Biodegradable Scaffolds for Tissue Engineering prepared from crosslinkable precursors

E. Schacht et al.

Polymer Chemistry & Biomaterials Research Group, U-Ghent"

i-SUP Symposium, 22 – 25 April 2008, Bruges

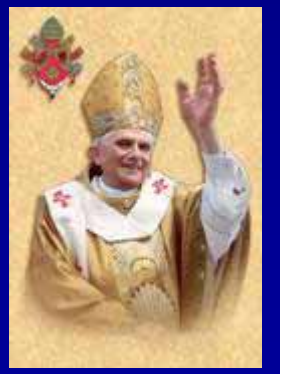
Theme 2: Biomaterials for improved quality of life

Innovation for Sustainable Production

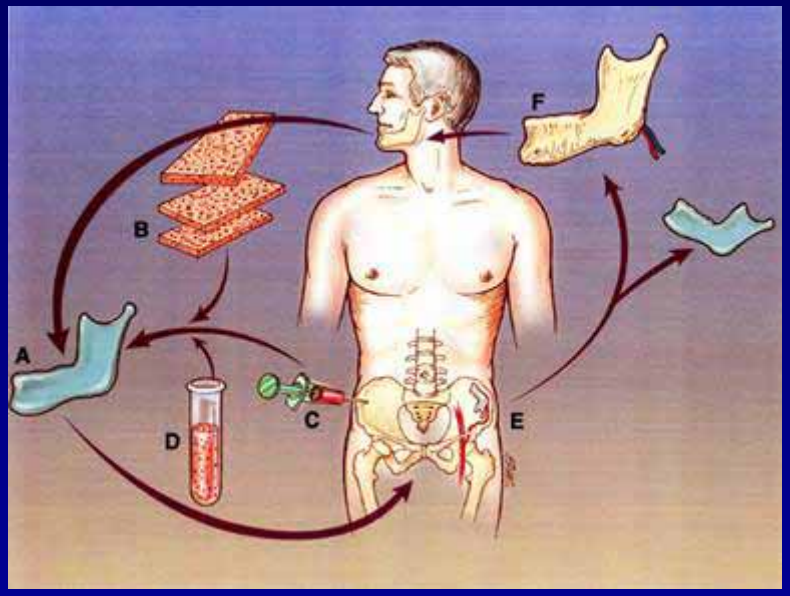
Sustainable production is based on :

- **Sustainable materials = result of science**
- **sustainable funding & career opportunities**
- **Sustainable scientists, people**

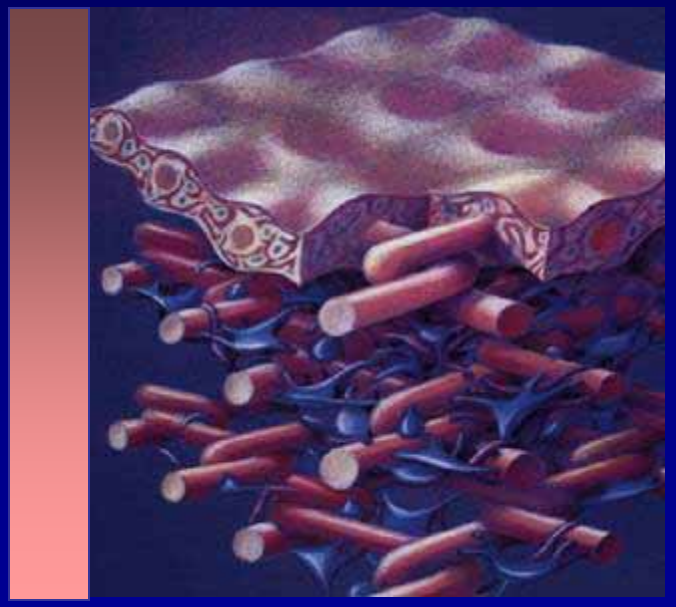
Sustainable people (Google)



TISSUE ENGINEERING OF COMPLEX STRUCTURES



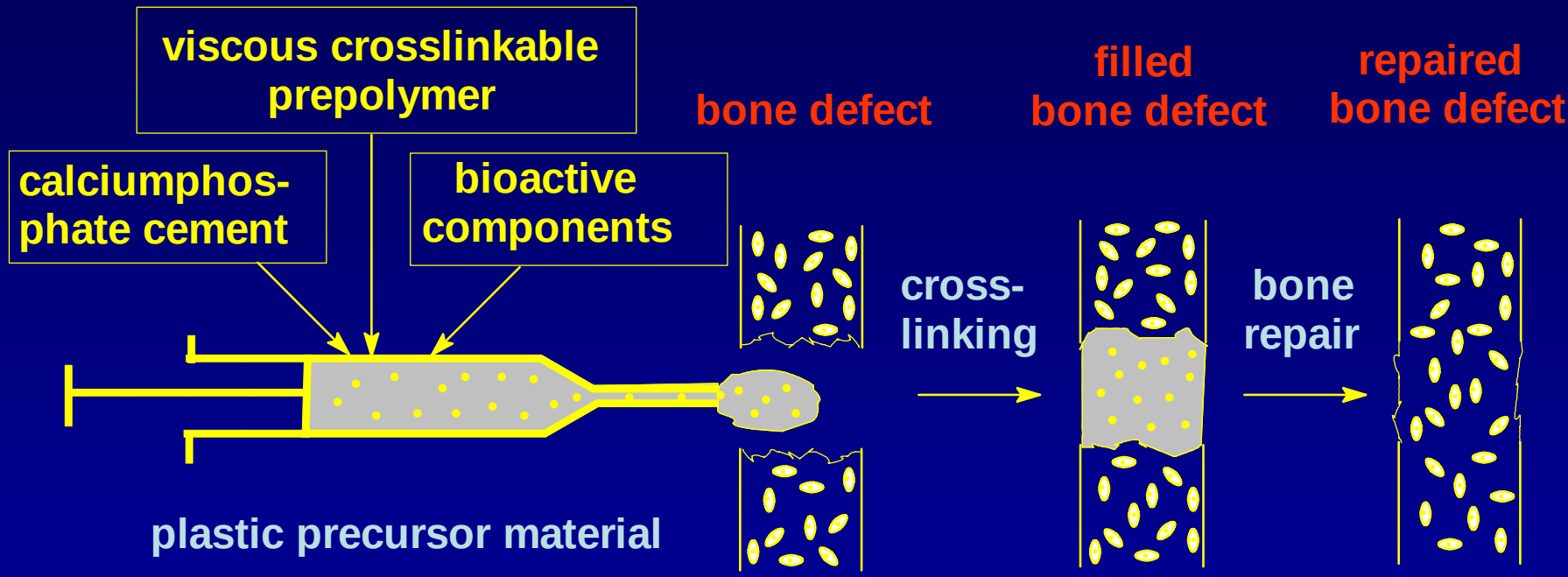
engineered tissue



complex organs

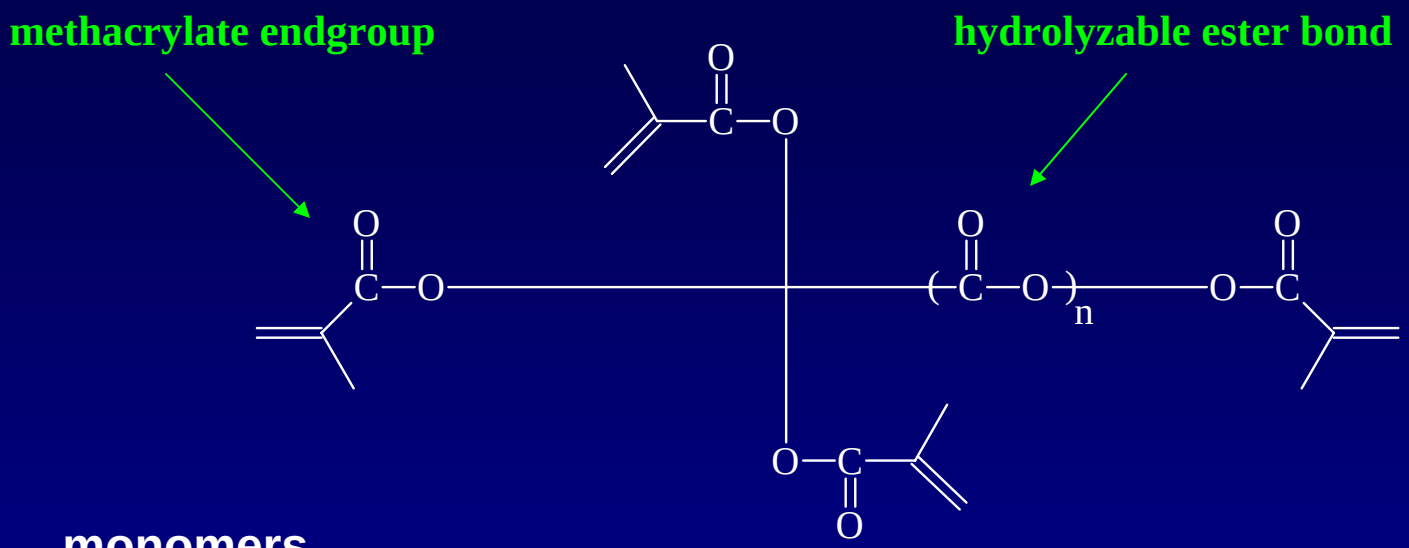
1. Porous scaffolds based on biodegradable polyesters

