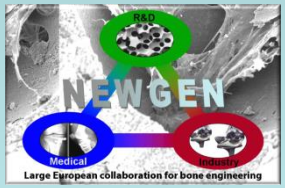
MP1301- NEWGEN
WG1

Nanocrystalline apatite: from fundamentals to bone substitute materials

Christèle Combes (INPT, CIRIMAT, France)

Margarida Almeida (University AVEIRO, CICECO, Portugal)

Paulo Quadros (Fluidinova, Portugal)



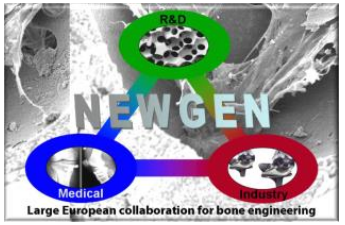
Nantes, 7th and 8th May 2014

Nanocrystalline apatites: from fundamentals to bone substitute materials

Christèle Combes, Christophe Drouet, Sophie Cazalbou, Stéphanie Sarda, Christian Rey

University of Toulouse, CIRIMAT, UPS-INPT-CNRS, ENSIACET, Toulouse, France

christele.combes@ensiacet.fr



Biological mineralisation properties

- Mineral elements and structures quite similar
  Silicates
  Calcium carbonates
  Calcium phosphates

- **Adaptable and elaborate architecture**
  (growth and autorepair)

- **Resistance and lightness**

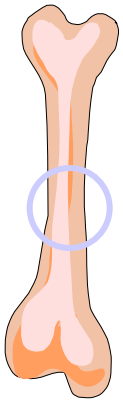
- **Saving energy**
  (low temperatures, recycling, renewal)

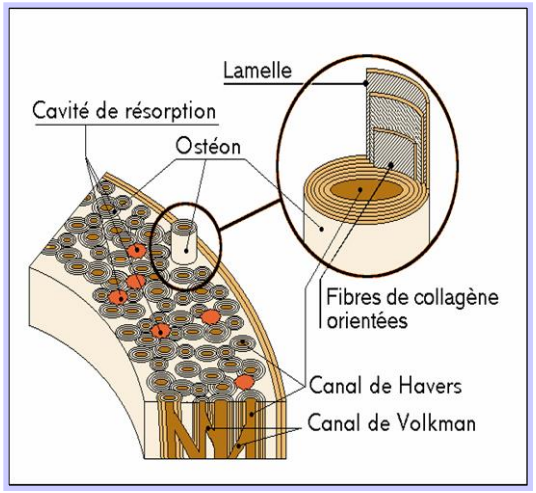
A model for material scientists !

Characteristics of biological mineralisations (vertebrates)

Bone	Tooth (enamel)
≠ Architecture	
Composition of tissue	
• 50-85 % mineral	• 97-99 % mineral
Crystal characteristics	
• Nanocrystals (20-60 x 10-20 x 2-5 nm) Platelets High specific surface area	• large crystals Needles low specific surface area

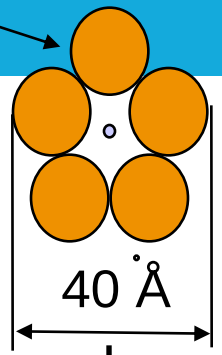
Structure and composition of bone tissue/mineral



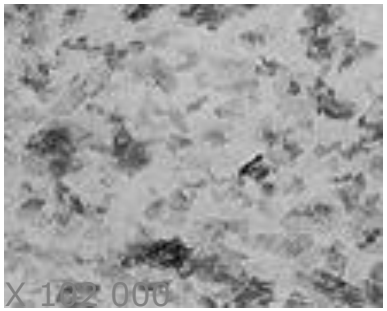


Compact bone structure
[A. Meunier, Galerne 2001]

Collagen fibers

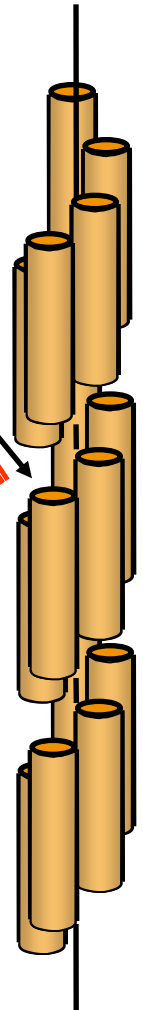


Empty space
(HAp location)



≅ 16x27 nm (4 y-o bovine)

[Kim *et al.* 1995]



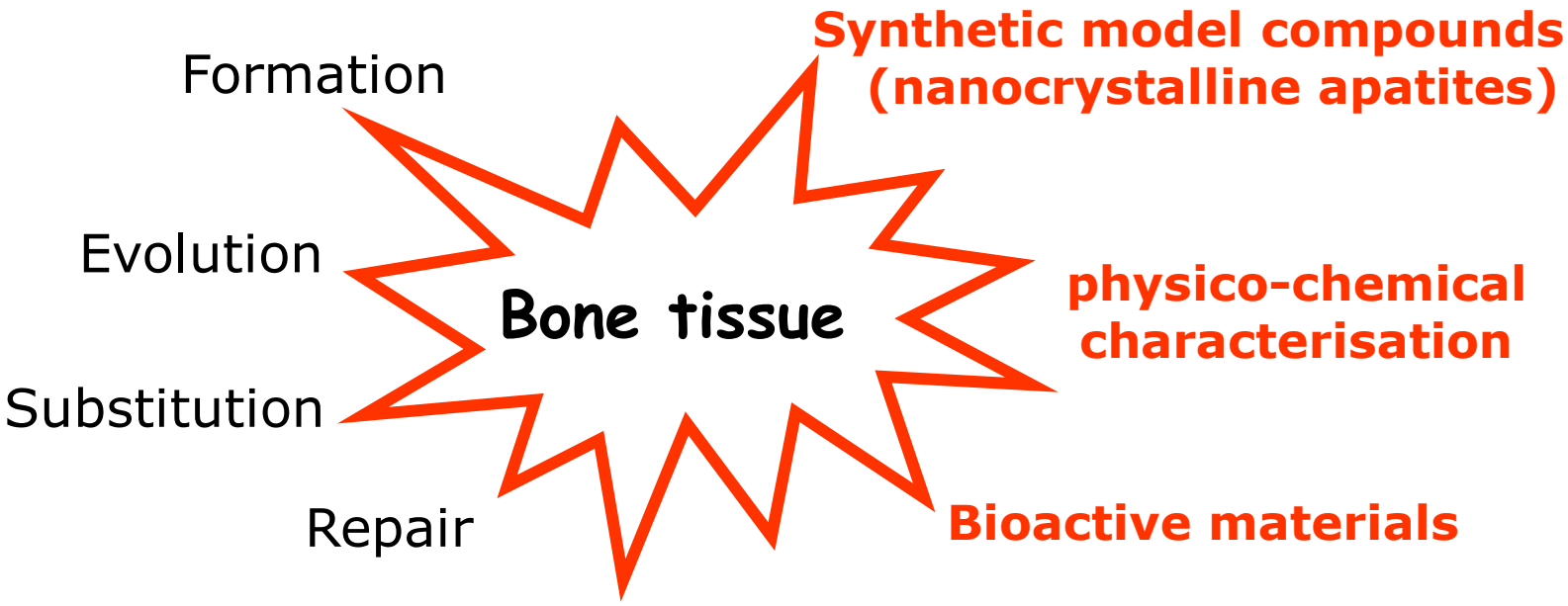
Ca_{10} $(PO_4)_6$ $(OH)_2$

CO_3^{2-}
HPO_4^{2-}

Bone mineral:
$Ca_{8,3}\ \square_{1,7}\ (PO_4)_{4,3}\ (HPO_4, CO_3)_{1,7}\ (OH)_{0,3}\ \square_{1,7}$

[Legros *et al.* 1986]

A biomimetic approach…



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Synthesis, properties and applications of nanocrystalline apatites analogous to bone mineral

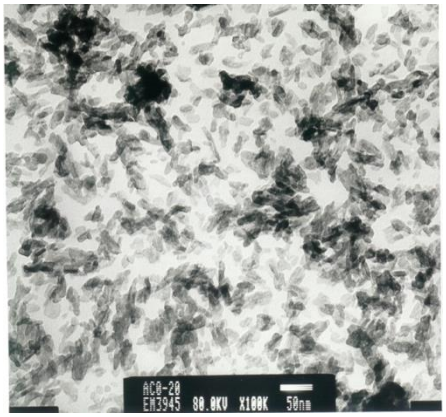
Nanocrystalline apatites analogous to bone mineral

Synthesis

$$Ca_{10-x}\,\square_x\,(PO_4)_{6-y}\,(HPO_4,\,CO_3)_y\,(OH)_{2-z}\,\square_z$$

$2x = y+z;\ x \leq 2$

$Ca(NO_3)_2$, 4 H_2O
$(NH_4)_2HPO_4$
ambient T
physiological pH

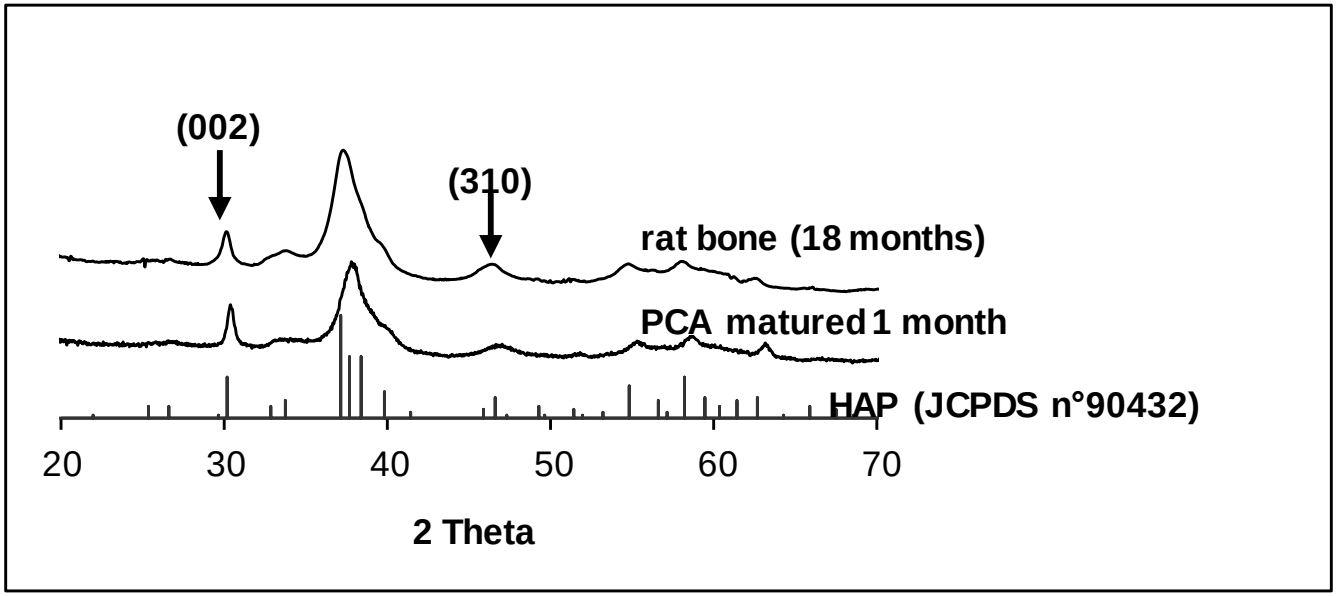


≅ 11x19 nm (1d maturation)

Nanocrystalline apatites properties:

