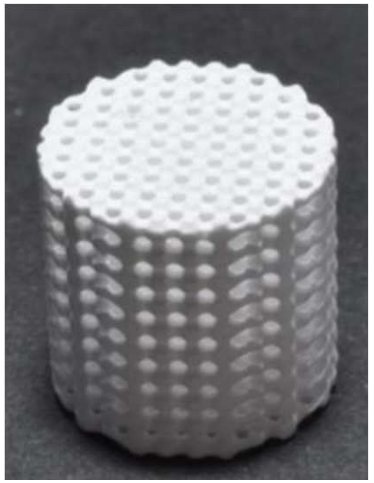
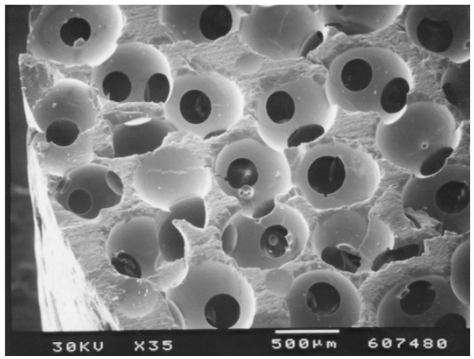
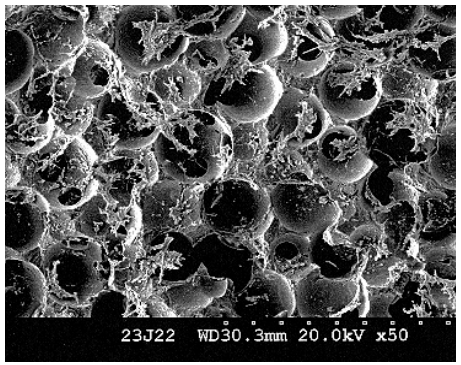
Training school: Non living materials meet living biology
Patras, Grece 9-12 May 2017

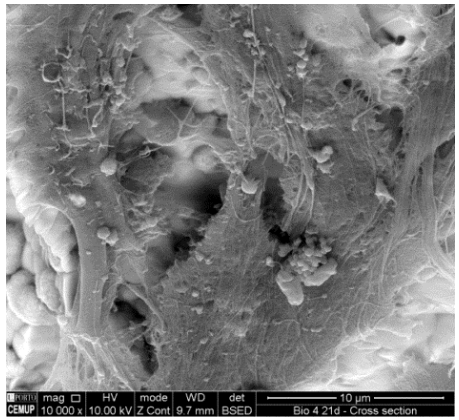
Scaffold design, processing, biodegradation and resorption



A.Leriche







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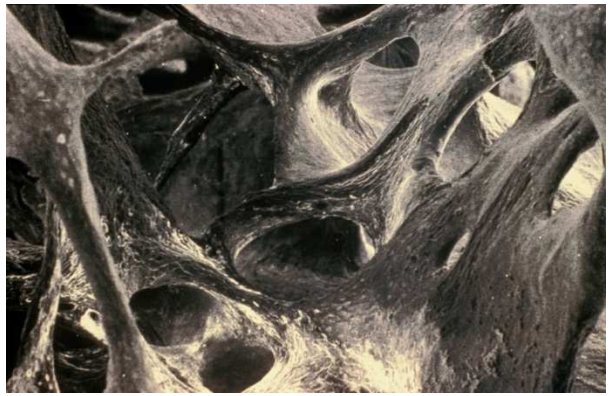
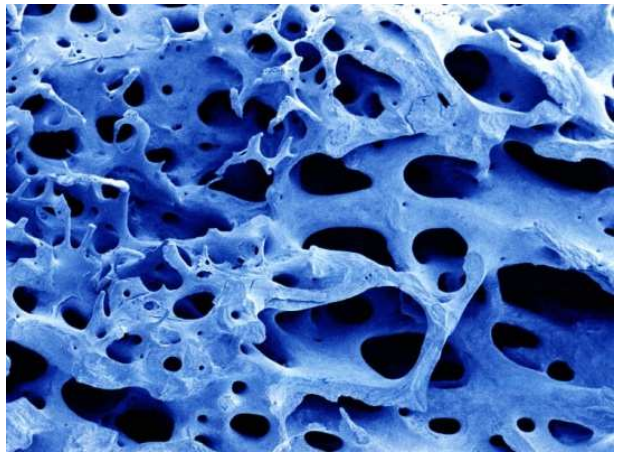
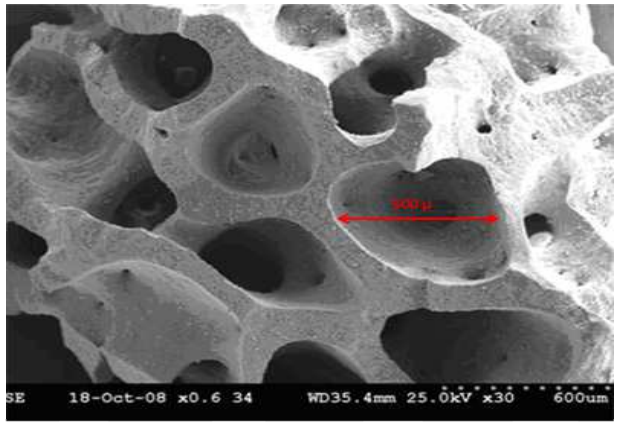






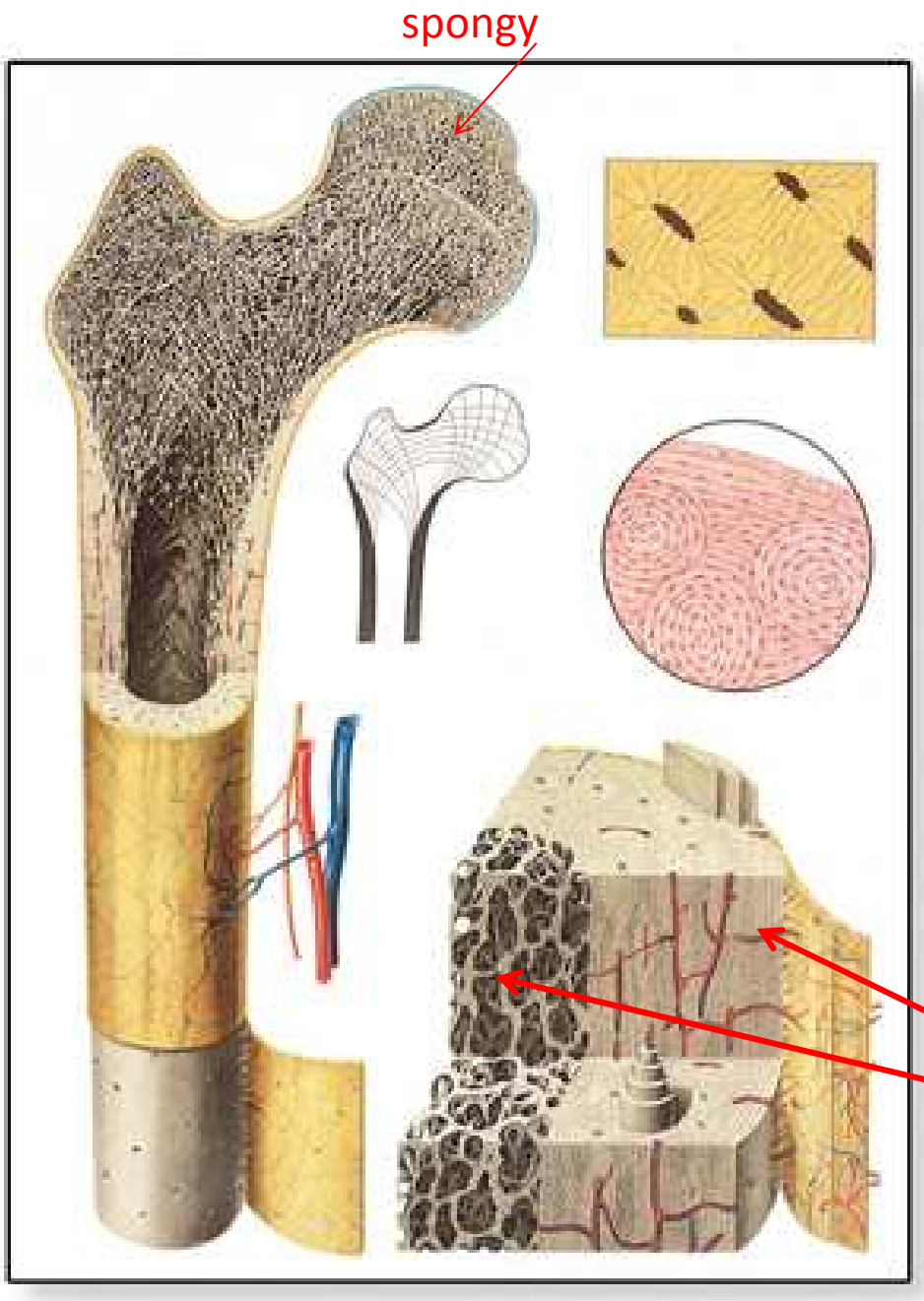
Regeneration of hard tissues (bone, cartilage and tooth) is one of the most demanding issues in medecine.

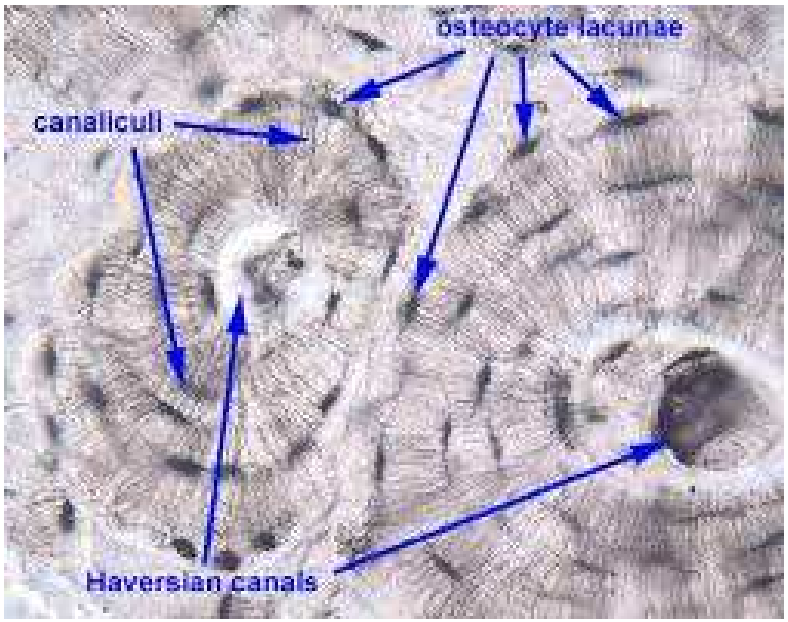
Synthetic bone scaffolds have advantages over bone grafts because they are not fraught with uncertainties (e.g. disease transmission, risk of infection, or immunogenicity).

However, these scaffolds have to show high mimicry of the host tissues and be able, with an appropriate porosity, to facilitate the progenitor cells in-growth and communication with neighboring cells to the regeneration of new functional tissue.

How to mimic natural bone for substitute processing?

Bone structure

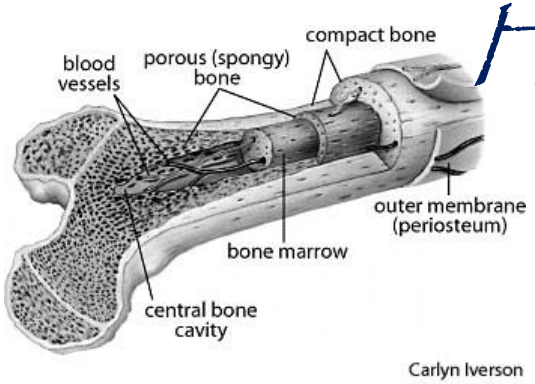


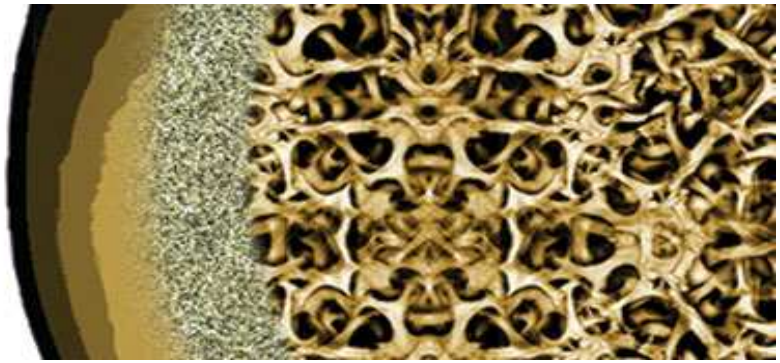


Natural composite of collagen and mineral (hydroxycarbonate apatite: HA)

2 types of porous structures:
- cortical with dense structure → compact bone *(usually outer layer)*
- cancellous or trabecular bone with high porosity → spongy bone *(usually interior)*

How to mimic natural bone structure?





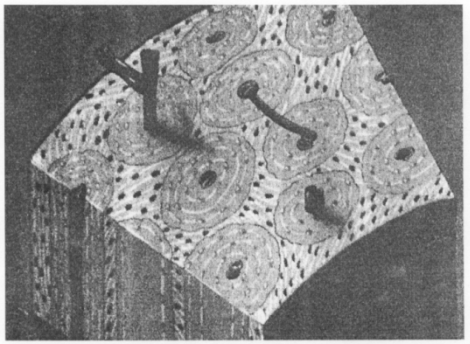
Compact bone

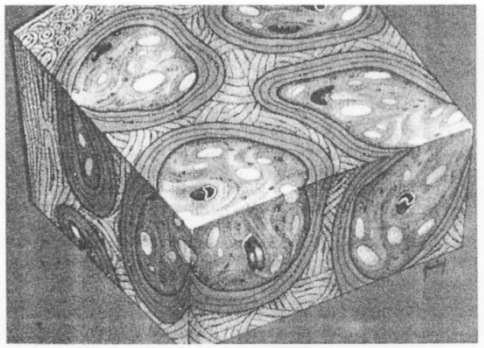
Porosity

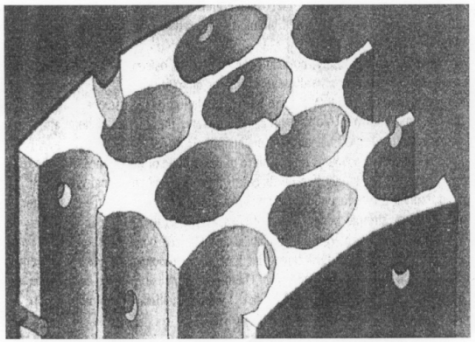
Φ : 190-230 µm

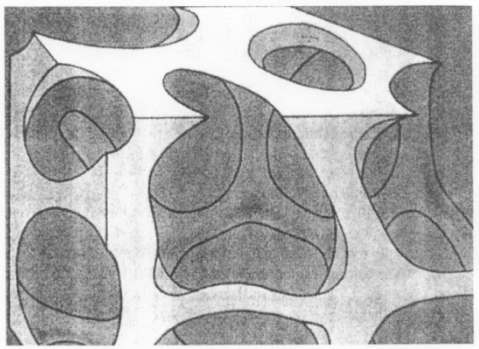
V : 10 - 30 %

σ_c = 80-200 MPa









Spongy bone

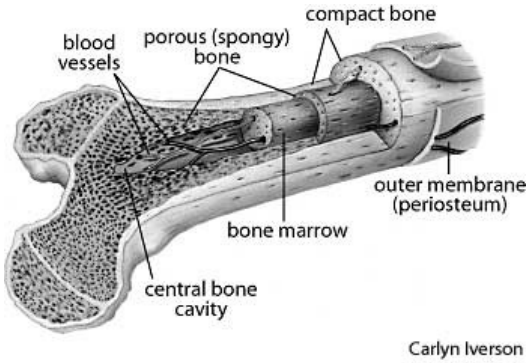
Porosity

Φ : 500-600 µm

V : 30 – 90 %

σ_c = a few tenths MPa

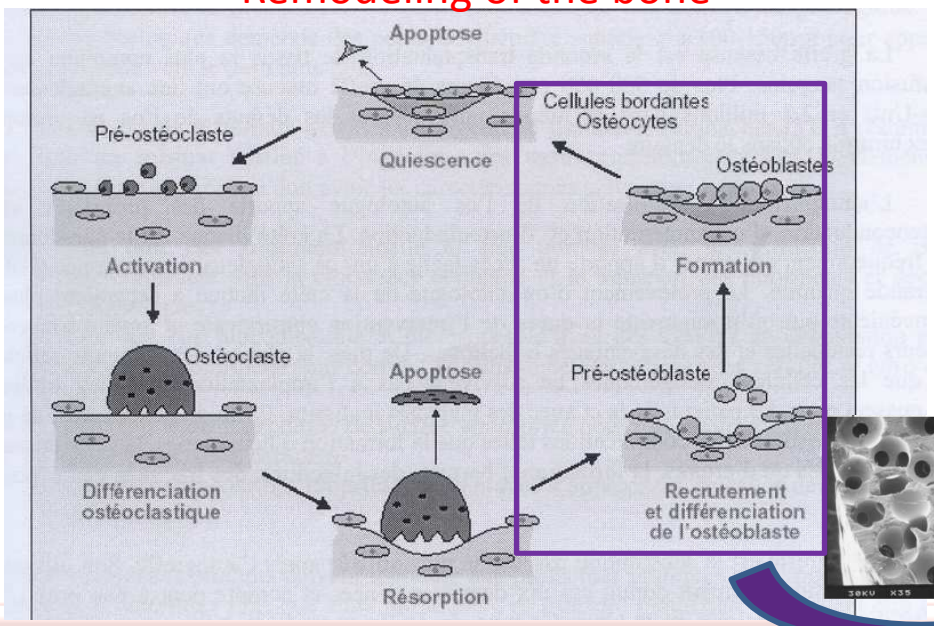
How to mimic natural bone structure?

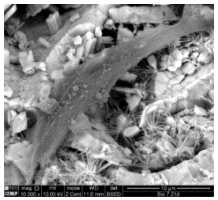


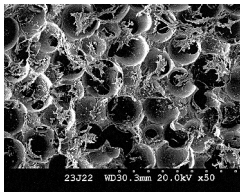
Bone scaffolds are porous frameworks with tailored chemical composition and porous architecture intended for inducing cartilage (hydrated proteoglycan hydrogel embedded into a type II collagen network) and bone tissue (type I collagen and hydroxyapatite) regeneration.

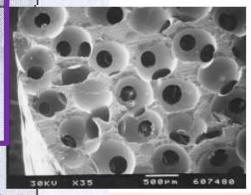
They may be referred to as 3D matrices that mimic the natural cellular environment for cells to grow, differentiate, and deposit biomimetic HA.

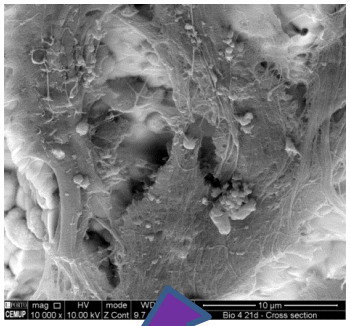
Remodeling of the bone



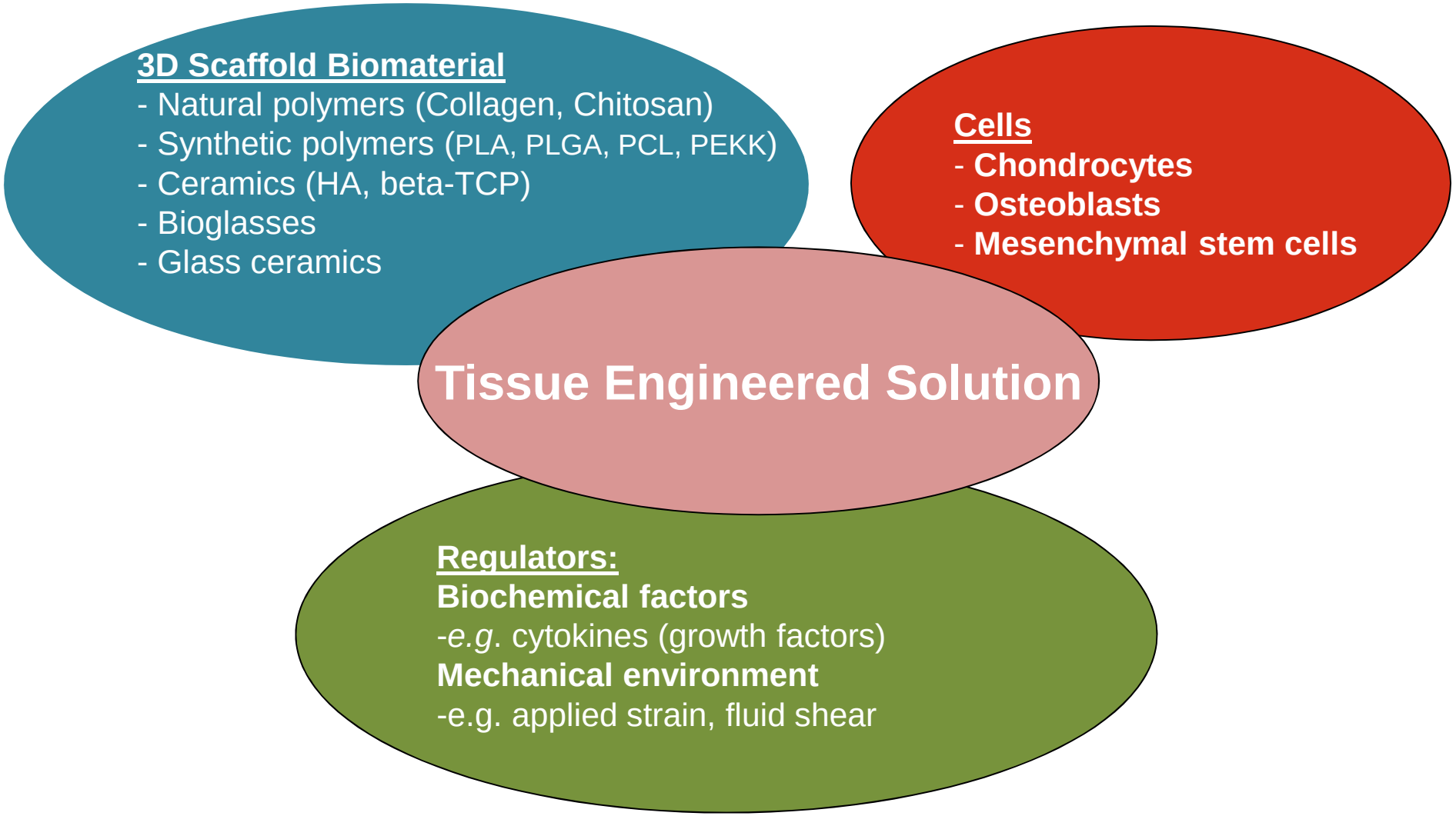




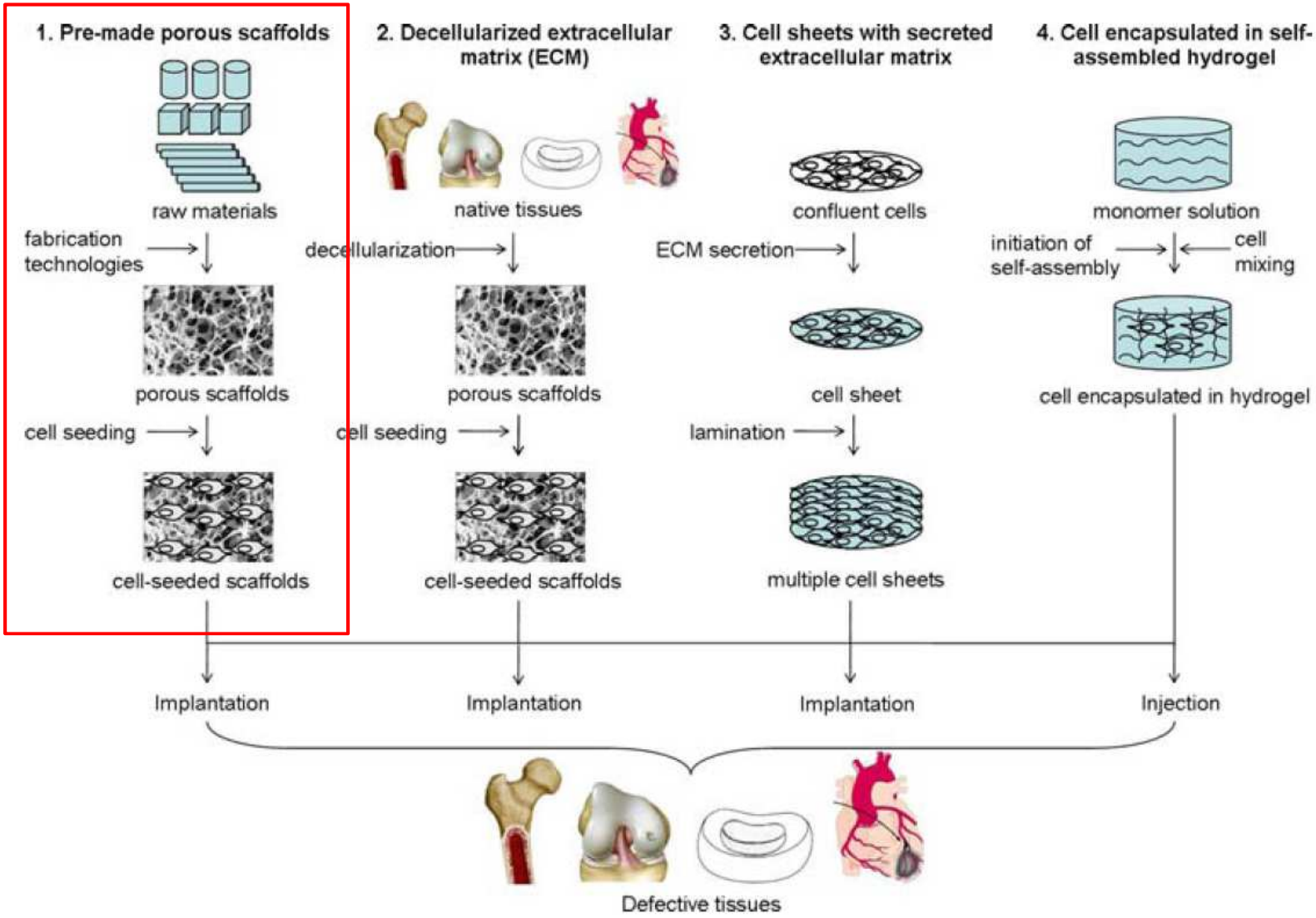




Therefore, bone tissue engineering is a complex and dynamic process that initiates with migration and recruitment of osteoprogenitor cells. This is followed by their proliferation, differentiation, and bone matrix formation.



Scaffold Approaches in Tissue Engineering



How to mimic natural bone?

Criteria for an ideal scaffold for bone regeneration:

[1] - Is made from a biocompatible material,

[2]- acts as template for tissue growth in 3 dimensions,

[3]- has an interconnected macro-porous network with **diameters > 100µm** to allow an ingrowth of cells and the vascularization and an efficient transport of morphogens, cytokines, growth factors, nutrients, oxygen, and waste products,

[4]- bonds to the host tissue

[5]- exhibits a surface texture favorable to cell adhesion

[6]- resorbs at the same rate as the tissue is repaired

[7]- is made from processing technique that can produce irregular shapes to match that of the defect in the bone of the patient,

[8]- exhibits mechanical properties sufficient to be able to regenerate tissue in bone in load bearing sites,

[9]- has the potential to be commercially producible to the required ISO or FDA standards.

J.R.Jones, L.L.Hench Current opinion in solid state and materials science 7 (2003) 301-307

How to mimic natural bone for substitute processing?

Part I: Requirements for ideal scaffolds

Part II: Fabrication technologies

Part III: Example of comparative study on cell colonization inside ceramic scaffolds presenting different architectures

How to mimic natural bone for substitute processing? 1st Material choice

Biocompatible material :

- Natural polymers: collagen, chitosan …
- Synthetic polymers: poly lactic acid (PLA), poly lactic-*co*-glycolic acid (PLGA), polycaprolactone (PCL), poly-ether-ketone-ketone (PEKK) …

Bioactive calcium phosphate ceramic scaffolds : HA, beta-TCP

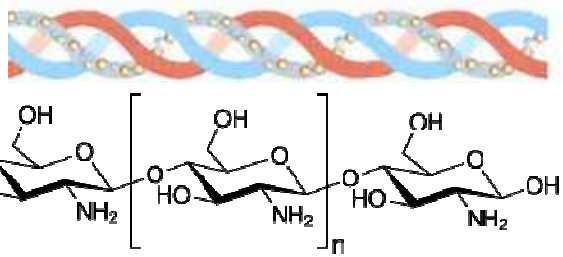
Bioglasses and Glass ceramics

Organic/inorganic composites

Biopolymers

Natural: alginates, collagen, chitosan, glycosaminoglycans (GAGs) and elastin, gelatin and fibrin

Collagen : protein constituted by polypeptide chains

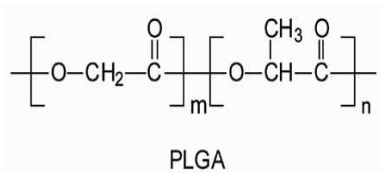


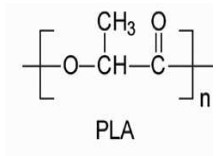
Chitosan: D-glucosamine extracted from shellfish skeleton

Synthetic:

PLA/PGA/copolymers PLGA, PLLA: Biodegradable : Weeks to Months

Main structure :





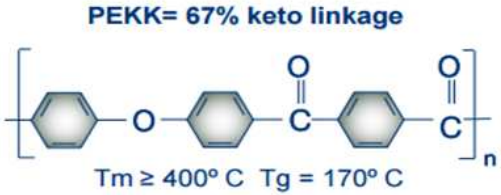
PCL: poly(caprolactone: Polyester with slower biodegradation than that of PLA.

Tm= 60°C Tg= -60°C

Biocompatible BUT no natural sites for cell adhesion and the in vivo degradation induces local reduction in pH with possible inflammation response.

PEKK (Poly-ether-ketone-ketone): a thermoplastic polymer

Use for long-term implantable human devices: unlimited (years)



Orthopedics, neurological, dental, spinal and cardiovascular implants; PEKK-based 3D-printed bones

How to mimic natural bone for substitute processing? 1st Material choice

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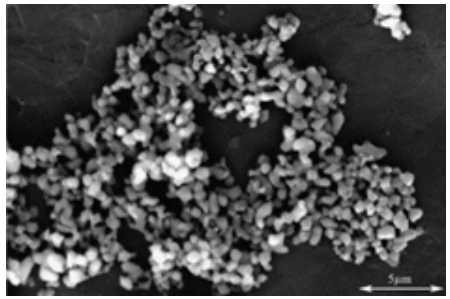
CaP-based scaffolds allow new bone formation and biomineralization by surface-induced CaP crystallization and contact osteogenesis.

Organic/inorganic composites

Bioactive calcium phosphate ceramics: CaP



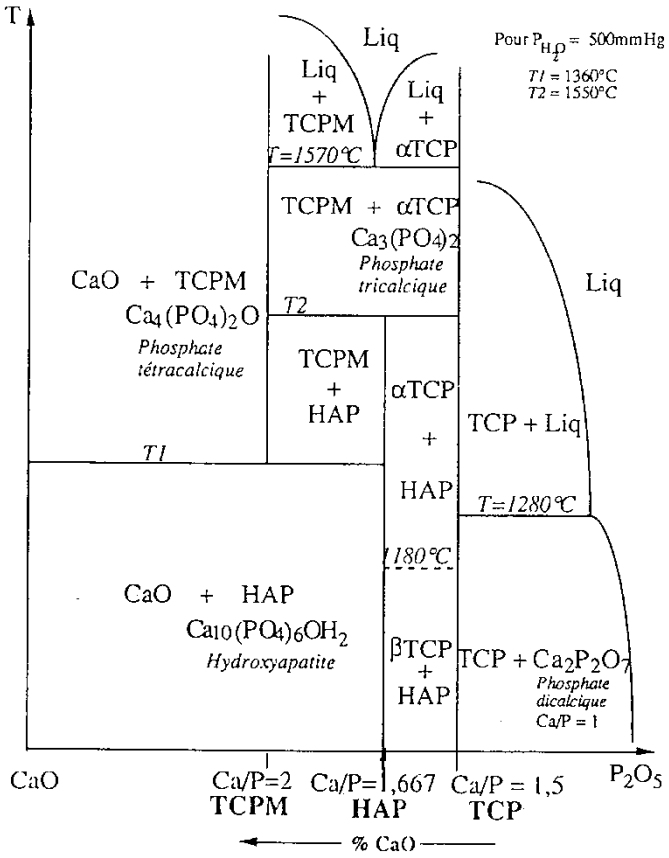


Fig. 4 Diagramme de phases du système CaO-P_2O_5 phases cristallines des différents phosphates calciques présents en fonction de la composition et de la température.

Composition of biological apatite (wt%) in adult human

$Ca_3(PO_4)_2$ $Ca_{10}(PO_4)_6OH_2$

	Enamel	Dentine	compact bone	TCP	HAP
Ca	36,1	35	35,5	38,8	45,2
P	17,3	17,1	17,1	20,0	21,0
CO_2	3	4	4,4	-	-
Ca/P (molar)	1,61	1,58	1,60	1,50	1,667

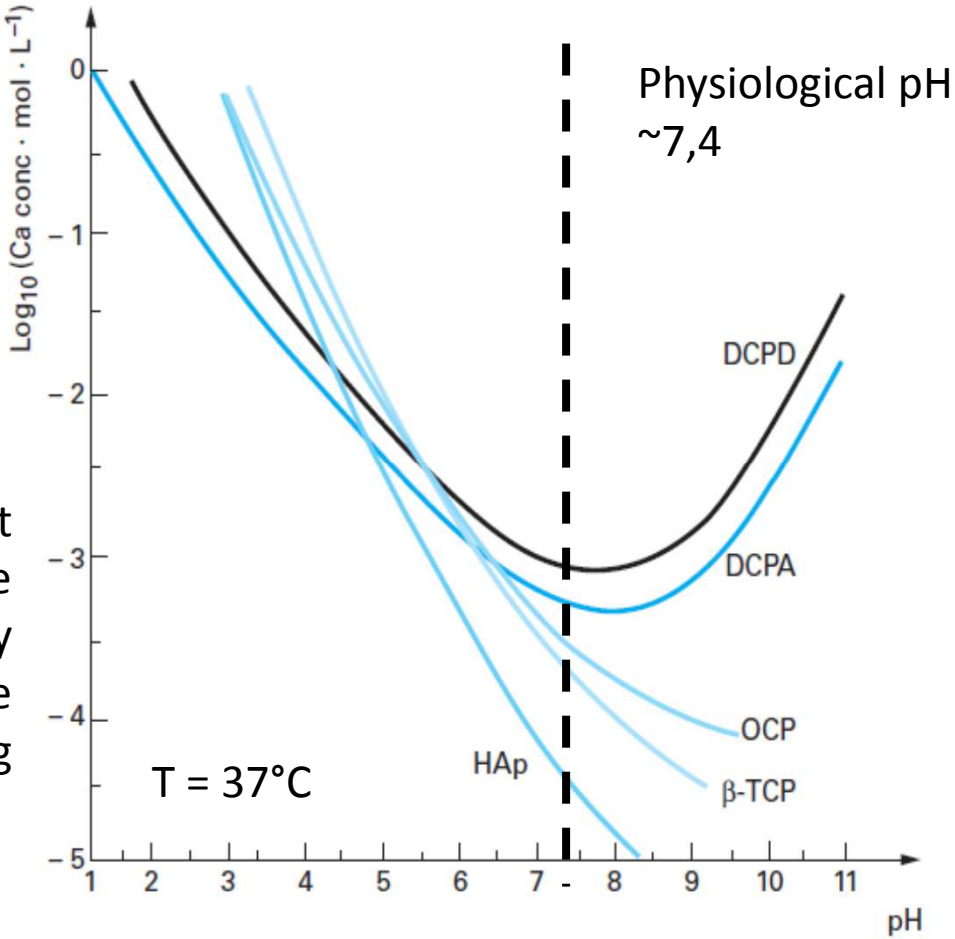
2 phases: α and β

Resorption rate: HAP < β-TCP < α-TCP

➢ Bio-degradability is depending on the solubility of CaP (+ enzymatic activity)

Materials	Log K_{ps} at 25°C
HA	116,8
α-TCP	25,5
β-TCP	28,9

- **HA** : no biodegradability
- **α-TCP** : too large solubility!
- **β-TCP** and **HA** won't dissolve at physiological pH, but acid pH are reached during inflammatory reactions or during bone regeneration, then involving dissolution of β-TCP.

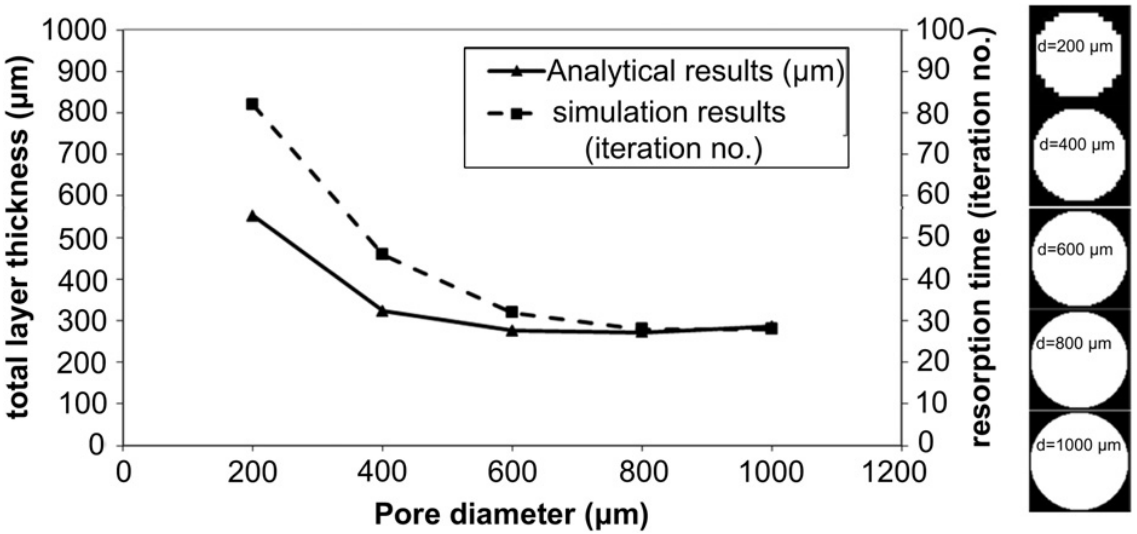


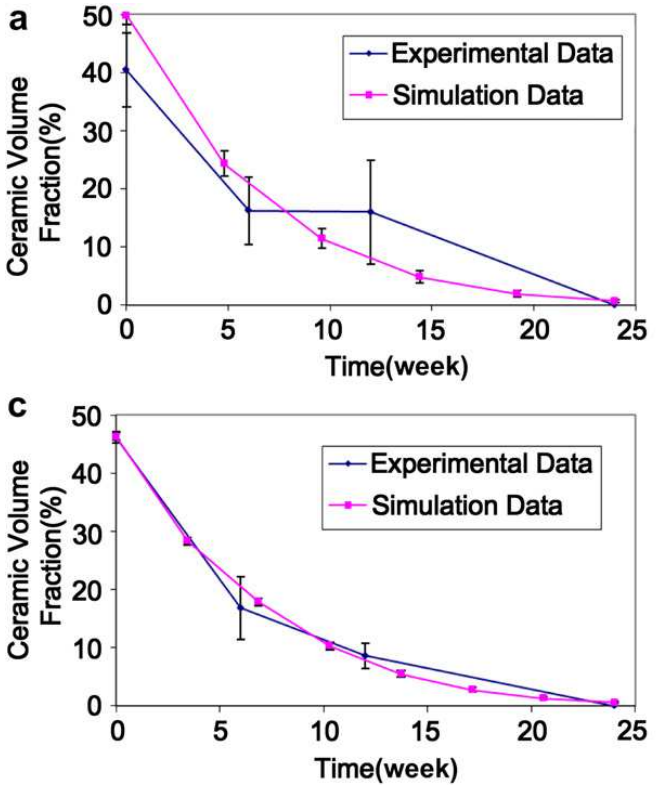
C.Combes and C.Rey *Biomatériaux à base de phosphates de calcium*, Techniques de l'ingénieur 2013
J.C.Elliot. – *Structure and Chemistry of the Apatites and Other Calcium Orthophosphates,* Elsevier (1994).