Concept: in situ curable composite

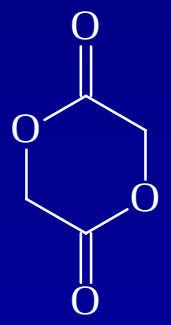


injection in a bone defect

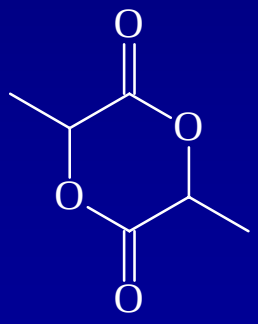
Curable polymers : methacrylates of polyesters



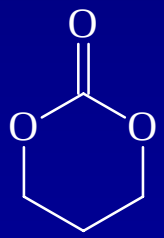
monomers



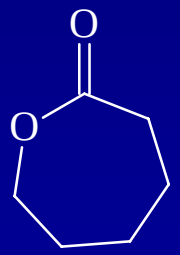
glycolide



D,L-lactide

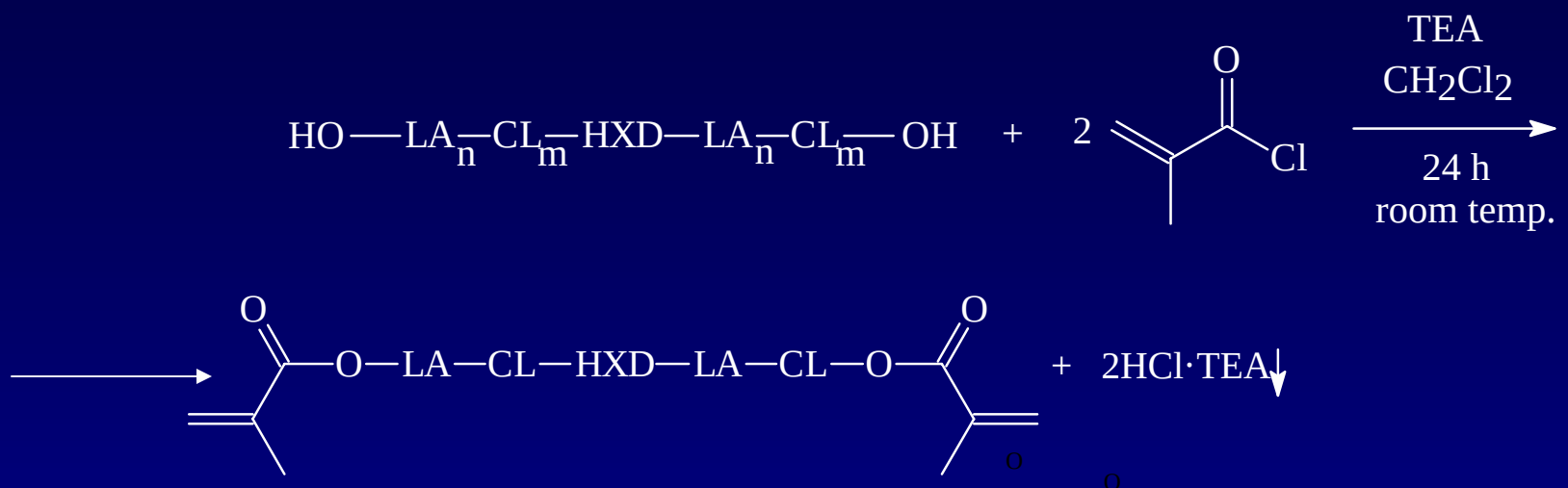


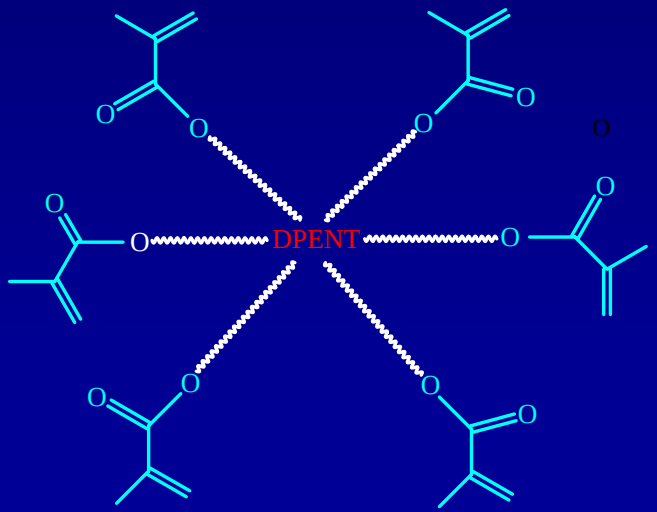
trimethylene carbonate

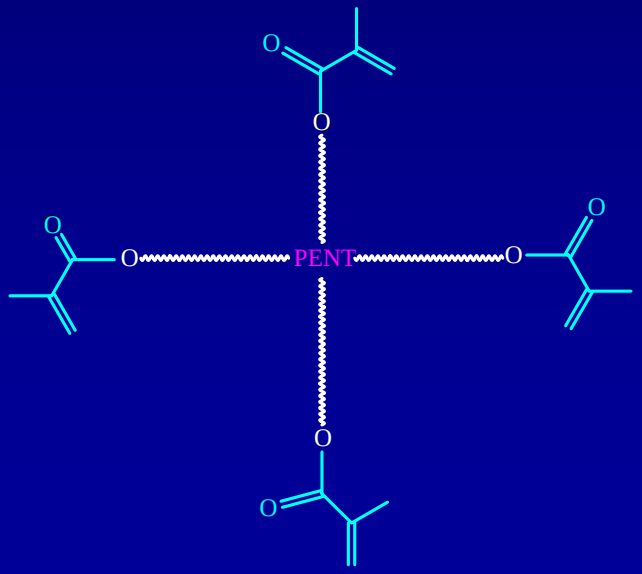


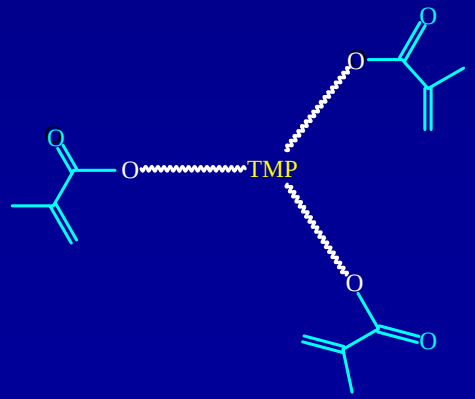
ε-caprolactone

derivatization of the hydroxyl endgroups into methacrylate esters

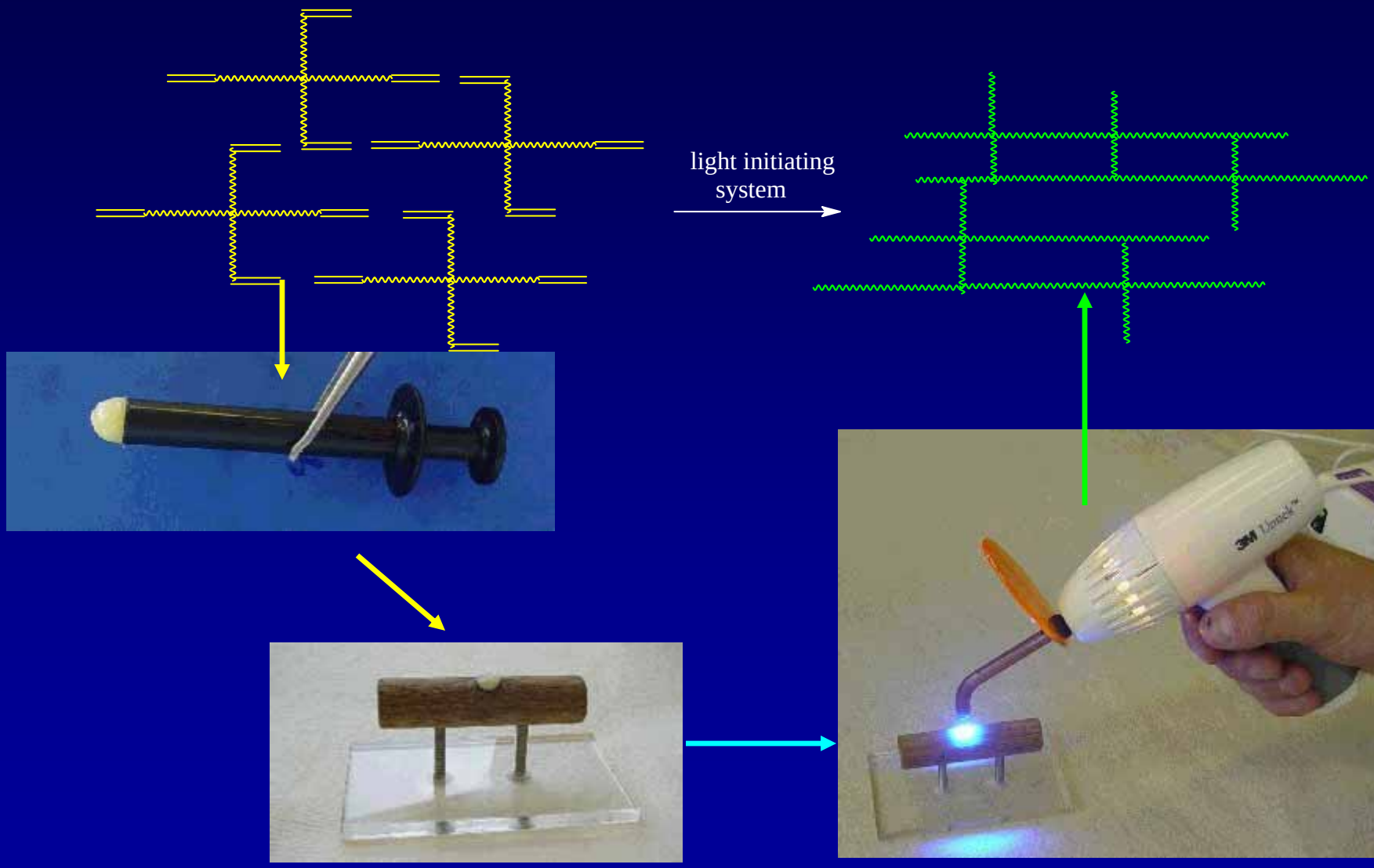




