NanoBioEurope 2008 – 9-13 juin – Barcelona, Spain

NACBO *– Novel and Improved Nanomaterials, Chemistries and Apparatus for Nano-Biotechnology*

CONTROL OF ORDERED/DISORDERED POROUS STRUCTURES IN SIO_2 MONOLITHS AND SiO_2/TiO_2 COMPOSITES FOR APPLICATIONS IN BONE TISSUE ENGINEERING

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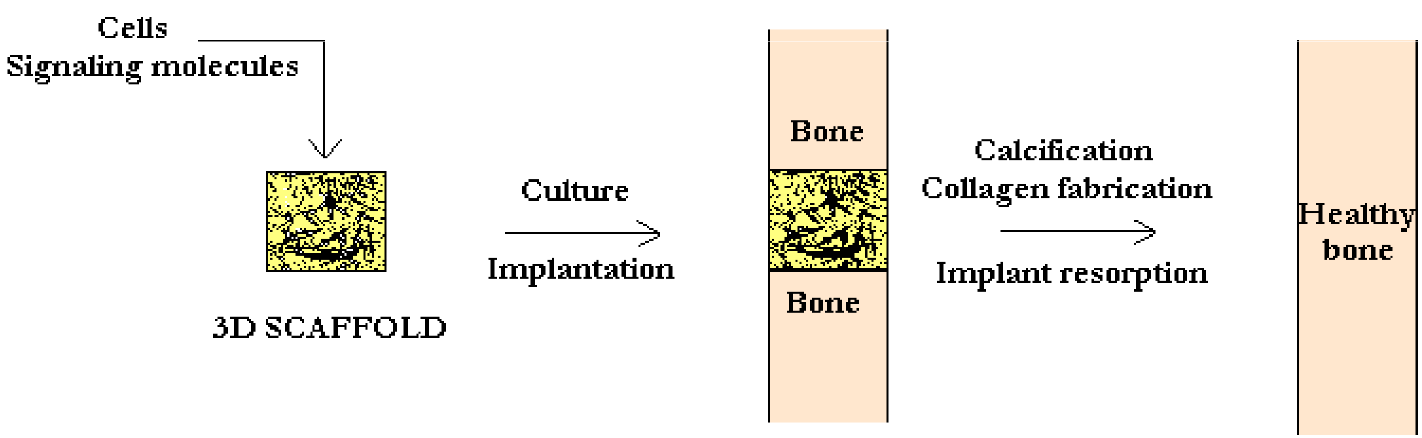
European Commission

Sixth Framework Programme

Tissue Engineering & Bone Regeneration

Development of viable substitutes that restore and maintain functions of human tissues
(bone, cartilage, blood vessels, …)

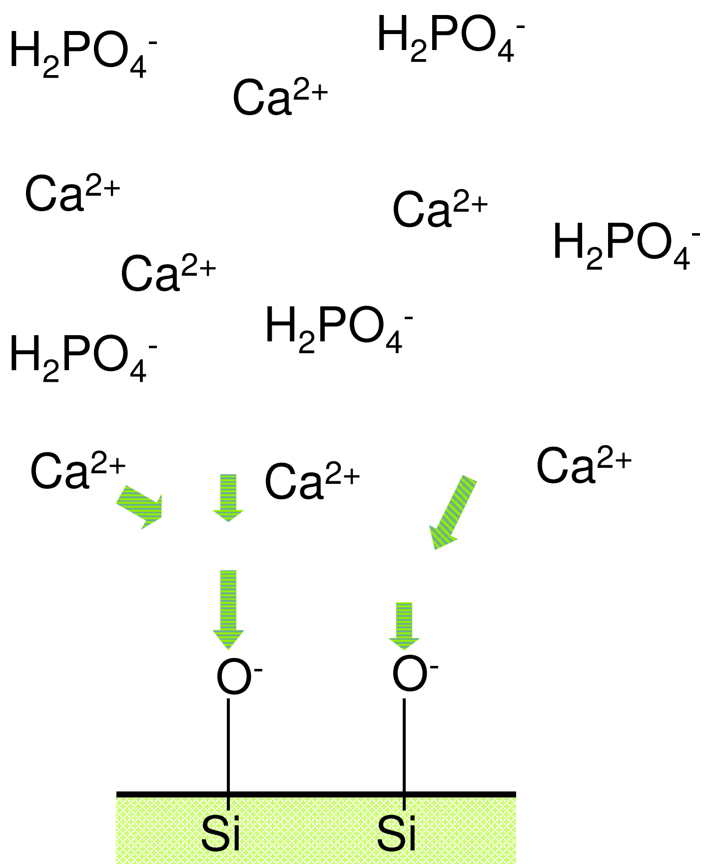
A combination of cells, engineering and materials synthesis, as well as suitable biochemical and physico-chemical factors are used to improve or replace biological functions



Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds, are often critical to recreate the *in vivo* environment and allowing cells to influence their own microenvironments.

Bioactivity / Bone regeneration process

HAp nucleation



SiO_2 ISOELECTRIC POINT pH 2
PHYSIOLOGICAL pH = 7.4 → SiO^-

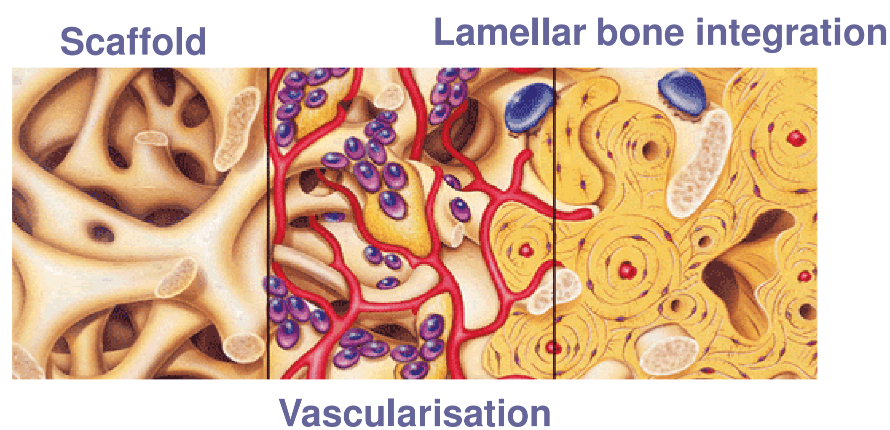
Negatively charged surface

Film surface roughness (nanoscale)
Apatite nucleation, cell adhesion
Surface density of functional groups

Mesoporous Structure
Apatite nucleation, drug delivery
Pore size and accessibility

Macroporous structure >100µm
cell infiltration, bone growth, scaffold vascularisation
Low mechanical properties