- **Maturation (h, d, m)**
- **Ion exchanges**
- **Protein adsorption**

X-ray diffraction characterisation



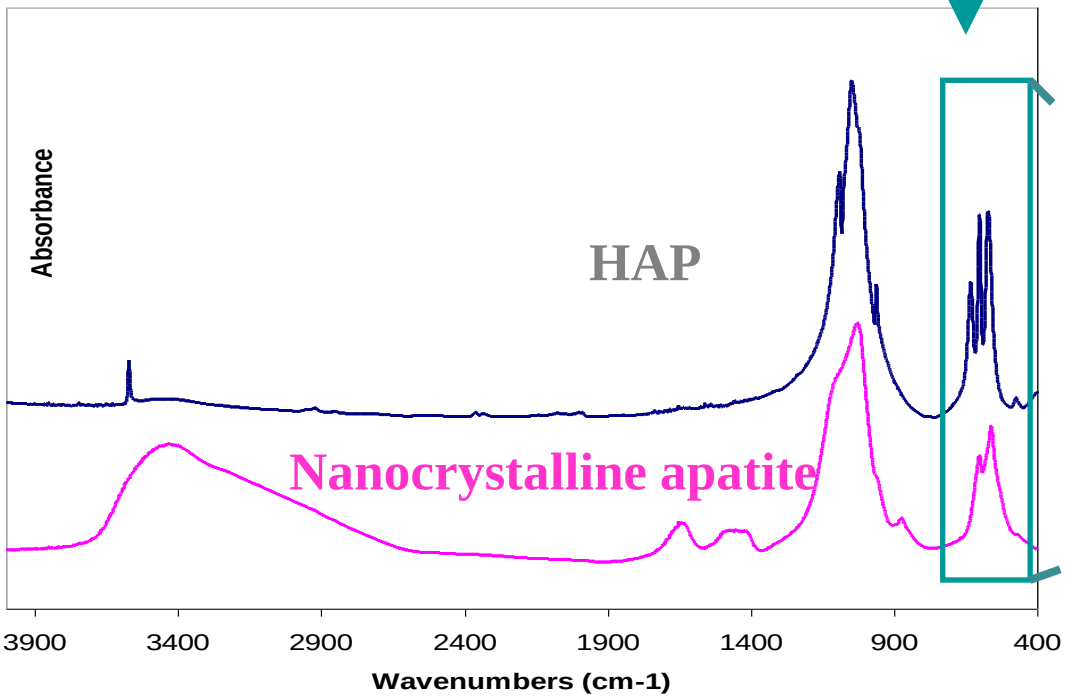
Estimated crystallite size for natural and synthetic apatites

[Eichert, PhD thesis, INP Toulouse 2001]

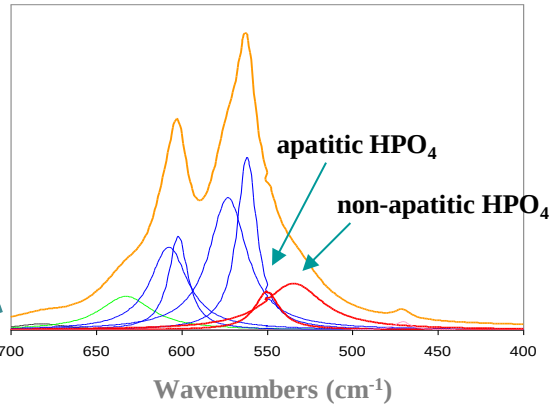
Sample	L(002) ± 3 Å (length)	L(310) ± 3 Å (width/thickness)
Chicken bone	207	66
Rabbit bone	190	Not evaluated
Adult human cortical bone	213	68
Synthetic apatite matured for 3 months	282	72

FTIR spectroscopy analysis

ν_4 PO_4 domain



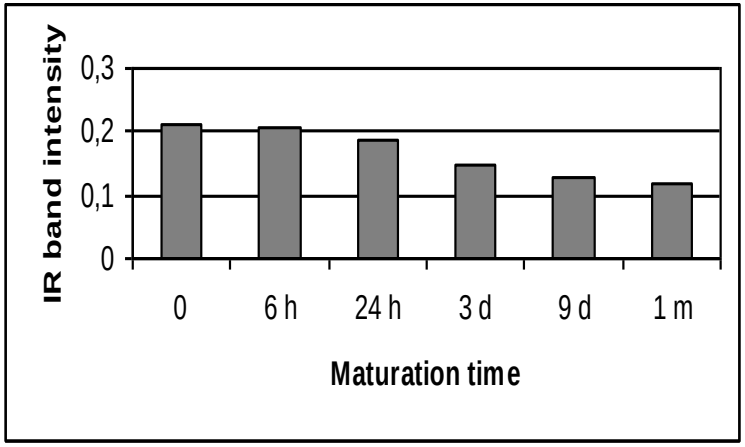
HPO_4^{2-} = important marker of NCA maturation



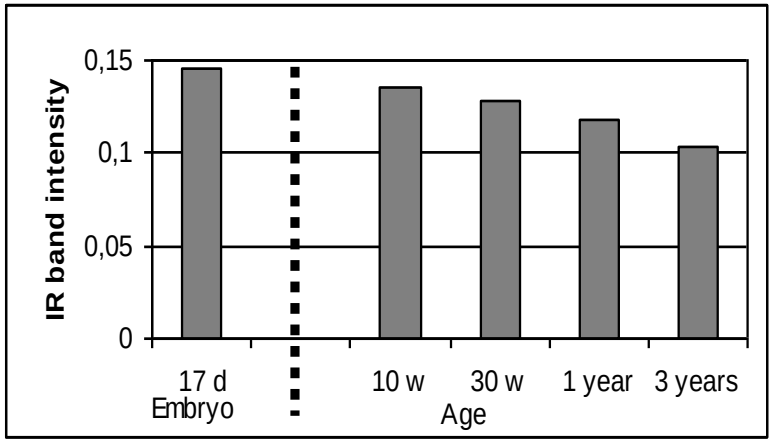
Apatites maturation

HPO_4^{2-} = important marker of apatite maturation

Synthetic apatites



Biological apatites (chicken bone)

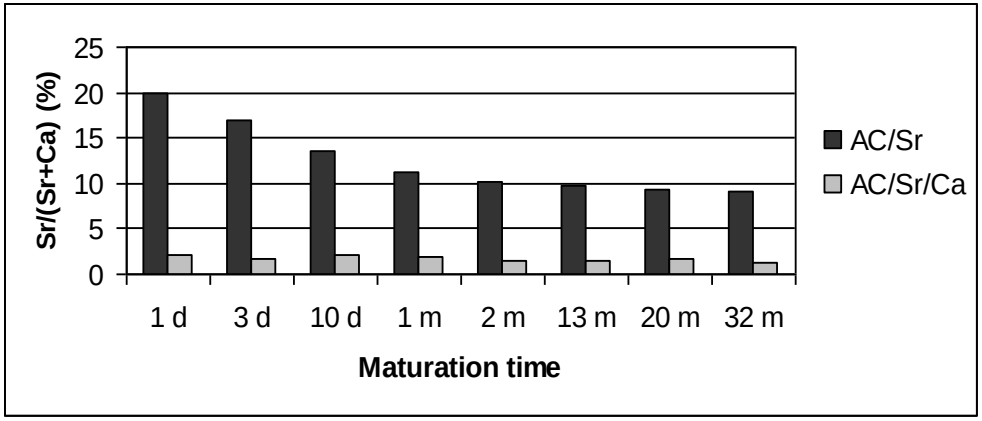


HPO_4^{2-} ↘ with maturation/ageing

[Cazalbou *et al., J. Mater. Chem.,* 2004]
[Cazalbou *et al., J. Bone Miner. Metab.,* 2004]

Ion exchange properties of nanocrystalline apatites

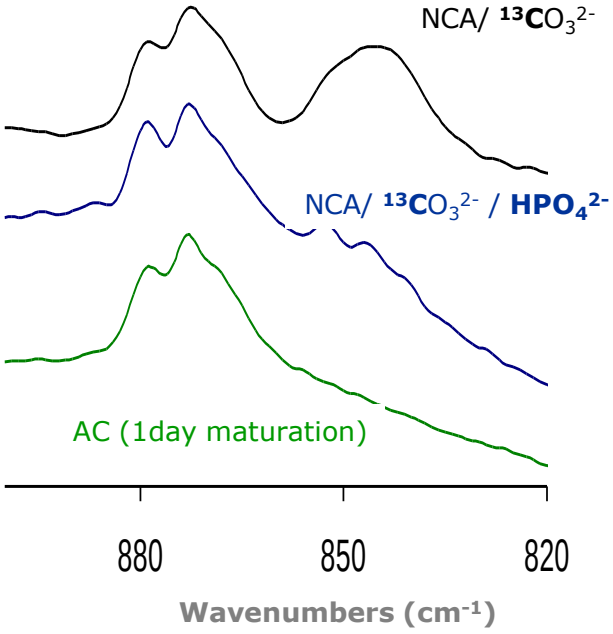
Cation exchanges
Sr^{2+}/Ca^{2+}



fast and reversible exchanges

exchange ability ↘ with maturation

Anion exchanges
CO_3^{2-}/HPO_4^{-}

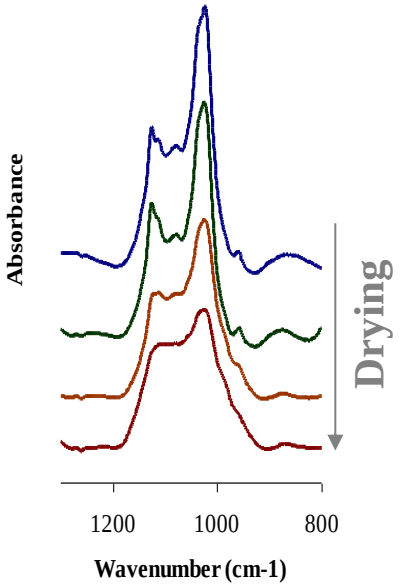


Surface divalent ions involved in these ion exchanges

[Cazalbou *et al., J. Mater. Chem.,* 2004]

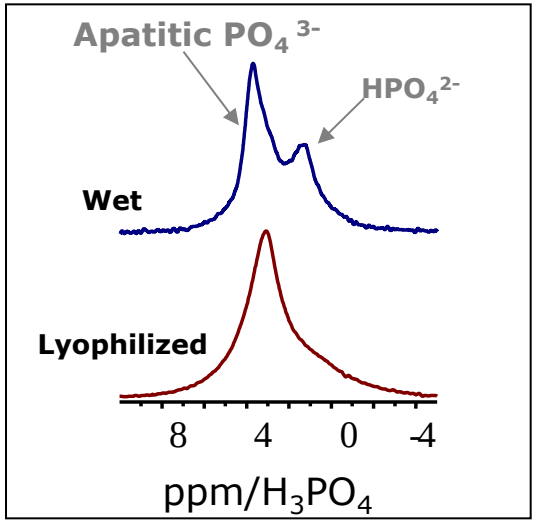
Role of water ?