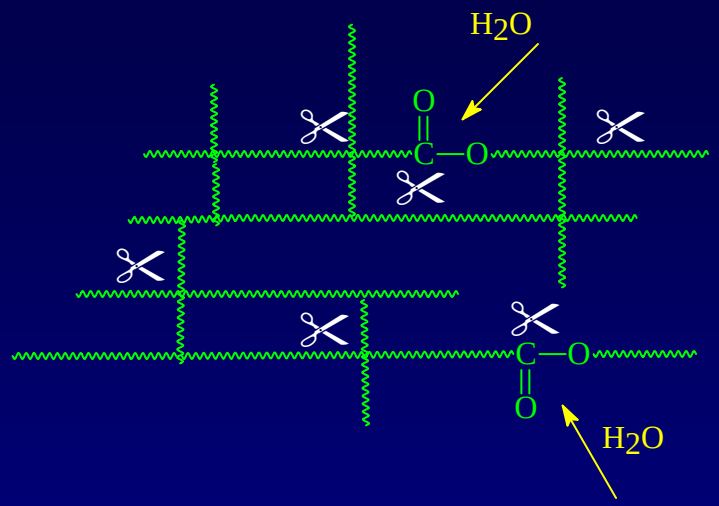


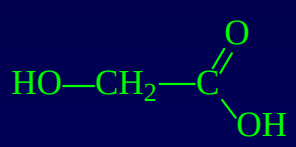


in situ photopolymerization - formation of the 3-D polymer network

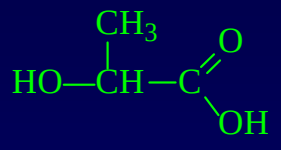


Hydrolytic degradation of the 3-D polymer network

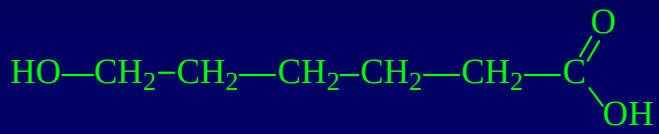




glycolic acid



lactic acid



6-hydroxycaproil acid

CO_2

Controlling of degradation rate:

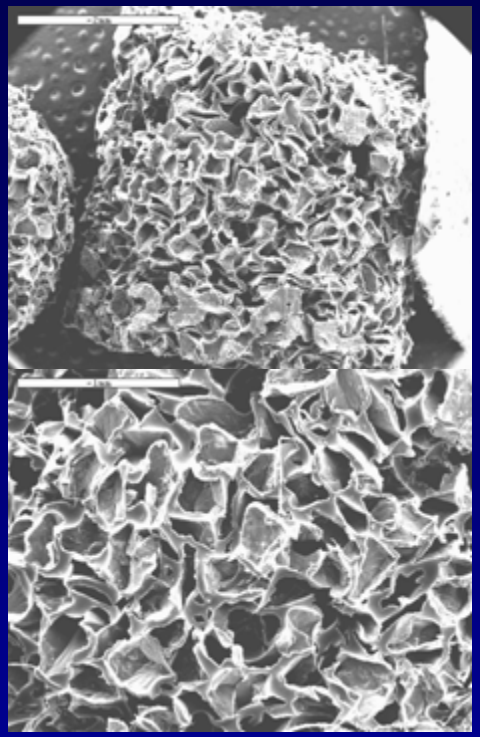
- polymer composition

- crosslinking density

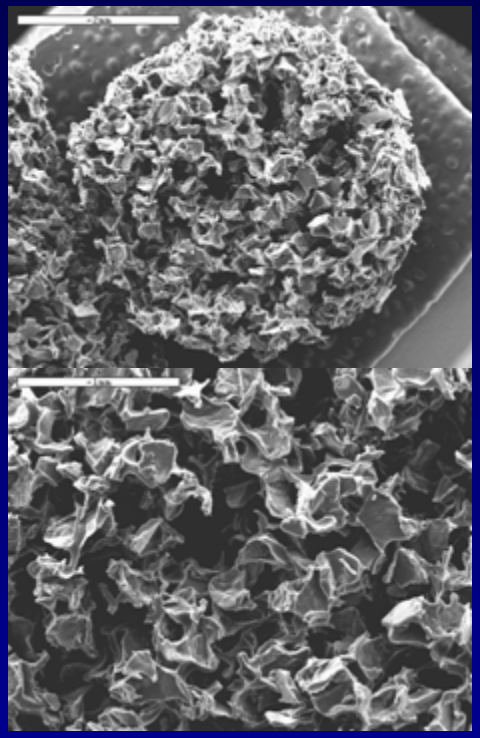
- type and amount of additives

Porous polymer scaffolds

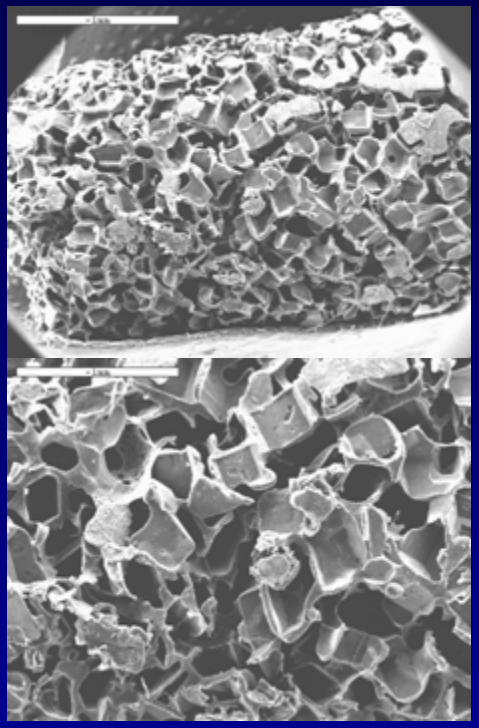
Porogen: gelatin, NaCl, sugar 250-355 μm



