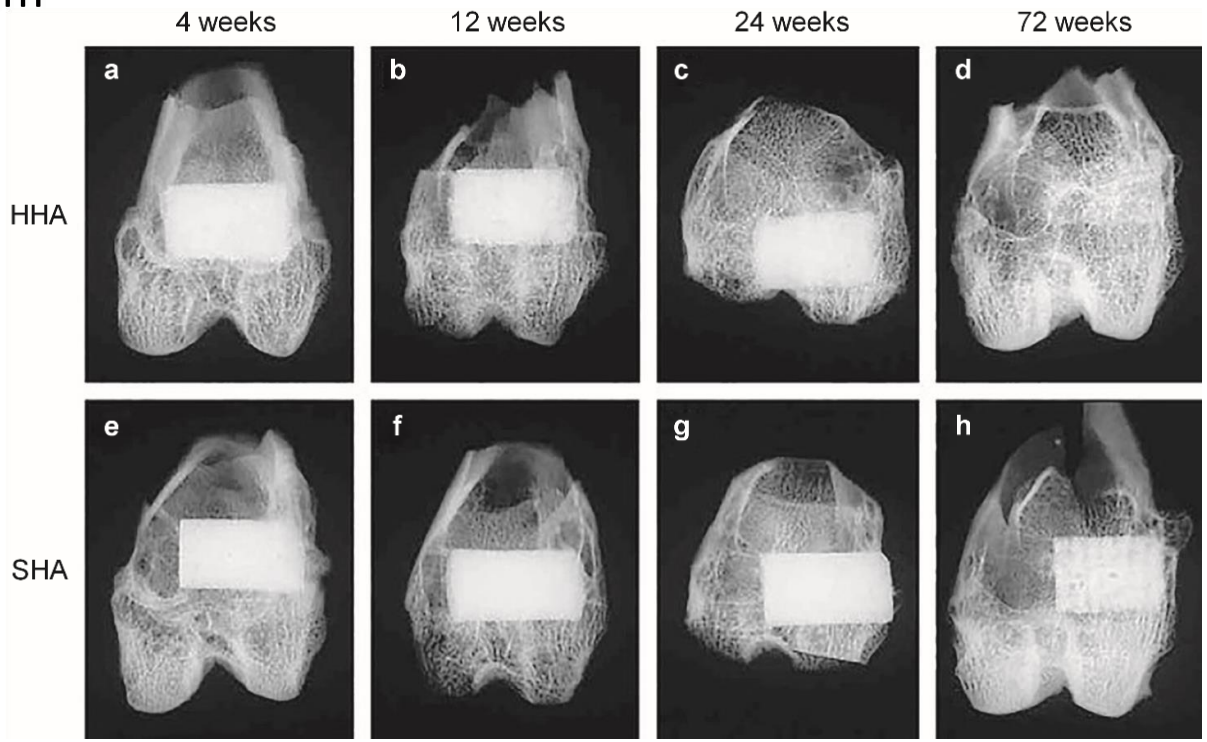


Figure Soft X-ray photographs of the operated portion of the rabbit femur. (a) 4 weeks, (b) 12 weeks, (c) 24 weeks and (d) 72 weeks after implantation of HHA; (e) 4 weeks, (f) 12 weeks, (g) 24 weeks, and (h) 72 weeks after implantation of SHA. Involvement of HHA in bone tissue was evident at 72 weeks (h). Reproduced with permission from T. Okuda, K. Ioku, I. Yonezawa, H. Minagi, Y. Gonda, G. Kawachi, M. Kamitakahara, Y. Shibata, H. Murayama, H. Kurosawa, T. Ikeda, *Biomaterials*, 2008, **29**, 2719. ©2008, Elsevier

Controlled Degradation

FACTOR THAT INFLUENCE DEGRADATION RATE

1-Chemical susceptibility of the material

- Hydrated form forms such as hydrated calcium sulphate degrade faster than their nonhydrated counterparts.

Controlled Degradation

FACTOR THAT INFLUENCE DEGRADATION RATE

2. Amount of crystallinity

- Ceramic degradation depend on water penetration.
- A more tightly packed crystalline material is less susceptible to dissolution than a ceramic that is mainly amorphous (unstructured).
- Polycrystalline ceramics degrade more quickly than single crystal ceramic due to presence of grain boundaries.
- Ceramic contain many smaller crystals is more susceptible to dissolution than one with fewer, larger crystal.

Controlled Degradation

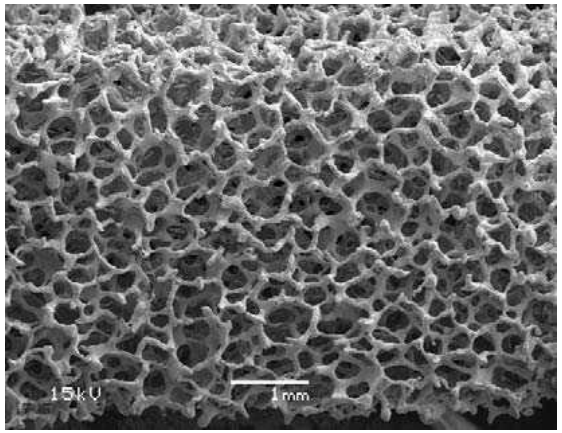
FACTOR THAT INFLUENCE DEGRADATION RATE

3. Amount of media (water) available

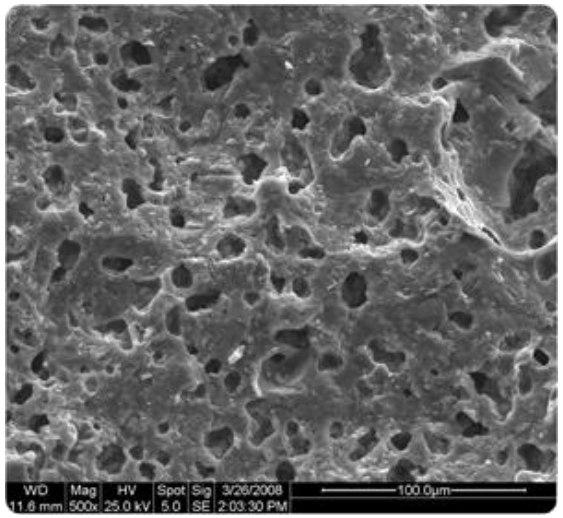
- High amount of water → increase degradation rate
- Low amount of water → slower degradation rate

4. Material surface area to volume ratio

- Highly porous ceramic will dissolve more quickly than the same ceramic with fewer pores due to increase in area for interaction with the environment.



Highly porosity



Low porosity

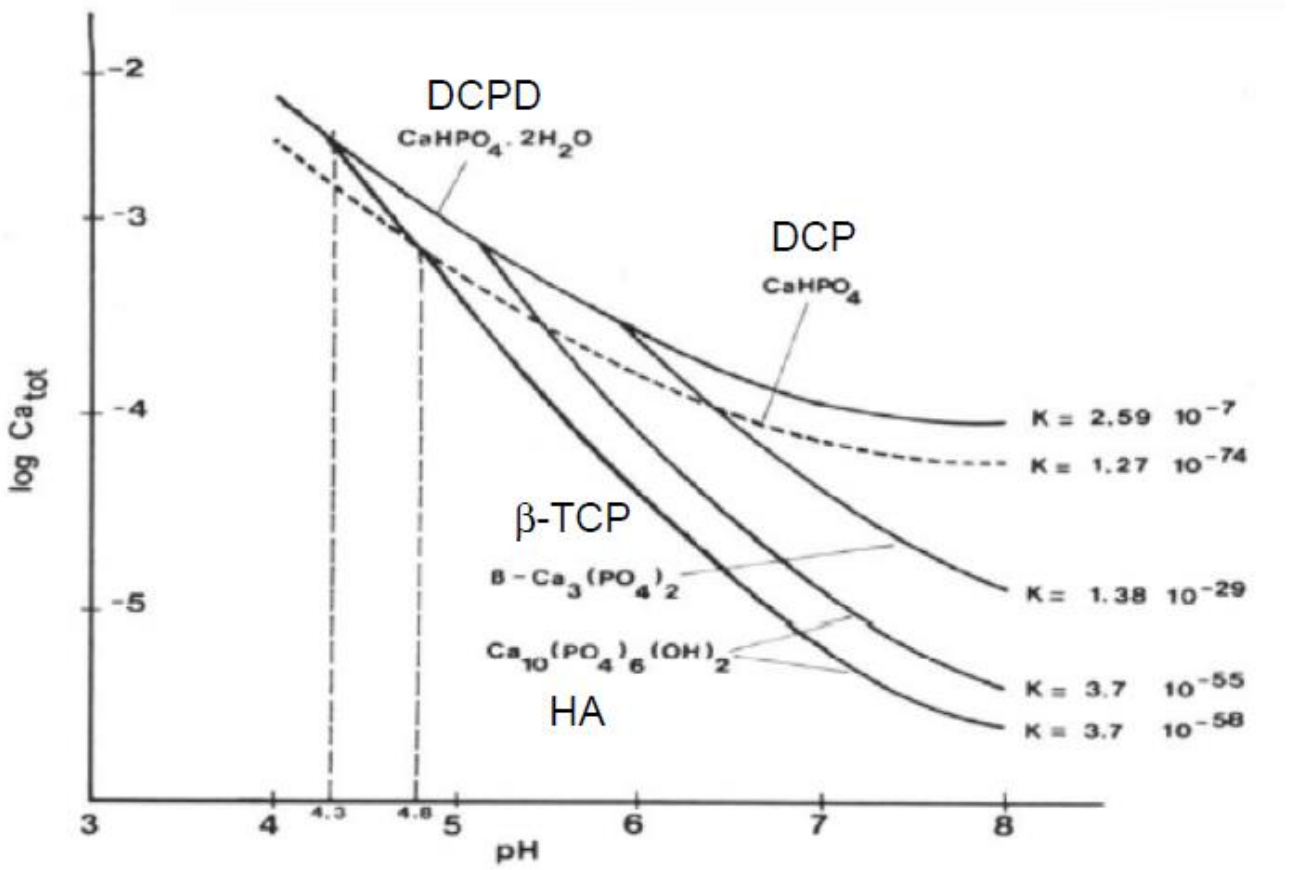
Controlled Degradation

5. Mechanical environment

- Ceramic degradation is encouraged in areas with high mechanical stress, either due to
  - Implant site location
  - Presence of stress raiser in the device
  - Production of wear particles will caused inflammatory response → pH drop → accelerate degradation of material

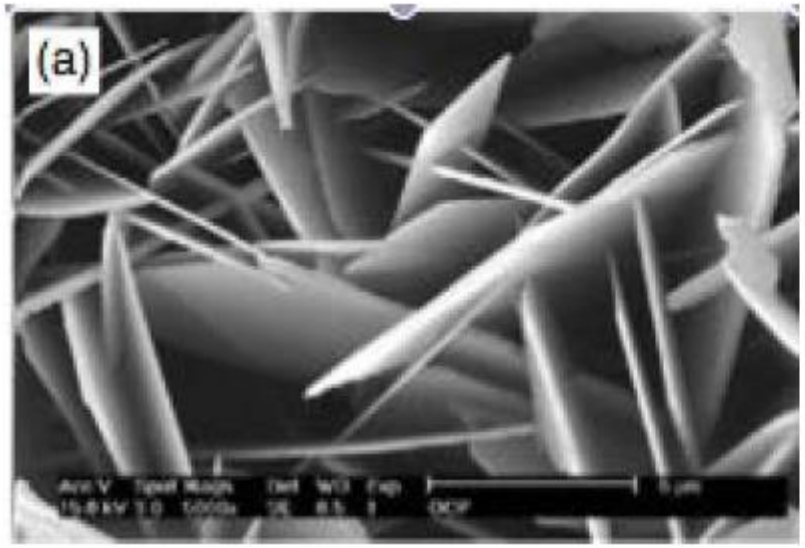
Order of solubility

- Fluoroapatite
- Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$
- Beta-Tricalcium Phosphate $Ca_3(PO_4)_2$
- Alpha- Tricalcium Phosphate $Ca_3(PO_4)_2$
- Amorphous Calcium Phosphate
- Tetracalcium Phosphate $Ca_4P_2O_9$

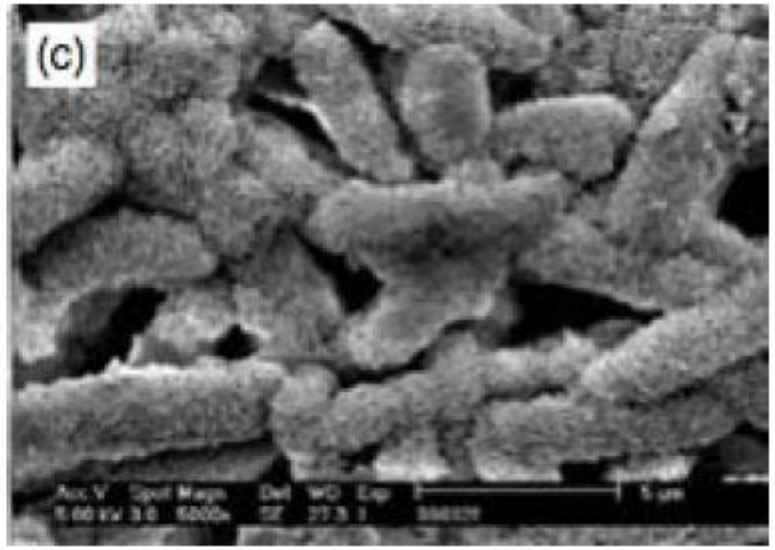


DCPD >> DCP >> β-TCP >> HA

- biodegradation mechanisms:
  - physico-chemical degradation
    - dissolution/precipitation



octacalciumphosphate at t = 0 weeks



octacalciumphosphate at t = 2 weeks

[reproduced from: Van Blitterswijk, C. **(2008)**, *Tissue Engineering*, Academic Press Oxford, UK.]

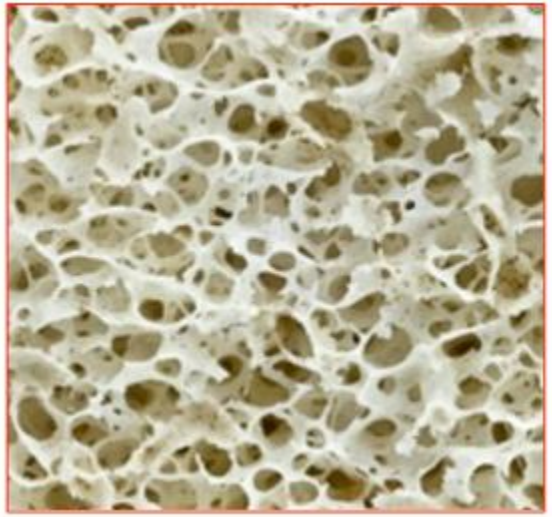
Select the right CaP material

- The only calcium phosphate that is stable in contact with water is hydroxyapatite (HA), and this is the form found naturally as bone mineral.
- In applications where a stable, but biocompatible material is desired, HA is the material of choice.
- If the goal is remodeling, then more soluble forms might be of interest.
- Regardless of the composition, all calcium phosphates are osteoconductive.
- Osteoconduction helps to increase the activity of bone forming cells (osteoclasts).
- Bone cells preferentially grow inside of porous materials. An ideal scaffold consists of interconnected pores in the size range of 50 to 400 microns.
- In contrast, for applications where inertness is required such as soft tissue augmentation, dense, nonporous, nearly insoluble HA particles with a minimal surface area are ideal.

Natural foams

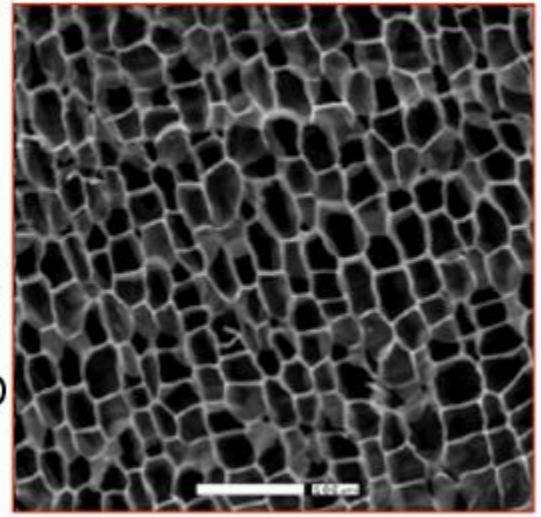
Marine (porifera)

Bone tissue

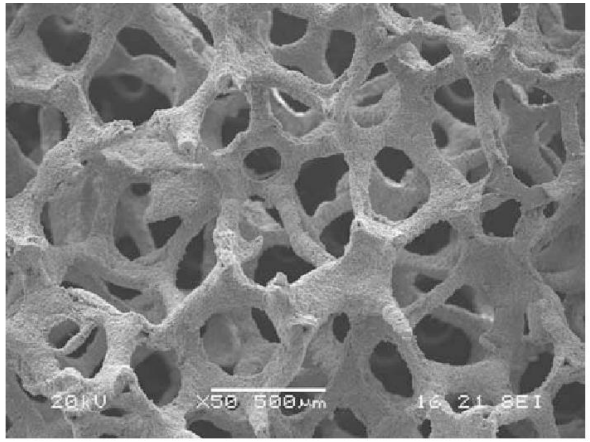


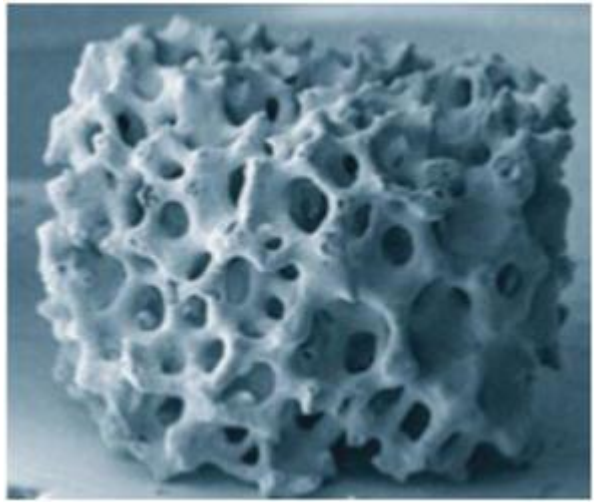
Bread

Vegetable



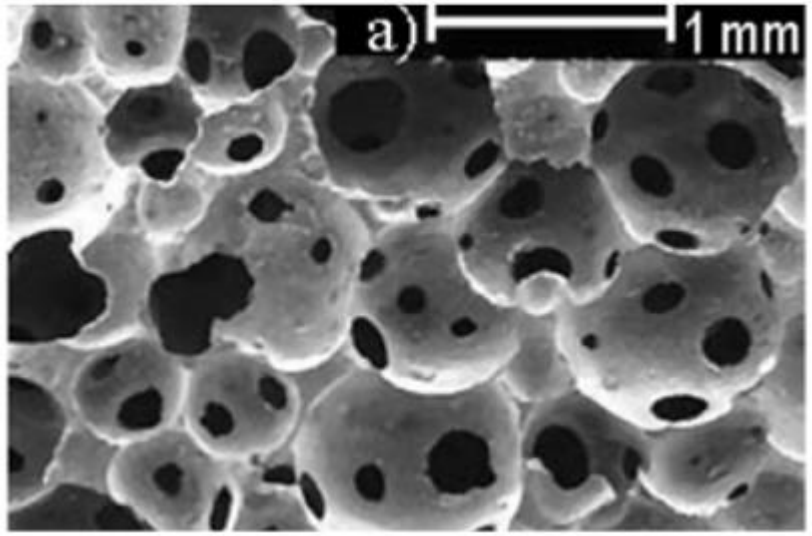
Macroporous foams



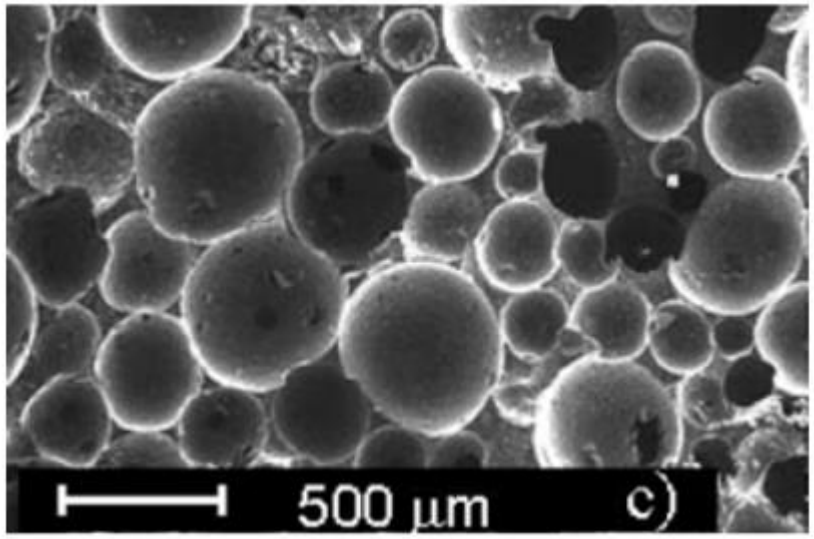


Classification of ceramic foams

- Open cells

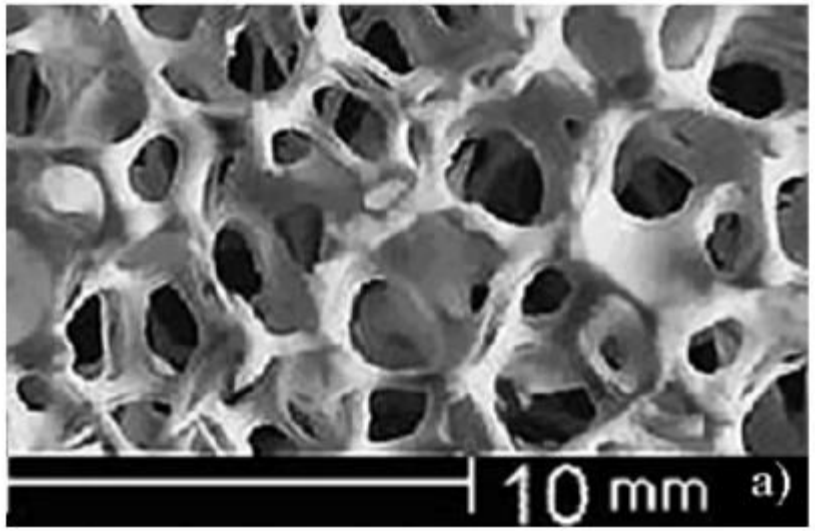


- Closed cells

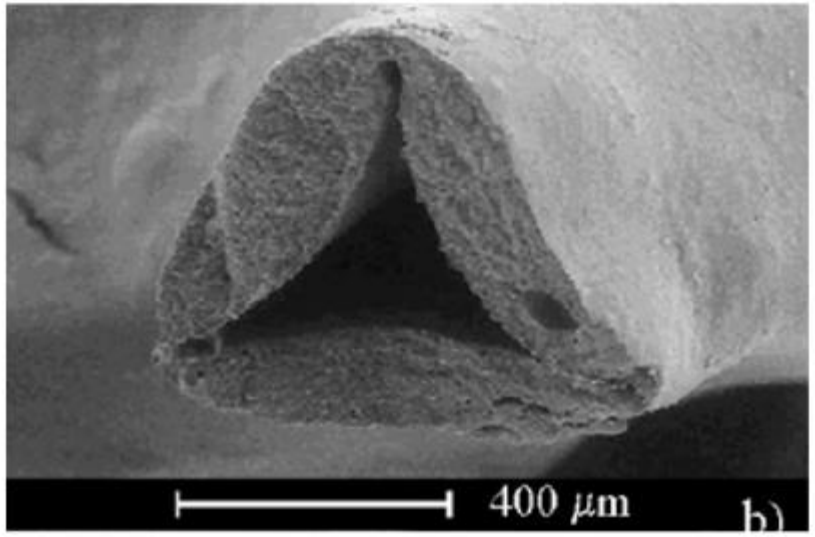


Classification of ceramic foams

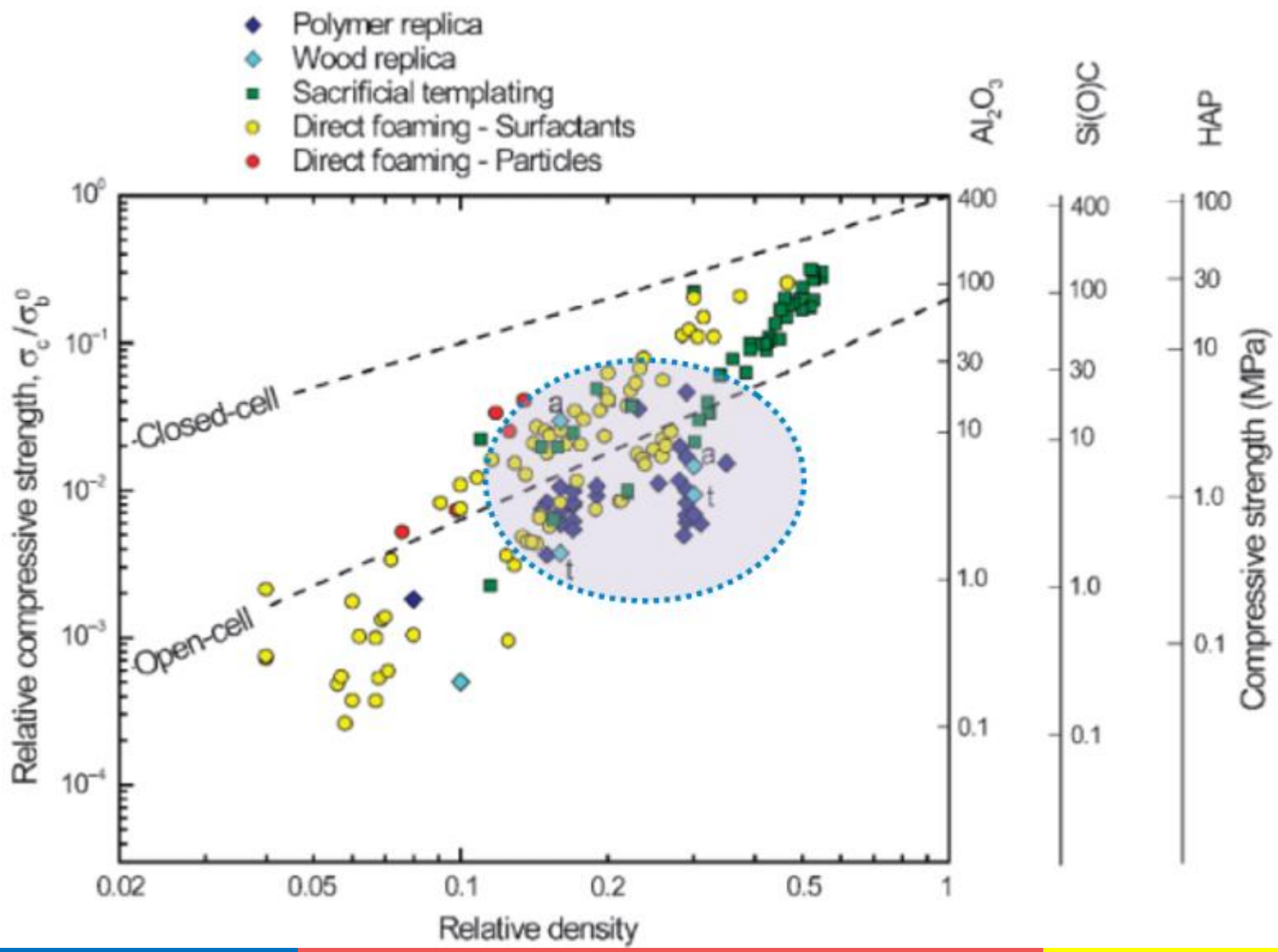
- ceramic strut



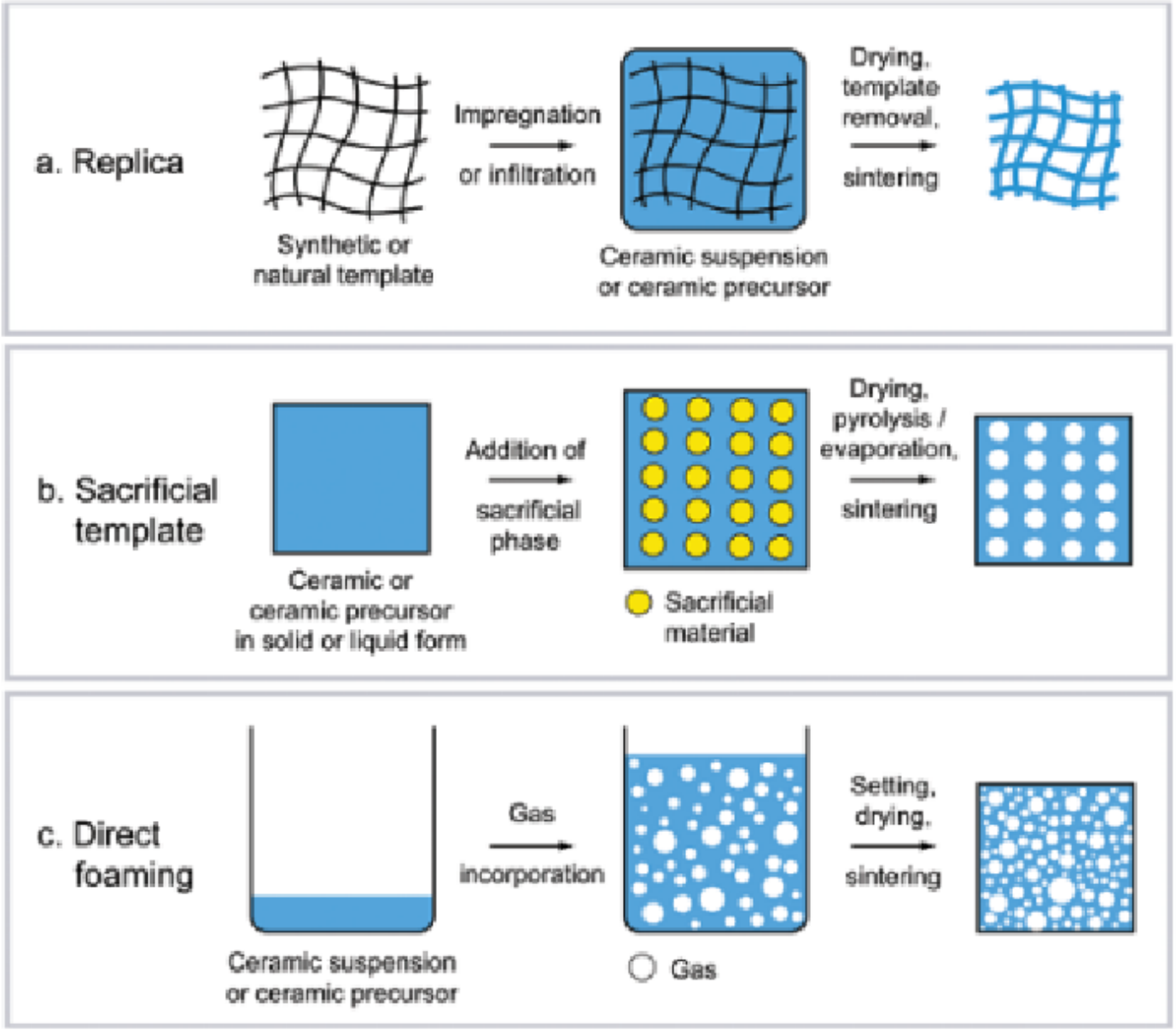
- Strut detail showing cavity left by templating material



Mechanical properties



Foams manufacturing



Design criteria of the scaffold

MORPHOLOGY

- High degree of porosity (> 80%)
- Highly interconnected pore
- Control pore size

Large pores:

for adhesion and cell proliferation (eg osteblast):

- 150-300μm
- 100-400μm

Small pores:

For the transport of nutrients and removal of waste substances and release of growth factors

- 0.1-10 μm

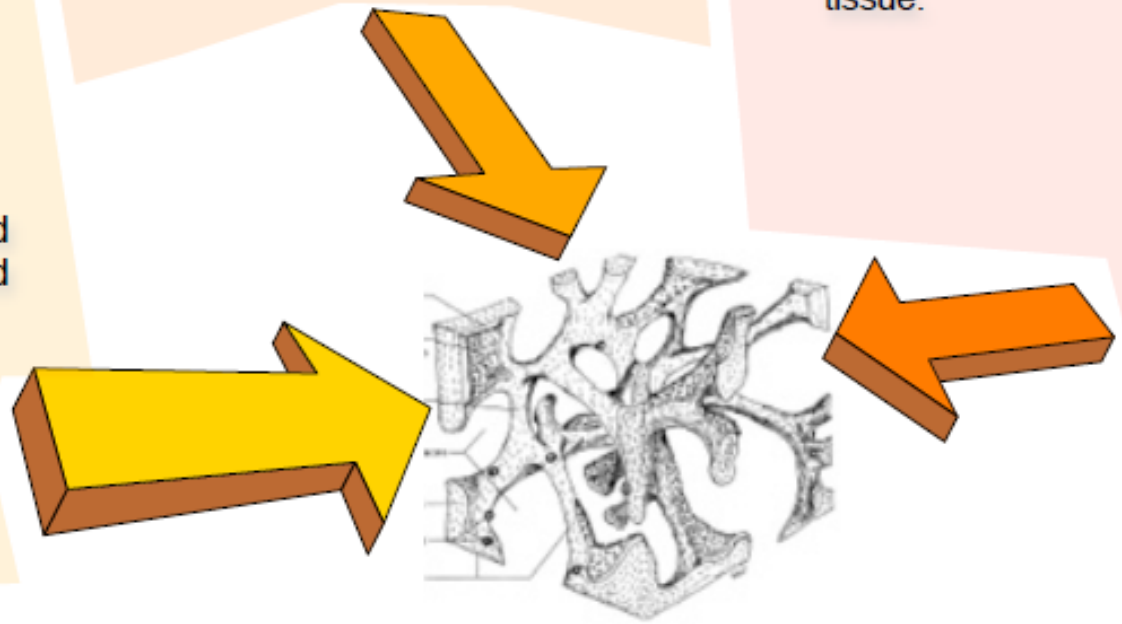
MECHANICAL PROPERTY

Mechanical response adequate to support the hydrostatic pressure and to preserve the geometry of the pores required for cell growth during the phases of in vitro culture (or the efforts in vivo).

