RECHERCHE

INSTITUT NATIONAL DES SCIENCES APPLIQUÉES DE LYON

Élaboration de verres bioactifs poreux par métallurgie des poudres et application à la substitution osseuse

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NORAKER

Innovative synthetic bone substitutes

membre de UNIVERSITÉ DE LYON

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Bio-active ceramics / Bone substitution

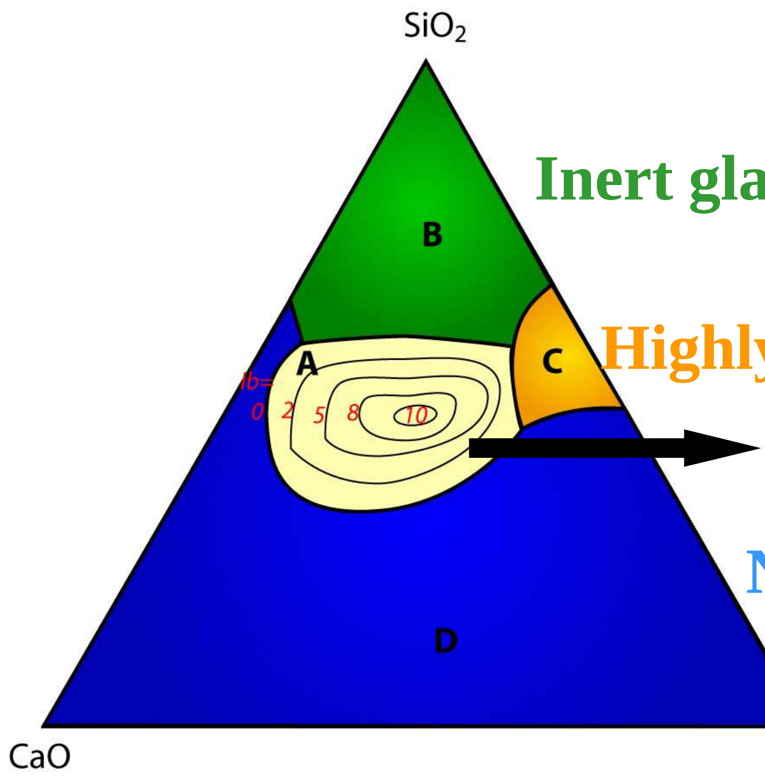
Bone substitutes in revision surgery

- Role:
  - fill bone defects
  - guide bone ingrowth and accelerate reconstruction (*osteocondution*)
  - provoke bone formation (*osteoinduction*)
- Materials
  - mostly calcium phosphates
    - Sintered and “macroporous”
    - cements

Bio-active glasses

An alternative to calcium phosphates:

Glasses in the system SiO_2 - CaO - Na_2O - P_2O_5



Inert glasses

Highly resorbable (too much!)

Bio-active

Not attainable by fusion / quenching

Diagram for 6 wt% P_2O_5

I_B : index of bioactivity: higher when faster bone bonding

Bioactive glasses

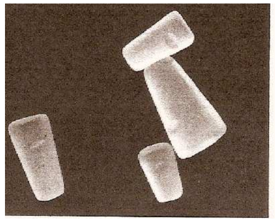
Bioactive glasses properties: Formation of a crystallized carbonated hydroxyapatite, identical to the mineral part of bone, at the implant surface in vitro and in vivo ⇨ Chemical bond between bone/Implant

45S5 Bioactive glass (Bioglass ®): Developed in 1969 by L.L. HENCH

Weight composition: 45% SiO_2; 24,5% Na_2O; 24,5% CaO et 6% de P_2O_5.

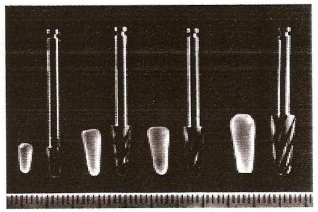
Interests:

- It is the most bioactive glass obtained by the traditional method. It allows a fast implant/tissue bonding.
- It favors stem cells differentiation into osteoblasts. Only known osteoinductive ceramic
- Bactericid effect



Applications: middle ear and dental implants…

(dense blocks or beads)



Objectives

<u>Porous bioactive glass pieces</u> :

- An alternative to calcium phosphate ceramics for orthopedic applications.
- A choice candidate for tissue engineering.

composition
system, cristallinity level

architecture
size, amount of porosity, morphology

surface properties
specific surface area

⇨

Mechanical properties
stress and strain to fracture, crack resistance

Biological properties
cell viability, adhesion, proliferation, bone growth

Synthesis of bioglass®

POWDERS MIXTURE
(SiO_2, Na_2CO_3, $CaCO_3$, P_2O_5)