Gelatin (250-355μm)
porosity ~ 60%



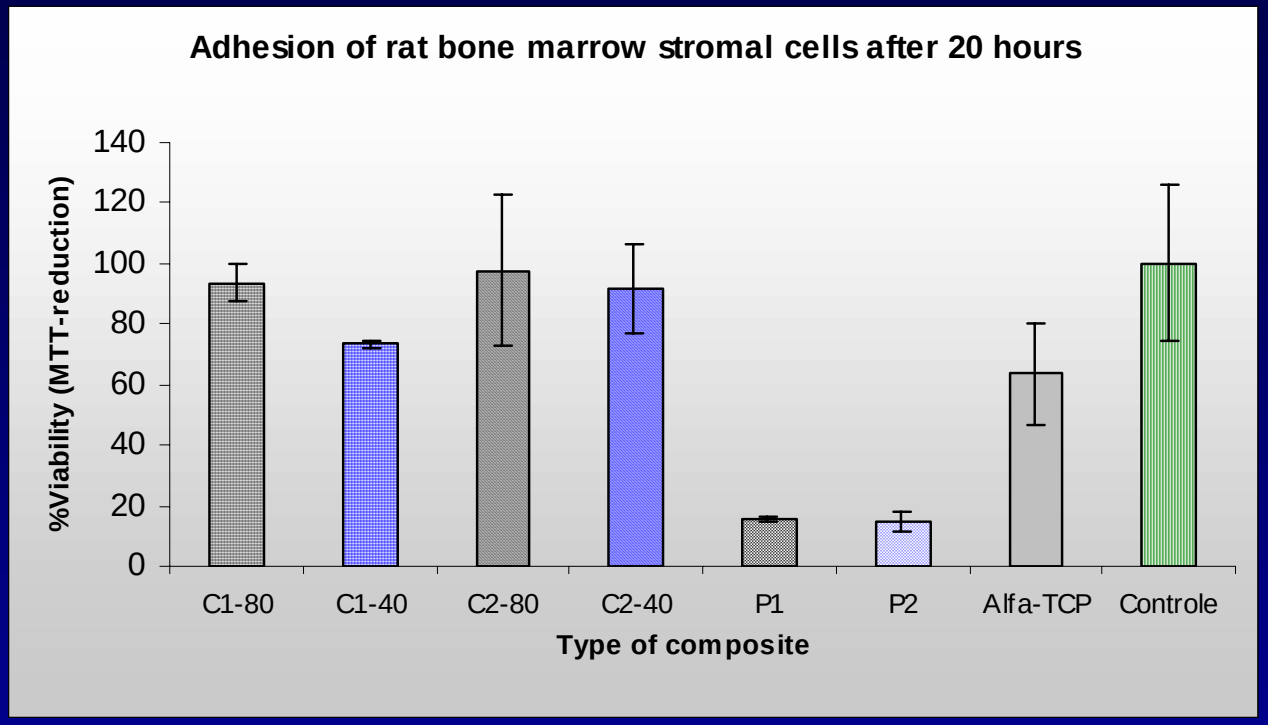
Gelatin (250-355μm)
porosity ~ 80%



Sugar (250-355μm)
porosity ~ 70%

The porogen can be leached out leaving open cells with a pore size and morphology defined by the porogen particles and providing osteoconductive properties of the composites.

Adhesion of rat bone marrow stromal calls



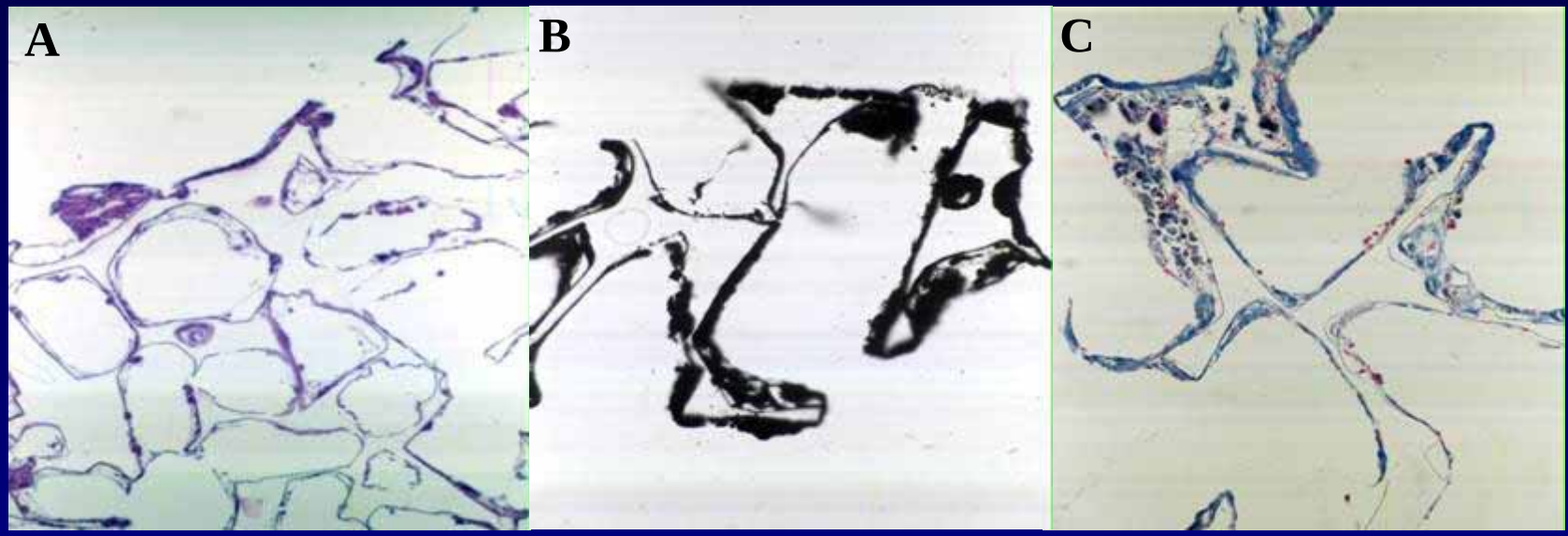
C1- 40 (C1 - 80) - $PGA_{30}CL_{70}HXD_{20/1}$ + 15wt% HEMA + 40wt% alfa-TCP (80wt% alfa TCP)

C2 - 40 (C2 - 80) - $PLA_{50}CL_{50}HXD_{20/1}$ + 15wt% HEMA + 40wt% alfa-TCP (80wt% alfa TCP)

P1 - $PGA_{30}CL_{70}HXD_{20/1}$ + 15wt% HEMA

P2 - $PLA_{50}CL_{50}HXD_{20/1}$ + 15wt% HEMA

In vitro - rat bone marrow cells

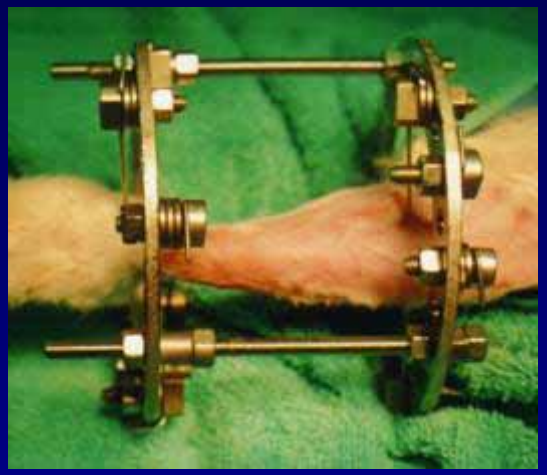


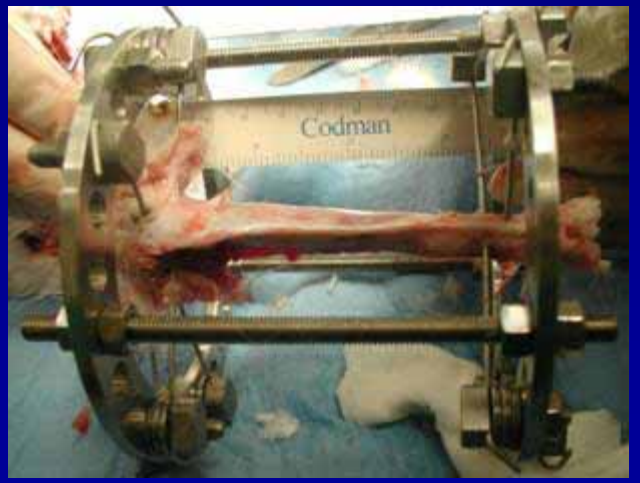
(A) Hematoxylin and eosin stain, (B) phosphate deposits by Von Kossa and (C) collagen by Trichrome Masson