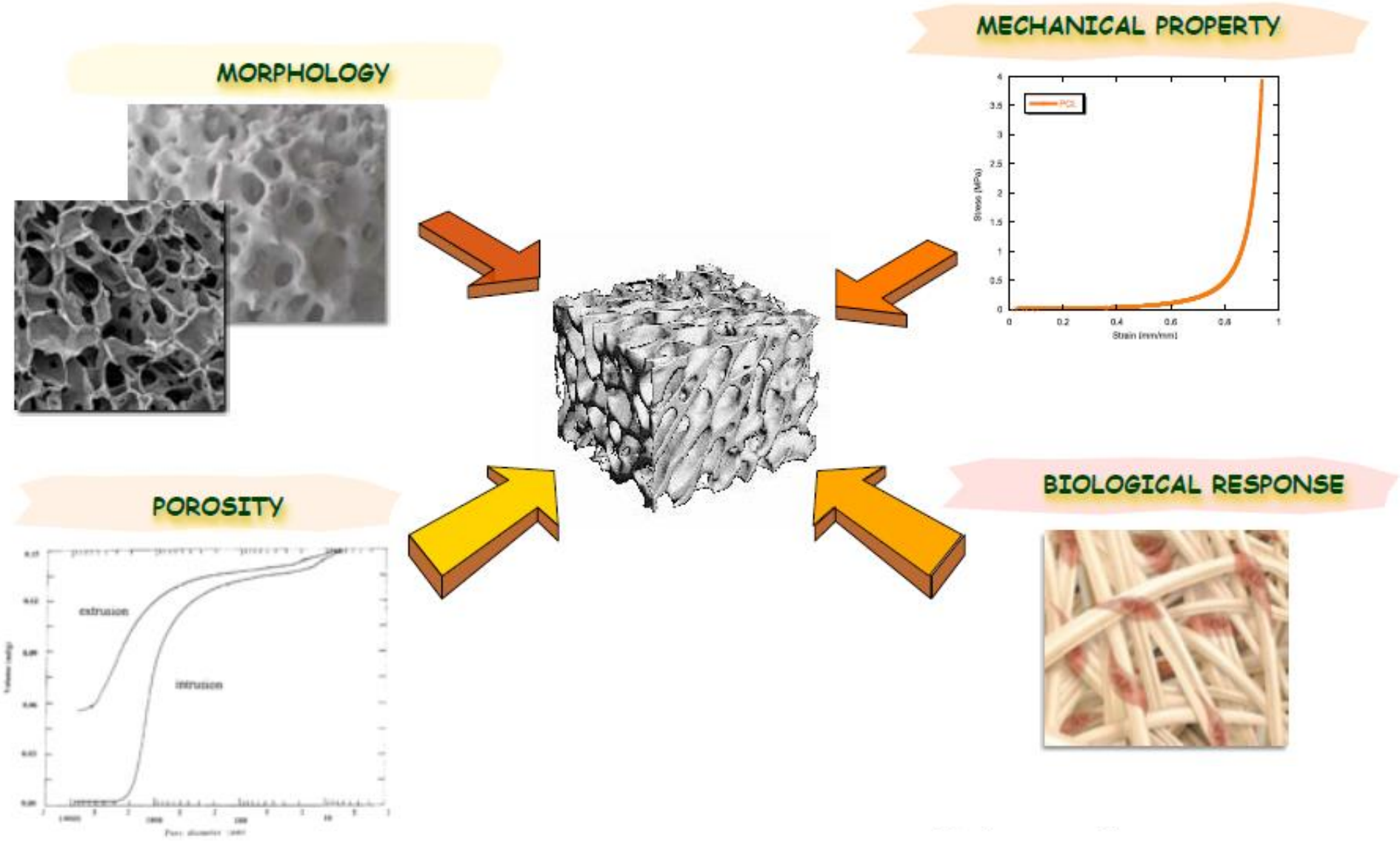
BIOLOGICAL REQUIREMENTS (Chemical Composition)

- Biocompatibility
- Osteoconductive
- Osteoinductive
- Degradation rate comparable with the rate of formation of new tissue.

bioactivity



Design criteria of the scaffold



Advantages

- ✓ Biological compatibility and activity
- ✓ Less stress shielding
- ✓ No disease transmission
- ✓ Unlimited material supply

Disadvantage

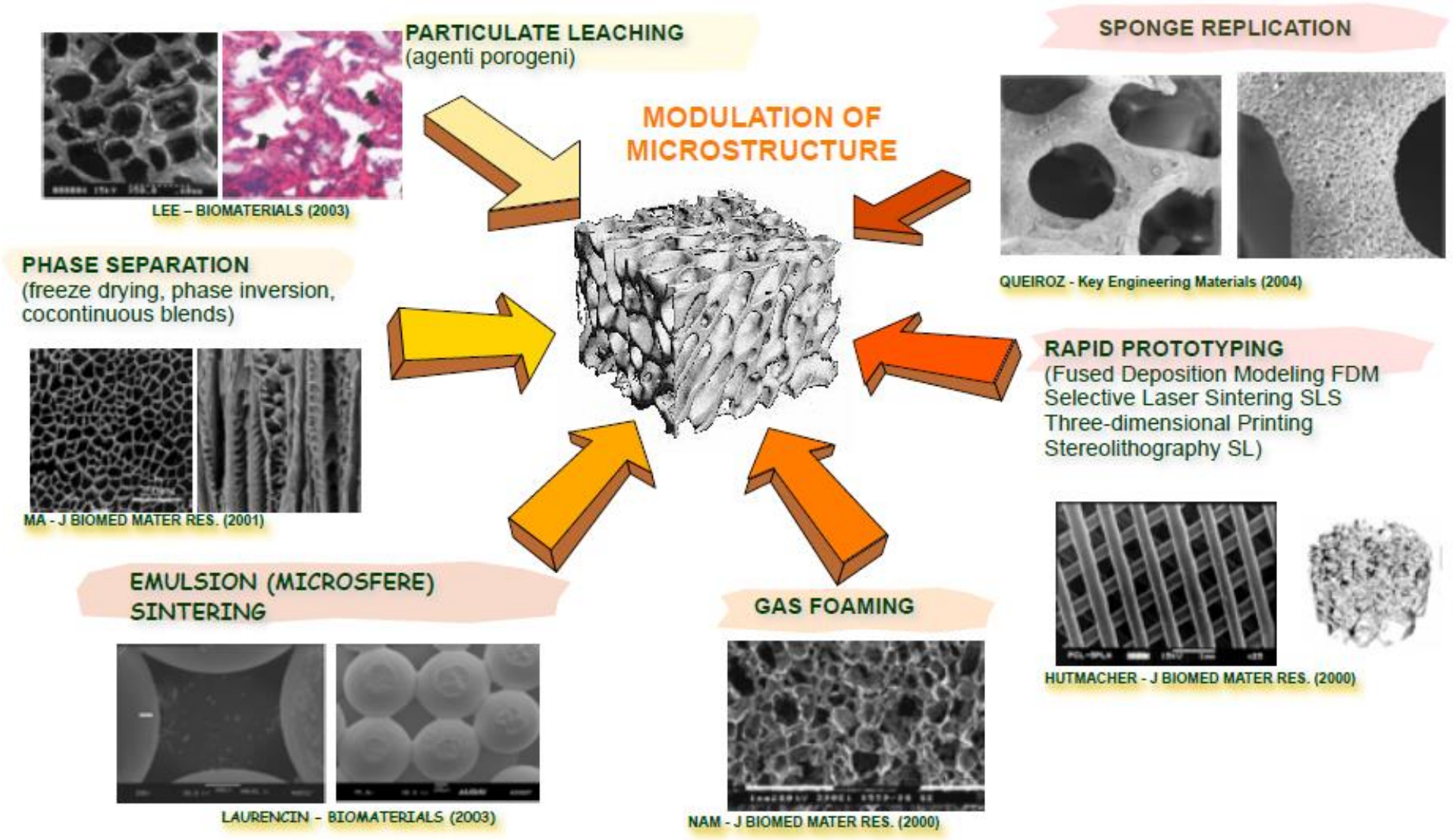
- ✓ Brittleness – not for load bearing applications

Materials commonly used

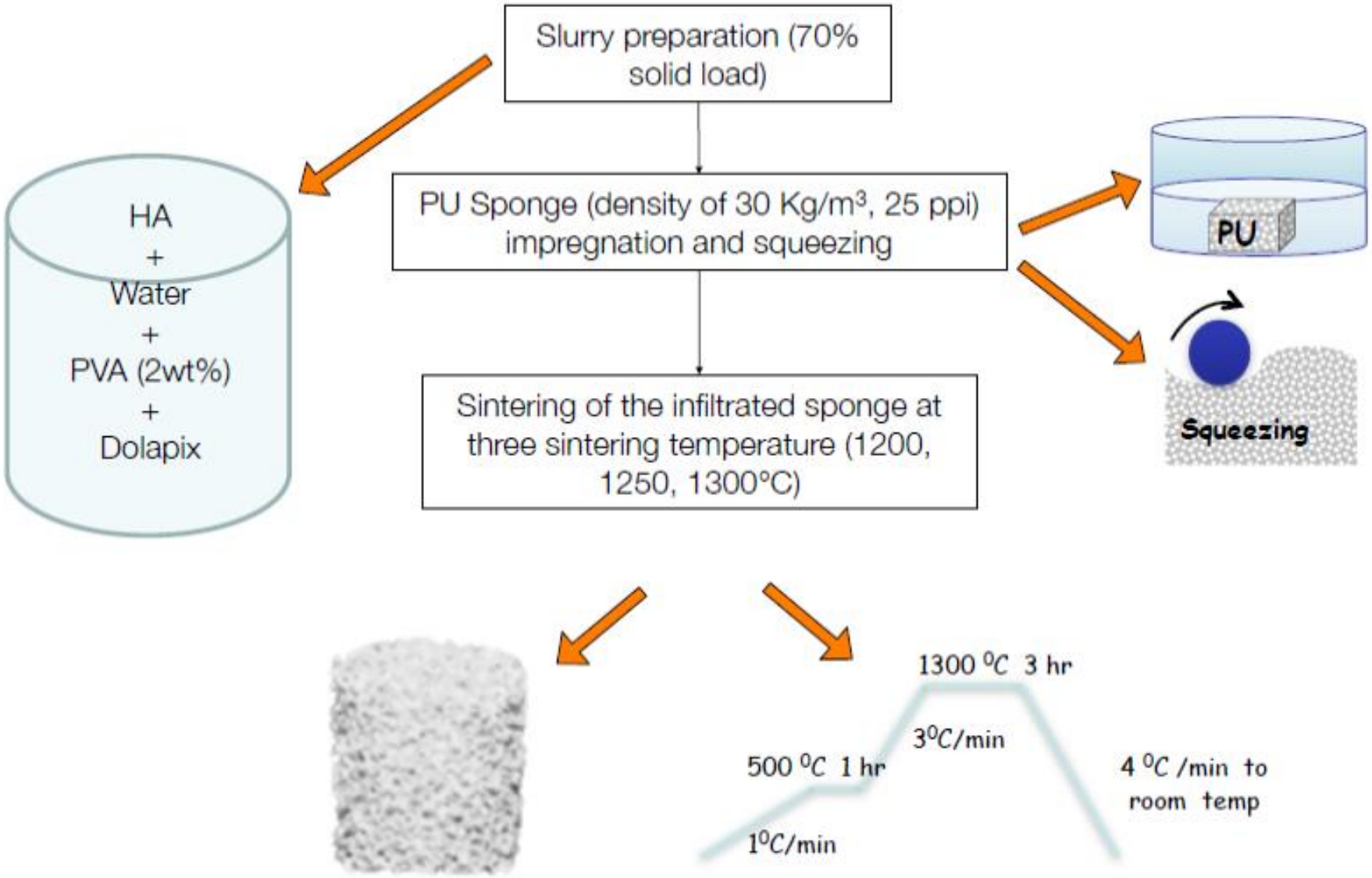
- ✓ Hydroxyapatite
- ✓ Tricalcium phosphate
- ✓ Glass ceramics (Ca-P-Si system)

- $Ca_{10}(PO_4)_6(OH)_2$
- Main component of bone and tooth minerals
- Ca/P ratio : 1.67
- Excellent biocompatibility
- Chemically similar to bone

Fabrication Processes for Porous Scaffolds

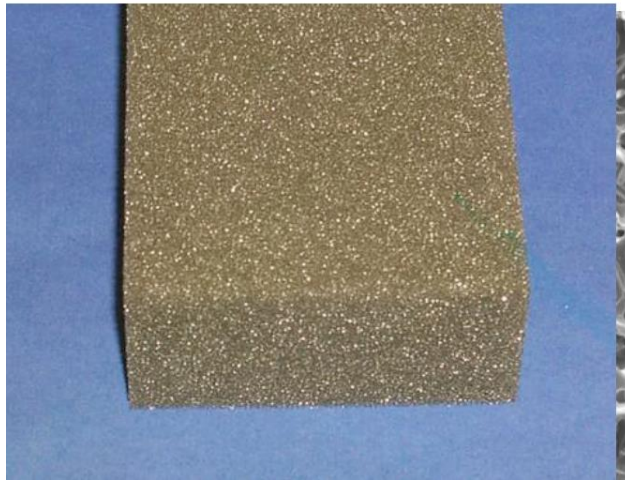


HA Scaffold: Sponge replica method

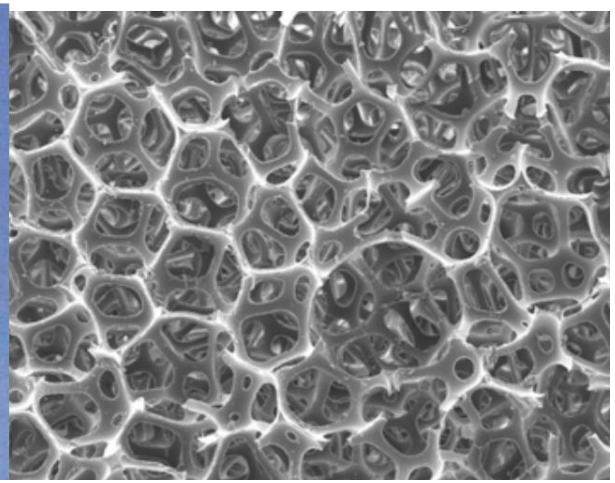


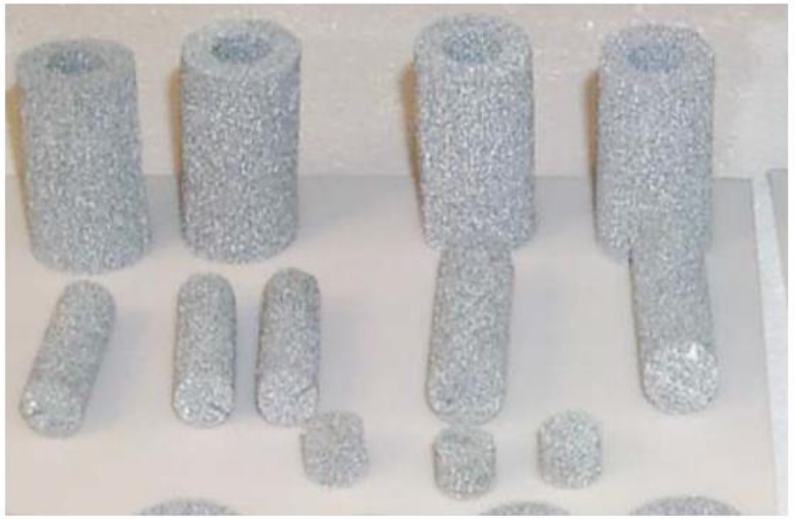
Manufacturing with sponge replica method

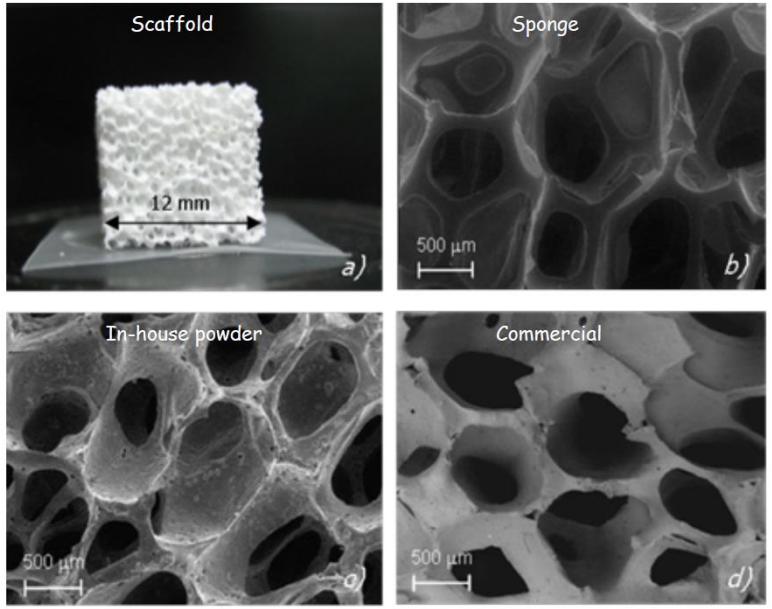
Poliurethan sponge

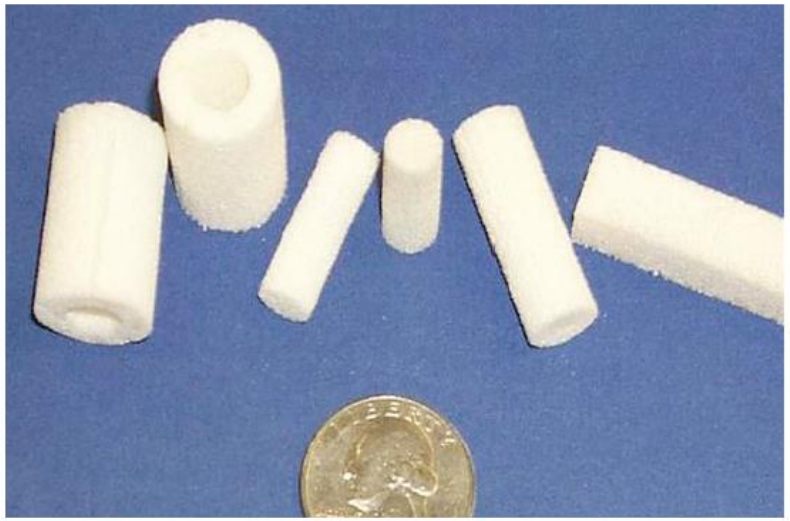


SEM micrograph

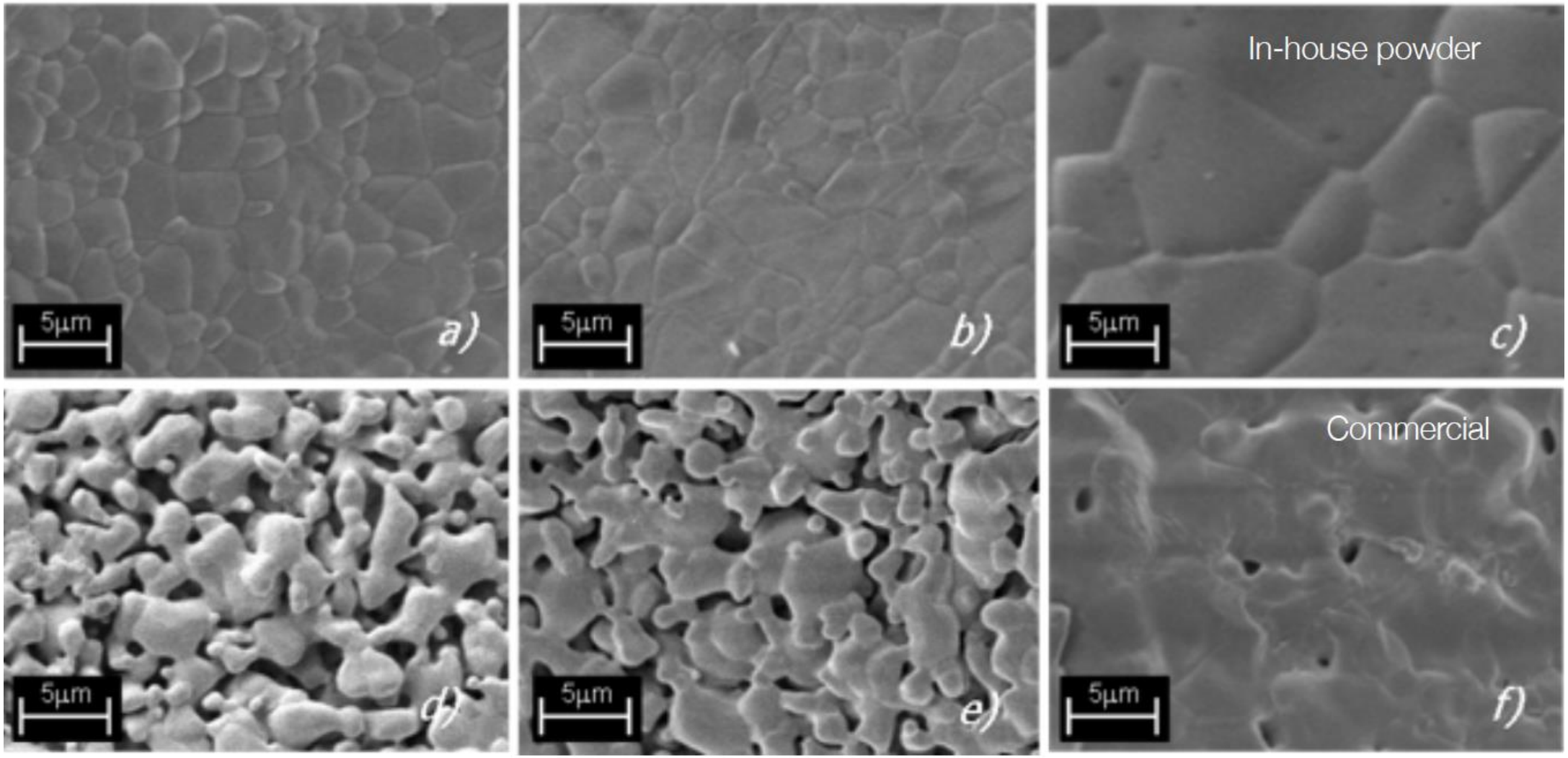






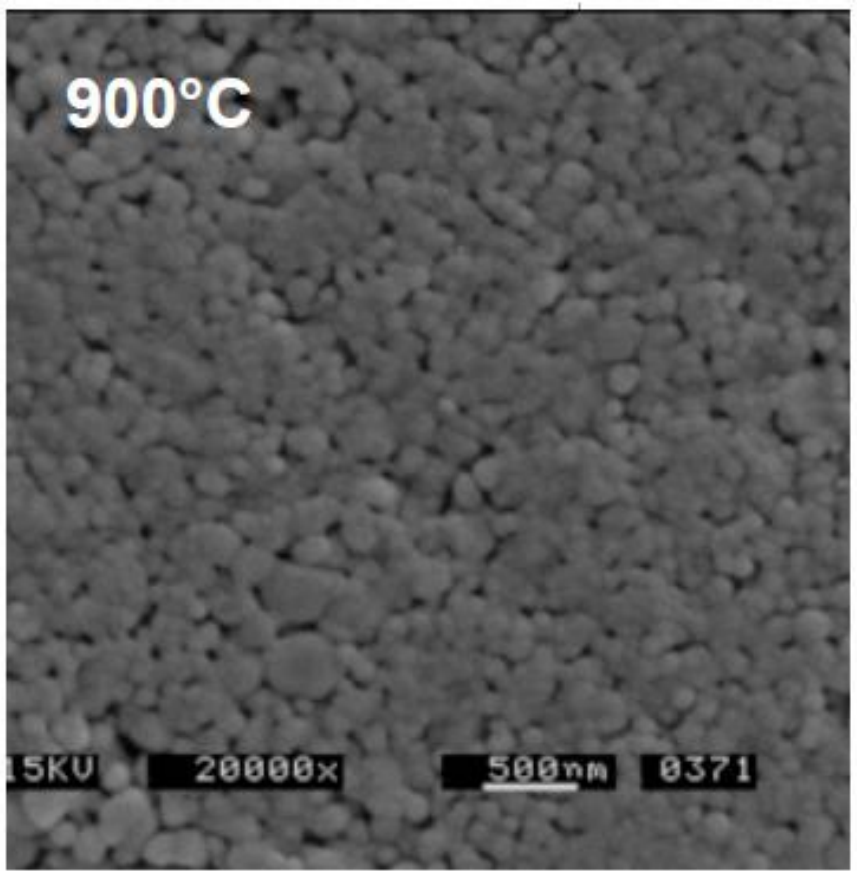


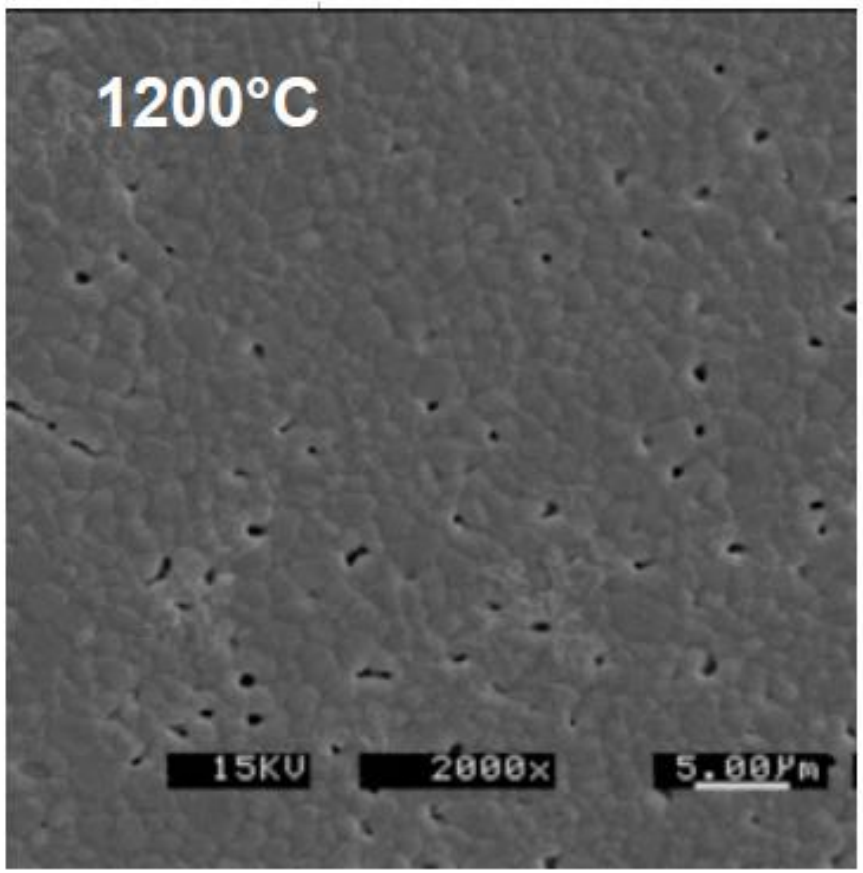
HA morphology vs temperature



a) and d) T_{sint}=1200°C b) and e) T_{sint}=1250°C c) and f) T_{sint}=1300°C

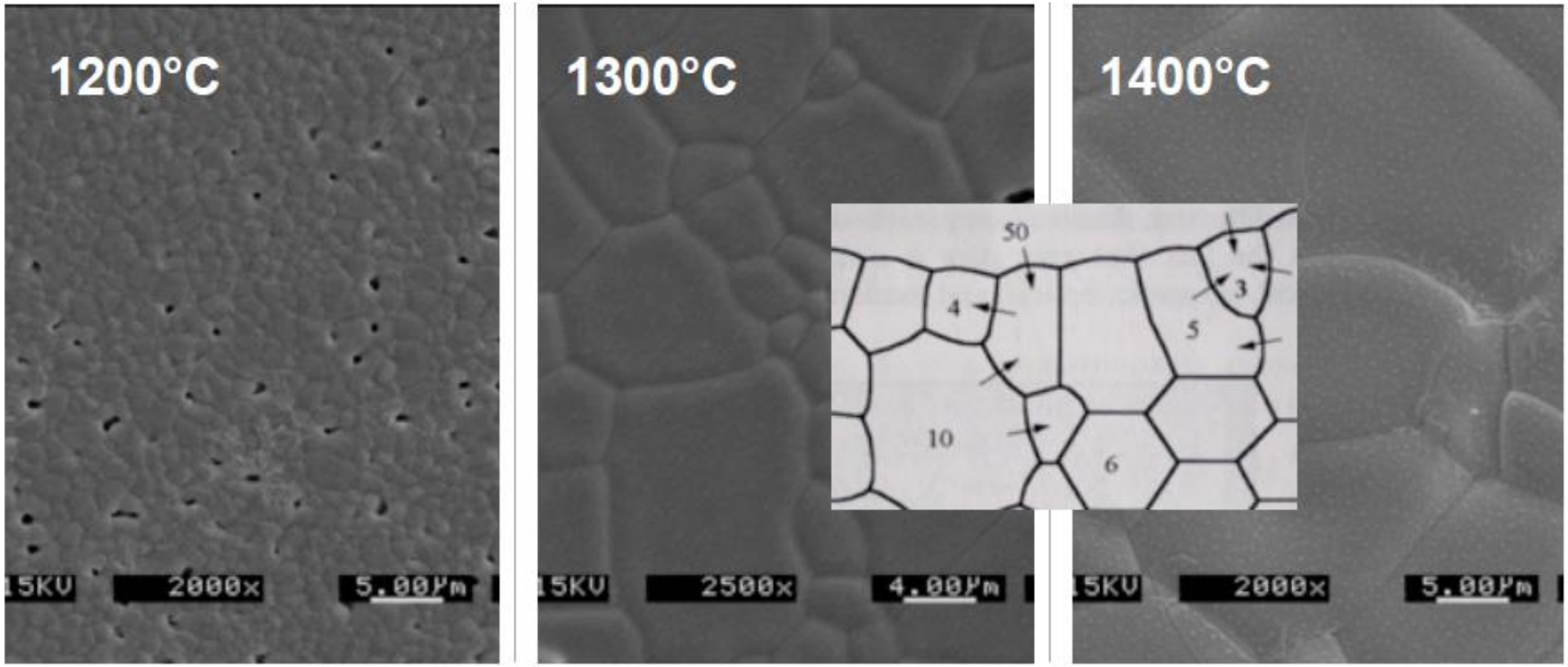
Microstructure of hydroxyapatite before and after the onset of sintering