FTIR spectroscopy



Wet samples exhibit
fine structural details
which disappear on drying

^{31}P NMR



Drying:

Loss of water

- ⇨ disordered ionic layer
- ⇨ alteration of short range order
- ⇨ **Non-apatitic environments**

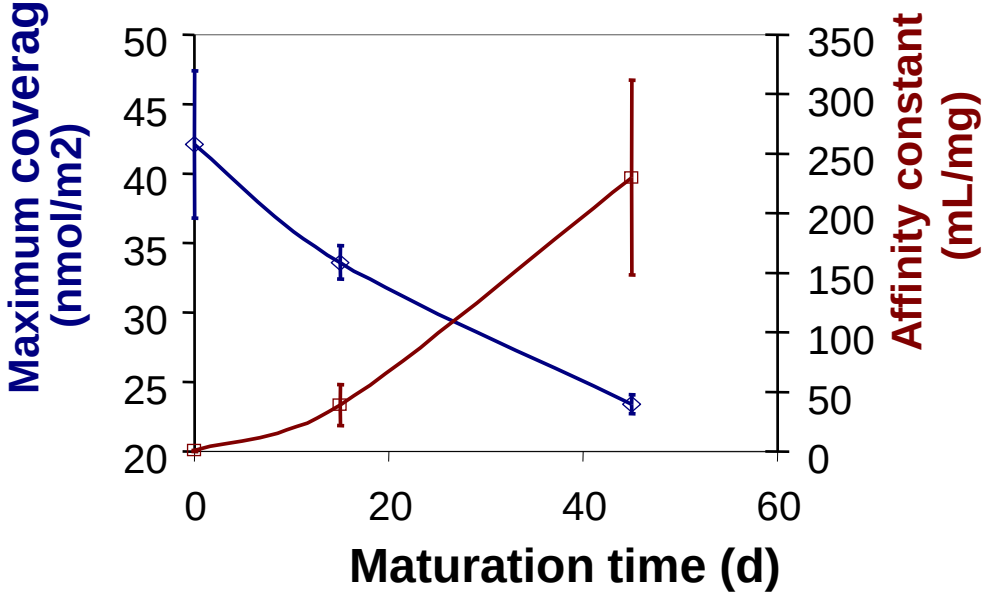
Very fragile
surface hydrated layer structure

Some analogy
but **≠ OCP and DCPD**

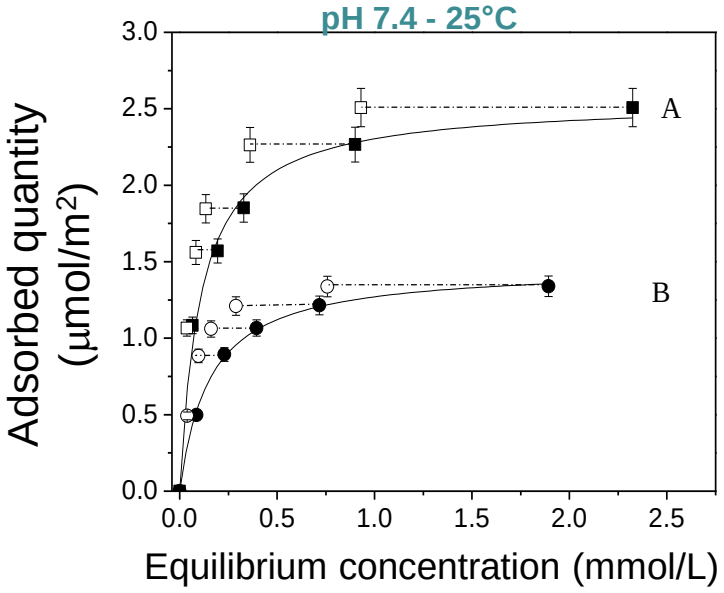
[Eichert *et al.*, Bioceramics 16, 2004]

Adsorption properties of nanocrystalline apatites

BSA



Bisphosphonate



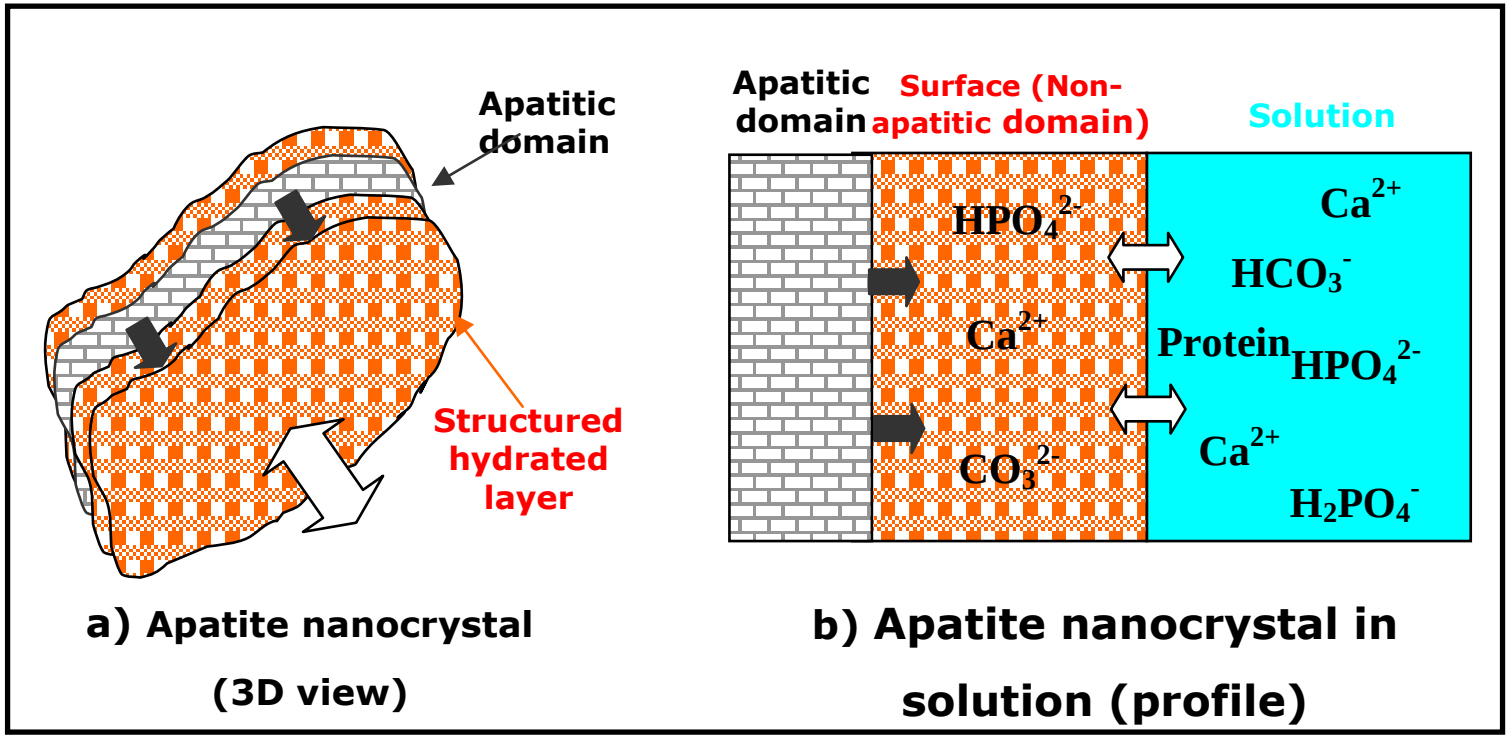
A: nanocrystalline apatite (30 d)
B: stoechiometric HAP

■ ● adsorption
□ ○ desorption

Adsorption properties vary according to the surface layer characteristics

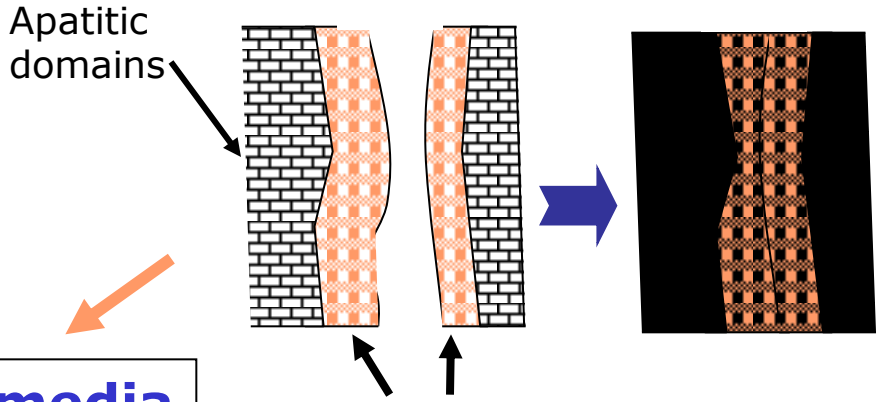
Specific structural characteristics of apatite nanocrystals

Structured hydrated layer at the surface of nanocrystals



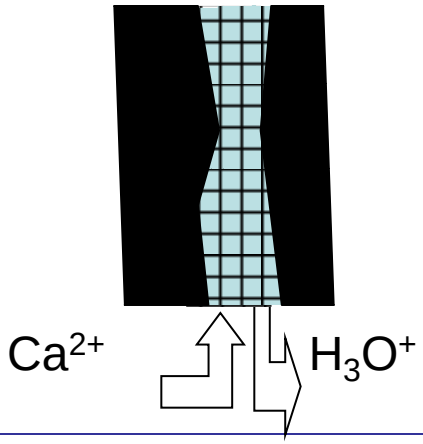
Rey *et al*., Osteoporosis Int., 2009

Consequences for biomaterials ?



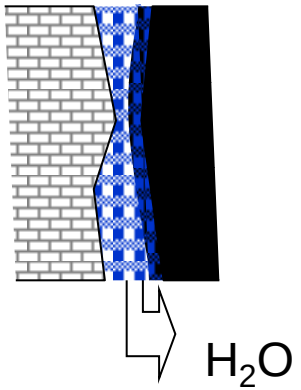
Crystal-crystal interactions

in aqueous media



- Crystal "fusion" (bone, enamel)
- **Setting reactions of biomimetic CaP cements**

by drying



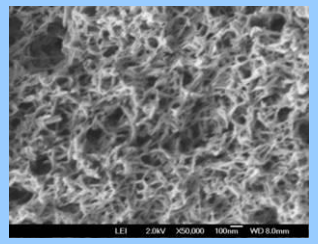
low temperature
- **Ceramics**
- **Coatings**
- **Composites**

Nanocrystalline apatites analogous to bone mineral → **Biocompatibility, biomimetism High surface reactivity**

Bone tissue engineering

- **Low temperature bioceramics** (**biomimetic cements**, SPS, gel casting, …)

Bioactivity
Resorbability → **Associations with drugs** (antibiotic, anticancer, growth factor, biologically active ions …) **for sustained release system**

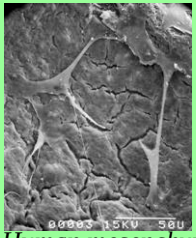


Ceramic prepared by SPS (Cryo SEM-FEG)

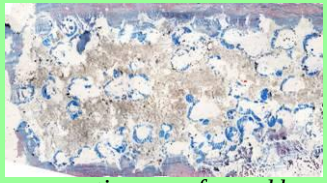
- **Nanocomposites** (nanocrystalline apatites + resorbable polymers)

Nanostructured coatings on implants (ceramics or metal)

+ association with biologically active molecules



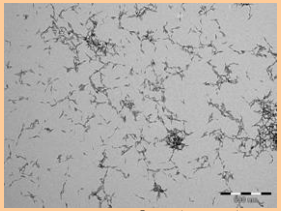
Human mesenchymatic stem cells on nanostructured coating



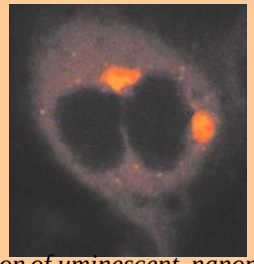
Histological cross section : neoformed bone (dark blue) in contact with a porous coated ceramic (+BMP-2) implanted in non osseous location (t= 3 months)

Luminescent nanoprobes for medical imaging

Colloïdal apatite nanoparticles (20-100 nm), luminescent (rare earth element), associated with a targeting agent towards cancer cellules



Nanoparticles (TEM)