↓

DECARBONATATION
950℃ – 5h

↓

MELTING 1400℃ – 4h

↓

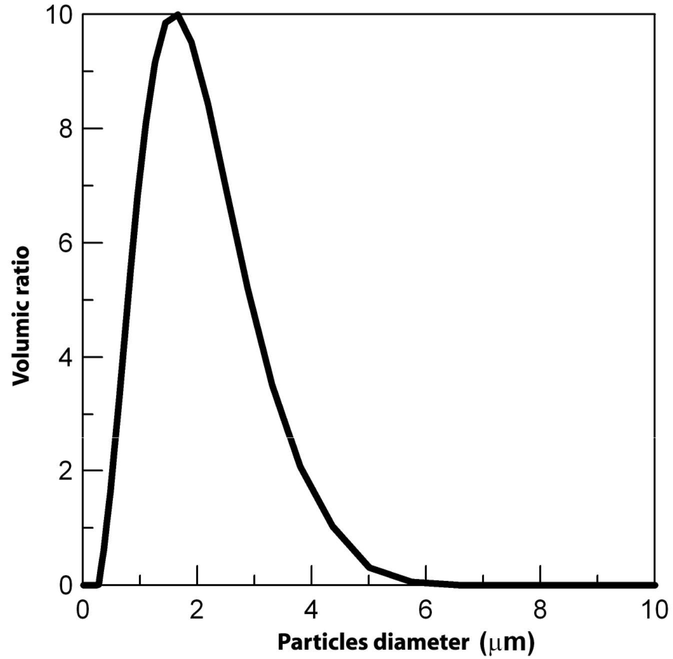
Quenching
In water

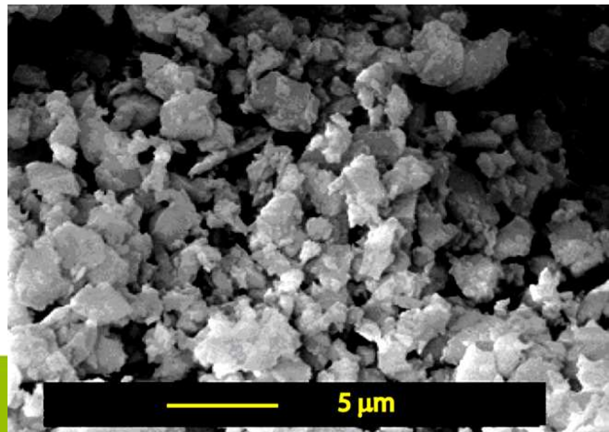
↓ **Milling**

Powder (1.6 µm)
D_{abs} = 2.69 g/cm^3
SSA = 2.4 g/m^2

Powders granulometric curve

Monomodal distribution centered on 1.6 µm

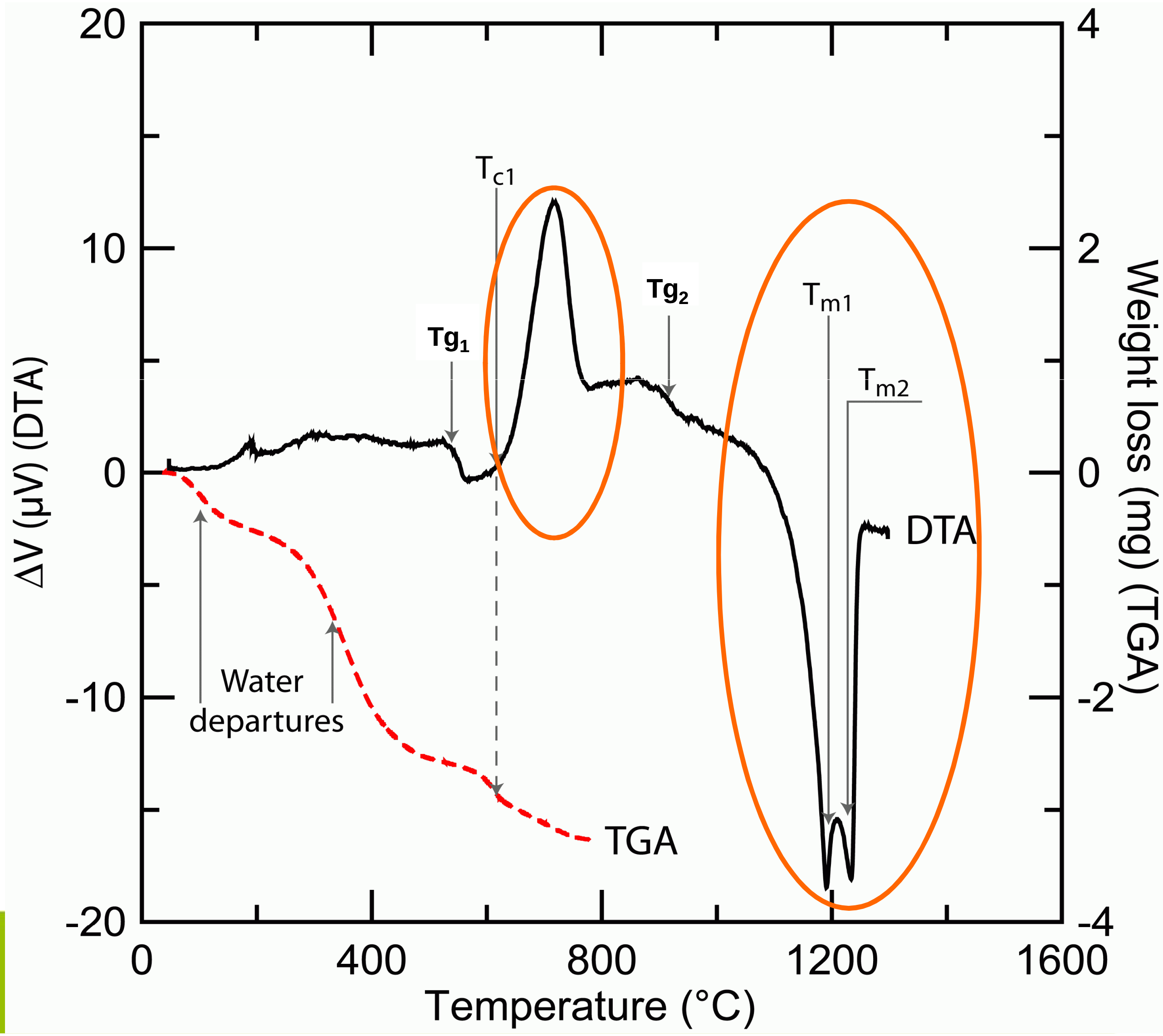




Powders SEM observations

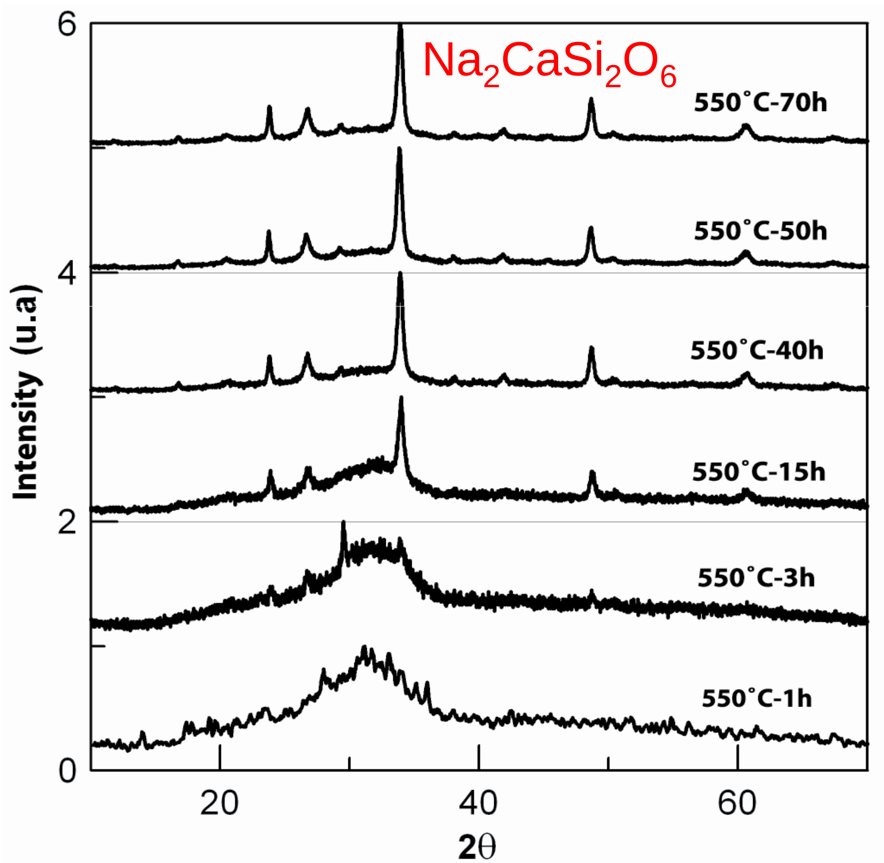