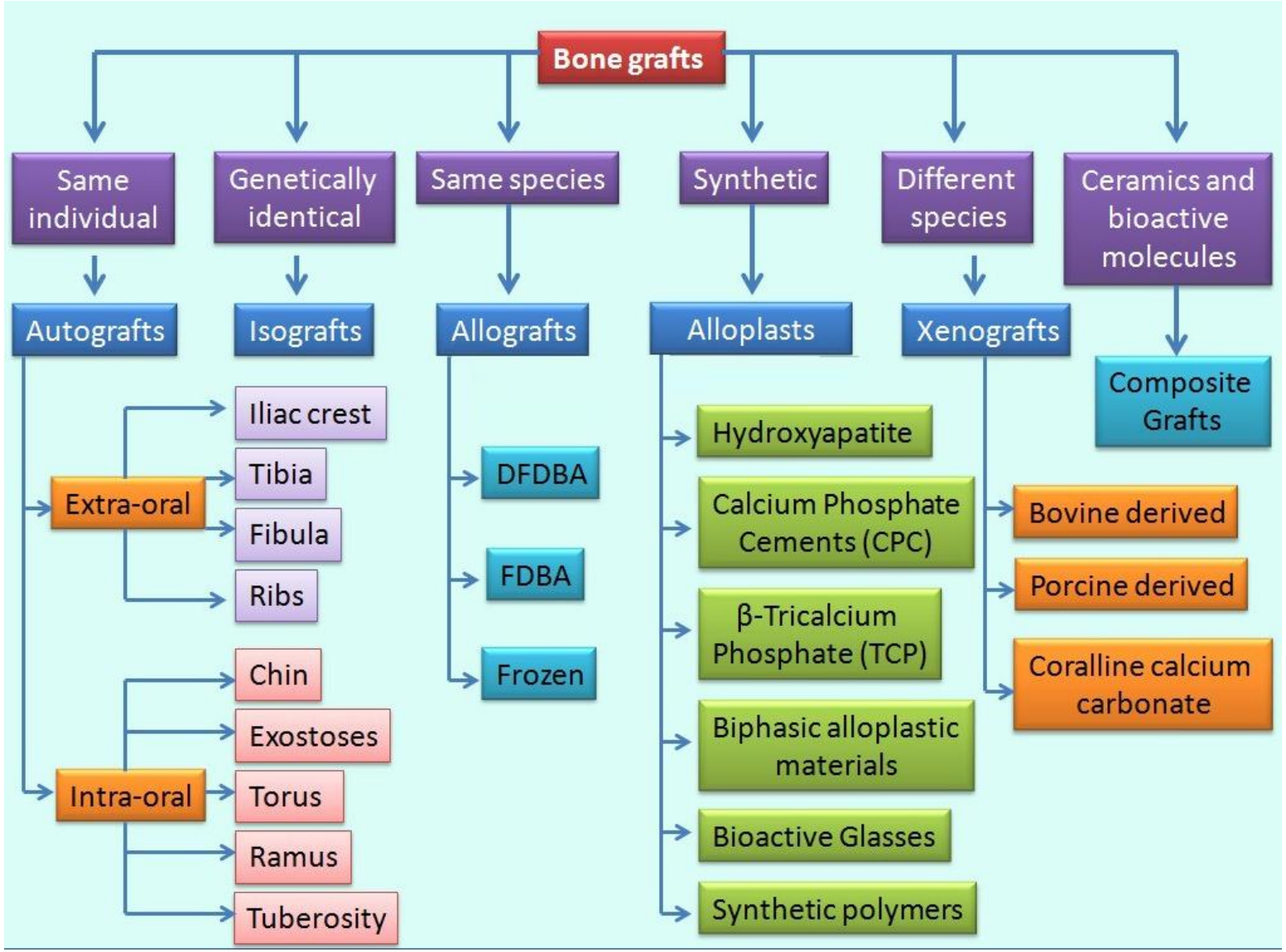
Microstructural evolution of hydroxyapatite as a function of sintering temperature, below 1000 ° C there is a substantial modification of the size of the powders

Sintering, grain growth and coarsening

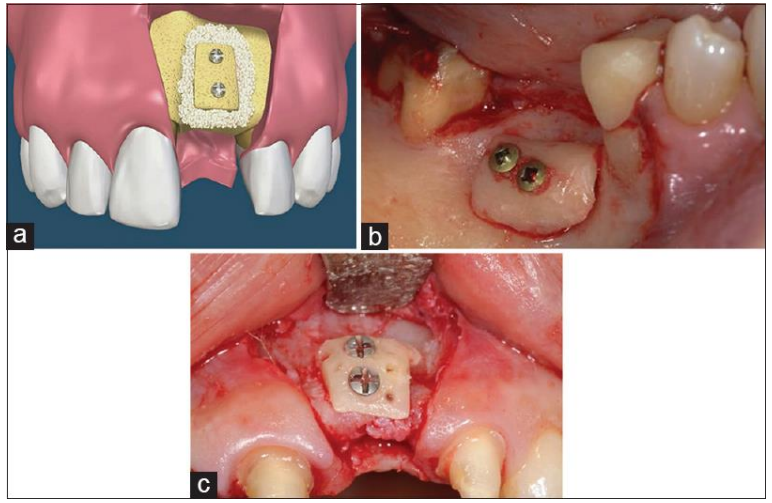


Microstructural evolution of hydroxyapatite obtained from a green of nanopowders (Different institution) varying the sintering temperature, evidence of abnormal growth of the grains from 1300 ° C

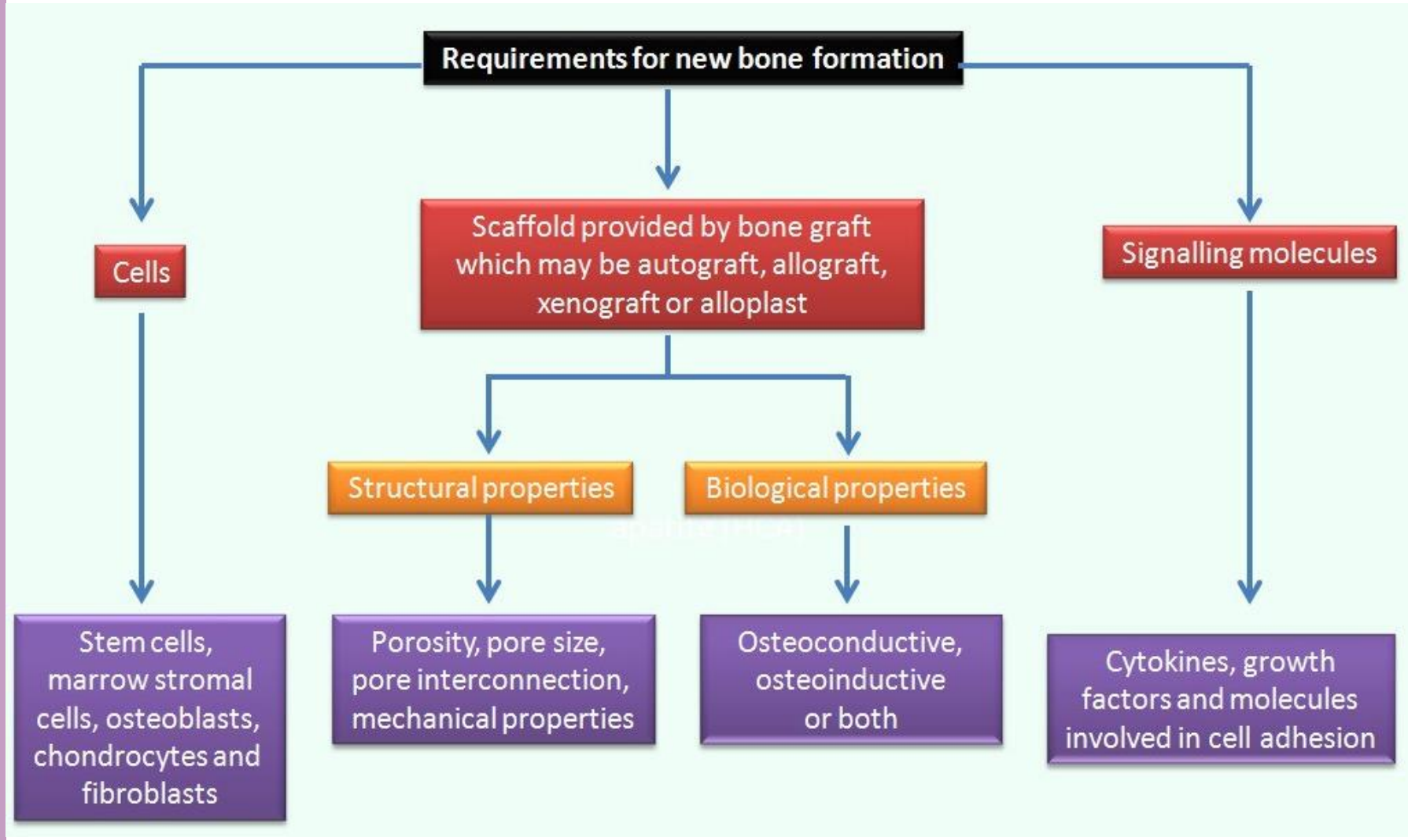
Bone Grafts


Bone grafts
Same individual
Genetically identical
Same species
Synthetic
Different species
Ceramics and bioactive molecules
Autografts
Isografts
Allografts
Alloplasts
Xenografts
Composite Grafts
Extra-oral
Iliac crest
Tibia
Fibula
Ribs
Intra-oral
Chin
Exostoses
Torus
Ramus
Tuberosity
DFDBA
FDBA
Frozen
Hydroxyapatite
Calcium Phosphate Cements (CPC)
β-Tricalcium Phosphate (TCP)
Biphasic alloplastic materials
Bioactive Glasses
Synthetic polymers
Bovine derived
Porcine derived
Coralline calcium carbonate

bone defect
bone defect is filled with grafting materials
finally all is covered with a membrane

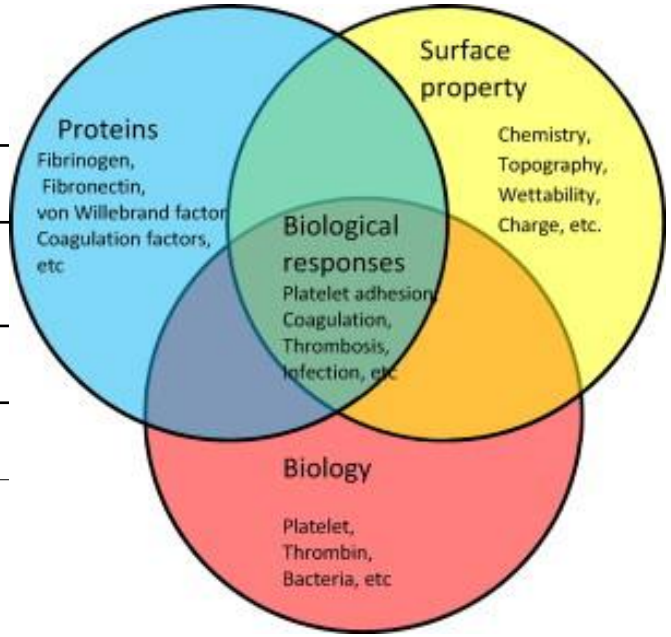

a
b
c

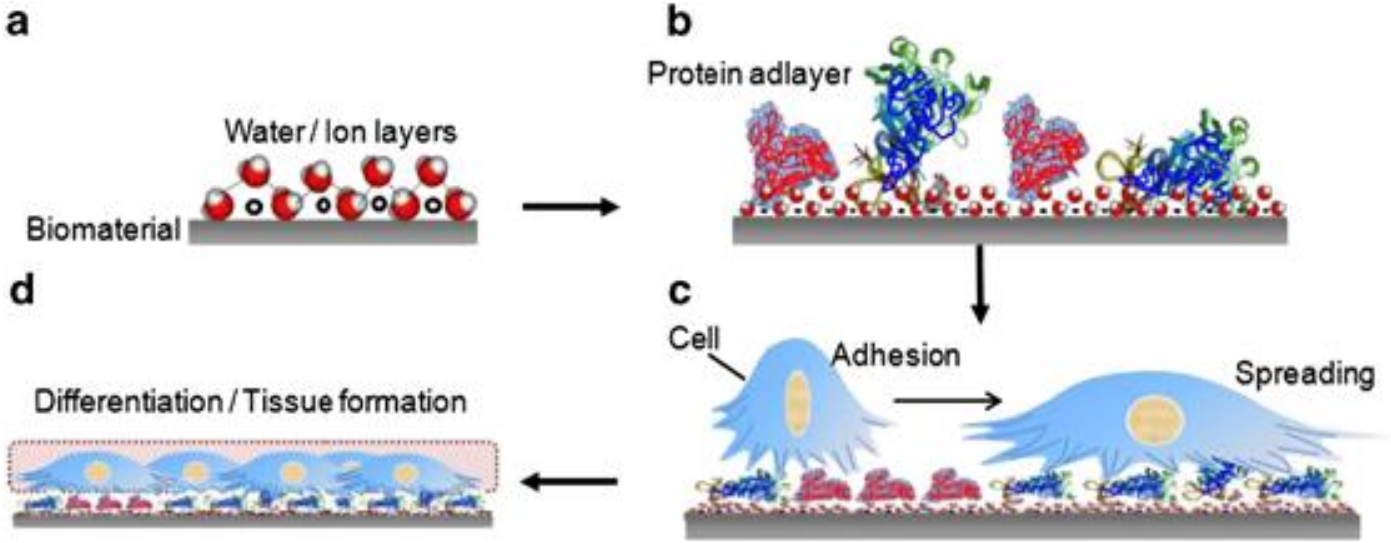
Bone Grafts for BTE



Implant-Tissue Responses

Implant-Tissue Rxn	Consequence
Toxic	*Tissue dies*
Bioinert	Tissue forms a fibrous capsule around the implant
Bioactive	Tissue forms an interfacial bond with the implant
Dissolution of implant	Tissue replace implant

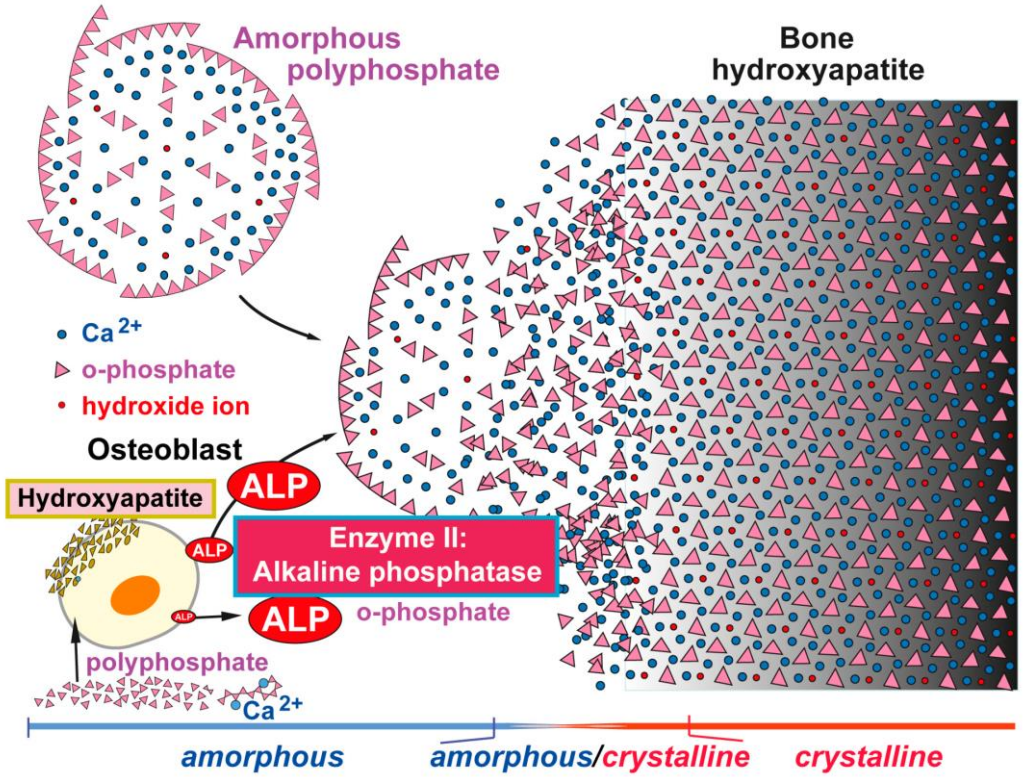


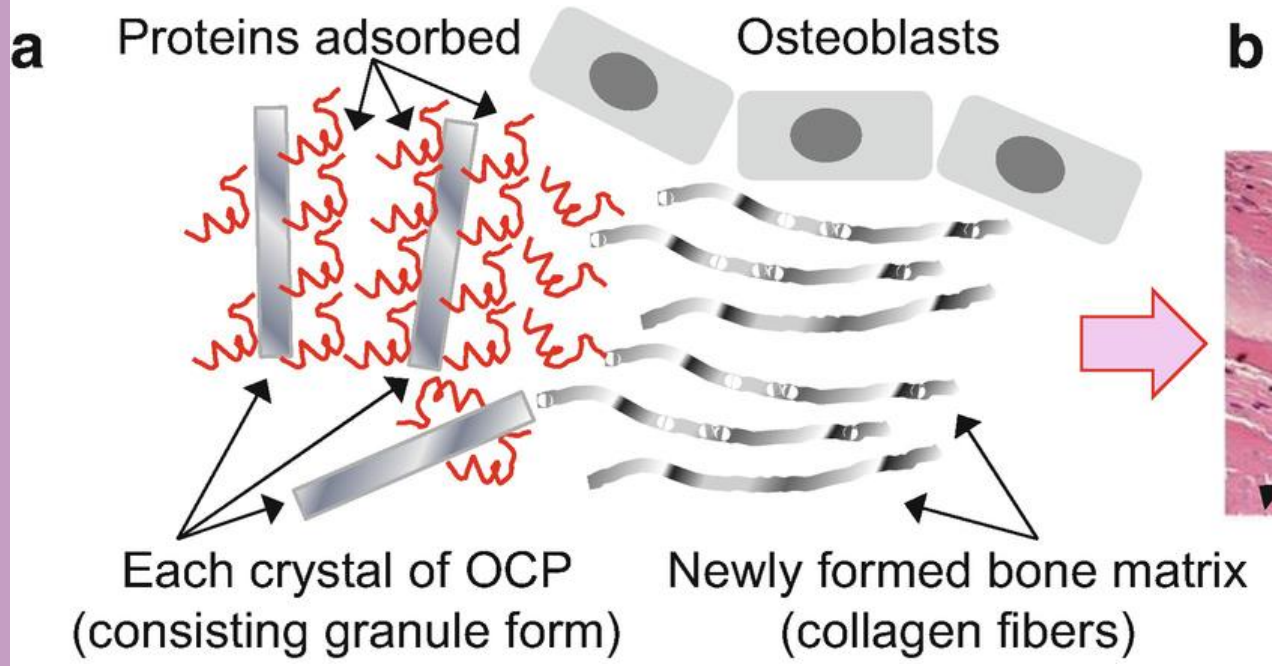


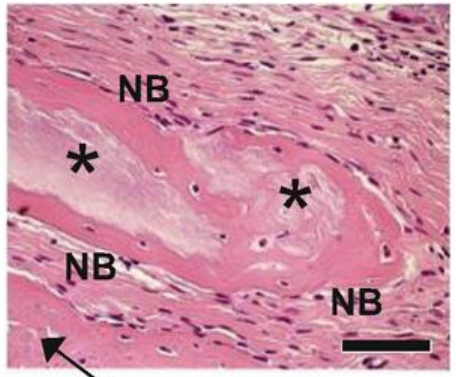
Bond formation between bone and HA

Bond formation between bone and HA

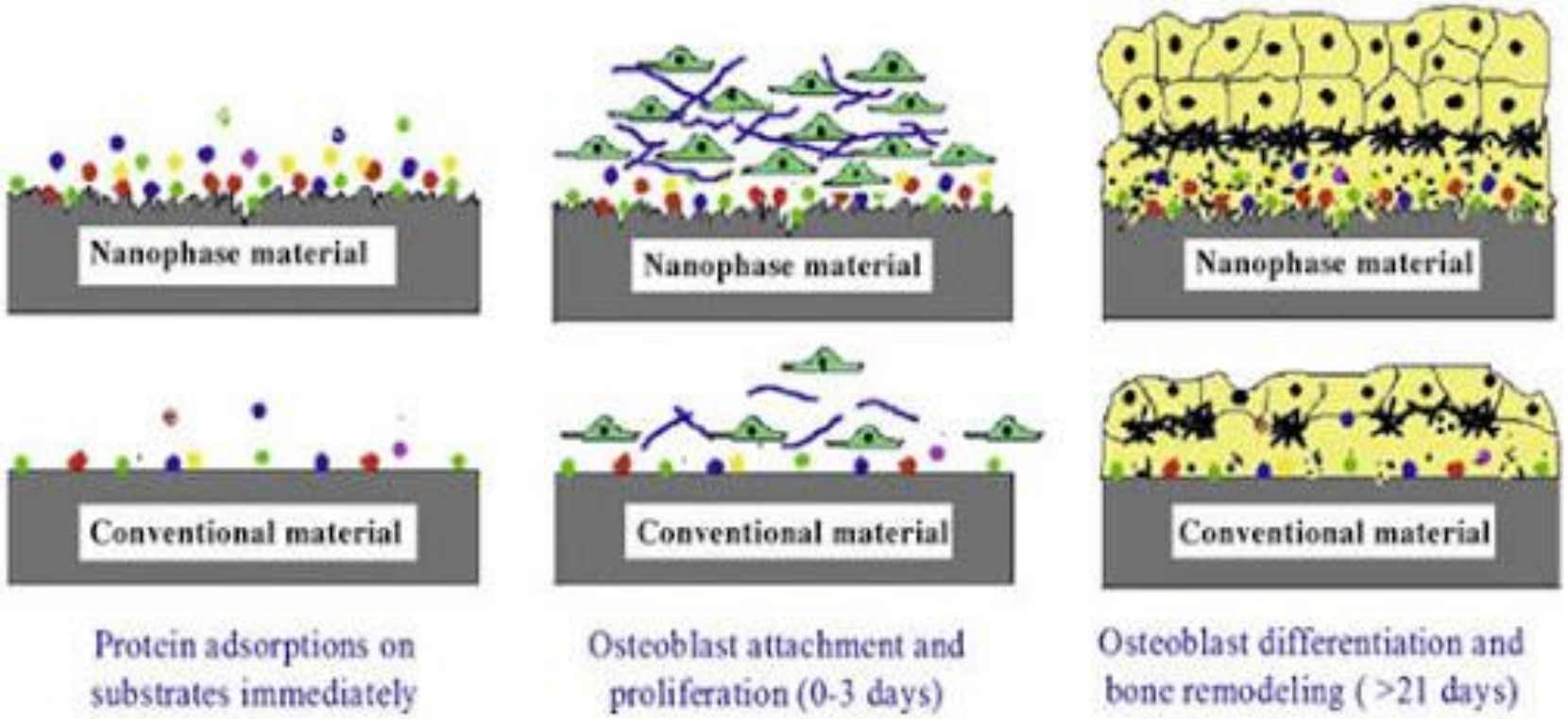
1. Bone cells formation at the interface
2. Formation of amorphous HA at the interface (3-5 µm)
3. Collagene formation between cells and amorphous HA
4. Epitaxial growth of bone (natural HA) on the implant
5. Reduction of the interface thickness (< 1 µm)

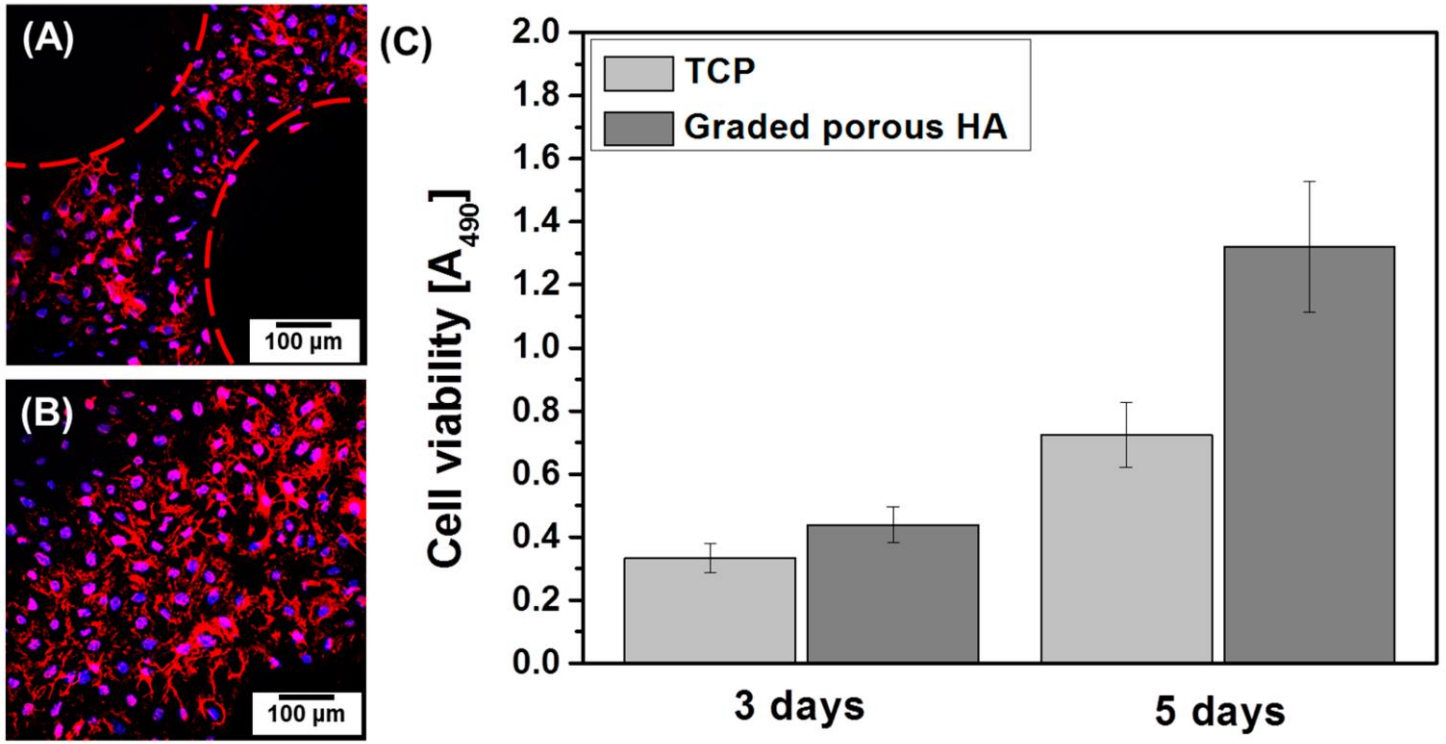






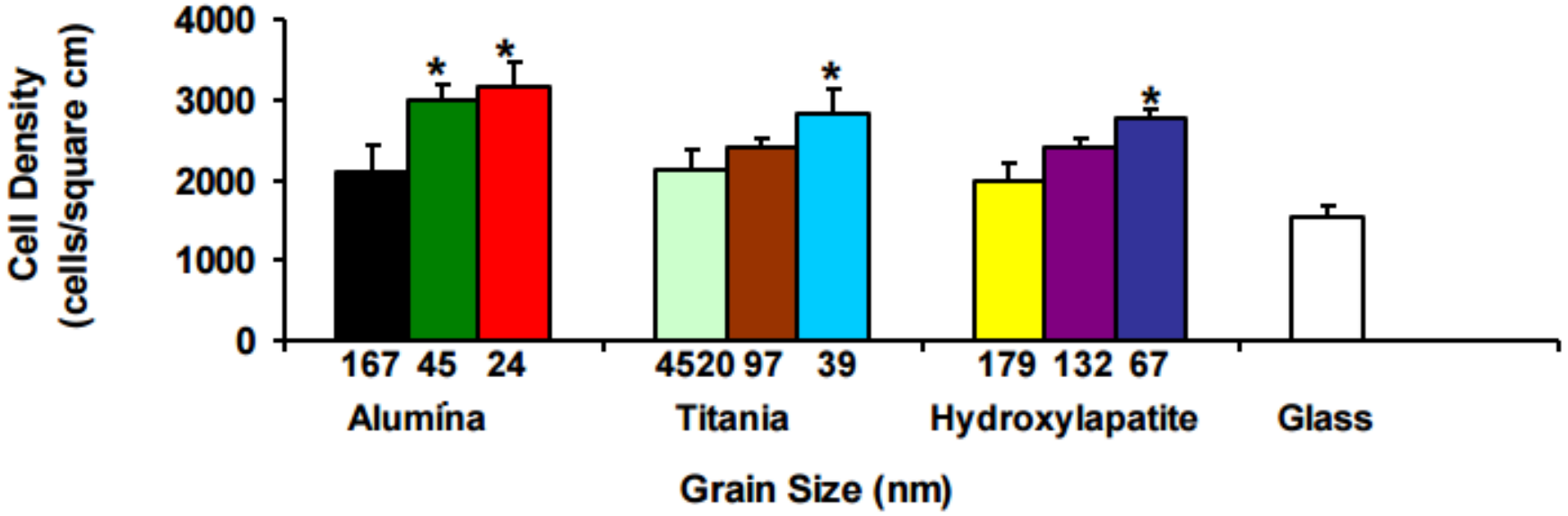
Bond formation between bone and HA





CLSM images of preosteoblast cells cultured for 5 h on porous HA scaffolds with bone-like structures: (**A**) porous part and (**B**) dense part; (**C**) Cell viabilities of the preosteoblast cells cultured for three and five days on porous HA scaffolds with bone-like structures.