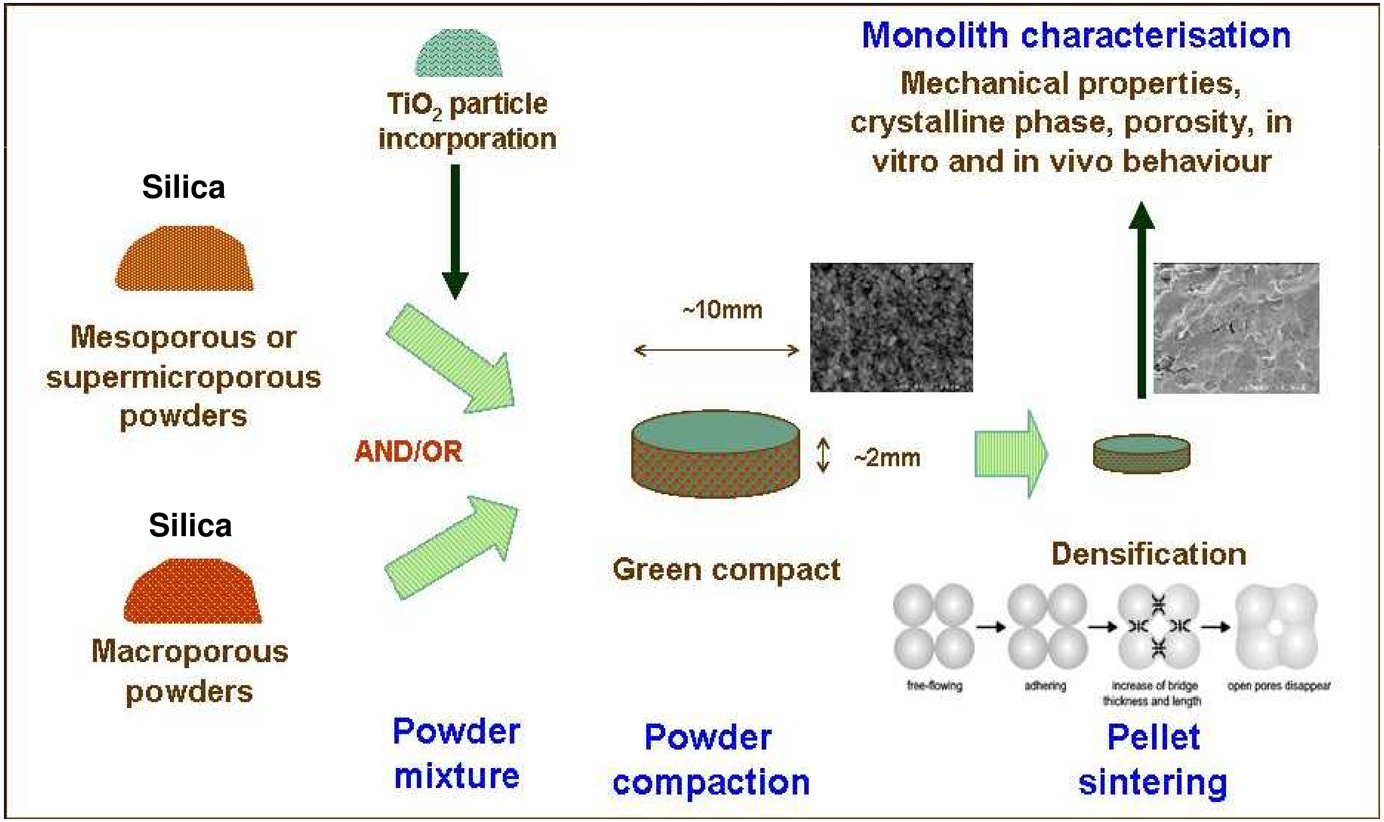
Osteoblast/osteoclast activity



Principle

Preparation of monolithic materials with suitable properties

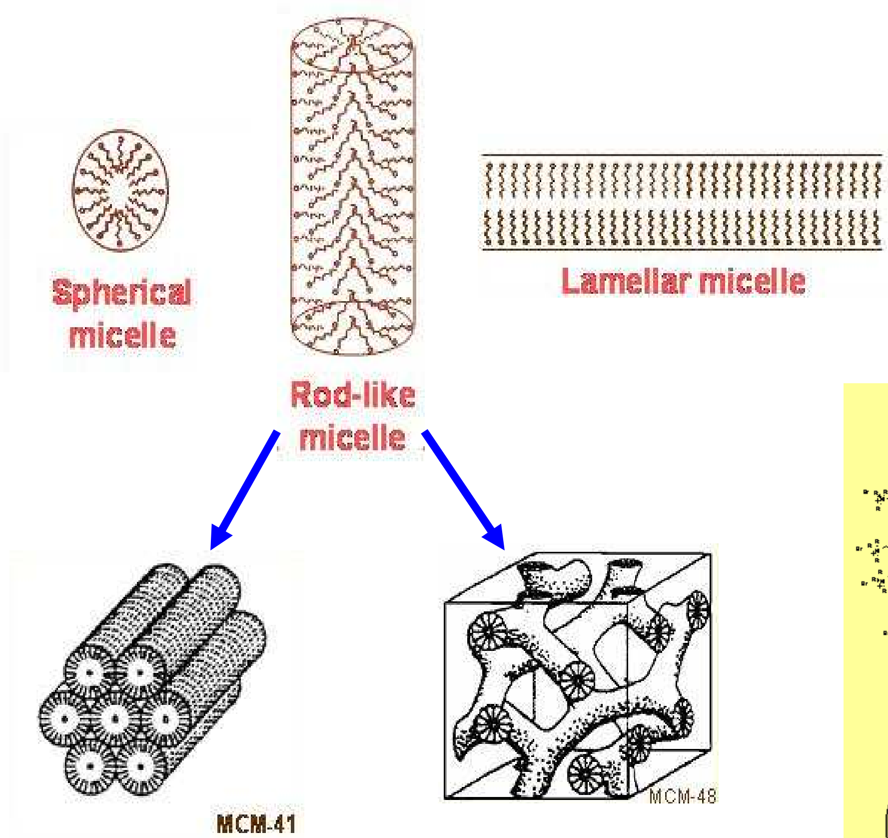
Surface functions: SiO_2 and TiO_2
Ordered mesoporous structure: SBA-15 silica
Macroporous structure: PS-templated silica
Mechanical properties: TiO_2 nanoparticles