Scaffold: $PLA_{50}CL_{50}HXD_{20/1}$+ 15wt% HEMA - porosity 70%

Cell growth inside the porous structure was observed and starting mineralization was detected by microscopical and histological analysis

Experiment on rabbit with 20 x 6 mm porous polymeric scaffold seeded with periost cells





PH scaffolds

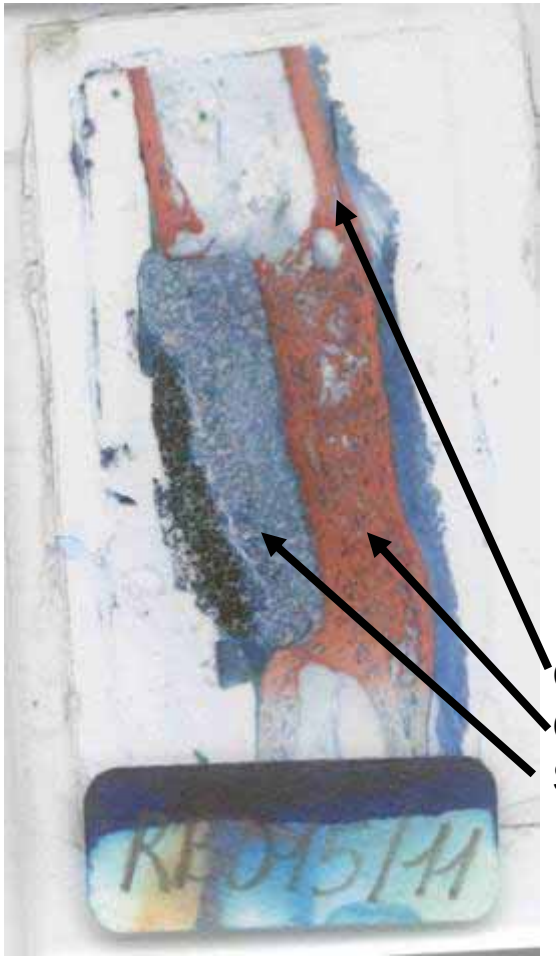
Composition:
PLA50CL50DPENT20/1-HM
+ 15 wt% HEMA

Scaffold size:
5 mm diameter and
3 mm height

6 mm diameter and
20 mm height

Pore size: 250-355 micrometer
Porosity: 70 or 80%

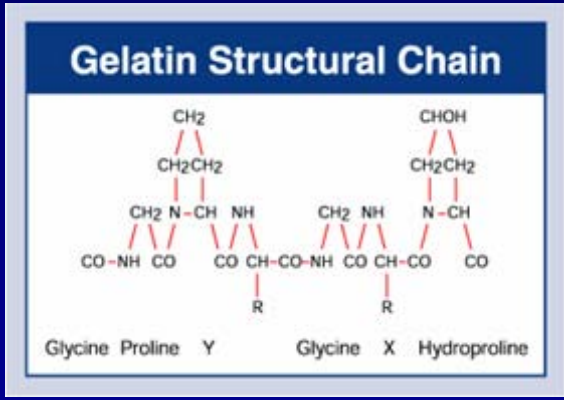
after 6 weeks

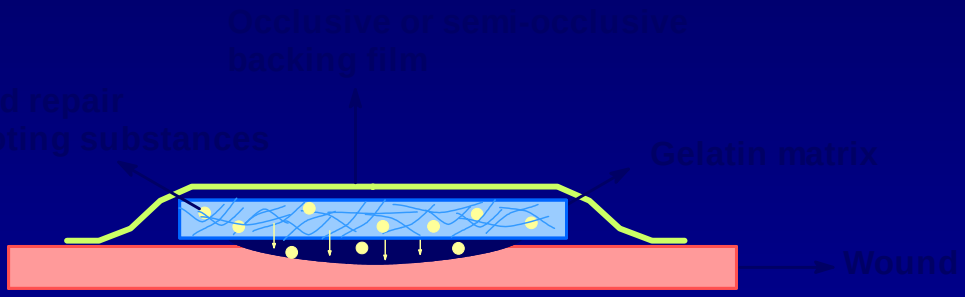


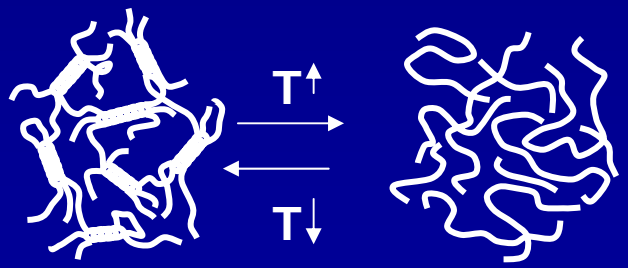
2. Cryogenic prepared porous gelatine scaffolds with controlled pore morphology

- preparation & characterisation
- cell interaction studies

Former work : Gelatin hydrogels for wound treatment
An Van Den Bulcke, Ilse De Paepe