Thermal behavior

DTA/TGA curves

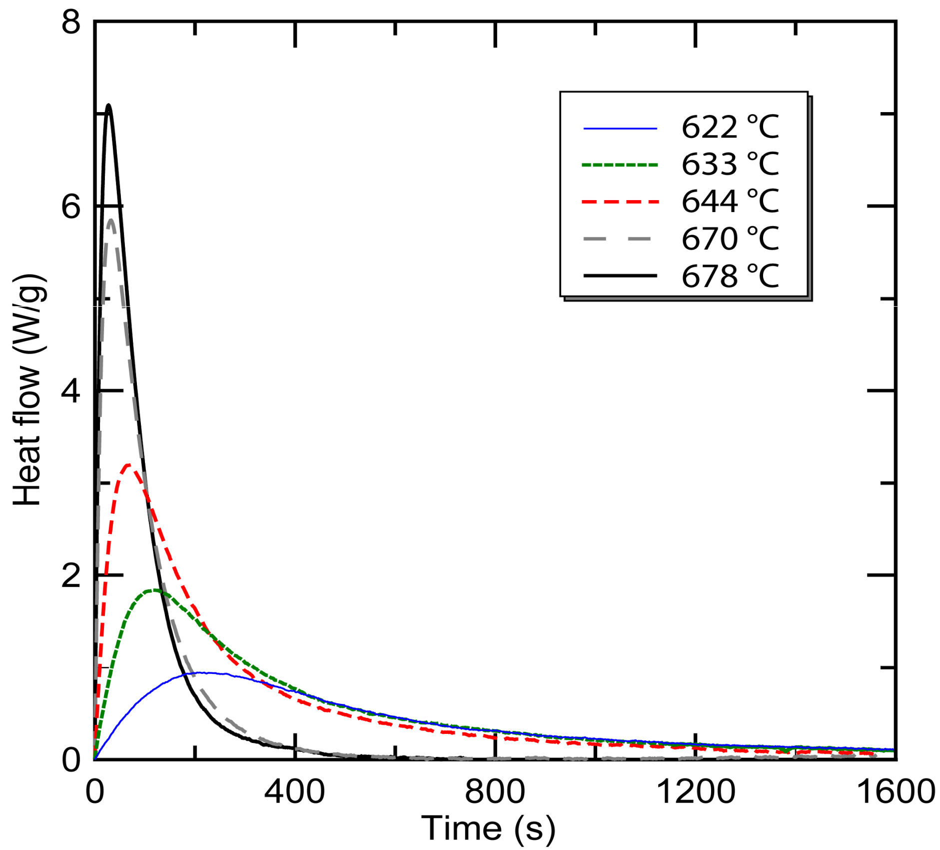


Crystallization kinetics

Kinetics between 550℃ and 580℃: XRD

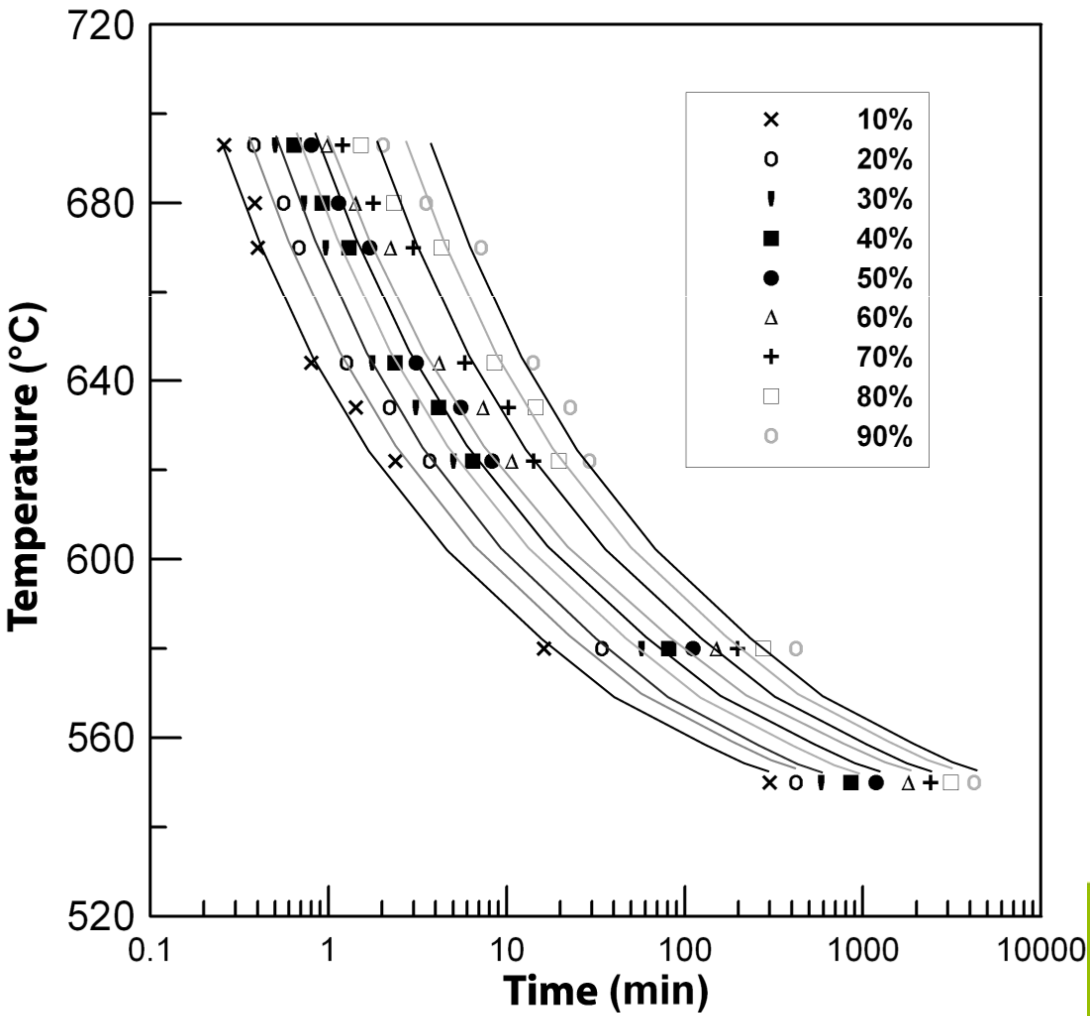


kinetics between 622℃ and 678 ℃: isothermal DSC



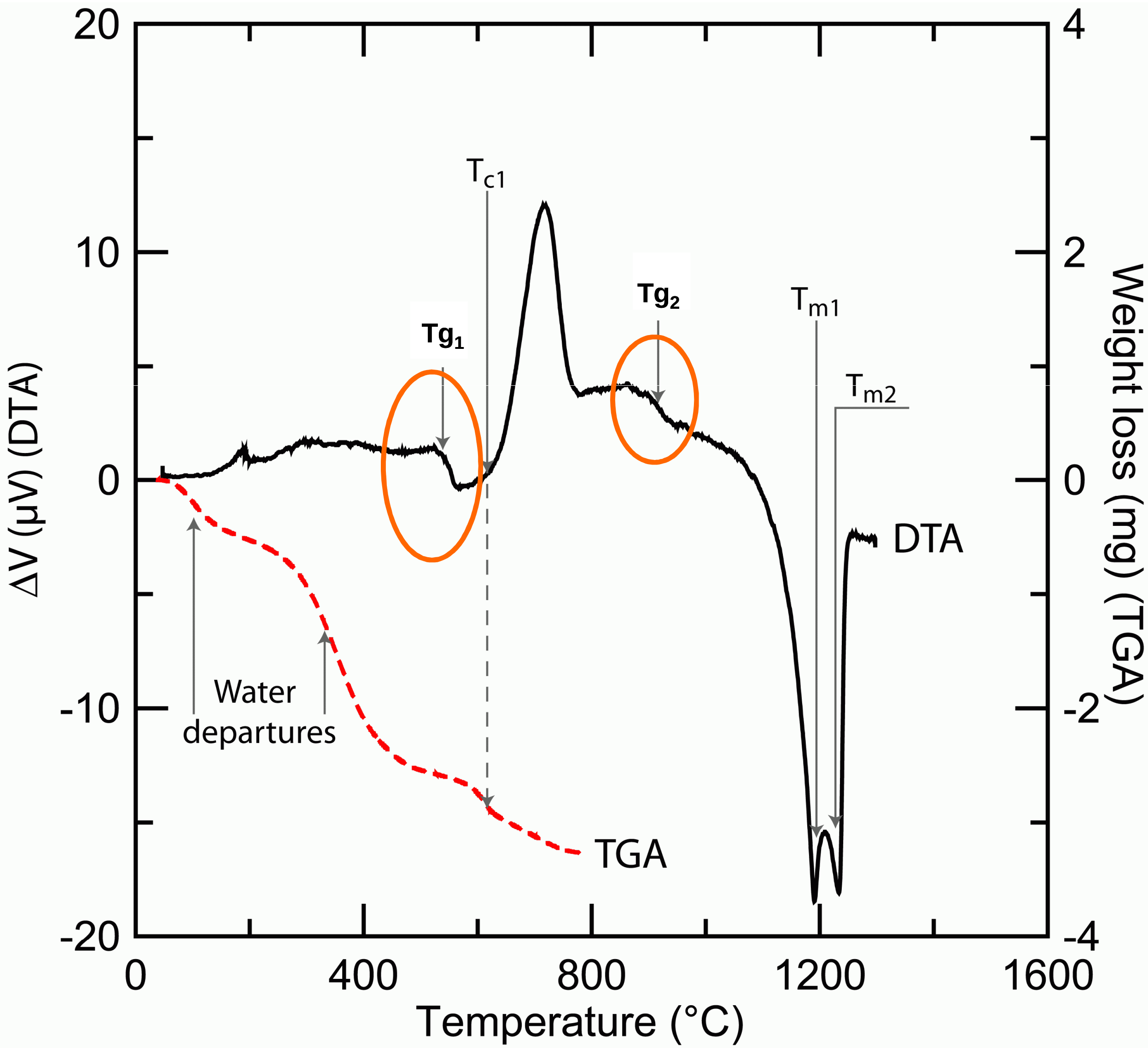
TTT curves

Temperature-Time-Transformation curves of 45S5 bioglass:
(transformation ratio relative to 80% maximum crystallinity)