ENHANCED OSTEOBLAST ADHESION ON NANOPHASE CERAMICS

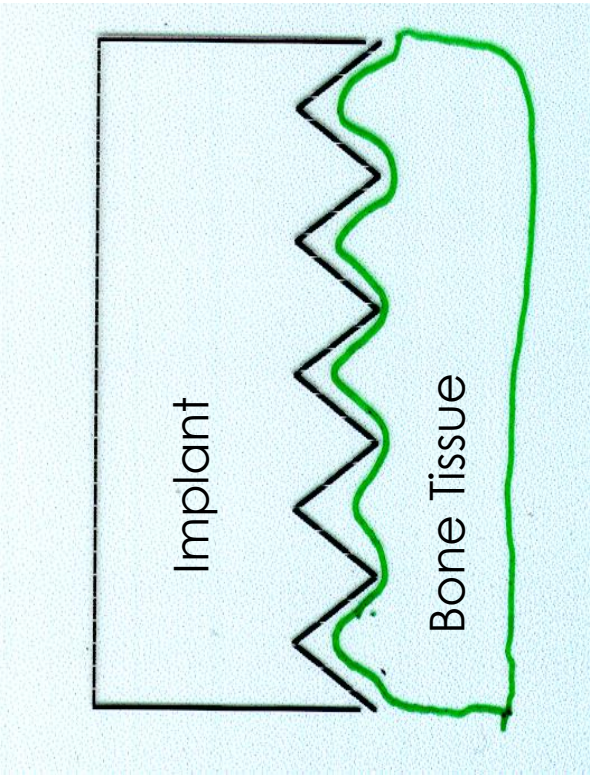


Culture media : DMEM supplemented with 10% fetal bovine serum.
Values are mean ± SEM; n = 3;
*p<0.01 (student t-test compared to respective conventional grain size ceramic).

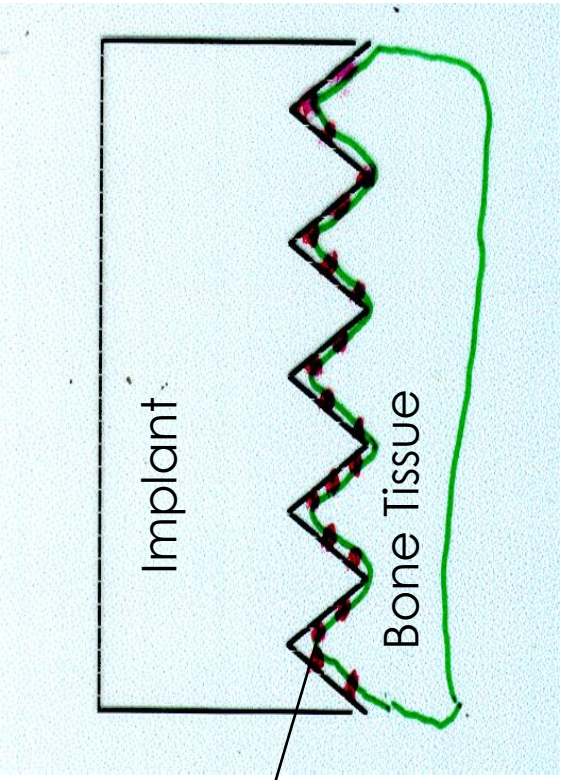
Reference: Webster, T.J., C. Ergun, R.H. Doremus, R.W. Siegel, R. Bizios. *Biomaterials* 21: 1803-1810 (2000)

Interactions of Bioceramic-Tissue

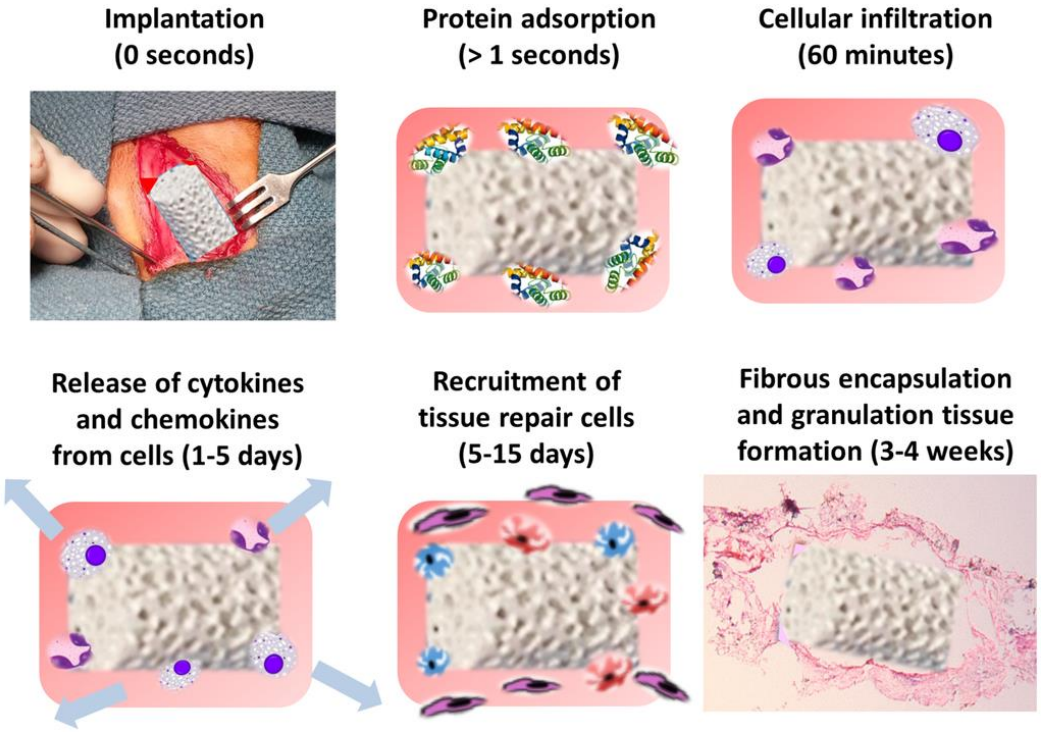
Bioinert



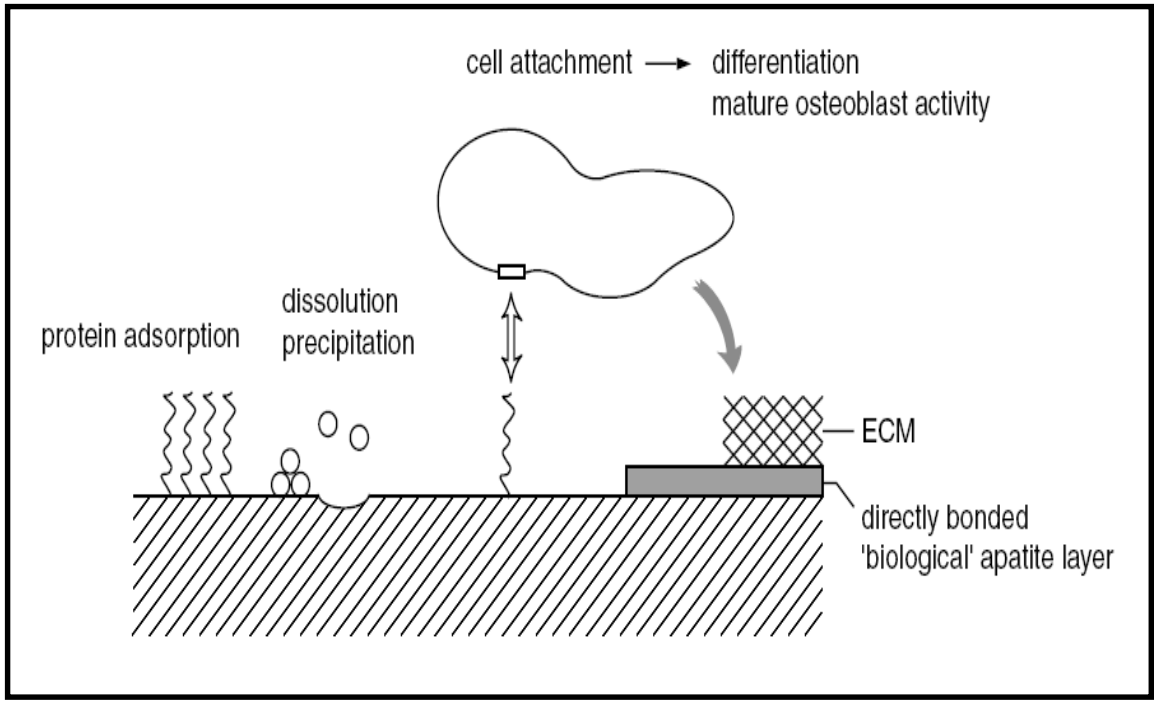
Bioactive

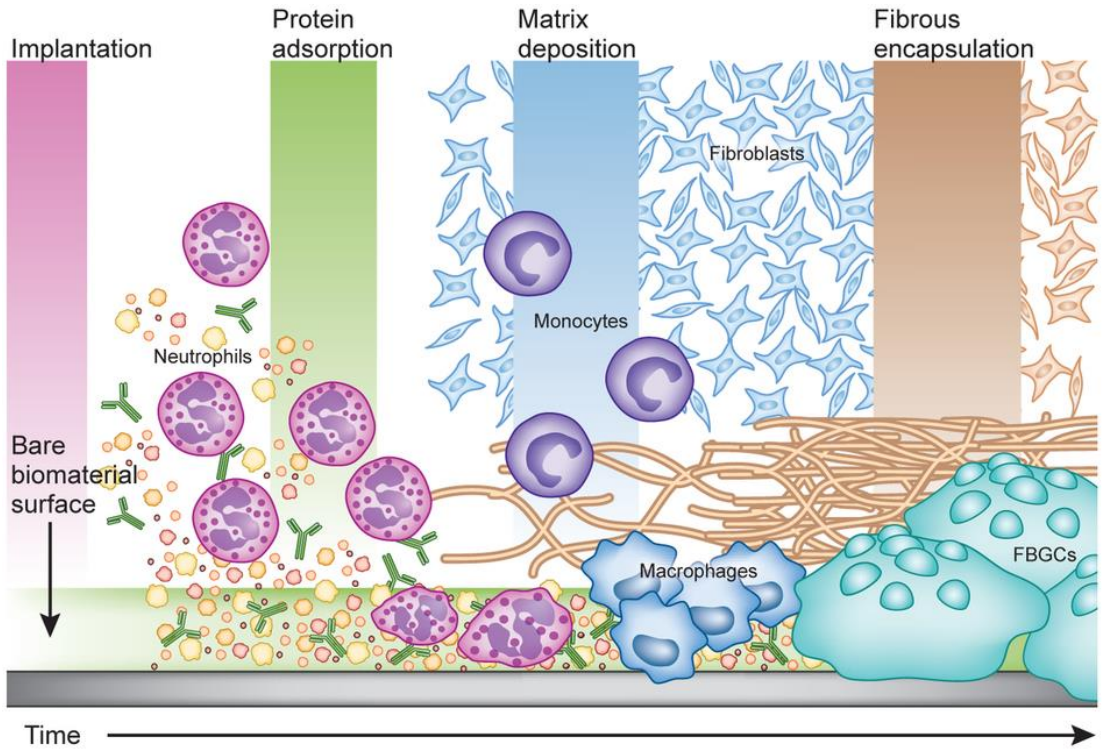


Cell adhesion

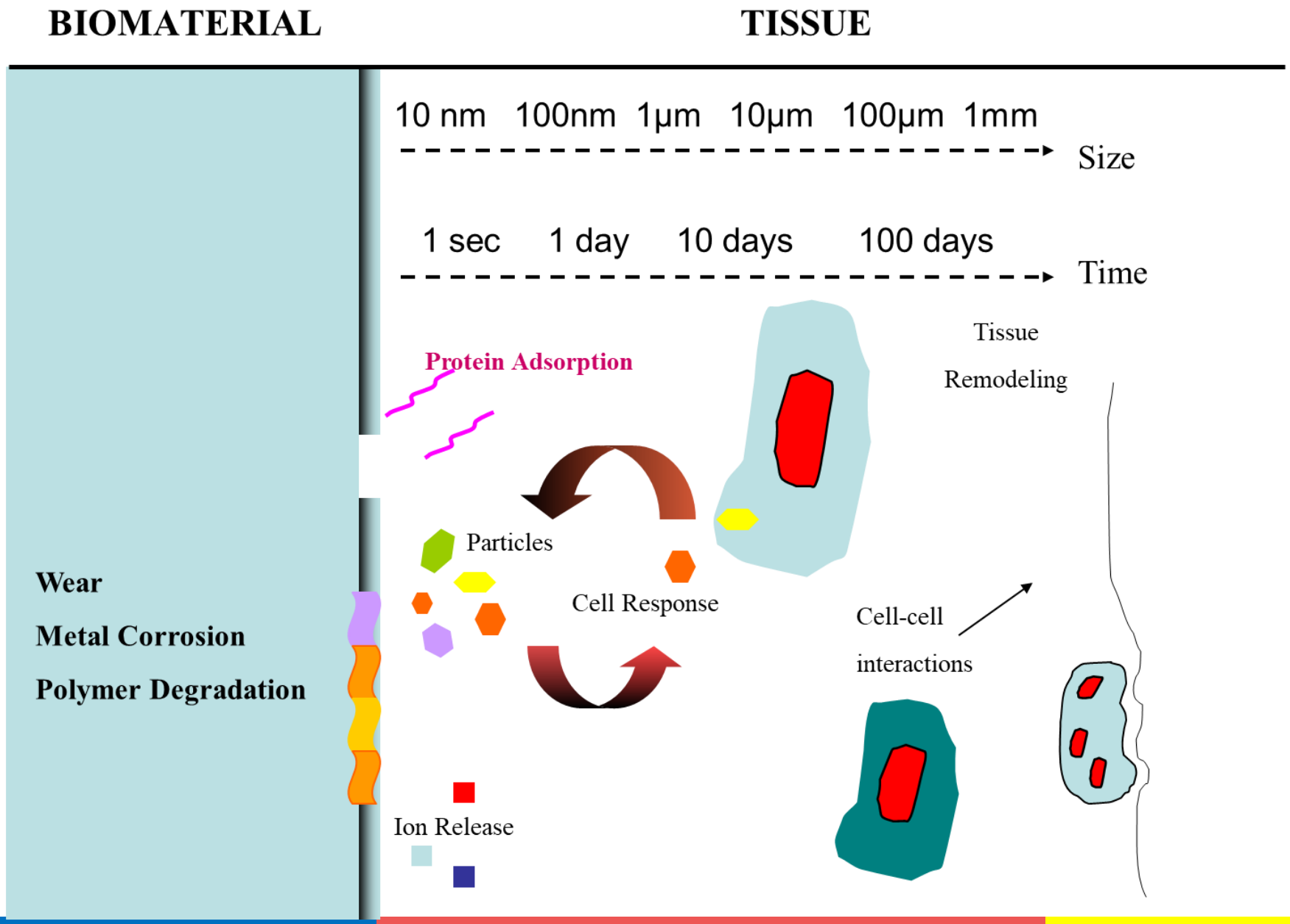


Interactions of Bioceramic-Tissue

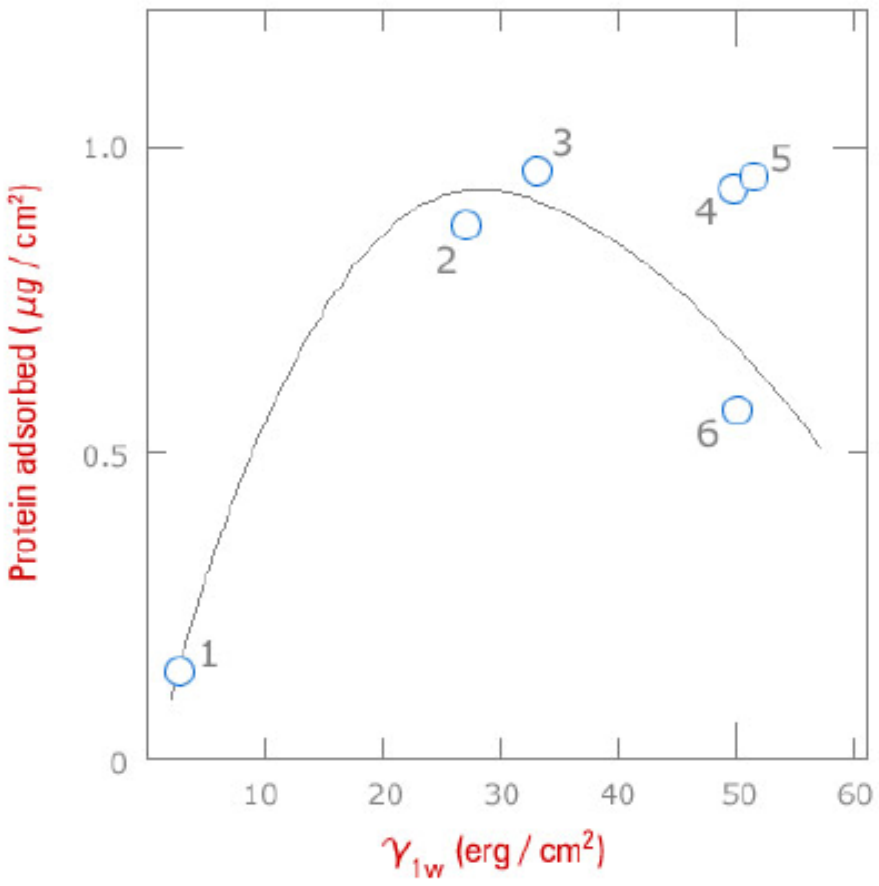




1. The surface interacts with the ionic solution,
2. *Proteins* adhere to the *surface* and *cells* interact with the *adsorbed* proteins.
3. Differentiation
4. Mature osteoblasts produce the extracellular matrix (ECM).


BIOMATERIAL
TISSUE
10 nm
100nm
1µm
10µm
100µm
1mm
Size
1 sec
1 day
10 days
100 days
Time
Protein Adsorption
Tissue
Remodeling
Particles
Cell Response
Cell-cell
interactions
Wear
Metal Corrosion
Polymer Degradation
Ion Release

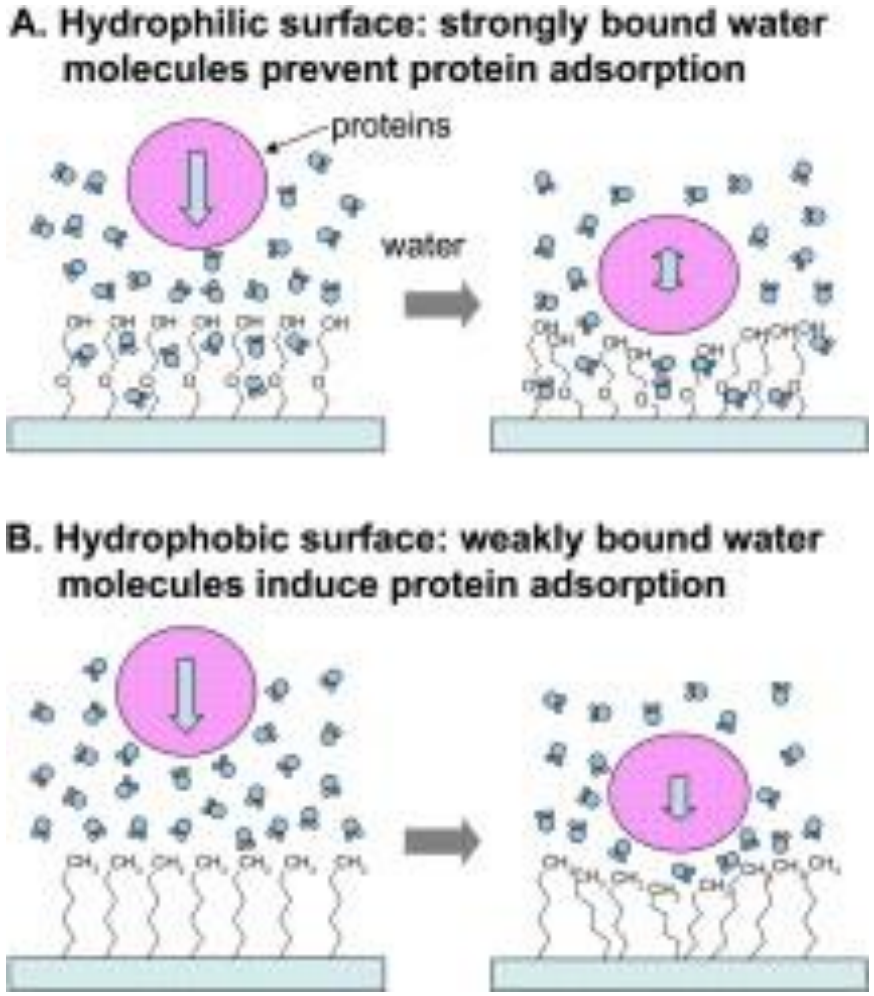
BSA adsorption on various polymers at 37°C.



Hydrophilic surfaces are currently seen as the most promising strategy for controlling protein deposition on biomaterials.

1. PVA
2. PMMA
3. PET
4. PP
5. HDPE
6. PTFE

(after Y. Ikada et al., *Polymers as Biomaterials*, Plenum Press, NY 1984)



Surface Properties: Hydrophobicity / Hydrophilicity

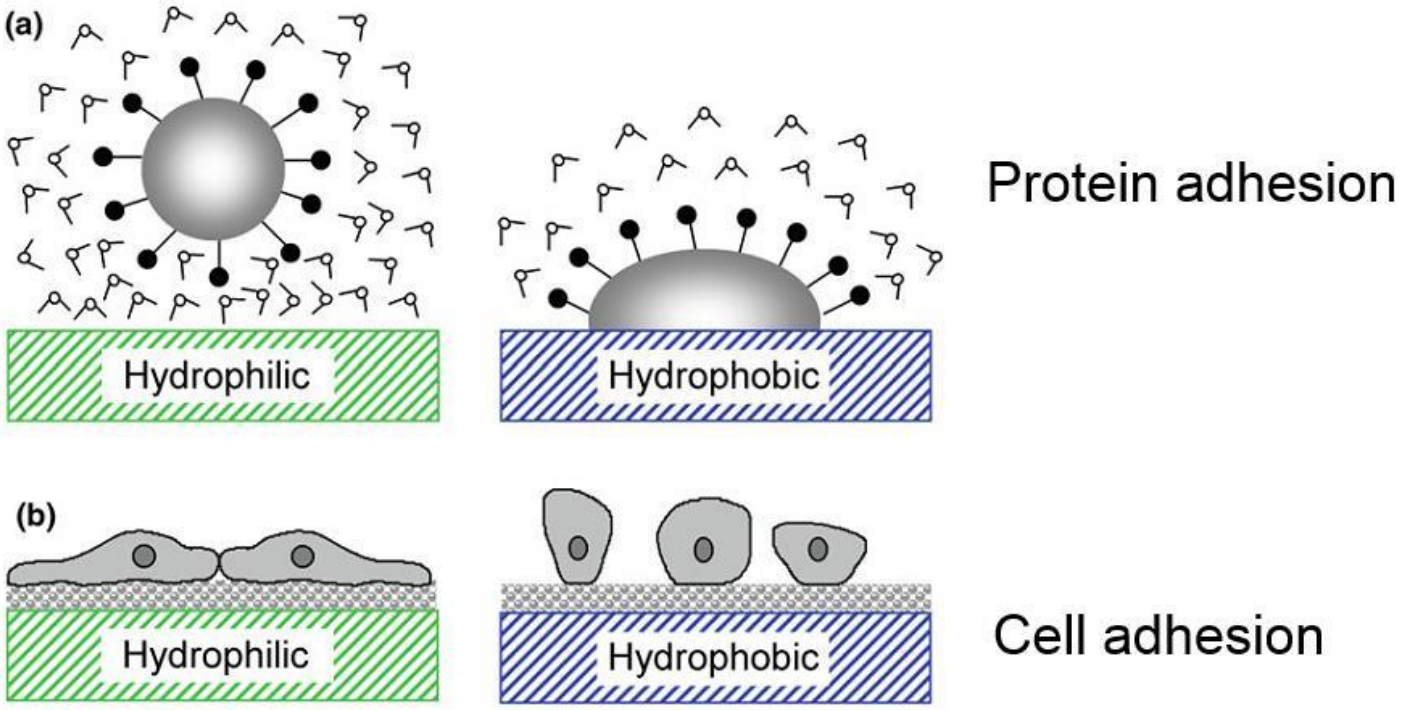
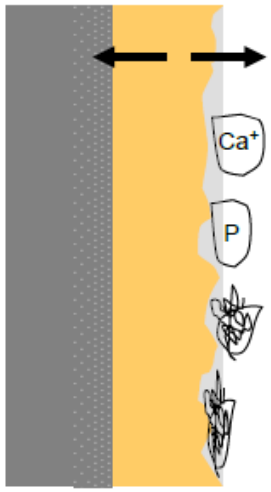
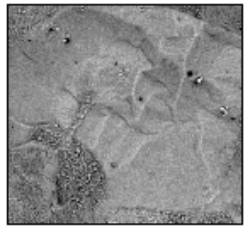


Fig. 1 Hydrophilic or hydrophobic in vitro surfaces in contact with a biological environment. a Protein adsorption, b cell adhesion (water molecules, polar amino acid side chains)

Michaelis et al Adv Biochem Engin/Biotechnol (2012) 126: 33–66

Molecular level events at implant surface

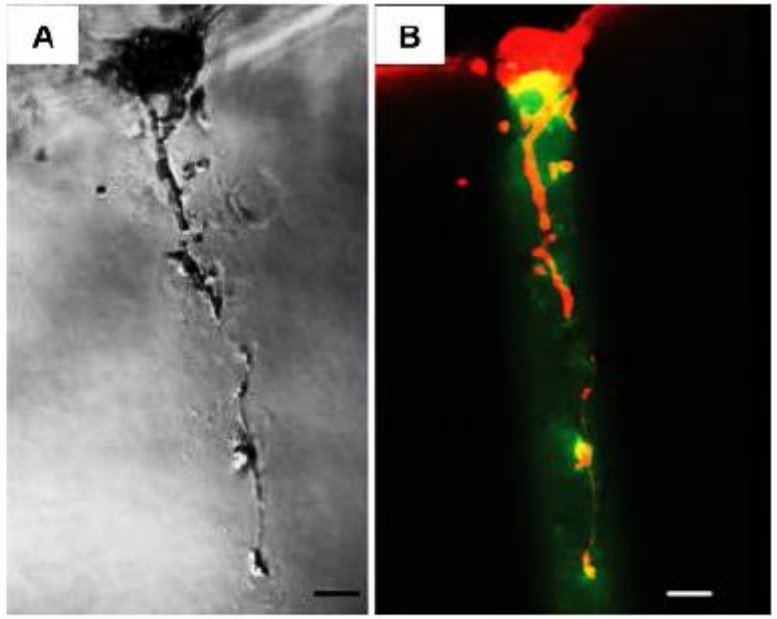




- ***Chemistry*** – determines the types of intermolecular forces, governing interaction with proteins
- *Hydrophobicity* – hydrophobic surfaces often bind protein more strongly (can limit cell adhesion)
- *Heterogeneity* – surface non-uniformity, domains interact differently with proteins
- *Potential* – influences ion distribution & interaction with proteins (dependant upon topography / chemistry)
- ***Topography*** – greater texture exposes discontinuities for interaction with proteins

Biological modifications

- Goal is to control cell and tissue responses to an implant by immobilizing biomolecules on biomaterials
- RGD peptides deposited on surface promote cell attachment →
- Heparin/heparin-sulfate binding peptides enhance cell adhesion
- Attachment of growth factors



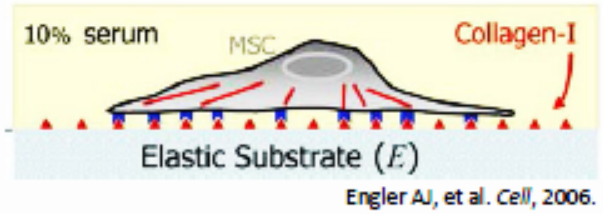
Dorsal root ganglion (DRG) outgrowth into 3-dimensional RGD-modified channels

Importance of proteins for biomaterials?