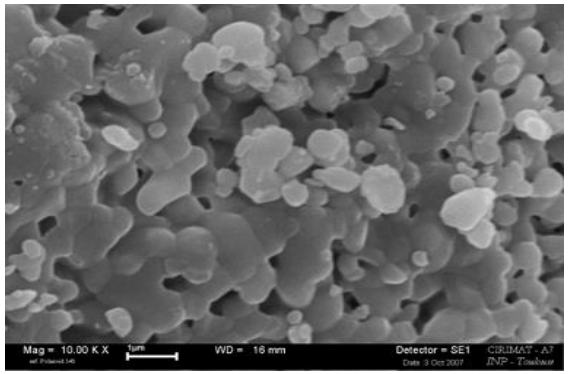


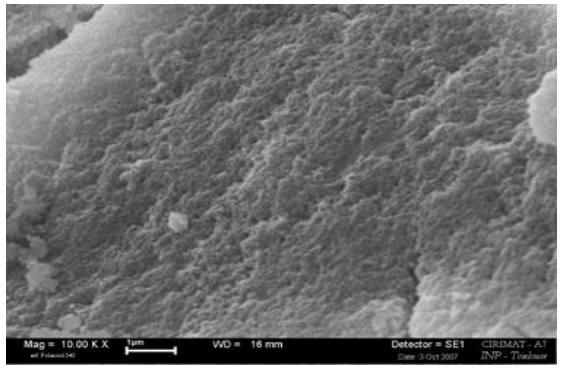
Internalisation of uminescent nanoparticles l Within a human pancreatic cell

Biomimetic apatite coating

HA-βTCP porous granules (Teknimed)

- with or without biomimetic apatite coating
- with or without adsorbed rh-BMP2





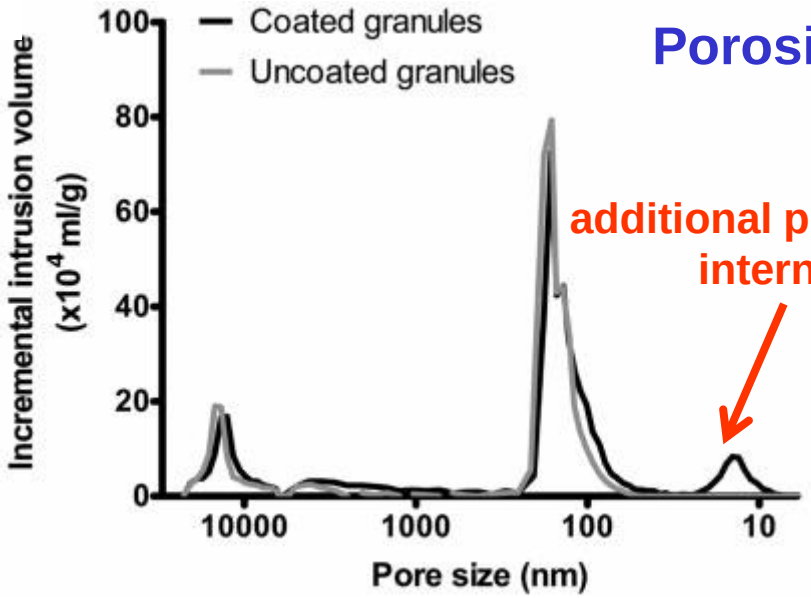
Scale bar = 1 µm

Raw HA-βTCP ceramic

Coated HA-βTCP ceramic

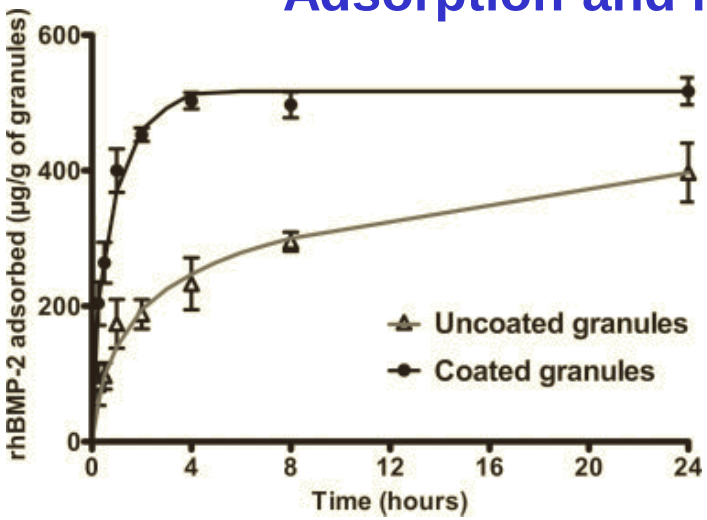
Application: Biomimetic apatite coating

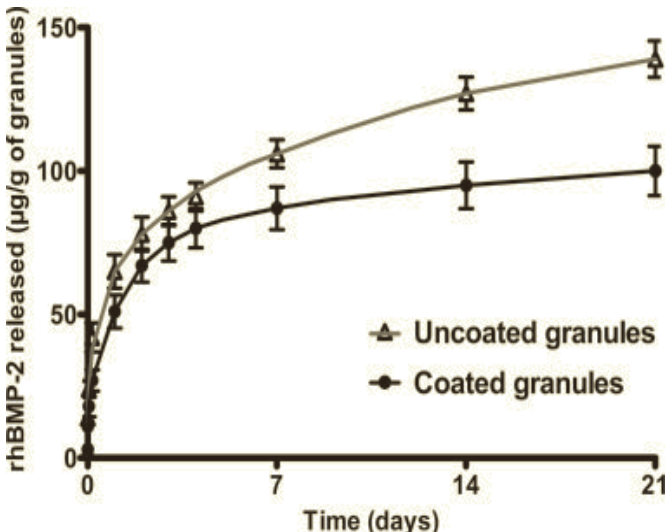
Porosimetry



additional porosity corresponding to internanocrystal spaces

Adsorption and release properties of rh-BMP2



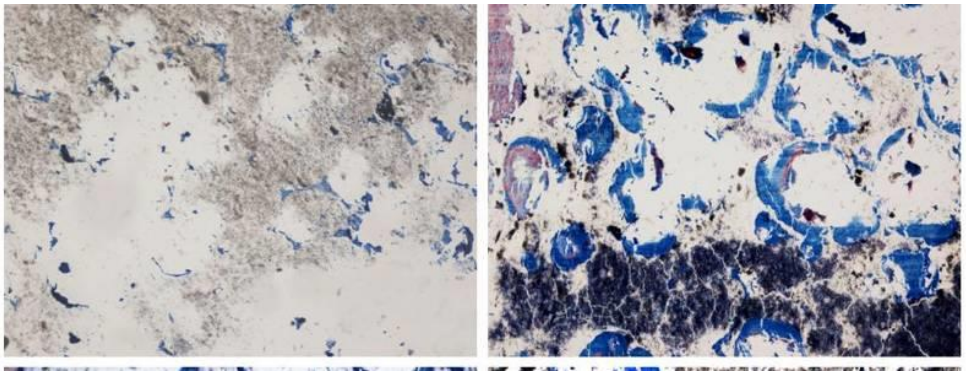


H. Autefage et al. J. Biomed. Mater. Res. B (2009)

In vivo study

Intramuscular implantation in sheep for 3 months

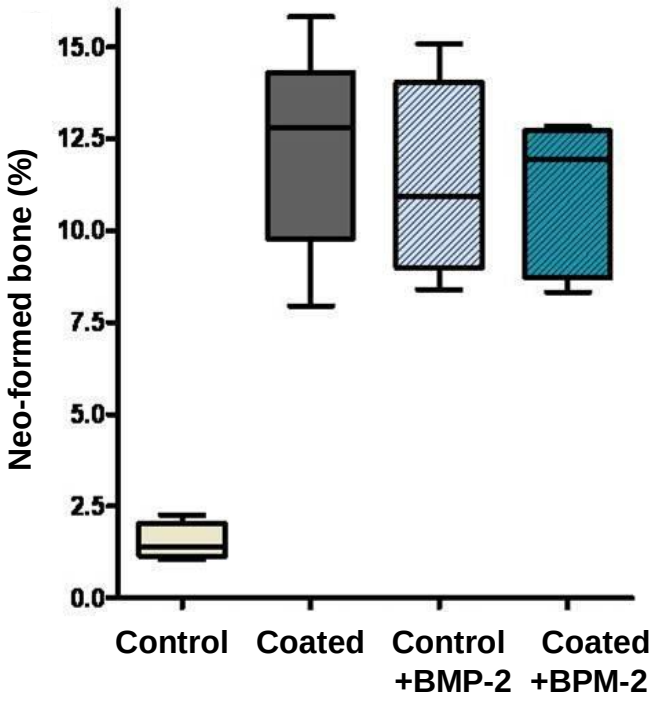
Histological study: after 3 months



Raw HA-βTCP ceramic

Coated HA-βTCP ceramic

without rh-BMP-2



Osteoinduction potential of the nanocrystalline coating alone … but WHY ?!!

nanostructure ? nanoporosity ? composition ? Multiple parameters ? ….

… a question to investigate within NEWGEN network ?

See Margarida Almeida talk (effect of [nanoHA] on ALP and BMP-2 expression)

Biomimetic apatite coating: Technology transfer

Coll. Teknimed S.A - CIRIMAT:

Ceraform®Revolution is a porous bioceramic with a nanocrystalline carbonated apatite coating enhancing surface reactivity leading to better bone reconstruction

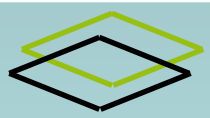
H. Autefage et al. US patent n°12/487,101 (2009)

Conclusion

- Bone mineral is a very reactive complex structure
- The evolution of bone apatite with age corresponds to a physical-chemical maturation process
- Nanocrystalline apatites exhibit specific surface structure in aqueous media
- This hydrated surface layer is unstable
- Although the structure of the hydrated surface layer of apatites is unknown it offers ion exchange and protein adsorption capabilities
- The surface reactivity thus obtained is utilised by living organisms
- **We can take advantage of this surface reactivity for making new bioactive bone substitute materials at low temperature**

Tailoring calcium phosphate nanostructured materials for biomedical applications

Elizabete Costa (elisabete.costa@ua.pt)
Margarida Almeida (margarida@ua.pt)

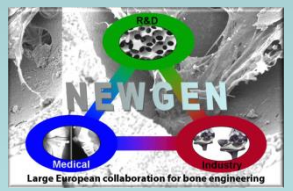


CICECO
Centre for Research in Ceramics and Composite Materials

Department of Materials and Ceramic Engineering

UNIVERSITY OF AVEIRO

PORTUGAL



MP1301 NEWGEN
WG1

MOTIVATION

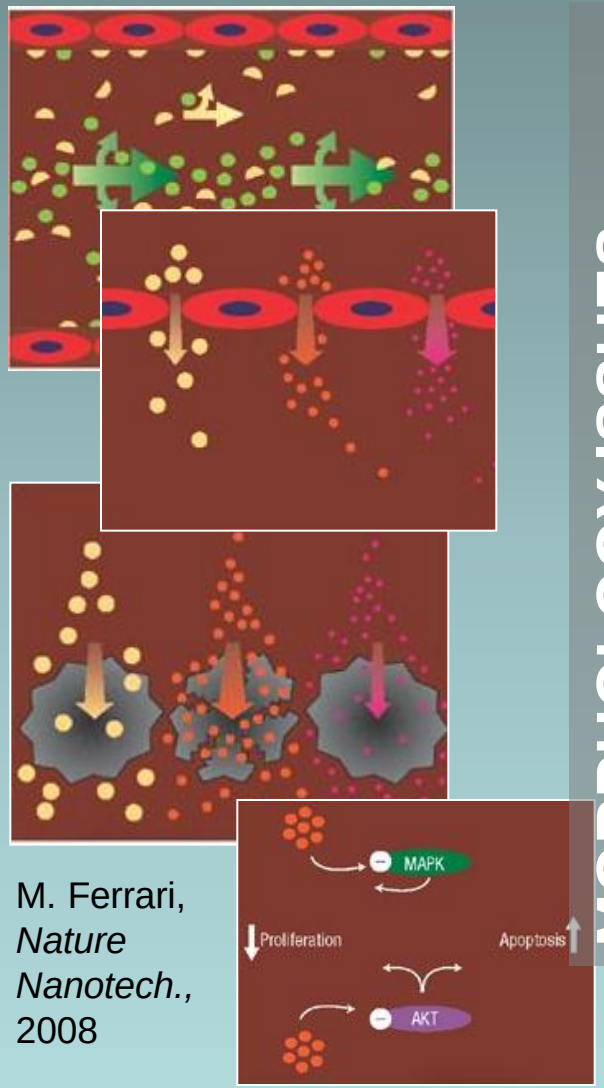
DRUG DELIVERY SYSTEMS DDS

PARTICLE REQUIREMENTS

Morphology accurately engineered is a prerequisite for a predictable performance of **DDS**

Synthesis procedures able to prompt particle design with **size and shape surgically tailored**

MORPHOLOGY ISSUES



M. Ferrari, *Nature Nanotech.*, 2008

MOTIVATION

what´s the issue?