By adjusting the content of β-tricalcium phosphate (β-TCP), CaP-based scaffolds can be made partially degradable.

Bioactive calcium phosphate ceramics: CaP

The resorption rate of the scaffolds depends also on the pore size





Increase with an increase of pore size (or a decrease of surface area)
with as consequence a decrease of mechanical properties.

Mahdieh Bashoor-Zadeh, Biomaterials 32 (2011) 6362-6373

Experimental data (mCT analysis) and simulations of samples implanted in bone defects of sheep for 6, 12 and 24 weeks. (a) 128 µm, (c) 365 µm

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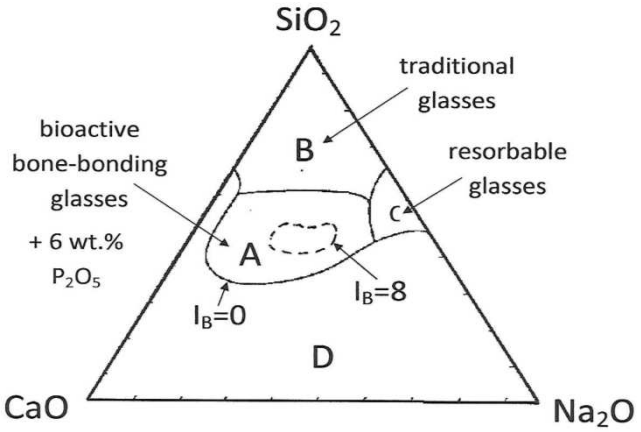
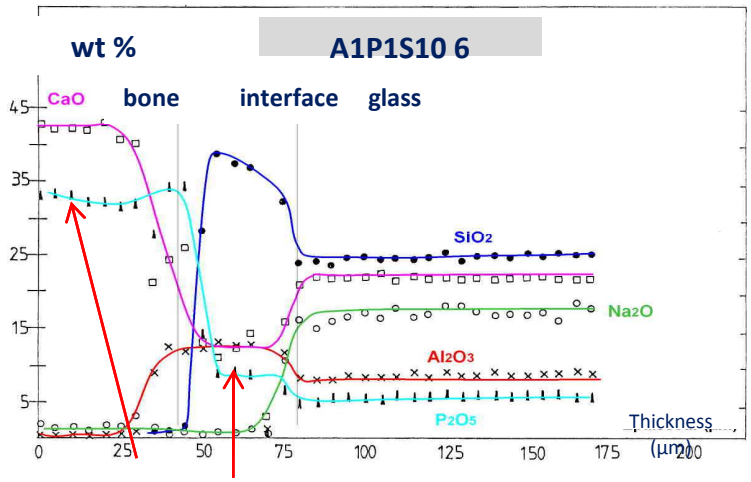
- Bioglasses and Glass ceramics:

Organic/inorganic composites

Bioglasses and glass ceramics





Ca/P ratio in the interface is identical to bone

Bioglass scaffolds release calcium and phosphate ions from their surface after implantation; this promotes formation of a hydroxycarbonate apatite (HCA) layer on them, most probably by a surface-induced CaP crystallization mechanism. This HCA layer can significantly enhance osteoblast activity, and also adsorb proteins and growth factors that facilitate new bone formation in vivo.

Bioglass® 45S5 of composition (in mol%): 46.1 % SiO_2, 24.4% Na_2O, 26.9% CaO and 2.6% P_2O_5

Low mechanical properties of bioglasses.

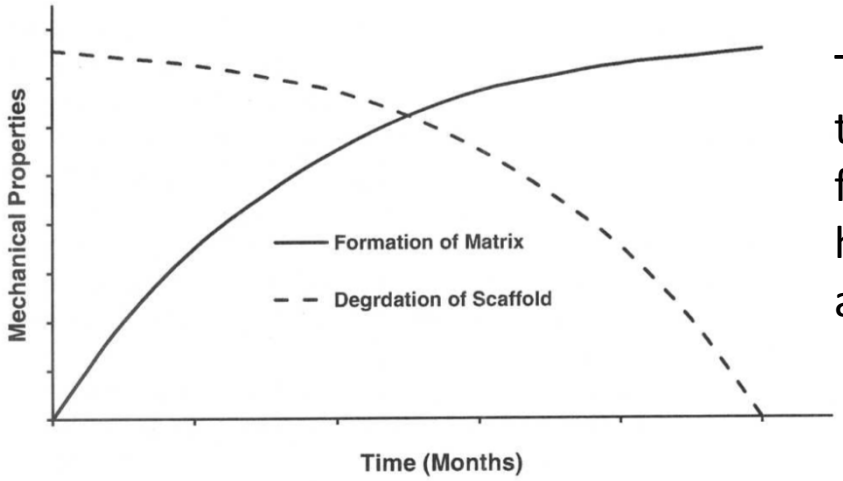
→ Glass ceramics

Property	Cerabone	Bioverit I	Highly bioactive glass-ceramic	Bioglass 45S5
Bioactivity class	B	B	A	A
Machinability	Low	Good	Fair	Low
Density (g/cm³)	3.1	2.8	2.6	2.66
Three-point flexural strength (MPa)	215	140-180	210	42
Young's modulus (GPa)	120	70-90	70	35
Vickers hardness (HA)	680	500	600	460
Fracture toughness (MPa√m)	2.0	1.2–2.1	0.95	-

S.Hampshire in Advances ceramis biomaterials : materials, devices and challenges Elsevier 2017

Bioactive calcium phosphate ceramics: CaP

The low mechanical properties of calcium phosphate ceramics have to be overcome

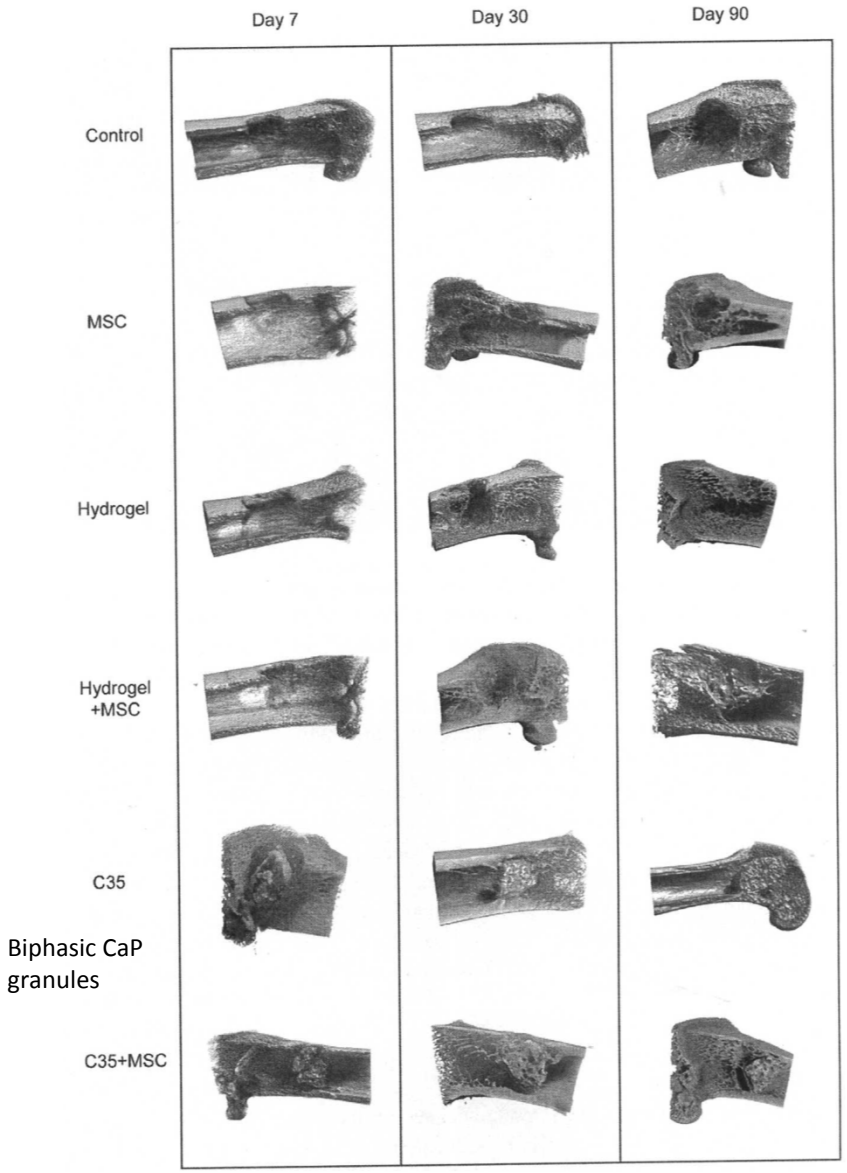


The mechanical properties decrease has to be balanced by the new bone formation. To date, no suitable solutions have been found for regenerating long and load-bearing bone segments.

Raghunath et al. (2007).

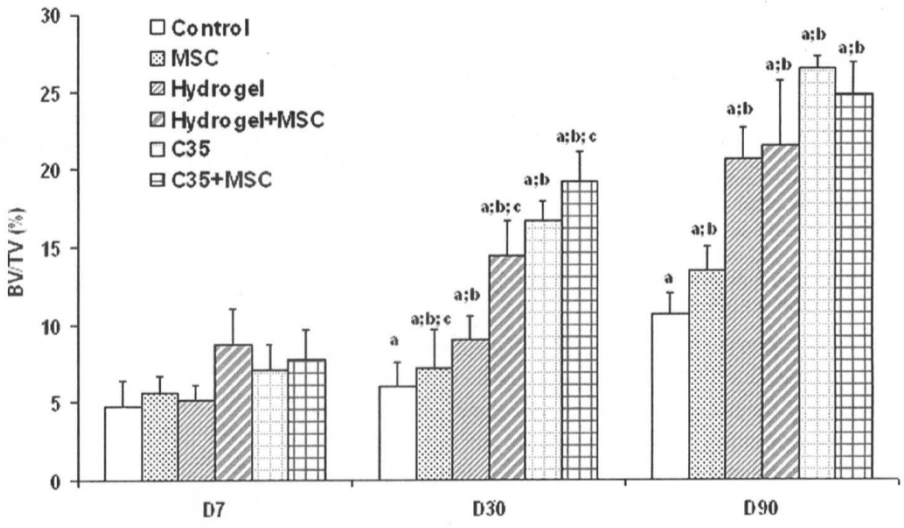
→ limited improvement of mechanical properties can be achieved by optimizing their pore architecture.

→ seeding the scaffolds with bone marrow stem cells (BMCs) and/or introducing and harboring drugs, genes, and different growth factors into the scaffolds.
Quarto R et al. 2001 N.Engl.J.Med. 344 (5) pp 385-386



Biphasic CaP granules

Combination of scaffolds with MSC osteoprogenitor cells



Bone volume fraction at 7, 30 and 90 days after surgery in the defect area.

S.Frasca et al J.Mater.Med. (2017) 28:35

Bioactive calcium phosphate ceramics: CaP

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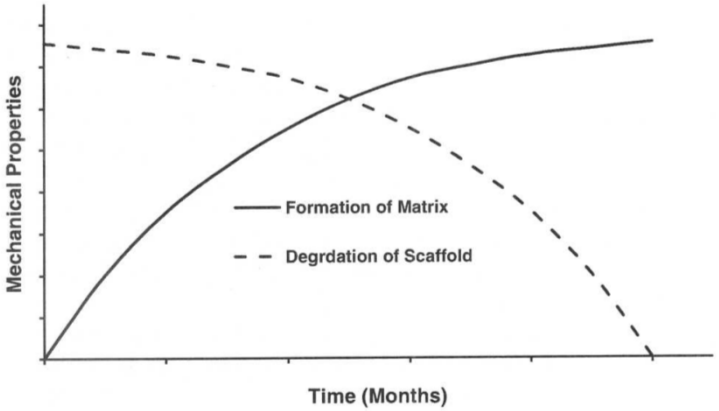

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→ employing a composite strategy:

* Sealing surface defects with a biodegradable polymer coating,
* or developing an interconnected CaP-polymer scaffold, to take advantage of both CaPs and polymers to meet the mechanical and physiological requirements of the host tissue.

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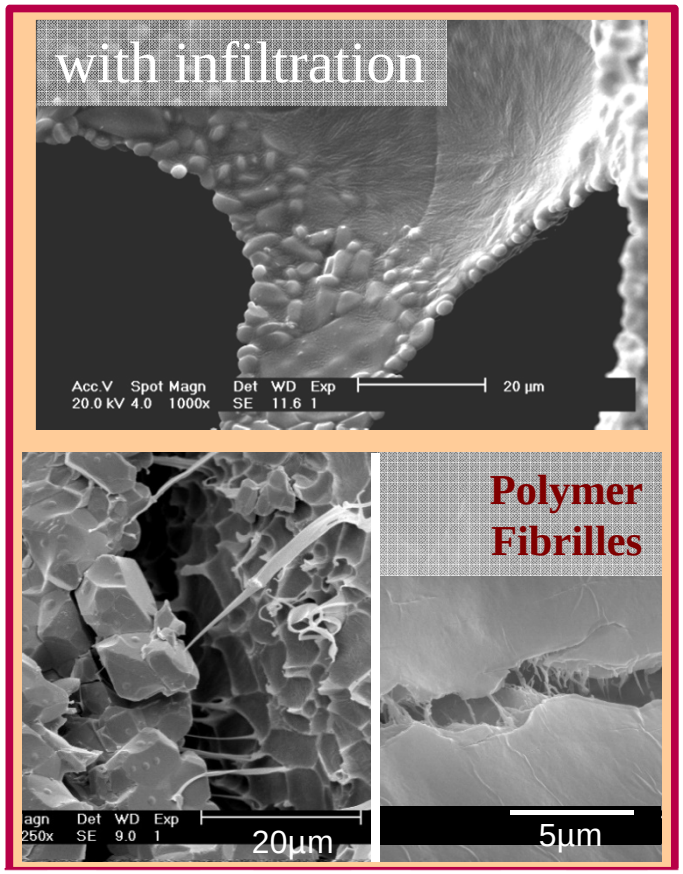
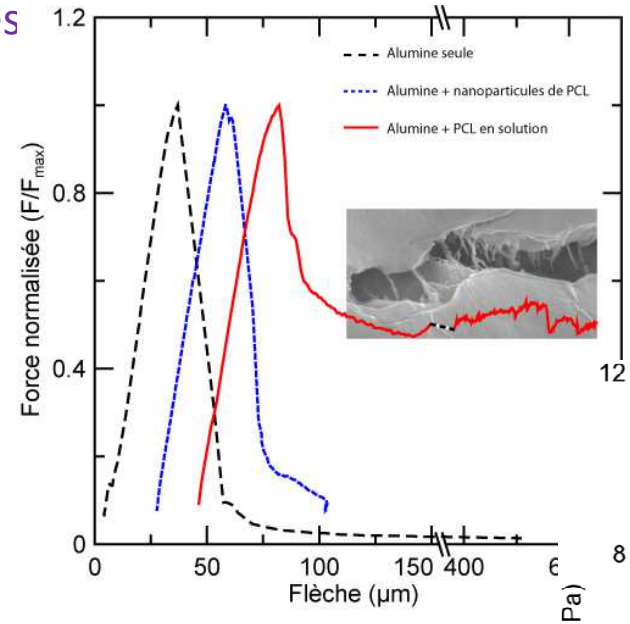
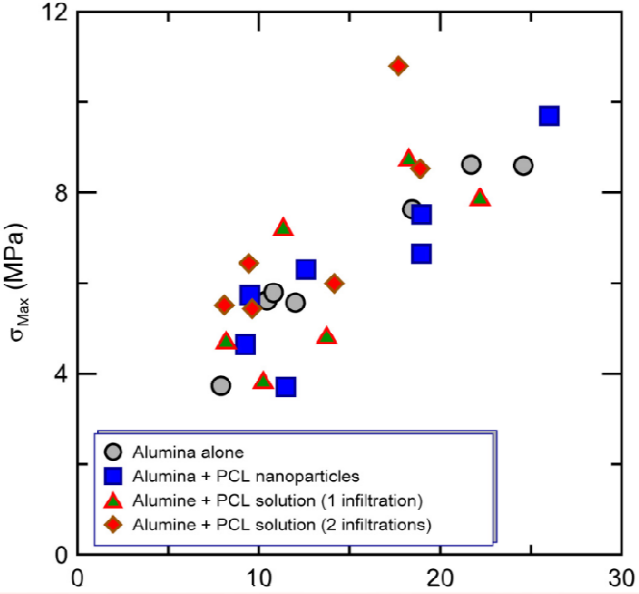




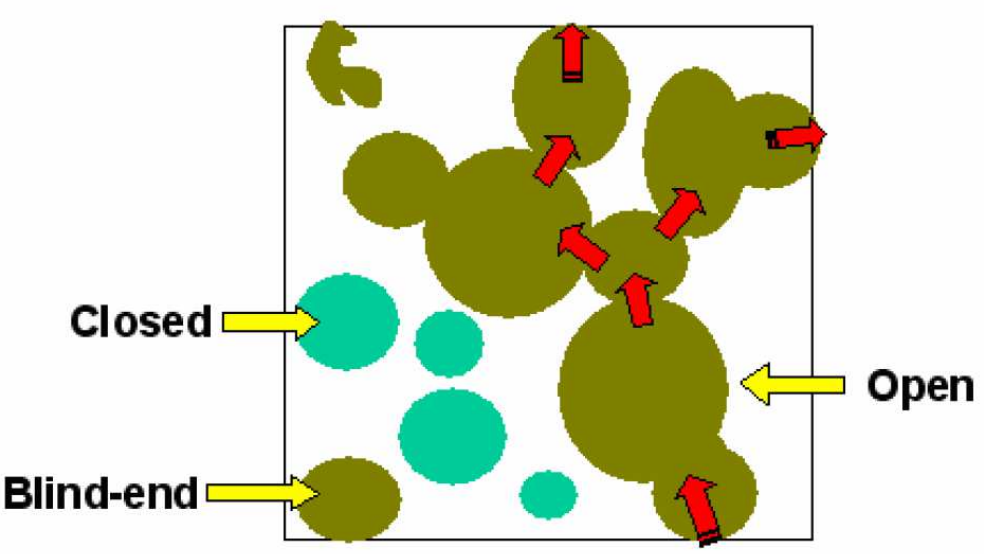


Nalla et al.Nature Materials, vol 2, March 2003

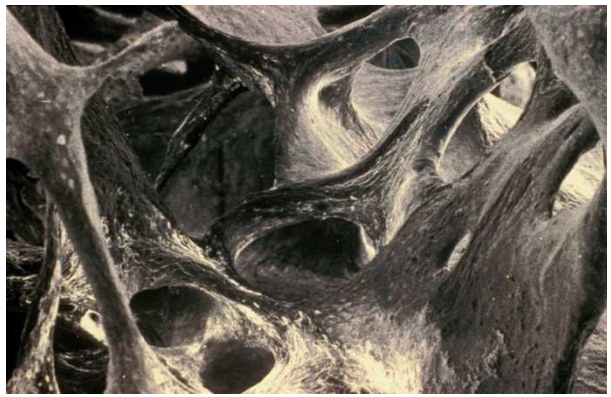
How to mimic natural bone for substitute processing? 2nd Porosity architecture

Porous scaffolds for bone regeneration require well-defined pore architectures to facilitate tissue in-growth:

✓ Interconnecting and tortuosity porosity



Schematic of the different pore types found in tissue engineering scaffolds

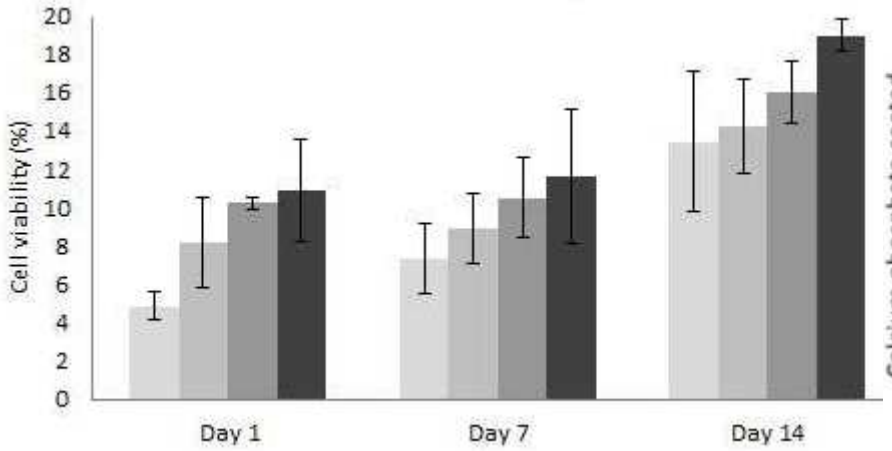


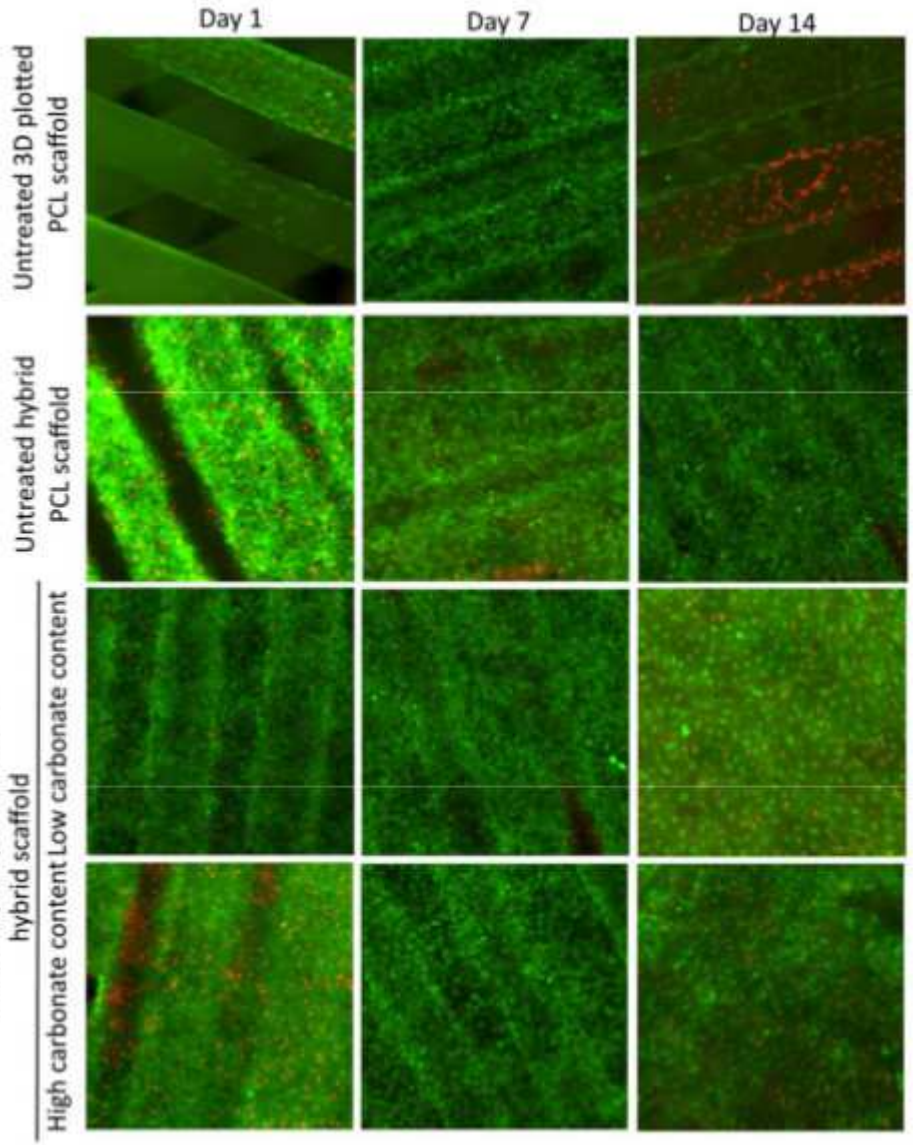
Porous scaffolds for bone regeneration require well-defined pore architectures to facilitate tissue in-growth:

✓ Communication with neighboring cells

In this context, fabrication of hybrid PCL scaffolds constituted by 3D plotted fibers (Ø192µm) with nanofibers (Ø331nm) deposited onto the fibers.

MC3T3-E1 Cell viability on untreated 3D plotted PCL scaffolds, untreated hybrid PCL scaffolds and hybrid scaffolds coated with HA with low and high carbonate content.





Nathalie Luickx, PhD thesis 2016, Gent University (B)

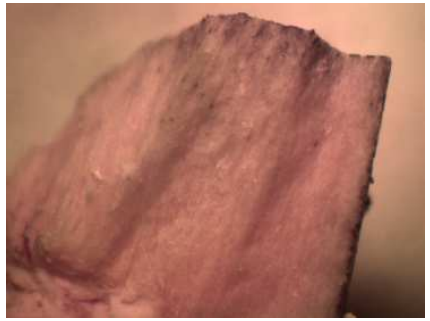
How to mimic natural bone for substitute processing? 2nd Porosity architecture

Porous scaffolds for bone regeneration require well-defined pore architectures to facilitate tissue in-growth:

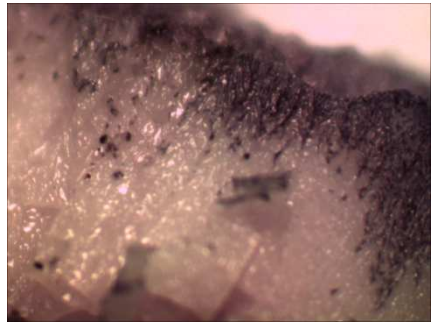
- ✓ Optimal pore size depending on cell type:
  - 5 μm for neovascularization
  - 5-15 μm for fibroblast ingrowth
  - 20 μm for hepatocytes
  - 200-350 μm for osteoconduction
  - 20-125μm for adult mammalian skin

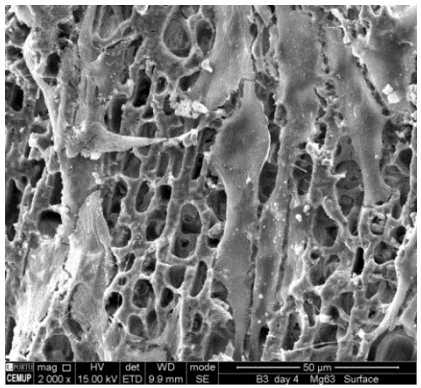
	Pore size (µm)		Lamellar thickness (µm)	Porosity (%)
	a	b		
IT 3	6 ± 2	13 ± 5	7 ± 3	53 ± 1
IT 7	44 ± 11	149 ± 60	43 ± 29	36 ± 1

6 -13 µm



44 -149 µm

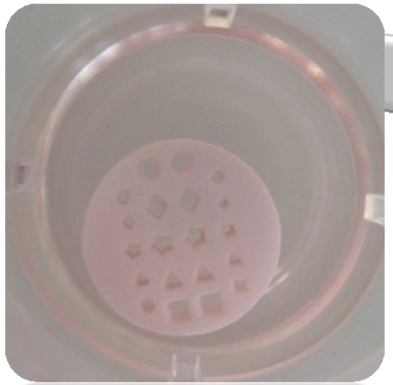




MG63 size: 10 µm width, 50 µm length

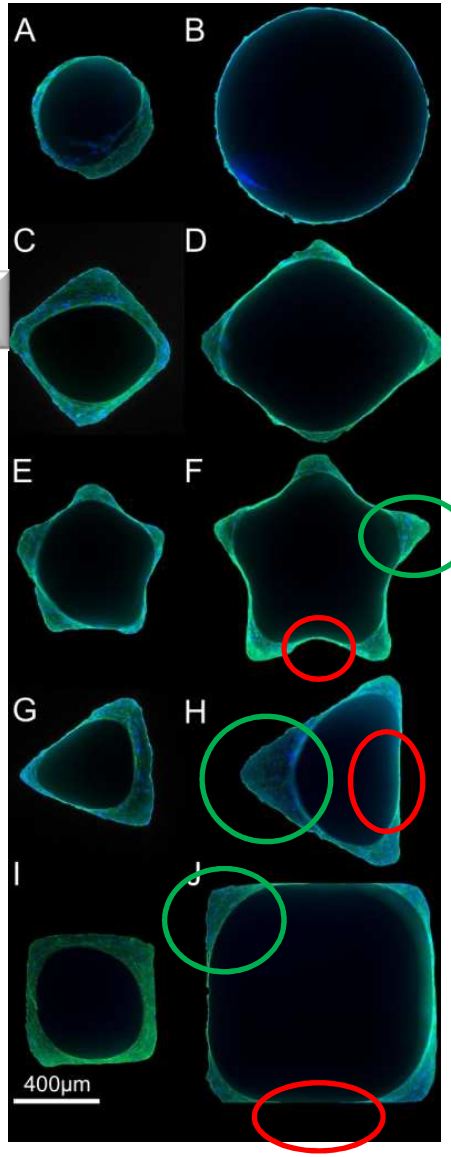
Porous scaffolds for bone regeneration require well-defined pore architectures to facilitate tissue in-growth:

✓ Shape porosity



In vitro cell colonisation

7 days



Favoured colonization in salient angles and concave areas

Flat or convexes areas → migration decrease

* collaboration with **Dr Urda Rüdrich**

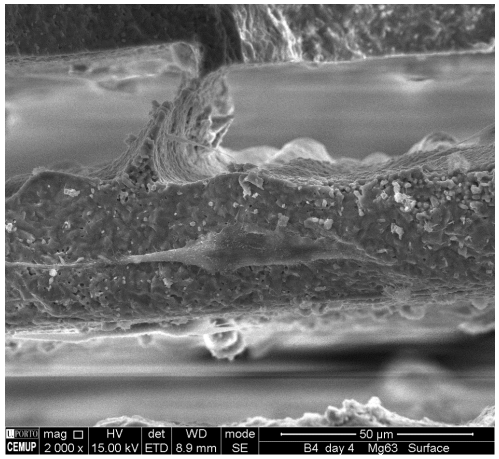
M.Lasgorceix, Ph.D. SPCTS Limoges 2014

How to mimic natural bone for substitute processing? 2nd Porosity architecture

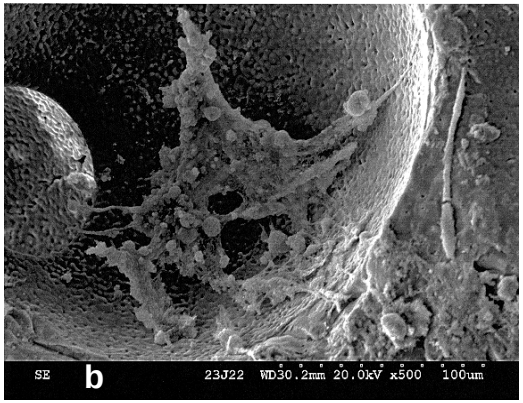
✓ Adequate surface properties:

Surface roughness modulates the biological response of tissues in contact with the implant. Direct influence in vitro as well as in vivo on:

- **cellular morphology,** - **proliferation,** - **phenotype expression**



Moving osteoblast morphology

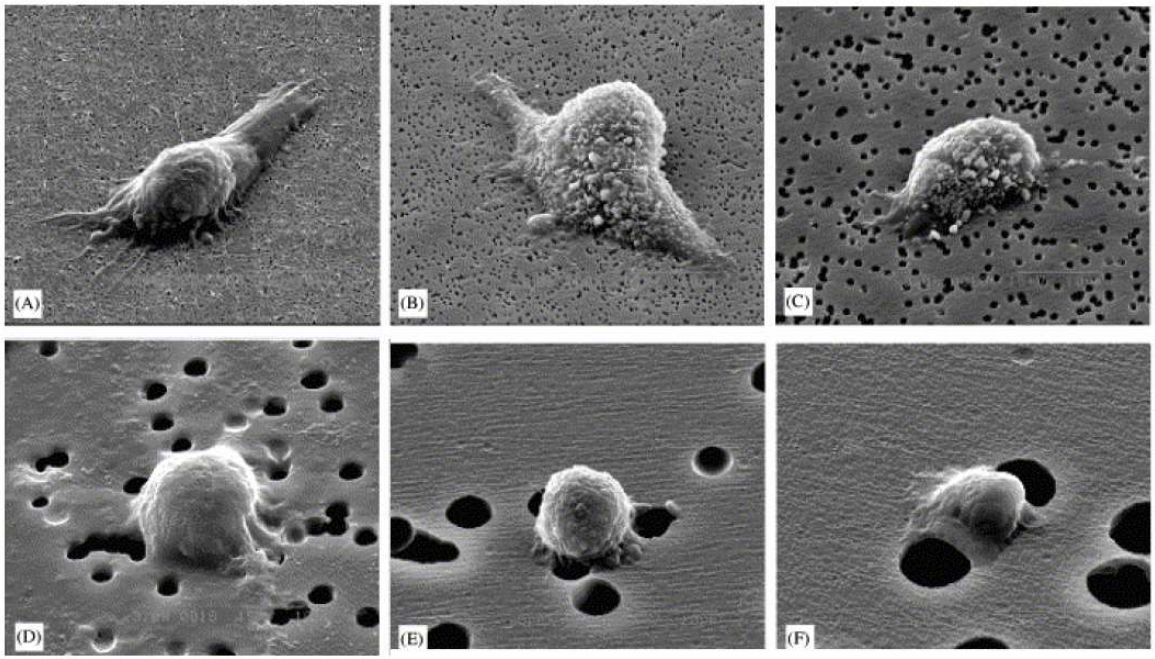


Static osteoblast morphology

How to mimic natural bone for substitute processing? 2nd Porosity architecture

Porous scaffolds for bone regeneration require well-defined pore architectures to facilitate tissue in-growth:

✓ *Adequate surface properties:*

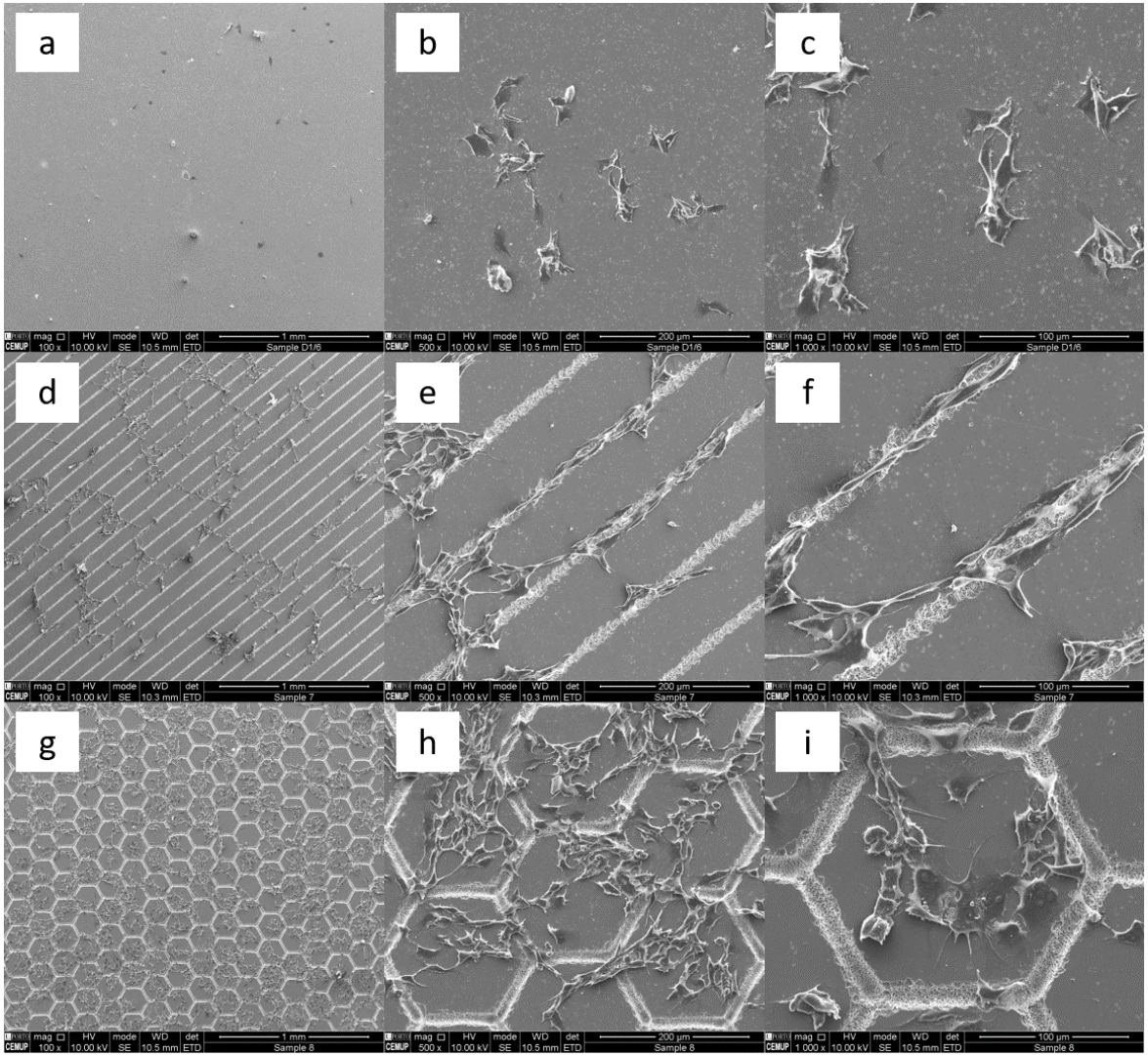


SEM pictures of MG63 cells attached to PC membrane surfaces with different micropore sizes: (A) 0.2, (B) 0.4, (C) 1.0, (D) 3.0, (E) 5.0, and (F) 8.0 µm. *S. J. Lee et al., 2004*

Cell adhesion and proliferation were progressively inhibited as the PC membranes had micropores with increasing size, probably due to surface discontinuities

✓ Adequate surface properties: SEM pictures of MG63 cells attached to HA surfaces treated by laser

D1



Surface: **polished**
Cell density: low
Morphology: random

Surface: **linear patterned**
Cell density: medium
Morphology: elongated, tendency of elongation along the grooves

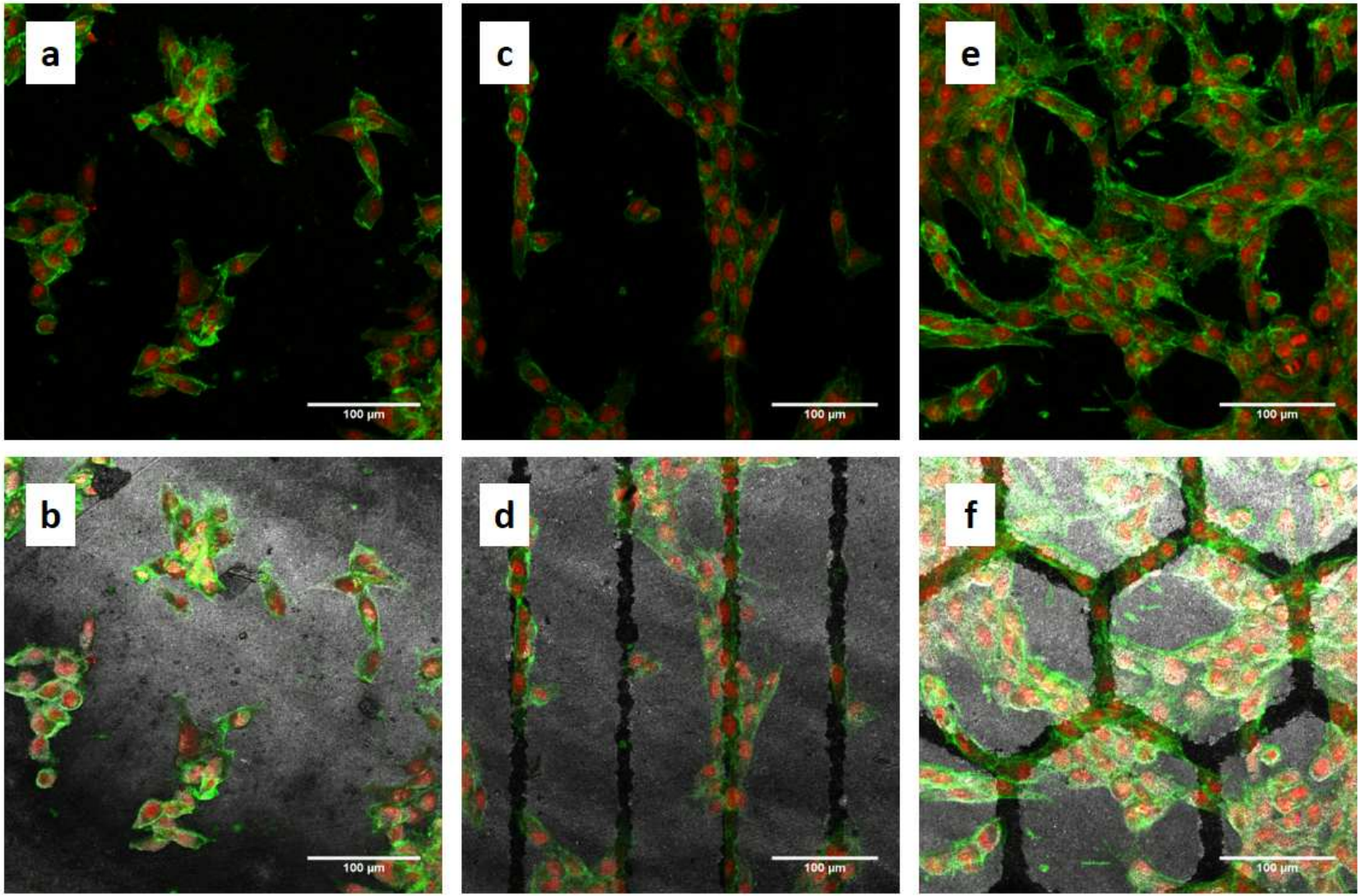
Surface: **honey patterned**
Cell density: high
Morphology: adjustable

M. Lasgorceix et al ,«Micropatterning of beta tricalcium phosphate bioceramic surfaces, by femtosecond laser, for bone marrow stem cells behavior assessment", sent to be published in JECS.