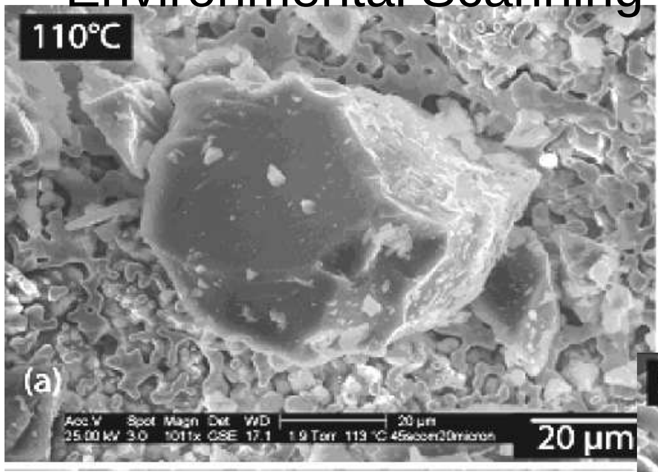
Thermal behaviour

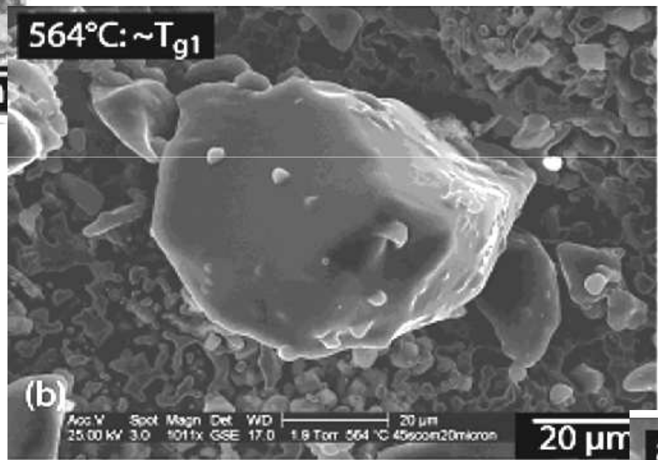
DTA/TGA curves

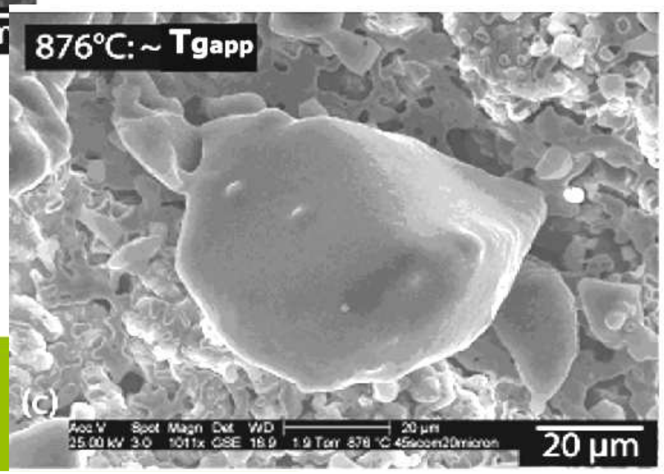


Glass transitions

Environmental Scanning Electron Microscopy with an in situ heating stage



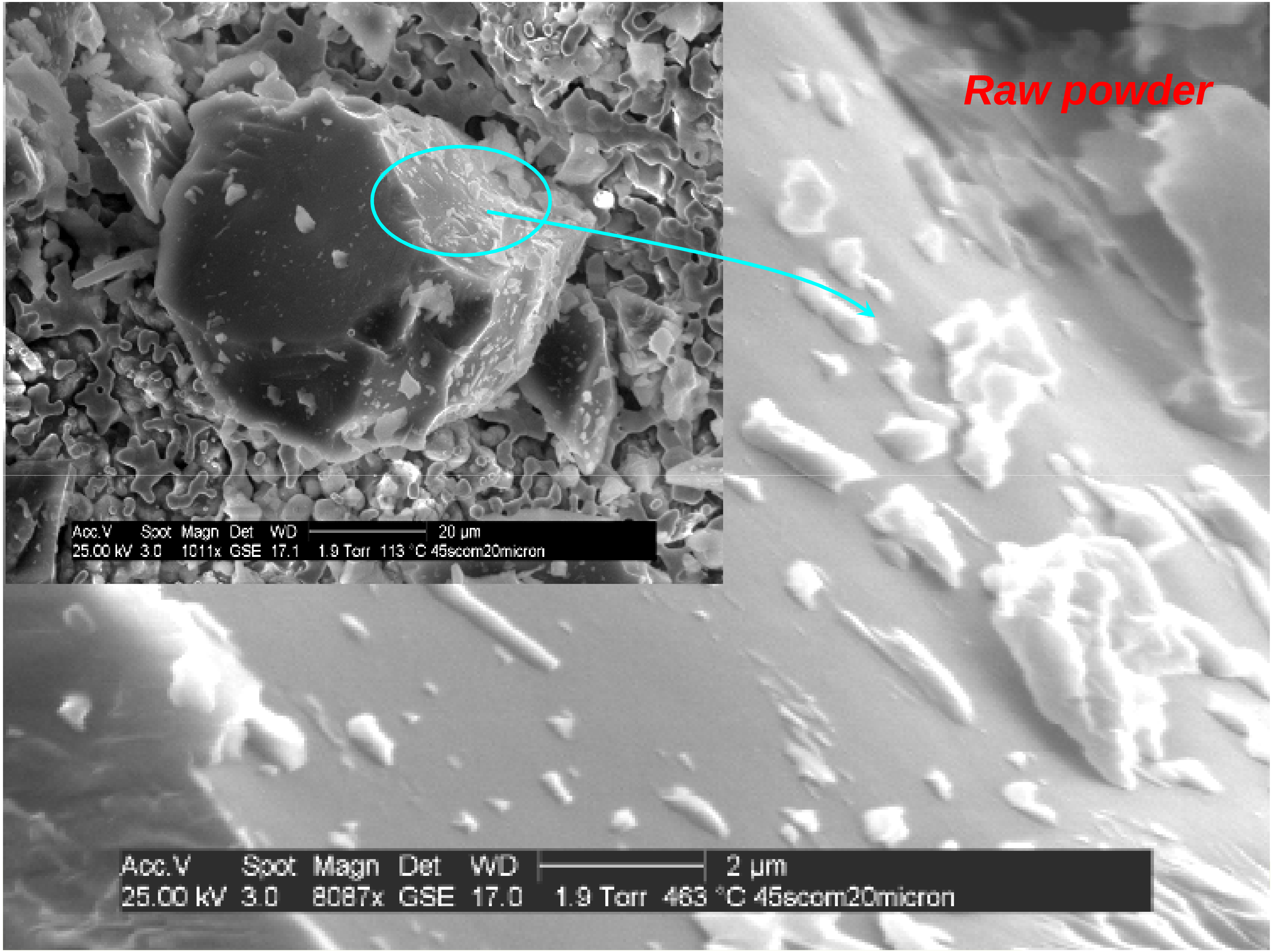




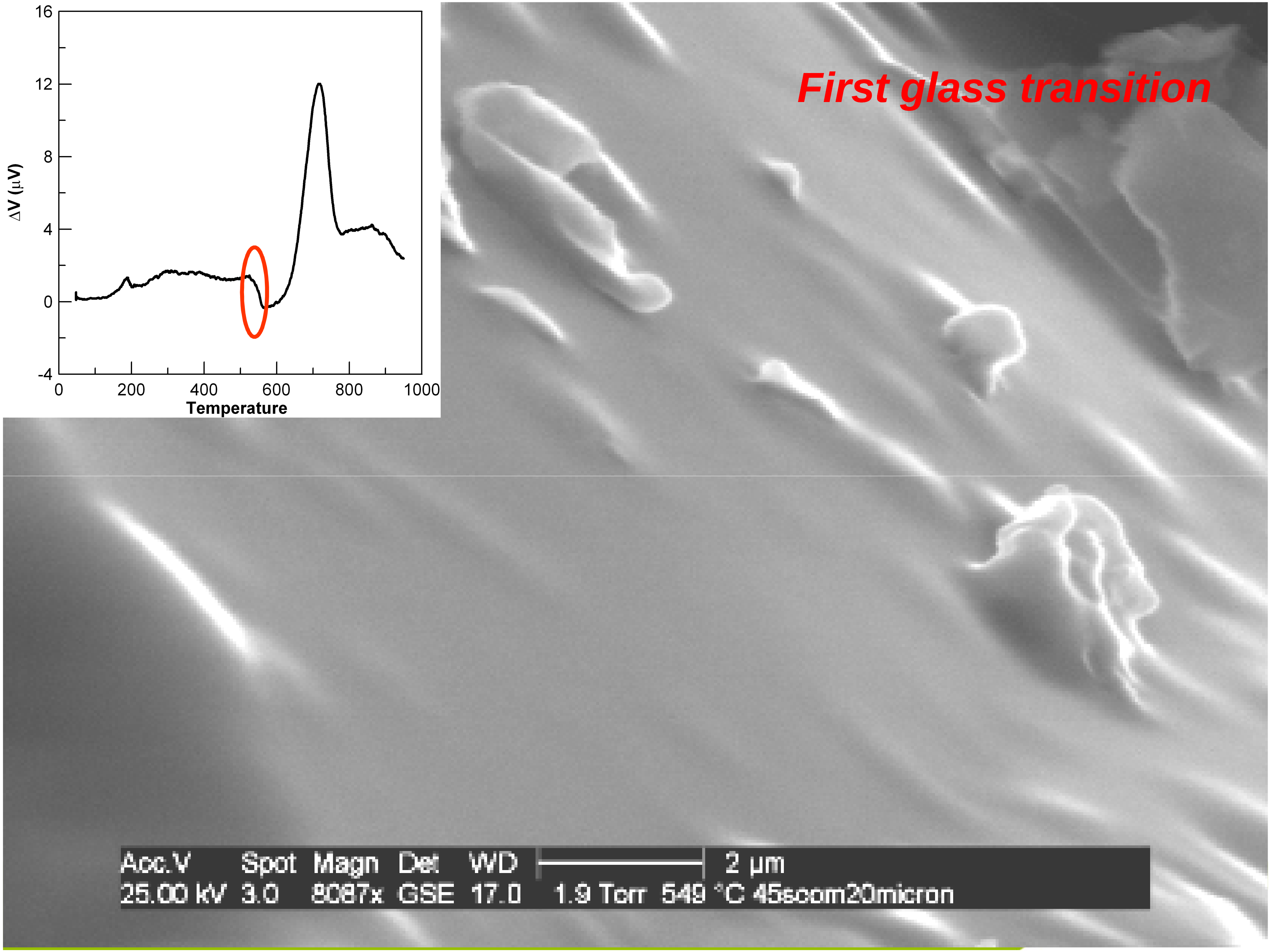
2 stages of particles rounding

⇩

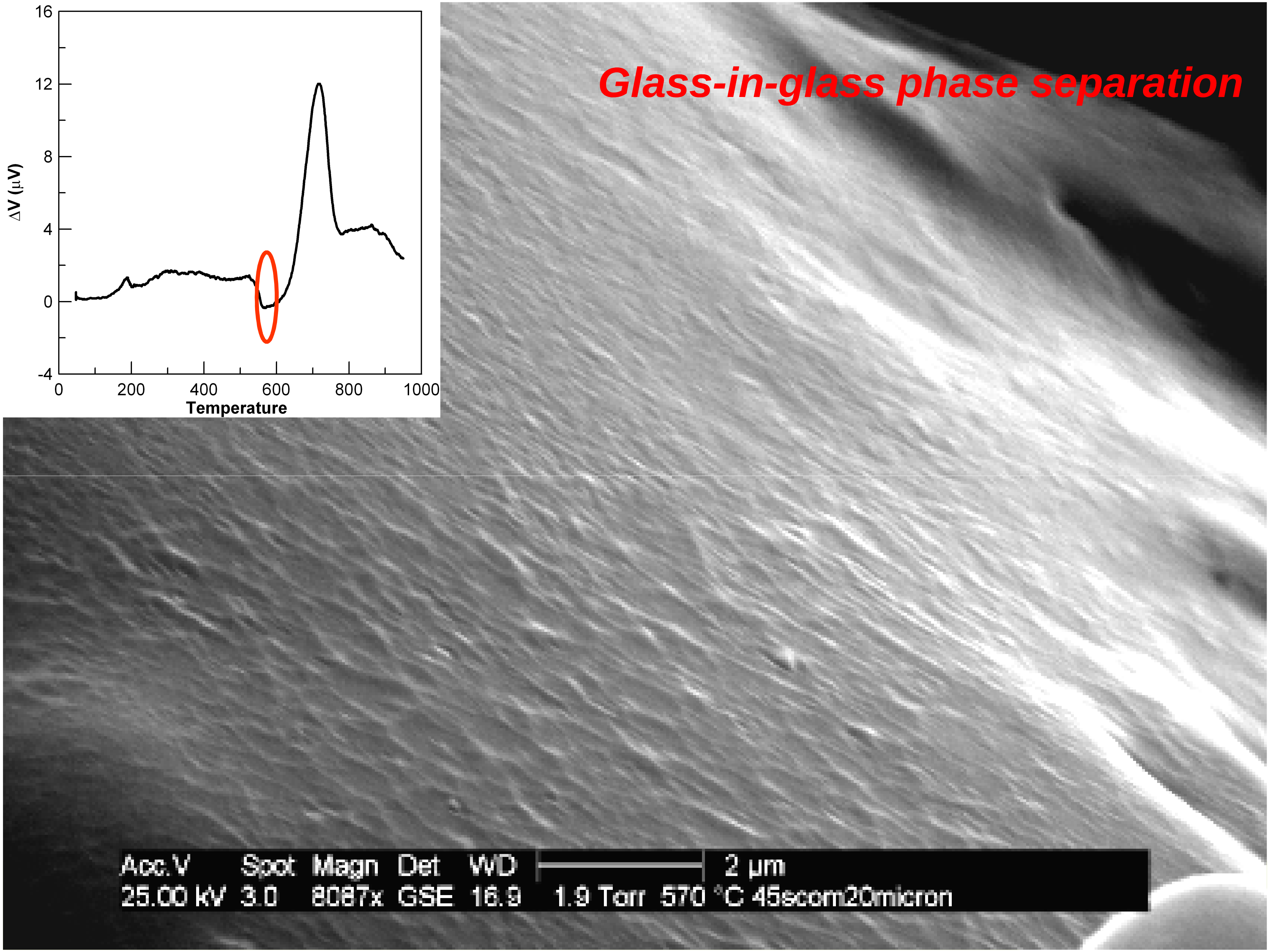
Confirmation of the 2 glass transitions



Raw powder
Acc.V Spot Magn Det WD 20 µm
25.00 kV 3.0 1011x GSE 17.1 1.9 Torr 113 °C 45sccm20micron
Acc.V Spot Magn Det WD 2 µm
25.00 kV 3.0 8087x GSE 17.0 1.9 Torr 463 °C 45sccm20micron

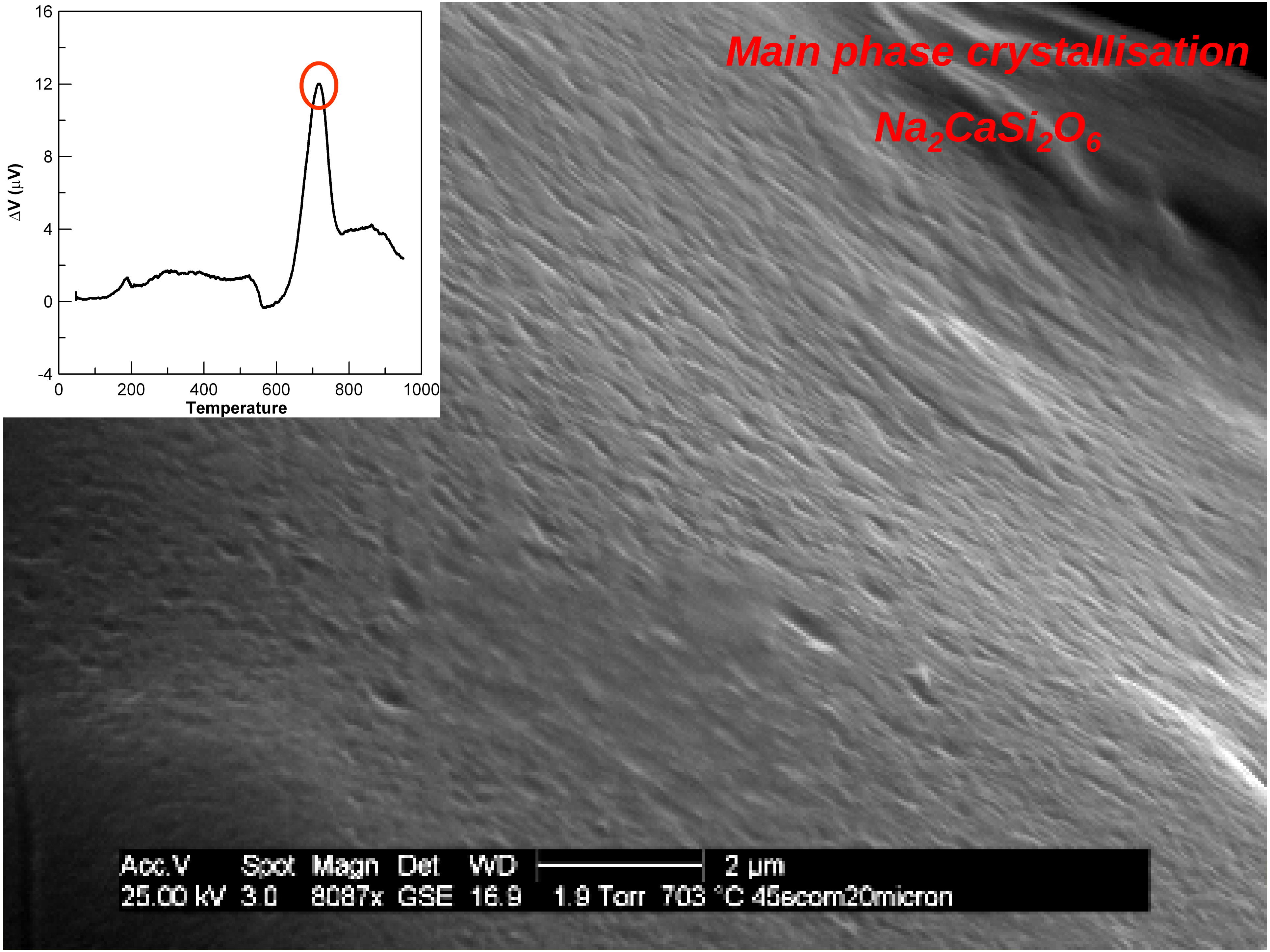

First glass transition
ΔV (μV)
16
12
8
4
0
-4
0
200
400
600
800
1000
Temperature
Acc.V Spot Magn Det WD
2 μm
25.00 kV 3.0 8087x GSE 17.0 1.9 Torr 549 °C 45scom20micron