…To exploit strategies based on

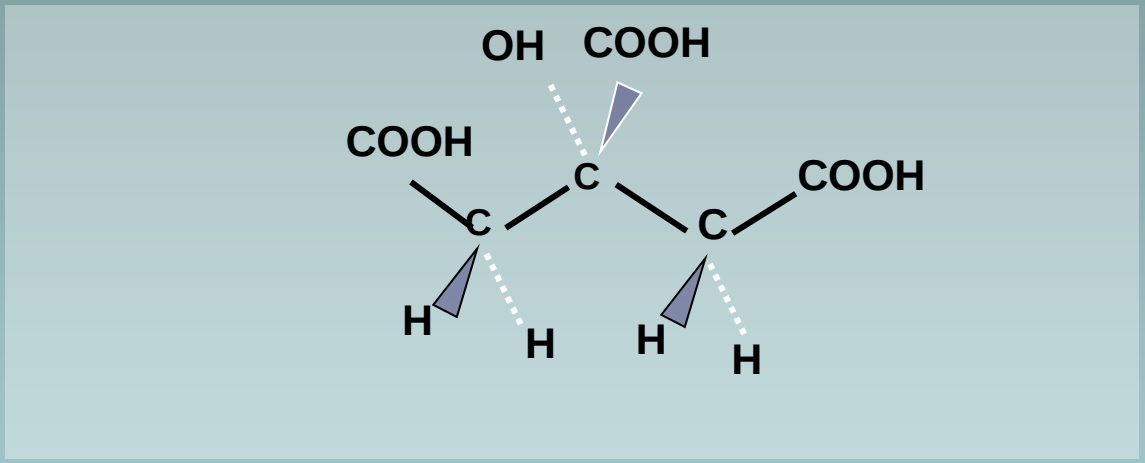
chelant assisted synthesis for producing apatite

nanoparticles targeted to biomedical devices

(drug delivery systems + bone tissue growth)

Chemical precipitation in presence of citrate

MOTIVATION



Citric acid $- x\,H^+ \longrightarrow$ **Citrate ion**

@ the lab. . .

Hydroxyapatite Synthesis Strategy

3 steps …

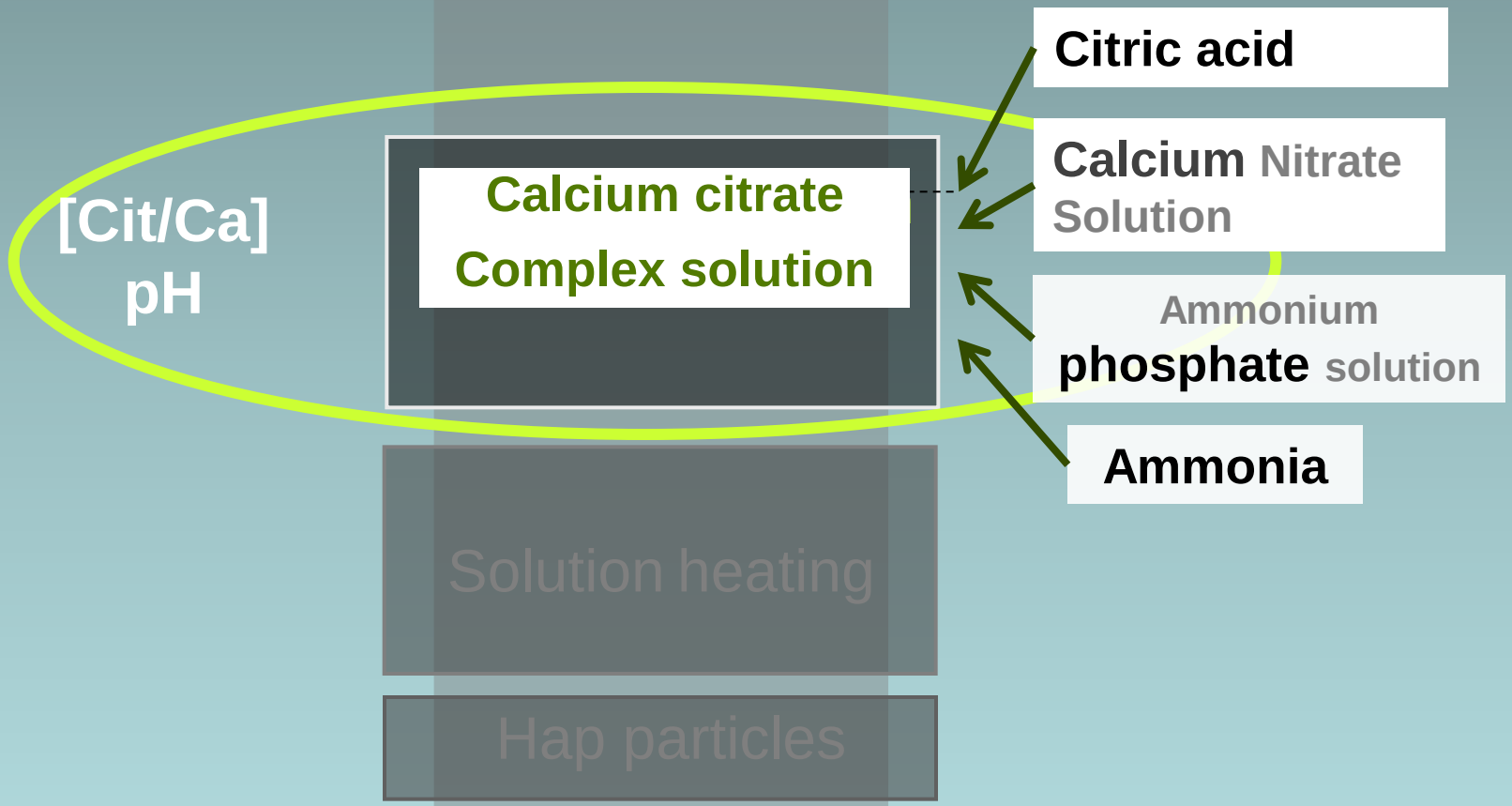
Precursor solution Preparation

Solution heating

HAp particles

@ the lab. . .

Preparing the precursor solution…



@ the lab. . .

Solution heating…

Precursor Solution

(Ca, P, Cit)

37ºC

(Physiological temperature)

or

180ºC

(Hydrothermal synthesis, Autoclave)

Hap particles

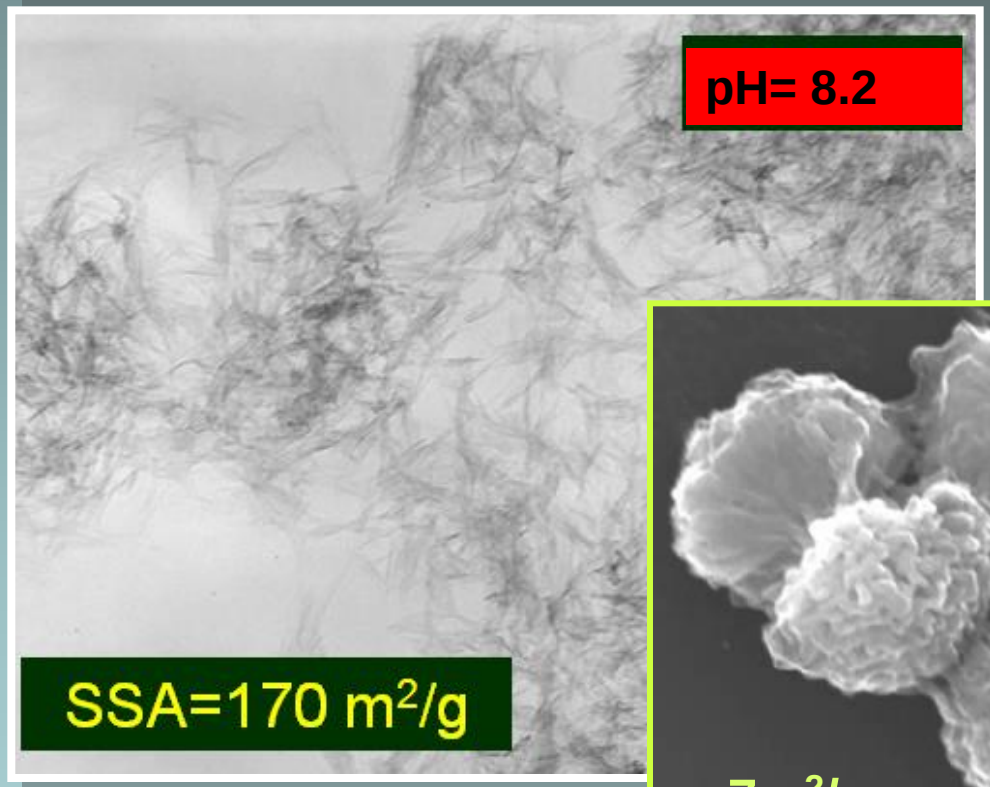
@ the lab. . .

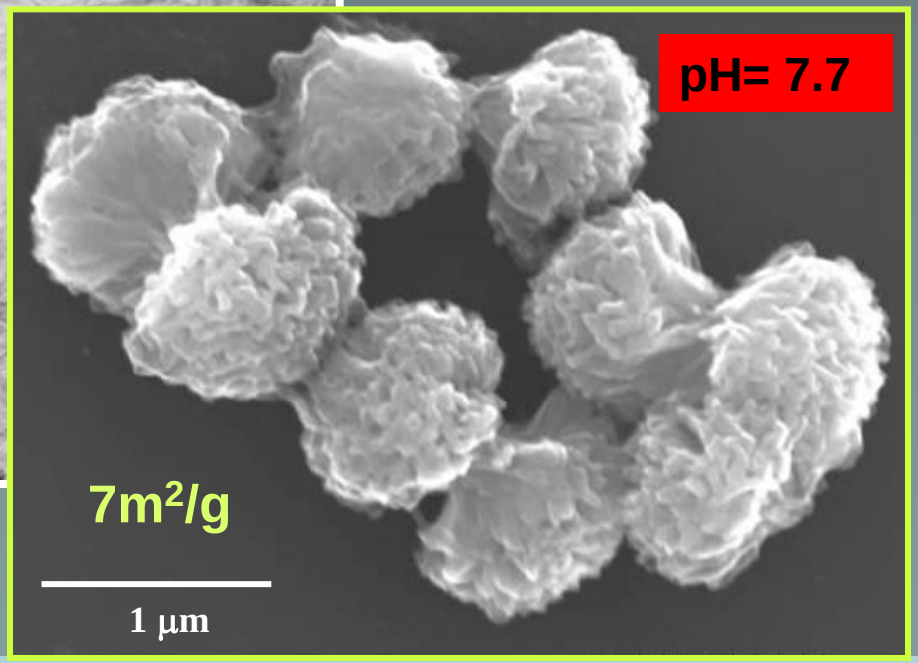
Characterizing the precipitates …

- **Chemical Composition (**ICP**)** and **Crystallinity (**X-ray Diffraction**)**
- **Morphology (Electronic Microscopy)**
- **Specific surface area (BET)**
- **Electrical surface charge (Zeta potencial)**
- **Adsorbed species (FTIR)**