✓ Adequate surface properties:

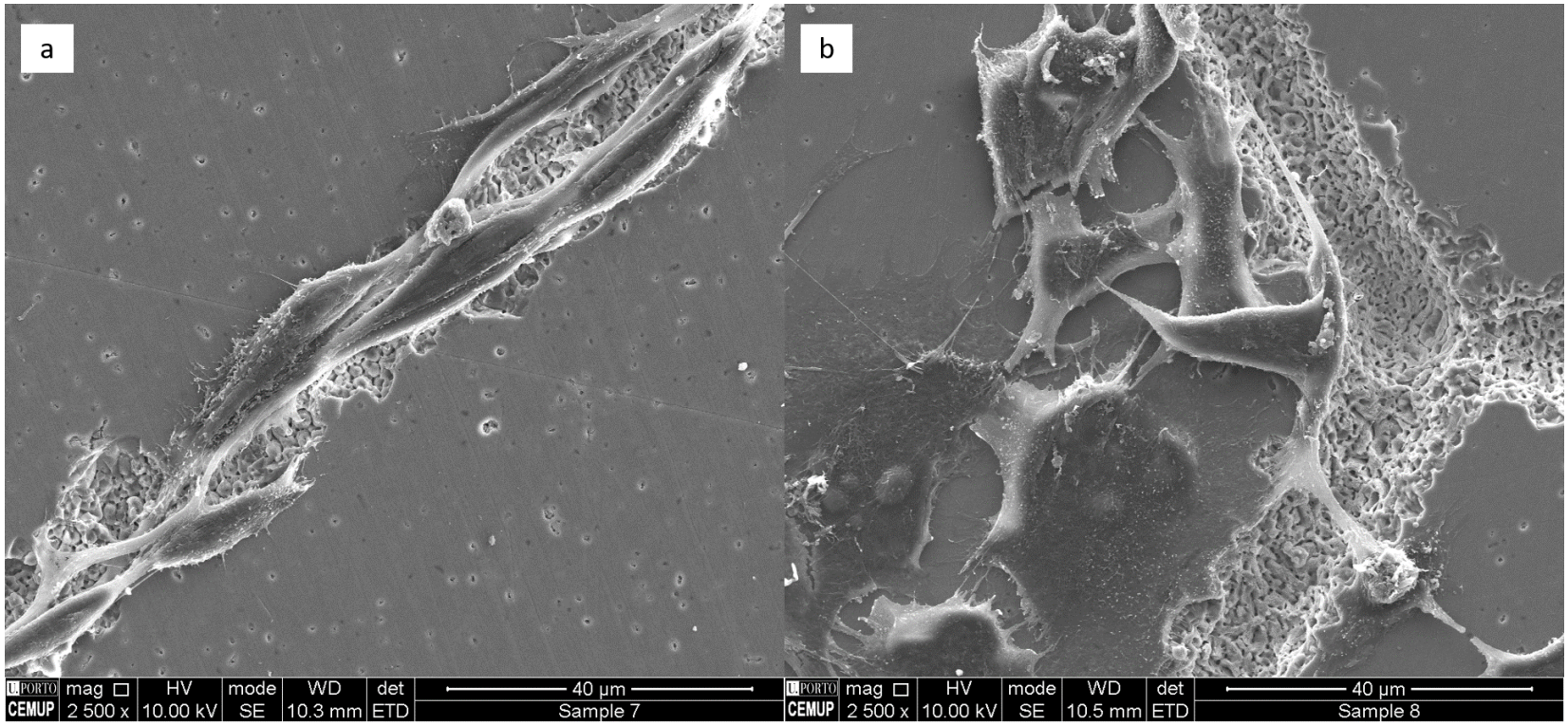
smooth linear honey

Day1



✓ Adequate surface properties:

	linear	honey
D1	Elongation along the grooves	Ability to adapt morphology to follow the design



Cell density remains sufficiently low to allow cell population growth after day 1

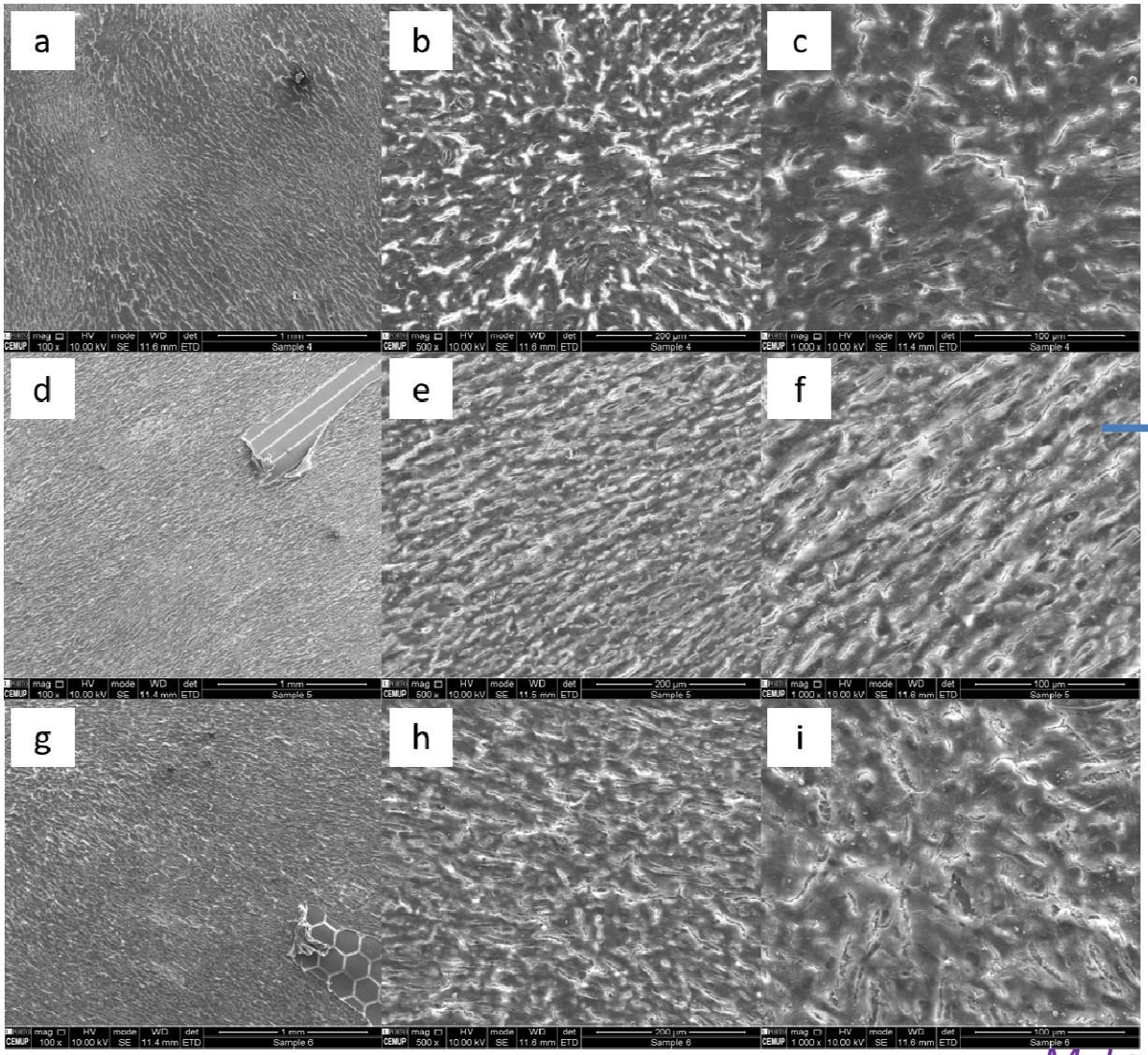
MG63 cell behaviour

D7

smooth

linear

honey



presence of several cell layers on all surfaces

orientation of the cells still clearly identified, even on the top cellular layer

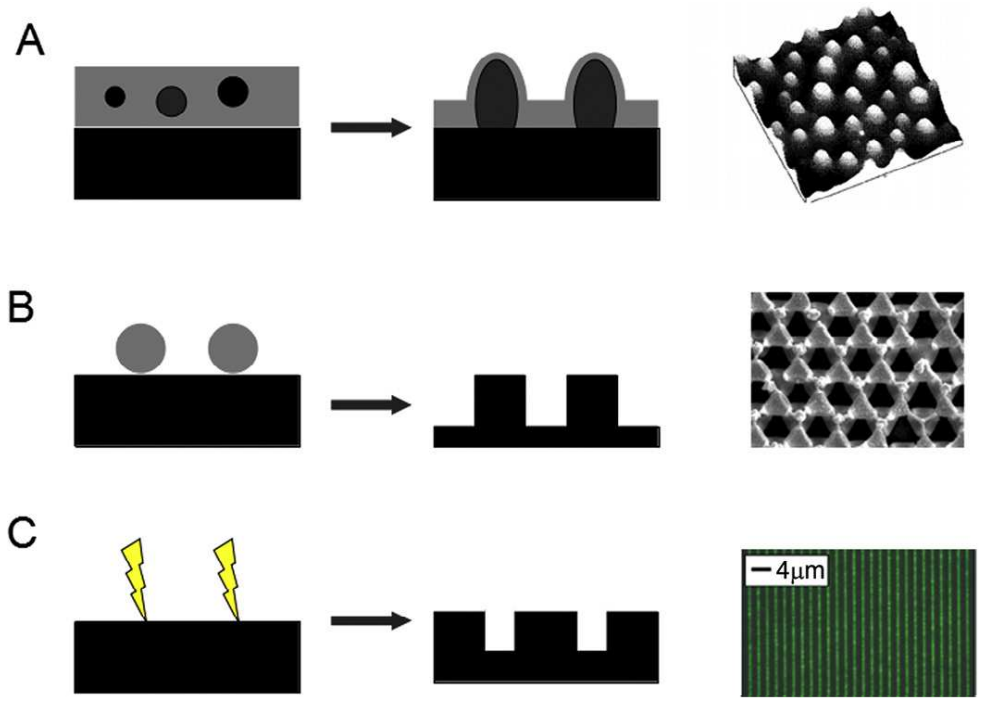
effect of the surface pattern is not limited at the first cell layer covering the surface

M. Lasgorceix, BCRC, 2017

How to mimic natural bone for substitute processing? 2nd Porosity architecture

Porous scaffolds for bone regeneration require well-defined pore architectures to facilitate tissue in-growth:

✓ Adequate surface properties:



Various techniques to prepare polymeric micro- or nano-patterned surfaces for cell engineering purposes:

(A) nanotopography formed via polymers spontaneously phase separate;

(B) colloids act as etch masks for topography formation;

C) Application of electron beam lithography on surface bio-patterning

How to mimic natural bone for substitute processing?

Part I: Requirements for ideal scaffolds

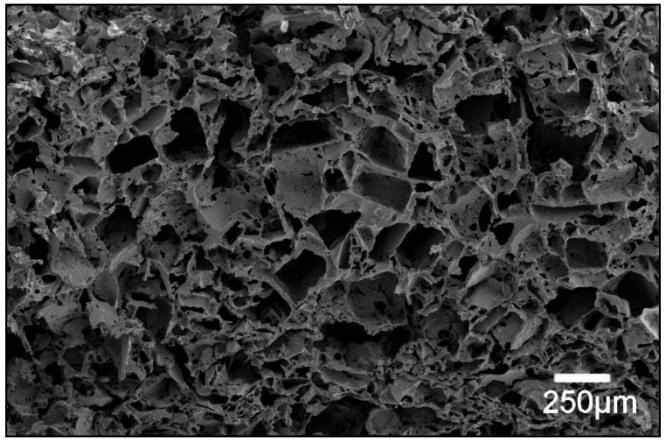
Porous scaffolds for bone regeneration require well-defined pore architectures (pore size , shape, adequate surface properties ...).
→ New processing concepts of free-form fabrication have been developed to prepare scaffolds with tailor-made interconnected macroporosity.
→ Scaffolds with customized external geometry may also be prepared on an industrial scale.

Part II: Fabrication technologies

Part III: Example of comparative study on cell colonization inside ceramic scaffolds presenting different architectures

1. Solvent casting & particulate leaching

- Simple and most commonly used method for fabricating polymeric scaffolds .
- Method involves mixing water soluble salt (e.g., NaCl) particles into a biodegradable polymer solution
  - Mixture cast into the mold of the desired shape.
  - Solvent removed by evaporation, vacuum or lyophilization
  - Salt particles are leached out by water to obtain a porous structure



Porous scaffold formed through particulate leaching technique

Advantages:

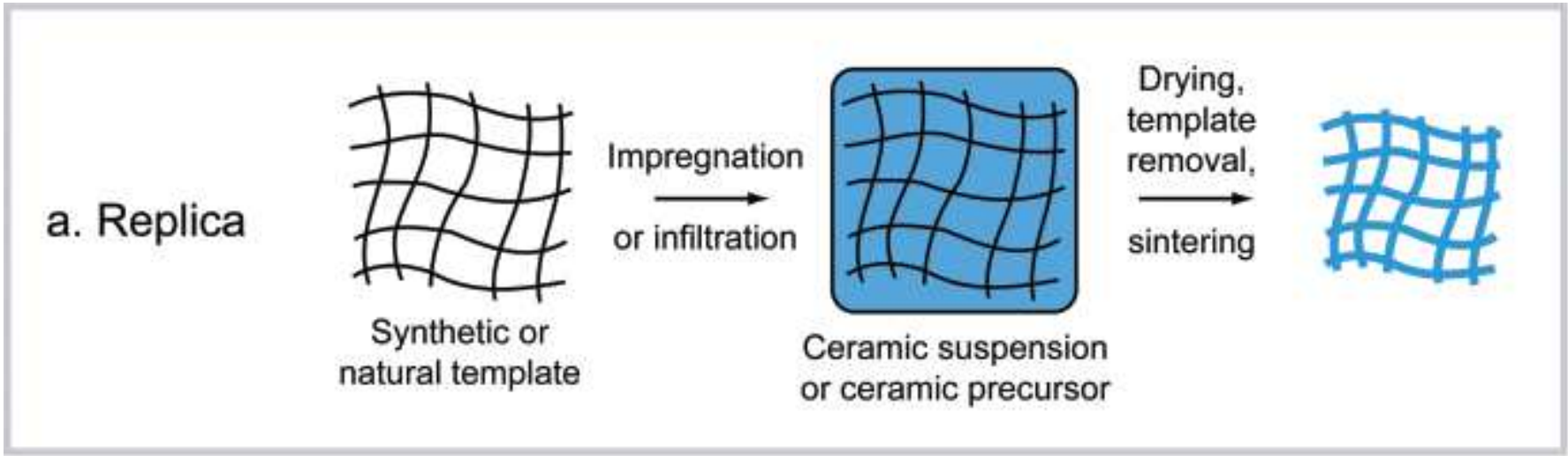
1. Simple operation
2. Adequate control of pore size and porosity by salt/polymer ratio

and particle size of the added salt, respectively

Limitations:

1. Cubic crystal shape of the salt
2. NaCl residues
3. Thickness: 0.5-2 mm
4. Issues with pore interconnectivity

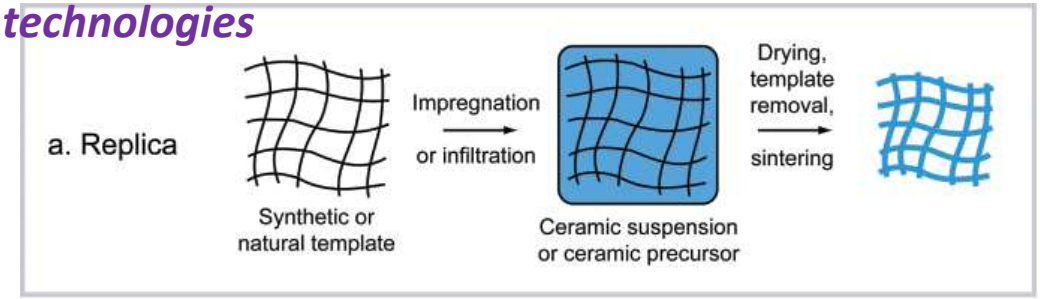
2.Replica techniques



Replica technique is based on the impregnation of a cellular structure with a ceramic slurry in order to produce a macroporous ceramic exhibiting the same morphology as the original porous material.

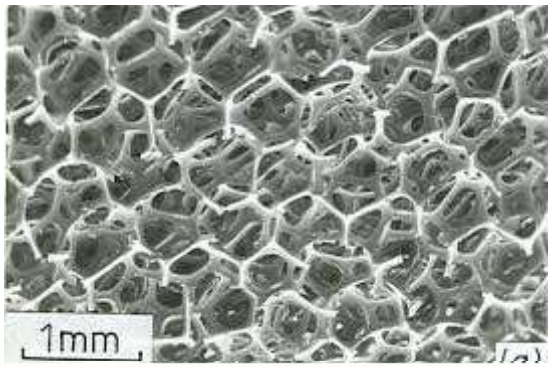
Many synthetic or natural cellular structures can be used as templates.

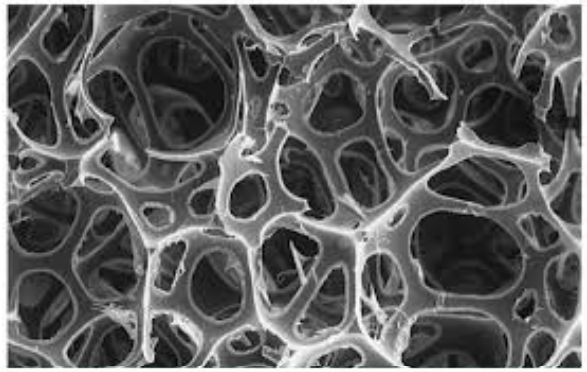
Scaffold Fabrication technologies



Synthetic templates:

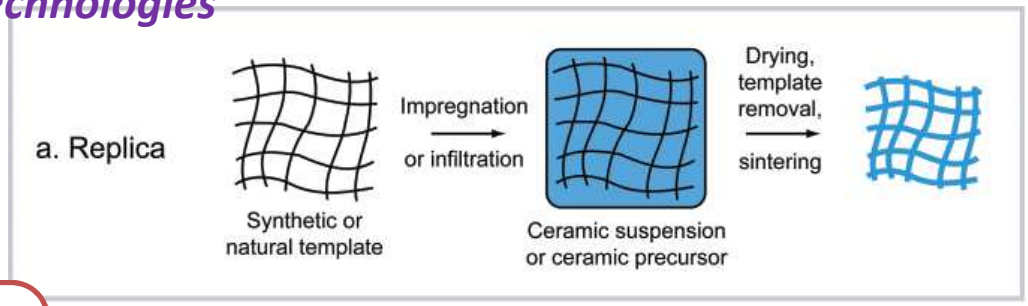
-Highly porous polymeric sponge (typically polyurethane) is initially soaked into a ceramic suspension until the internal pores are filled in with ceramic material.
-The impregnated sponge is then passed through rollers to remove the excess suspension and enable the formation of a thin ceramic coating over the struts of the original cellular structure.
-The ceramic coated polymeric template is dried and pyrolysed and finally the ceramic coating is sintered.





Scaffold Fabrication technologies

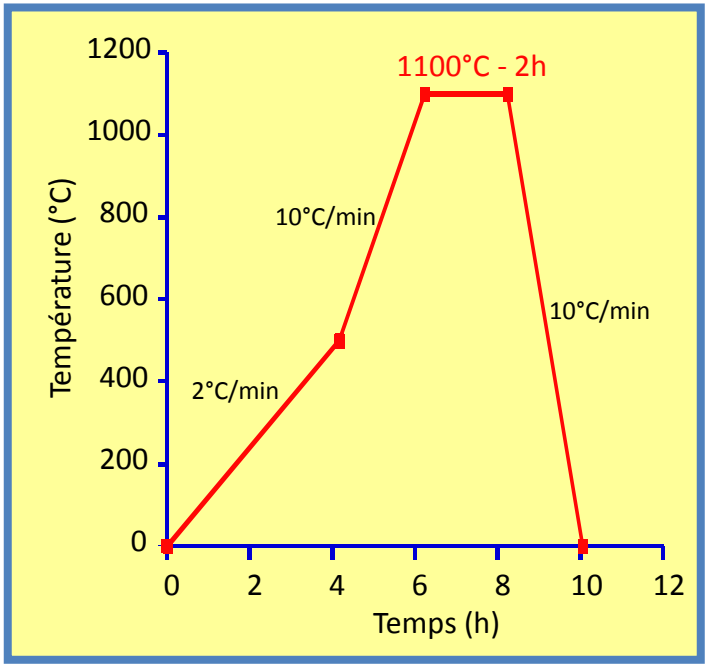
Replica from sponge



Impregnation of the foam by calcium phosphate slurry

Pyrolysis and sintering

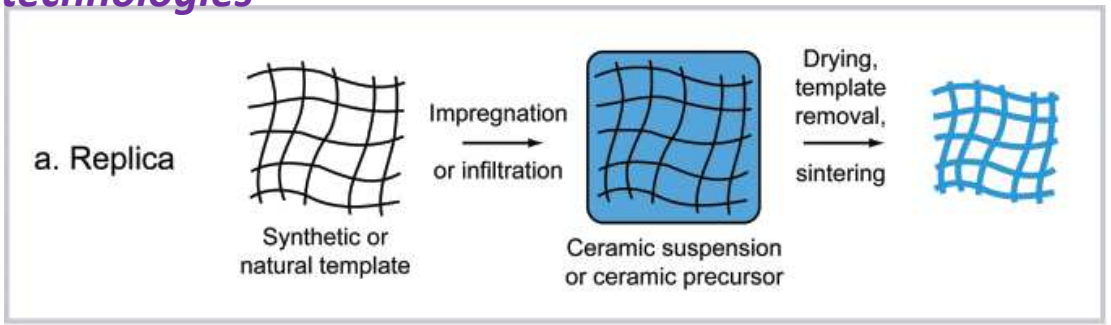
Sintered scaffold



►Macropore and interconnection sizes depend on foam characteristics and on CaP slurry properties

F. Lelièvre & A. Destainville , Thèses, Limoges, 1992 & 2005

Scaffold Fabrication technologies



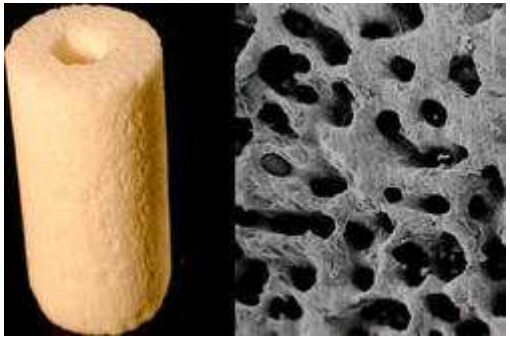
Natural templates: Coral $CaCO_3$

The coral exhibit the presence of both calcite and aragonite phases and presents a porous structure which depends on coral species:

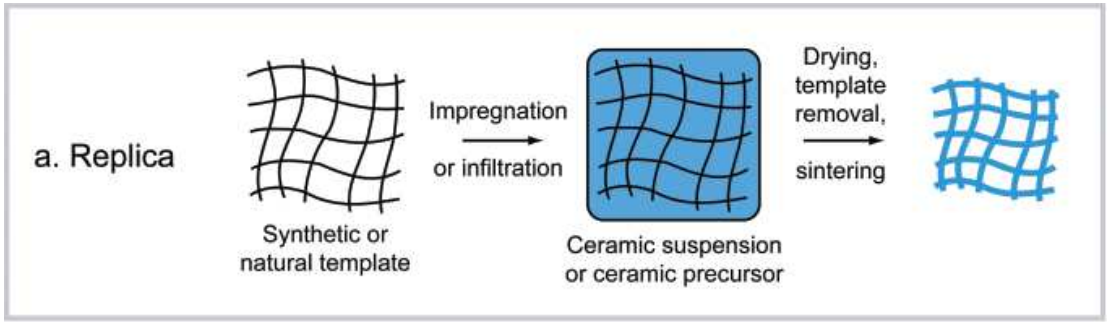
- Acropora (20% porosity) near to compact bone
- Porites (50% porosity) near to spongy bone

The pore size varies between 150 and 500 microns versus species.

→ coral can be directly used as bone substitute



Scaffold Fabrication technologies



Natural templates: Coral

First method: 1970 White et al. lost-wax method named "replamineform"

-The coral is first impregnated with wax under vacuum to obtain a negative form of the cellular form.

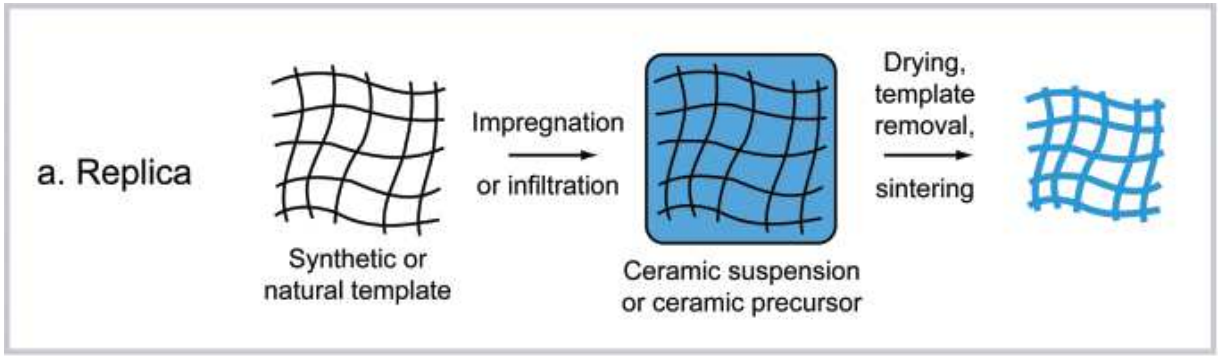
-After hardening the wax, the calcium carbonate of the coralline skeleton is leached out using a strong acidic solution.

-The wax model is impregnated with a ceramic suspension and subsequently removed by pyrolysis.

Second method: 1974 D. M. Roy and S. K. Linnehan

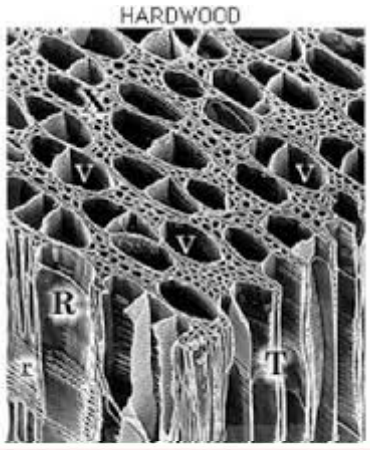
The coral is directly converted into macroporous scaffolds by hydrothermal treatments at high temperatures and pressures in a phosphate solution. The carbonate ions from the aragonite material ($CaCO_3$) originally present in the coral are partially or totally replaced by phosphate ions to form hydroxyapatite.

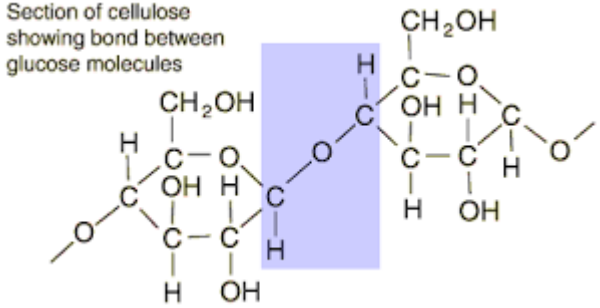
Scaffold Fabrication technologies

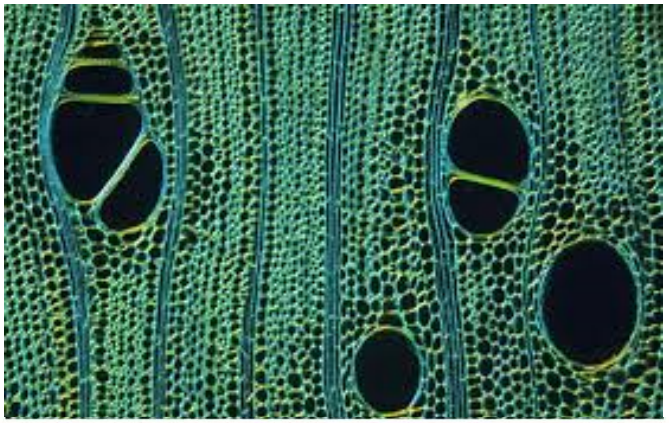


Natural templates: wood

The presence of oriented vessels in the structure of wood enables the preparation of macroporous ceramics with highly anisotropic aligned pores.





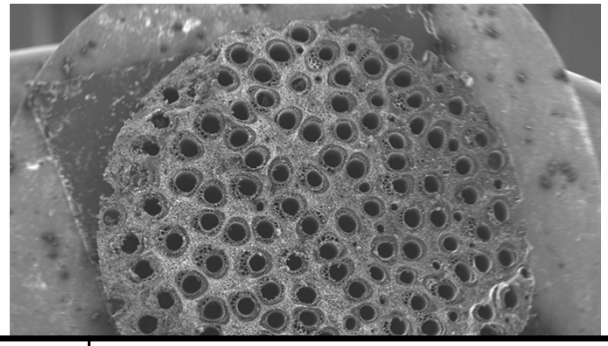


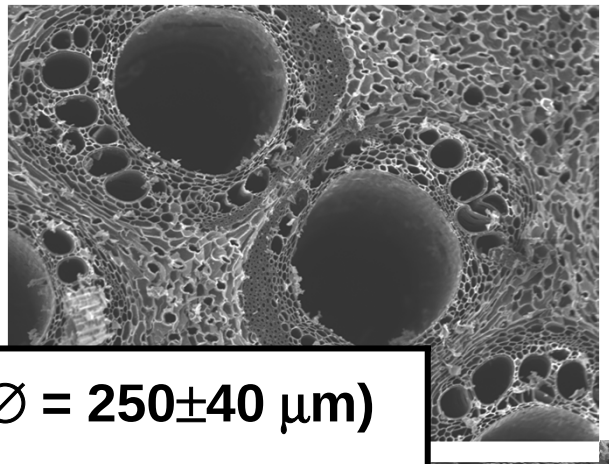
Scaffold Fabrication technologies

Replica from wood

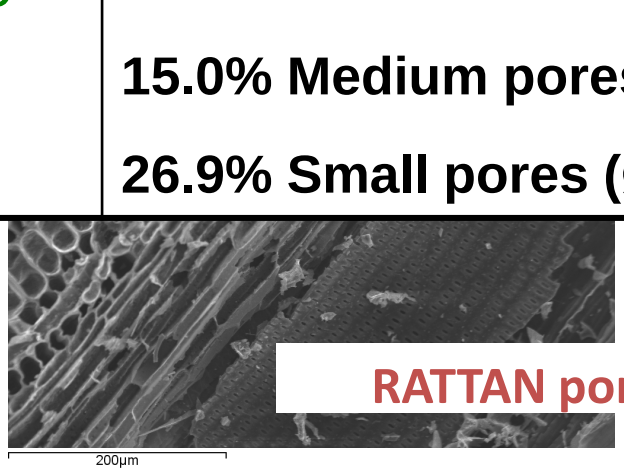
CRITERIA OF SELECTION:

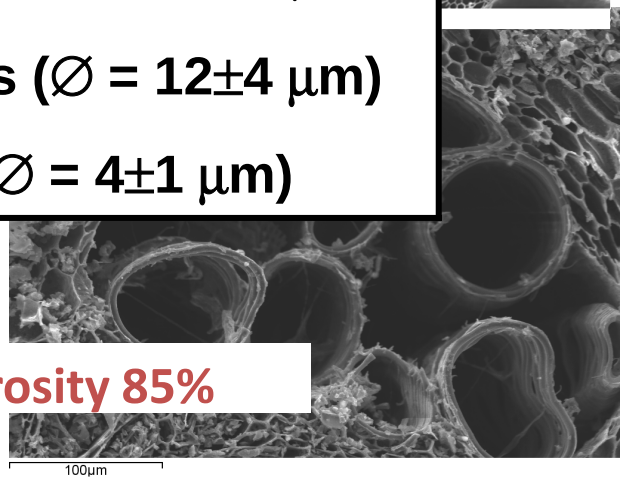
- **Hierarchical and anisotropic structure**
- **Microstructural properties (pore size and distribution, pore interconnection)**





Pores surface fraction distribution	**58.1% Large pores (∅ = 250±40 μm)** **15.0% Medium pores (∅ = 12±4 μm)** **26.9% Small pores (∅ = 4±1 μm)**

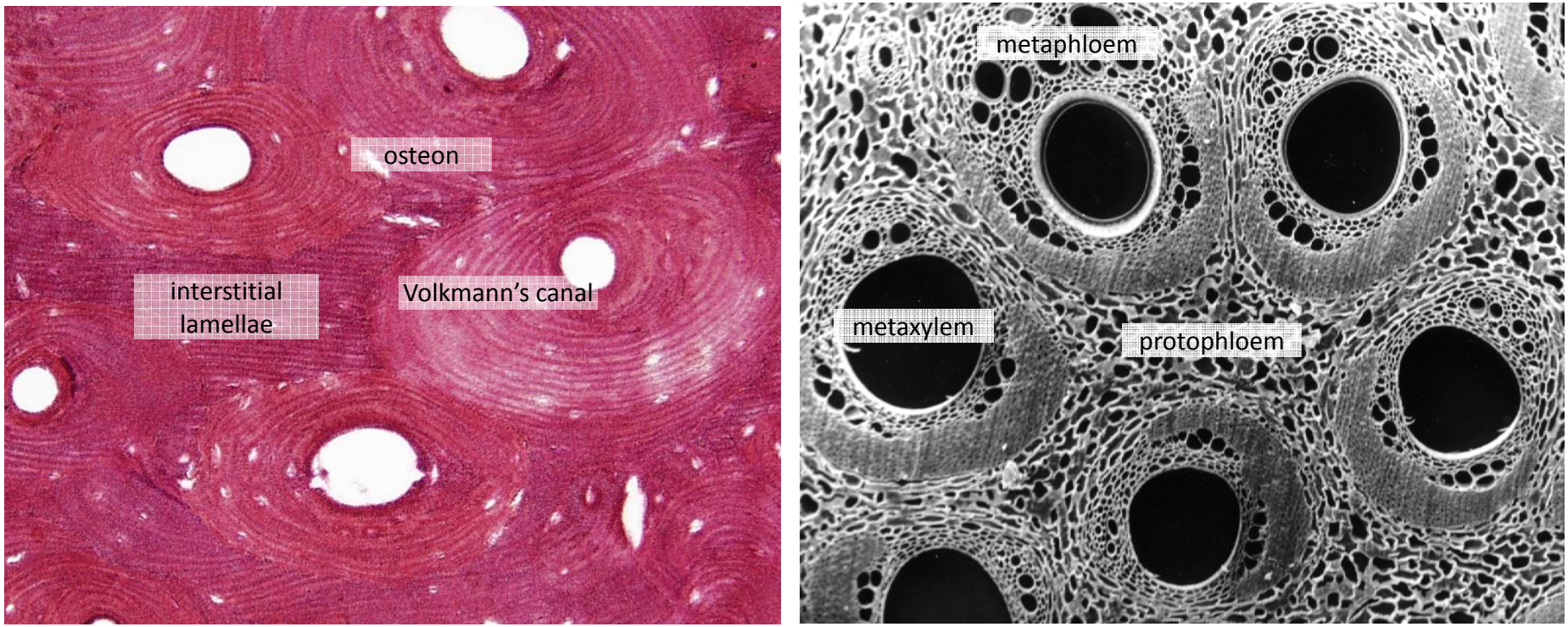




RATTAN porosity 85%

Replica from wood

RATTAN WOOD REPRODUCES SPONGY BONE



Bone

Rattan wood

Replica from wood

CERAMISATION PROCESS OF NATIVE WOOD

1) PYROLYSIS
Wood → Carbon

2) CARBURIZATION
Carbon → CaC_2

3) OXIDATION
CaC_2 → CaO

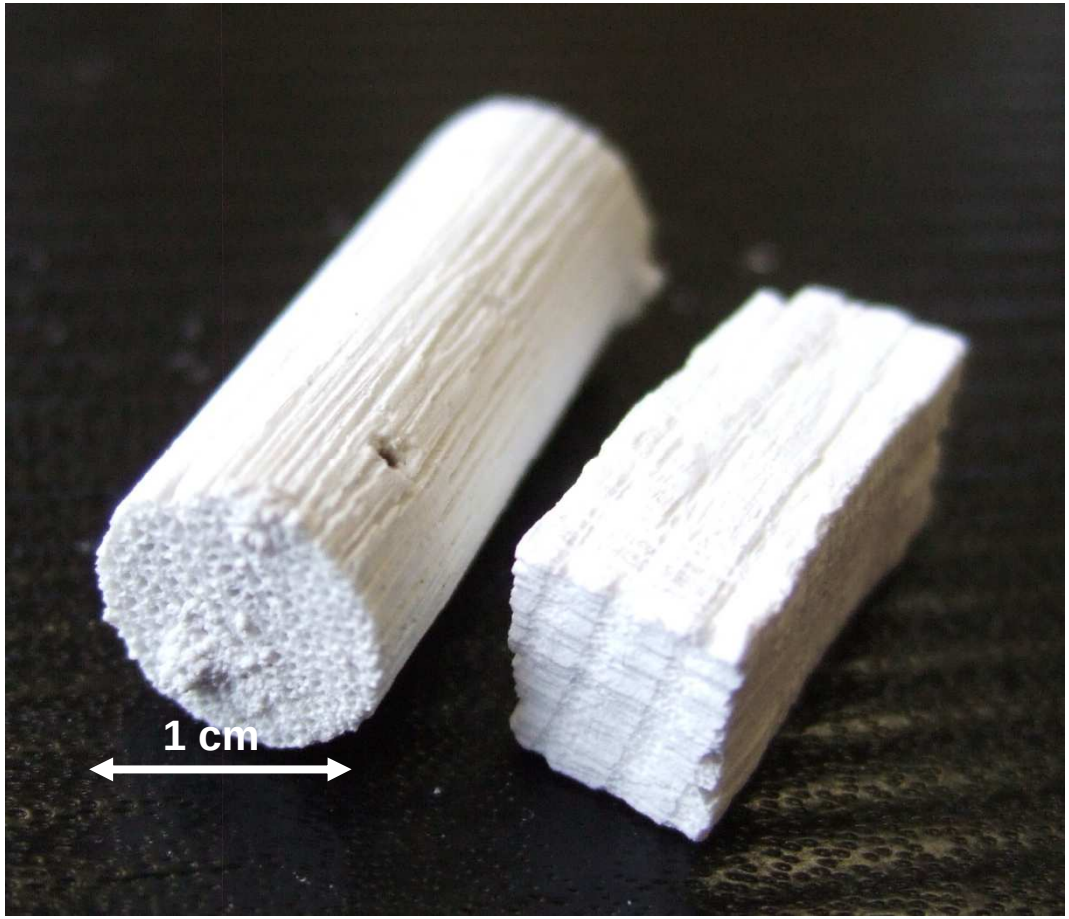
4) CARBONATION
CaO → $CaCO_3$

5) PHOSPHATION
$CaCO_3$ → Hydroxyapatite

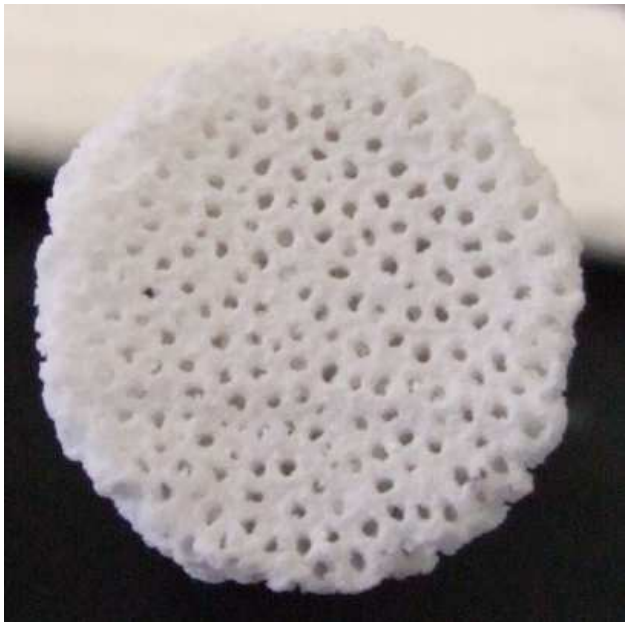
A.Tampieri et al. J Mater Chem (2009) 19, 4973
A.Ruffini et al (2012) Chem Eng J. 217: 150