Glass-in-glass phase separation



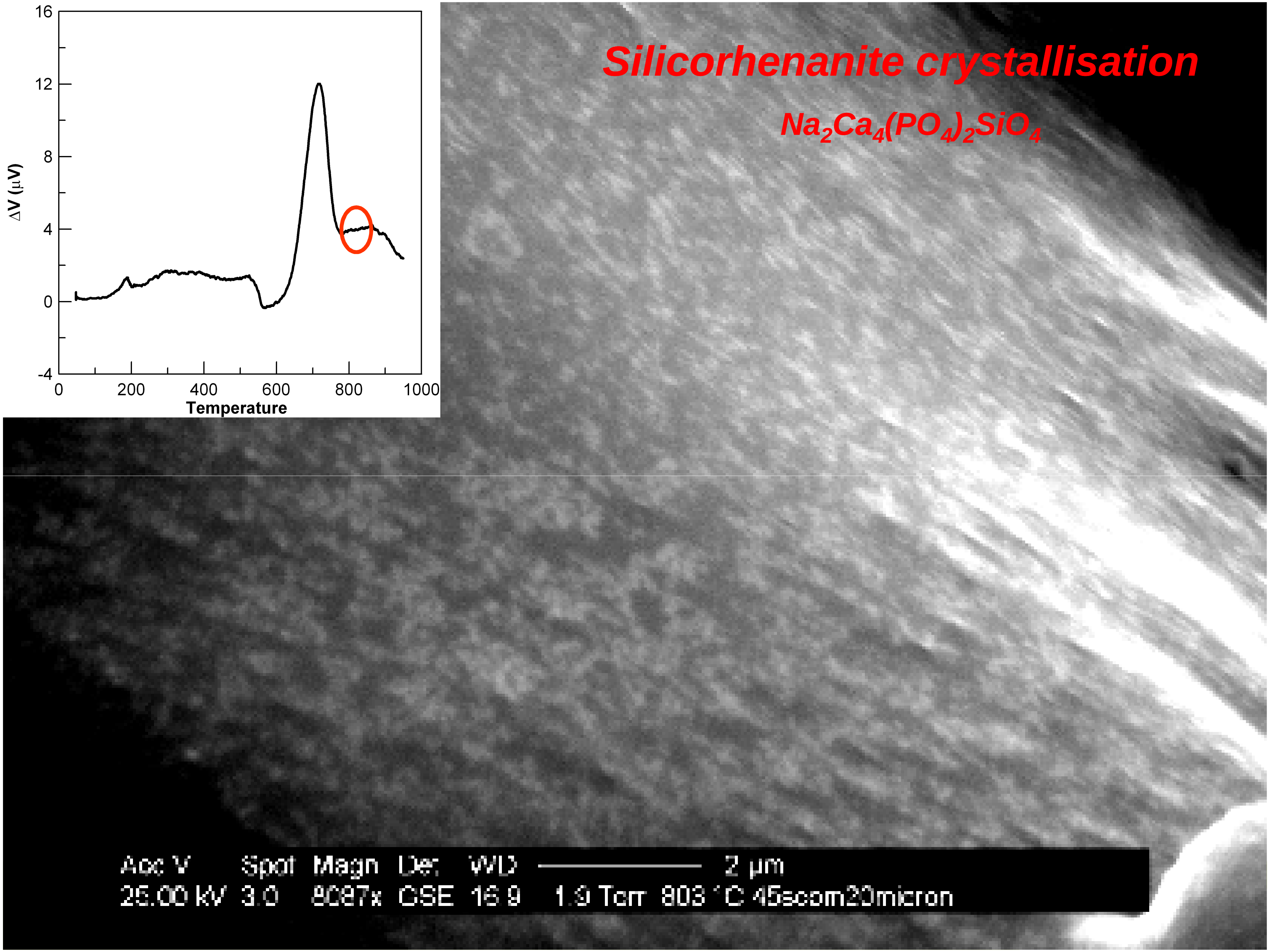
Main phase crystallisation

$Na_2CaSi_2O_6$

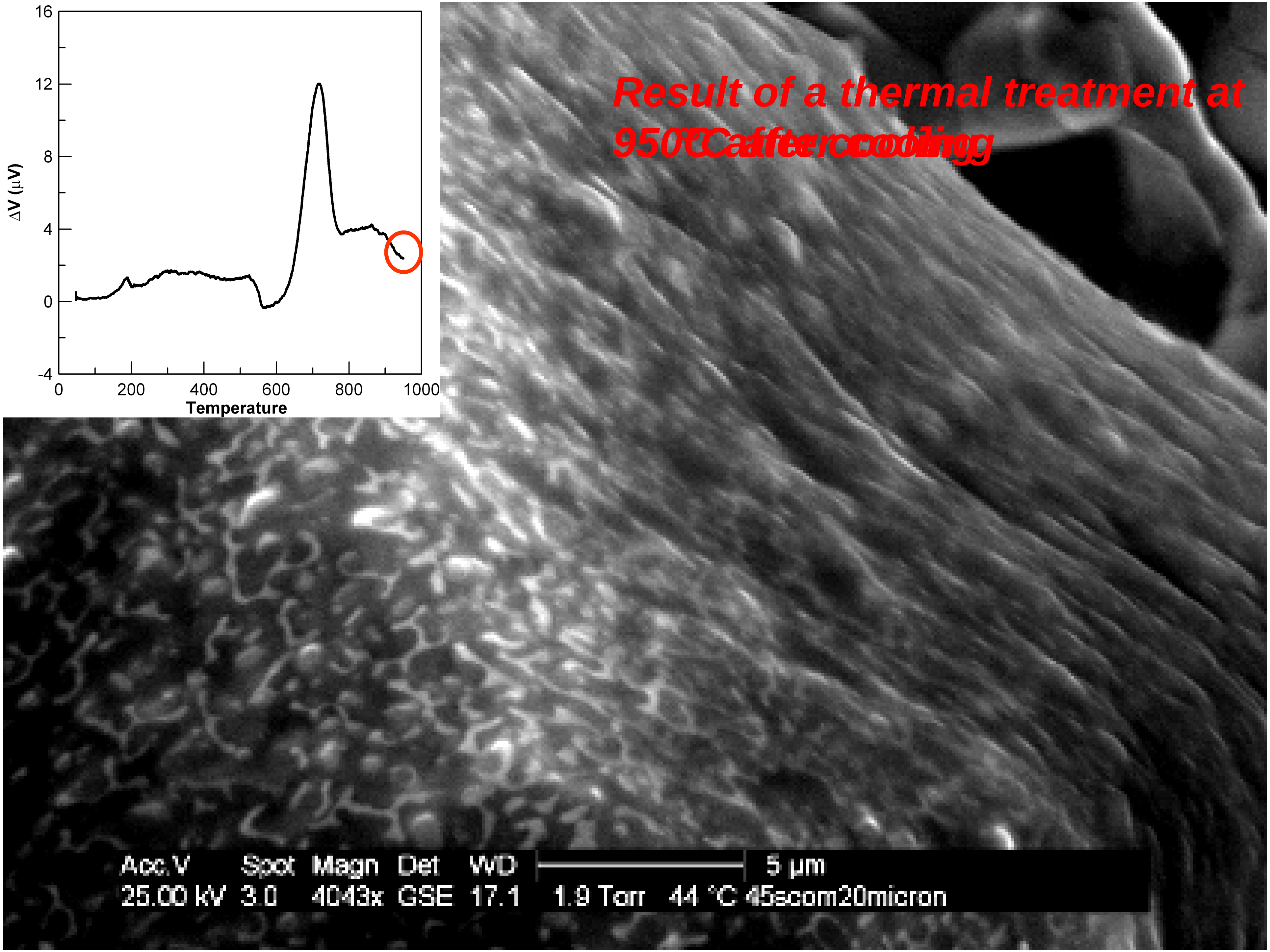


Silicorhenanite crystallisation

$Na_2Ca_4(PO_4)_2SiO_4$

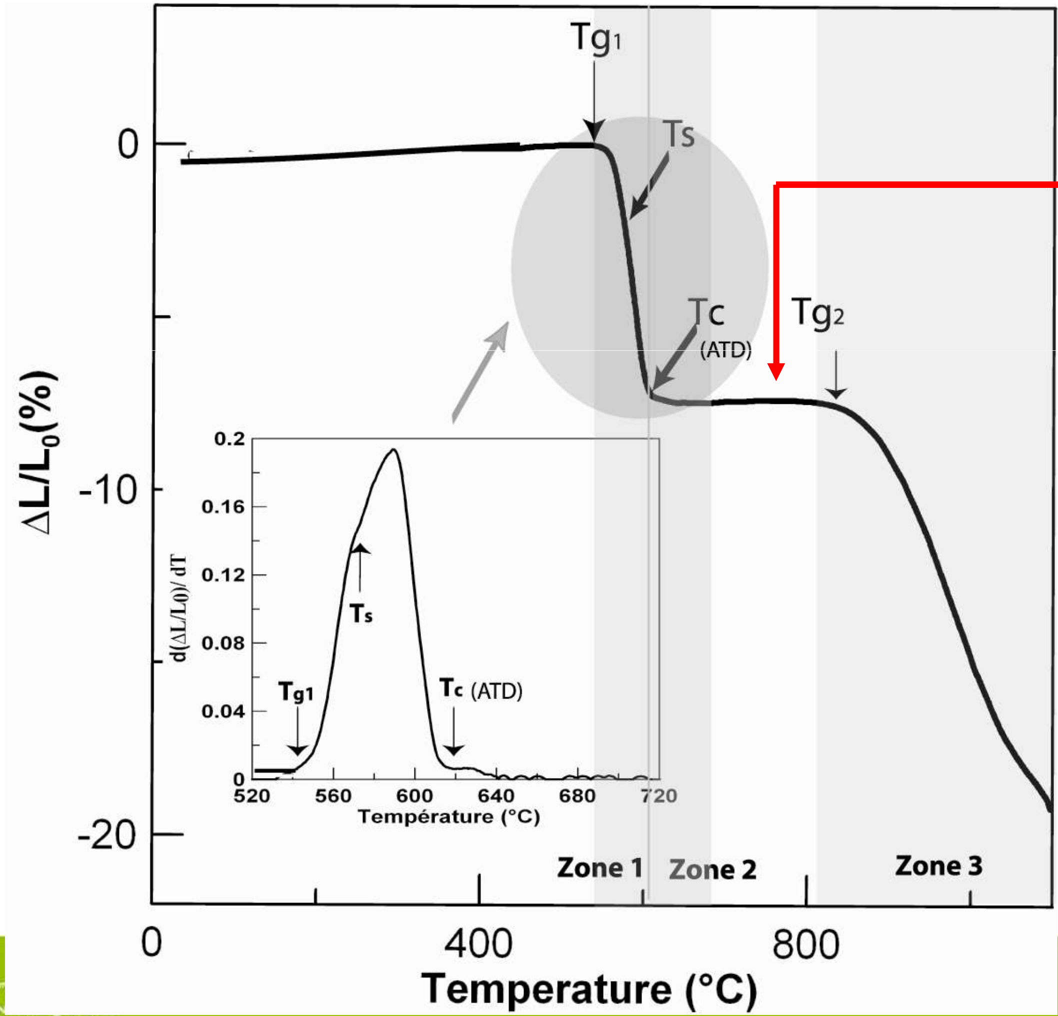


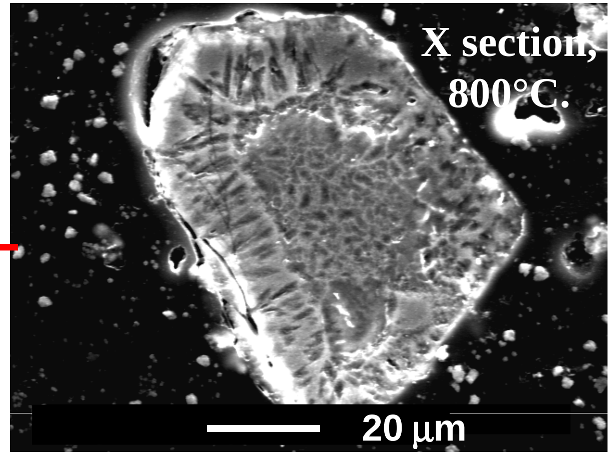
Result of a thermal treatment at 950°C after cooling