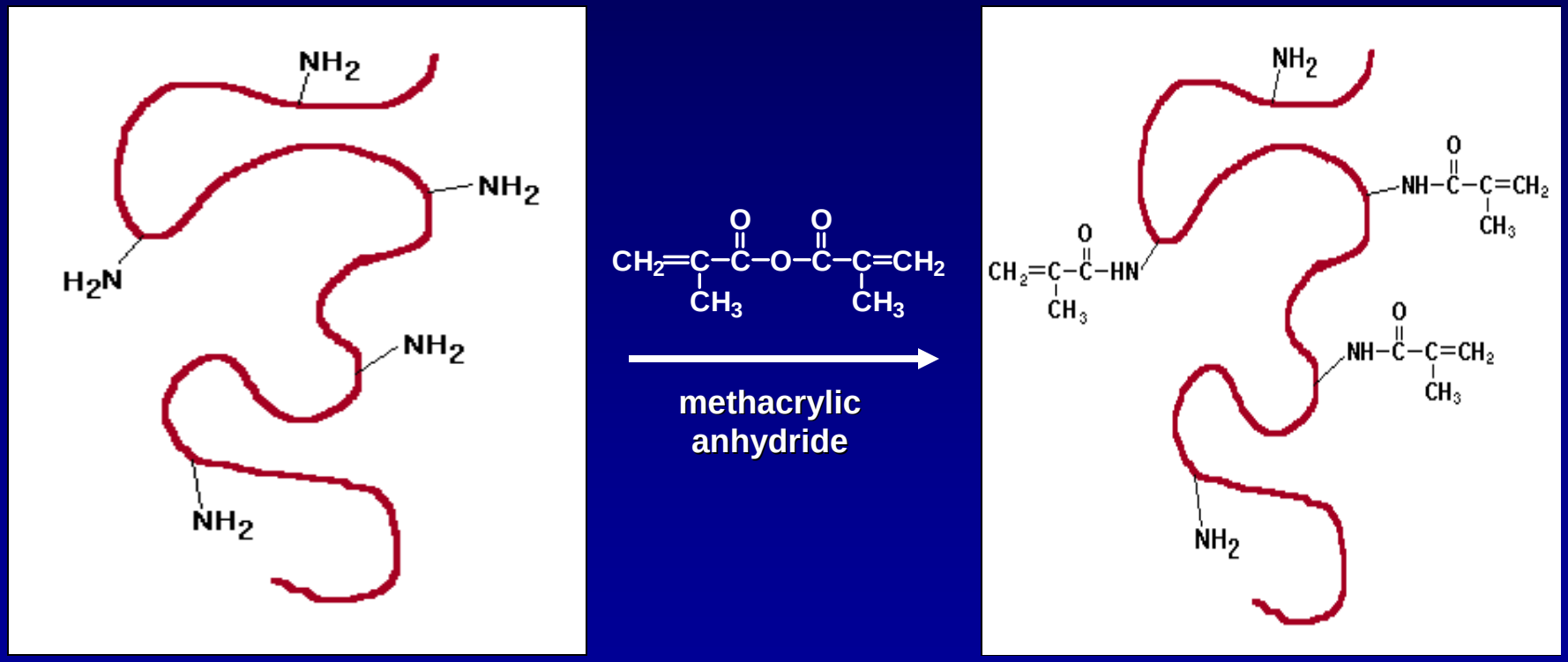
gel sol

thermoreversible gelation

transition temp 30-35°C

Chemical modification of Gelatin

Synthesis of gelatin methacrylamide

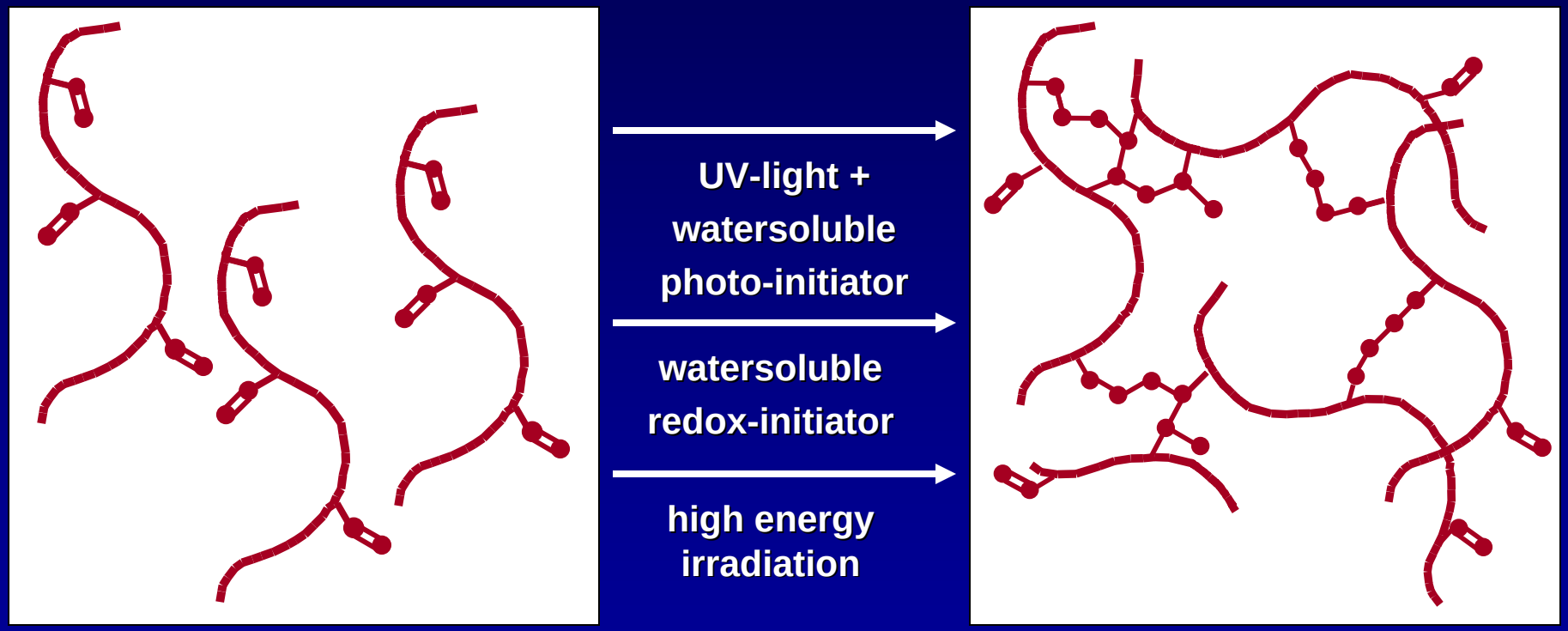


gelatin

gelatin methacrylamide

Hydrogel Preparation

Crosslinking of gelatin methacrylamide

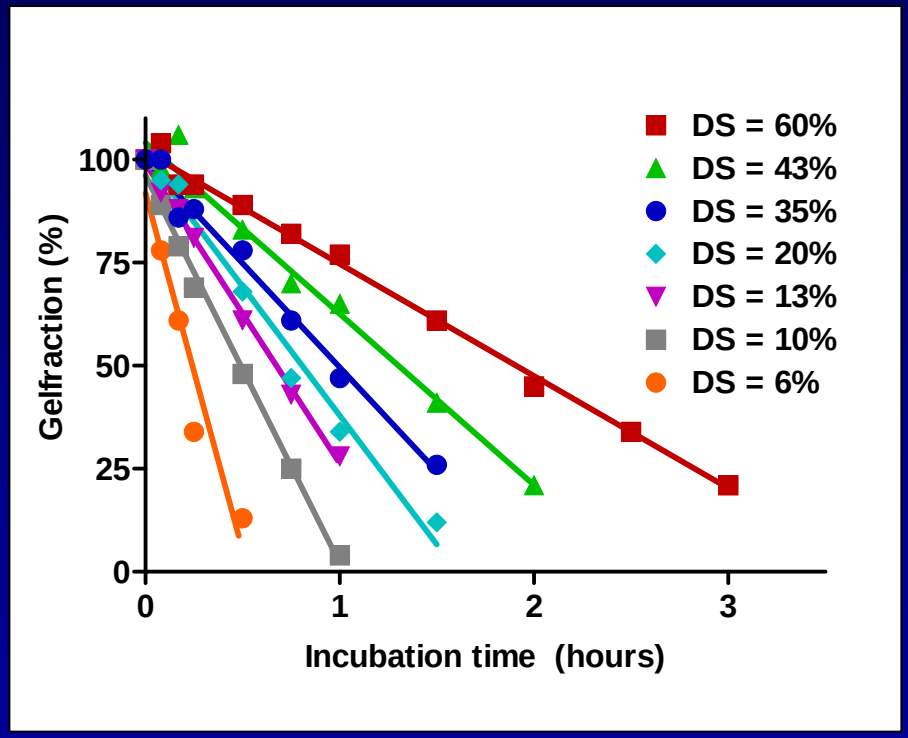


gelatin methacrylamide

hydrogel network

In vitro degradation

Gelatin methacrylamide hydrogels in a collagenase solution

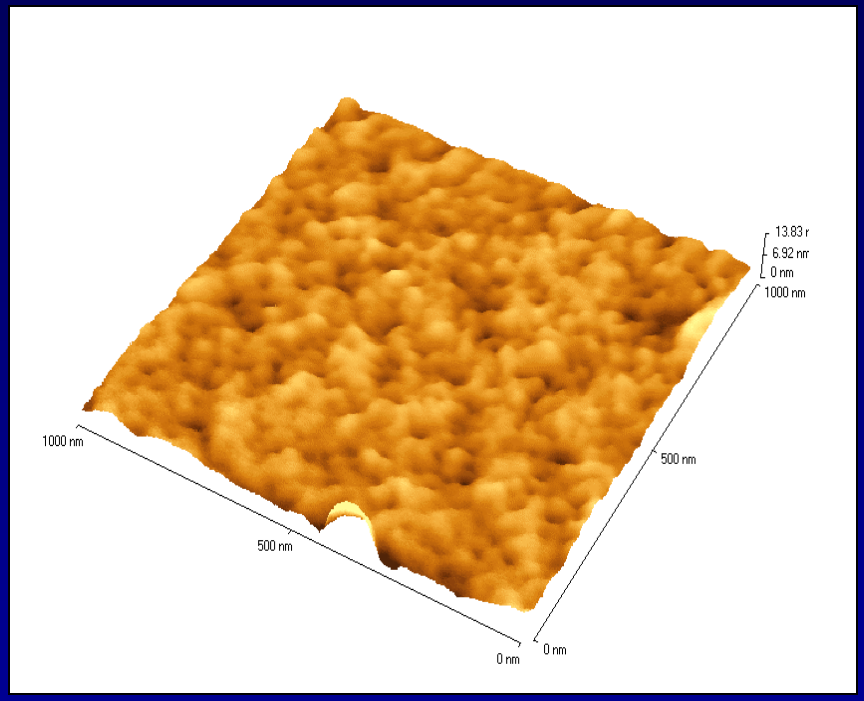