Transformation of the natural wood template and preserving its initial morphology

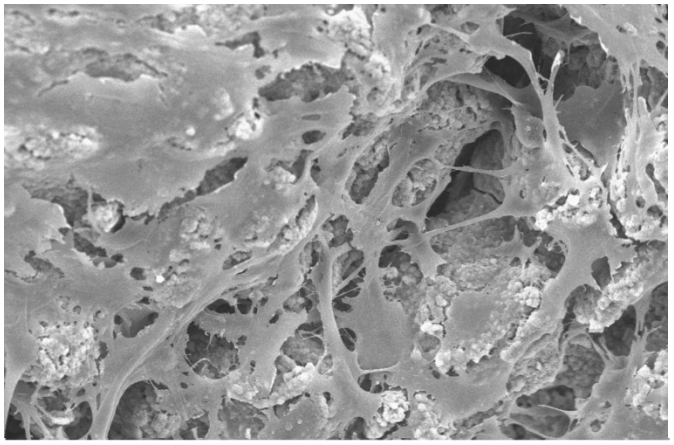
Scaffold Fabrication technologies



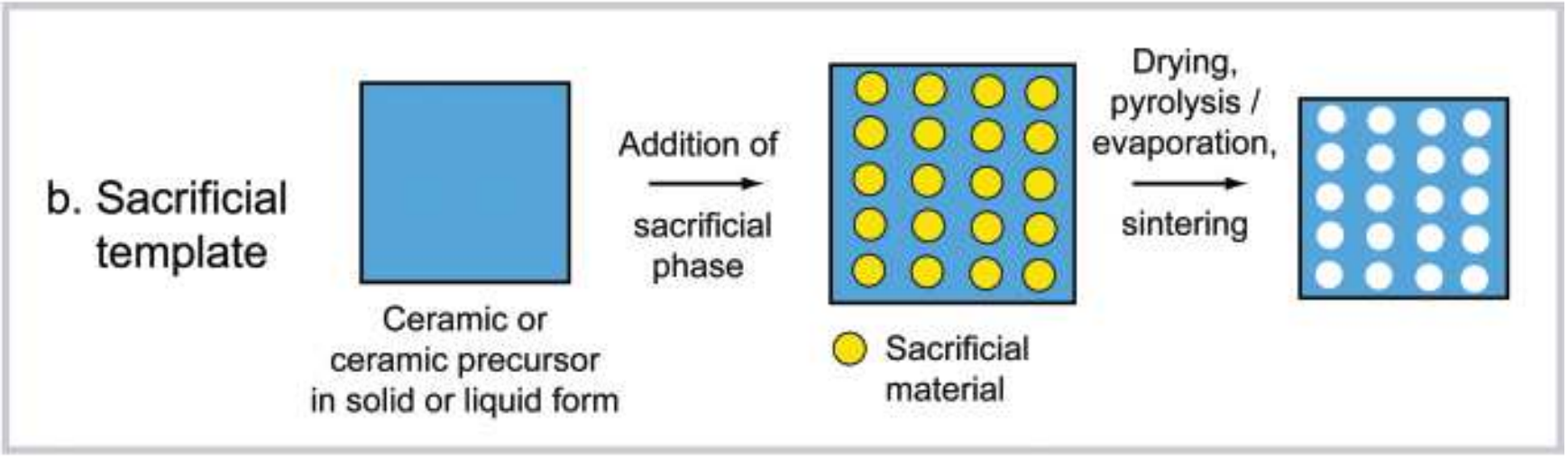
hydroxyapatite scaffolds derived by rattan for sheep implant



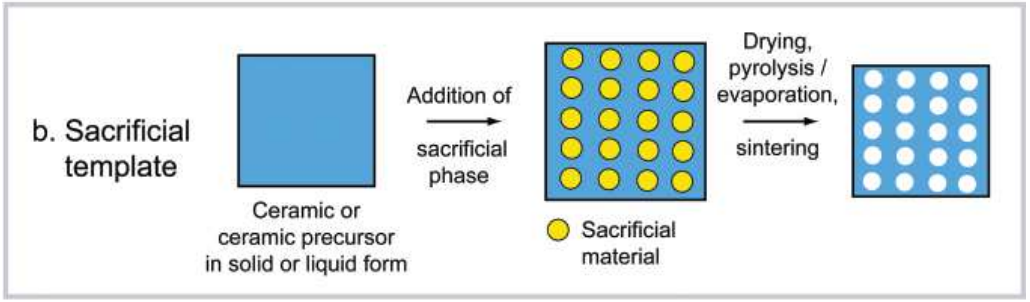
MG63 osteoblast-7 days



3.Sacrificial template technique



Sacrificial template technique consists of the preparation of a biphasic composite comprising a continuous matrix of ceramic particles and a dispersed sacrificial phase. This phase is extracted to generate pores within the structure. This method leads to porous materials displaying a negative replica of the original sacrificial template contrarily to the previous replica methods.



Wide variety of sacrificial materials:

synthetic organics : PVB beads, PMMA or PMMA-PEG beads, …

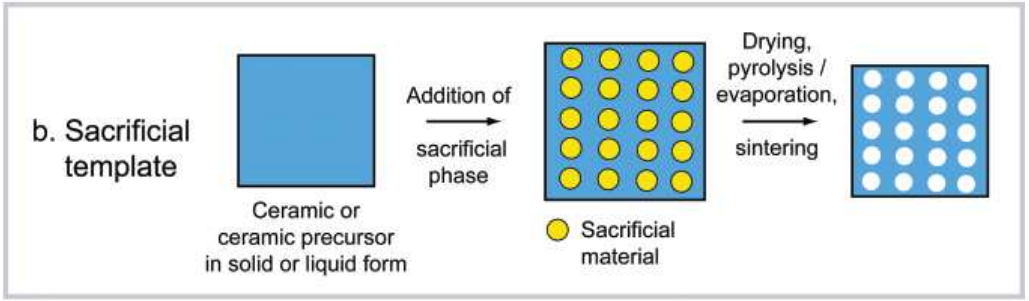
natural organics: sucrose, wax, starch…

The biphasic composite is prepared by various ways:

a) Pressing a powder mixture of the two components
b) Forming a two-phase suspension that is processed by wet colloidal routes such as slip or tape casting
c) Impregnating previously consolidated preforms of the sacrificial material with the ceramic suspension.

The organics are after extracted through pyrolysis by applying long heating times at temperatures between 200 and 600°C depending on organic species.

Scaffold Fabrication technologies



Wide variety of sacrificial materials:

synthetic organics : PVB beads, PMMA or PMMA-PEG beads, …

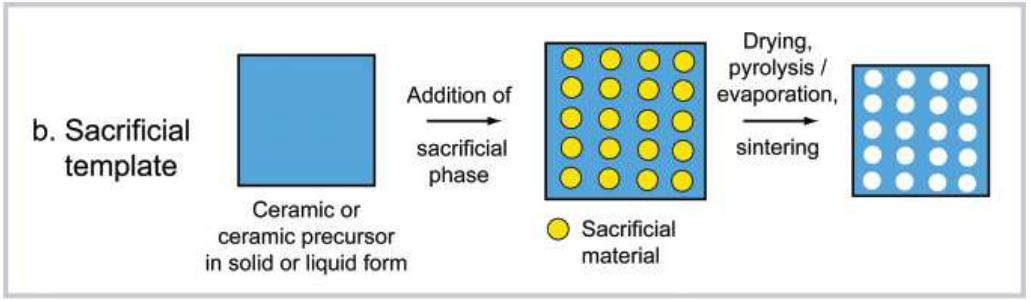
natural organics: sucrose, wax, starch…

The biphasic composite is prepared by various ways:

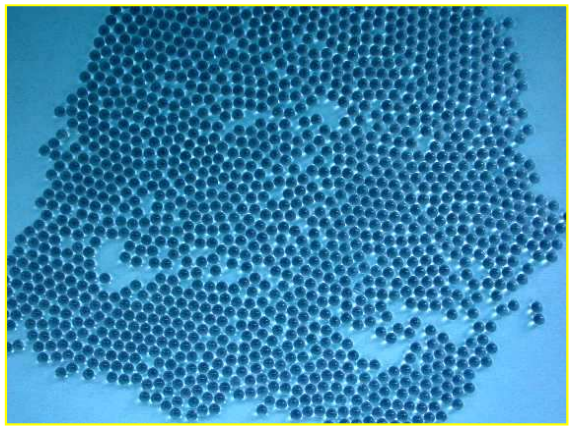
a) Pressing a powder mixture of the two components
b) Forming a two-phase suspension that is processed by wet colloidal routes such as slip or tape casting
c) Impregnating previously consolidated preforms of PMMA beads scaffold with the CaP powder suspension.

The organics are after extracted through pyrolysis by applying long heating times at temperatures between 200 and 600°C depending on organic species.

Scaffold Fabrication technologies

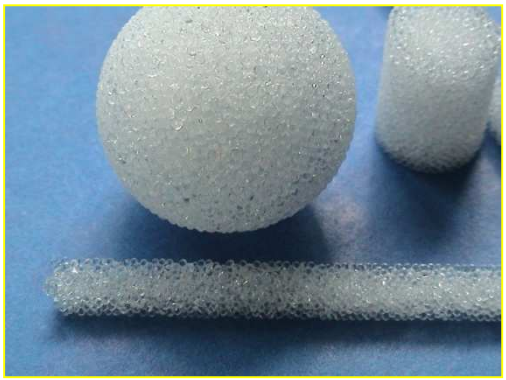


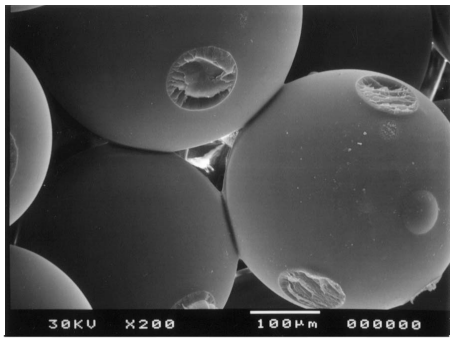
Impregnation of PMMA beads consolidated preforms with CaP powder suspension.



Chemical forming with acetone

under pressure

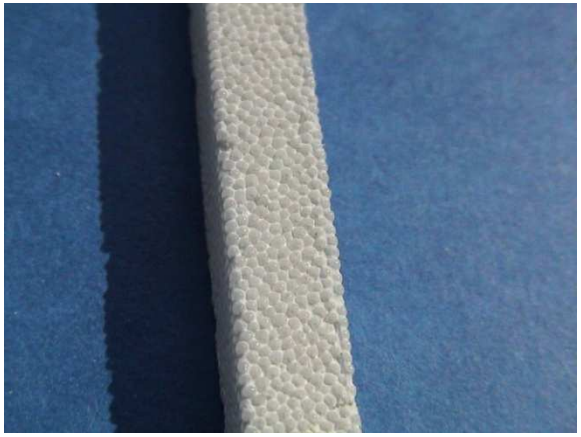




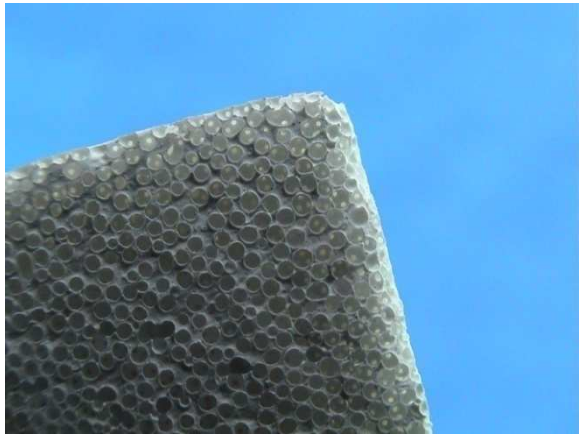
- Bonding between PMMA beads (scaffold)
- Controlled diameter bonding (Interconnection) depends on time, temperature, pressure

Impregnation of PMMA beads consolidated preforms with CaP powder suspension.

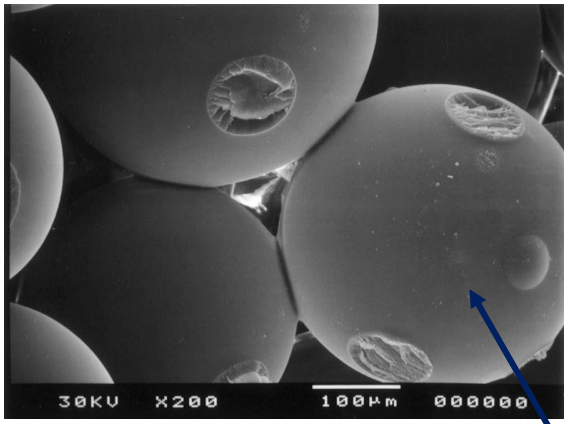
Shaping

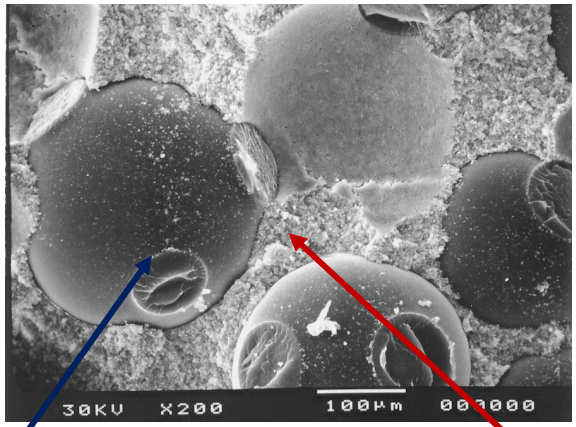


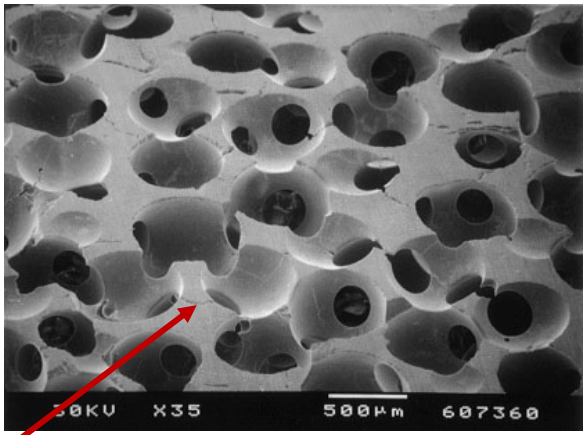
Impregnation



Debinding







PMMA beads

ceramic

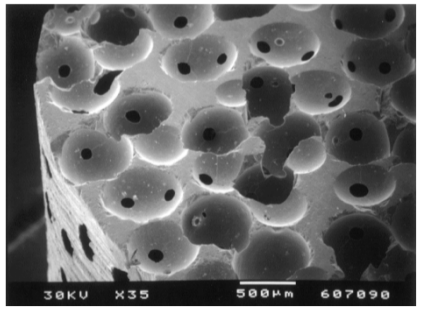
M. Descamps et al., JECS. 28 (2008) 149

Scaffold Fabrication technologies

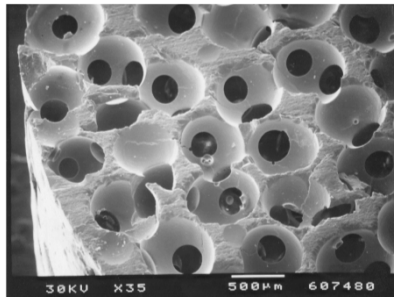
- Control of pore size depending on PMMA beads size
- Control of interconnection diameters: Id

PMMA beads (500 - 600 µm)

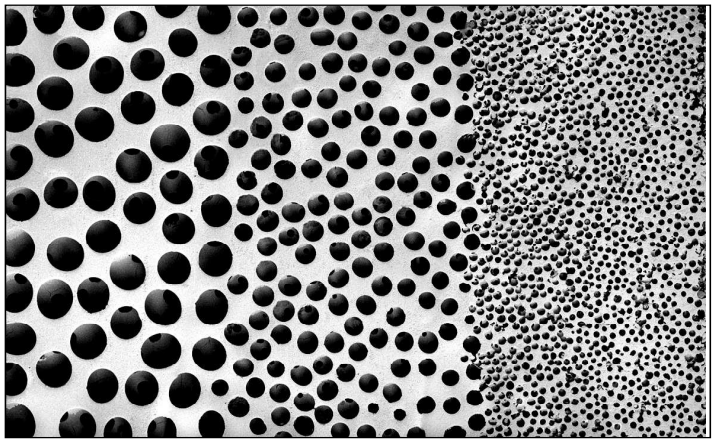
Id : 60 µm

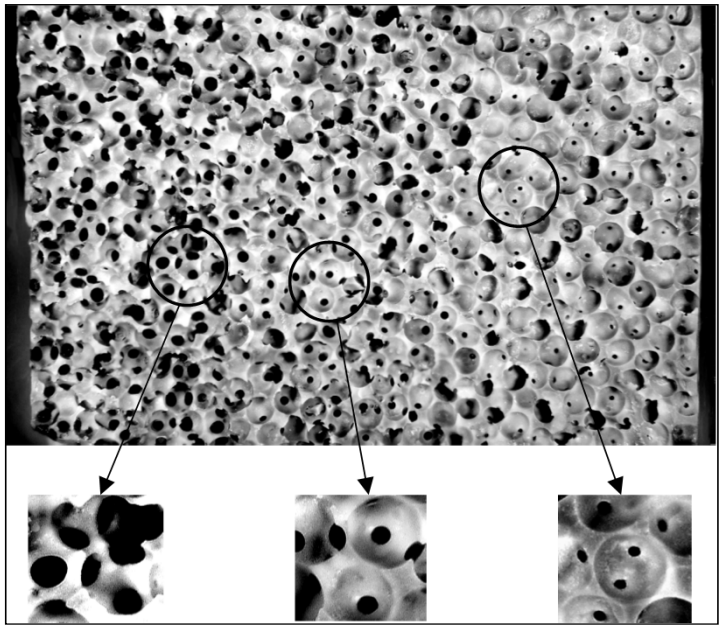


Id: 260 µm



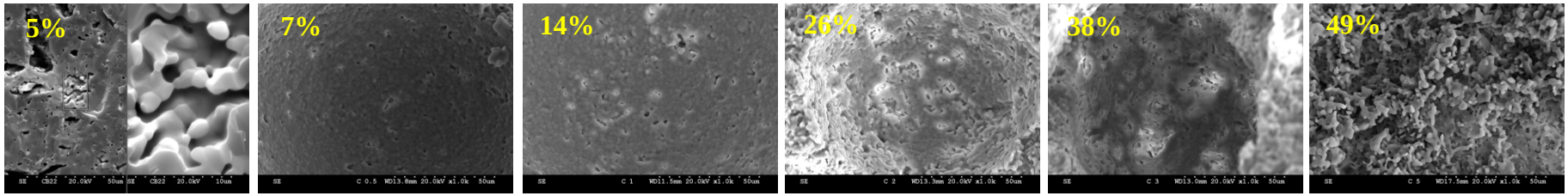
- Control of porosity gradient in pore size and interconnection size

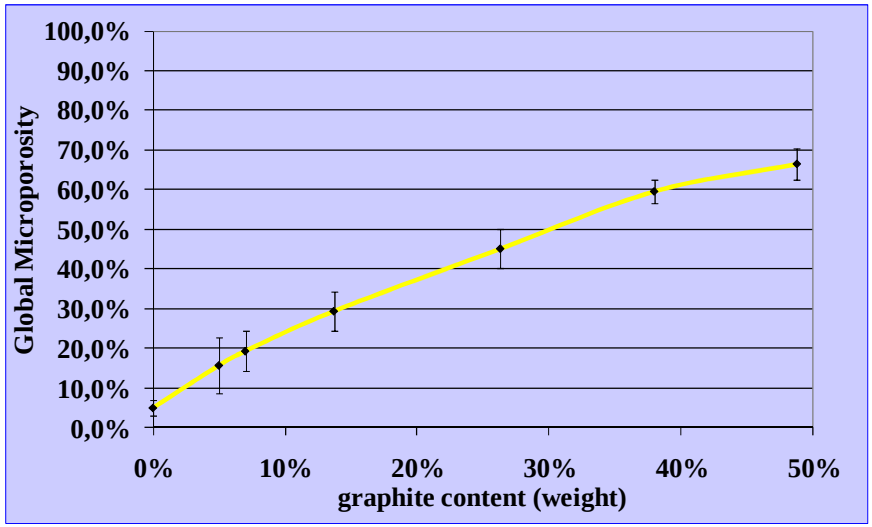


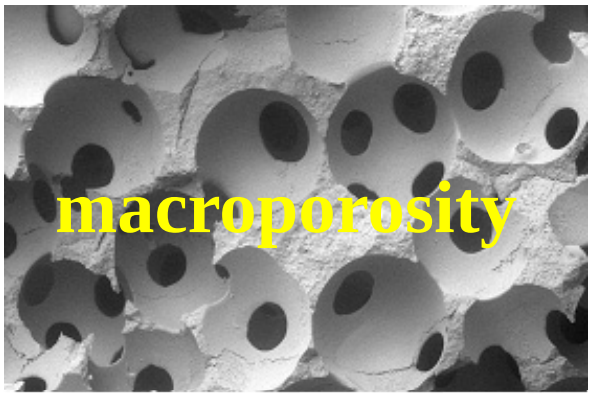
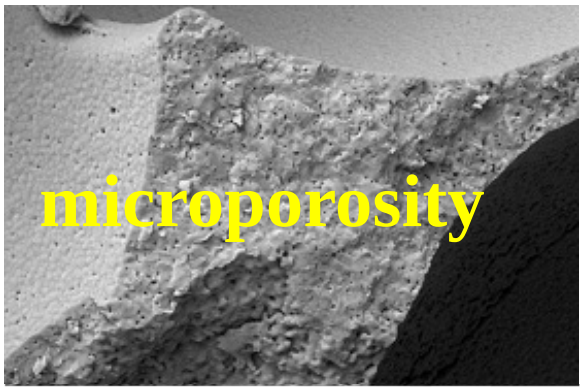


Scaffold Fabrication technologies

Possibility to add microporosity by mixing graphite as micropore forming agent.





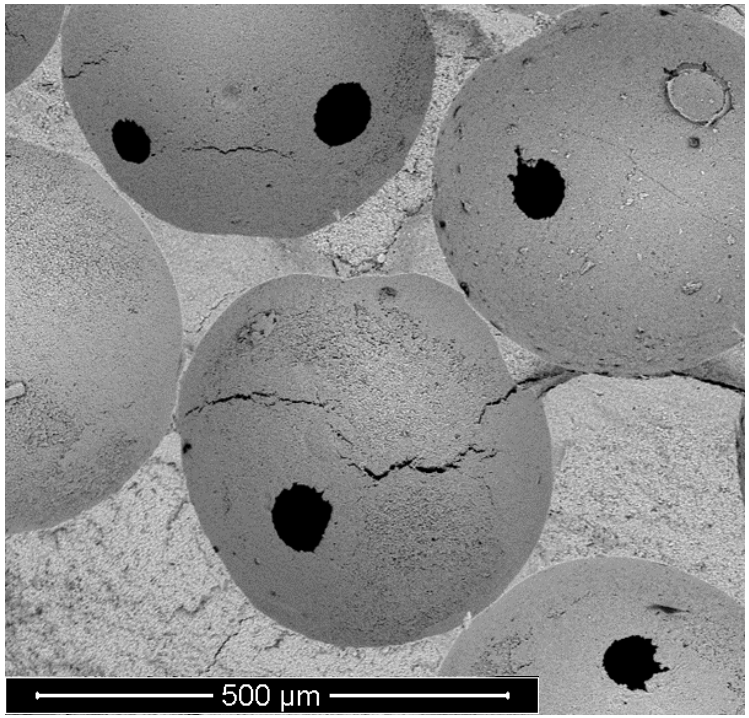
Controlled  **and** 

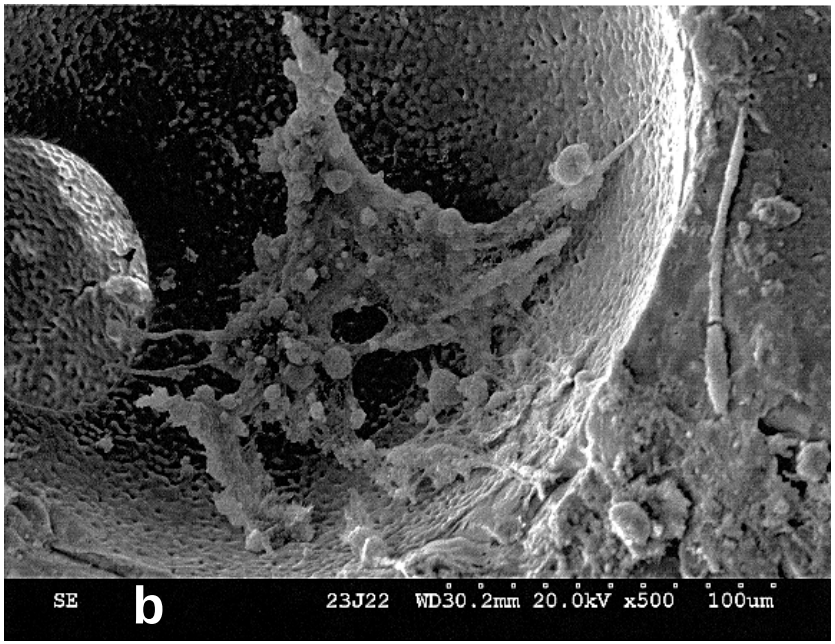
Scaffold Fabrication technologies

Impregnation of PMMA beads consolidated preforms with CaP powder suspension.

Debinding:
220°C 30 hours + 400°C 5 hours
Sintering: 1115°C 3 hours

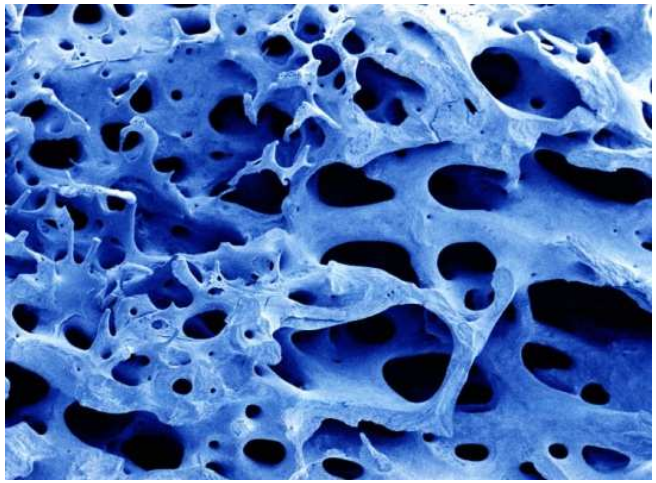
Density of ceramic walls = 99 %
Porosity = 65%
Spherical pores homogeneously distributed in space with several interconnecting holes.

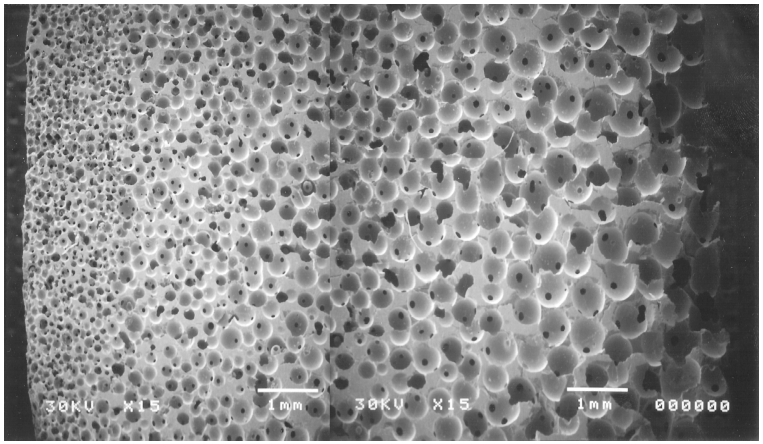


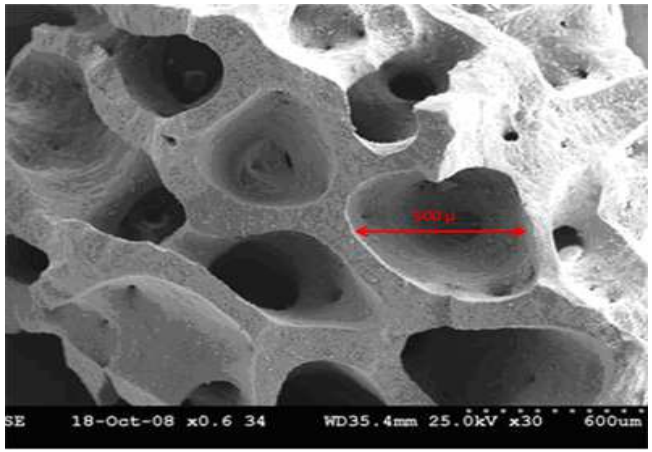


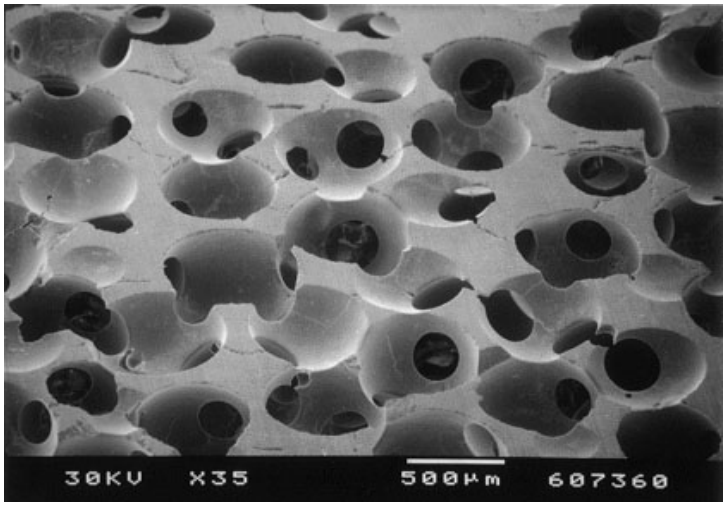
B550 **pore diameter 400 µm**
interconnection 45 - 65 µm

Impregnation of PMMA beads consolidated preforms with CaP powder suspension.





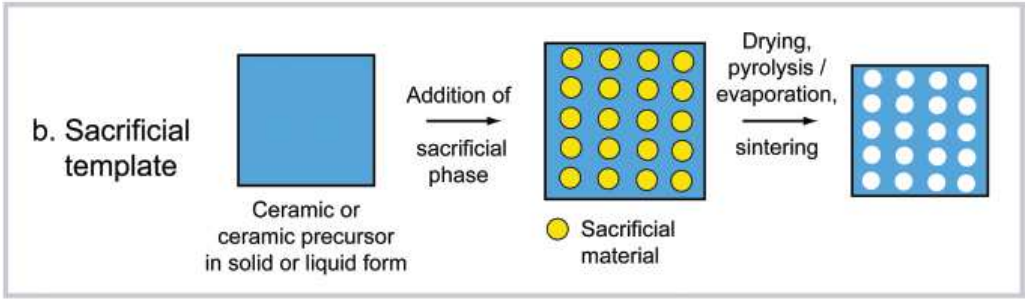




Human bone

Patent FR2823305, Biocetis SARL,
M. Descamps, P Hardouin, J Lu, F Monchau

Scaffold Fabrication technologies



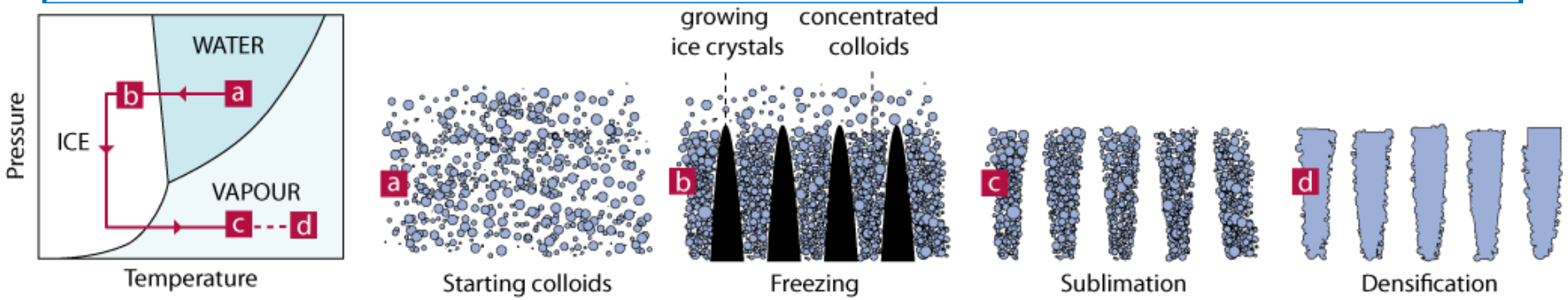
Wide variety of sacrificial materials:

synthetic organics : PVB beads, PMMA or PMMA-PEG beads, …

natural organics: sucrose, wax, starch…

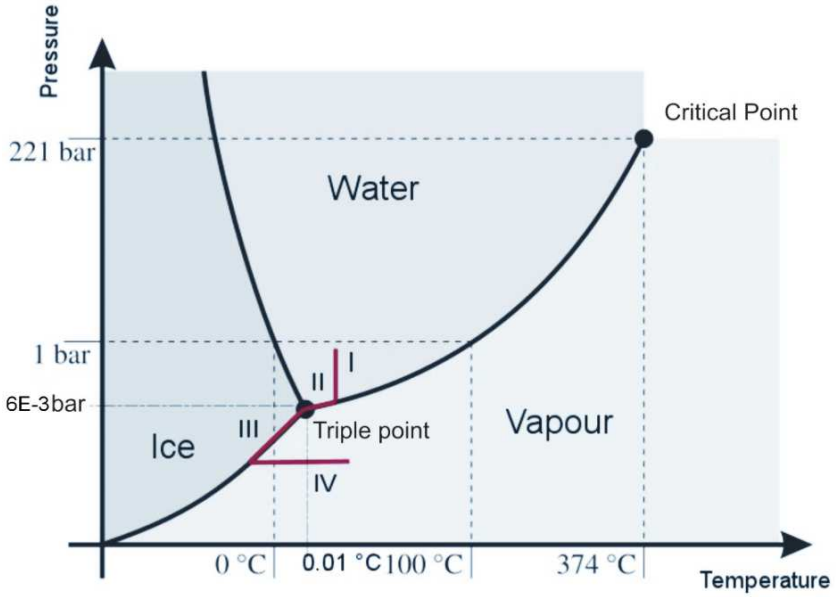
liquids: freeze-drying of water

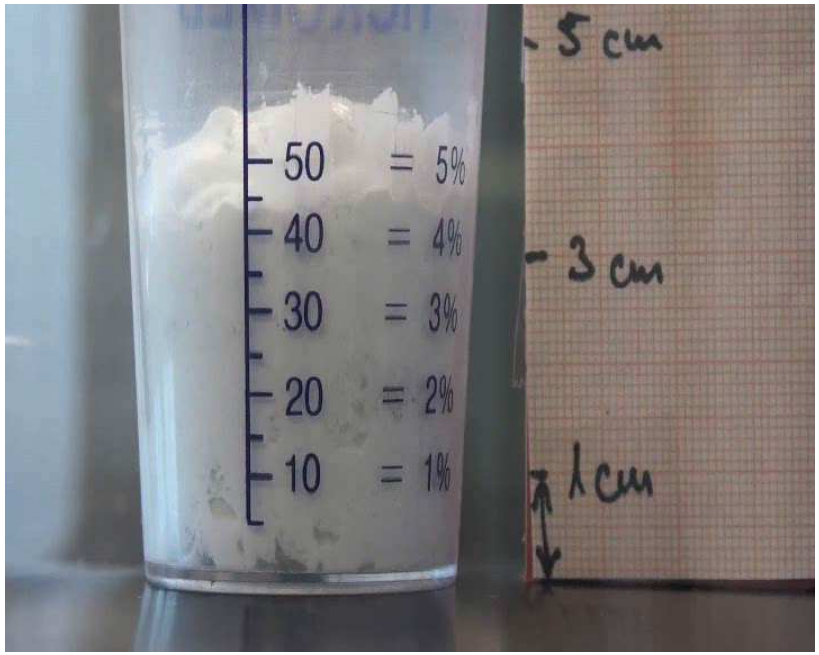
By using liquid pore formers such as water and oils, liquids and volatile oils can be evaporated or sublimated at milder conditions without generating toxic gases and excessive stresses during pore former removal.