Precipitation of HAp particles

HAp SYNTHESIS





T=37ºC

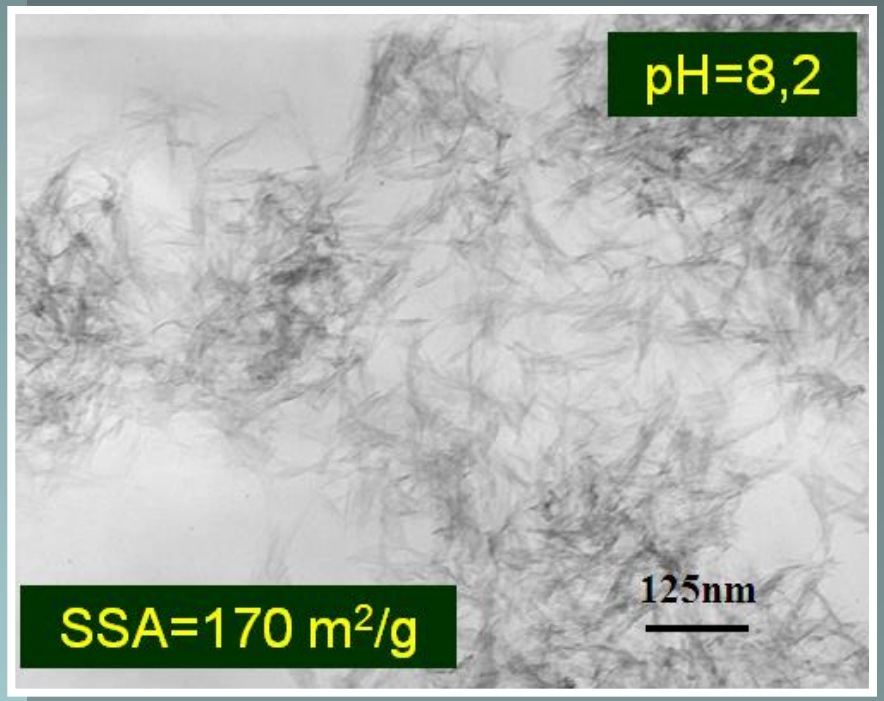
pH

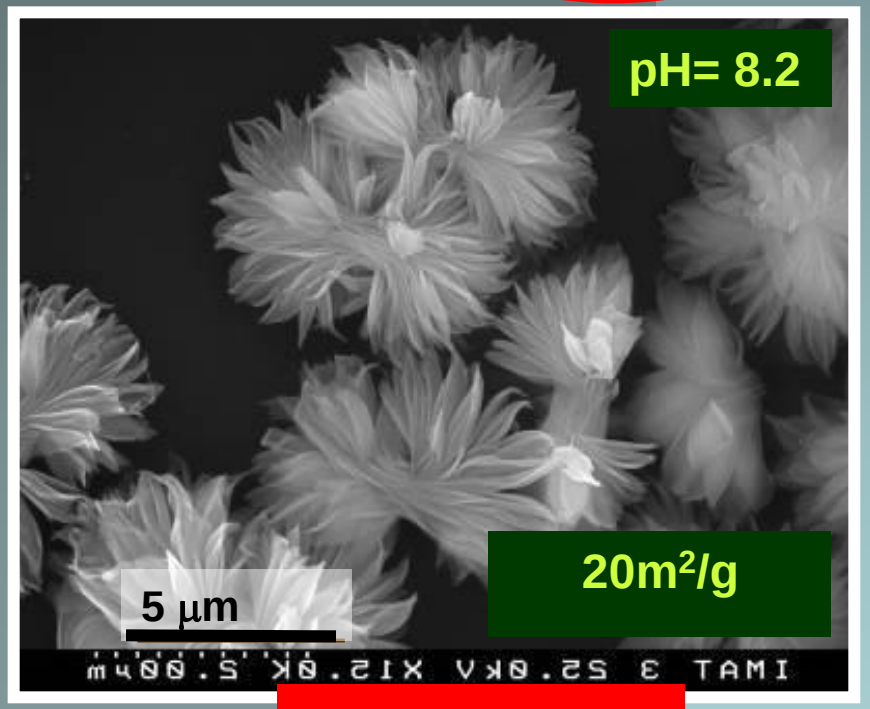
[1] M.Martins et al, J.Colloid and Interface Sci, 2008;

[2] C.Santos et al, Microscopy and Microanalysis, 2009

HAp SYNTHESIS

T=37ºC





Cit:Ca=3:1

Cit:Ca=5:1

[Citrate]

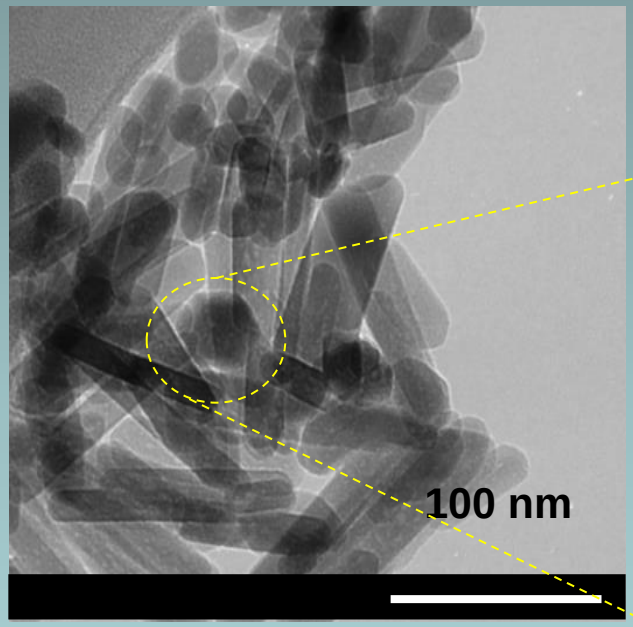
[1] M.Martins et al, J.Colloid and Interface Sci, 2008;

[2] C.Santos et al, Microscopy and Microanalysis, 2009

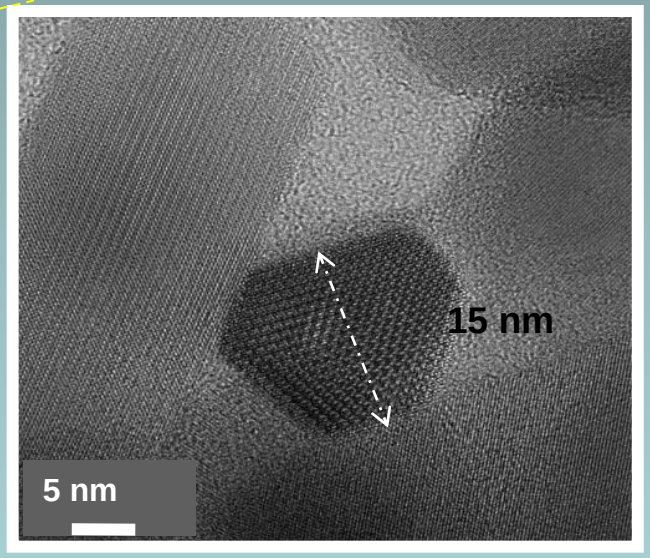
HAp SYNTHESIS

T=180ºC

55 m^2/g



Hexagonal prisms

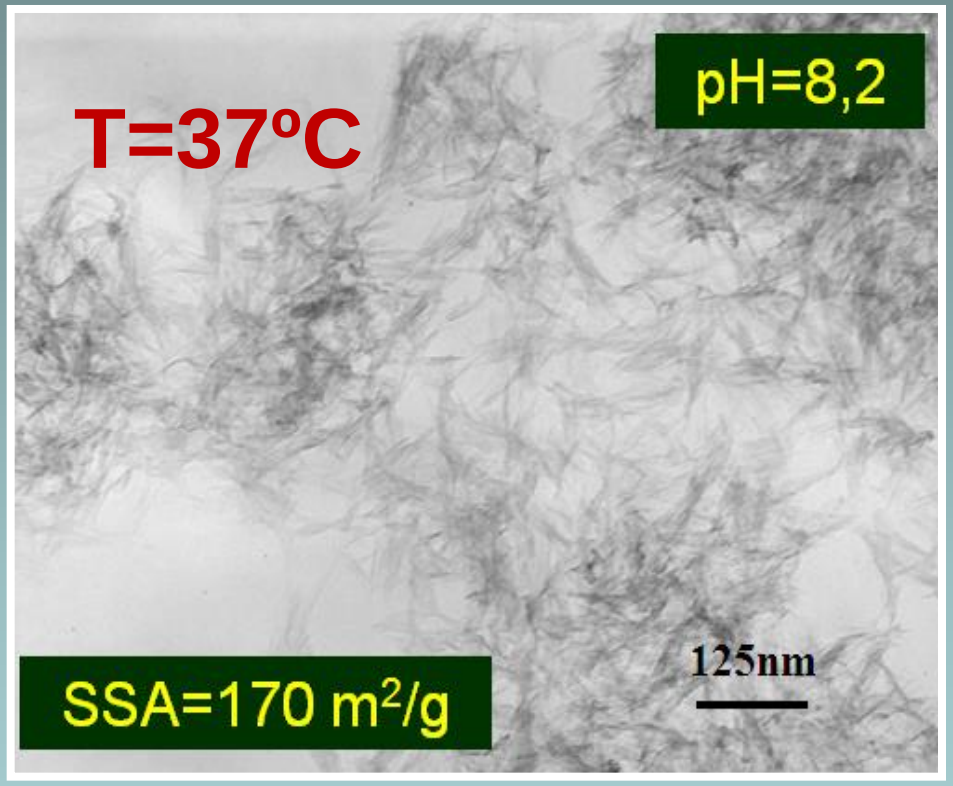


Cit:Ca=3:1

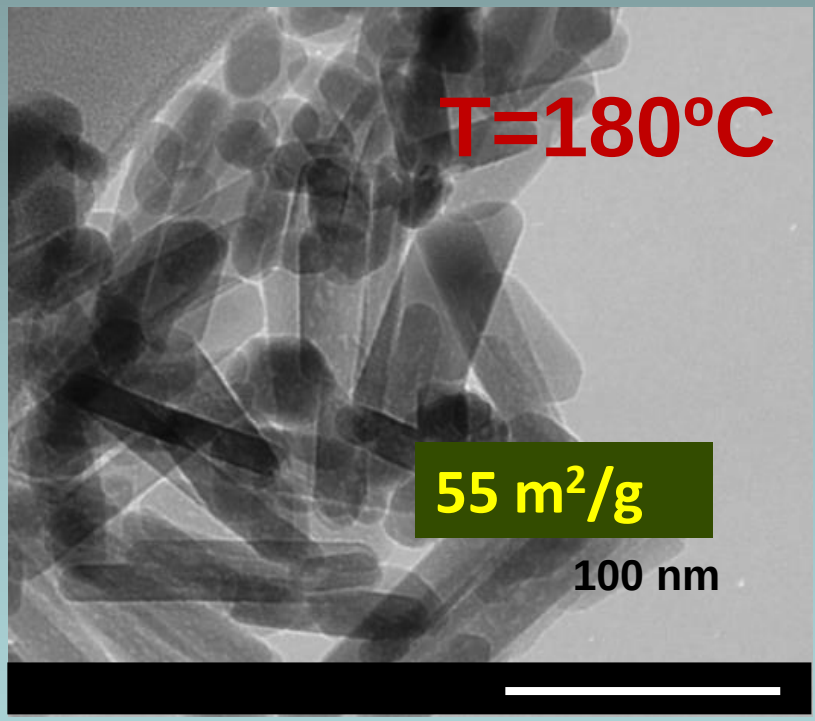
[1] M.Martins et al, J.Colloid and Interface Sci, 2008;