- 3 major ways (amongst others!) in which proteins are revelant in the field of biomaterials:

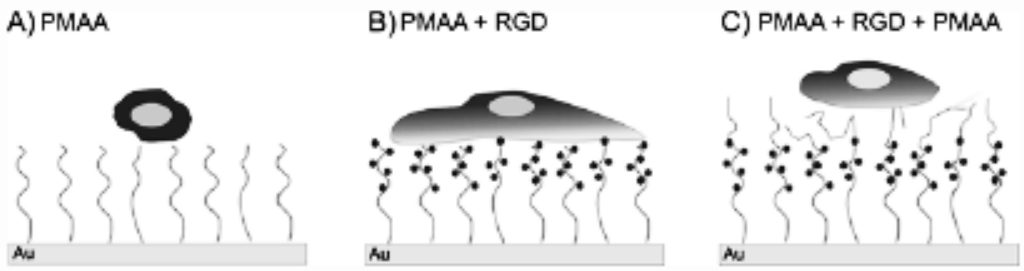
1) Surface modification of biomaterials with proteins or peptides:
   - Typically uses adsorption (proteins) or covalent coupling (peptides) to the surface

cell adhesion



Engler AJ, et al. *Cell*, 2006.

using proteins or fragments
(could also use fibronectin)

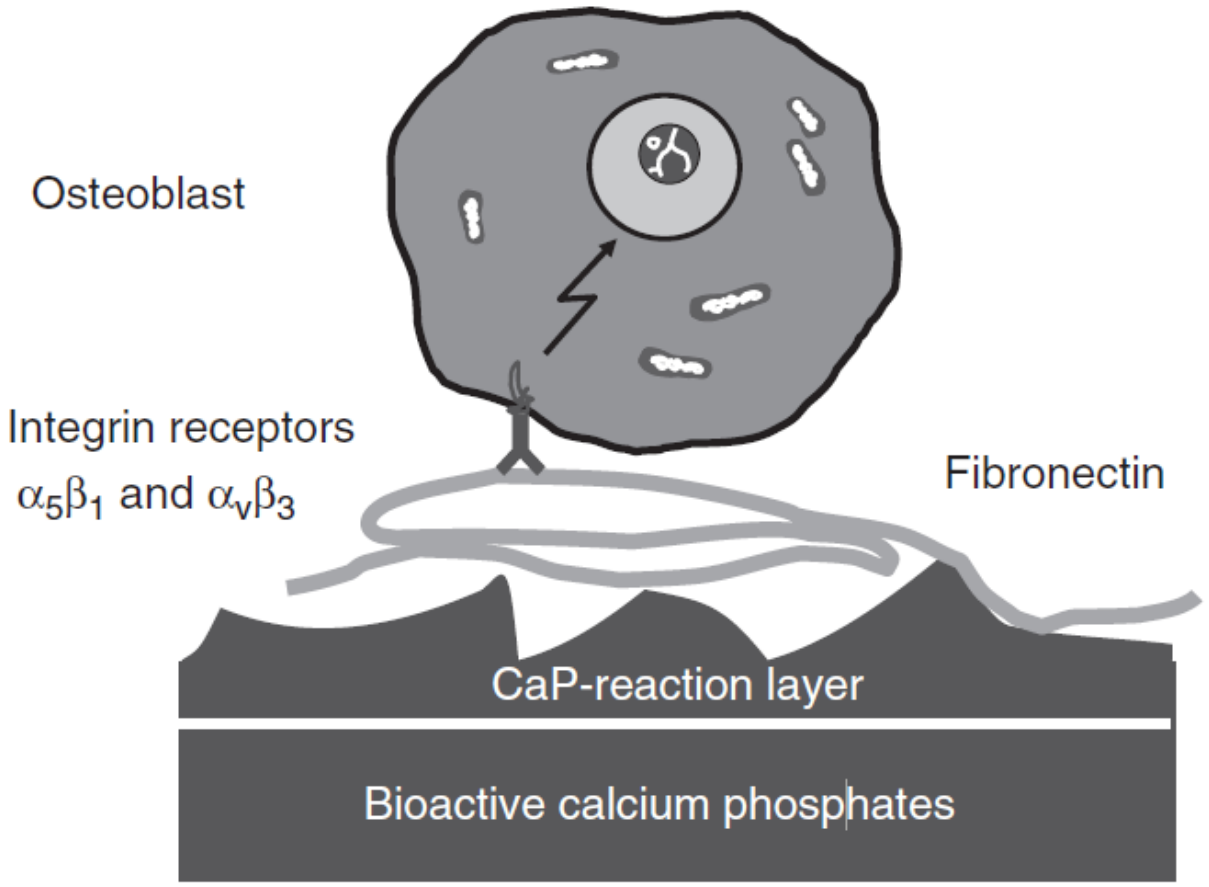


using peptides
(non-RGD sequences are also used)

2) Loading of proteins (e.g., growth factors, chemotherapeutics) into biomaterials (e.g., biodegradable microspheres) for controlled release
   - A challenge is maintenance of the bioactivity/stability of encapsulated proteins

3) Interactions between biomaterial surfaces and *in vivo* proteins following implantation into the body

Bioceramic- cell interaction



Schematic diagram illustrating the events that take place at the interface between the bioactive ceramics and the surrounding biological milieu and thereby establish bioactive behavior.

Cell Culture for Bone Tissue Engineering

Cells and Tissue Engineering

Over the last century, various ever-evolving models have been developed with the intention of reproducing the complex structure and functionalities of living human organs.

Which alternatives do we have?

"Lifecycle Biotechnologies" [Online]. Available: https://www.lifecyclebio.com/2017/05/15/choose-boval-cell-culture-needs/

2D Cell culture

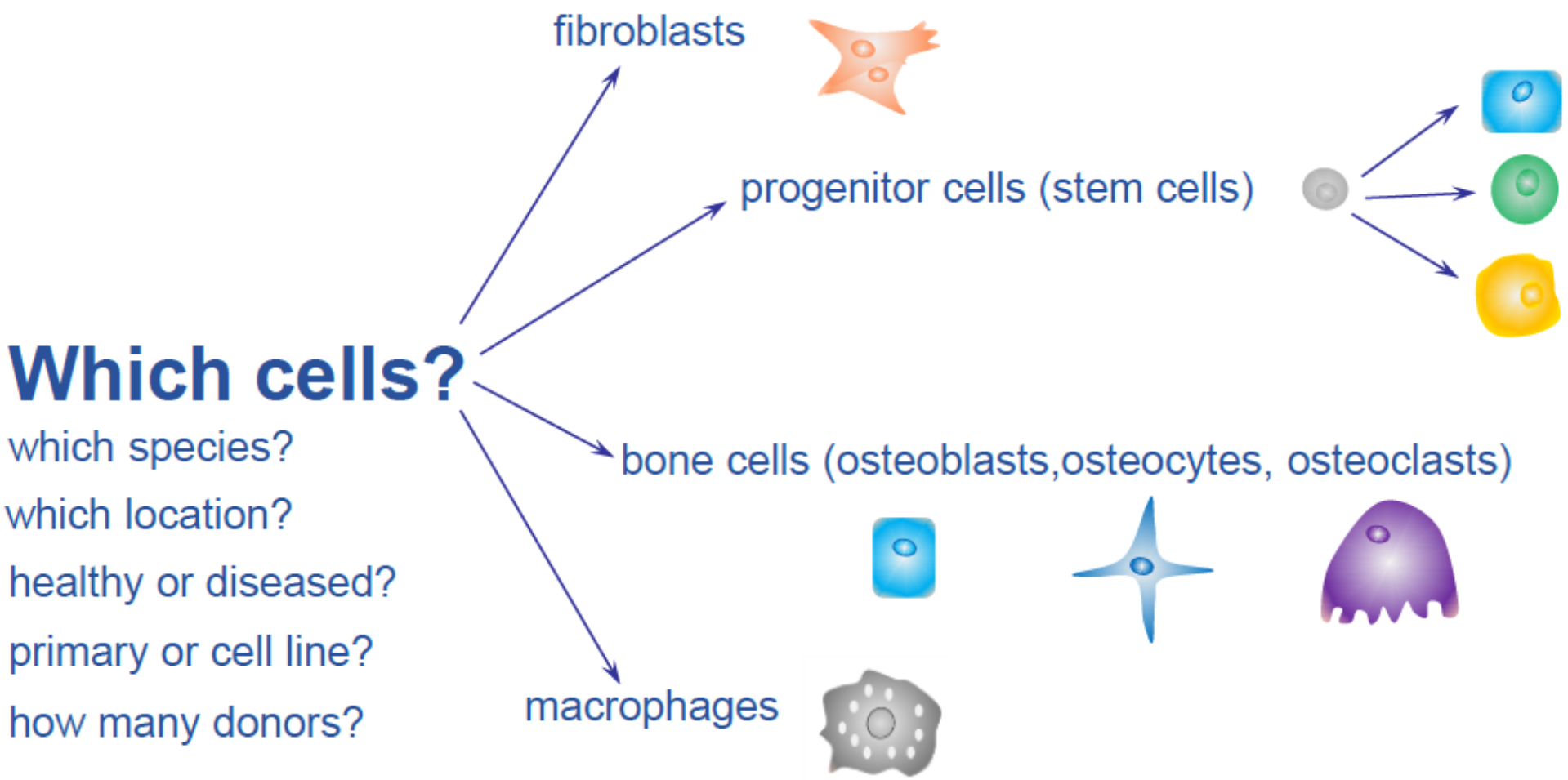
"INFORSHT" [Online]. Available: http://www.infors-ht.com/index.php/en/products/bioreactors

3D Cell culture

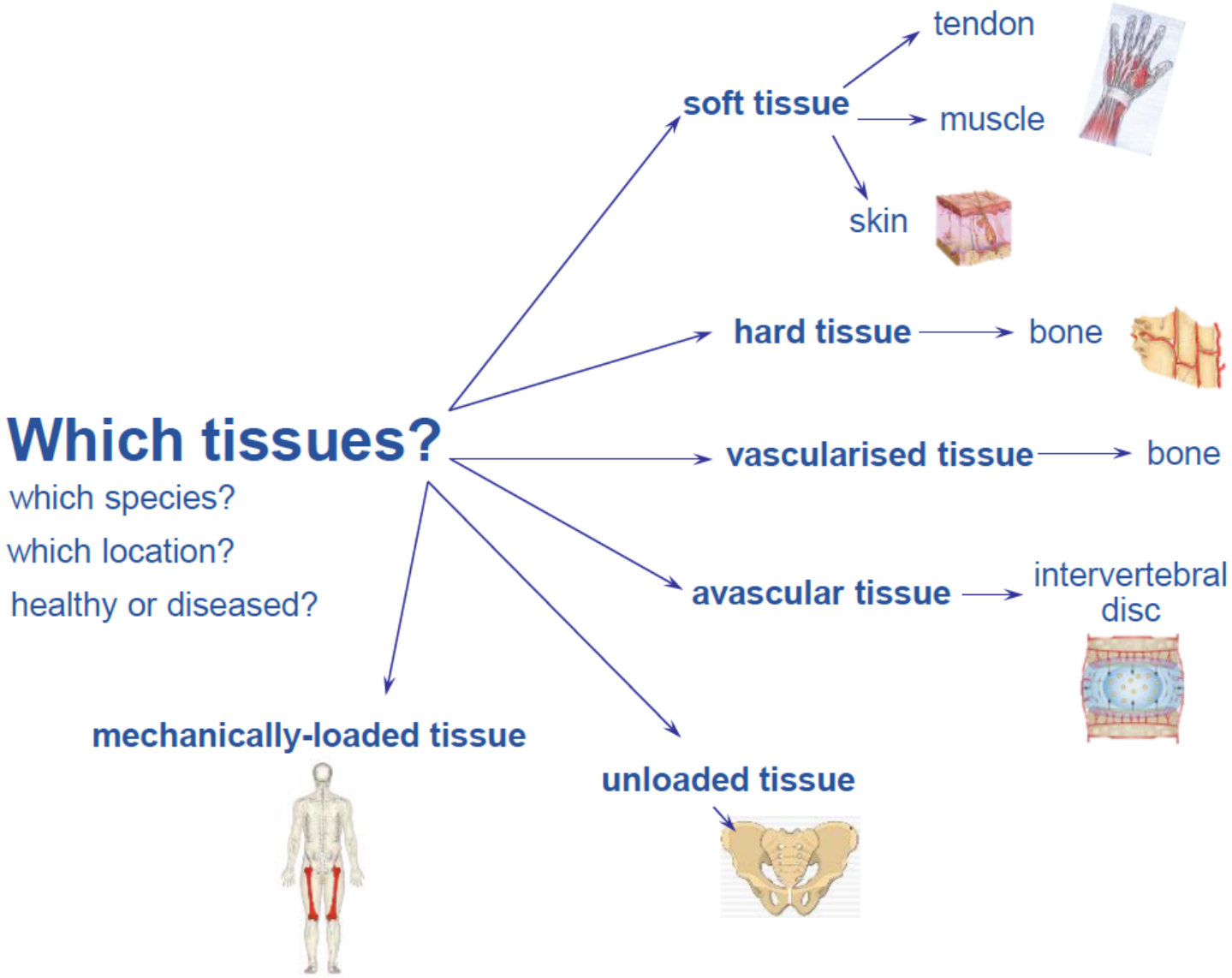
"FUNPOP" [Online]. Available: http://www.fanpop.com/clubs/against-animal-testing/images/29038373/title/no-more-experiments-photo

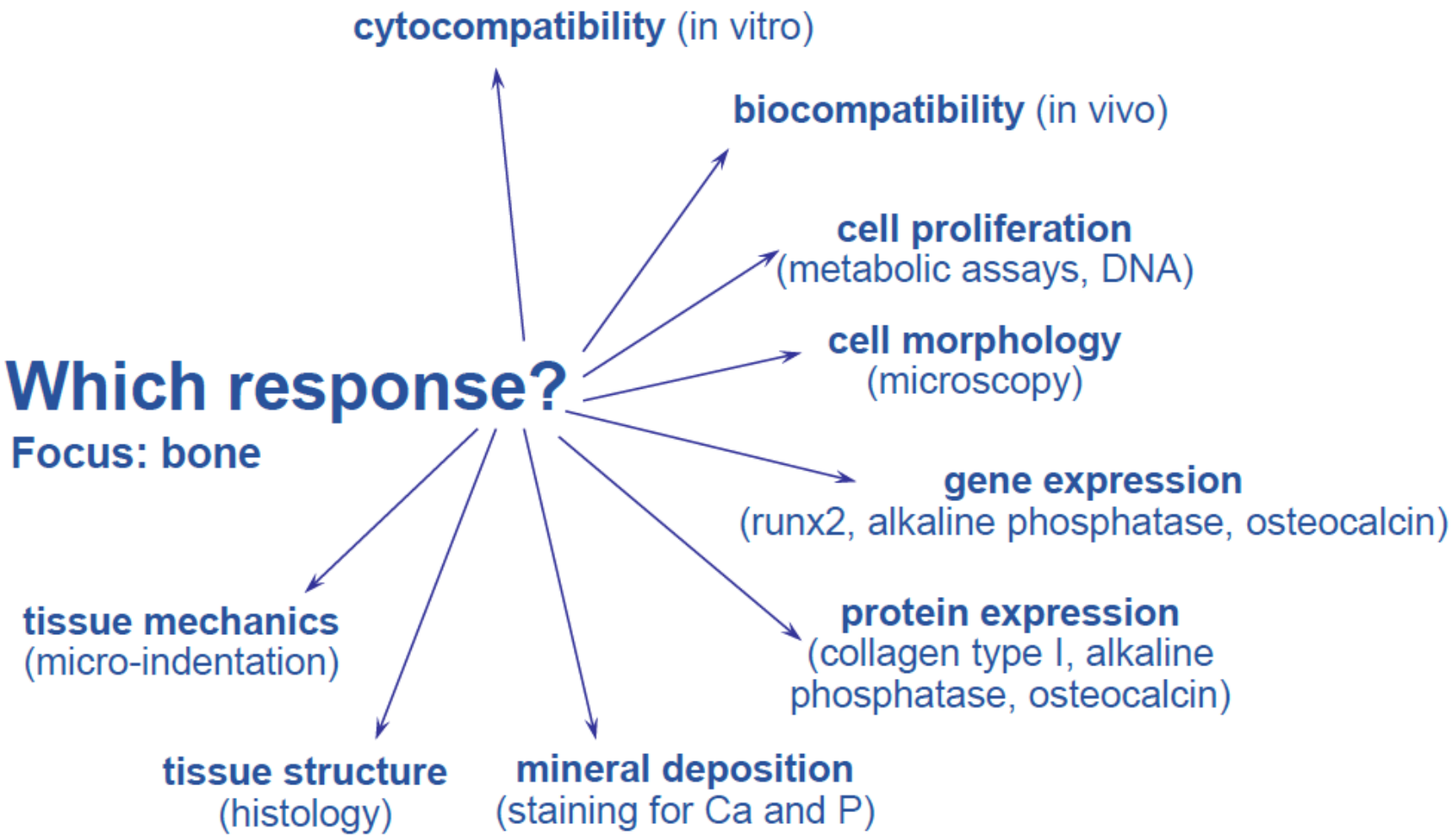
Animal Testing

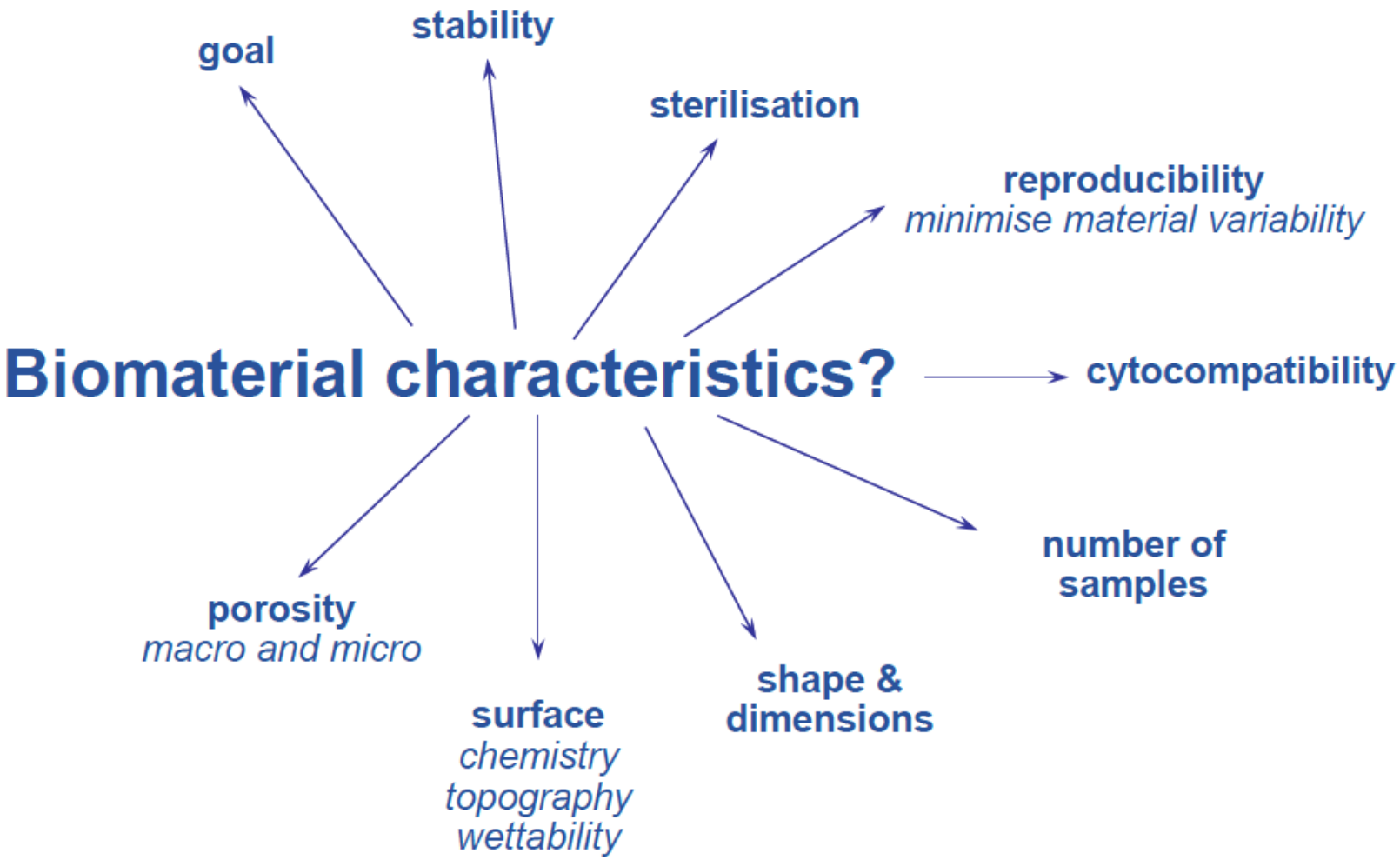
Cells and Tissue Engineering



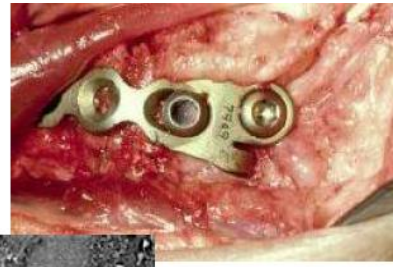
Cell Culture for Bone Tissue Engineering

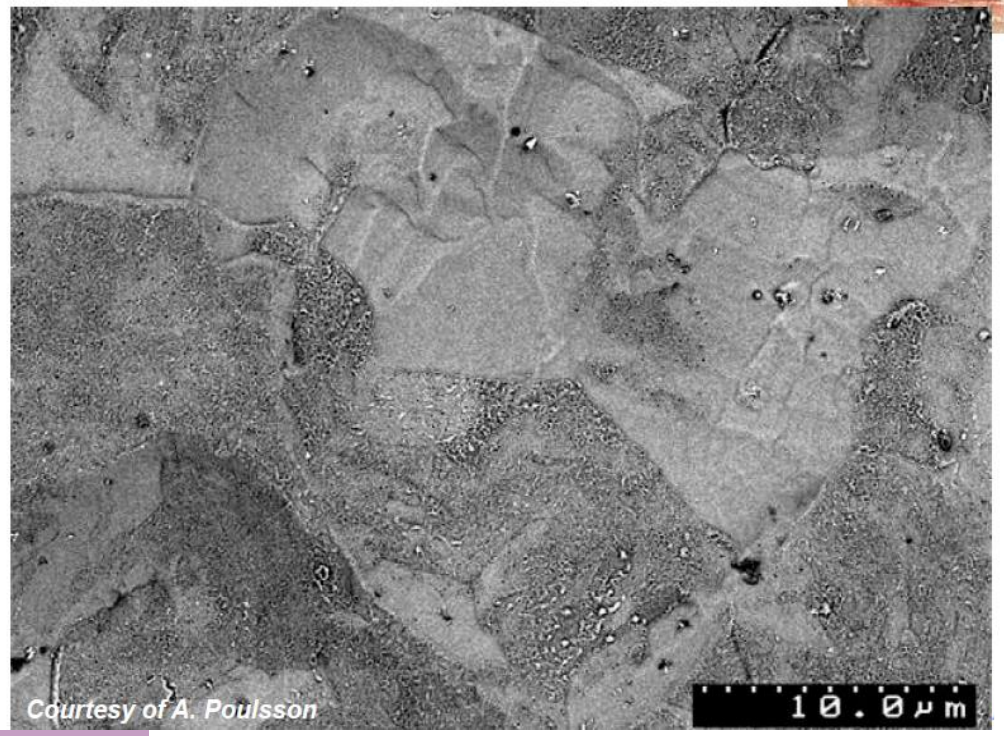



Which response?
Focus: bone
cytocompatibility (in vitro)
biocompatibility (in vivo)
cell proliferation
(metabolic assays, DNA)
cell morphology
(microscopy)
gene expression
(runx2, alkaline phosphatase, osteocalcin)
protein expression
(collagen type I, alkaline phosphatase, osteocalcin)
mineral deposition
(staining for Ca and P)
tissue structure
(histology)
tissue mechanics
(micro-indentation)

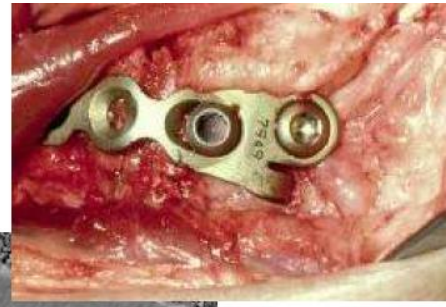

Biomaterial characteristics?
goal
stability
sterilisation
reproducibility
minimise material variability
cytocompatibility
number of samples
shape & dimensions
surface
chemistry
topography
wettability
porosity
macro and micro

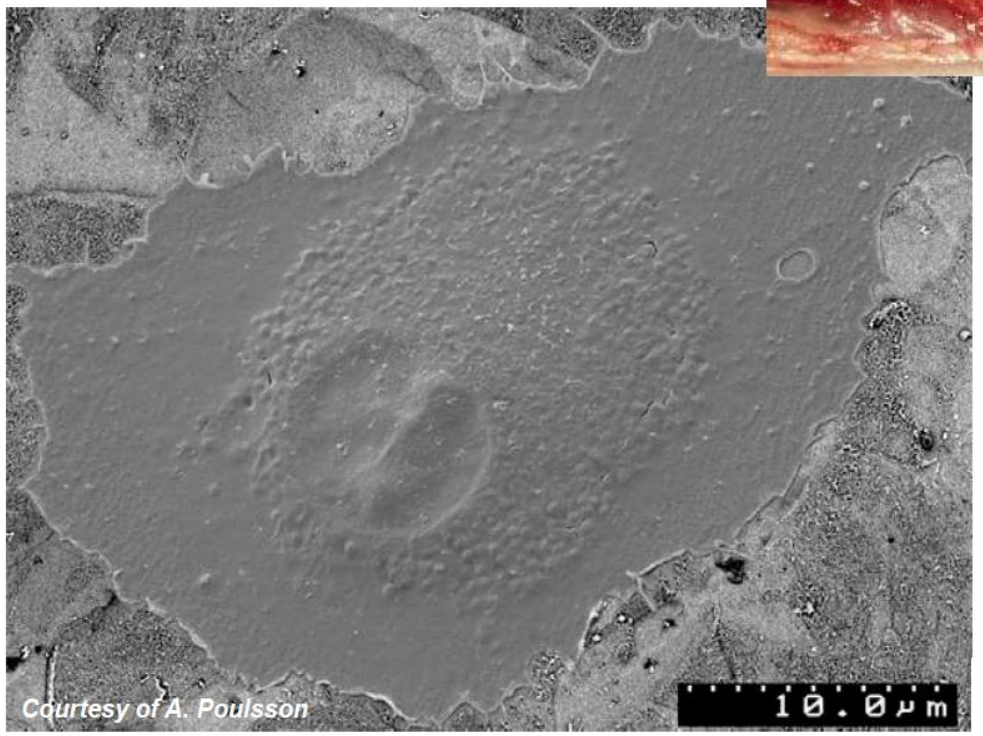
Surfaces… what do cells see ?



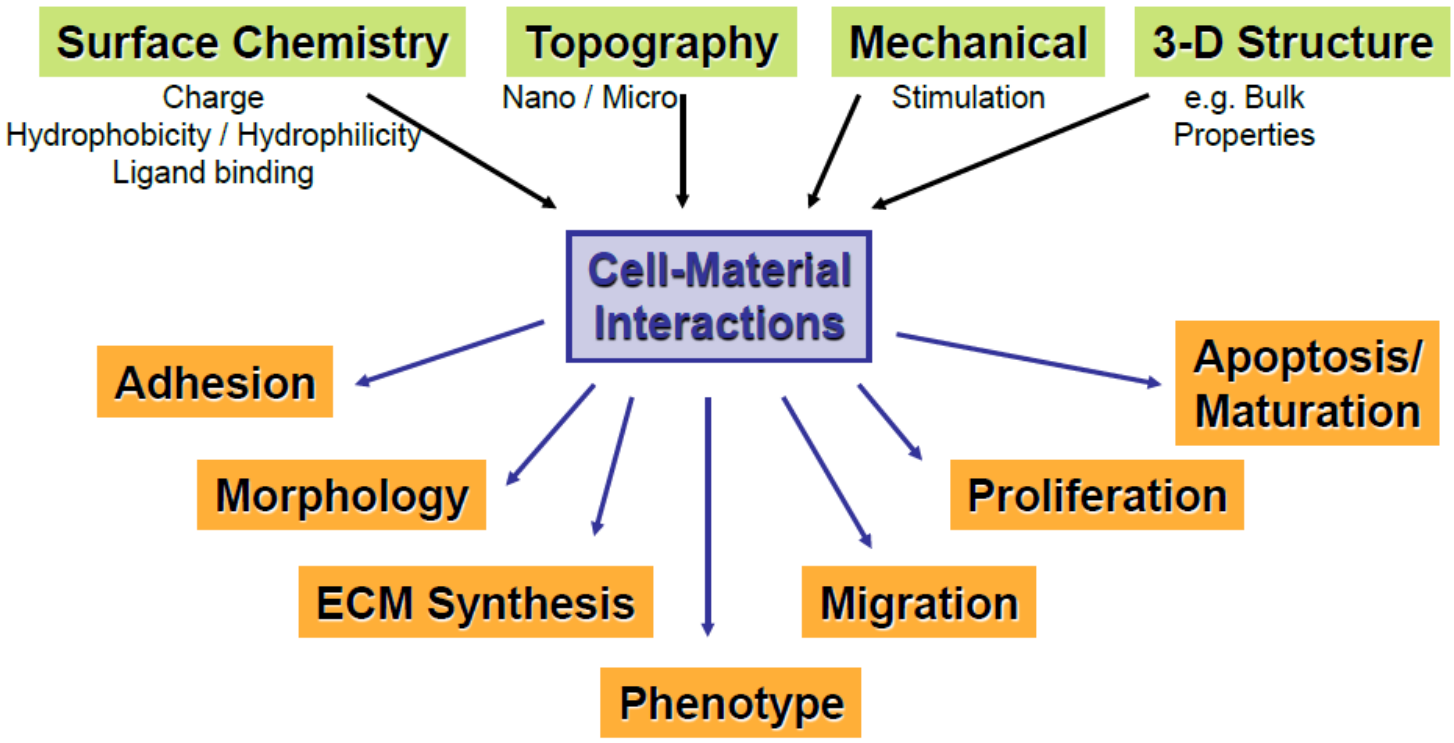


- Rough & smooth topography (micro/nano range)





Cell-material interactions



Masters K.S., Anseth K.S., *Advances in Chemical Engineering* **2004** 29: 7.