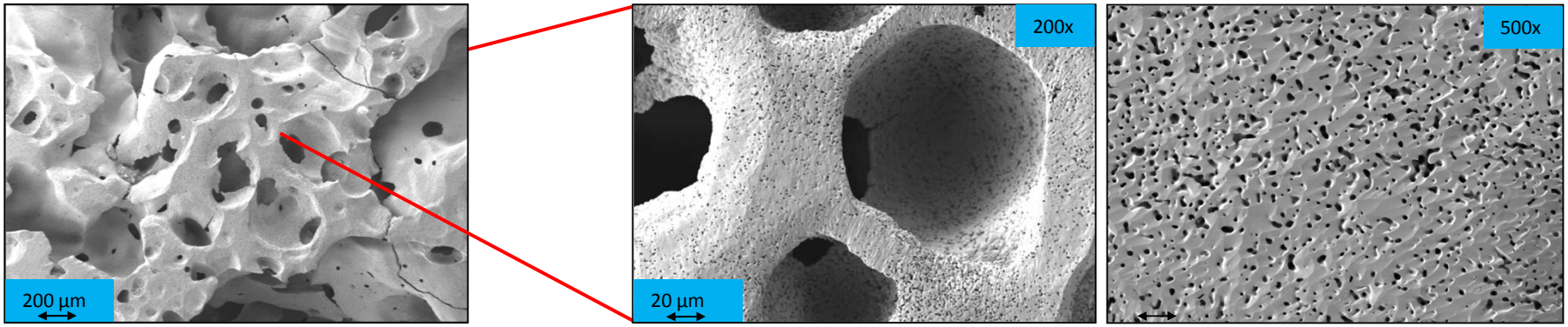
Freeze Foaming

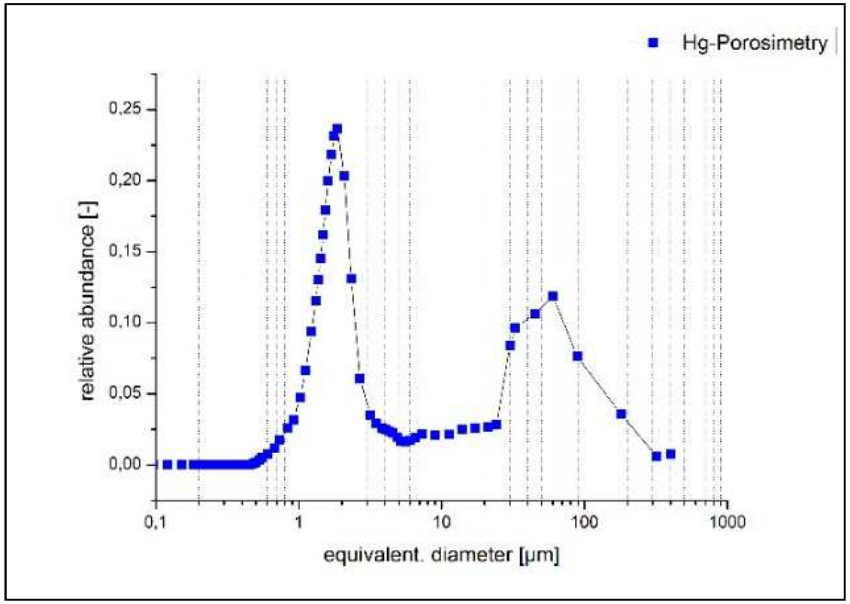
- Foaming process is based on pressure reduction in the vacuum chamber of a freeze dryer
- Pores through rising water vapor, procedural air and later sublimation of frozen water (aqueous suspensions)





Freeze Foaming : Advantages



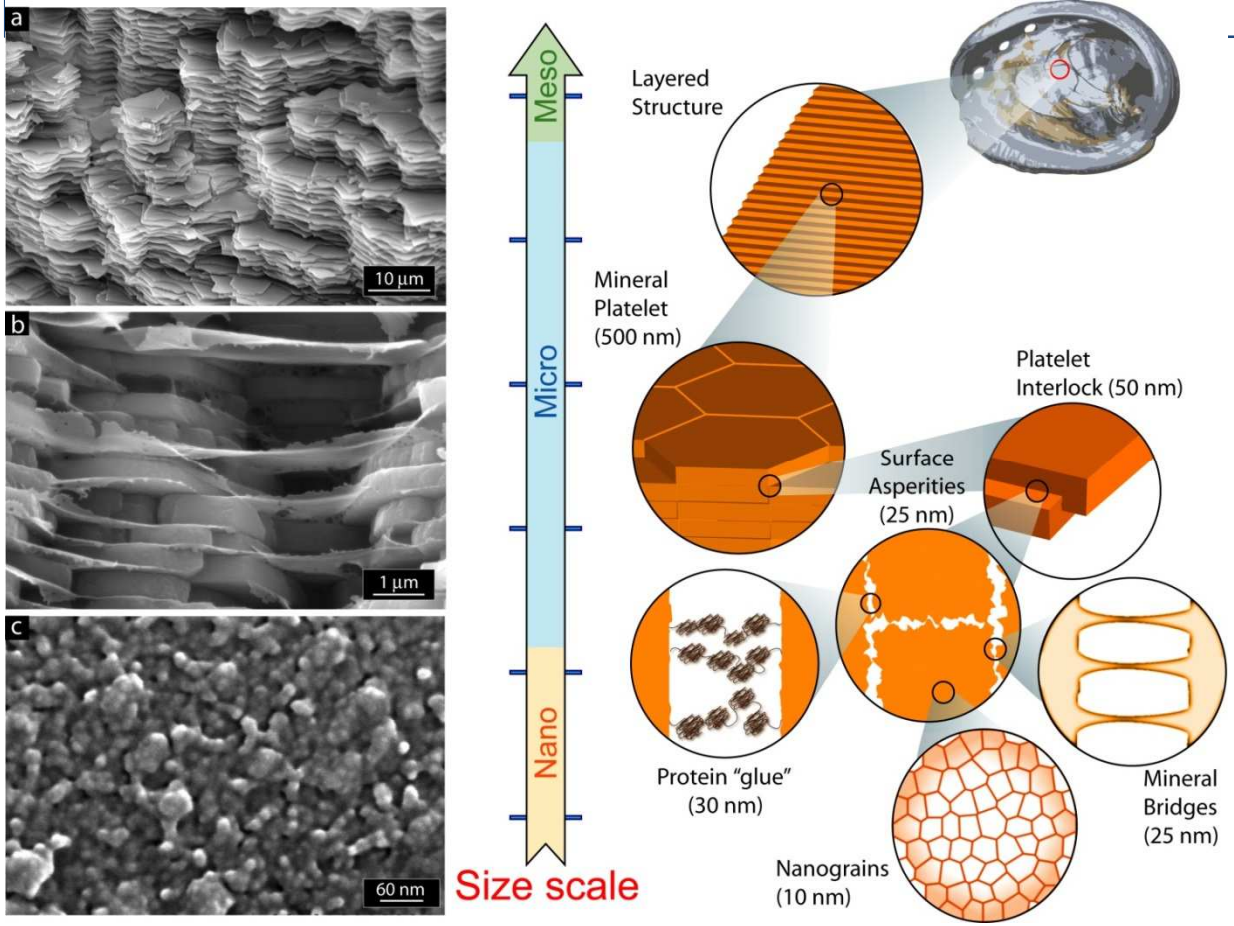


Patent: DE 10 2008 000 100, Tassilo Moritz

- No pore-forming agents needed (Replica or Placeholder Technique)→ environmentally friendly
- Foam with high amount of open porosity (50 - 95%) and bimodal pore size distribution
- Meso- to macropores
- Near-net shaping possibility → personalization
- Different materials → different product lines according to the needs.

Freeze casting – ice templating

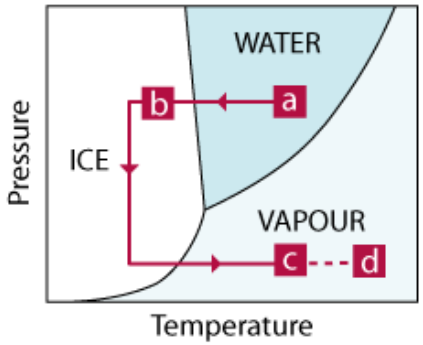
The objective was to mimic the nacre structure by using an oriented freezing process to allow oriented pore development.

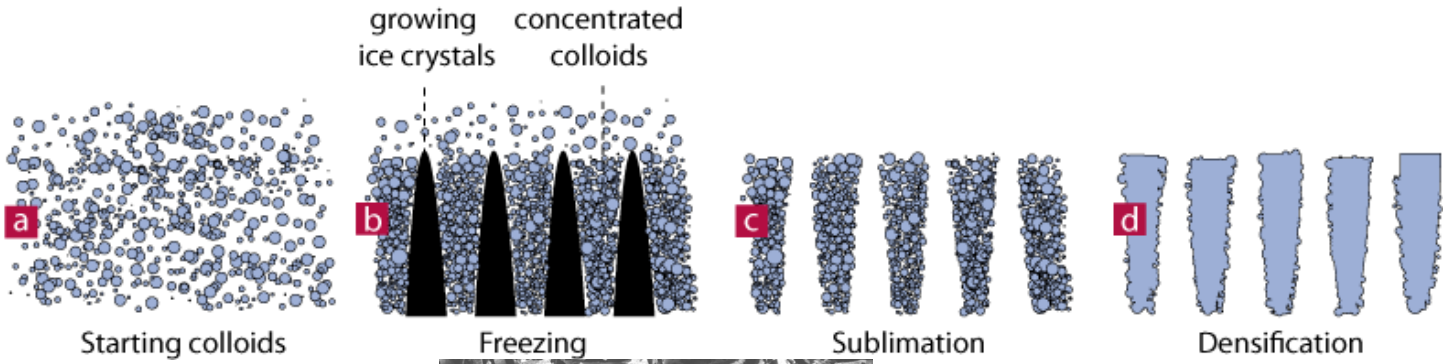


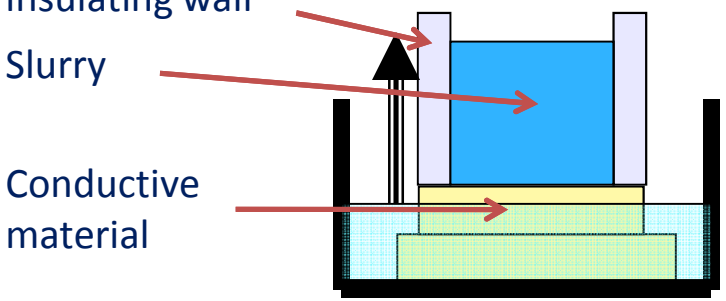
>95% $CaCO_3$

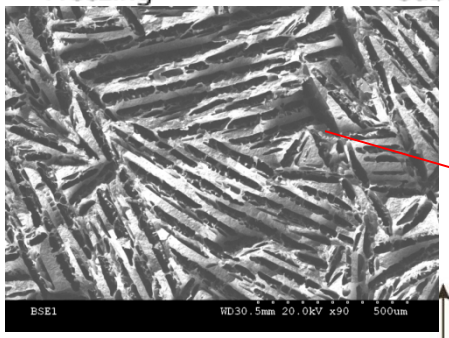
K_{IC} : $8 MPa\sqrt{m}$

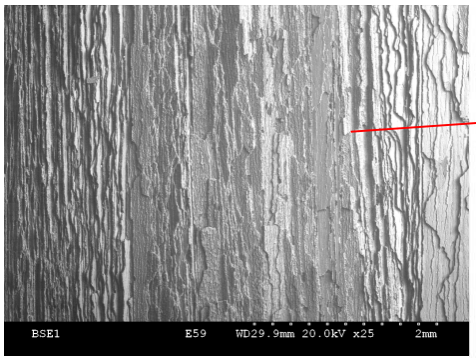
Freeze casting

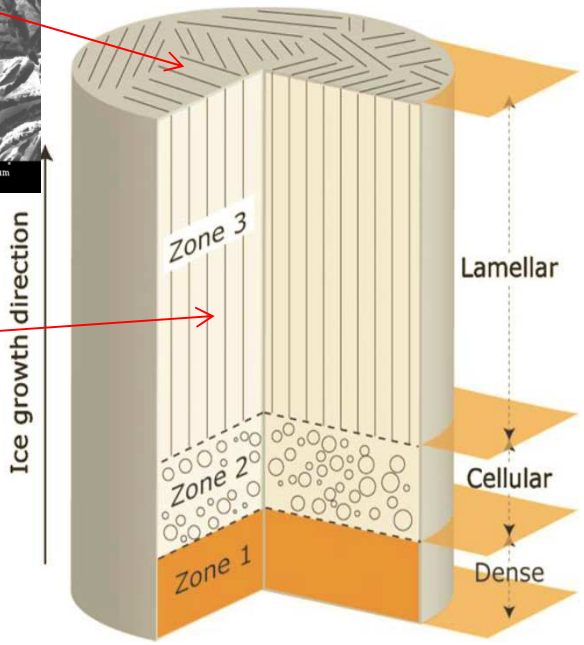








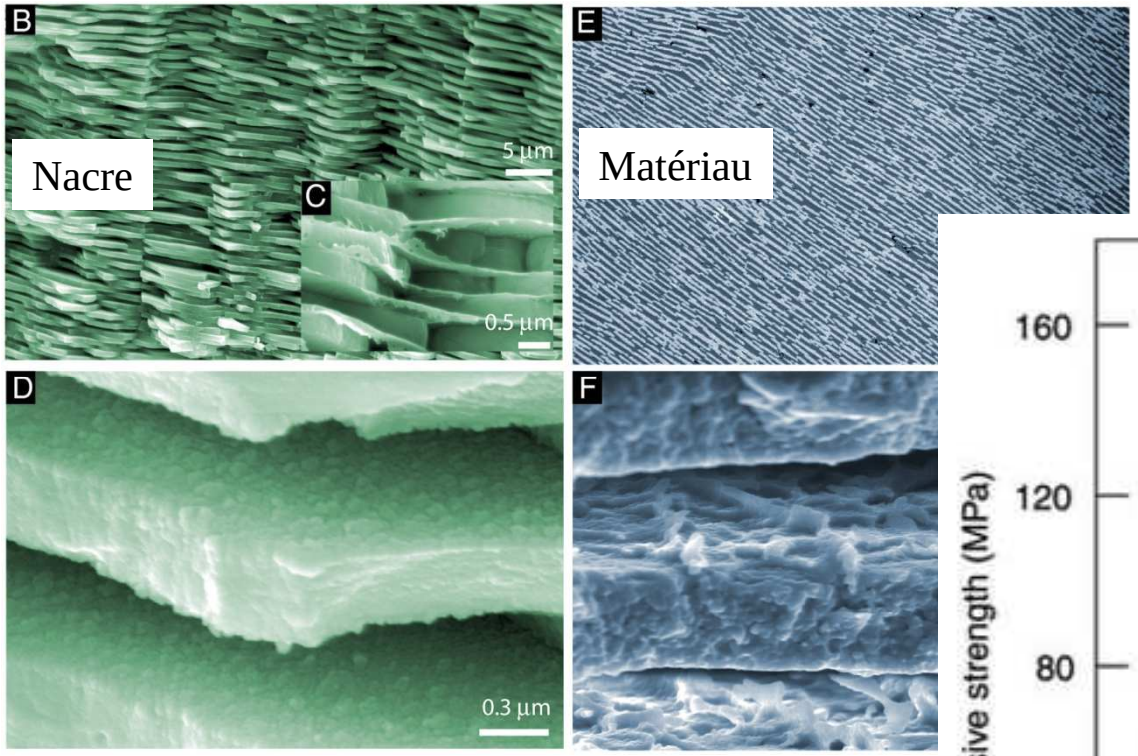


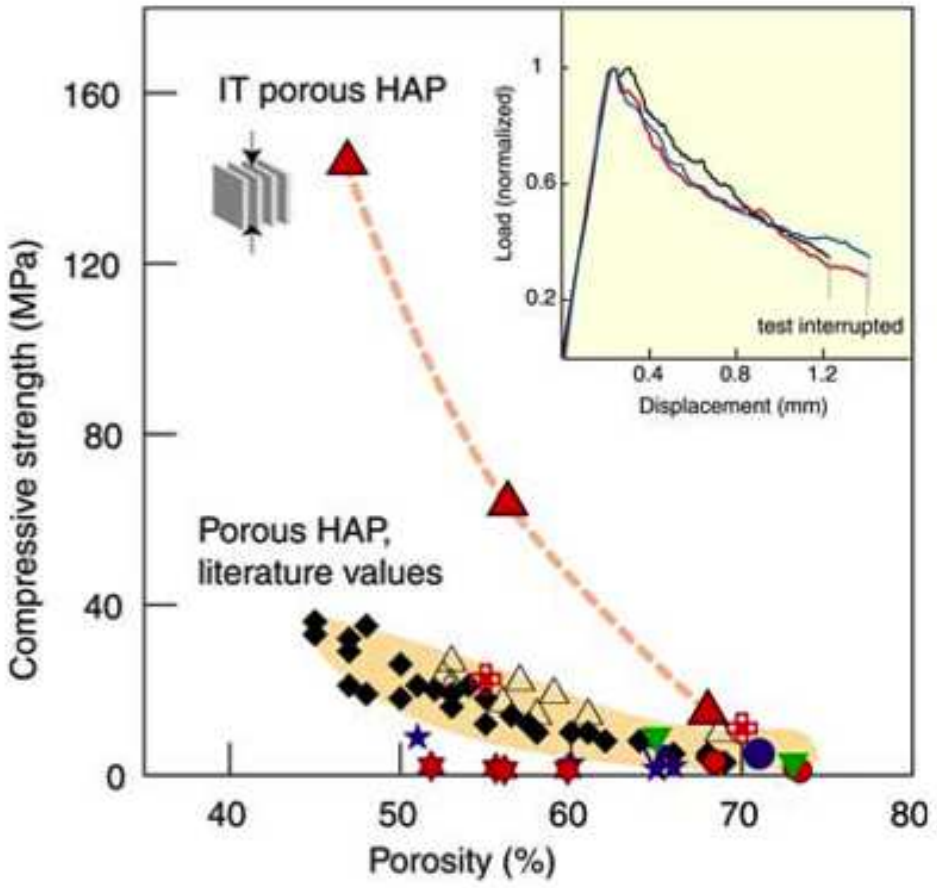


S. Deville et al,, Biomaterials 27 (2006) 5480–5489

Scaffold Fabrication technologies

Freeze casting

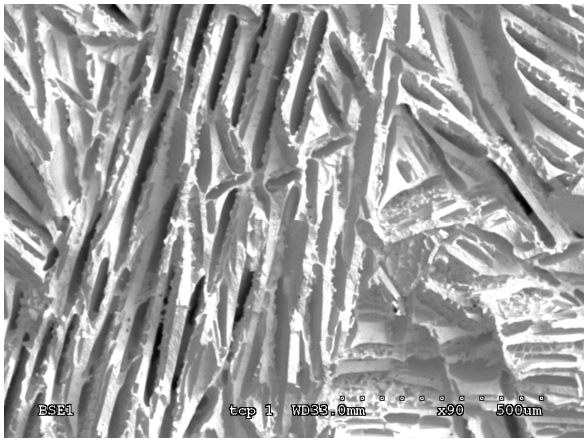


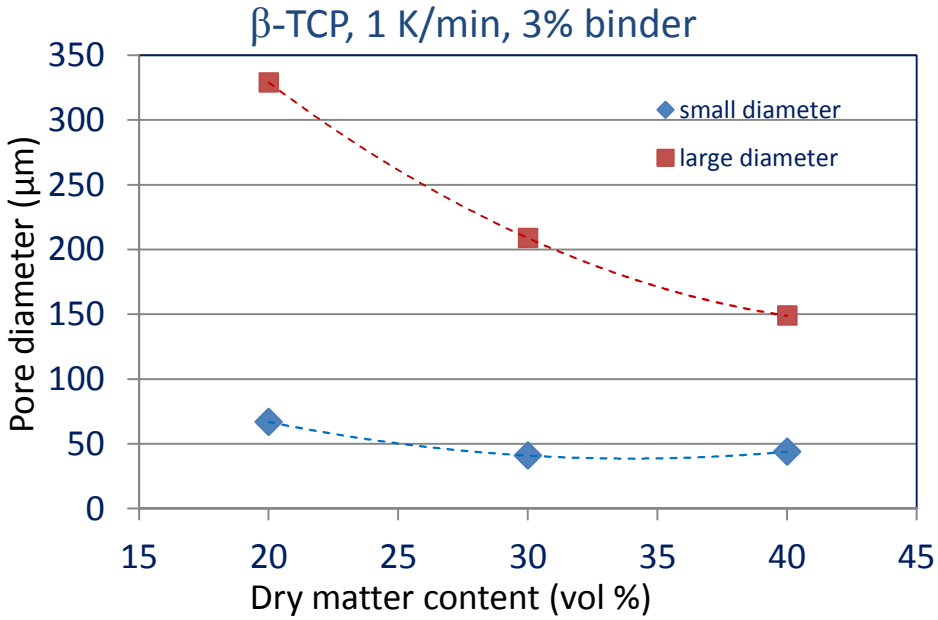


S. Deville et al., Science, 2006

Scaffold Fabrication technologies

Freeze casting

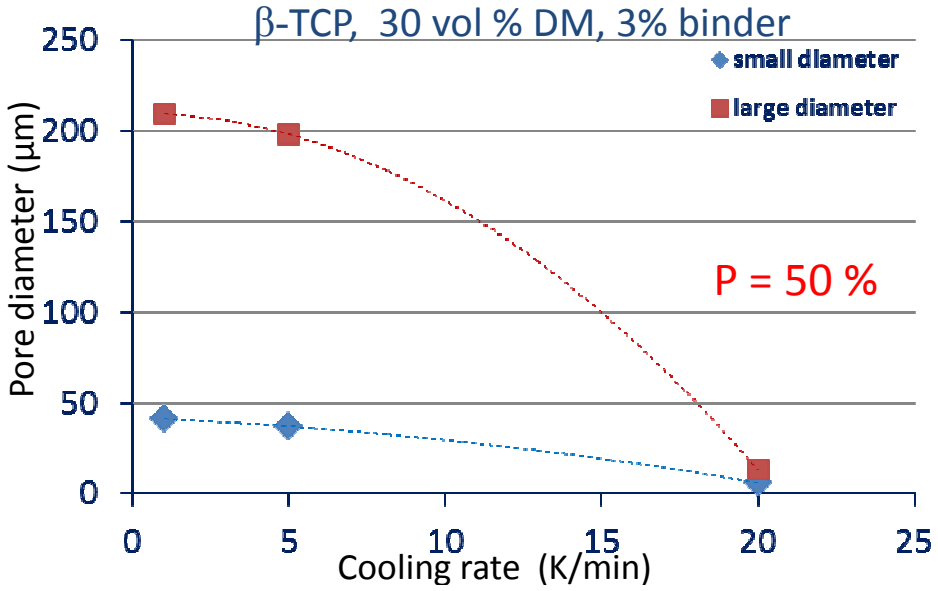




Pore long axis size:

- **between 150 and 340 µm versus dry matter content**
- **between 13 and 210 µm versus cooling rate .**

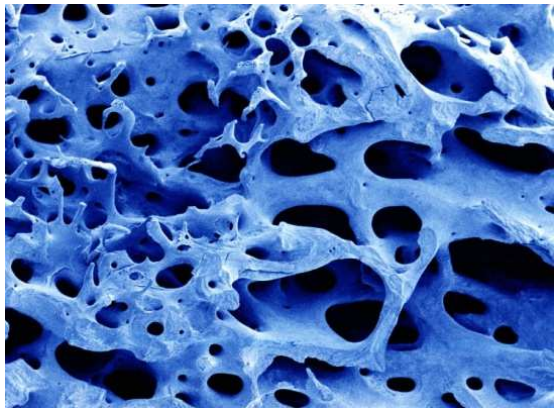
Total porosity:
36 to 67 % versus dry matter %



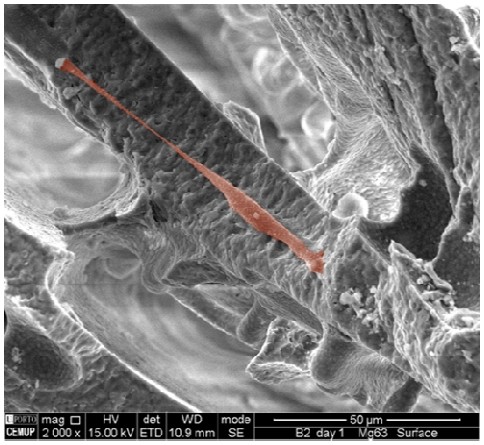
D. Hautcoeur Ph D UMons-BCRC Nov 2014

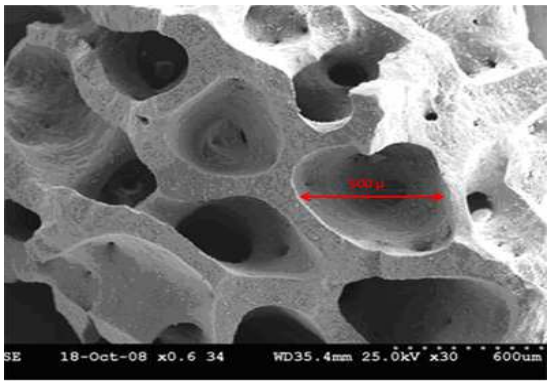
Scaffold Fabrication technologies

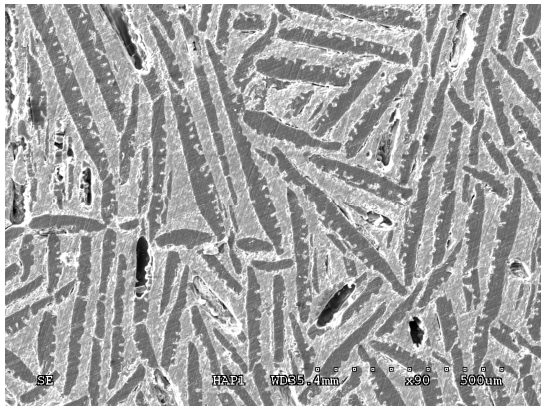
Human bone

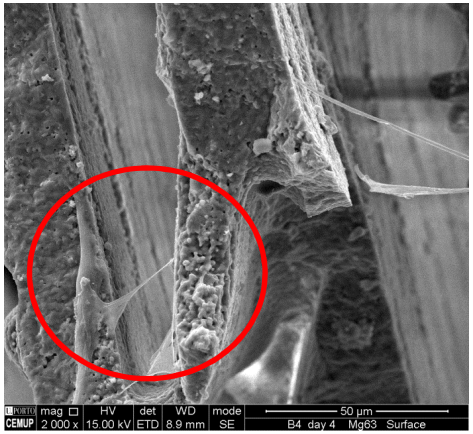


Freeze casting





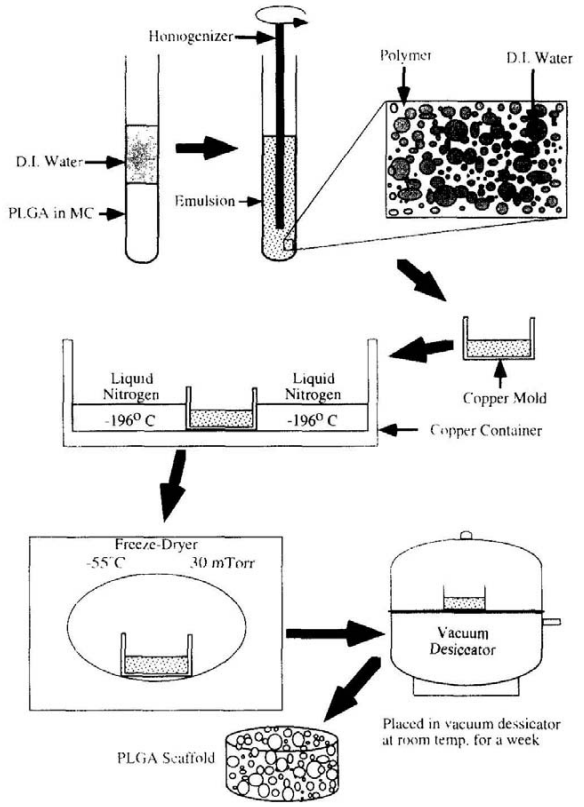


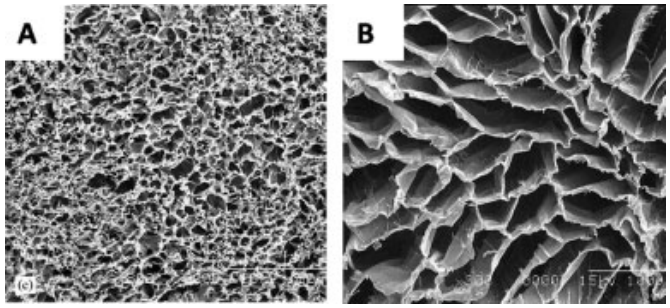


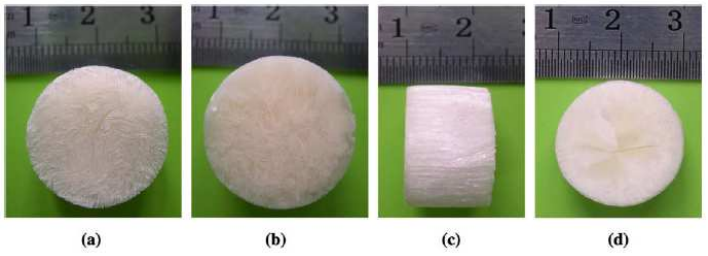
Freeze drying

Applied also to biopolymers

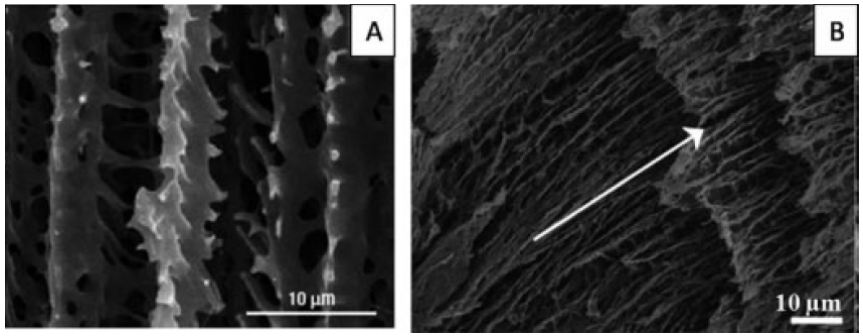
- Method consists of creating an emulsion by homogenization of a polymer solution (in an organic solvent) or water mixture
- Rapidly cooling the emulsion to lock in the liquid state structure
- Removing solvent (water) by freeze-drying
- Can control pore morphology (to some extent) by controlling rate of freezing and final freezing temperature.





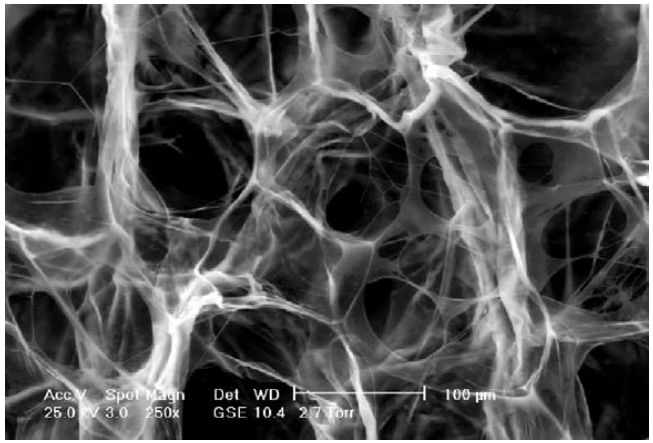


Freeze drying

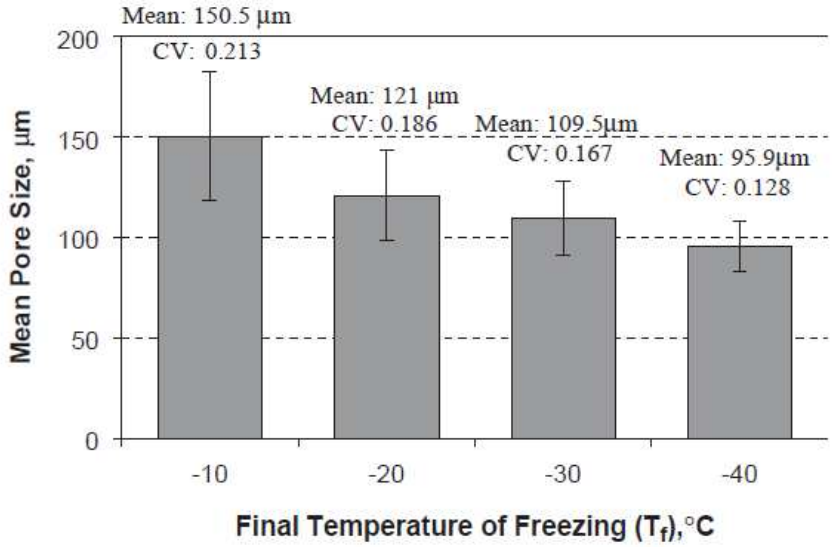


Aligned porous PVA scaffolds prepared by directional freezing

- Influence of freezing rate and final freezing temperature
- Constant cooling rate technique produced pores with a more uniform size and structure

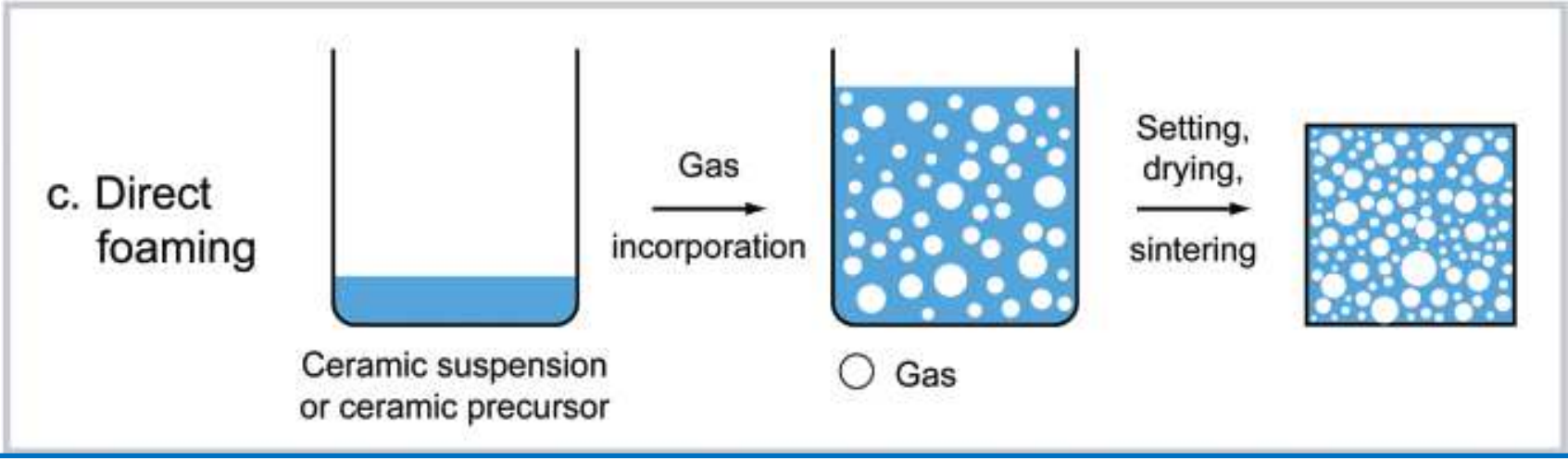


Porous collagen-GAG scaffolds obtained with cooling rate (0.9C/min), (*O'Brien et al 2004*)



Effect of freeze-drying temperature on mean pore size of CG scaffolds.*(O'Brien et al 2005)*

4.Direct foaming technique



Direct foaming technique consists of the incorporation of air into a suspension to create air bubbles.

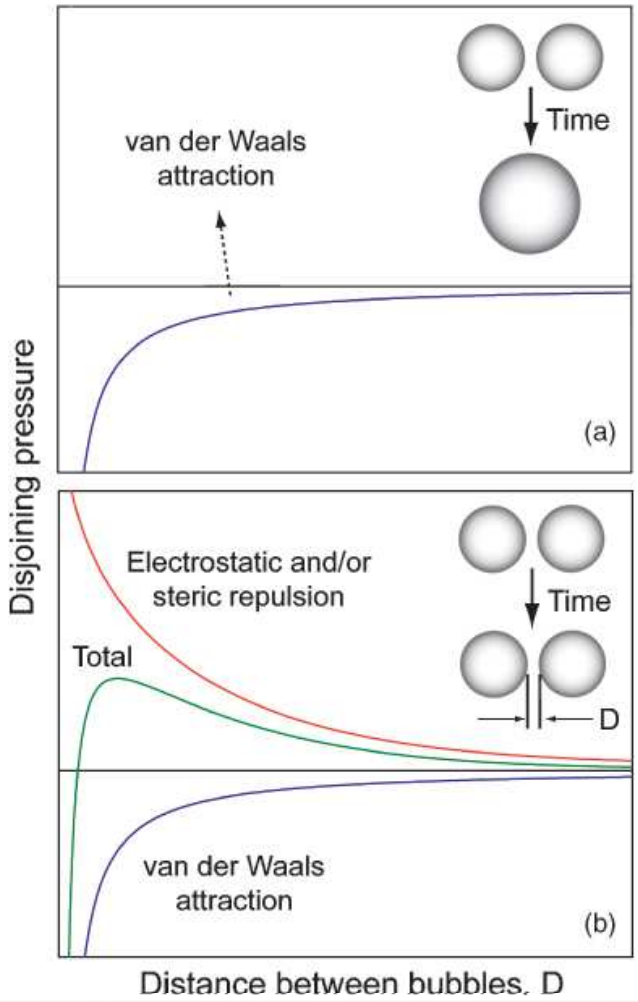
The incorporation of bubbles can be carried out by mechanical agitation or by chemical reaction accompanied by degassing.

The total porosity of sintered foamed ceramics is proportional to the amount of gas incorporated into the liquid and is between 40% - 97%.

The pore size depends on the stability of the wet foam before setting takes place → foam stabilization with surfactant is necessary to control bubble size and final pore size (10 µm - 300 µm).

Direct foaming technique

Schematic dependence of the disjoining pressure among two interacting gas bubbles as a function of their distance D.



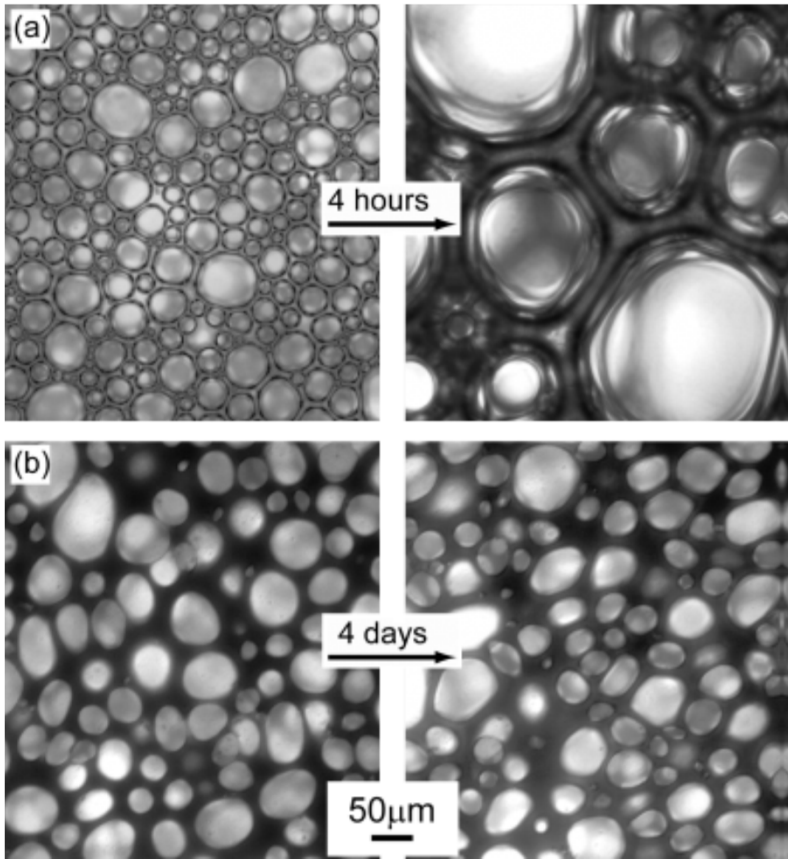
Coalescence is favored by attractive van der Waals forces (a)

and can only be hindered by providing steric and/or electrostatic repulsion among the interacting bubbles (b) by adding long-chain surfactants or proteins or by adding colloidal particles.