Principle

Synthesis of ordered mesoporous silica powders

Self-assembled surfactant systems and sol-gel chemistry



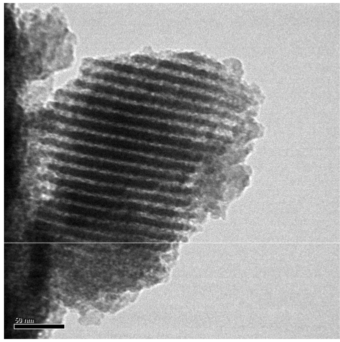
Hydrolysis

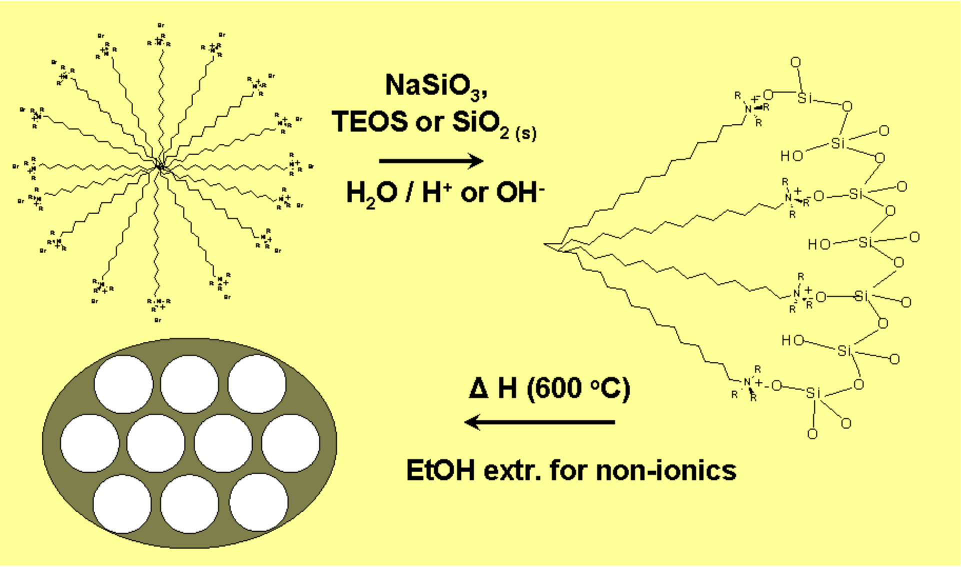
$$Si-OR + H-OH \rightarrow Si-OH + ROH$$

Condensation

$$Si-OH + HO-Si \rightarrow Si-O-Si + H_2O$$

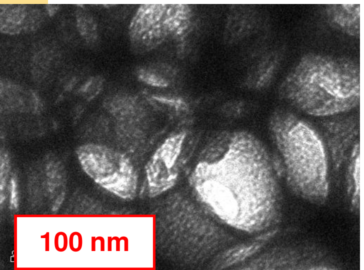
$$Si(OR)_4 + 2H_2O \rightarrow SiO_2 + 4ROH$$



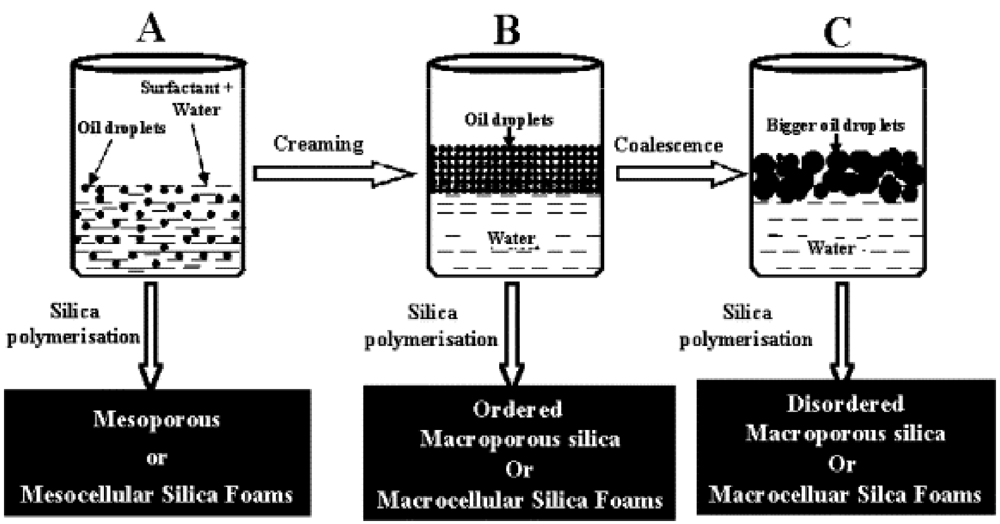


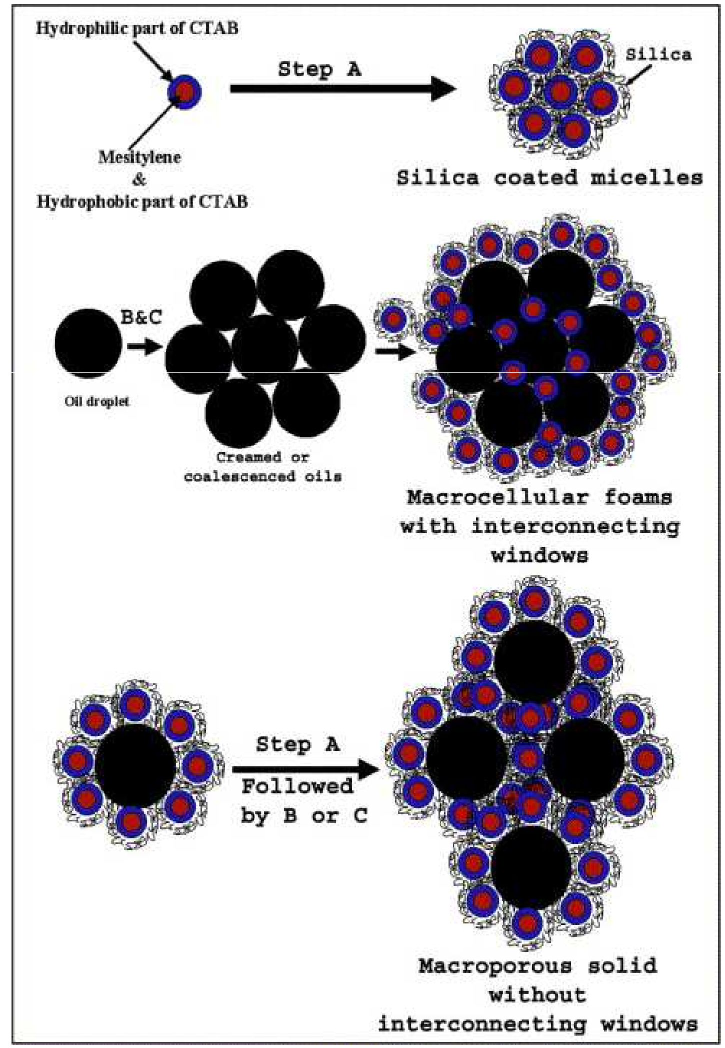
Principle

Synthesis of PS-templated meso/macroporous silica powders



Method proposed by Sen at al.* using both polystyrene bead and surfactant as templates and sol-gel process for TEOS polymerisation

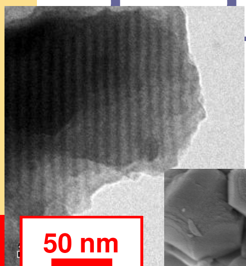


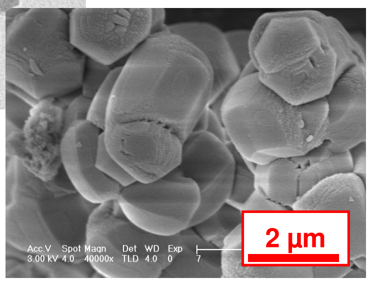


** T. Sen, G.J.T. Tiddy, J.L. Casci and M.W. Anderson, Meso-cellular silica foams, macro-cellular silica foams and mesoporous solids: a study of emulsion-mediated synthesis, Microporous and Mesoporous Materials 78 (2005) 255-263.*