Tissue-material interactions

micromotion

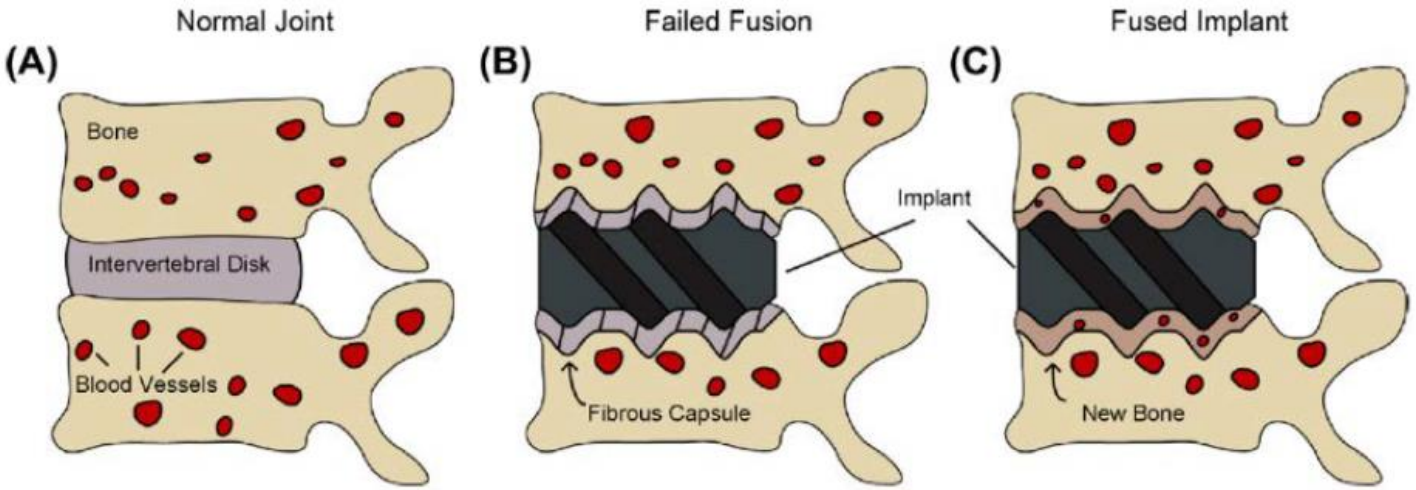
⇕

inflammation

⇓

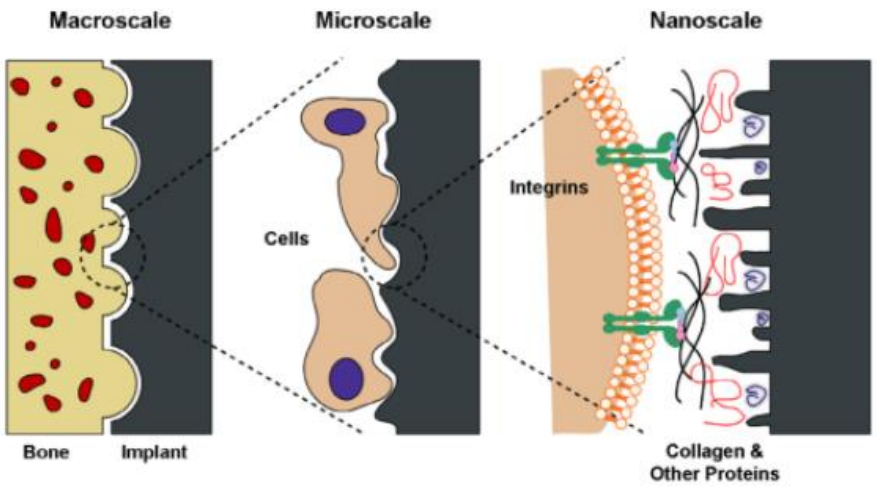
osteolysis

⇓



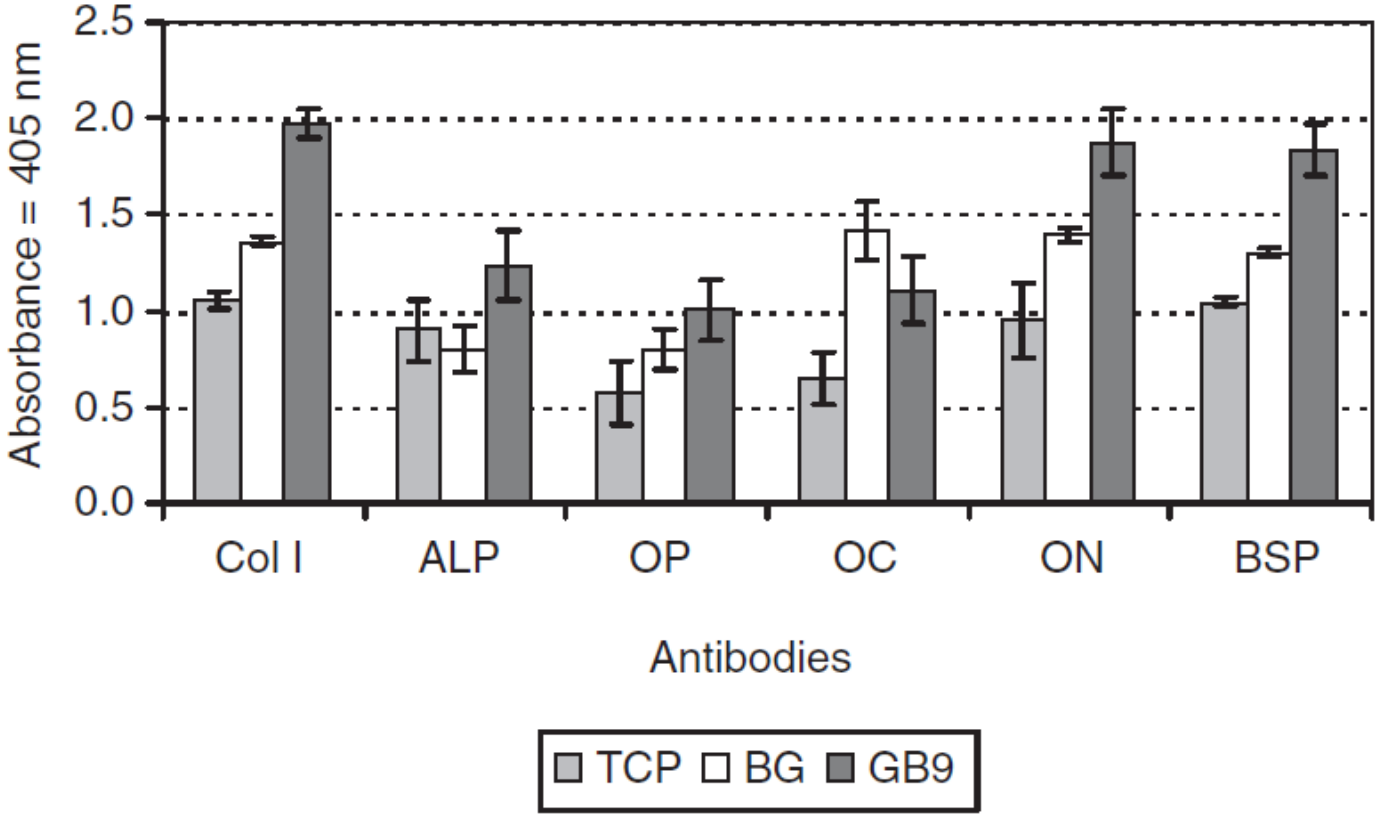
Gittens RA. *Acta Biomaterialia* **2014**
Implant osseointegration and the role of microroughness and nanostructures: Lessons for spine implants

Surface roughness



Gittens RA. *Acta Biomaterialia* **2014**
Implant osseointegration and the role of microroughness and nanostructures: Lessons for spine implants

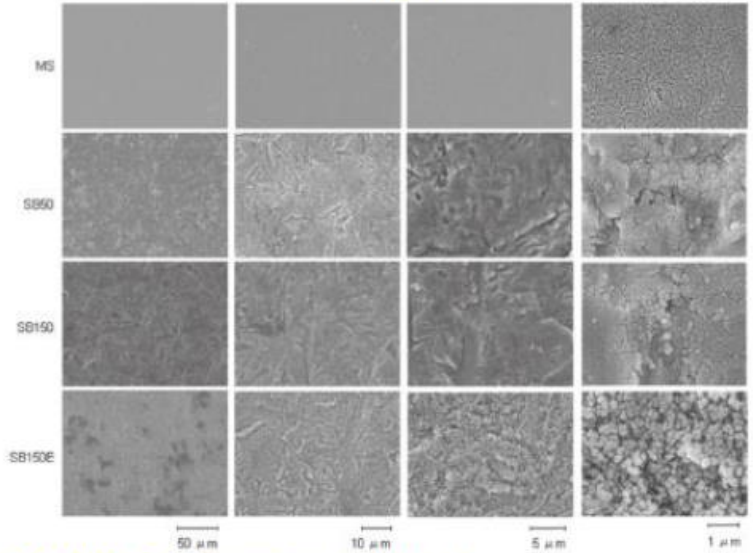
Osteoblast differentiation is defined by three principal biological periods: cellular proliferation, cellular maturation and matrix mineralization.



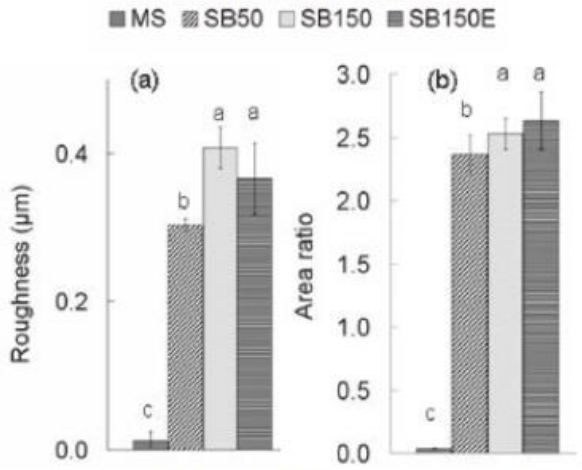
Type I collagen is expressed during the initial period of proliferation and extracellular-matrix synthesis, whereas *ALP* is expressed during the post-proliferative period of extracellular-matrix maturation, and the expression of *osteopontin*, *osteonectin*, ***osteocalcin*** and *bone sialoprotein* occurs later during the third period of extracellular-matrix mineralization.

Synergic effect of micro & nanoroughness

SEM



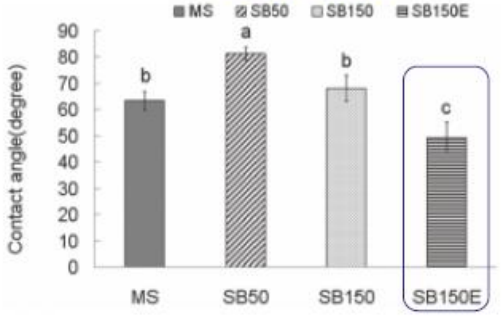
Profilometry



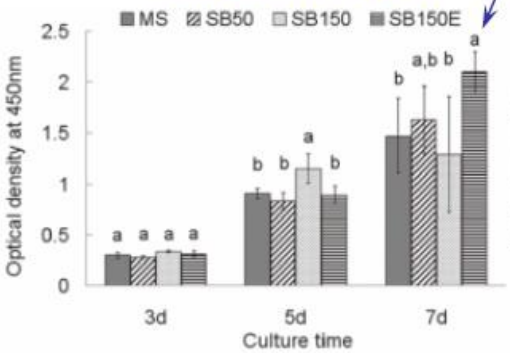
MS: mirror-polished, SB50: sand-blasted 50 μm
SB150: sand-blasted 150 μm, E: etched

MC3T3-E1 (murine cell line)

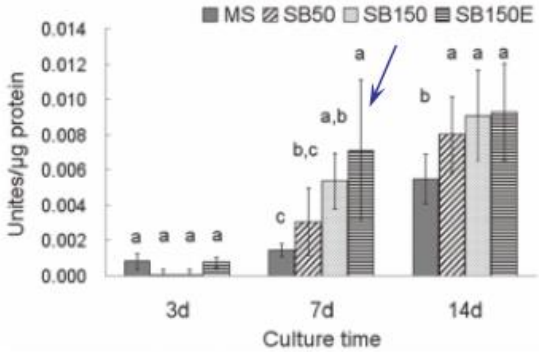
Wettability



Cell proliferation



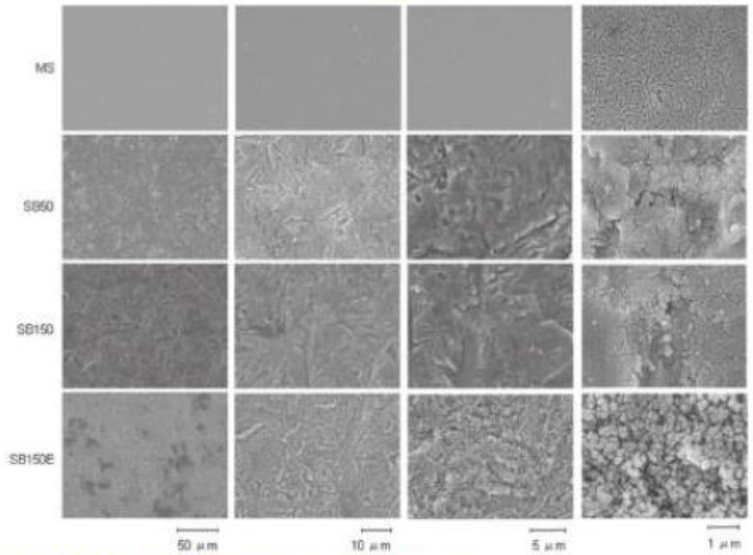
ALP activity



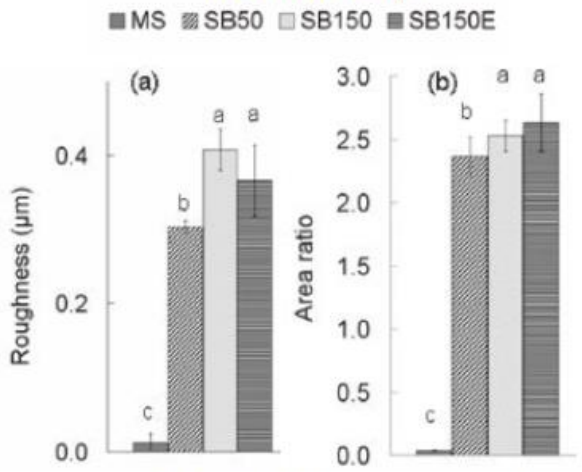
Ito H. *Dent Mater J* **2013**
Response of osteblast-like cells to zirconia with different surface topography

Synergic effect of micro & nanoroughness

SEM



Profilometry



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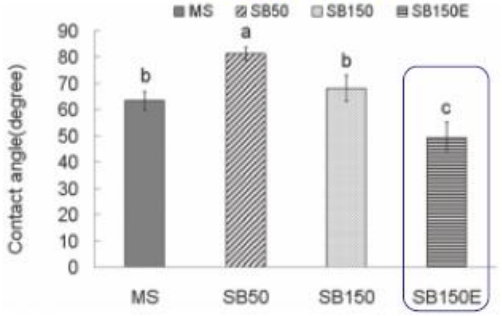
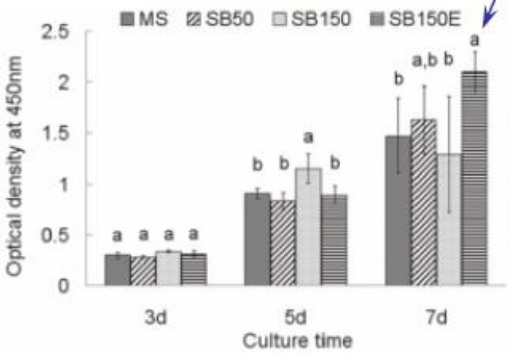
MC3T3-E1 (murine cell line)

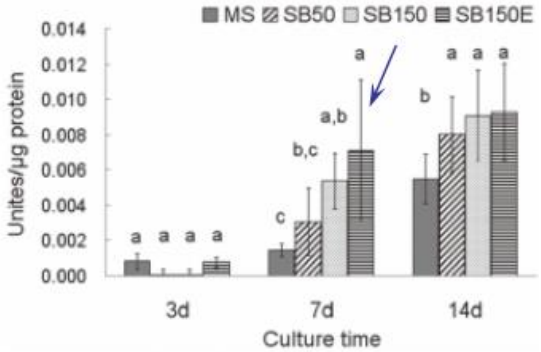
Wettability



Cell proliferation



ALP activity



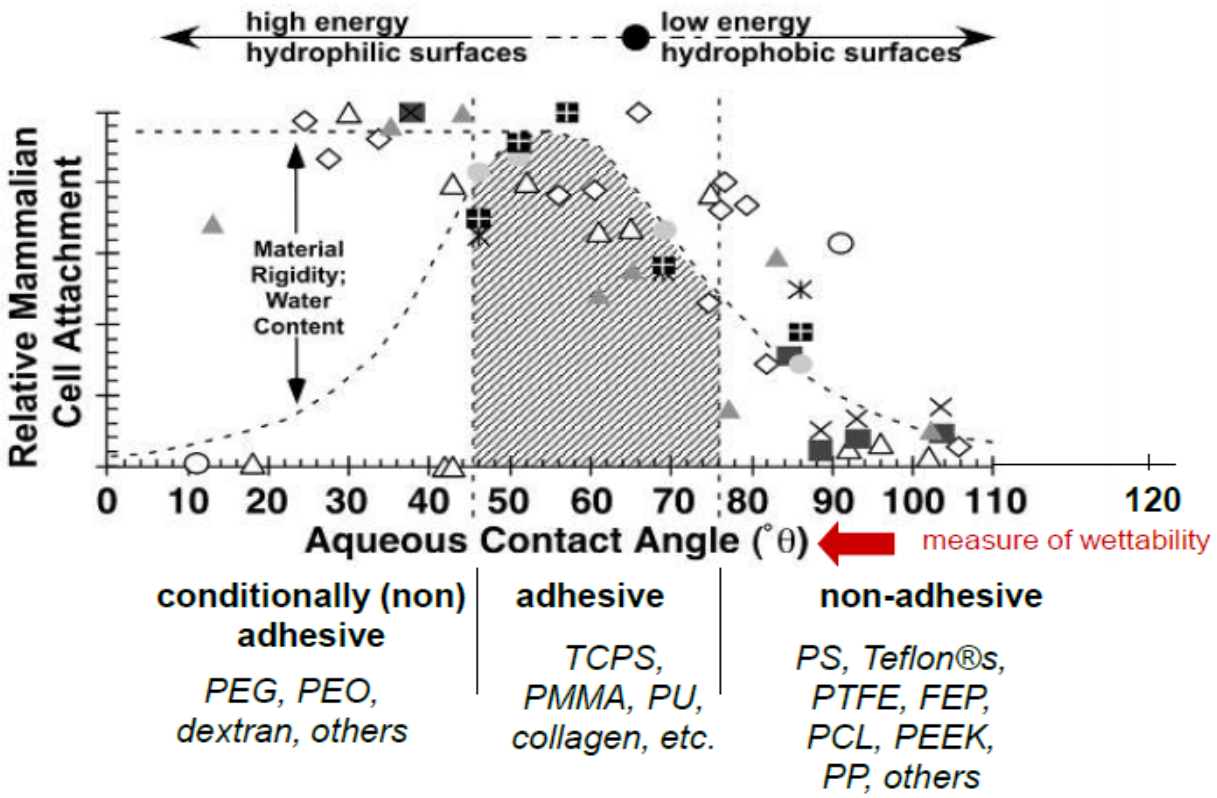
Ito H. *Dent Mater J* **2013**
Response of osteblast-like cells to zirconia with different surface topography

Plasma-modified PEEK:
in vitro

Survey of cell adhesion to materials (hydrophobicity on very smooth surface)

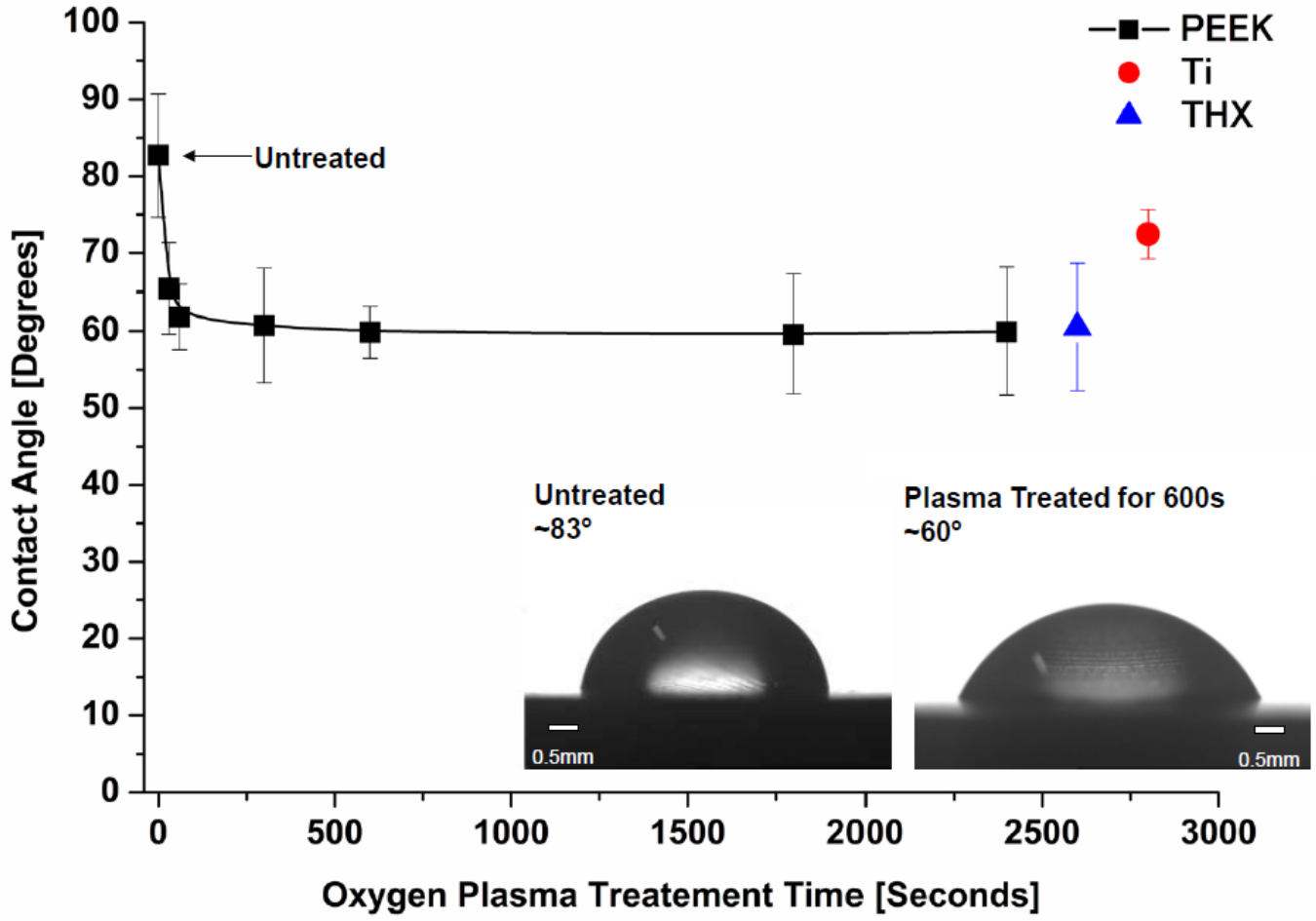
cells attach best to surfaces that are neither too hydrophobic or too hydrophilic

Manufacturing & contamination moves these boundaries

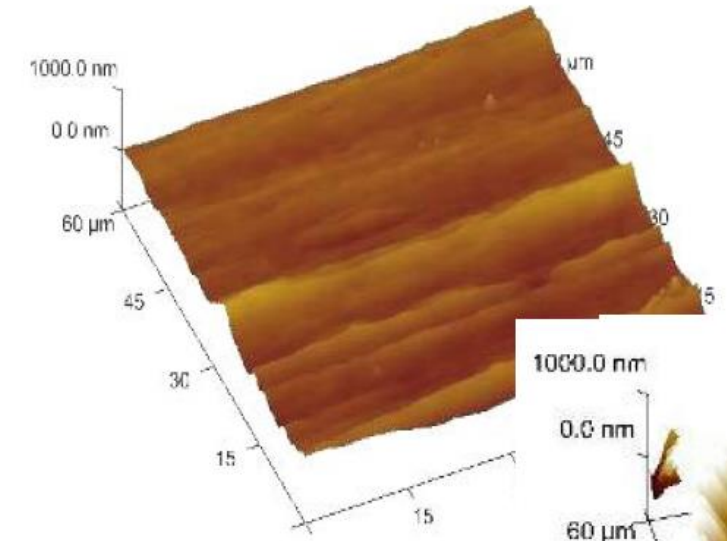


Adapted graph courtesy (Harbers, G. M., & Grainger, D. W.)