A.R.Studart et al JACS 89 [6]1771-1789 (2006)

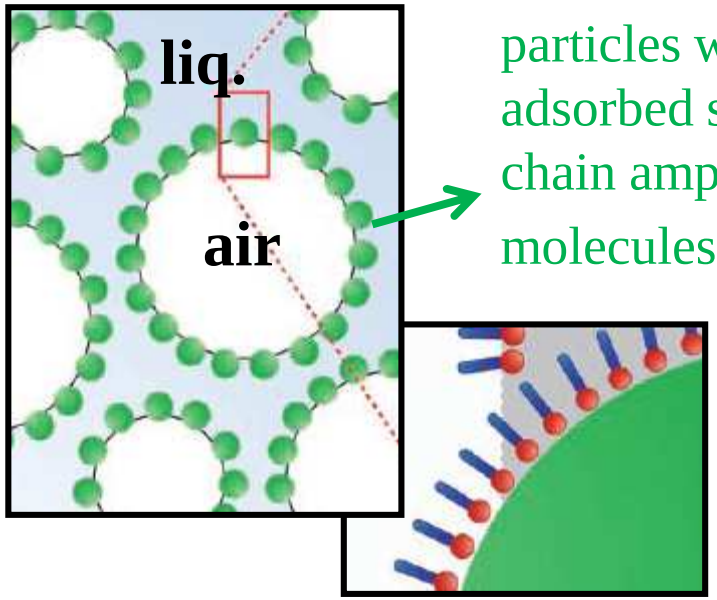
Direct foaming technique

Direct foaming with long chain surfactant



Direct foaming with colloidal particles

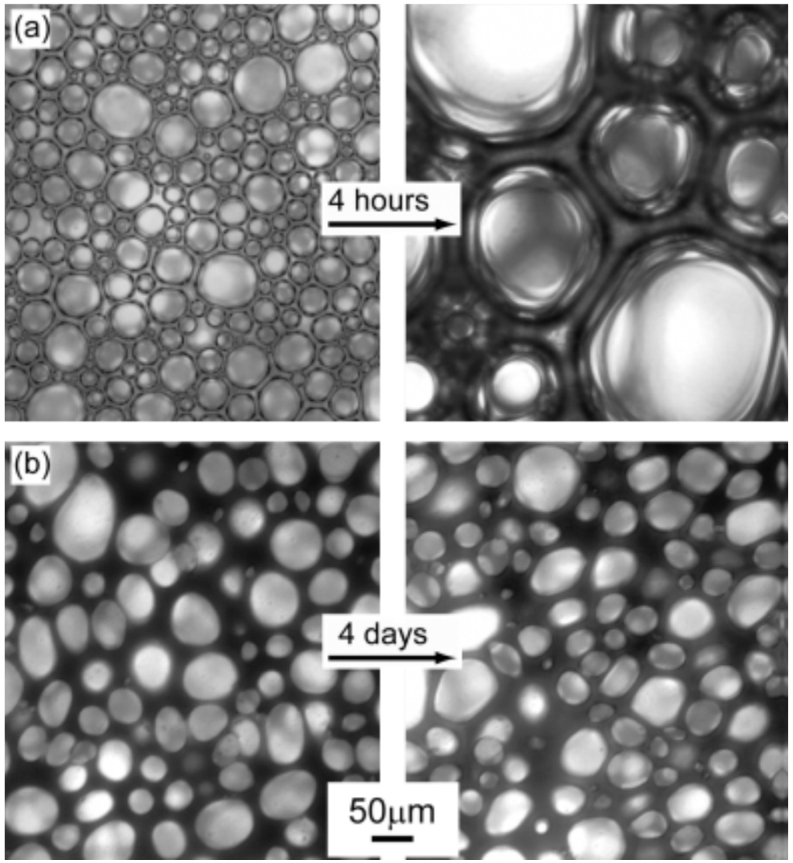
The stabilisation with colloidal particles produces macroporous ceramics with smaller pore sizes (10 to 100 µm instead of 35 to 1200 µm) .



particles with adsorbed short-chain amphiphilic molecules

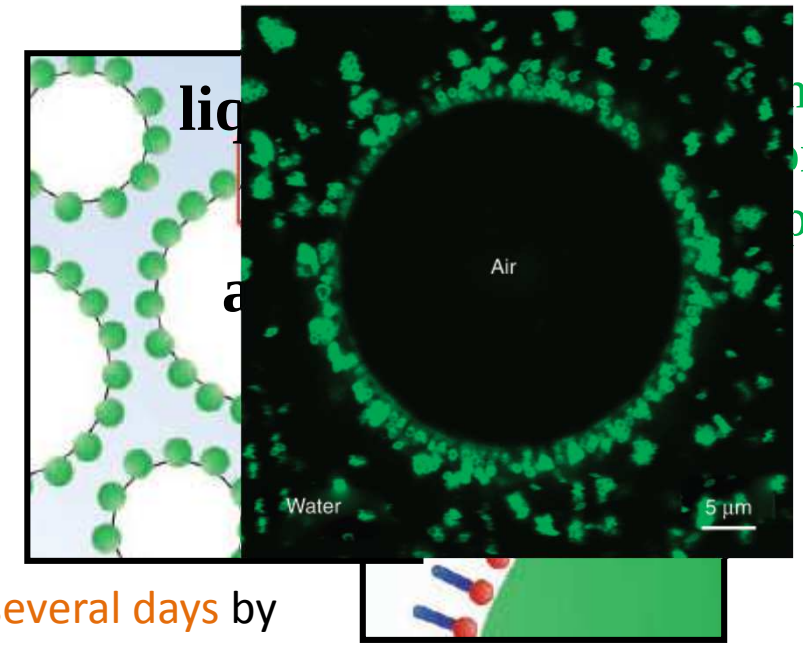
Direct foaming technique

Direct foaming with surfactant



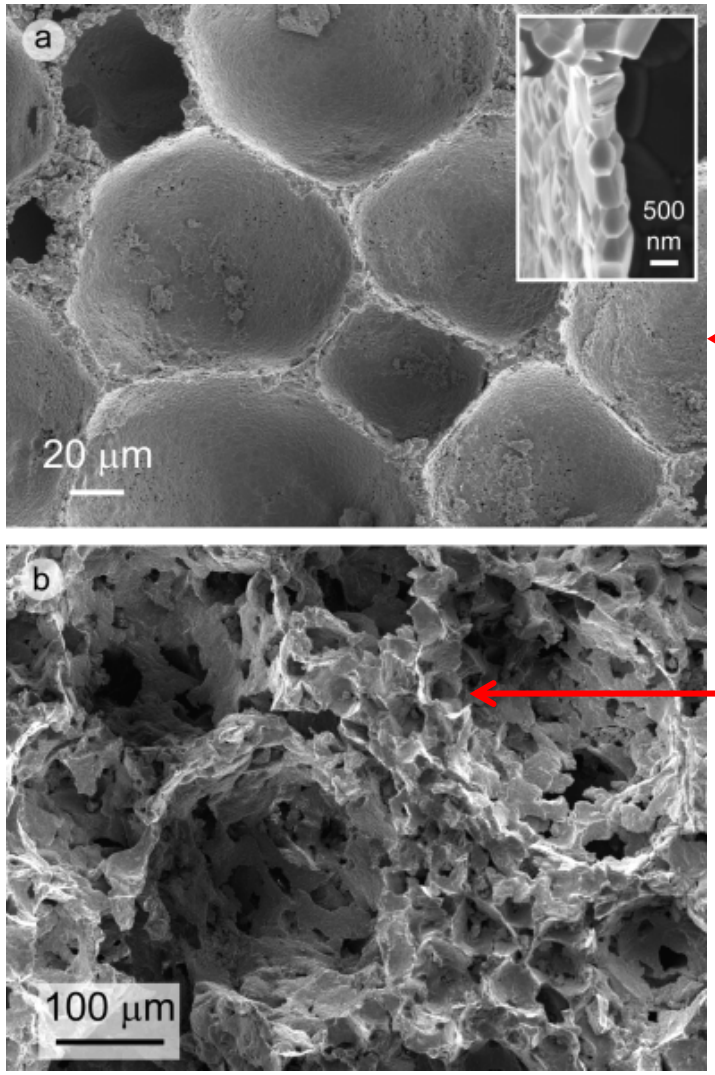
The foam lifetime can be increased to several hours by adsorbing long-chain surfactants.

Direct foaming with particles



The foam lifetime can be increased to several days by adsorbing colloidal particles in the air bubbles.

Direct foaming technique

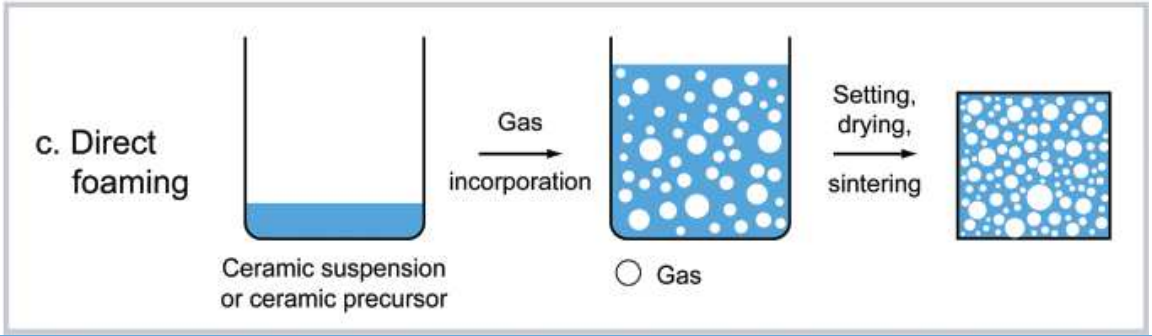


The direct foaming technique leads usually to **close porosity** (Fig a)

but **open porosity** ceramics can be obtained - by decreasing the concentration of stabilizing particles -or by adding minor amounts (<1wt%) of a sacrificial phase (e.g. graphite particles) (Fig b).

A.R.Studart et al JACS 89 [6]1771-1789 (2006)

Direct foaming technique



Direct foaming by gel casting technique

Another way to stabilise the bubbles is to gelify the slurry. Suspensions of CaP particles in water with dispersing agents and organic monomers are foamed by agitation with surfactant under a nitrogen atmosphere. In situ polymerisation of the monomers is initiated to provoke cross-linking and form a 3D polymeric network (gel) before casting. Porous samples are sintered. Foam volume (and hence porosity) could be controlled by the surfactant concentration in the slurry, producing pores of maximum diameter of 100–200 µm.

Direct foaming technique

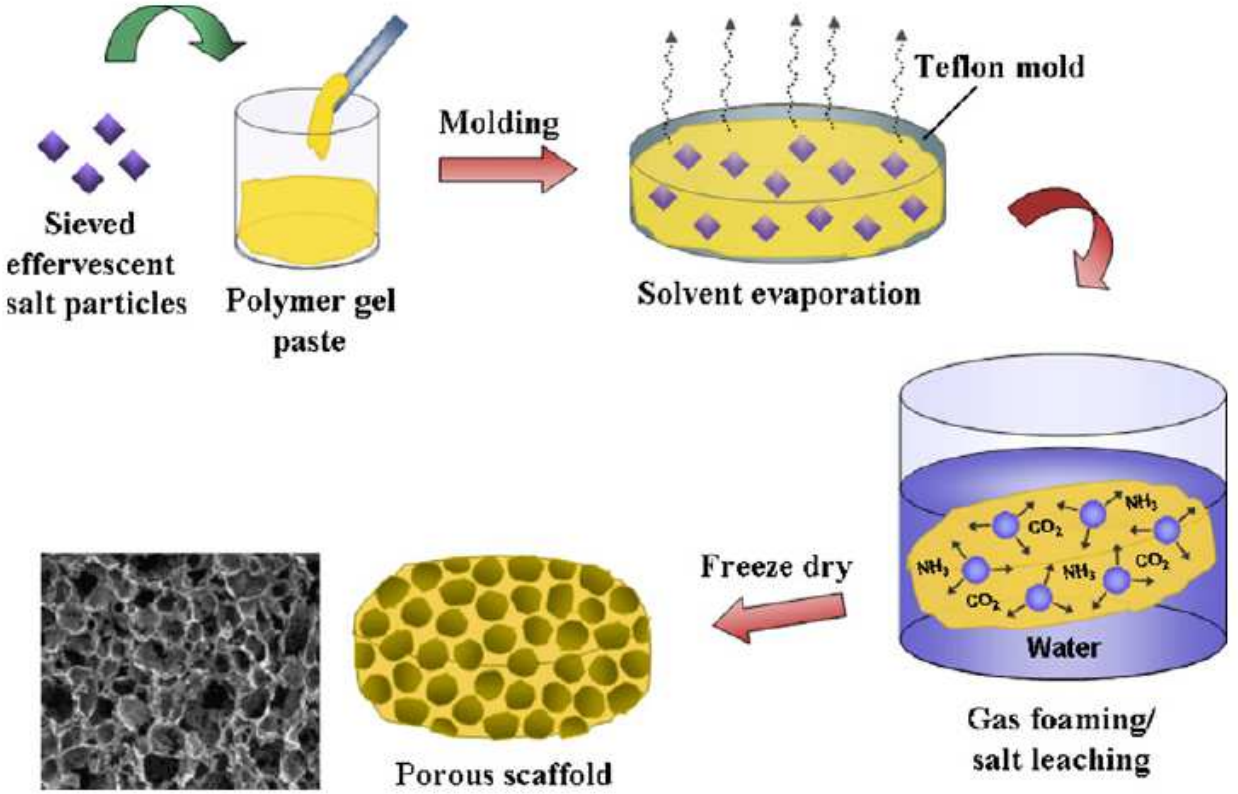
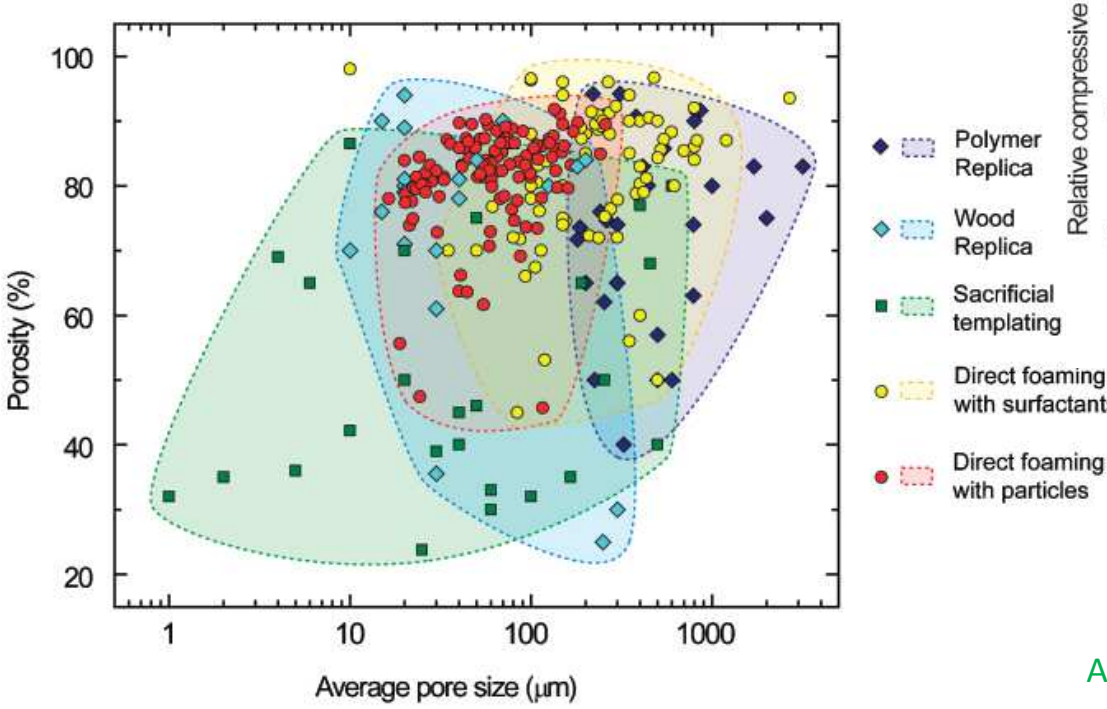
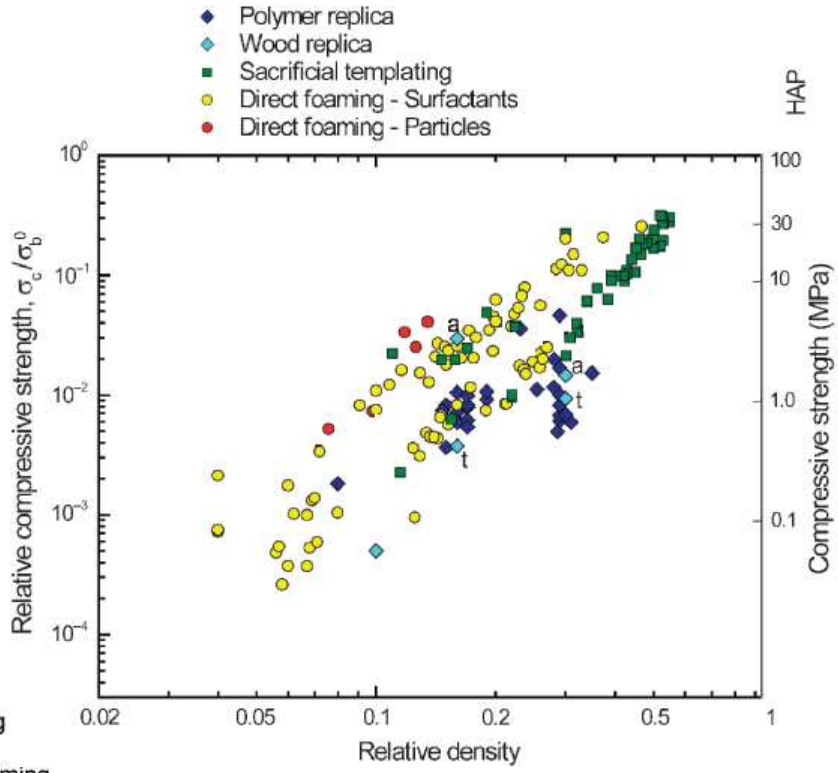


Fig: Fabrication of porous scaffolds by gas foaming/particulate leaching: Sieved effervescent salt particles are dispersed in polymer gel paste, cast on a Teflon mold for solvent evaporation, immersed in water for gas foaming/salt leaching, and freeze dried. (*Jung Chung 2007, Adv Drug Delivery Reviews*)

Scaffold Fabrication technologies

Comparison of porosity and compressive strength between various techniques





A.R.Studart et al JACS 89 [6]1771-1789 (2006)

Scaffold Fabrication technologies

	Method	Porosity (%)	Pore size (μm)	Pore size distribution	Pore shape	Space distribution
Replica	PU sponge	40 to 95	150 to 1300	Wide	Random	Anisotropic
	Coral	20 to 50	150 to 500	Wide	Random	Anisotropic
	Wood	25 to 95	10 to 300	Trimodal	Elongated	Columnar
Sacrificial templates	PMMA beads	25 to 90	250 to 1000	Monomodal or multimodal	Spherical	Isotropic
	Freeze foaming	50 to 95	2 to 90	Bimodal	Spherical	Isotropic
	Ice-templating (freeze casting)	30 to 65	5 to 200 width 10 to 500 length	Monomodal	Ellipsoidal	Columnar
Direct foaming	With surfactant	40 to 95	30 to 1000	Wide	Spherical	Isotropic
	With particles	40 to 90	20 to 300	Wide	Spherical	Isotropic
	Gel casting	40 to 90	100 to 1000	Wide	Spherical	Isotropic

5. 3D-Additive manufacturing technique

3D Additive manufacturing technique consists of production of highly complex 3D objects using data generated by computer aided design (CAD) systems.

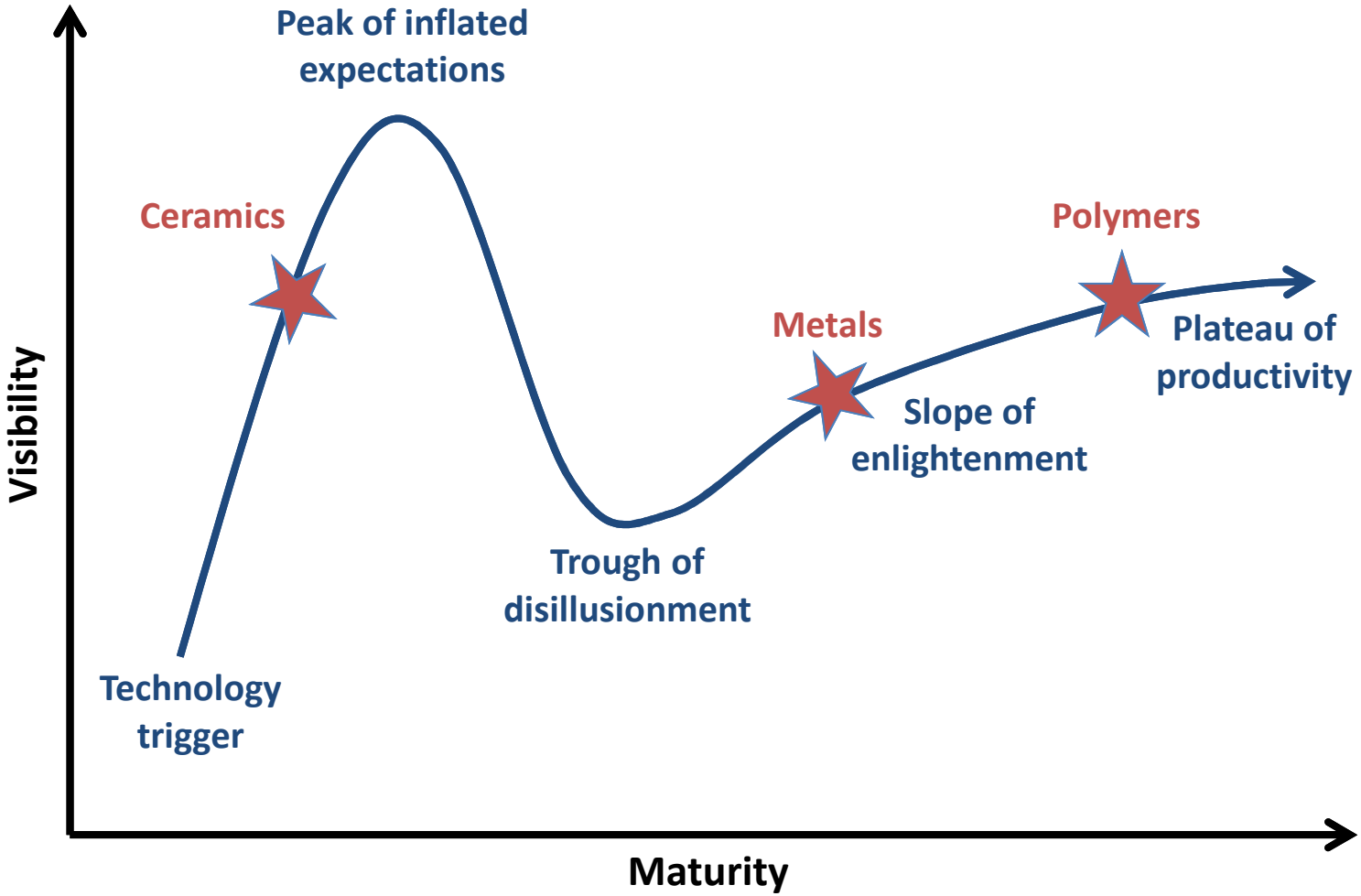
An image of a defect in a patient can be taken (e.g. by X-ray microtomography, CT scan), which is used to develop 3D CAD computer model. The computer can then reduce the model to slices or layers.

The 3D objects are constructed layer-by-layer using rapid prototyping techniques :

- Paste extrusion techniques,
- Selective laser sintering,
- Binder jetting,
- Stereo lithography.

These techniques are traditionally applied to polymers and recently extended to ceramics.

Additive Manufacturing



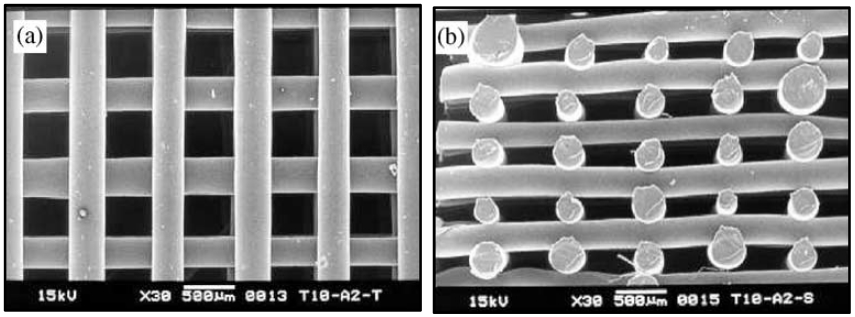
1.Paste extrusion

FDM : fused deposition modeling

- Incandescent material extruded through a nozzle
- The solidification of each layer takes place instantly in contact with the previous one

3DPlot : threedimensional-plotting

Liquid or paste extruded through a mobile head, using compressed air

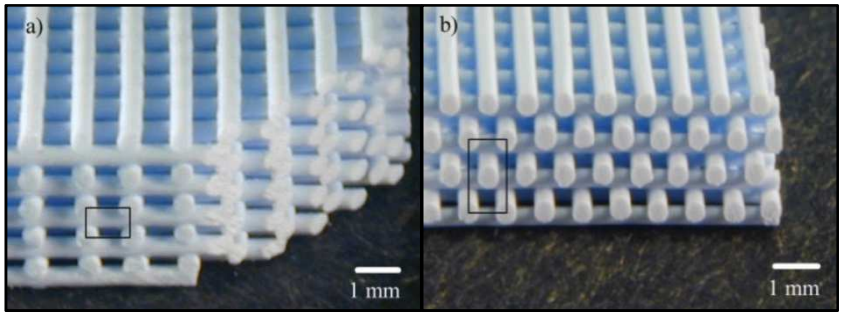


Polymer *[Zein et al., Biomaterials. 2002;23:1169–85]*

MJS : multiphase jet solidification

- Binder-powder mixture heated and extruded through a nozzle by a pumping system
- The nozzle scans horizontally to deposit the melting loading

[Kupp et al., Proceedings of the SFF Symposium. 1997]

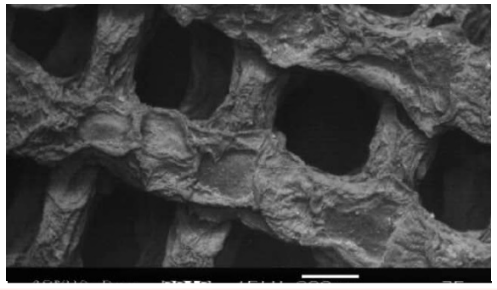


Hydroxyapatite

[Dellinger et al., J Biomed Mater Res. 2007;82A:383–94]

ROD : robotic dispensing

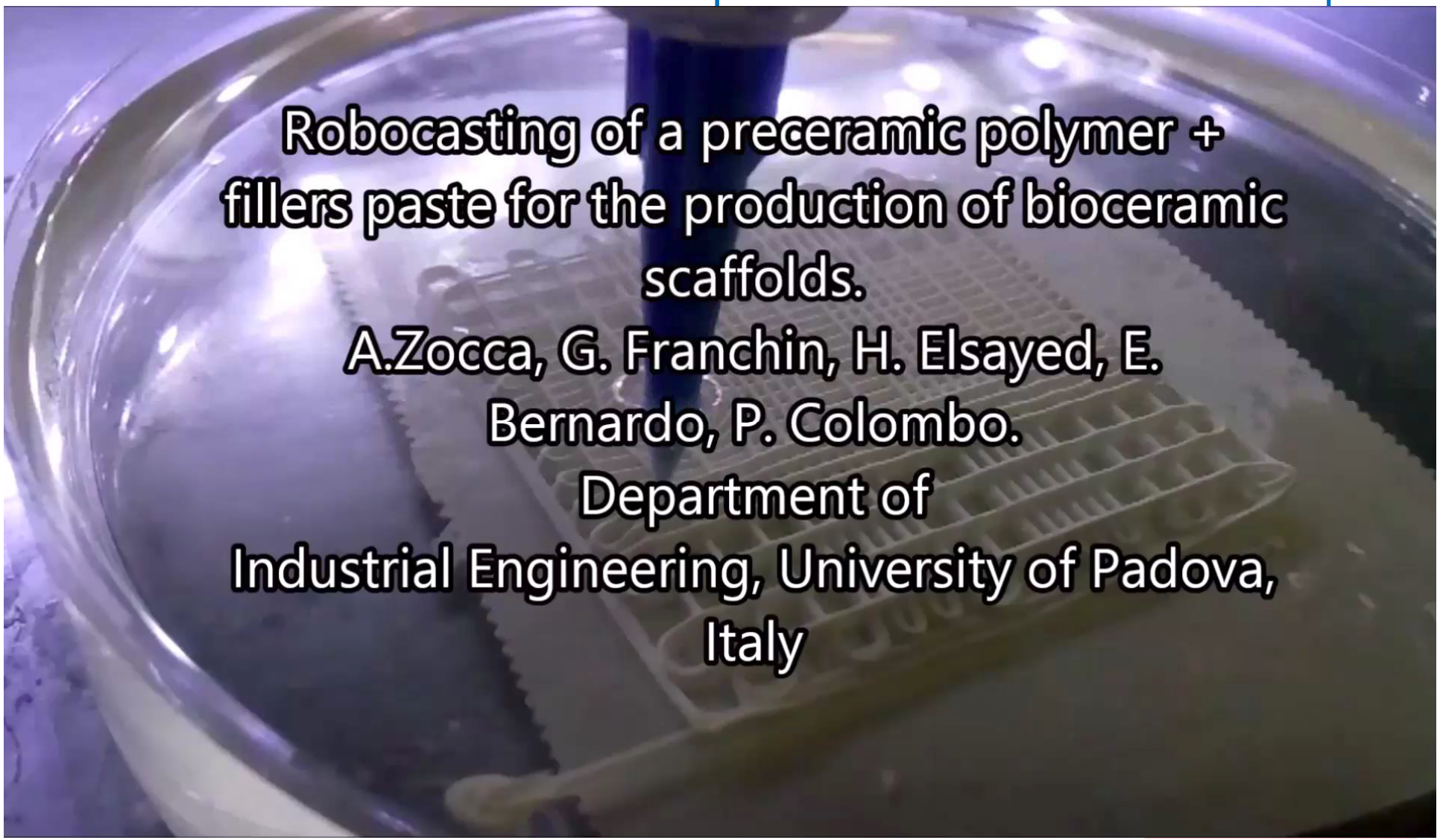
- Ejection of a slurry in a solvent to induce precipitation
- Freezing and lyophilizing



HA/Chitosan

[Ang et al., Mater. Sci. Eng. 2002;20:35–42]

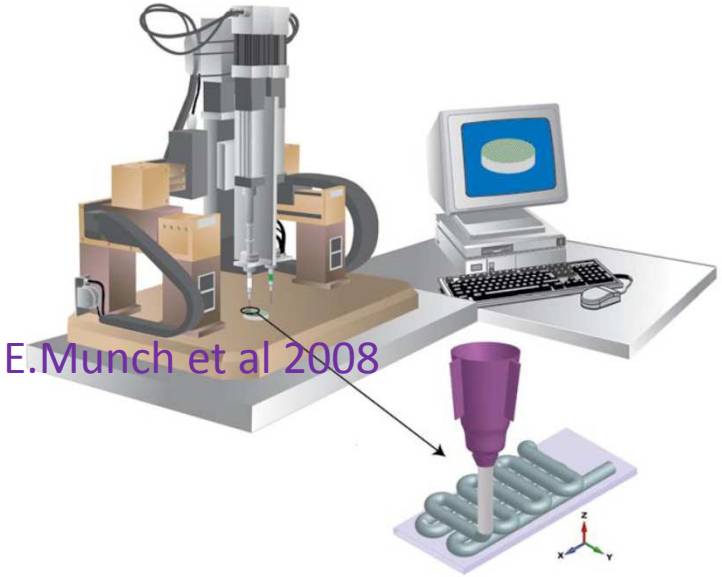
Robocasting



Paste extrusion technique

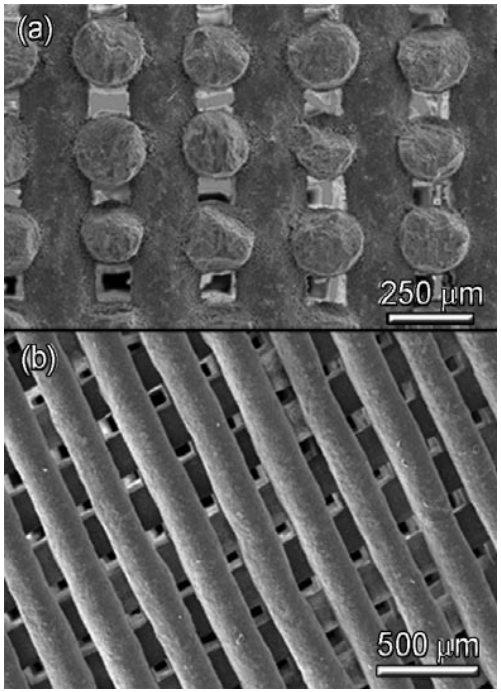
Robocasting

Robotic-assisted deposition consists of the robotic deposition of inks capable to fully supporting their own weight during assembly.



E.Munch et al 2008

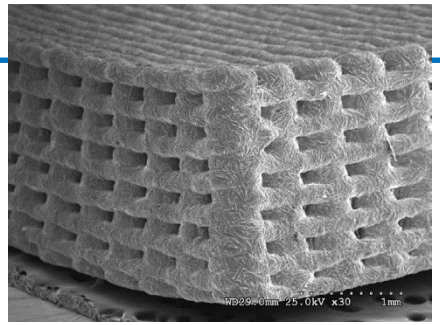
Cross section



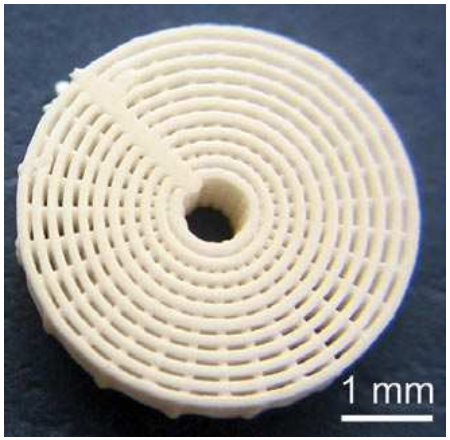
Surface

Strut thickness : 200 to 500 µm

Line spacing : 75 to 500 µm



β-TCP scaffold *(P. Miranda González)*



HA part with a gradient in porosity after printing and sintering (P=45%, σ_c = 25-40 MPa)

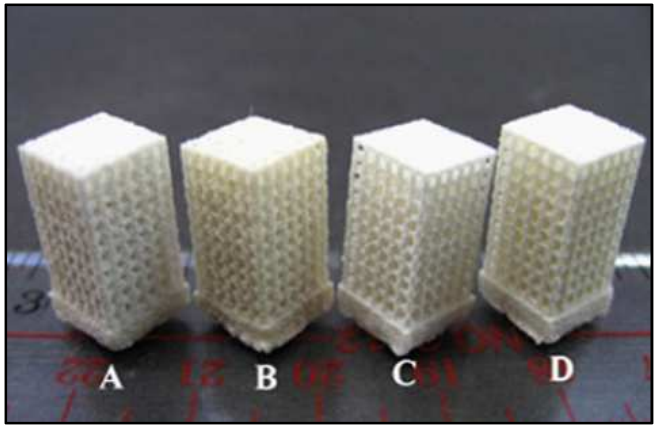
2.Selective laser consolidation

SLS : selective laser sintering

- A laser beam scans the surface of a powder bed, mixed with a binder

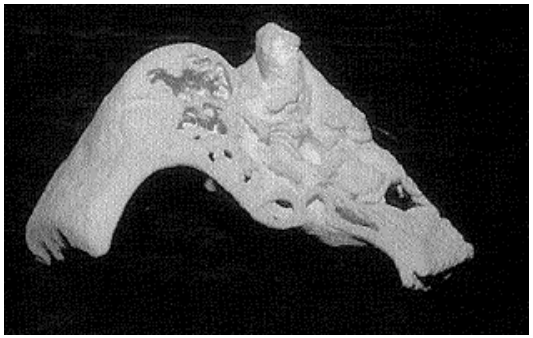
  → formation of a layer of material by selective sintering

- The non-sintered powder is then removed by brushing and / or blowing



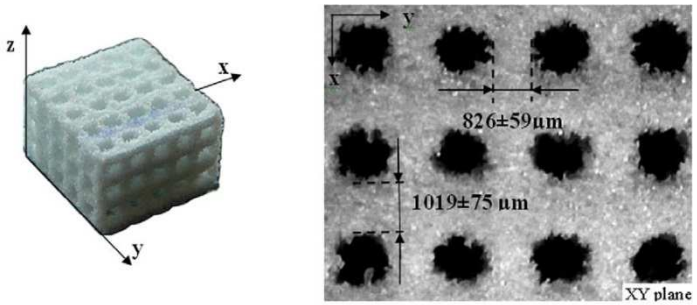
Polymer ; CaP ; polymer/CaP composite

[Duan et al., Acta Biomater. 2010;6:4495–505]



CaP craniofacial implant

[Lee et al., Proc. Solid Free. Fabr. Symp. 1994;191–7]



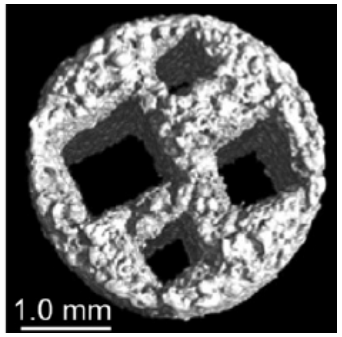
Polymer/HA composite

[Eosoly et al., Acta Biomater. 2010;6:2511–7]

2.Selective laser consolidation

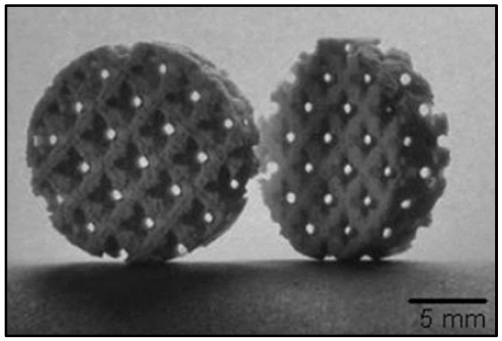
SLM : selective laser melting

The powder is melted under the laser irradiation



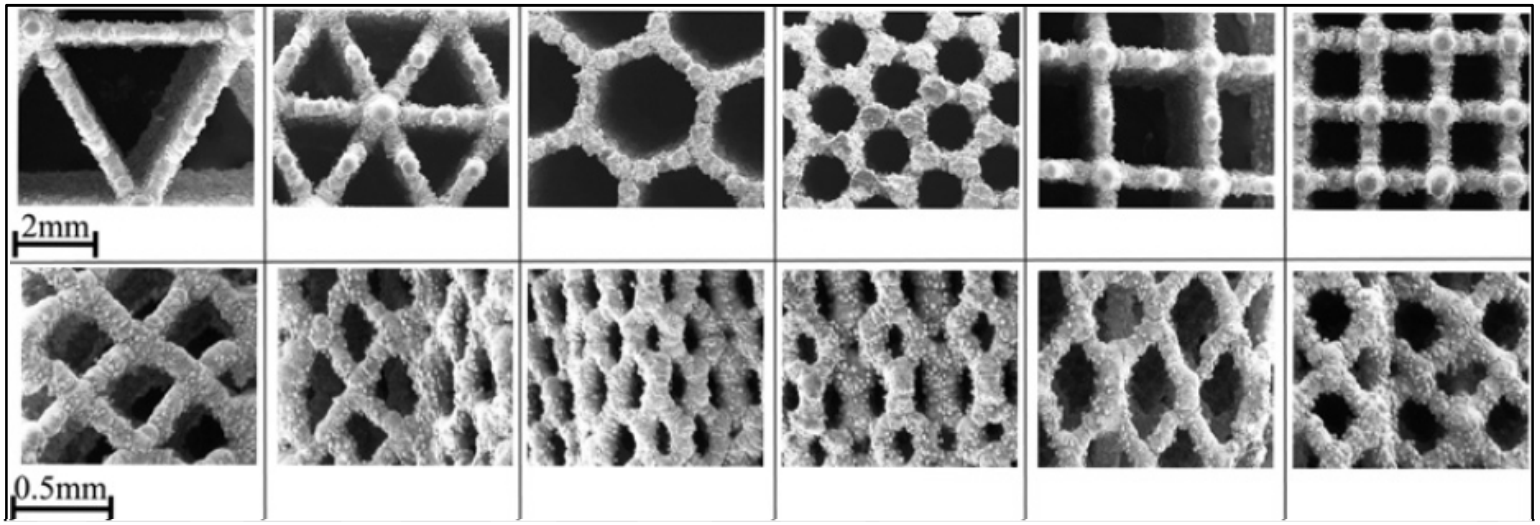
Titanium

[Fukuda et al., Acta Biomater. 2011;7:2327–36]