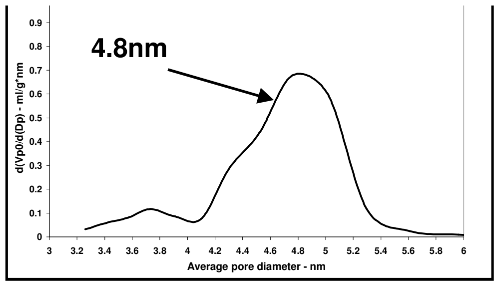
Characteristics of powders used for monolith preparation

SBA-15 ordered mesoporous silica powders

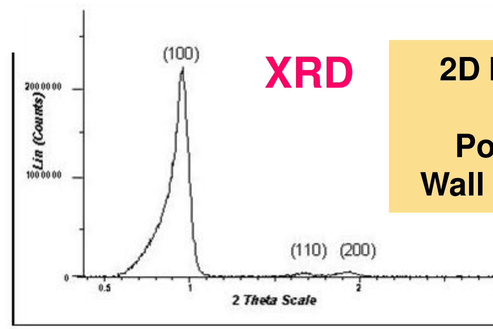




Pore volume distribution

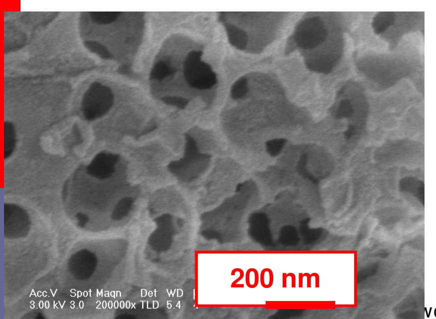


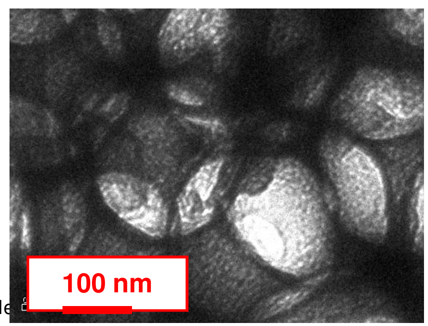
XRD



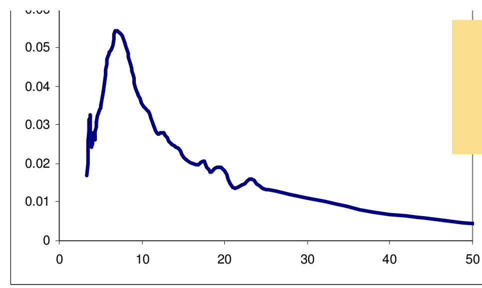
2D hexagonal ordering
S_{BET} = 800m^2/g
Pore size = 4.5 - 5nm
Wall thickness = 5 – 6 nm