[2] C.Santos et al, Microscopy and Microanalysis, 2009

HAp SYNTHESIS



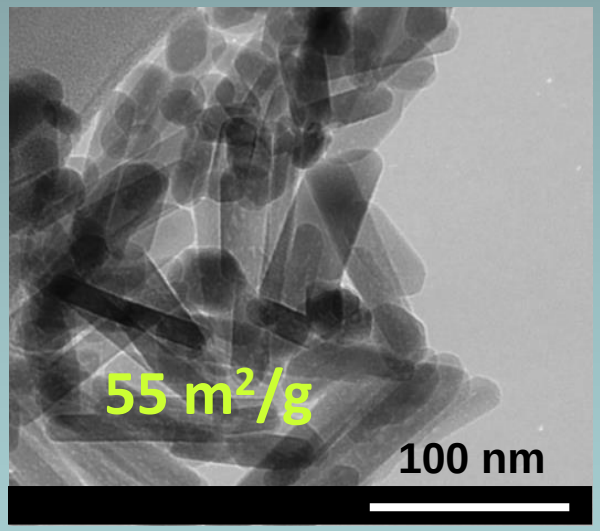
Cit:Ca=3:1



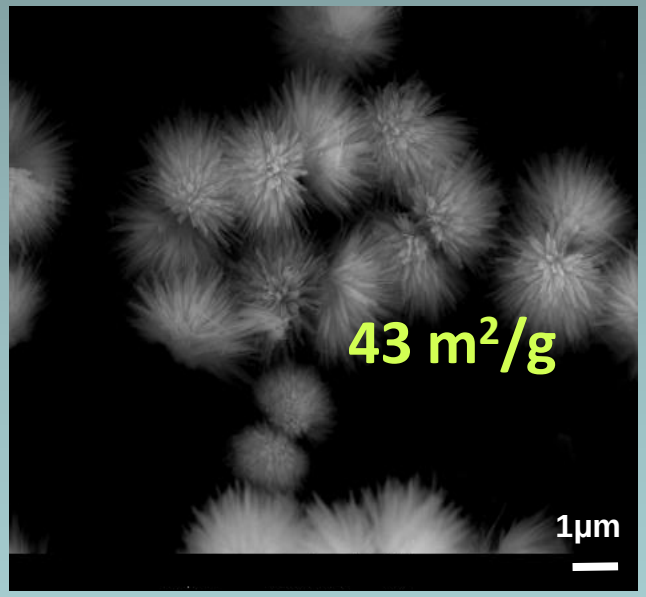
HAp SYNTHESIS

T=180ºC

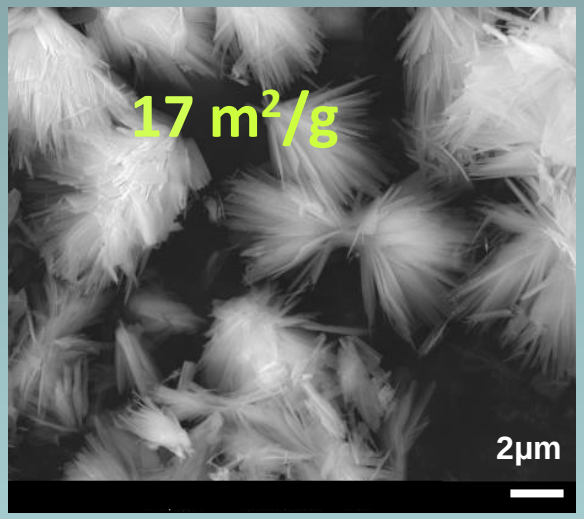
Rods



Hedgehogs



Bundle-like

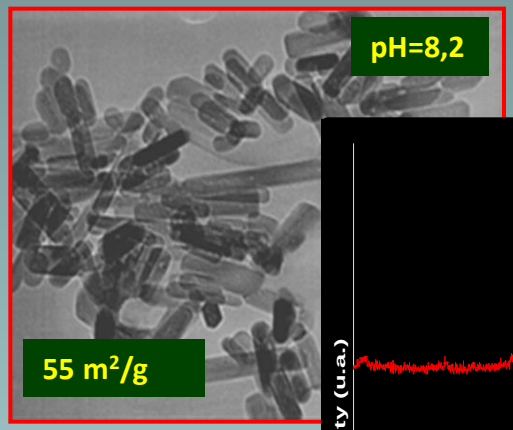


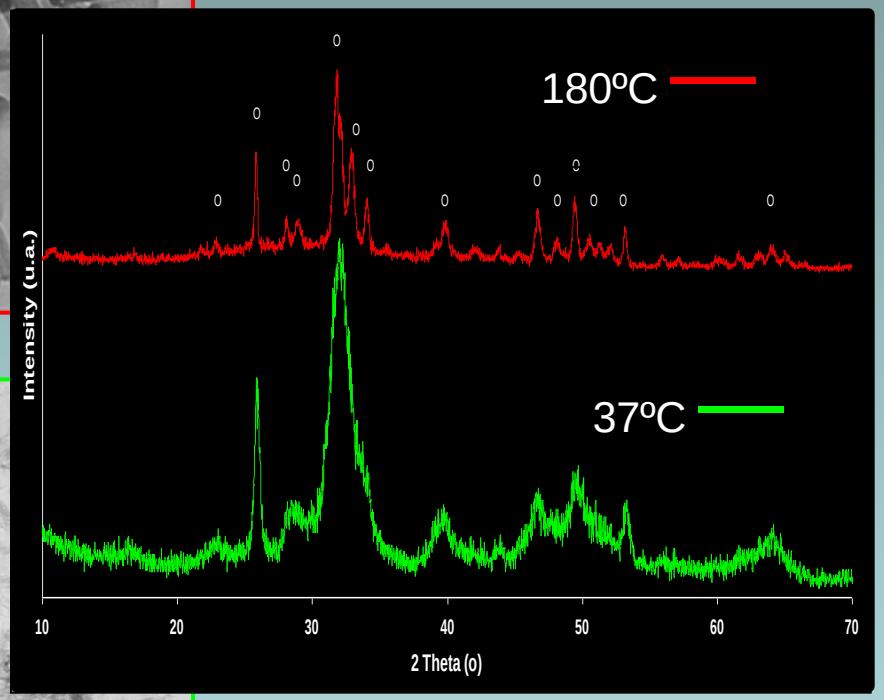
3:1 — 4:1 — 7:1

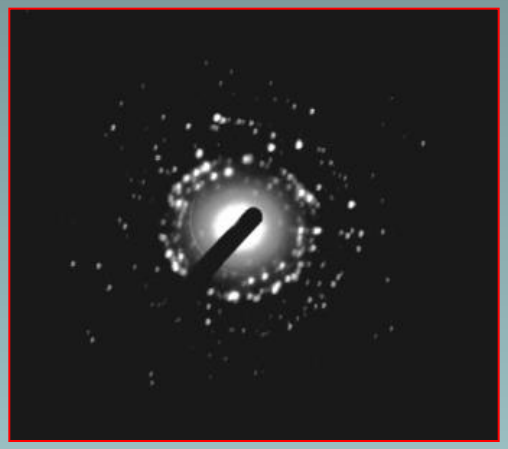
[Citrate/Calcium] Ratio

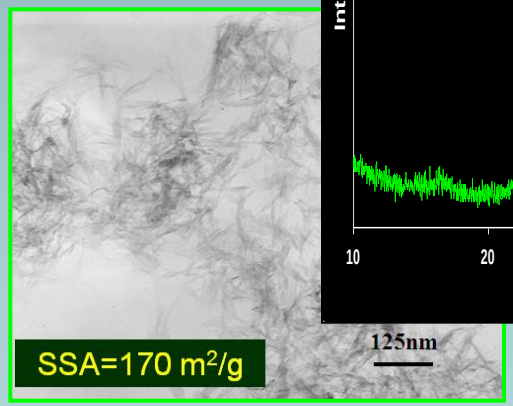
Comparing HAp nanoparticles

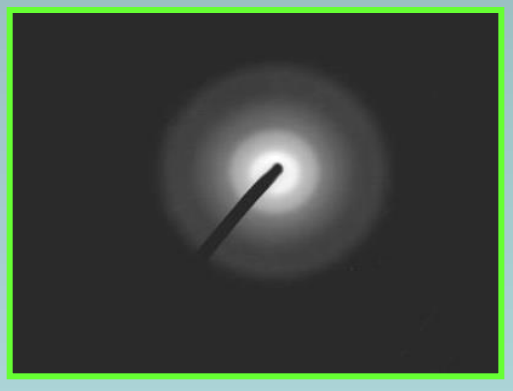
HAp nanoparticles : 180ºC *versus* 37ºC









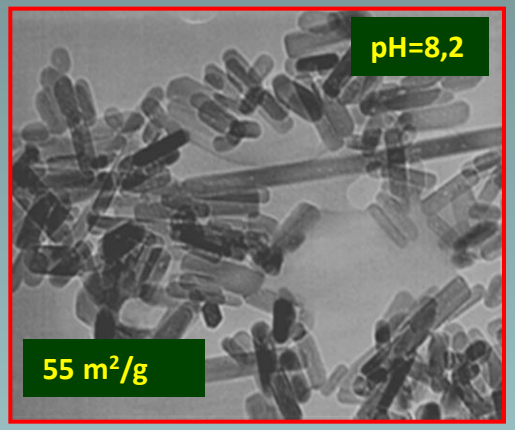


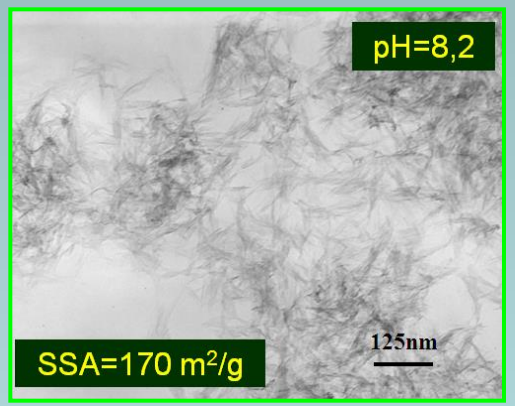
[1] M.Martins et al, J.Colloid and Interface Sci, 2008;

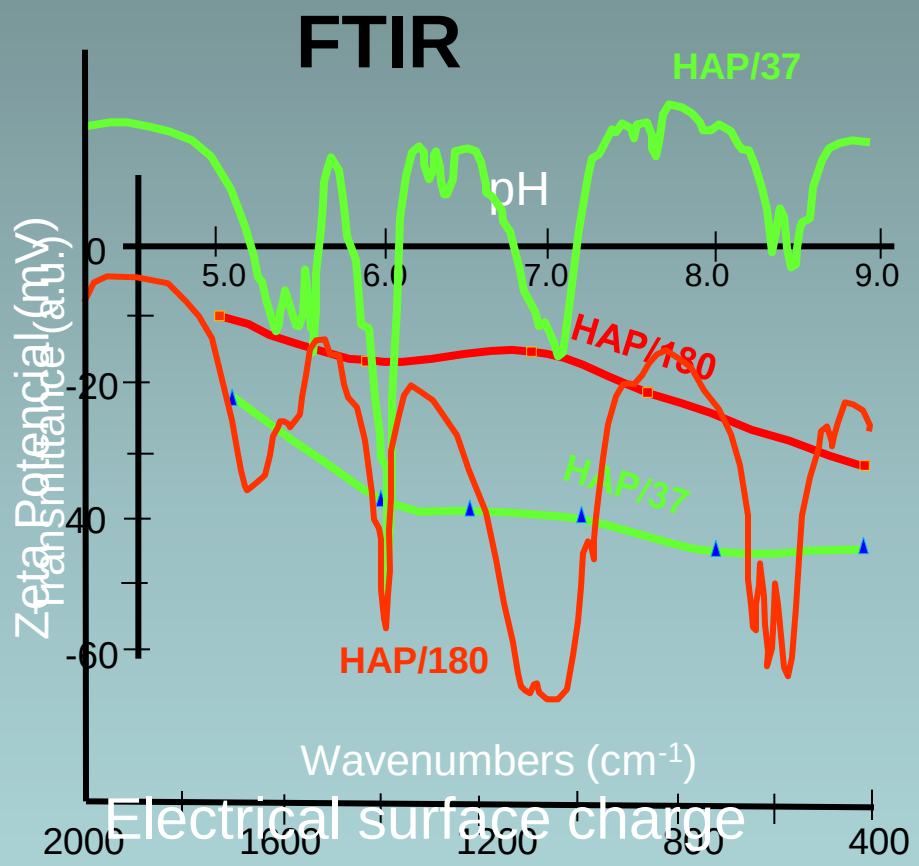
[2] C.Santos et al, Microscopy and Microanalysis, 2009