β-TCP / PDLLA composite

[Lindner et al., J. Biomed. Mater. Res. A. 2011;97:466–71]



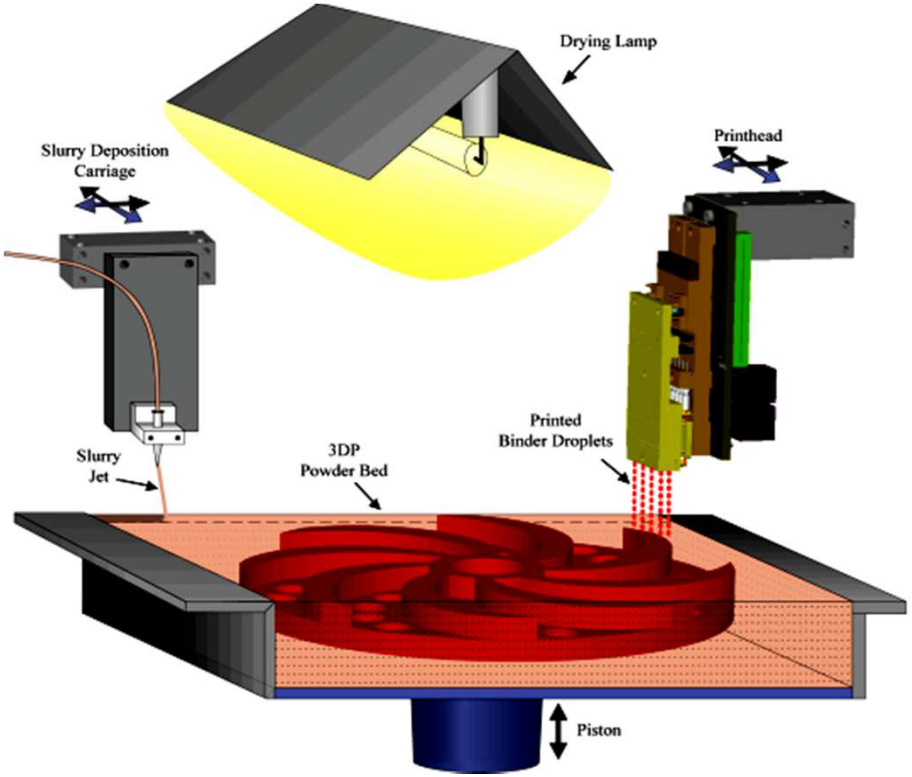
Ti-6Al-4V

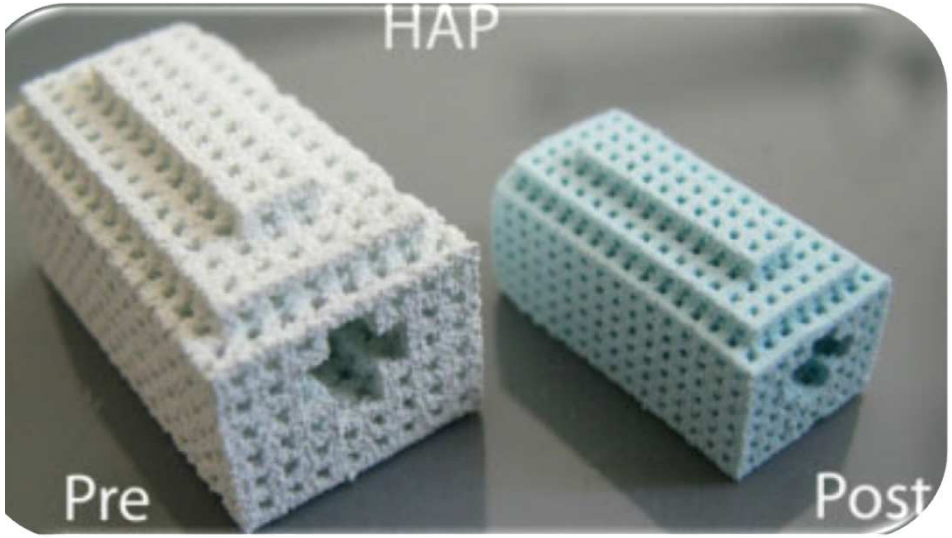
[Van Bael et al., Acta Biomater. 2012;8:2824–34]

3.Binder jetting

- Binder jetting – a liquid bonding agent is selectively deposited to consolidate a powder bed

3DP : threedimensional-printing





Before sintering

Hydroxyapatite

After sintering

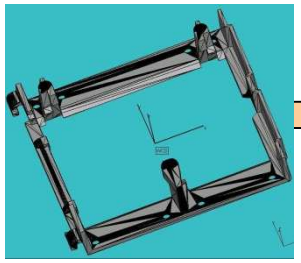
[Warnke et al., J. Biomed. Mater. Res. B Appl. Biomater. 2010;93:212–7]

4.Stereolithography (SLA) and microstereolithography (μ-SLA)

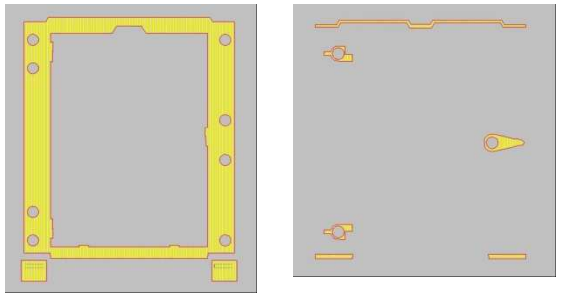
suspension of ceramic particles in UV sensitive monomer/oligomer

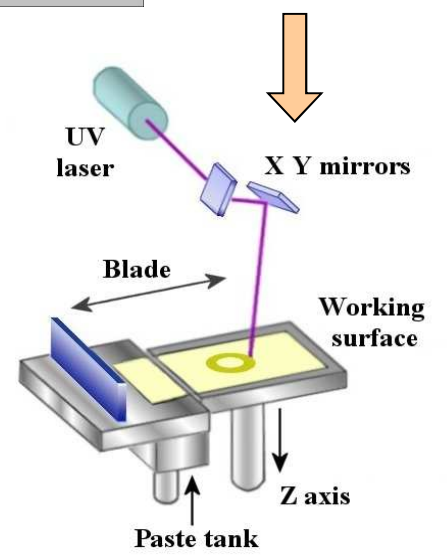
Creation of CAD 3D file of the part

Creation of .STL file

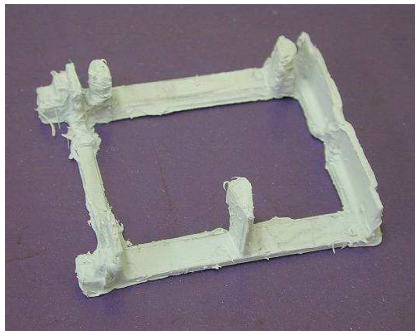


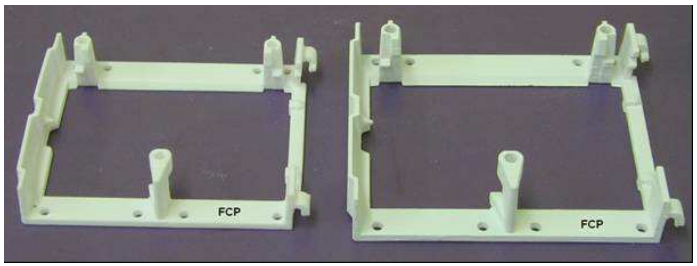
.STL file sliced in cross-sectional layers (interplane spacing = deposited layer thickness)





Cleaning



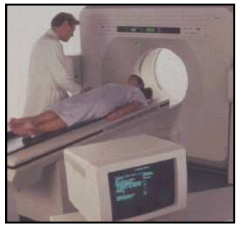


Sintered part **Green part**

SL: resolution 80 μm
μ-SL: resolution 10 μm

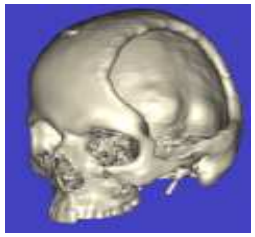
4.Stereolithography (SLA) and microstereolithography (µ-SLA)

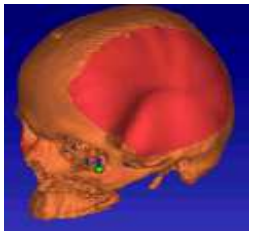
HAP Biactive Implant



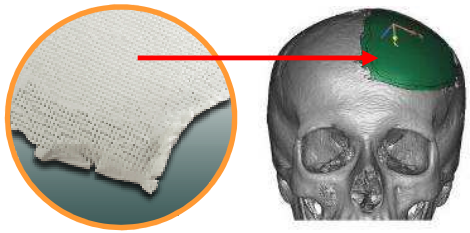
Scanner data of the patient

Simulation / creation of the implant Surgeon demand



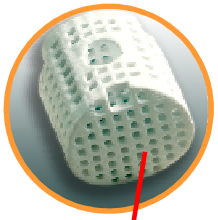


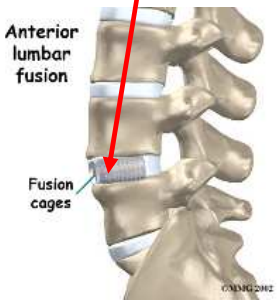
3D implant realized from scanner data

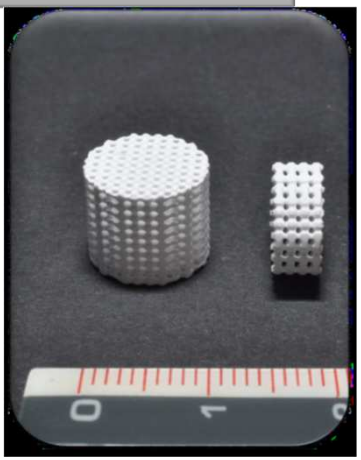


3DCERAM Advanced Ceramics

Intervertebra bone substitute







Lithoz GmbH

Occular implant

HAP parts implanted at Limoges Hospital

CHU Centre hospitalier universitaire Limoges

3D AM techniques	Tolerance	Advantages	Limitations
Material extrusion	0.5 to 1mm	- Ease of support removal - Good mechanical properties - No material waste	- Precision limited by the filament diameter (about 1mm)
Binder jetting	0.05 to 0.1mm	- Wide variety of materials - Simple technology	- High roughness of the surface - Expensive technology - Poor mechanical properties - Use of toxic organic binders
Selective laser consolidation	0.2 to 0.5mm	- High production rates possible - Complex designs - Low costs - Good surface finishing	- High roughness of the surface - Poor mechanical properties - Limited to materials which absorb IR light
Stereolithography	0.01 to 0.1mm	- Complex designs - Good surface finishing - Good mechanical properties - High accuracy	- Expensive photosensitive resins - Cleaning step necessary - Control of the vertical accuracy

Review: Additive Manufacturing to Produce Complex 3D Ceramic Parts
T. Chartier, C. Dupas,M. Lasgorceix, J. Brie,E. Champion,N. Delhote, Chr. Chaput

J. Ceram. Sci. Tech., **xx** [xx] xx (2015)
DOI: 10.4416/JCST2014-00040

How to mimic natural bone for substitute processing?

Part I: Requirements for ideal scaffolds

Part II: Fabrication technologies

Many techniques are today employed to produce macroporous bioceramics with varying structural and mechanical properties.
Most of them are already commercially used.
The more recent 3D-manufacturing methods are promising to producing specific interconnected scaffold architectures with various pore size and morphologies and pore size gradient, not achievable by the usual techniques .

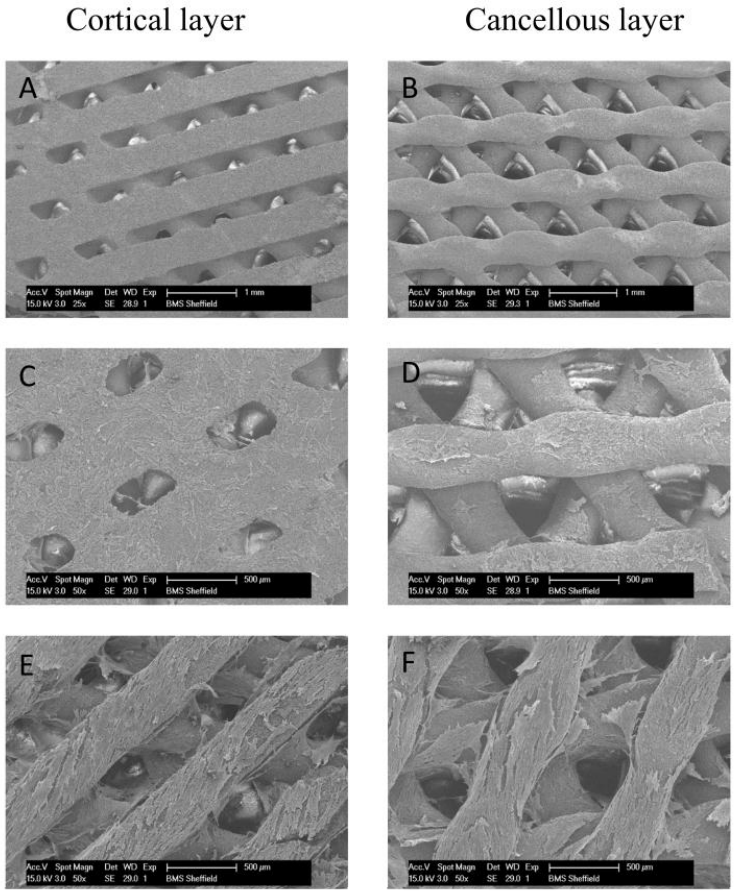
How to mimic natural bone for substitute processing? 2nd Porosity architecture

✓ Porosity gradient

3D- printing

TCP paste was extruded from Simulation of cortico-cancellous bone structure by 3D printing (3D bio plotting system EnvisionTEC, Germany) of bilayer calcium phosphate-based Scaffolds

Thafar Almela et al Bioprinting 2017
DOI: http://dx.doi.org/10.1016/j.bprint.2017.04.001

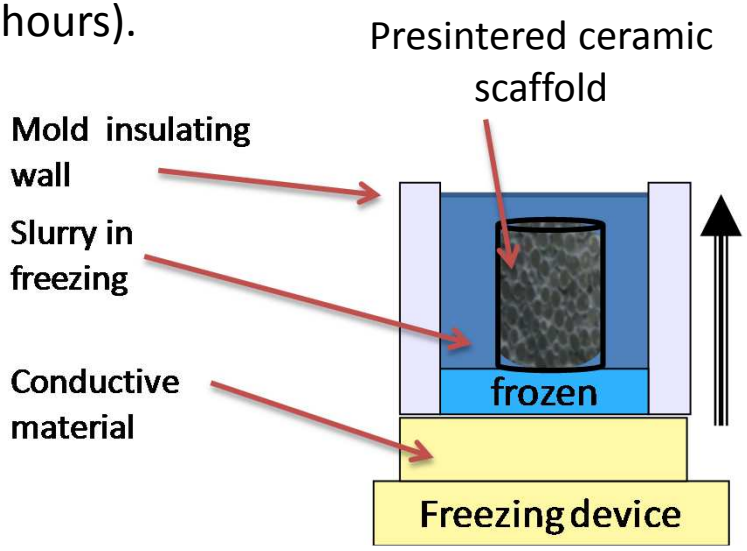


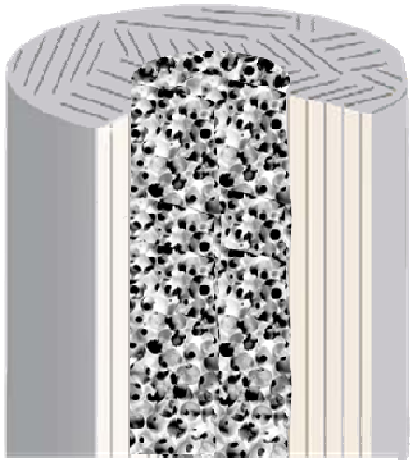
How to mimic natural bone for substitute processing? 2nd Porosity architecture

✓ Porosity gradient

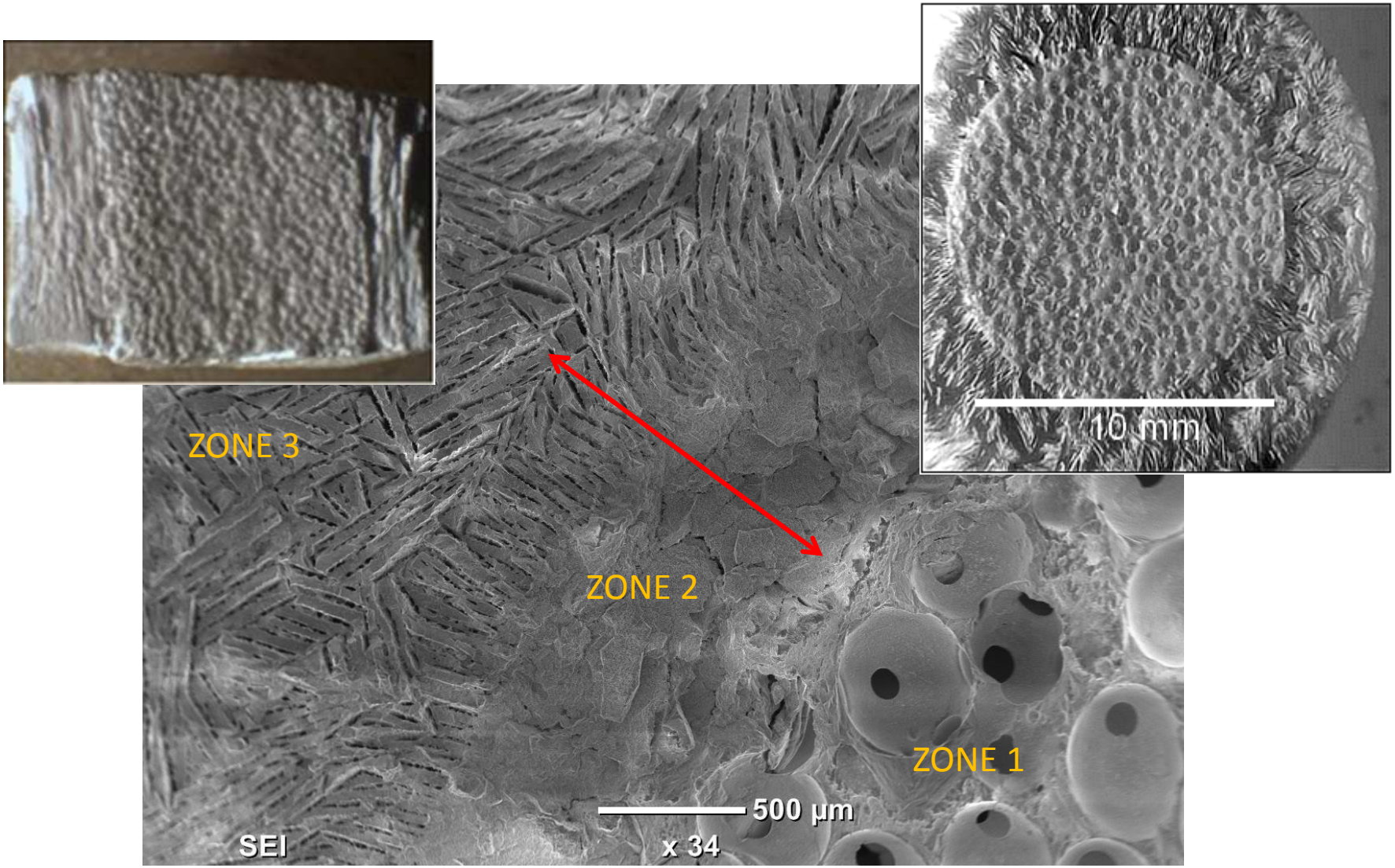
Freeze casting by ice-templating + PMMA route

•Add few mL of slurry inside the mould at -20°C
• Wait few minutes to freeze and place at the centre the pre-sintered ceramic scaffold.
• Wait until it is blocked by ice before adding more slurry and wait for a total freezing (24 hours).





Freeze casting by ice-templating + PMMA route



S.Chamary et al Journal Ceramics International 2017

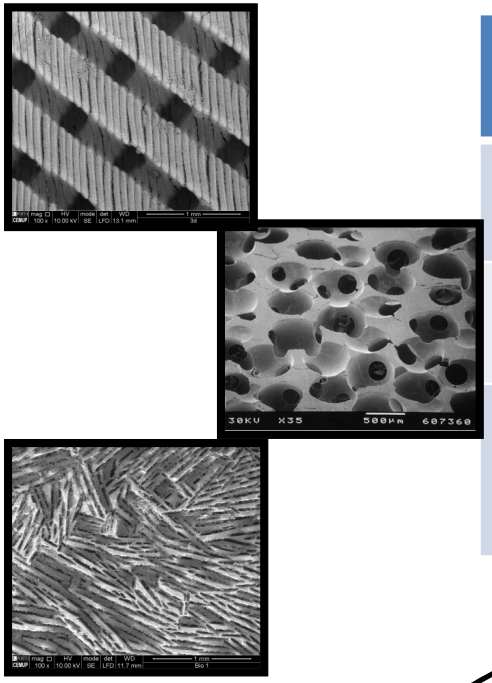
How to mimic natural bone for substitute processing?

Part I: Requirements for ideal scaffolds

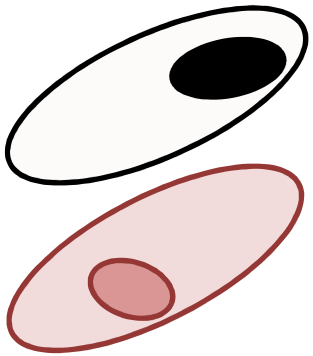
Part II: Fabrication technologies

Part III: Example of comparative study on cell colonization inside ceramic scaffolds presenting different architectures

Objectives: Study of influence of β-TCP porous architecture on cell invasion, proliferation and osteogenesis



Samples	Shaping techniques	Porosity (%)	Ø pore and interconnexion (µm)
3D	stereo lithography	50	500 / 100
PS	Polymer	65	400 – 500 / 100
BIO 1 BIO 4 BIO 7	Freeze casting	50 50 36	150 / 40 360 / 55 150 / 45

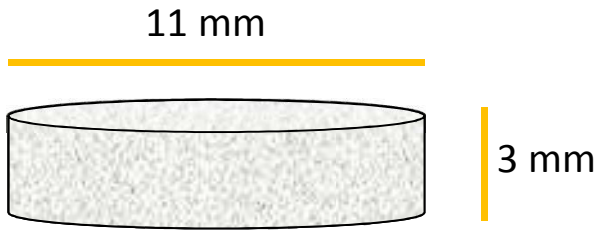


MG63
Osteoblasts

HMSC
Osteoprogenitors

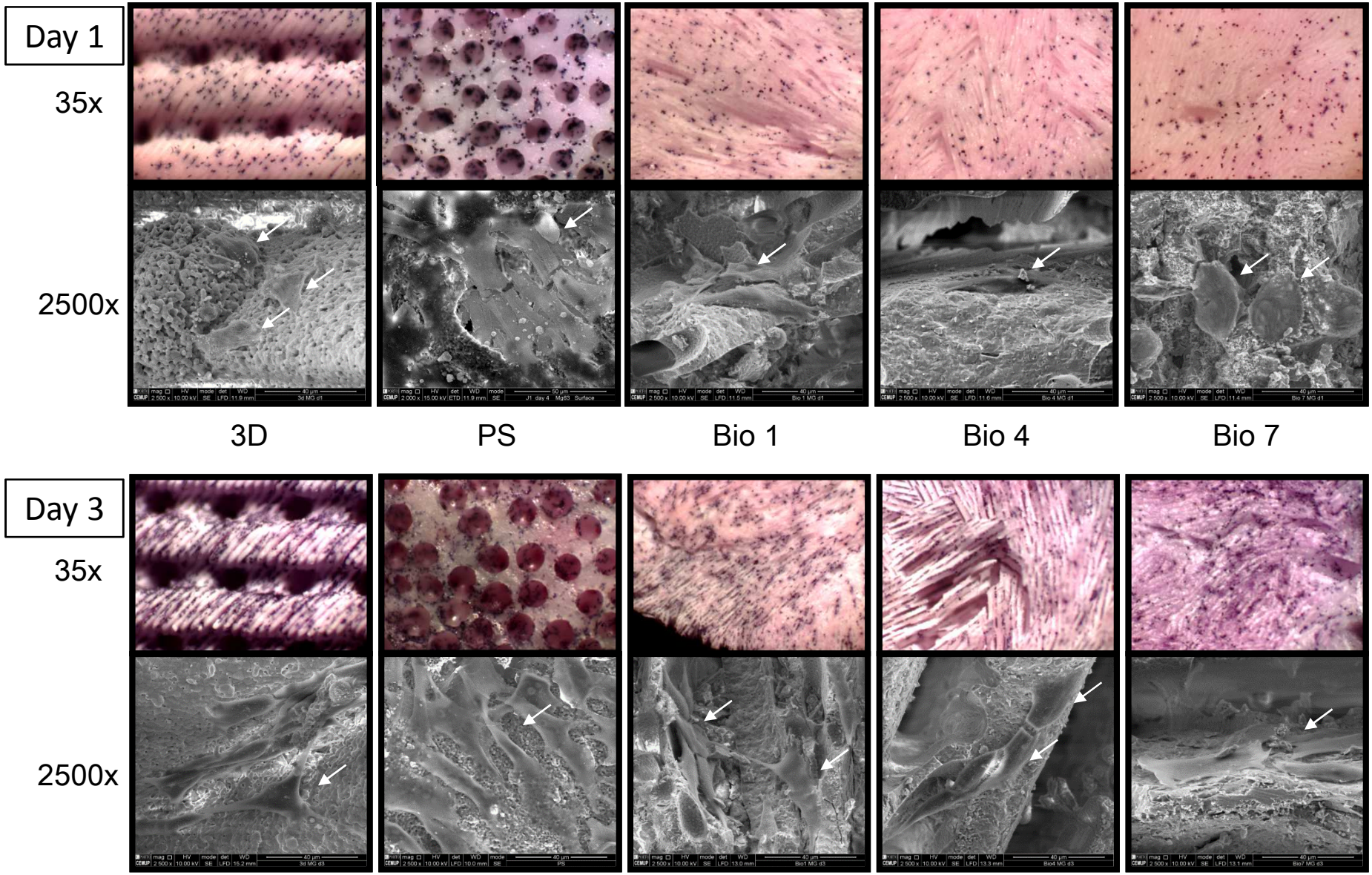
2×10^5 cells/well

2×10^4 cells/well



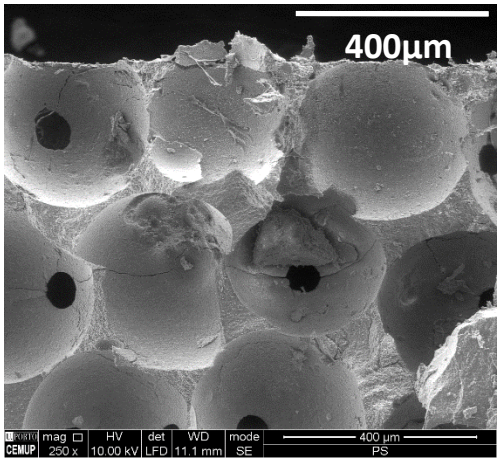
- Cell morphology: OM and SEM and Cell activity : MTT et Resazurine
- Proteic activity: alkaline phosphatase – biochemical marker of cellular differenciation

Cell colonization with MG63 osteoblasts

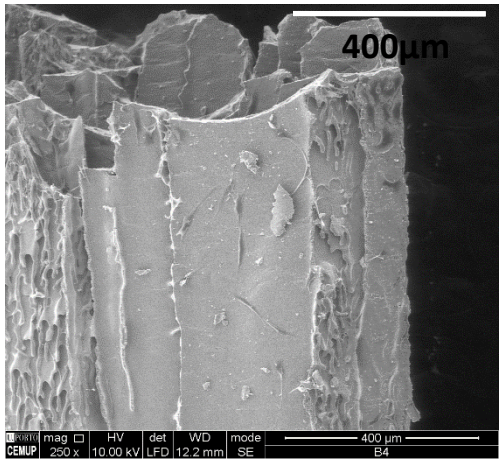


4 days

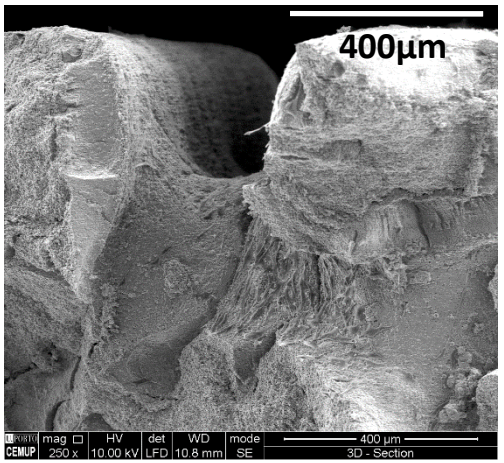
PMMA beads
450µm

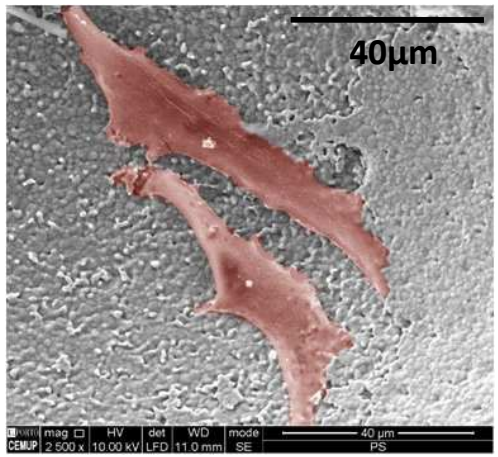


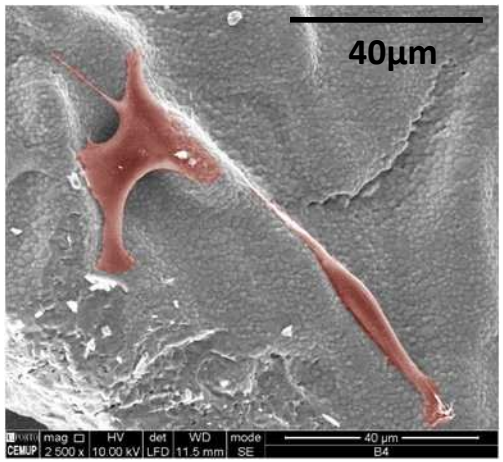
Ice-templating
366/54 µm, P= 51%, t=52µm

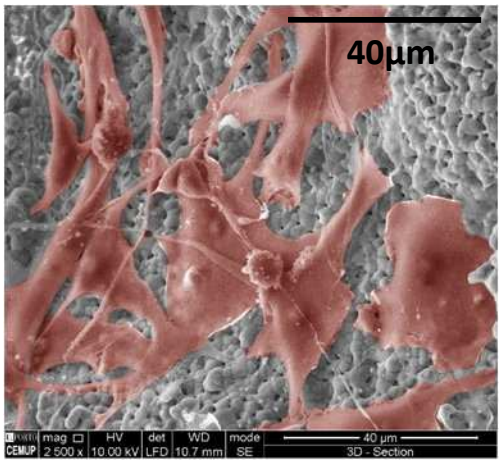


Stereolithography
450µm









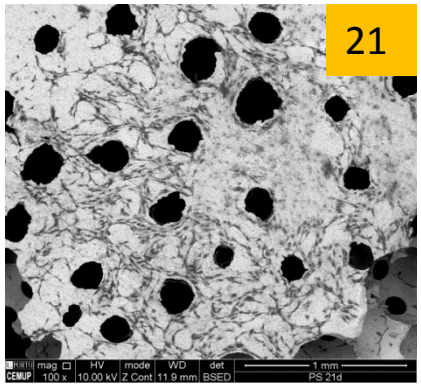
HMSC
Osteoprogenitors

Proliferation

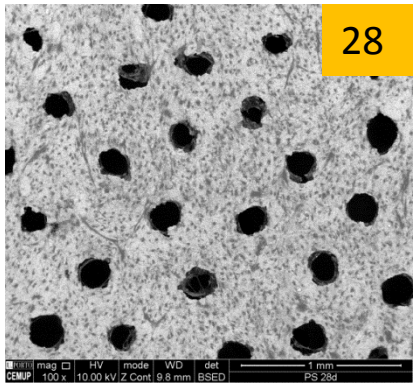
PS

400-500
Id=100
65%

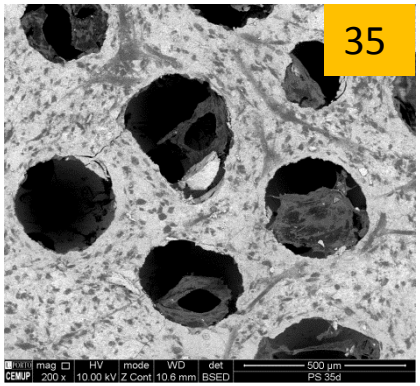
100x



100x



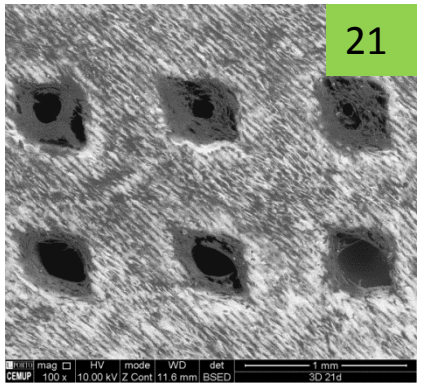
200x



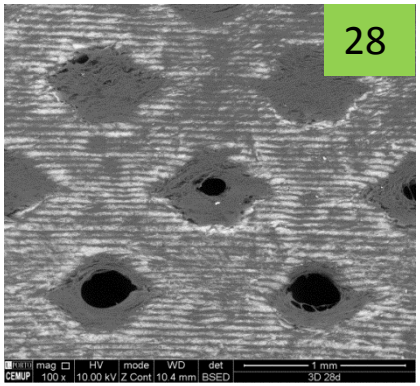
3D

500 µm
Id=100
50%

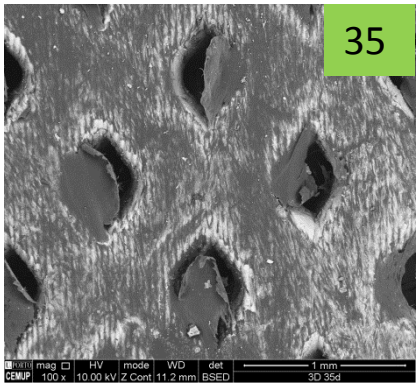
100x



100x

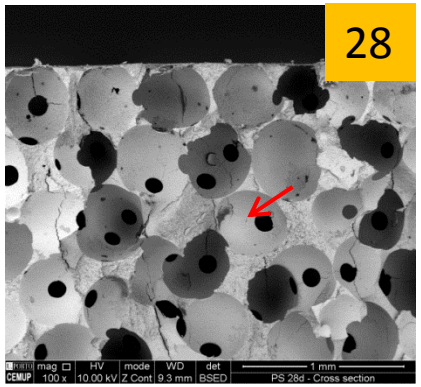


100x

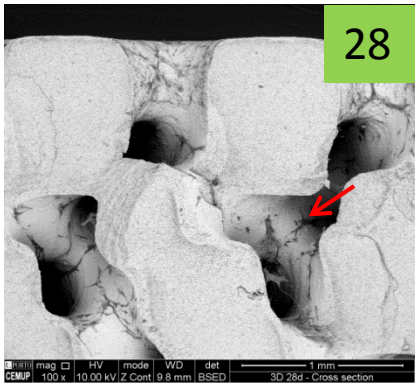


Penetration

100x

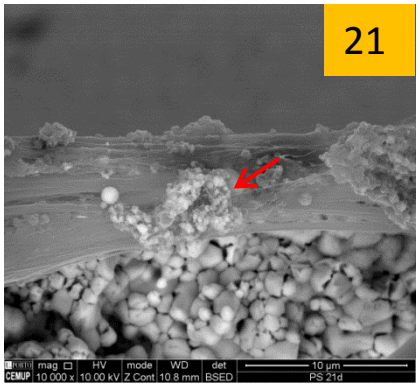


100x



Mineralisation

10000x



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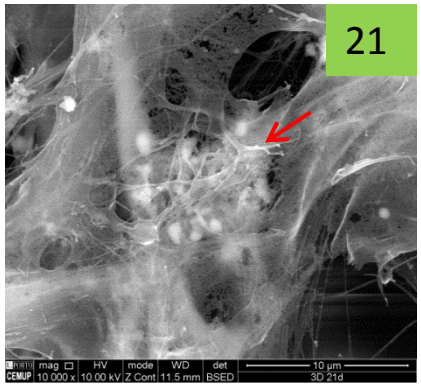


HMSC Osteoprogenitors

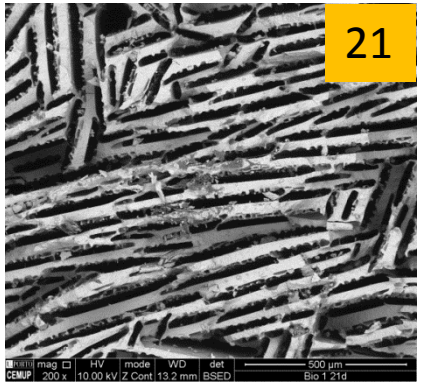
Proliferation

Penetration

Bio1

150/40
50%

200x



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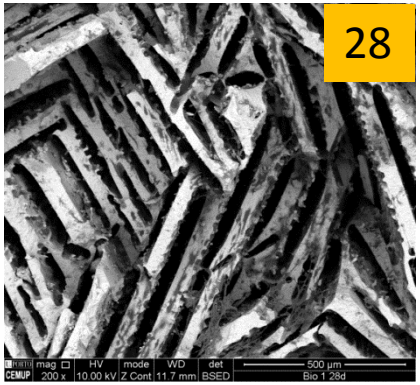

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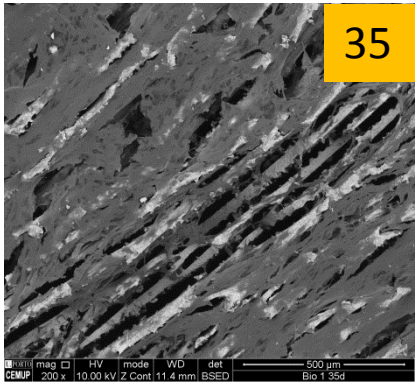
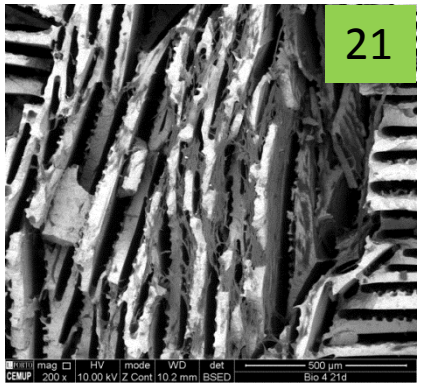