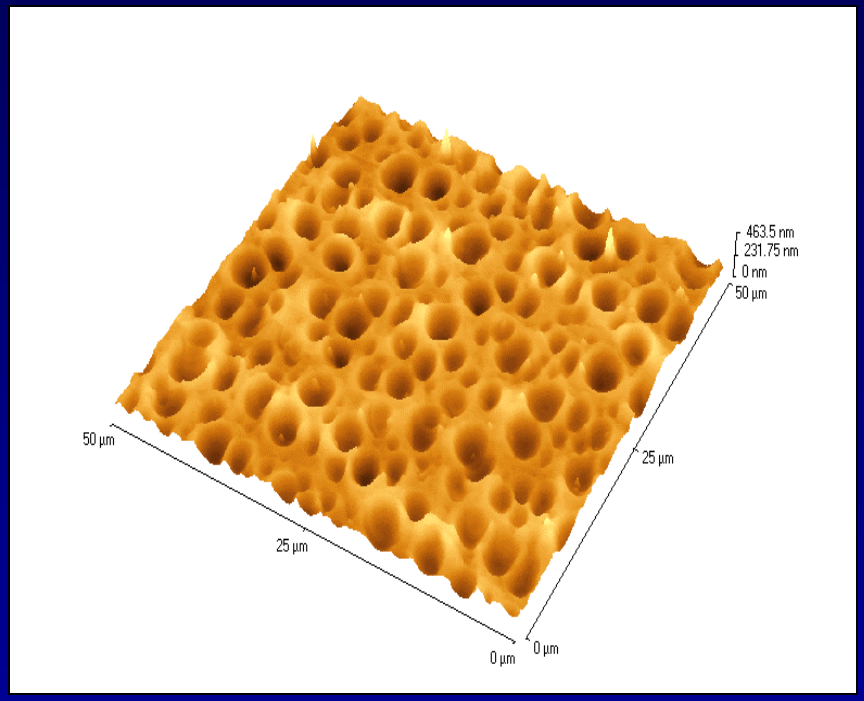
Surface erosion

Size reduction

AFM analysis

Gelatin hydrogels : effect of cryogenic treatment

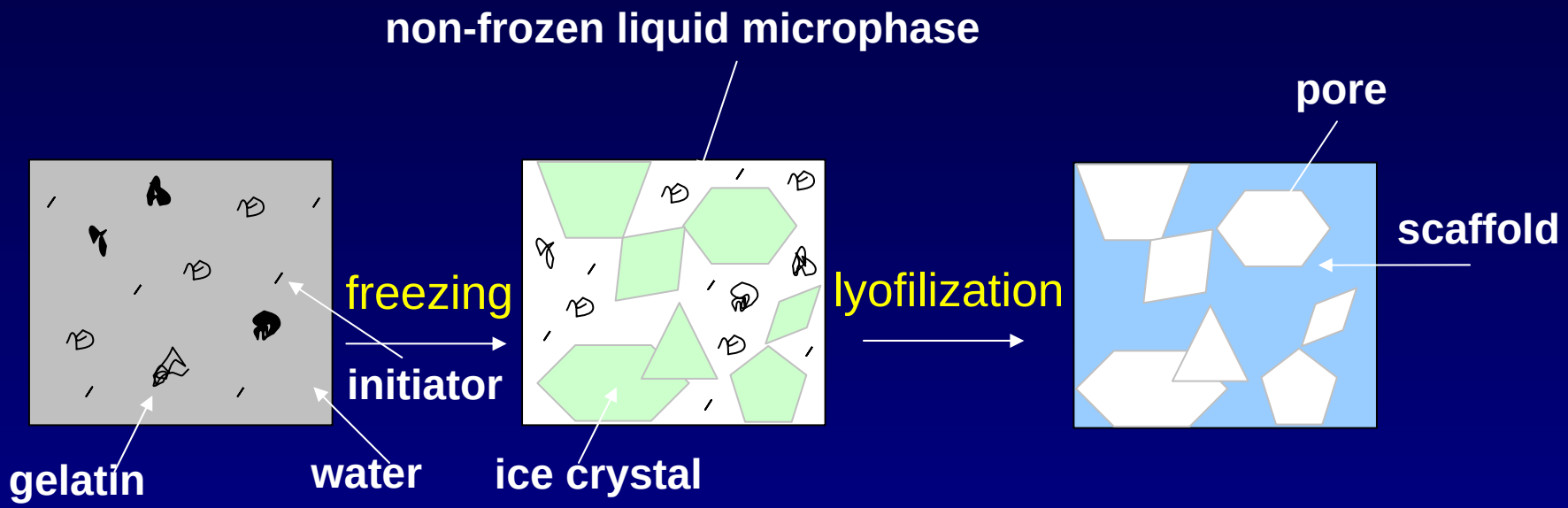




4°C

- 20°C

Cryogenic Treatment

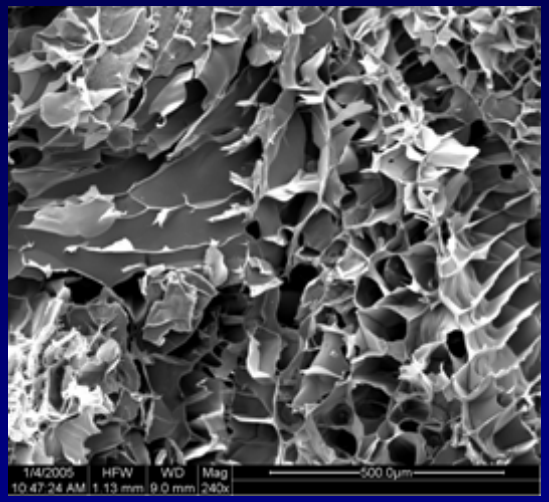


Porous scaffolds obtained by means of cryo-unit

1) gelatin concentration
2) cooling rate
3) temperature gradient ($\Rightarrow$ pore gradient)

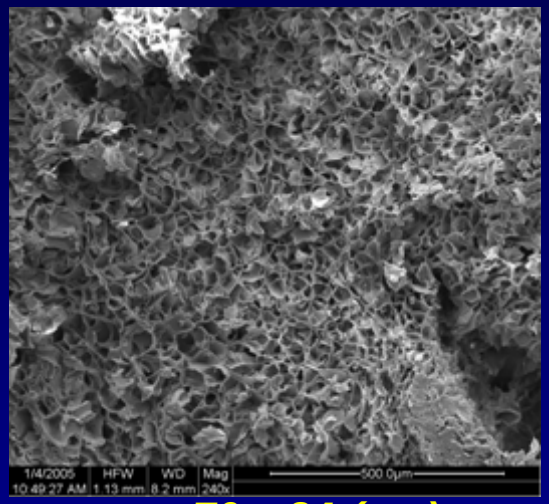
Influence of gelatin concentration

5 w/v% ↔ 15 w/v%

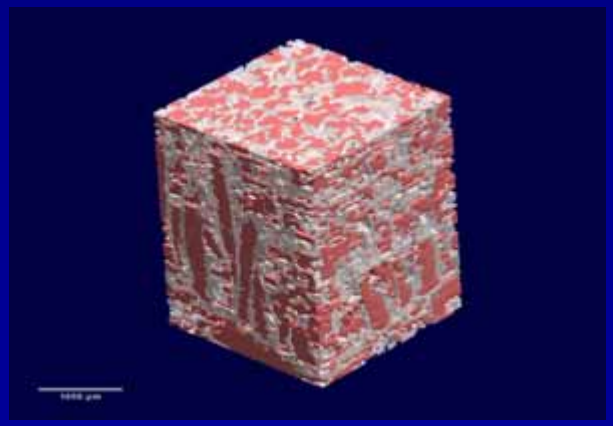


147 ± 41 (µm)