Comparing HAP properties

HAp nanoparticles : 180ºC *versus* 37ºC





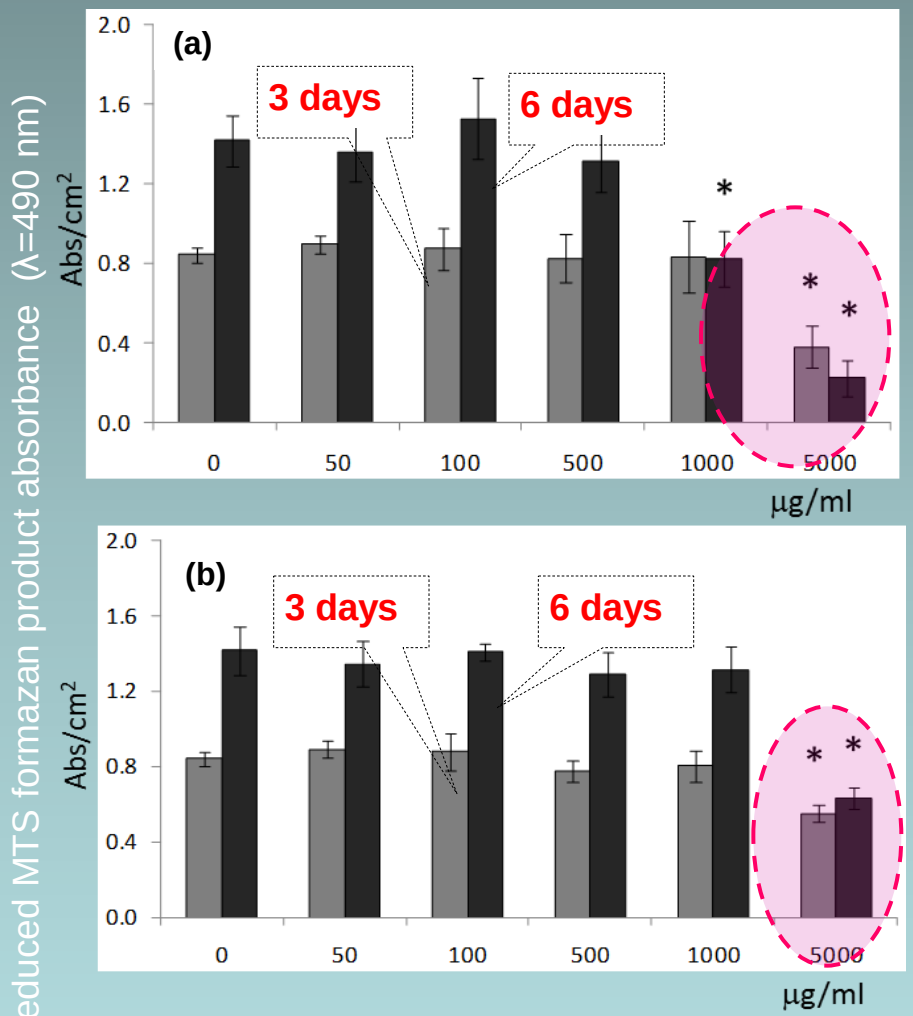


Citrate derived carboxilic groups (COO^-) coordinated to HAp under different configurations

Santos, C. *et al.* Journal of the Royal Society Interface, 2012

Interaction with cells

MG63 osteoblast-like cell response to HAp nanoparticles*



*Faculty of Dental Medicine, U. Porto

HAp / 37ºC

MG63 Cell viability

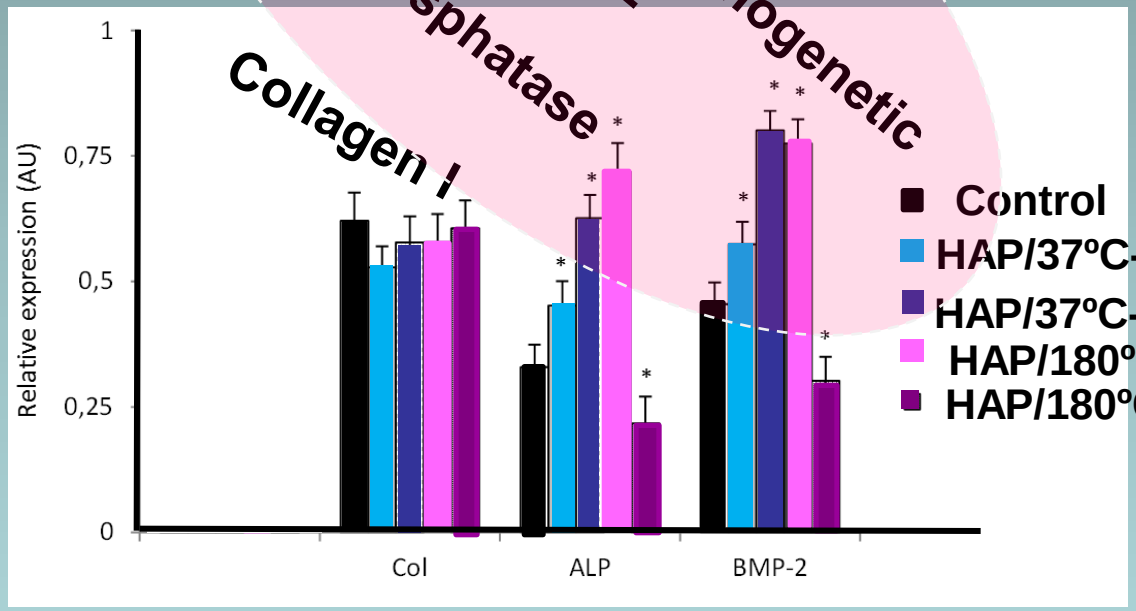
HAp / 180ºC

Low levels do not affect cell viability.
Deleterious effects observed for the higher HAp concentrations

Santos, C. *et al.* Journal of the Royal Society Interface, 2012

Interaction with cells

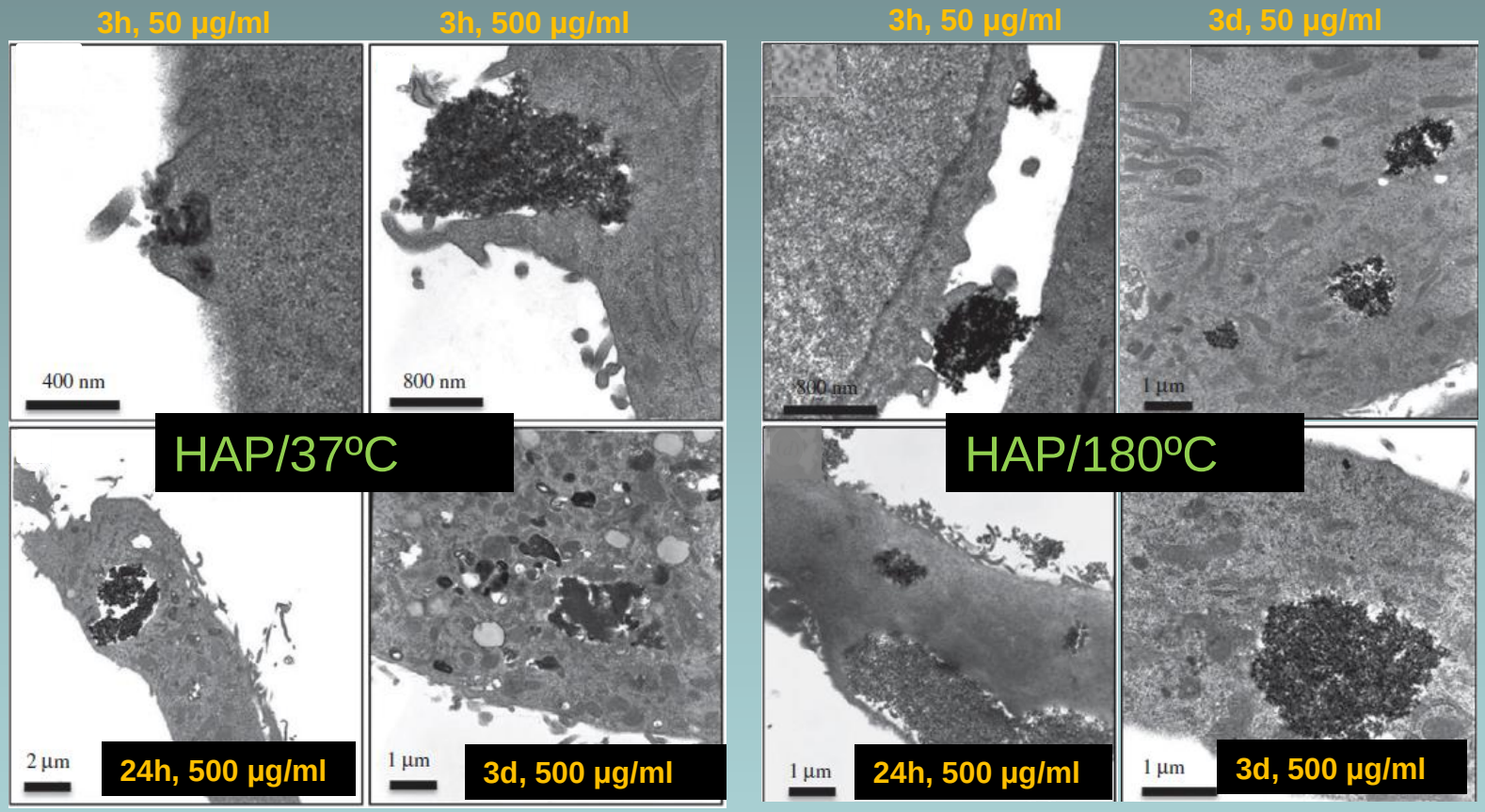
MG63 cell cultures exposed to HAP nanoparticles , 50 and 500 µg/ml, 3 days.



Low levels (not toxic) increased the expression of ALP and BMP-2

Santos, C. *et al.* Journal of the Royal Society Interface, 2012

Interaction with cells



NanoHap are readily internalized by MG63 cells.
Toxic effects observed at high concentrations reflect the vesicular entrapment.

Santos, C. *et al.* Journal of the Royal Society Interface, 2012

Concluding …

- HAP nucleation and growth phenomena are strongly dependent on precursor solution chemistry { pH, [cit] } and temperature. Manipulating these variables allows controlling the shape, size and surface properties of HAP nanoparticles (nanoHAp).

- nanoHap are readily internalized by MG63 osteoblastic cells. Low [nanoHAp] do not affect cell viability/proliferation and enhance ALP and BMP-2 expression → interesting profile for bone tissue applications.

Thank you

Acknowledgments

UNIVERSITY OF AVEIRO

CICECO
Centro de Investigação em Materiais Cerâmicos e Compósitos

COMPETE
PROGRAMA OPERACIONAL FACTORES DE COMPETITIVIDADE

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL
PORTUGAL 2007.2013

Laboratório de Farmacologia e Biocompatibilidade Celular
Pharmacology & Biocompatibility

FCT Fundação para a Ciência e a Tecnologia
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