Sintering behaviour

Dilatometric curve taken at 5℃/min of Bioglass® wi th its derivative

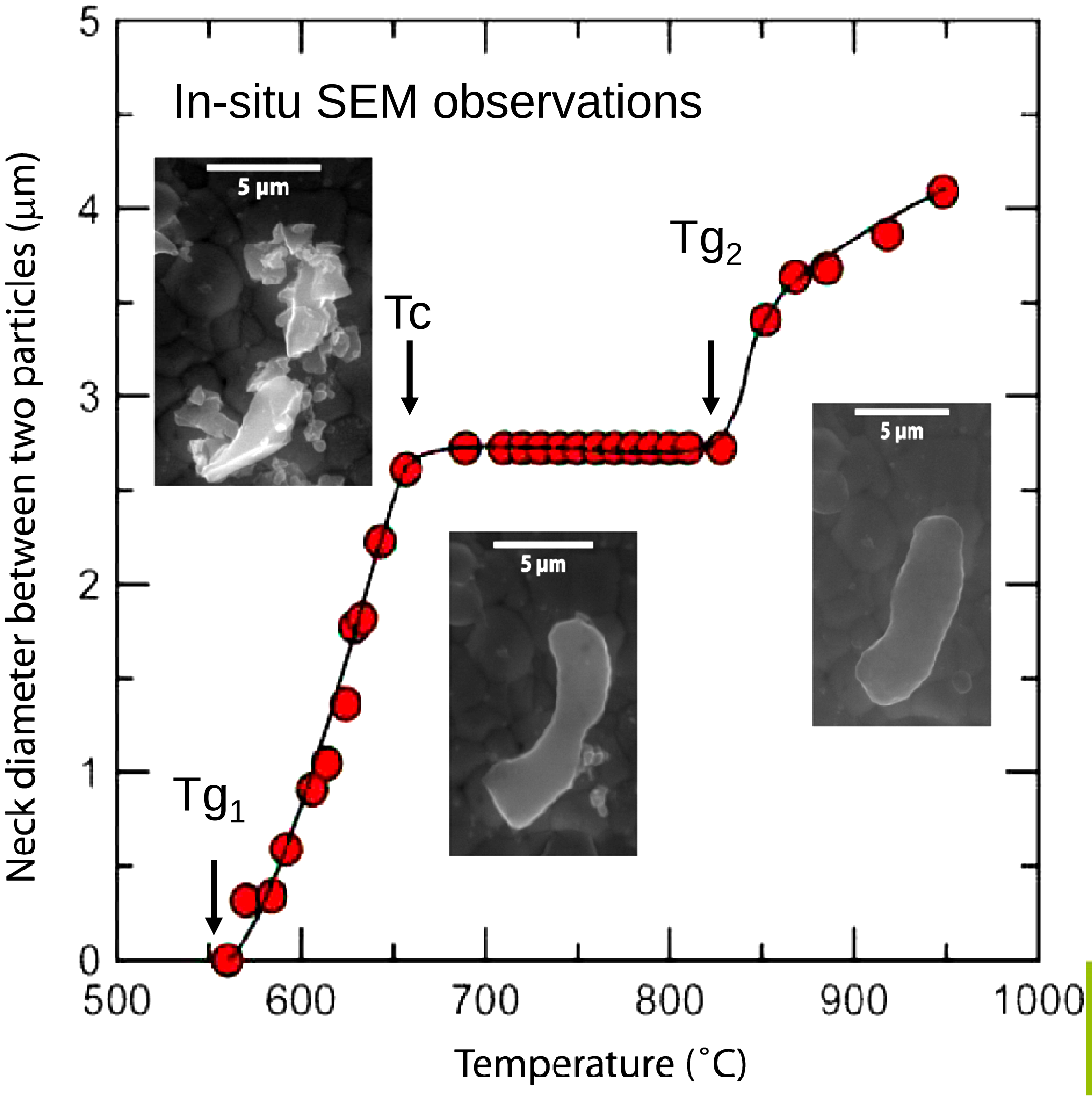




- **2 major steps of shrinkage**
- **Hindering of viscous flow with crystallisation**

Formation of a neck between 2 particles

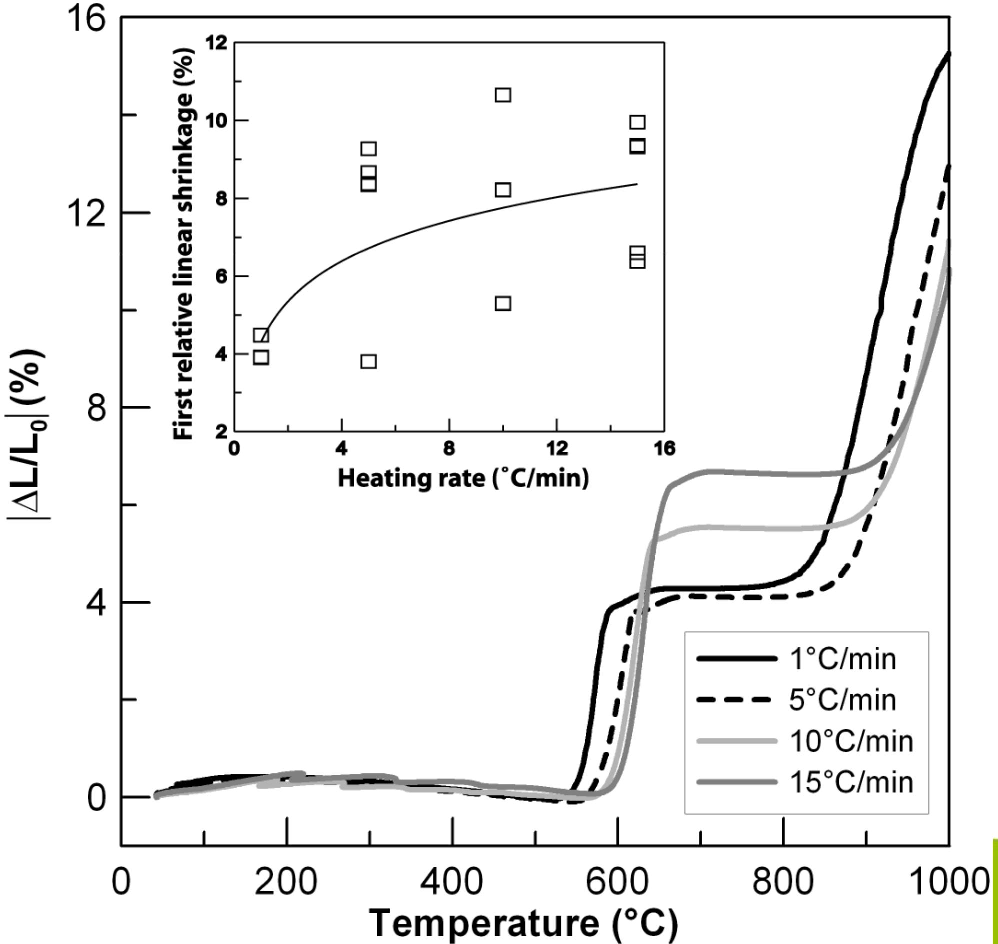
Evolution of the neck diameter between two particles
versus temperature (at 5℃/min)

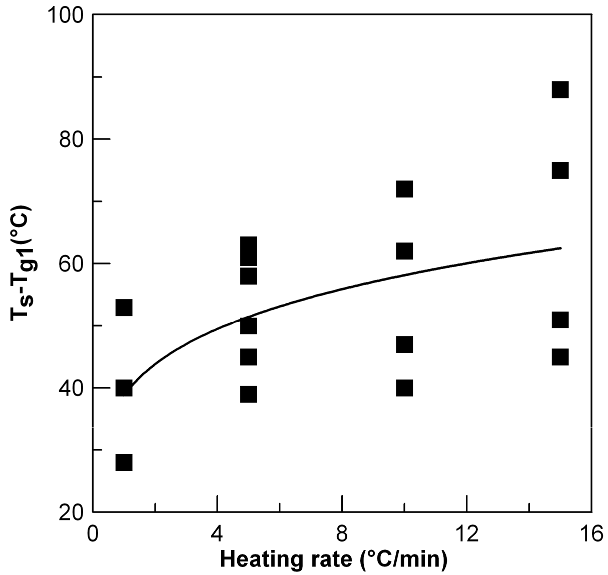


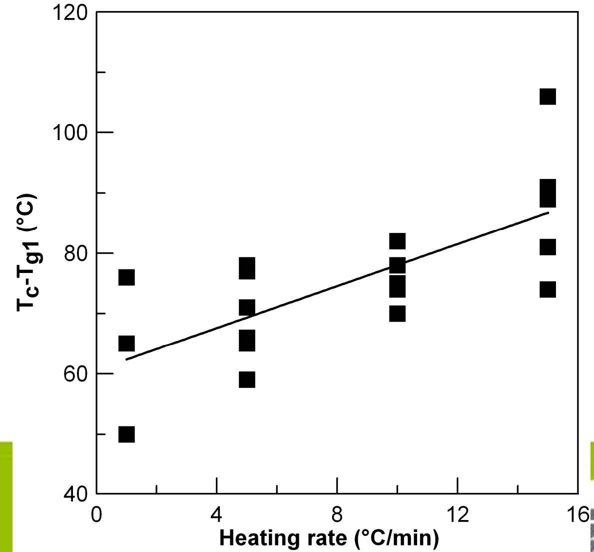
- **Same sintering steps as on dilatometric measurements**
- **Same temperature spans**

Sintering: influence of the heating rate

- increase of (Ts - Tg1) and (Tc – Tg1) with heating rate
- => increased shrinkage during the first step with heating rate