PS-templated meso/macroporous silica powders



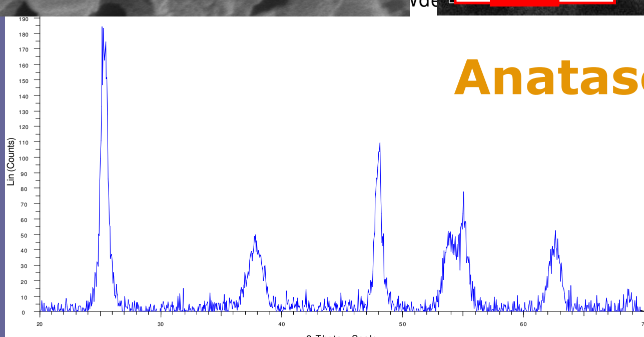


Pore volume distribution

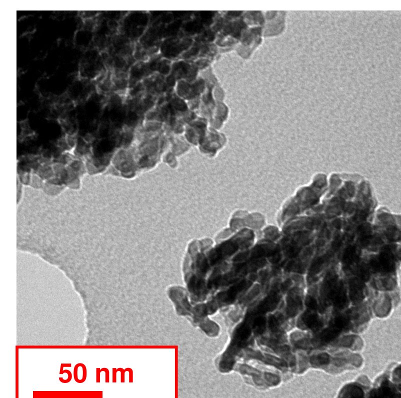


Mesoporosity but not ordered
Mesopores sizes: 4 and 7nm
Macropore size: ~ 300nm

Anatase TiO_2 nanoparticles - Aldrich

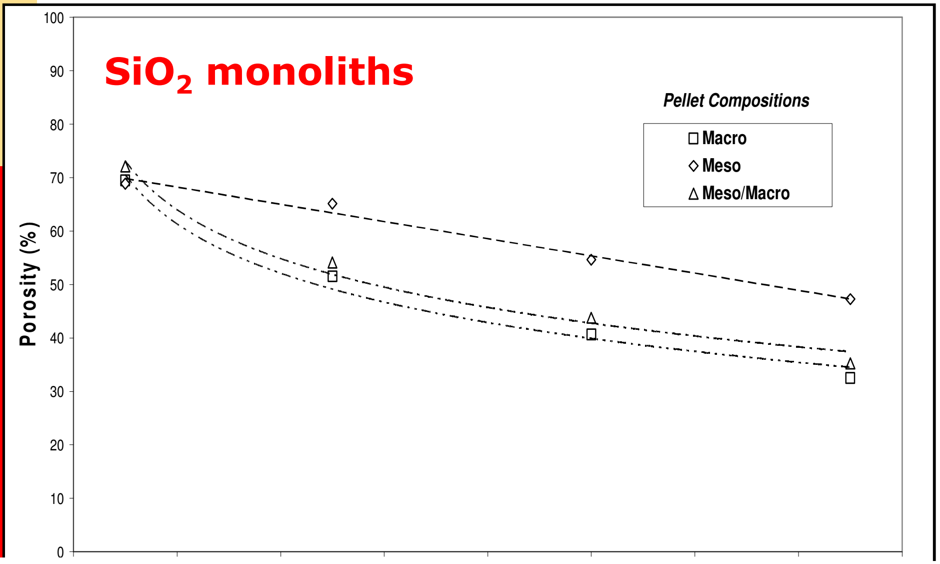


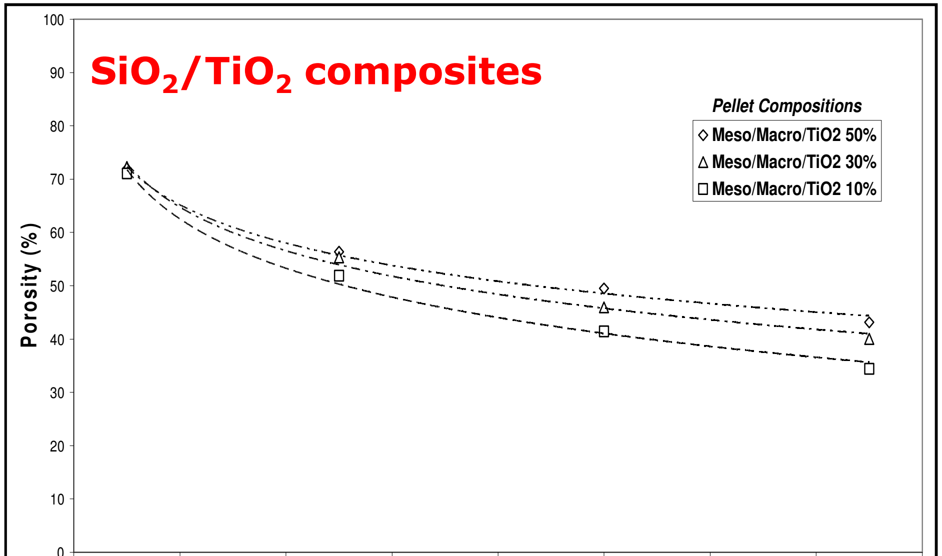
Aggregated nanoparticles
Nanoparticles size < 25nm
S_{BET} > 200m^2/g



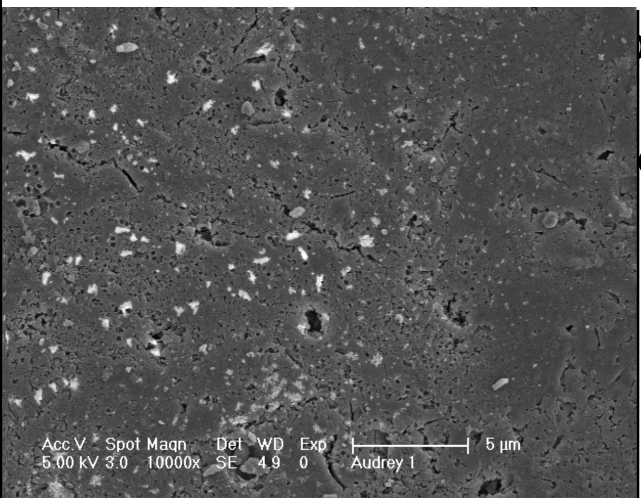
Control of the monolith porous structure