Surface wettability of plasma treated PEEK

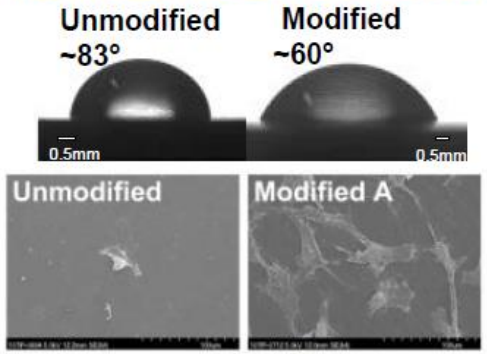


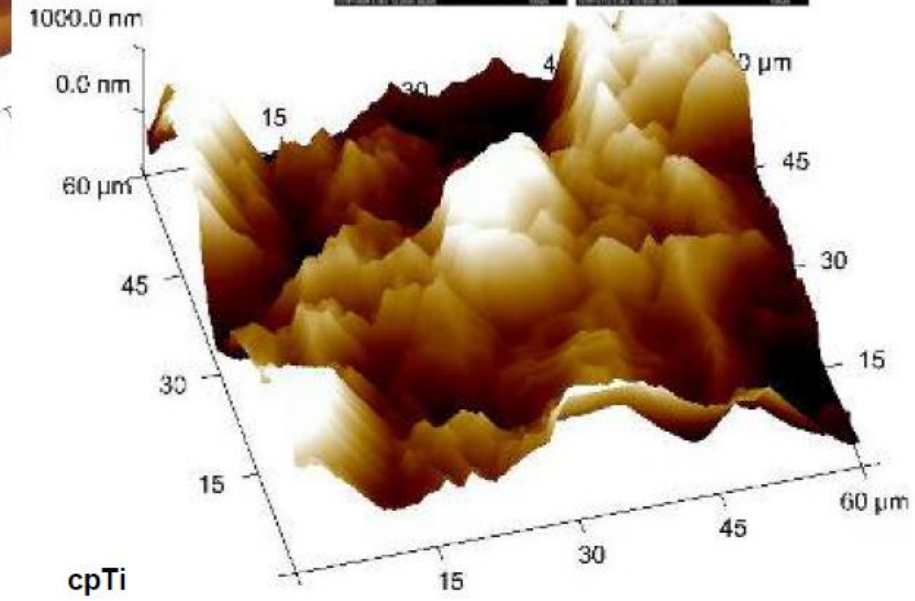
AFM of evaluation of surface topography



Inj. Moulded PEEK

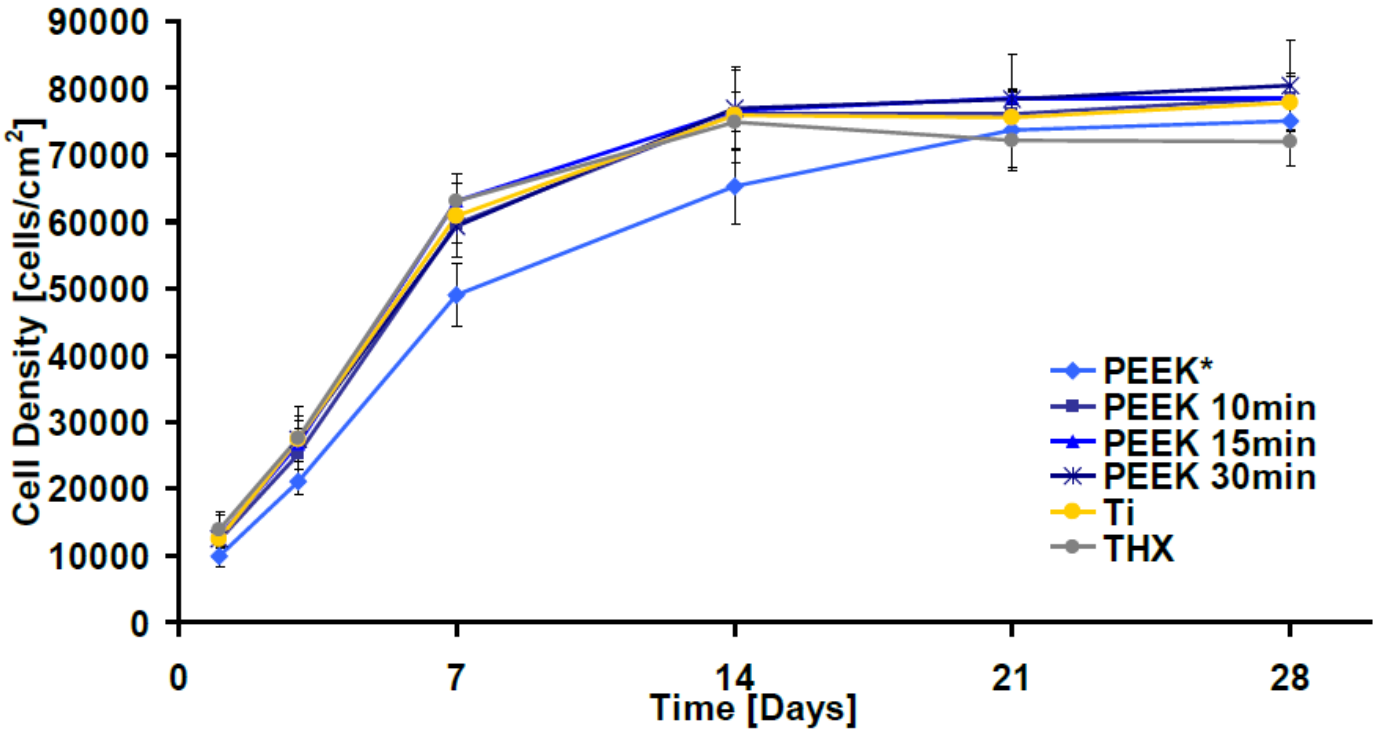
Plasma surface modification





cpTi

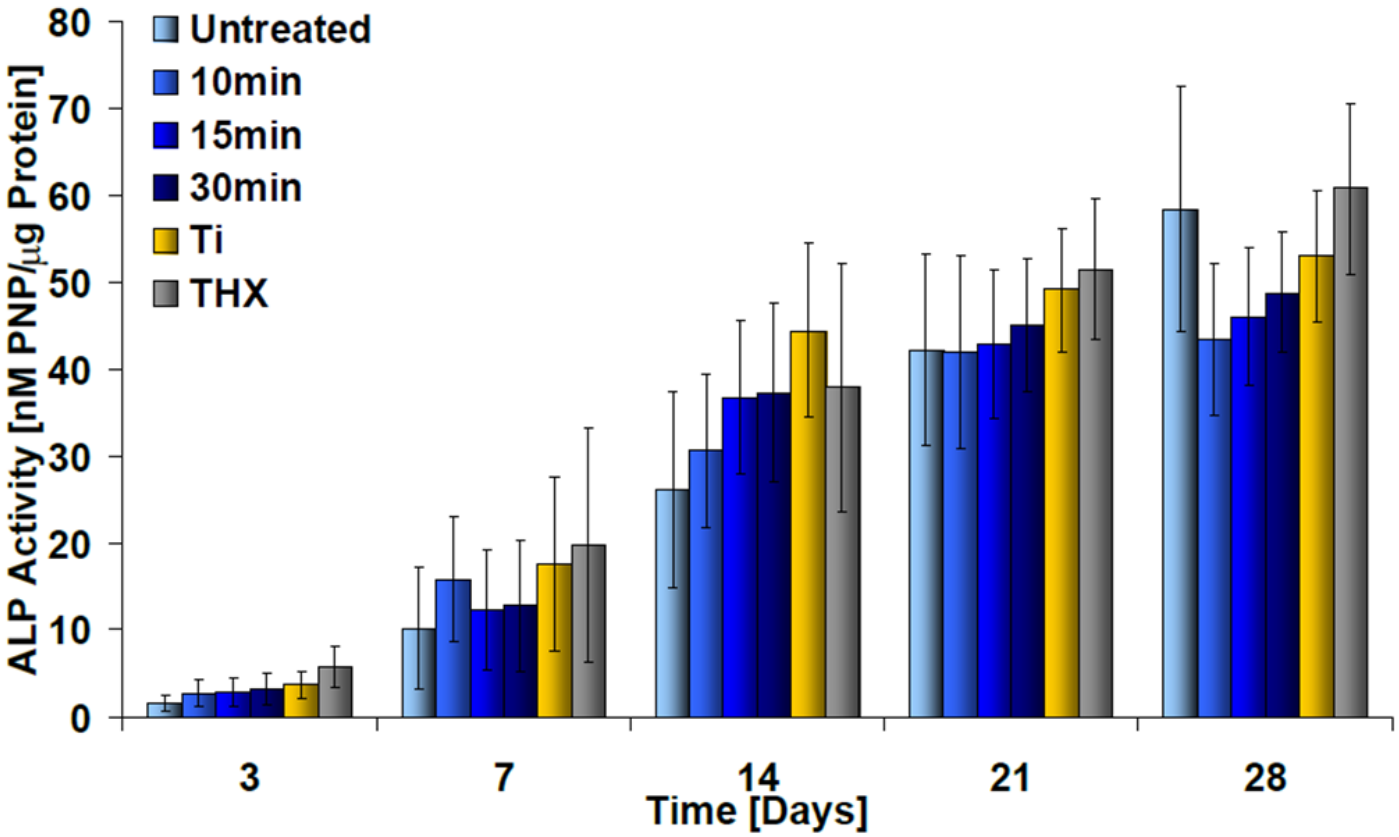
Cell proliferation on PEEK



Poulsson AHC in PEEK Biomaterials Handbook (Kurtz SM, Elsevier ed). **2012**
Surfaces to control tissue adhesion for osteosynthesis with metal implants.

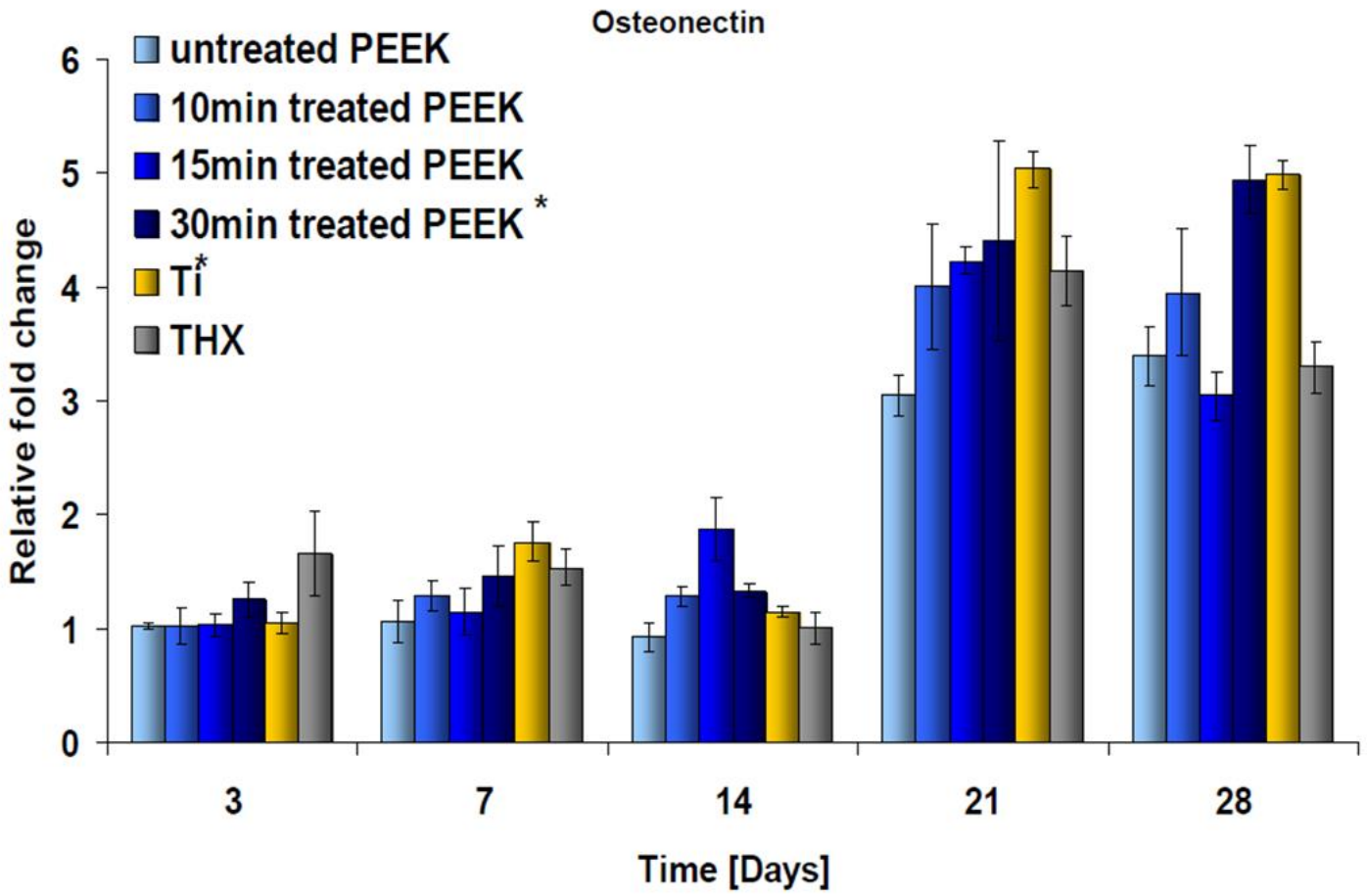
Data from 5 independent femoral heads, ± st. dev. GLM ANOVA with Tukey *post-hoc*, significance $P<0.05$

Alkaline phosphatase activity on PEEK



Poulsson AHC in PEEK Biomaterials Handbook (Kurtz SM, Elsevier ed). **2012**
Surfaces to control tissue adhesion for osteosynthesis with metal implants.

Gene expression profile of HOB on PEEK



Poulsson AHC in PEEK Biomaterials Handbook (Kurtz SM, Elsevier ed). **2012**
Surfaces to control tissue adhesion for osteosynthesis with metal implants.

Cells and Tissue Engineering

Cell cultures: equipment

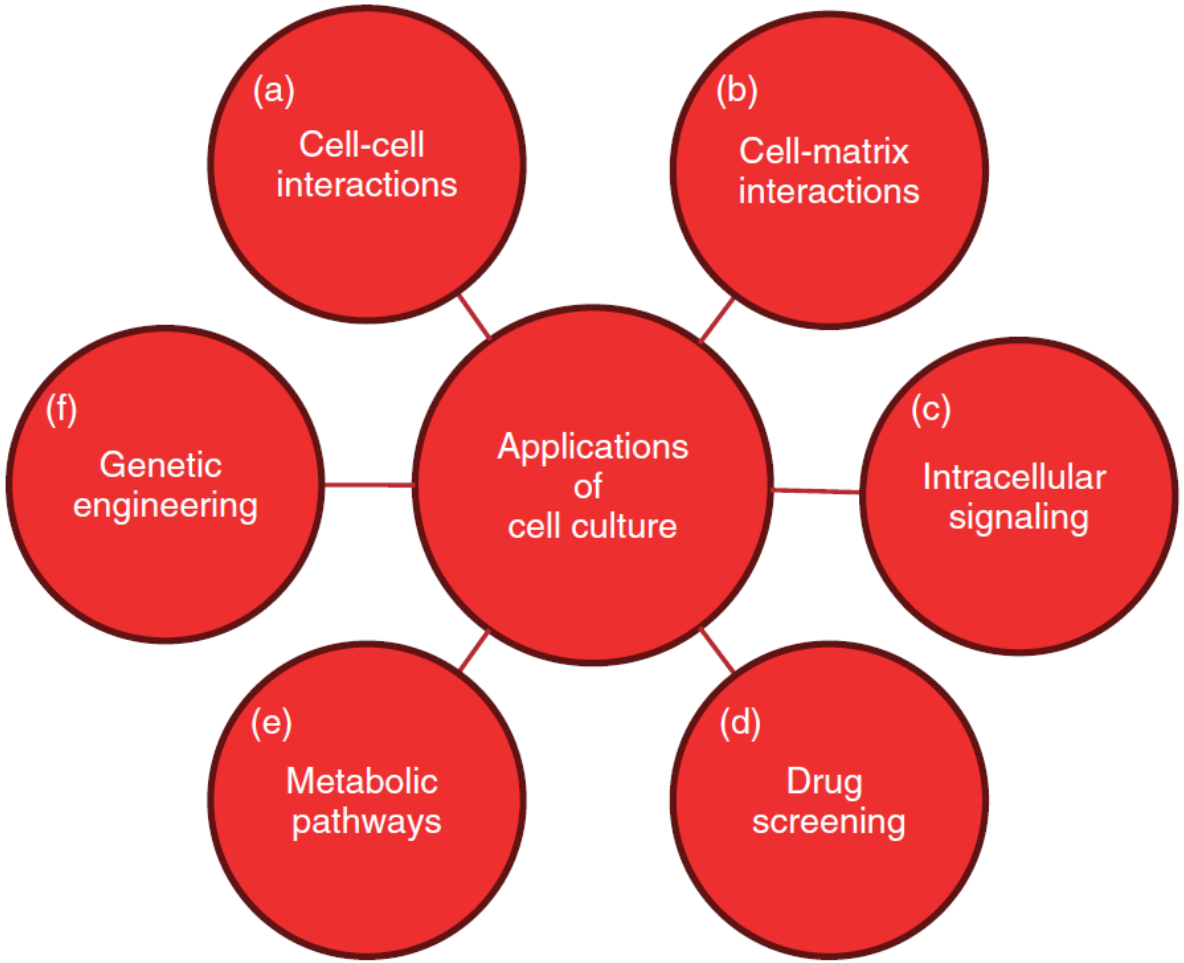
Safety Cabinet

Incubator
37 °C; humidified atmosphere
5% CO_2 / ar

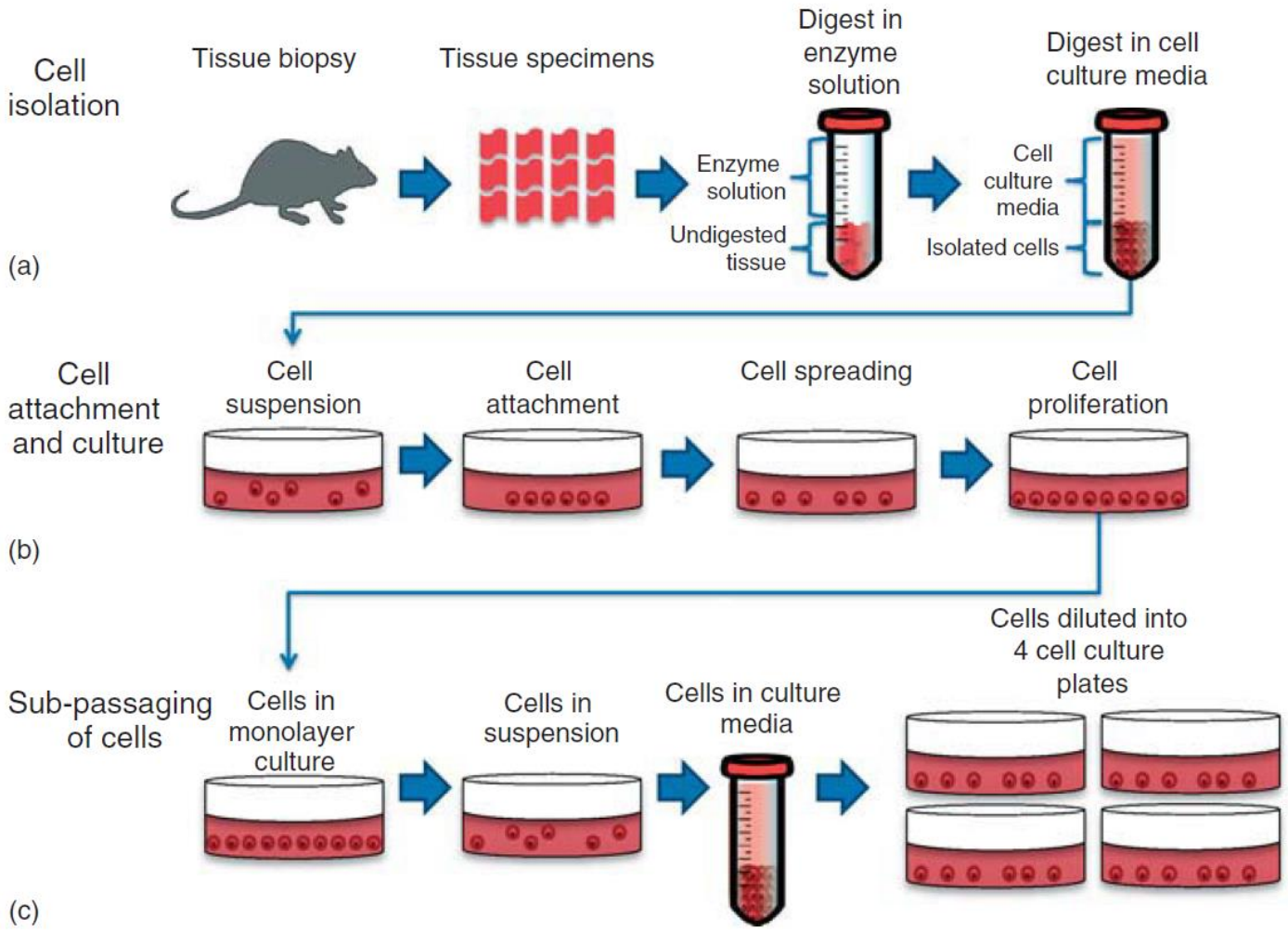
Appropriate culture medium

Culture flasks and plates

Applications of Cell Culture for TE



The Cell Culture Process

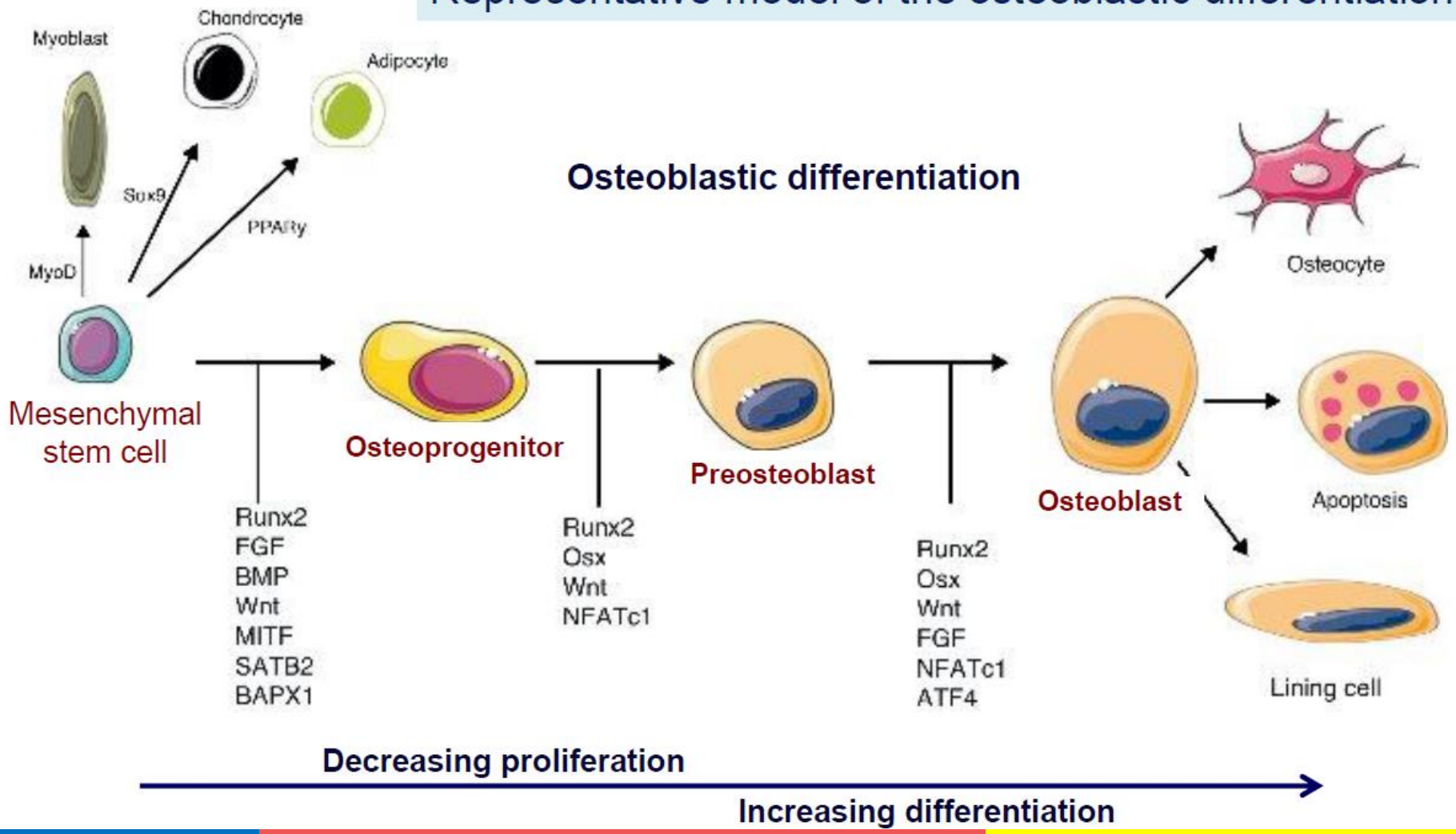


(a) Cell Isolation—A tissue specimen from a biopsy is cut into small pieces and subjected to an enzymatic digestion process to yield isolated cells.
(b) Cell Attachment and Culture—The primary cells are cultured on the surface of tissue culture plates where they attach, spread, and proliferate.
(c) Cell Expansion—Upon confluency, cells are subjected to treatment with trypsin and then replated to promote cell expansion.

Cells and Tissue Engineering

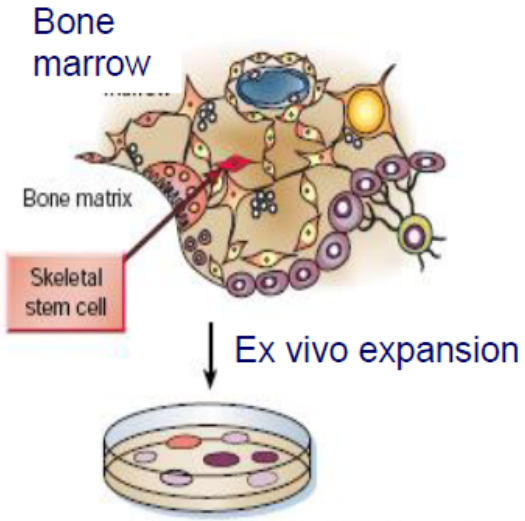
Osteoblastic cell cultures

Representative model of the osteoblastic differentiation



Cells and Tissue Engineering

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

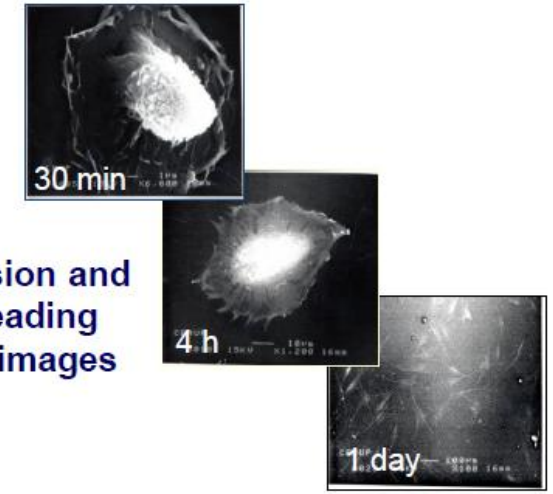
Biochemical, histochemical, immunohistochemical and molecular methodologies; SEM, CMSM

Cells and Tissue Engineering

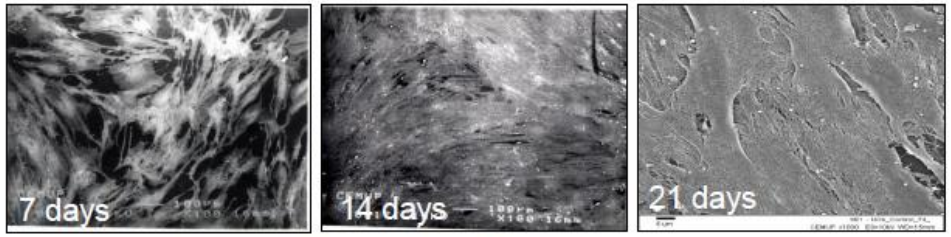
Osteoblastic cell cultures: Proliferation / differentiation pathway

Human bone marrow-derived osteoblastic cell cultures

Characterization of the cell behaviour



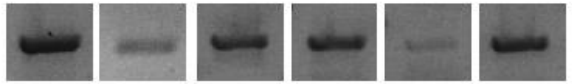
Adhesion and spreading SEM images



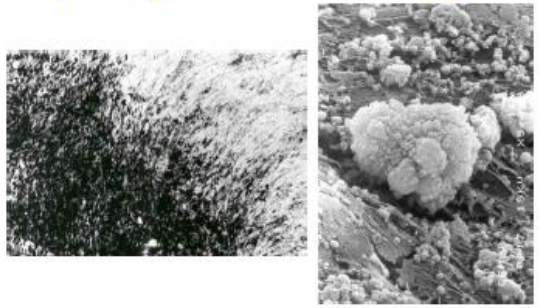
Cell proliferation (SEM images

Alkaline phosphatase staining

Gene expression profile



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

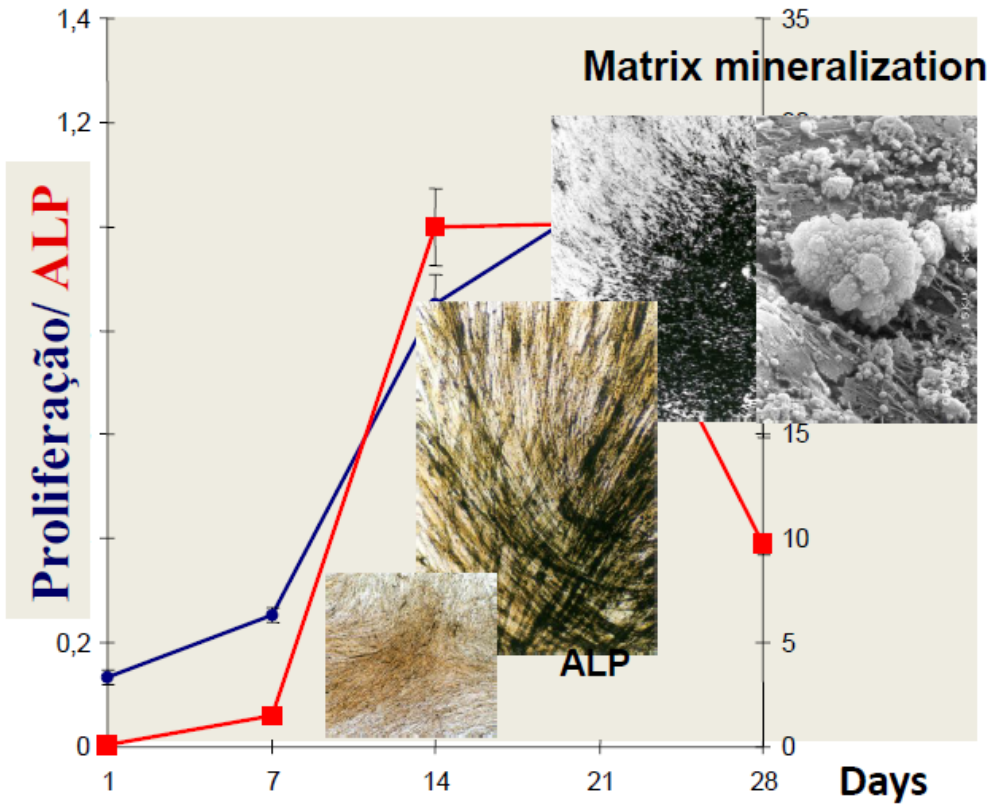


Matrix mineralization (SEM)

Cells and Tissue Engineering

Osteoblastic cell cultures: Proliferation / differentiation pathway

Human bone marrow-derived osteoblastic cell cultures



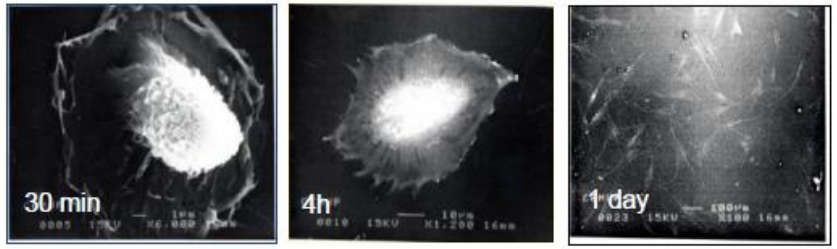
Inverse relationship between proliferation and differentiation

Cells and Tissue Engineering

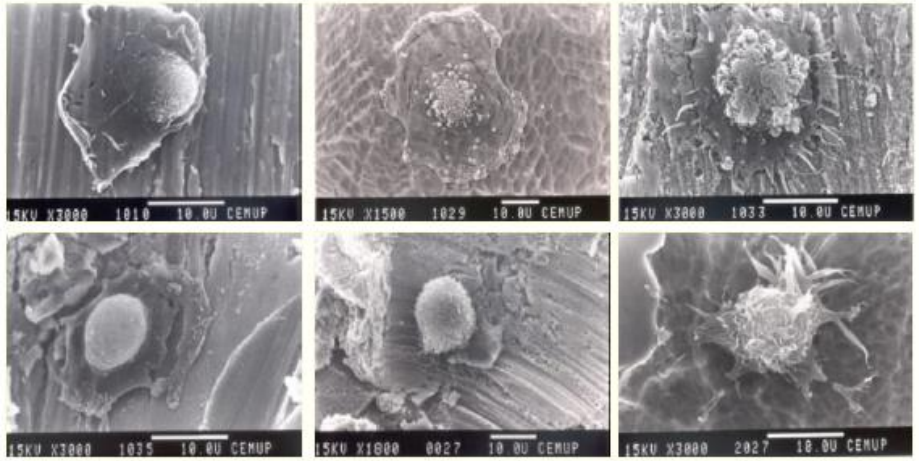
Osteoblastic cell cultures: cells / biomaterials interactions