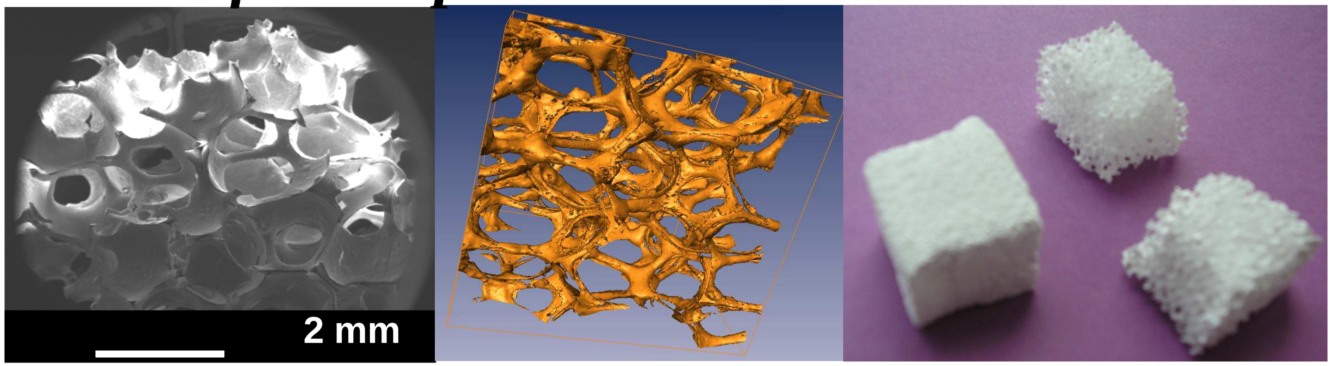






Fabrication of porous blocks

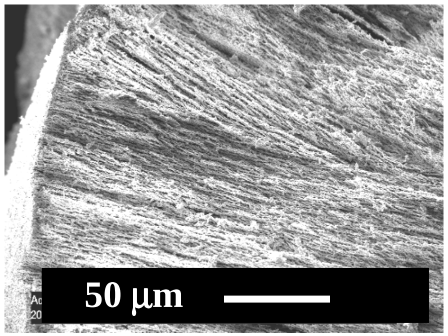
PU foam replication

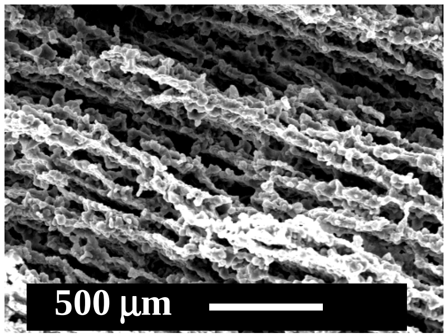


- Homogeneous and interconnected structure
- Control of the macropore size

- Debinding is necessary => difficult control of the phase transformations

Freeze Casting





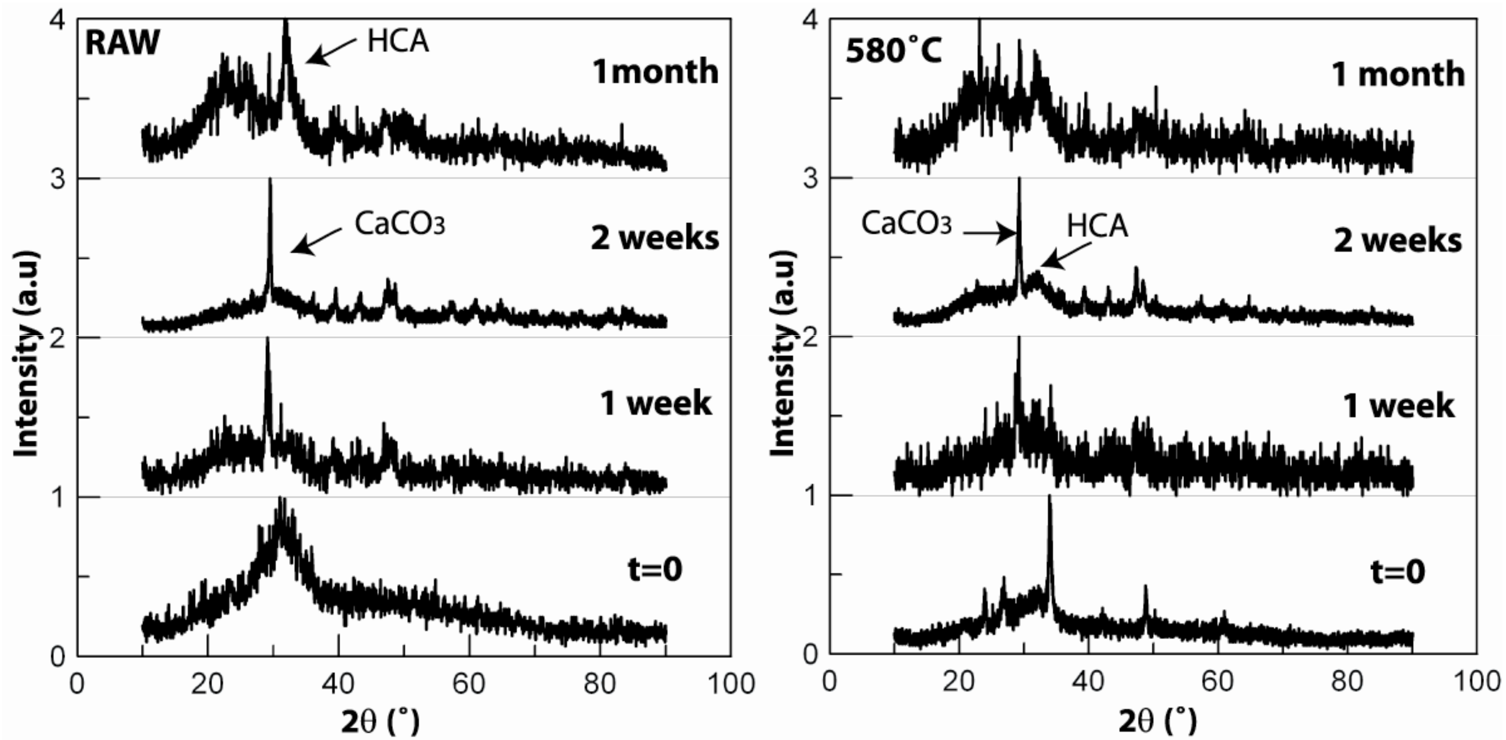
Possibility of controlling:

- porosity amount
- freezing direction => pore morphology
- size of macropores
- structural transformations

1 mm

Bioactivity of thermally treated powders

XRD diffractograms of raw and thermally treated (580℃) bioactive glass powders immersed in a Simulated Body Fluid for different times

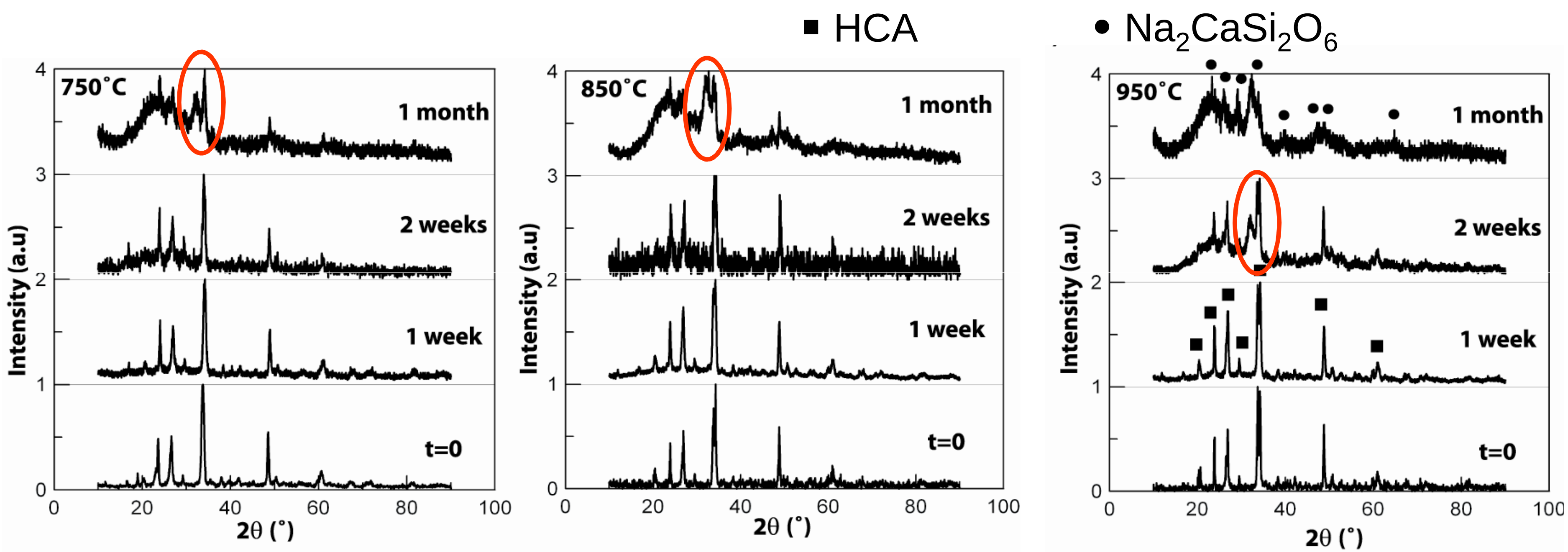


Formation of $CaCO_3$ + HCA

1 month: HCA predominant

Bioactivity of thermally treated powders

XRD diffractograms of powders thermally treated at 750℃, 850℃ and 950℃, and immersed in a SBF for different times

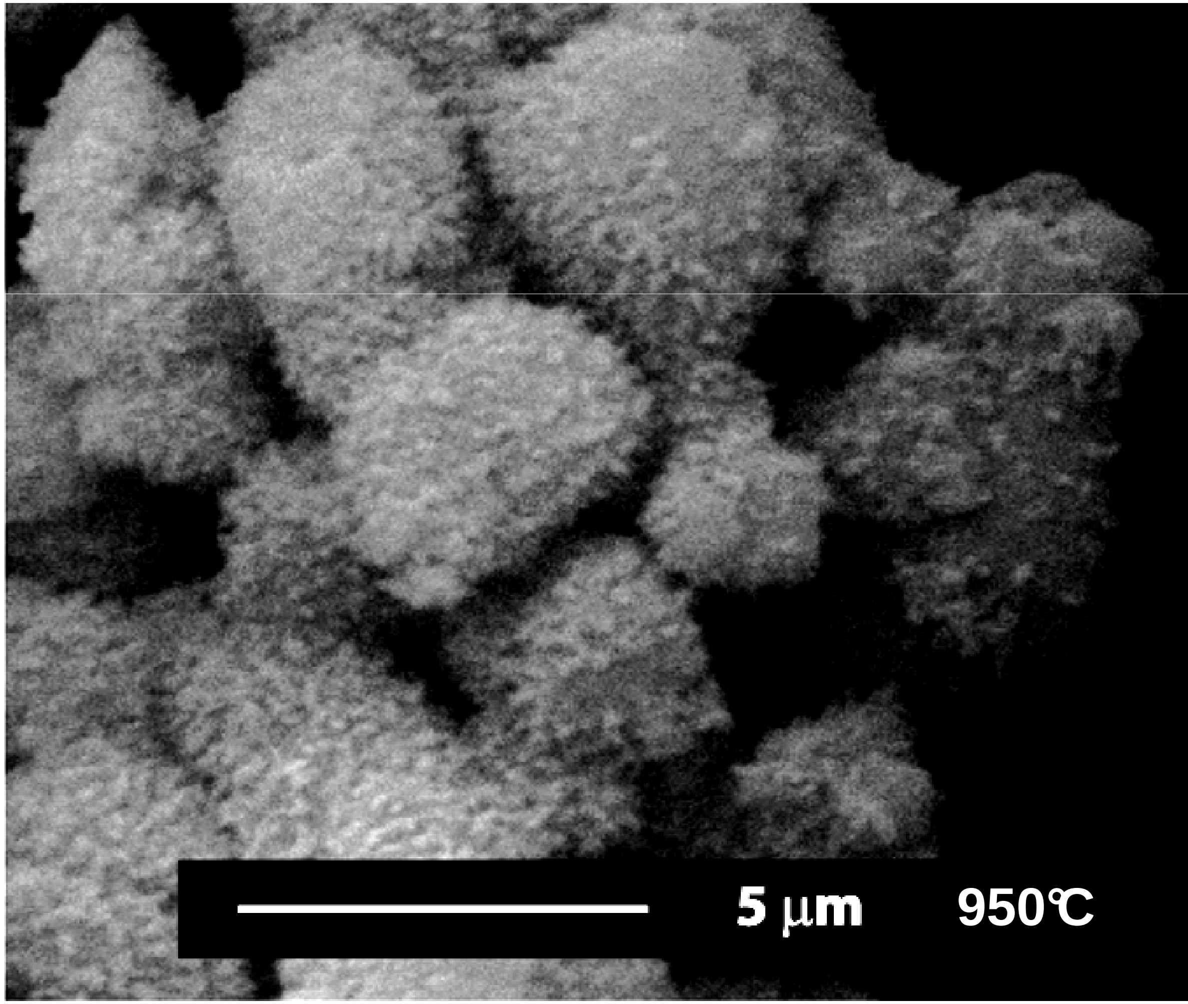


•***2 weeks***: **HCA formation faster for powders treated at 950°C**

•***1 month***: **Increase of I_{HAC} / $I_{Na2CaSi206}$ ratio with thermal treatment temperature**

Bioactivity of thermally treated powders

Formation of needle-like HCA crystals on thermally treated bioactive glass powders after 1 month immersion

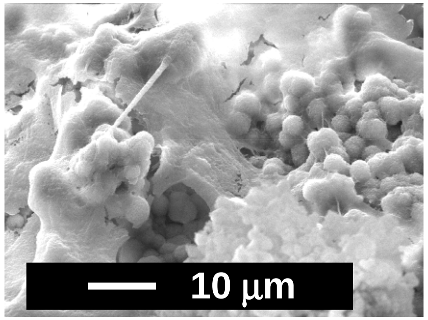


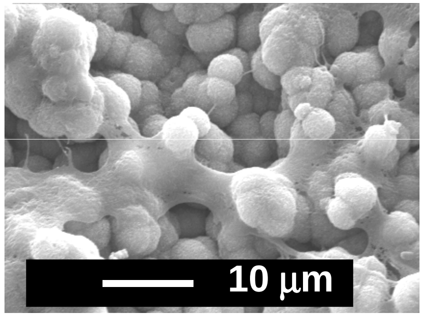
Cell culture (osteoblasts) on porous blocks

***Protocol*:** **Dissolution of 45S5 Bioglass® => increase of pH => death of osteoblasts**

- **pre-treatment (function of sintering temperature and porosity)**
- **Osteoblast culture is possible**

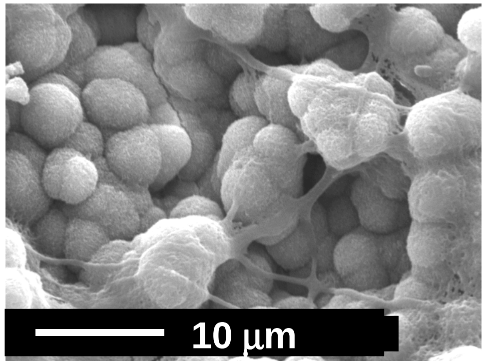
After 13 days of culture:

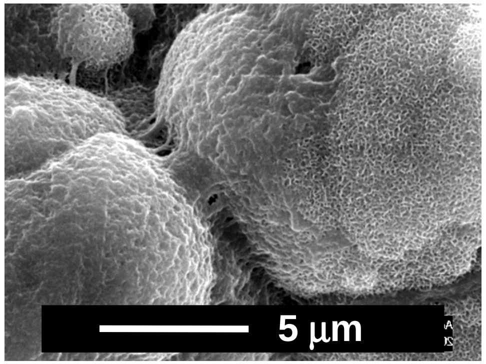


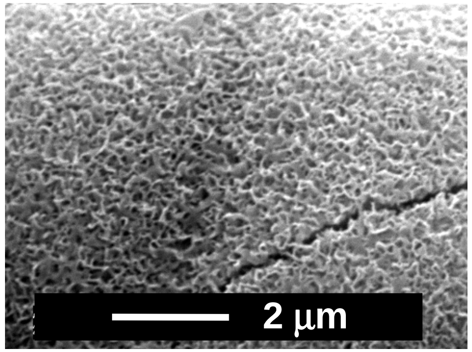


Osteoblasts form a cellular layer on the surfaces of the porous blocks

cytoplasmic extensions





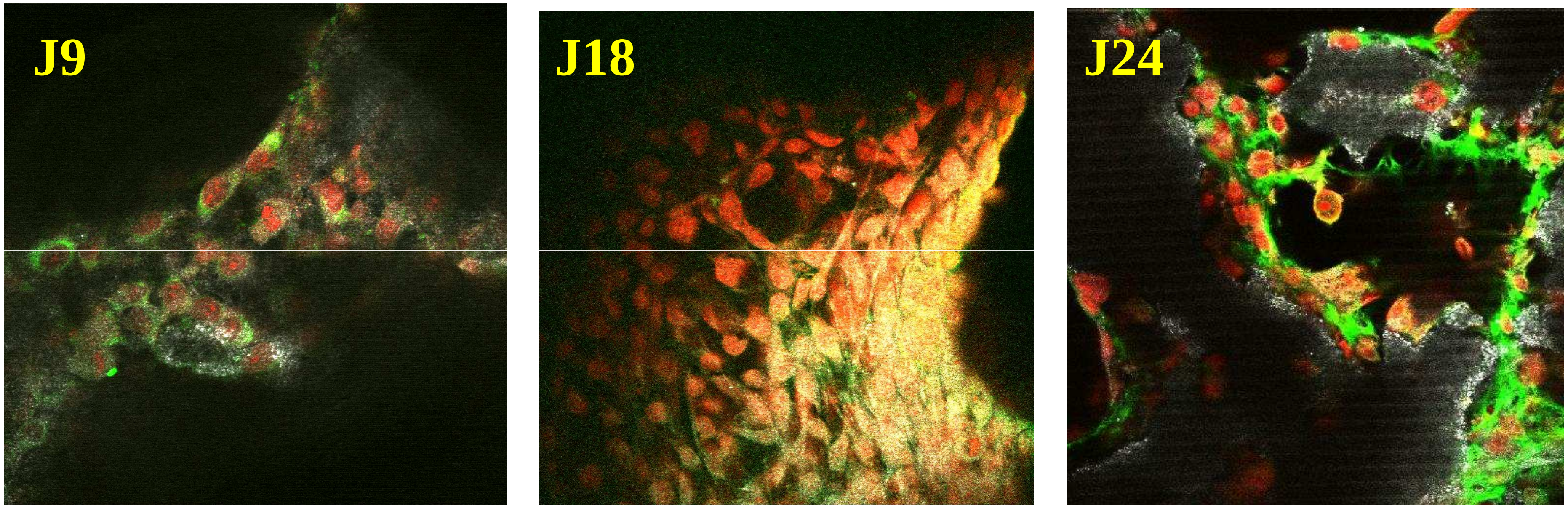


The microporosity of the HA layer helps a fast attachment of the cells.

Cell culture (osteoblasts) on porous blocks

CONFOCAL MICROSCOPY

Confocal micrograph of cells proliferation and metabolism kinetics on bioactive glass 45S5 porous blocks



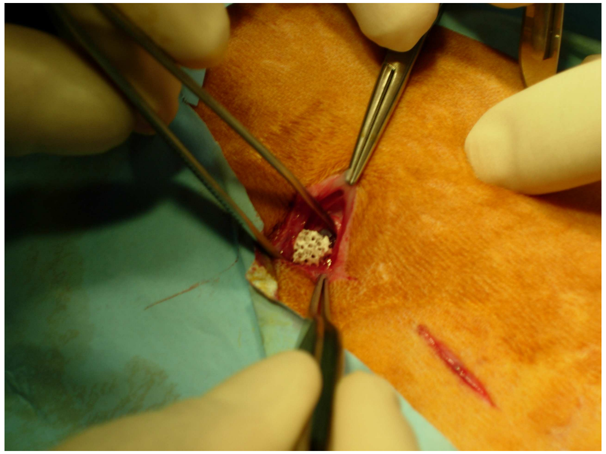
Porous block   Cells   Type I Collagen

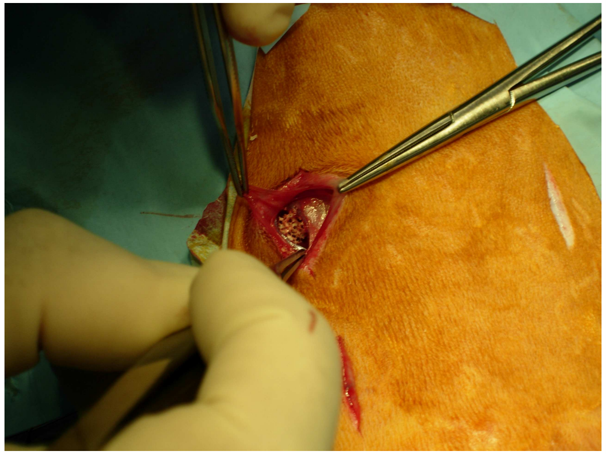
- **Cell adhesion and proliferation**
- **Cells synthesize type I Collagen (extra-cellular matrix)**
- **Cells observed until 660 µm deep**

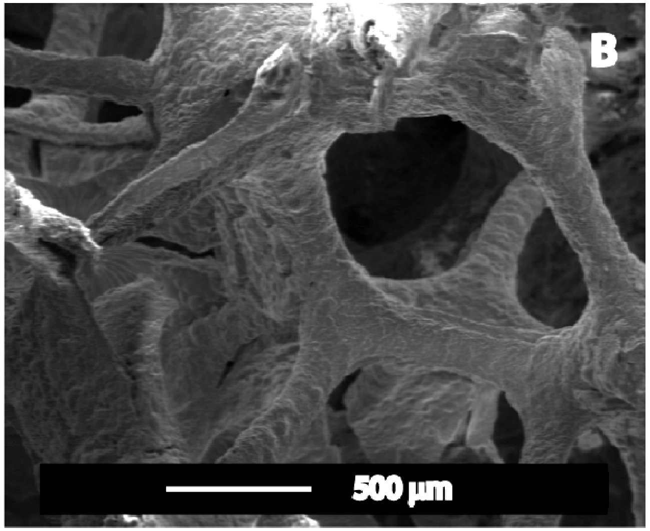
Coll: R. Maksoud, univ Lyon 1

In vivo studies on rabbits

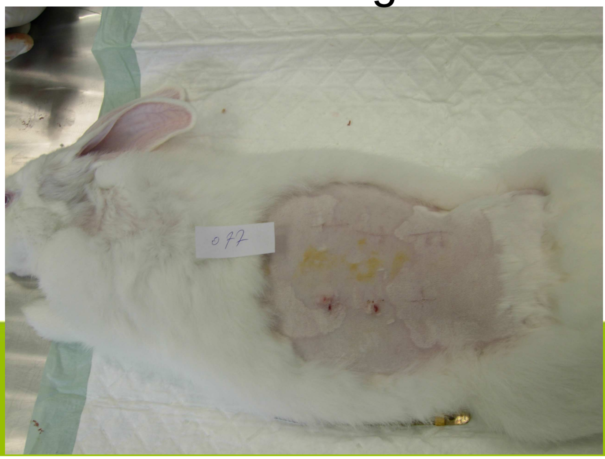
<u>Intramuscular implantation</u> of porous bioglass blocks obtained by the sponge replication technique

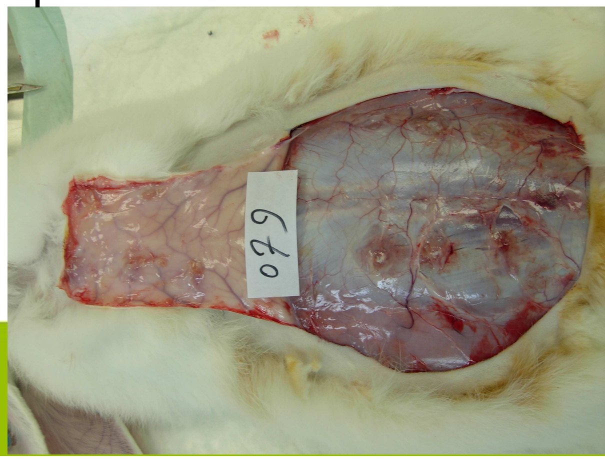


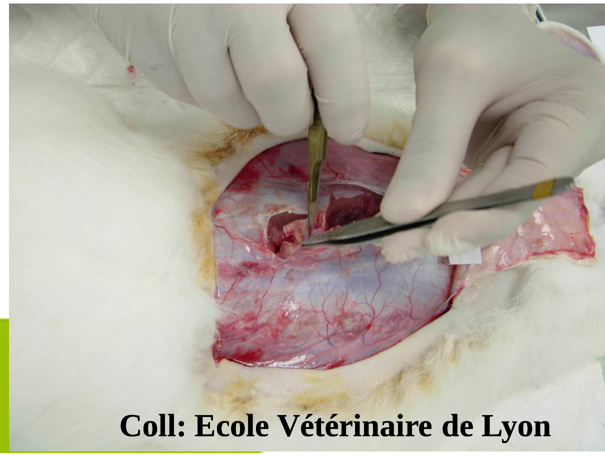




- Conclusions after 1 month
  - Biocompatible, some cases of slight superficial inflammation but no infection
  - Good integration of the porous blocks







Coll: Ecole Vétérinaire de Lyon

Summary :
process – structure – properties relations

Temperature	*Room*	*550*	*610*	*Tg2*	*1000*
Crystallinity	0% →	→	↗ 80%	→	
Microporosity	44% →	↘ 37%	→	↘ 15%	
Macroporosity Architecure	Depend on the processing technique				

Mechanical properties

Transformations	**Raw**	**Glass-in-glass phase separation**	**Main phase crystallisation**		**Crystallisation of silicorhenanite**
			α < 60% *	**α > 60% ***	
Bioactivity	**++++**	**(+++) ??**	**+++ ↘ +**	**+**	**++**

Biological properties

*** *Peitl et al.***

Perspectives

- New uses of bioglass:
  - As fillers in polymer-based composites
  - As bioactive additives in calcium phosphate or sulfate – based cements
  - As scaffolds in ceramic-based polymer-ceramic composites

- Extended control of cristallinity:
  - By new shaping methods (without debinding step)
  - By new compositions?