Influence of the composition

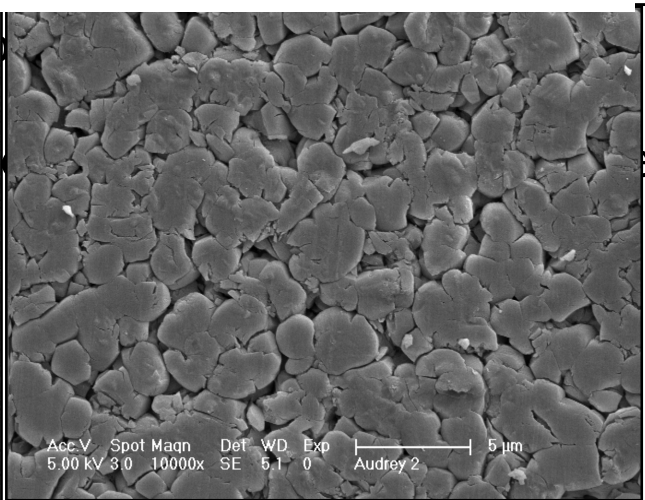




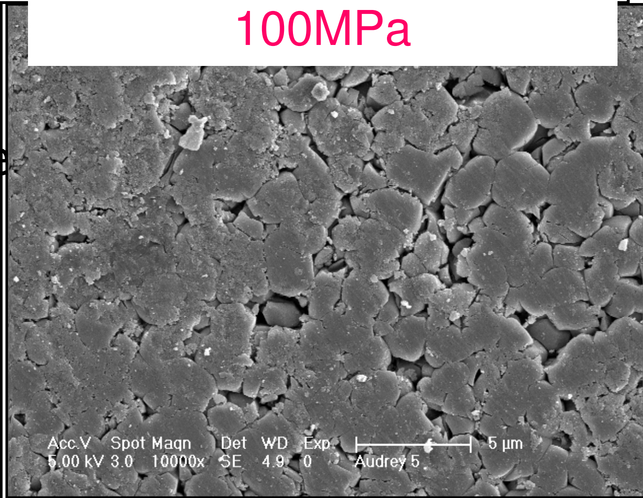
MACROPOROUS 100MPa



MESOPOROUS 100MPa

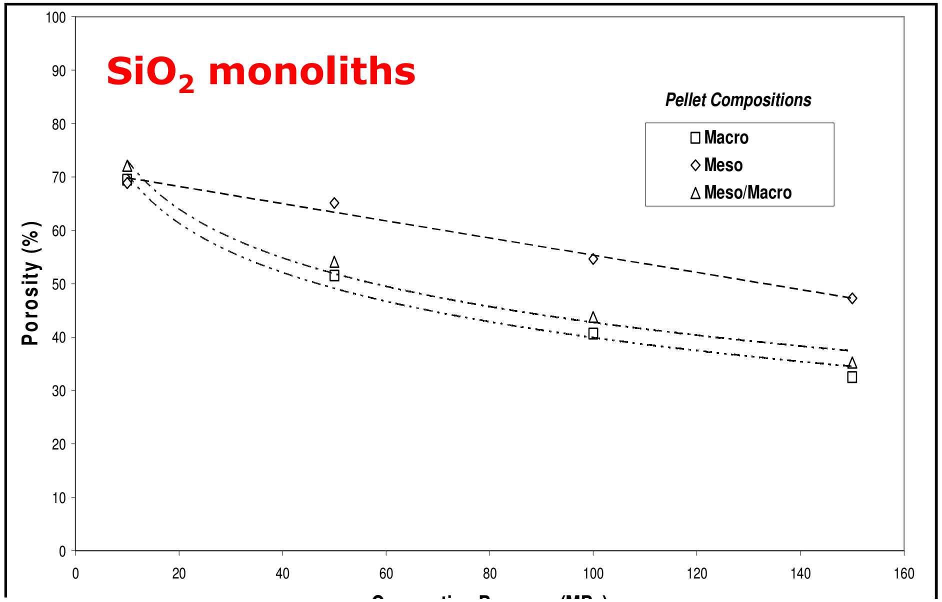


MACRO/MESOPOROUS 100MPa

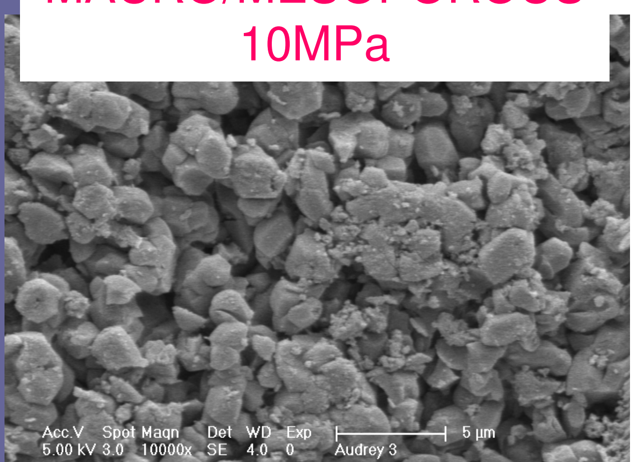


Control of the monolith porous structure

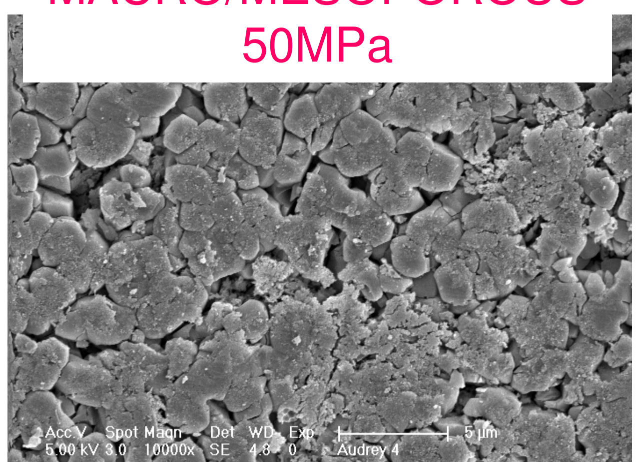
Influence of the compaction pressure



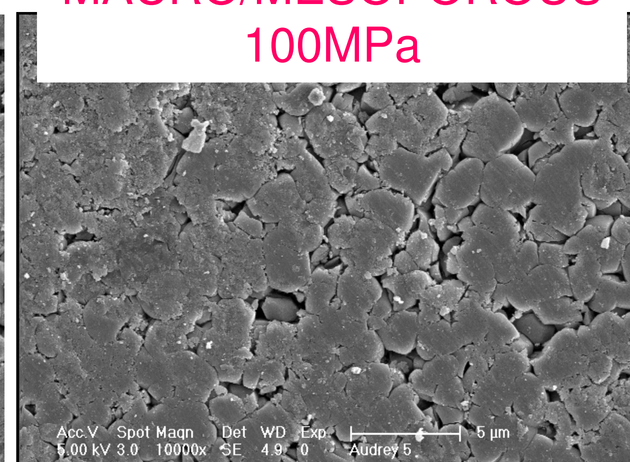
MACRO/MESOPOROUS 10MPa



MACRO/MESOPOROUS 50MPa

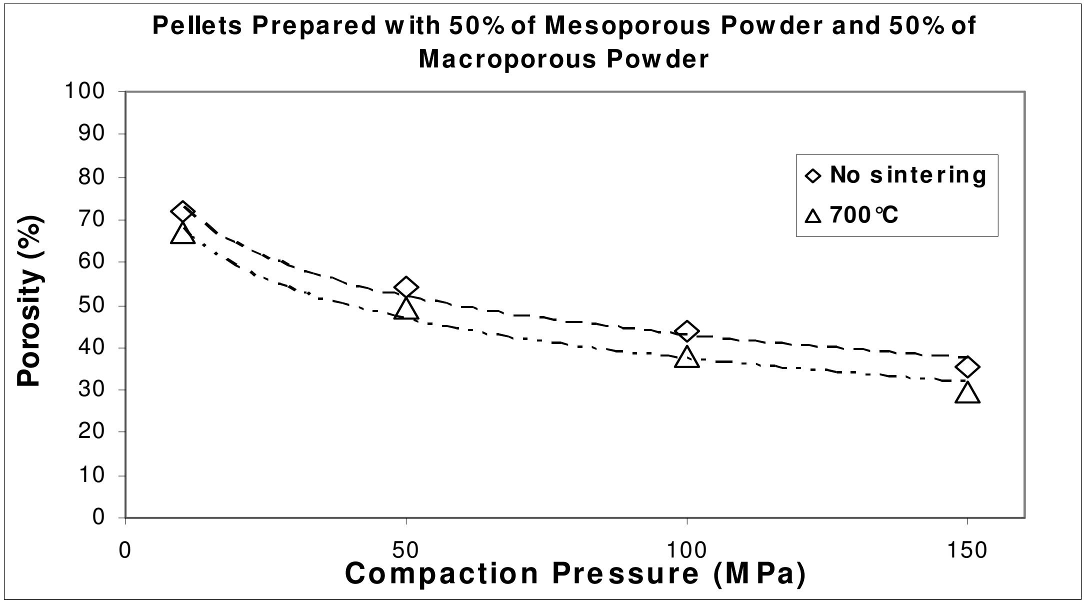


MACRO/MESOPOROUS 100MPa

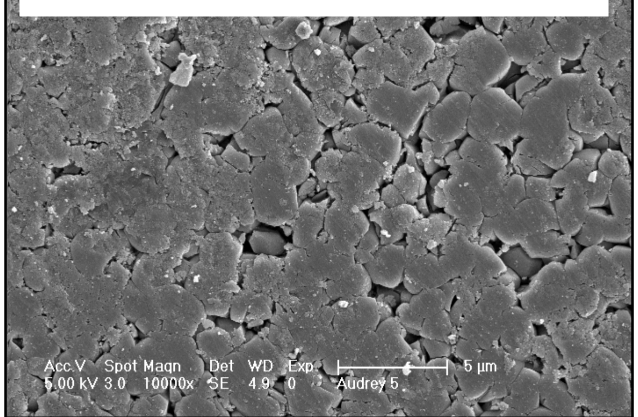


Control of the monolith porous structure

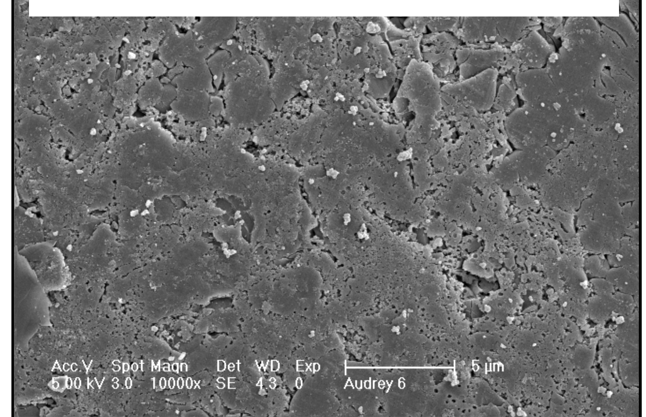
Influence of low sintering temperature



MACRO/MESOPOROUS
100MPa



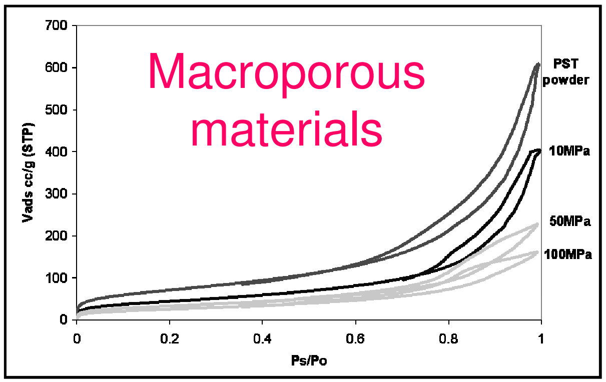
MACRO/MESOPOROUS
100MPa - 700°C

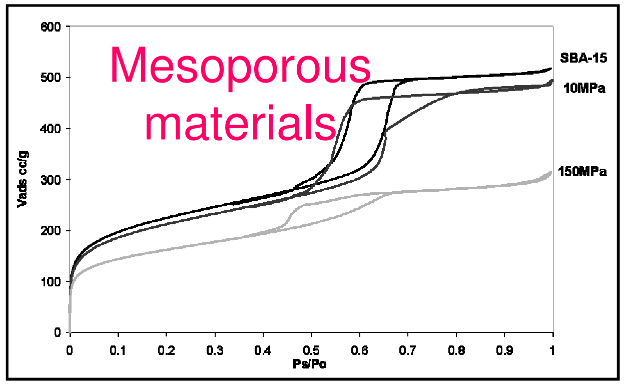


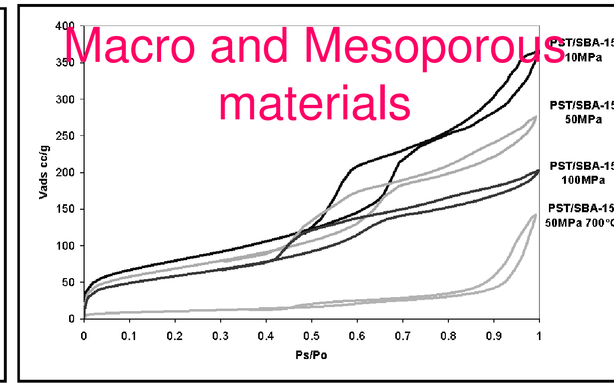
Control of the monolith porous structure