Human bone marrow-derived osteoblastic cell cultures

Cell ahesion: standard tissue culture plates

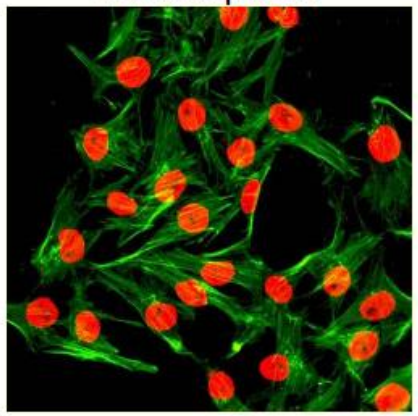


Ahesion to HA substrates with different topographies; 30 min

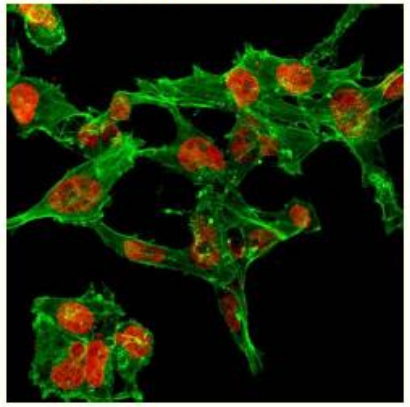


Cell morphology

Culture plates

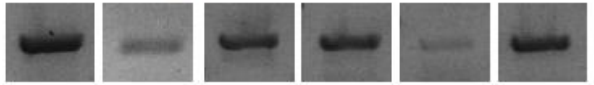


Degradable ceramic



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

Gene expression profile

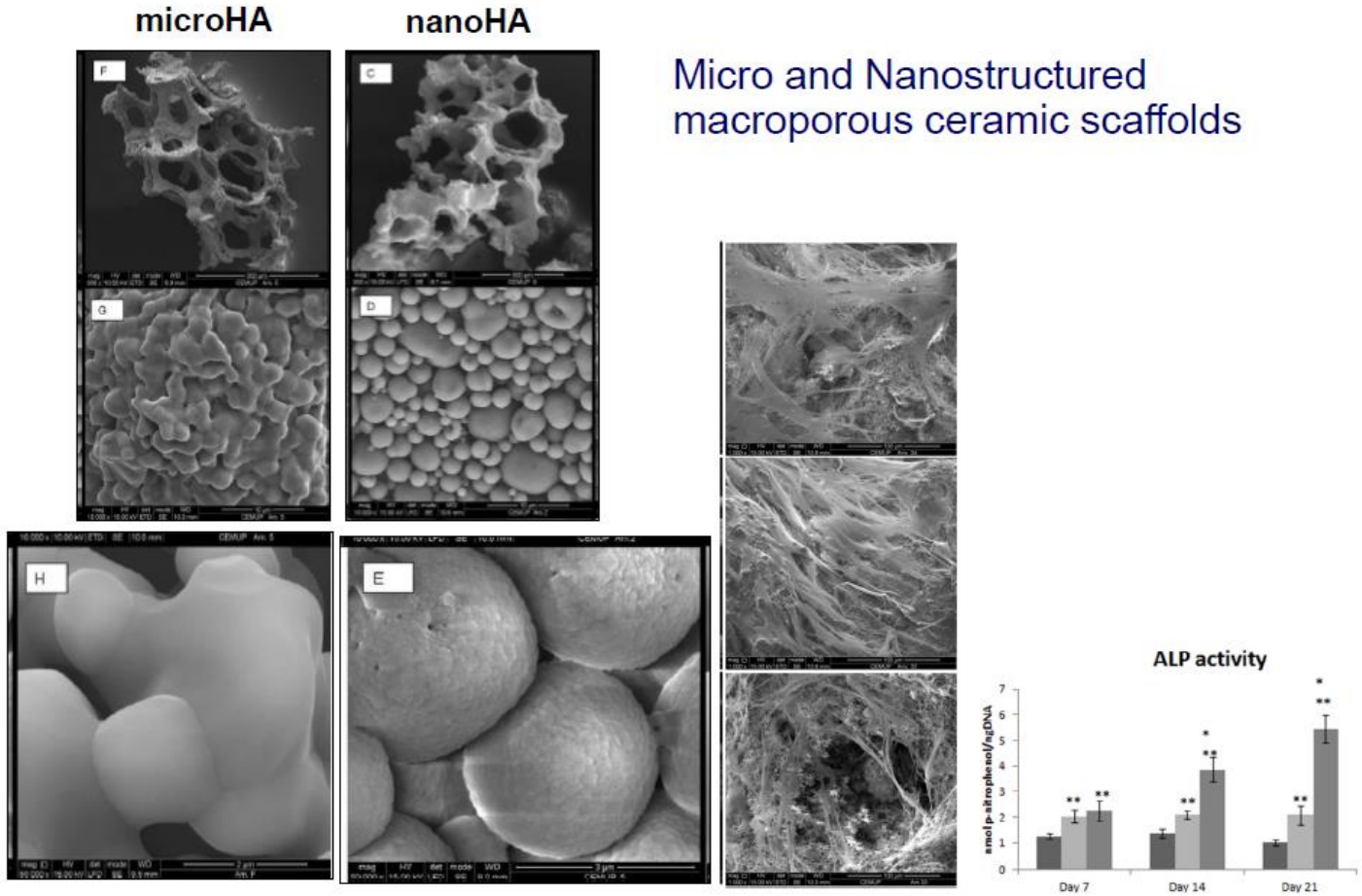


Runx-2; Col-1; ALP; OC; OPG; RANKL; .....

Cells and Tissue Engineering

Osteoblastic cell cultures

Illustrative study



Micro and Nanostructured macroporous ceramic scaffolds

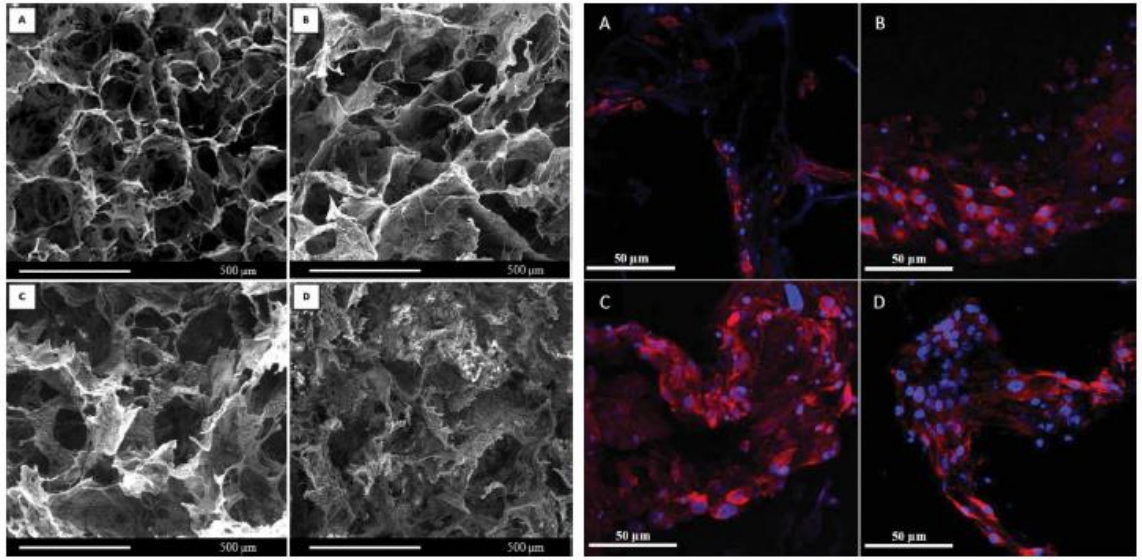
J Biomed Mater Res A 101: 1080-1094 (2013)

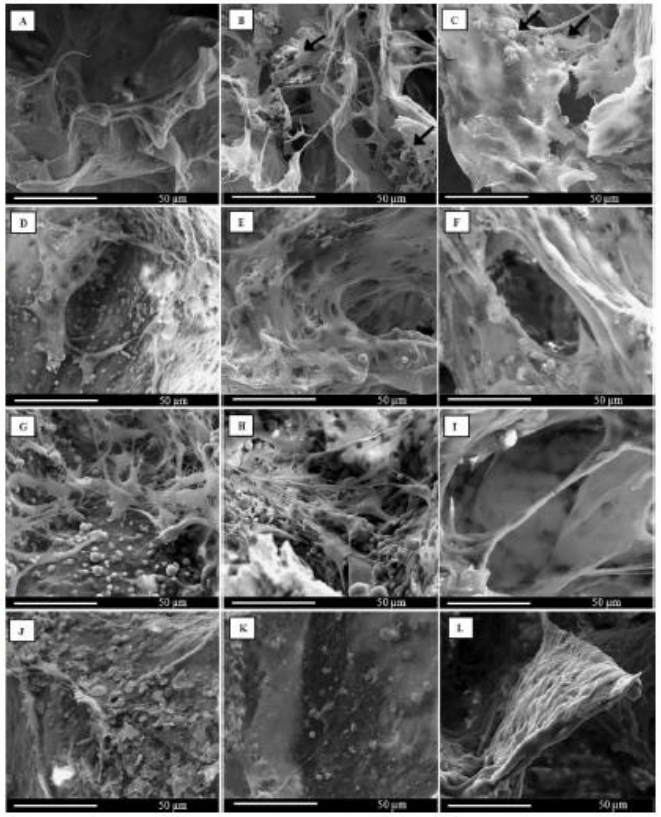
Cells and Tissue Engineering

Osteoblastic cell cultures

Illustrative study

Collagen-nanohydroxyapatite biocomposite scaffolds



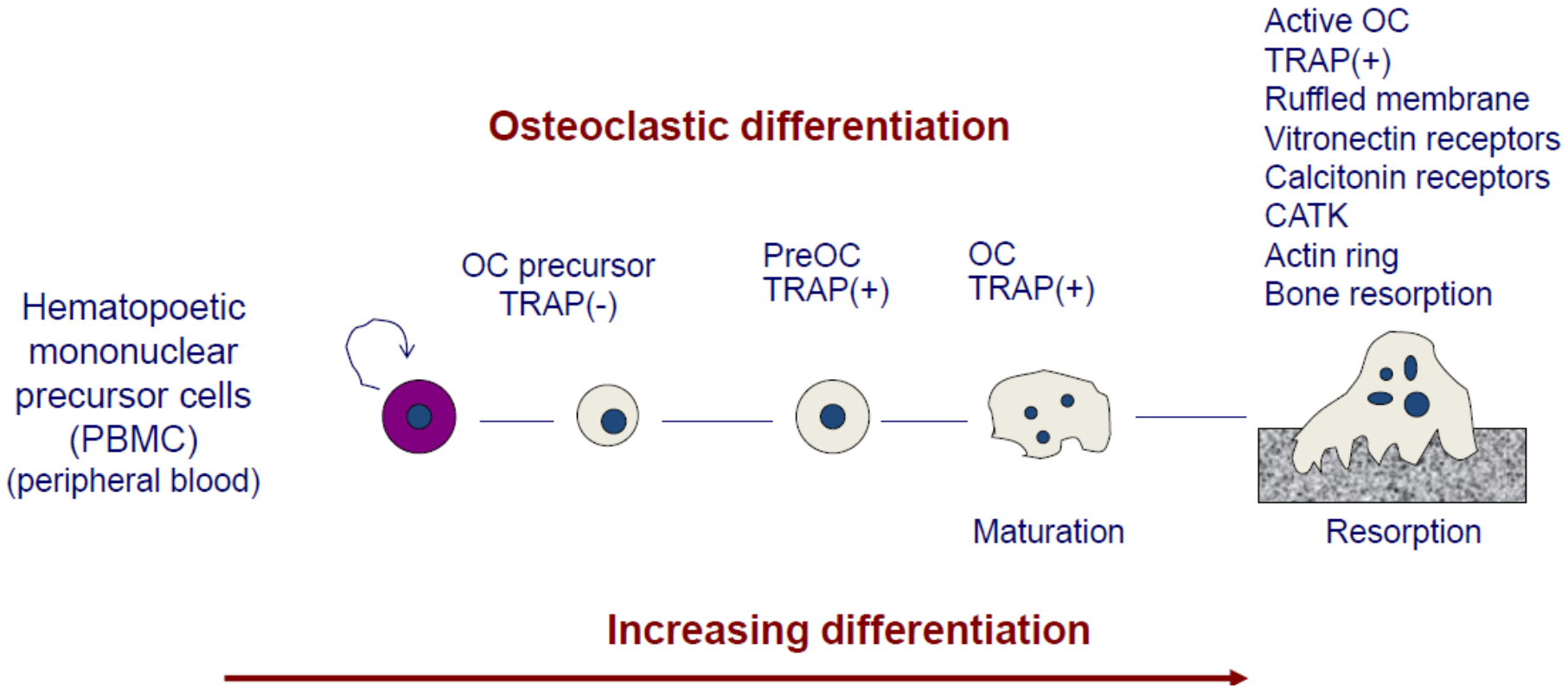


J Biomed Mater Res Part A: 101A: 10–1094 (2013)

Cells and Tissue Engineering

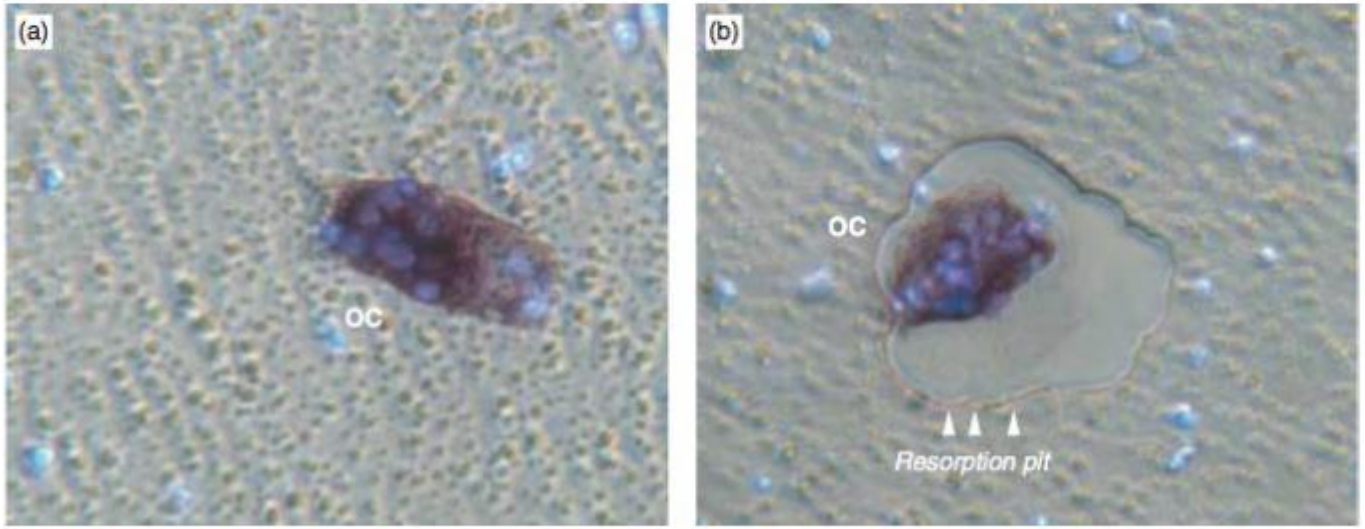
Osteoclastic cell cultures

Representative model of the osteoclastic differentiation



Cells and Tissue Engineering

- biodegradation mechanisms:
  - physico-chemical degradation
  - cell-mediated degradation
    - osteoclasts (and macrophages)



before resorption **after resorption**

[reproduced from: Van Blitterswijk, C. **(2008)**, *Tissue Engineering*, Academic Press Oxford, UK.]

Cells and Tissue Engineering

- biodegradation mechanisms:
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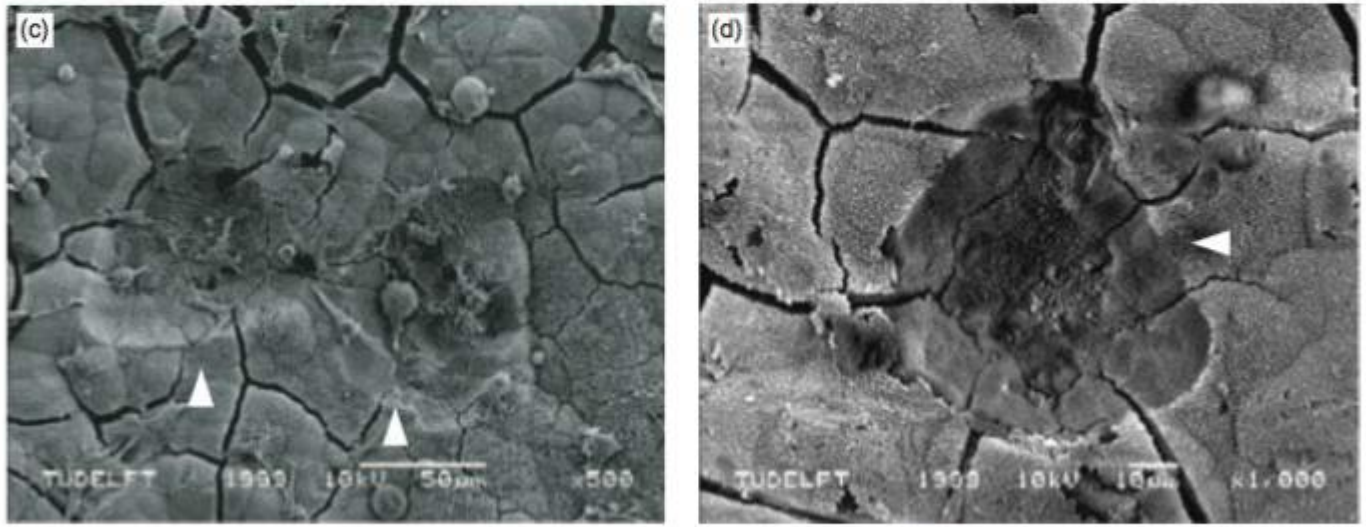
  - cell-mediated degradation
    - osteoclasts (and macrophages)



resorption pits **magnified resorption pit**

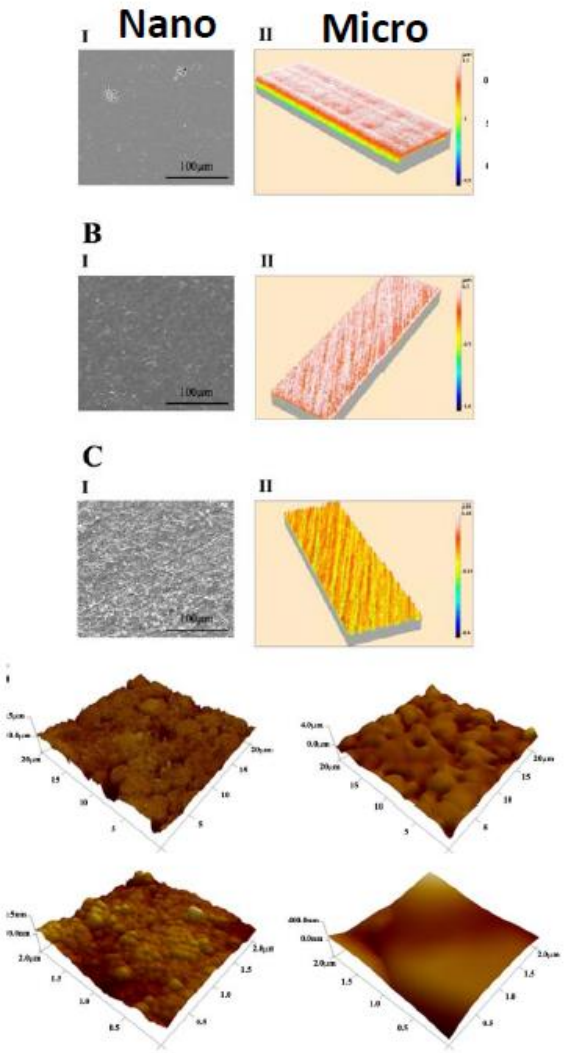
[reproduced from: Van Blitterswijk, C. **(2008)**, *Tissue Engineering*, Academic Press Oxford, UK.]

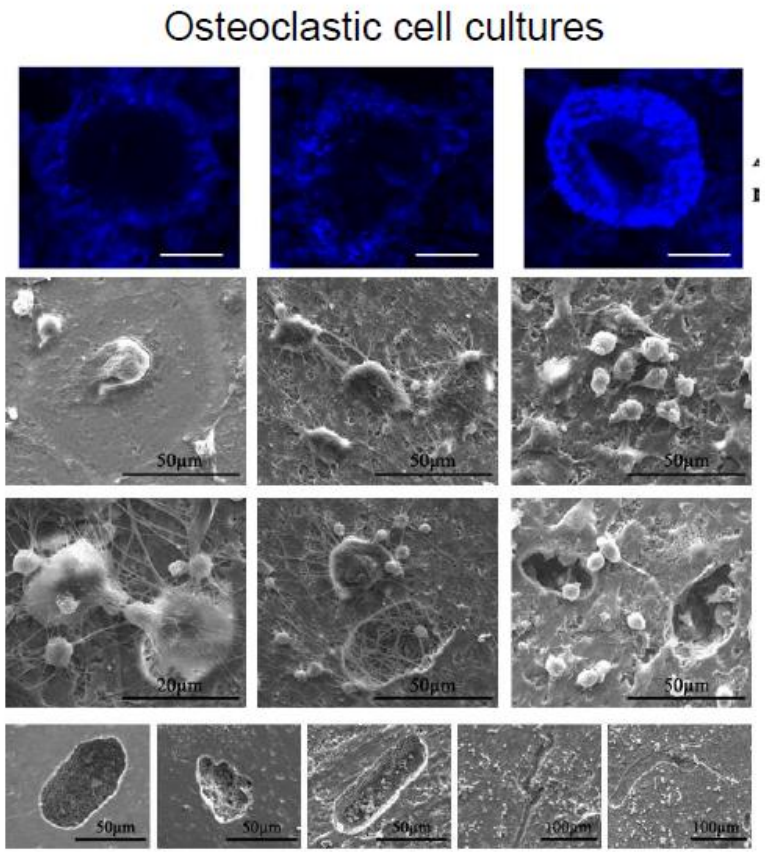
Cells and Tissue Engineering

Osteoclastic cell cultures

Illustrative study

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



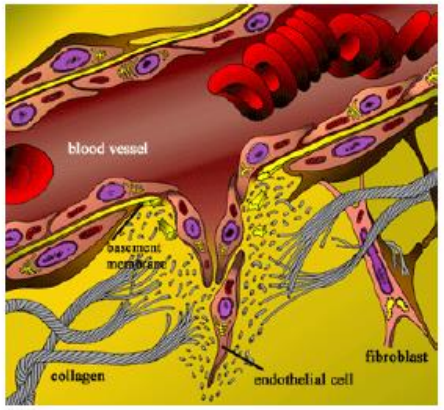


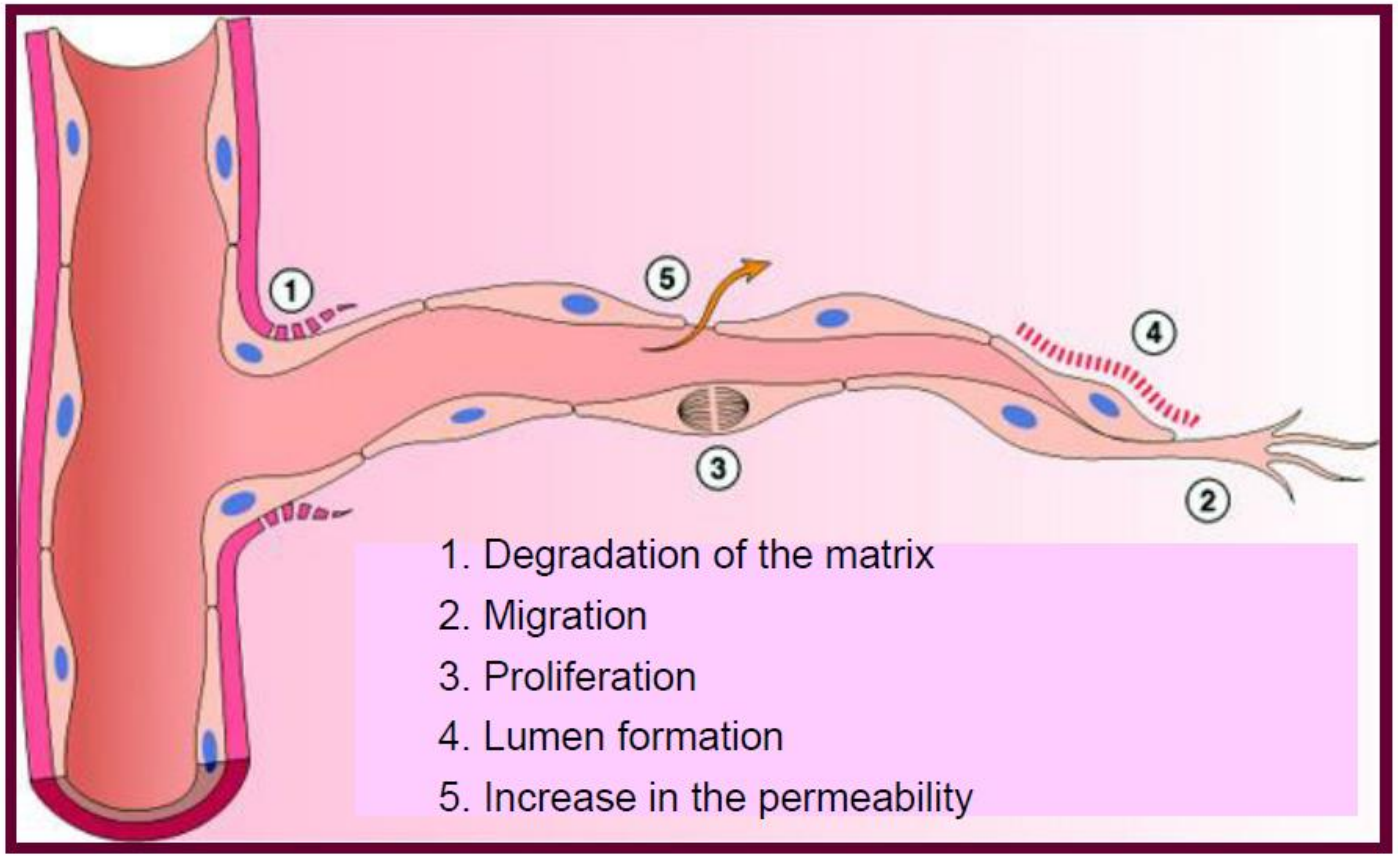
Acta Biomaterialia 8:1137-45 (2012)

Cells and Tissue Engineering

Endothelial cell cultures

Representative model of angiogenesis





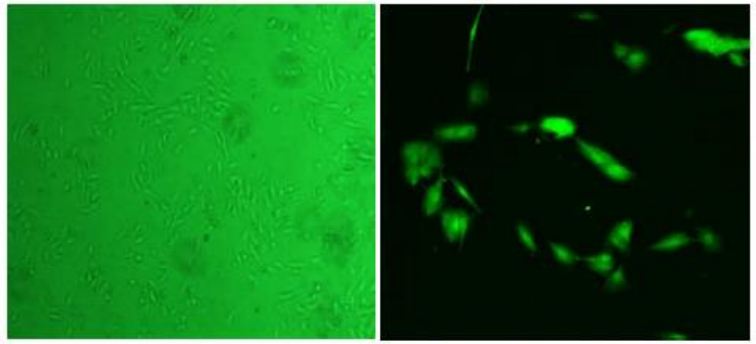
Cells and Tissue Engineering

Cells and Tissue Engineering

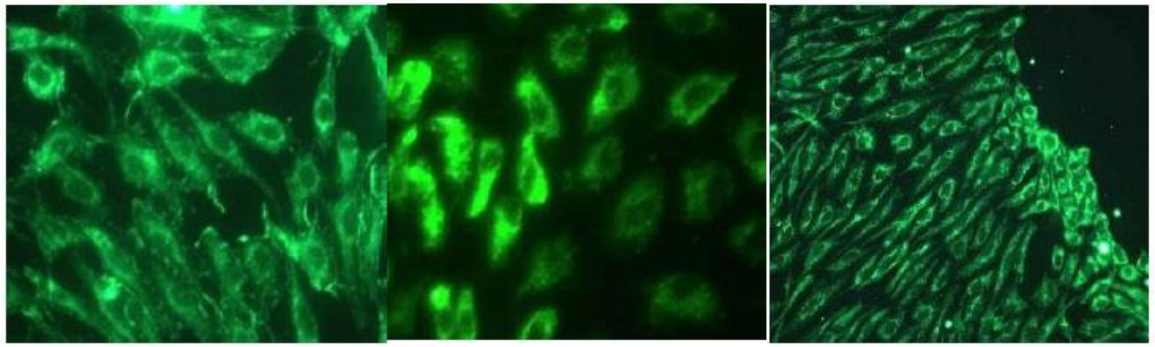
Endothelial cell cultures

Characterization of cell behaviour

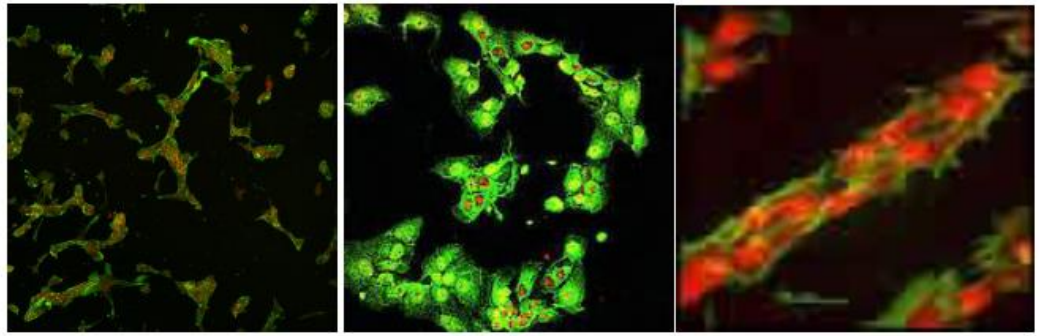
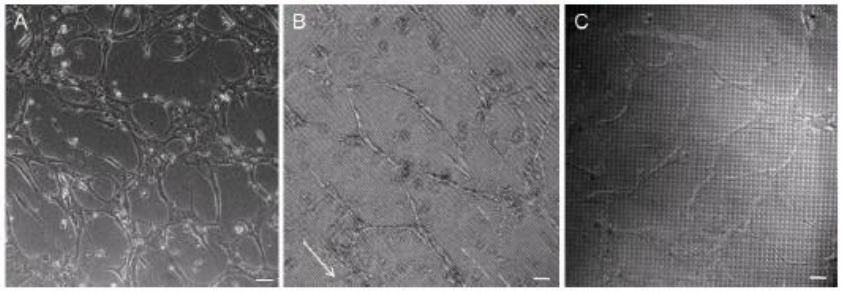
Circular pattern of cell proliferation



PECAM-1 factor vWB VE-cadherin



Capillary-like tube formation



Capillary-like tube formation