200x

Bio4

360/55
50%

200x



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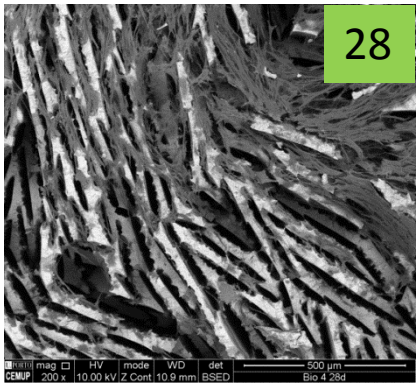

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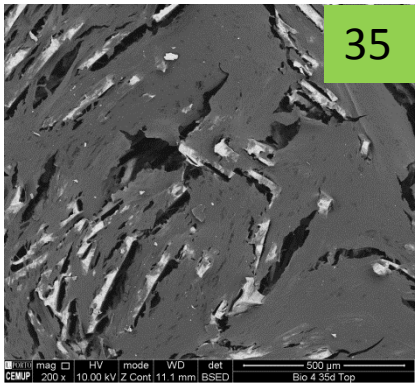

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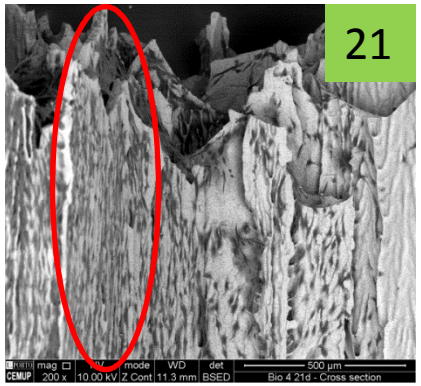
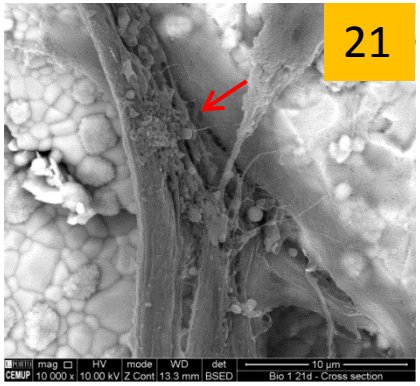


Mineralisation

10000x



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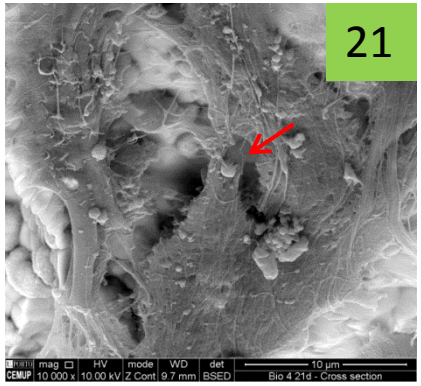


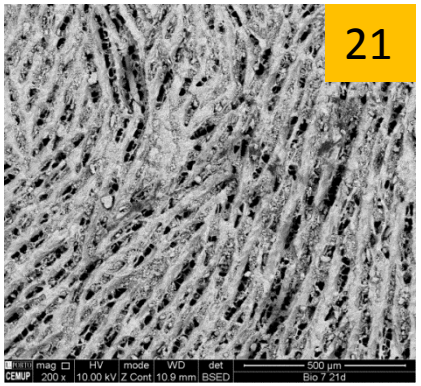
HMSC Osteoprogenitors

Cell proliferation

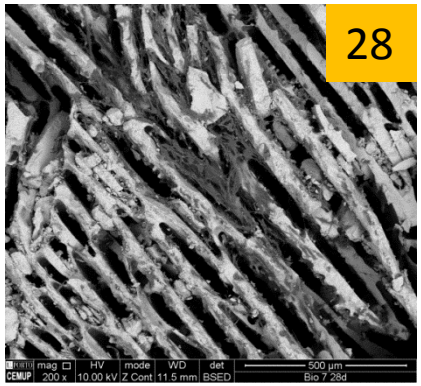
Bio7

150/45 µm
36%

200x



200x



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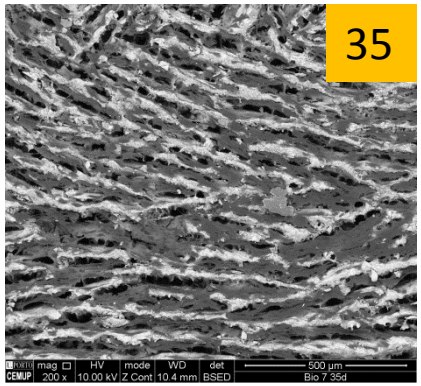
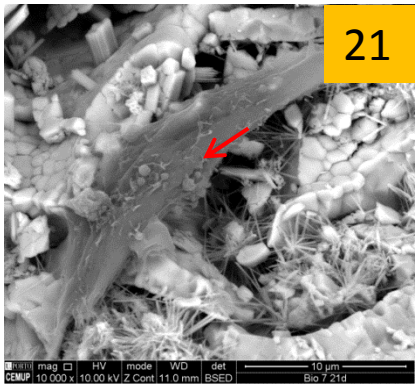


Mineralisation

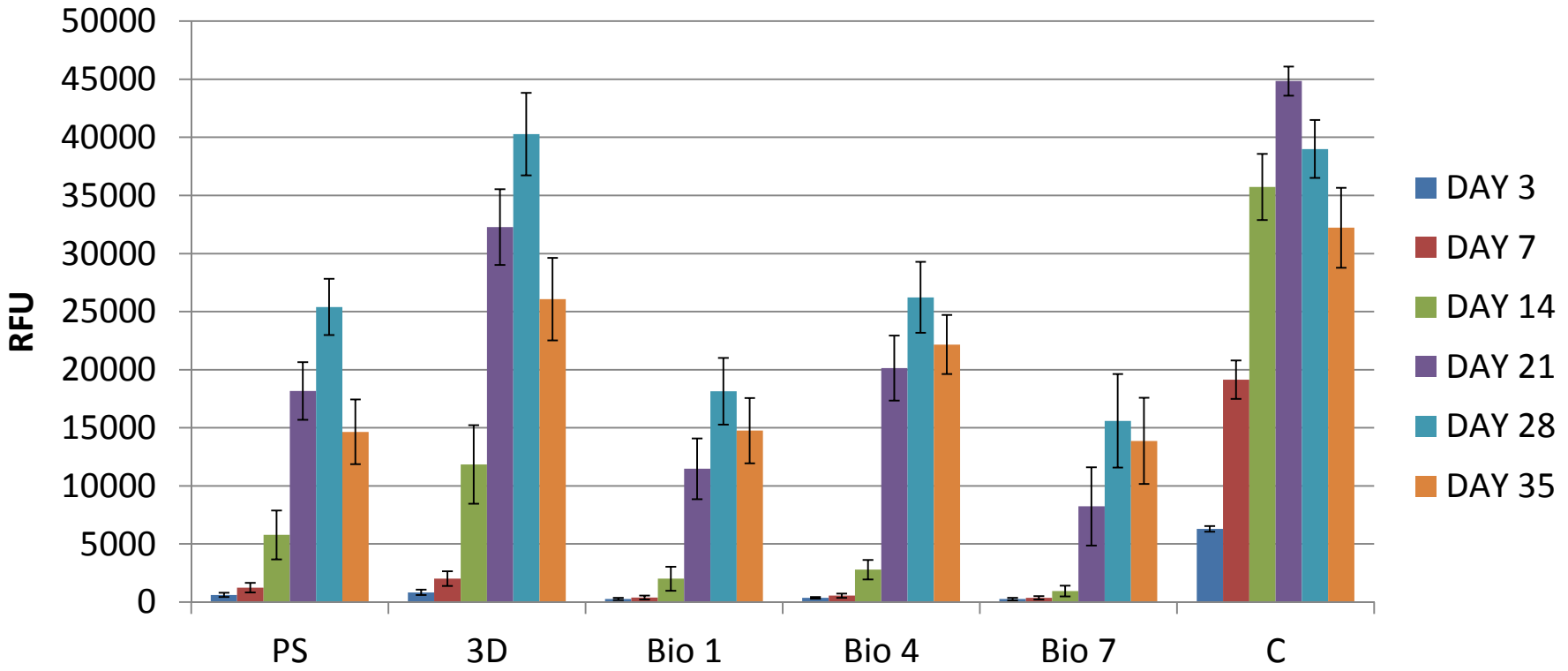
10000x



Cell penetration:
- not visible after 21 days
- Penetration starting at day 28 , increase at day 35 but much lower than for the other freeze cast scaffolds

Human Mesenchymal stem cells

- Cellular proliferation (Resazurine)

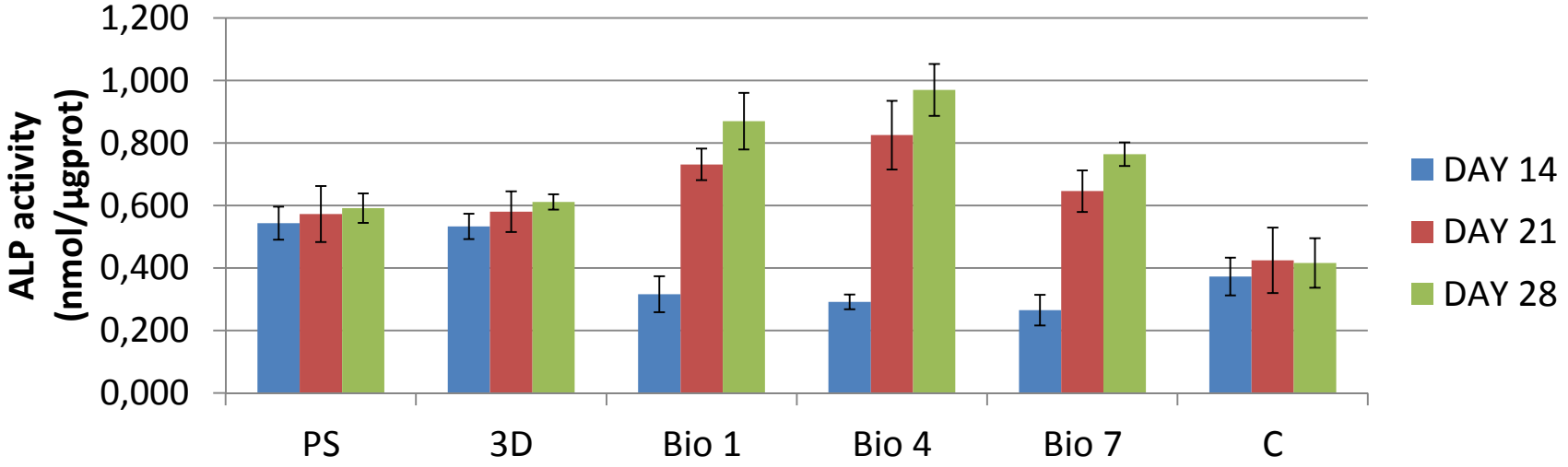


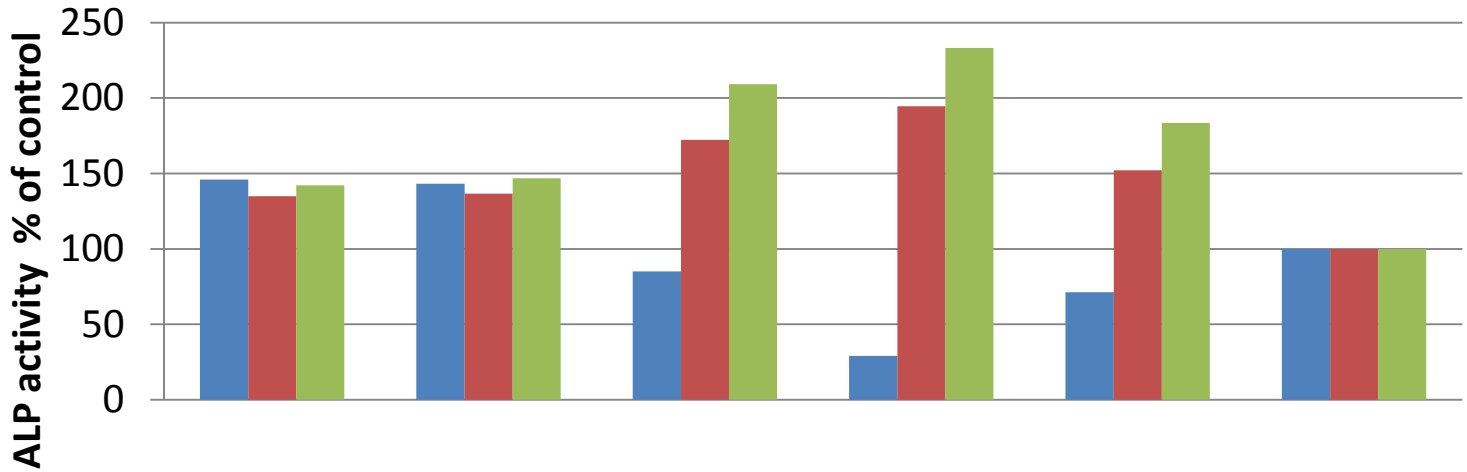
-Latence period followed by regular growth

-Confluence reached at day 28 for all the samples and at day 21 for the control

-PS and 3D are very good substrates for cell culture

Alkaline phosphatase - HMSC





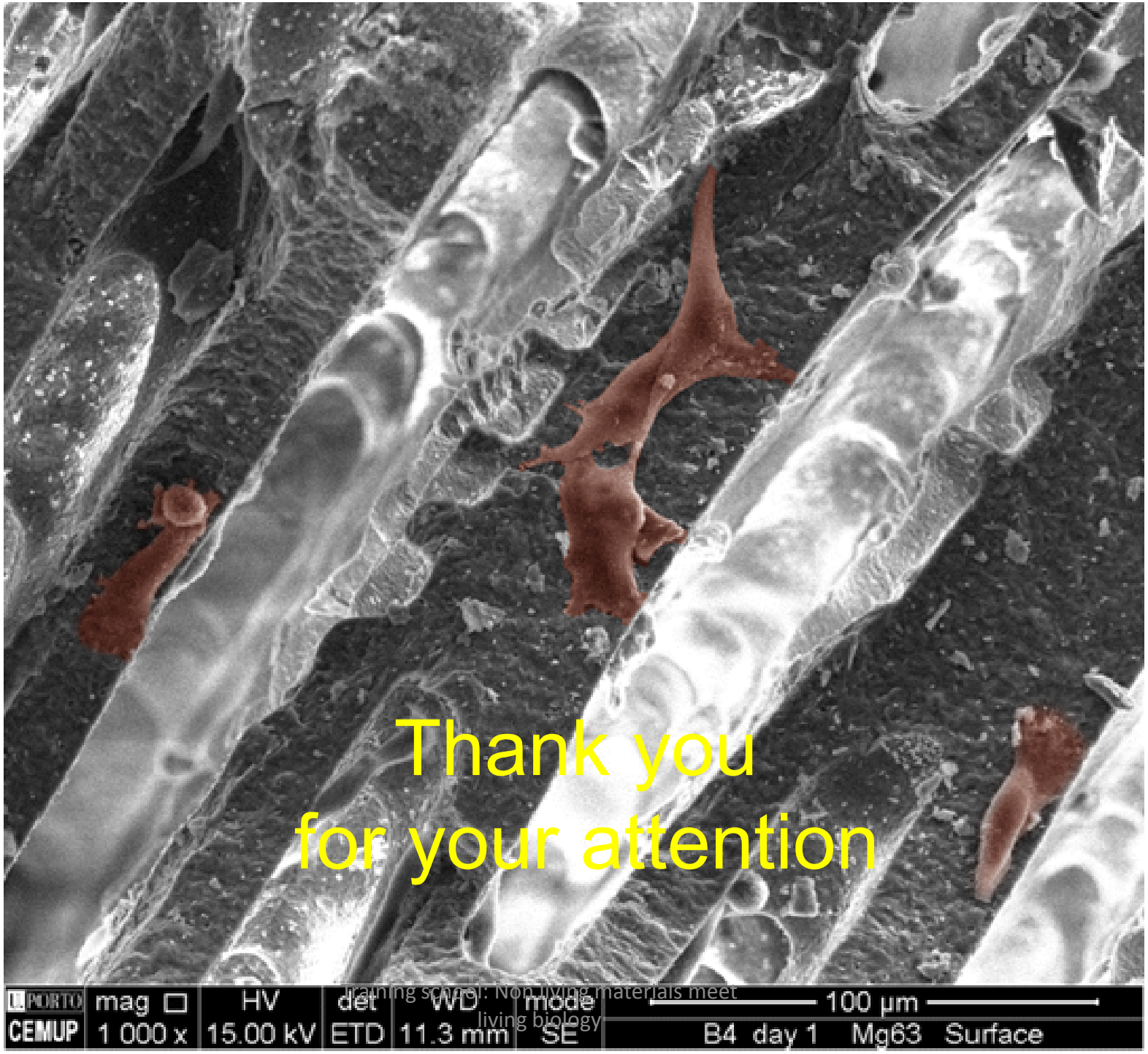
The freeze cast scaffolds due to their particular pore morphology present a more rapid cell differentiation into osteoblasts.

Conclusion

- The choice of the shaping technique has to be done according to the desired pore size range and architecture in relation to the application (implantation site : load or non load bearing area, defect size,…)
- The cell differentiation is influenced by pore architecture.
- The additive manufacturing techniques are very promising to achieve mimic porous architectures and hybrid composites constituted by both mineral and polymeric materials. These techniques can manufacture pore size and shape gradient favorable to a fast cell invasion into the center of scaffolds.

Acknowledgments

T.Chartier, M.Lasgorceix, *SPCTS Limoges*
A.Tampieri, *ISTEC Faenza*
M.Ahlhelm, *IKTS Fraunhofer*
A.Zocca, P.Colombo, *Dept of Industrial Engineering University of Padova*
D.Hautcoeur, S.Hocquet, F.Cambier, *BCRC Mons*
V.Sciamanna, M.Gonon, *University of Mons*
E.DeBarra, *University of Limerick, Ireland*
Conor Buckley, *Trinity College Dublin, Ireland*
K.Salim, *PCAS, Paris, France*
J.C. Hornez, F.Bouchart, S.Chamary, *LMCPA-UVHC*

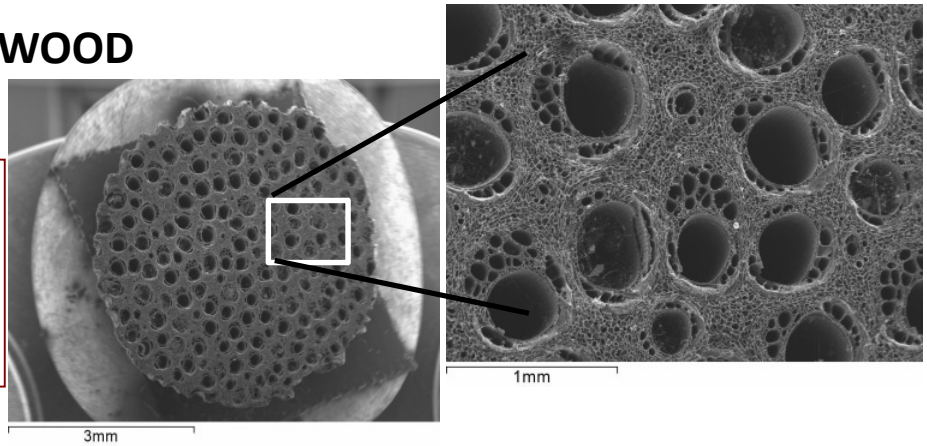

Thank you
for your attention
PORTO
CEMUP
mag
1 000 x
HV
15.00 kV
det
ETD
WD
11.3 mm
mode
SE
100 µm
B4 day 1 Mg63 Surface

Replica from wood

STAGES OF BIOMORPHIC TRANSFORMATION OF WOOD

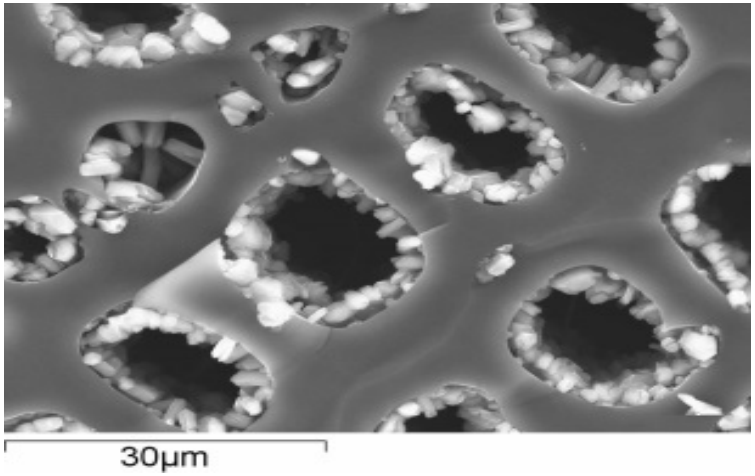
Pyrolysis
Wood → Carbon

Slow heating/cooling (1°C/h) to decompose the organic component and maintain the wood structure



Carburization
Carbon → CaC_2

Highly controlled heterogeneous gas/solid reaction between gaseous Ca and solid carbon.



Oxidation
CaC_2 → CaO

Two chemical reactions are competing in the oxidation process.

$$CaC_2 + 2H_2O \rightarrow Ca(OH)_2 + C_2H_2$$
$$Ca(OH)_2 \rightarrow CaO + H_2O$$
$$CaC_2 + H_2O \rightarrow CaO + C_2H_2$$

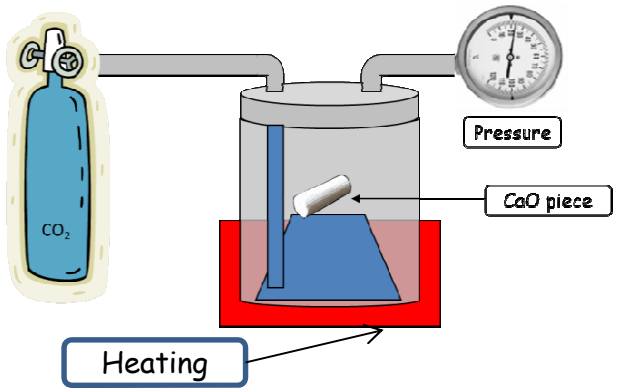
$$2CaC_2 + 5O_2 \rightarrow 2CaCO_3 + 2CO_2$$
$$CaCO_3 \rightarrow CaO + CO_2$$
$$2CaC_2 + 5O_2 \rightarrow 2CaO + 4CO_2$$

STAGES OF BIOMORPHIC TRANSFORMATION OF WOOD

$$CaO + CO_2 \rightarrow CaCO_3$$

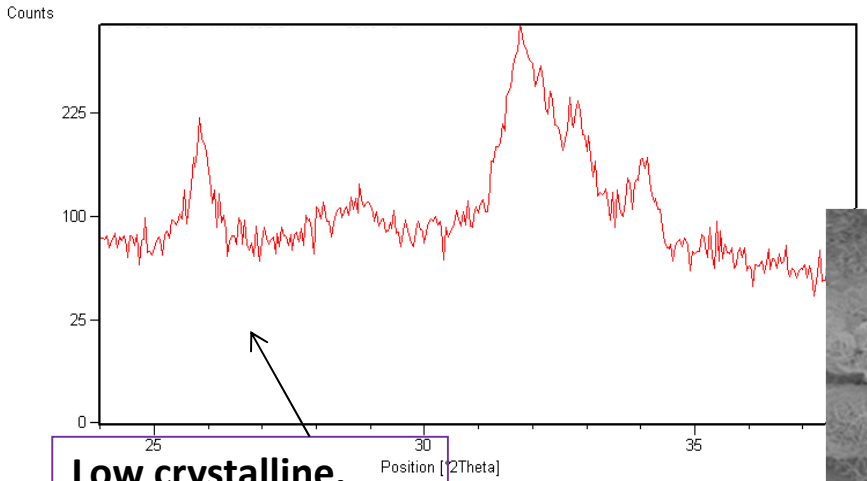
Carbonation
$CaO \rightarrow CaCO_3$

Carbonation of CaO is carried out at high temperature and CO_2 pressure to achieve effective CO_2 diffusion in the whole scaffold.

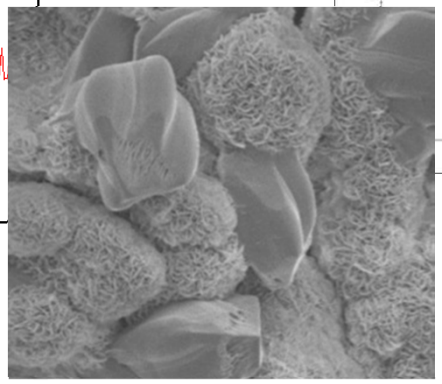


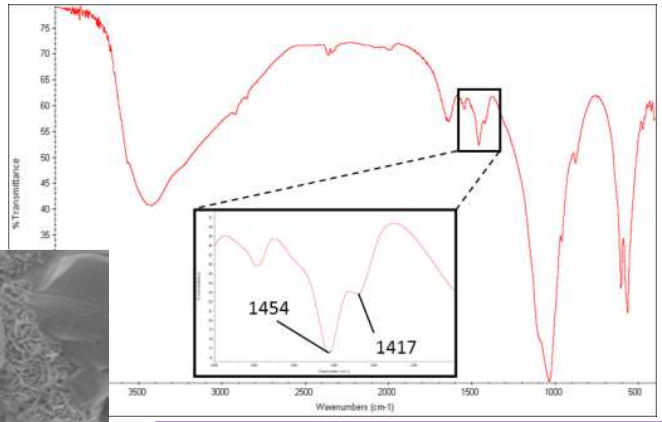
Phosphatization
$CaCO_3 \rightarrow HA$

$$10CaCO_3 + 6(NH_4)_2HPO_4 + 2H_2O = Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)_2CO_3 + 4H_2CO_3$$



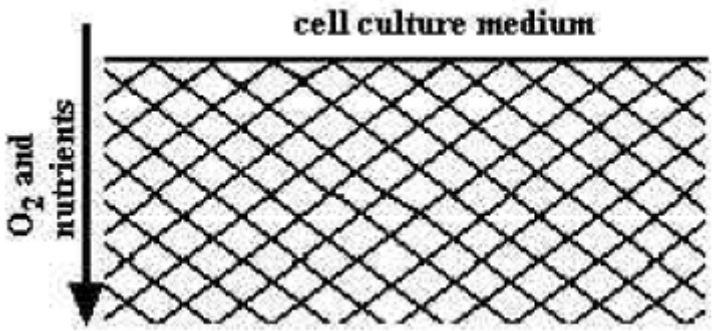
Low crystalline, bone-like HA phase



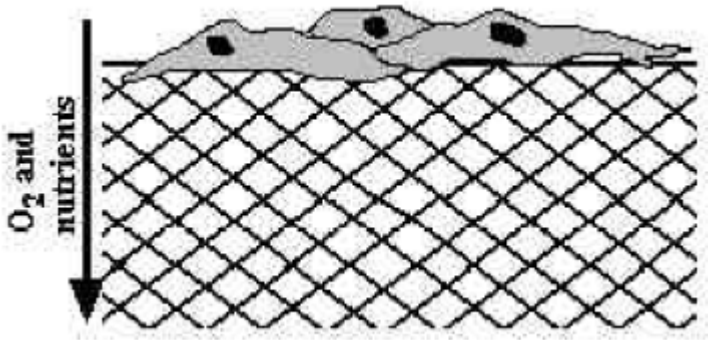


B-site carbonation (2-4 wt%)

Diffusion Constraints of Scaffolds

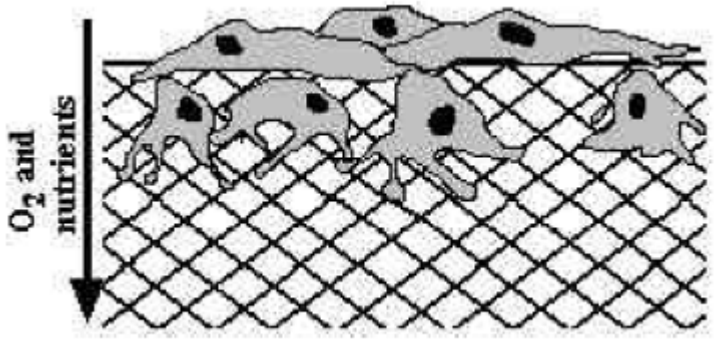


1. Tissue engineering **scaffold** is typically an **open-cell foam structure**. Oxygen and nutrients are supplied from the liquid cell culture medium via **diffusion**.

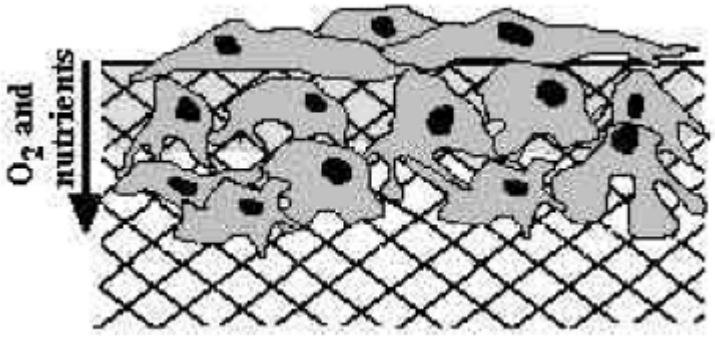


2. Cell seeding on scaffold. Many techniques available. Simplest form is pipetting.

Diffusion Constraints of Scaffolds

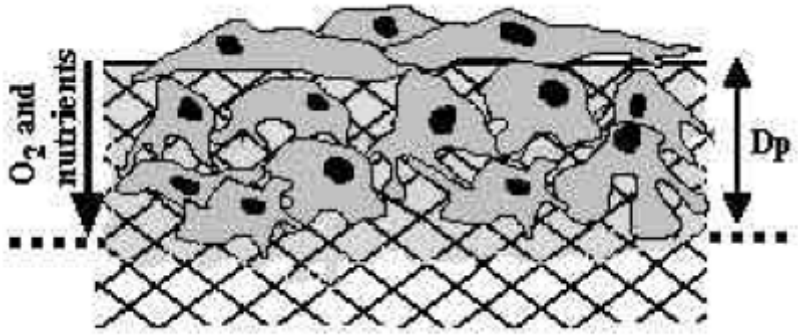


3. Cells start to proliferate and migrate into the pores of the scaffold.



4. Cells fully **colonise the pores** and start to **lay down** their own extracellular matrix **(ECM)**

Diffusion Constraints of Scaffolds



5. **Top layer of cells consume most oxygen and nutrients** in addition to limiting the diffusion of these components

6. Reducing amount available for pioneering cells migrating deep into the scaffold.

7. Eventually, **cellular migration/ECM deposition is halted** due to the lack of oxygen and nutrients supply.

8. **Layer of cells that can survive** on the diffusion of oxygen and nutrients from the medium is called the cellular penetration depth (Dp).

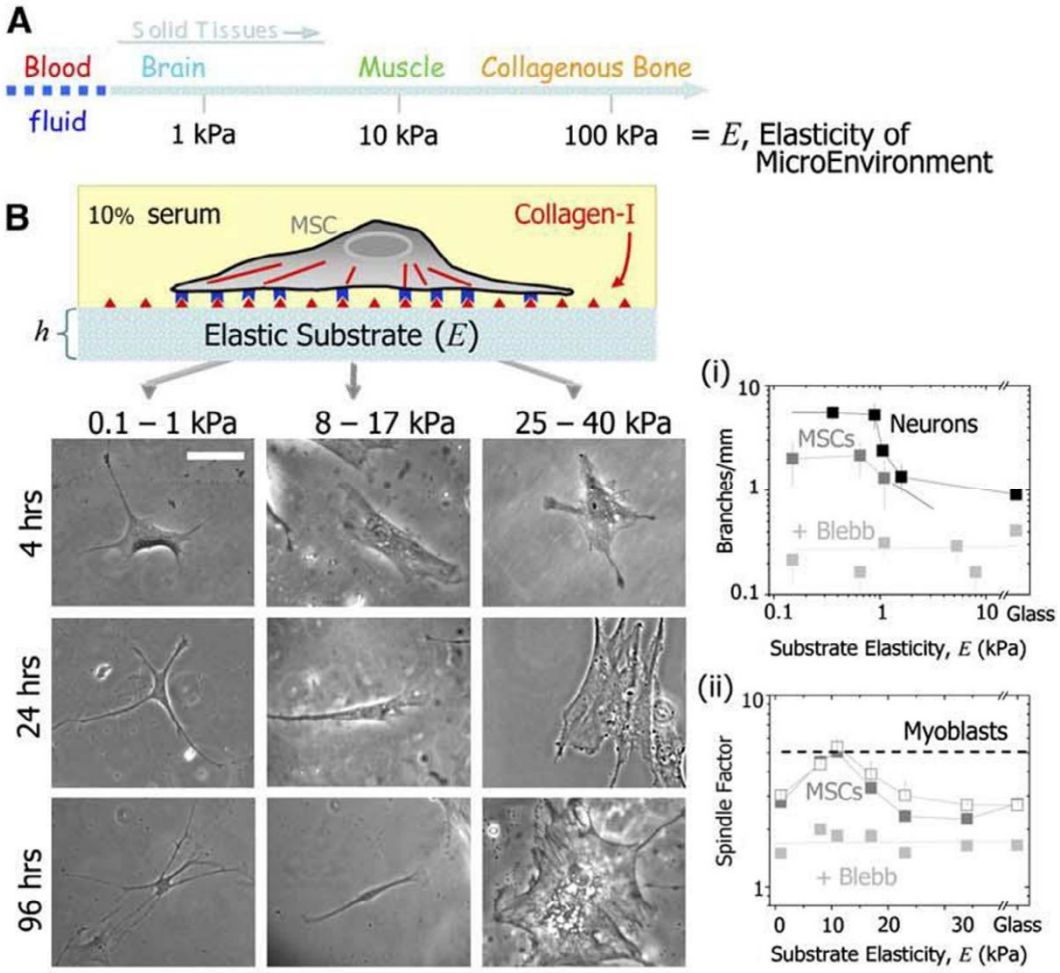
Scaffold Fabrication Techniques

Methods	Merits	Demerits	References
Solvent casting/ particulate leaching	Control over Porosity, pore size and crystallinity	Limited mechanical property, residual solvents and porogen material	Ma, 2007; Xiang et al., 2006
Porogen leaching	Controlled over porosity and pore geometry	Inadequate pore size and pore interconnectivity	Mano et al., 2007
Gas foaming	Free of harsh organic solvents, control over porosity and pore size	Limited mechanical property, inadequate pore interconnectivity	Ikada., 2006
Self assembly	Control over porosity, pore size and fiber diameter	Expensive material, complex design parameters	Zhang et al., 2003; 2006
Electrospinning	Control over porosity, pore size and fiber diameter	Limited mechanical property, pore size decrease with fiber thickness	Liang et al., 2007
Phase separation	No decrease in the activity of the molecule	Difficult to control precisely scaffold morphology	Smith et al., 2006

Scaffold Fabrication Techniques

Rapid prototyping	Excellent control over geometry, porosity, no supporting material required	Limited polymer type, highly expensive equipment	Hutmacher et al., 2000; 2001
Fiber mesh	Large surface area for cell attachment, rapid nutrient diffusion	Lack the structural stability	Chen et al., 2002
Fiber bonding	High surface to volume ratio, high porosity	Poor mechanical property, limited applications to other polymers	Mooney et al., 1996
Melt molding	Independent control over porosity and pore size	Required high temperature for non amorphous polymer	Thompson et al., 1995 a; b
Membrane lamination	Provide 3D matrix	Lack required mechanical strength, inadequate pore interconnectivity	Maquet & Jerome, 1997
Freeze drying	High temperature and separate leaching step not required	Small pore size and long processing time	Boland et al., 2004; Mandal & Kundu, 2008

How to mimic natural bone for substitute processing? 1st Material composition



Cells in various tissues can sense the elasticity of the matrix and transduce the mechanical signals into various physiological responds.

Therefore, biomaterials with matched mechanical properties with the defective tissue have good bioadaptability

A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Cell 126 (2006) 677–689.

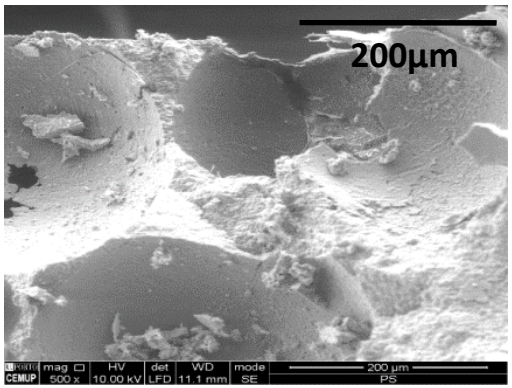
Tissue elasticity (A) and stem cell differentiation on glass matrix with various mechanical properties

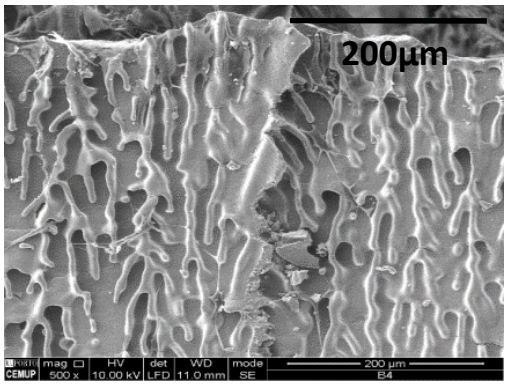
Comparison of the three different macroporosities

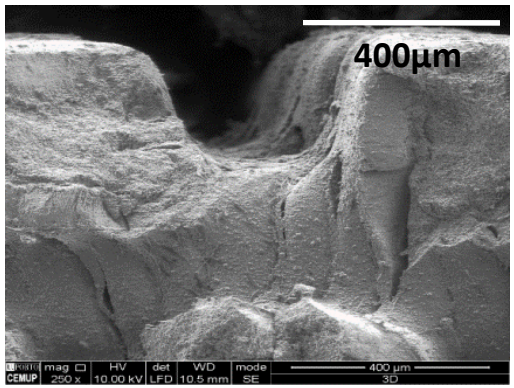
Cell colonization with MG63 osteoblasts?

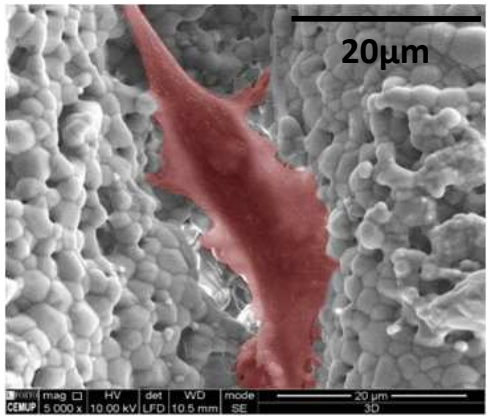
1 day

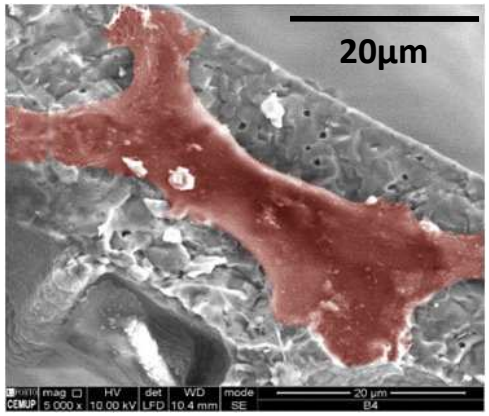
PMMA beads	Ice-templating	Stereolithography
450µm	366/54 µm, P= 51%, t=52µm	450µm

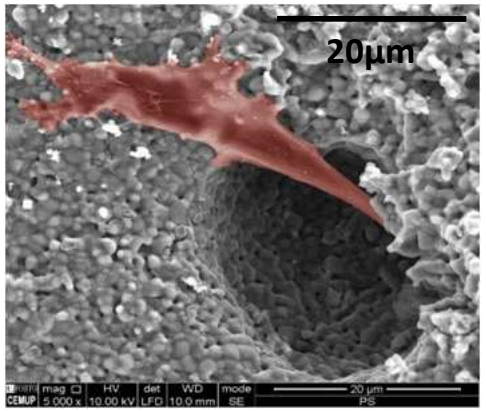












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