Influence of major parameters to control the mesoporous structure

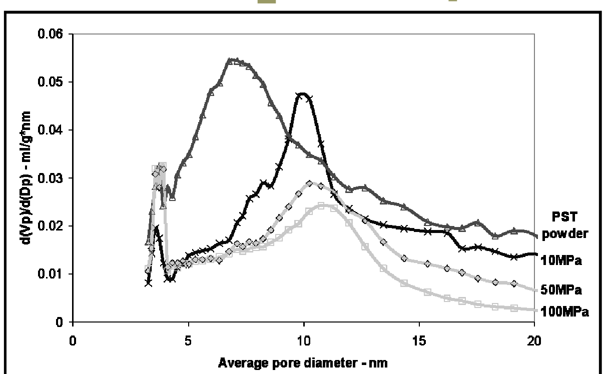
N_2 adsorption/desorption isotherm profiles

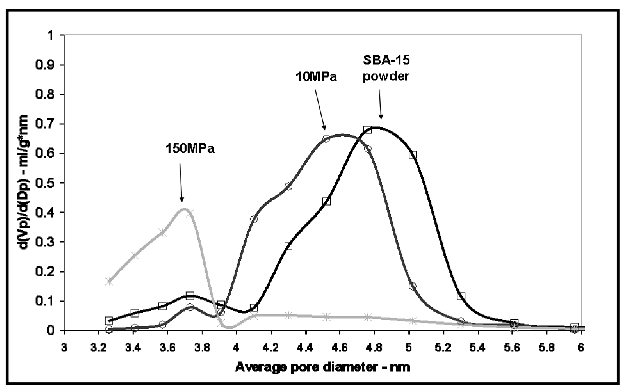


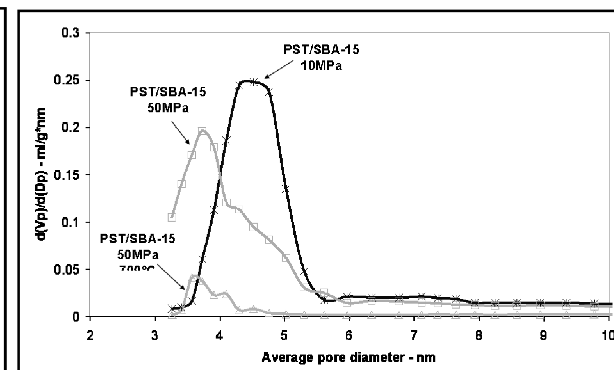




N_2 adsorption/desorption pore volume distribution







Main pore size>10nm

Main pore size from 3.4 to 4.6nm

Increase in mesopore wall thickness, Shrinkage or Collapsing of mesoporous structures

In-vitro characterisation of the materials

Cytotoxicity

Determination using indirect method:

Monoliths were soaked 24h in medium and removed

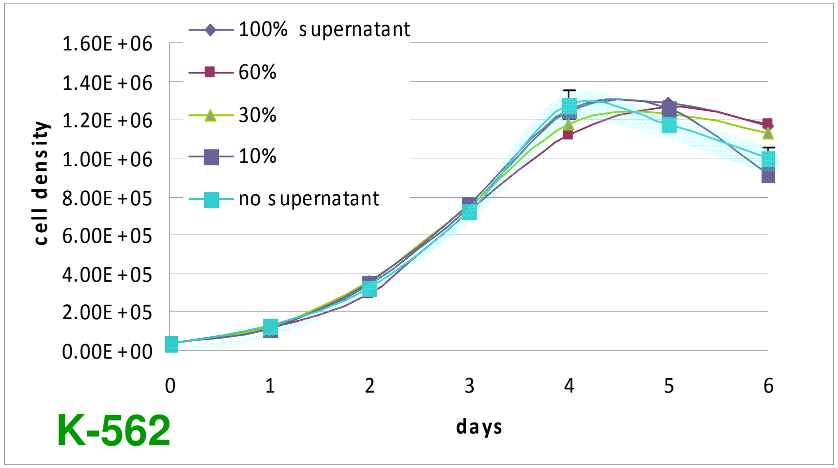
Pre-treated medium has been diluted at various ratios with fresh media (0%, 10%, 30%, 60%, 100%) and used for cell culture

Meso/Macroporous monoliths

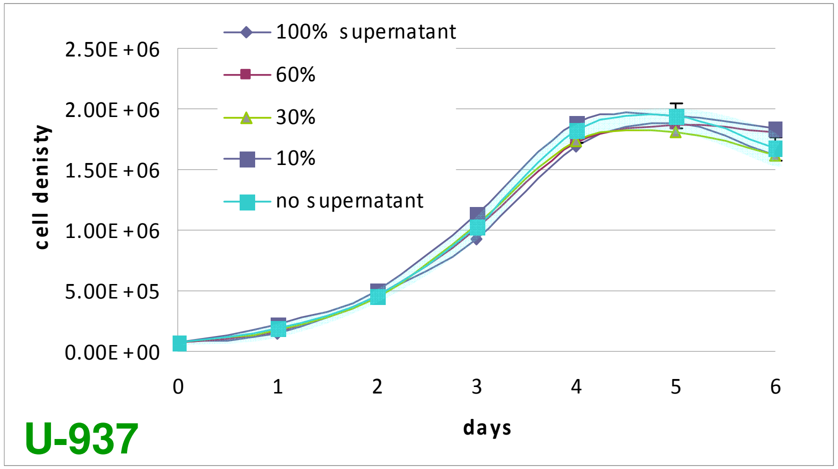
SBA-15/PST

No material toxicity has been evidenced after 6 days of culture using U-937 and K-562 cell lines

by comparison with a control sample: culture carried out in fresh medium (0%)



K562 (derived from a chronic myeloid leukaemia)



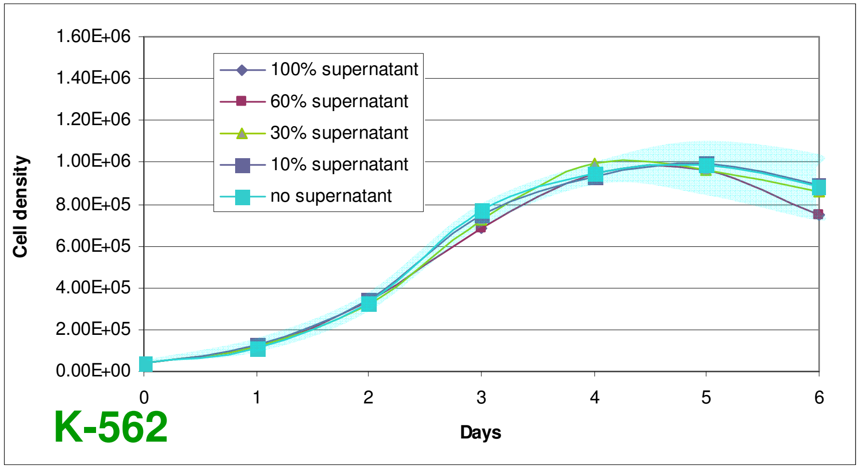
U937 (leukaemic cell)

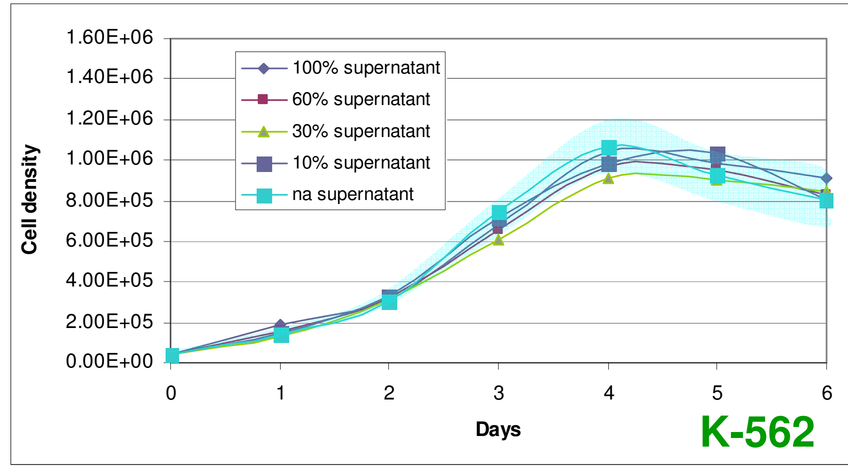
In-vitro characterisation of the materials

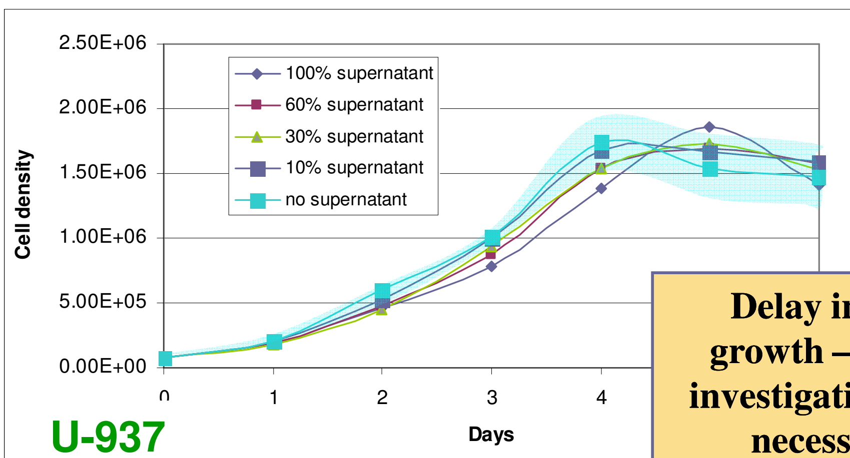
Cytotoxicity

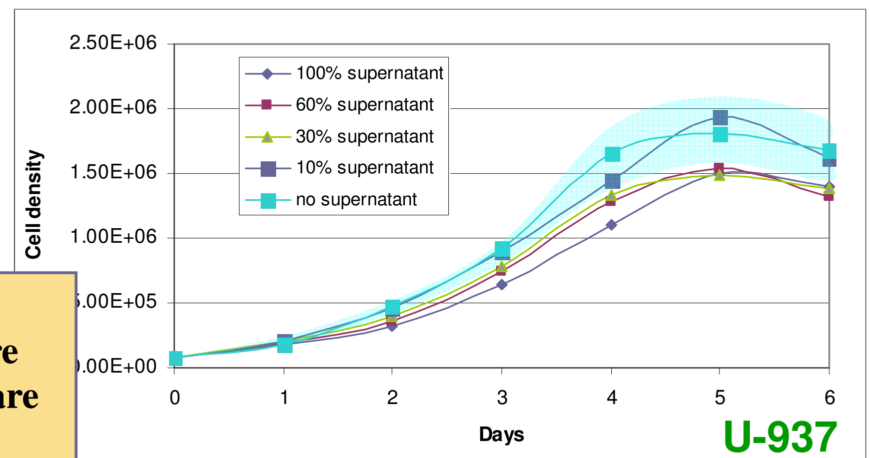
SBA-15/PST + 30% TiO_2 anatase nanopowder

soaked 24h in medium | **soaked 48h in medium**









Delay in cell growth – More investigations are necessary

Conclusions and Prospects

- Monoliths with suitable surface properties (SiO_2/TiO_2) and porous structure have been successfully prepared.
- Monolith characteristics are easy to control and adjust to obtain specific properties.
- Tested materials have been proved to be non toxic so far.

- *In-vitro* cytotoxicity tests will be continued and cell adhesion/proliferation assays will be carried out using MG-63 osteoblast-like cell lines. *In-vivo* tests are planned to start shortly.
- Study of hydroxyapatite nucleation using SBF treatment are in progress and interesting results are expected soon.
- Correlations between process parameters, structures and properties will be evidenced.
- Materials with bigger macropores will be prepared and characterised.

Acknowledgements

NACBO *– Novel and Improved Nanomaterials, Chemistries and Apparatus for Nano-Biotechnology*

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Ray Newsam (TEM)
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