SEM

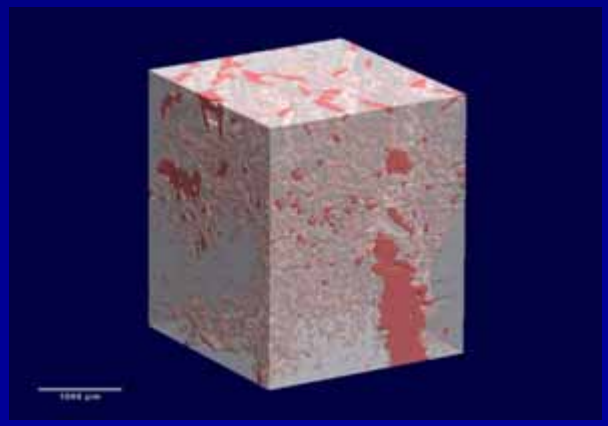


70 ± 24 (µm)



160 *(µm)*

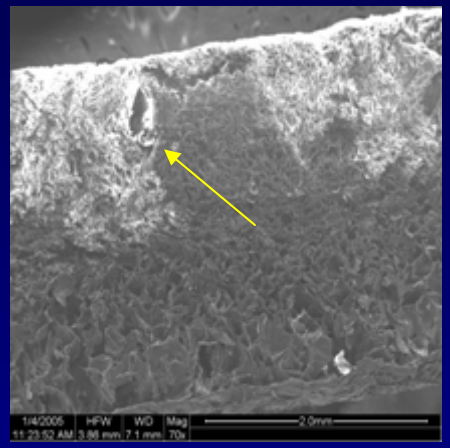
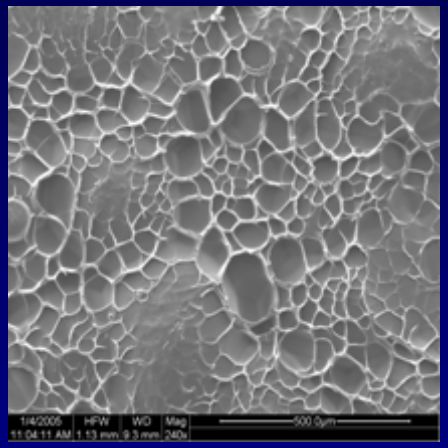
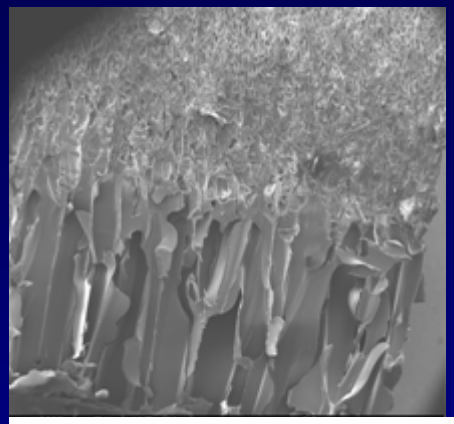
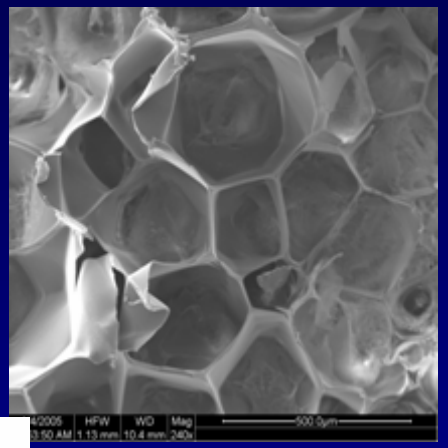
µCT



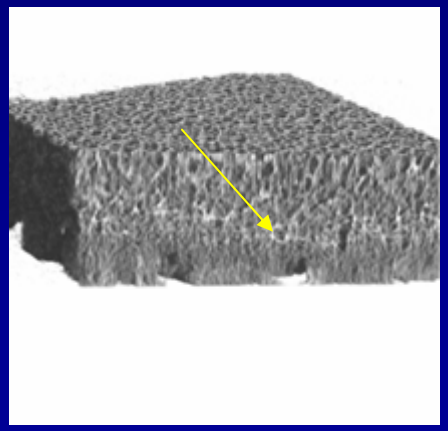
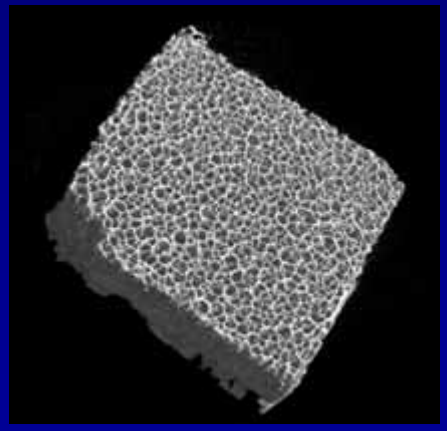
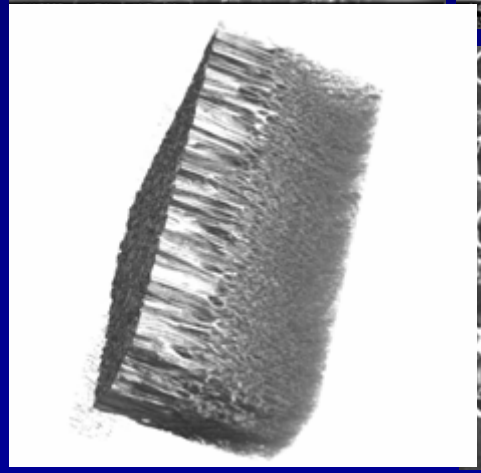
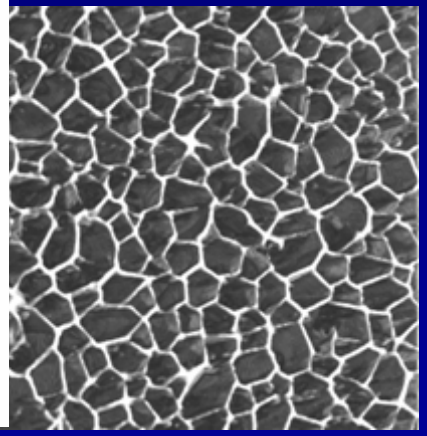
105 *(µm)*

Effect of T gradient :

10°C ↔ **30°C**

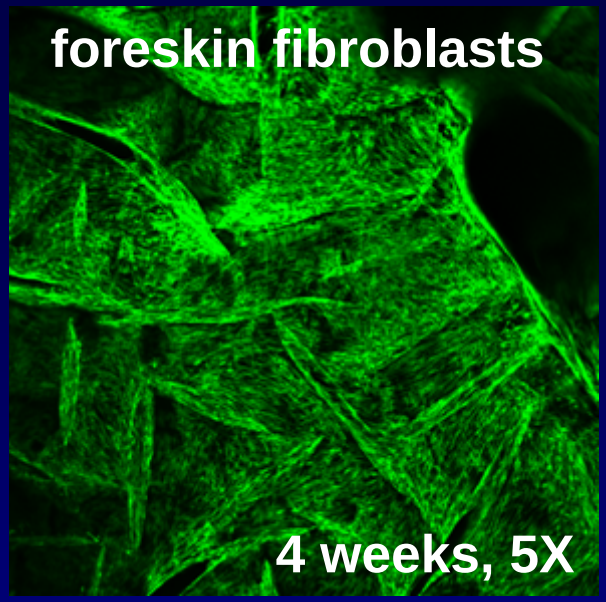
S E M

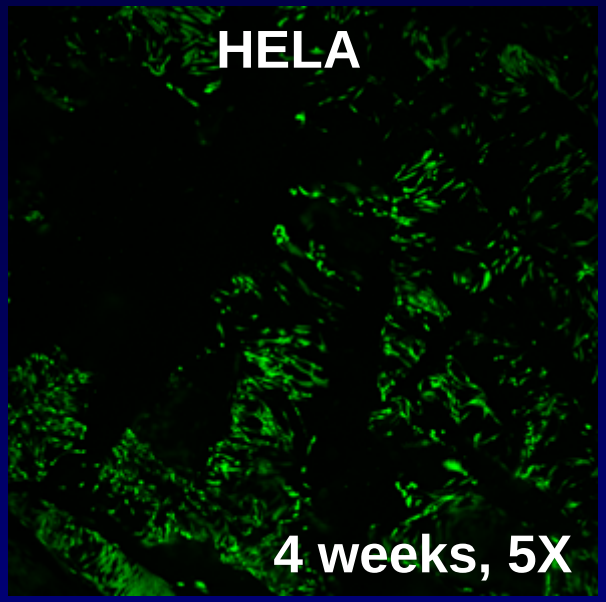





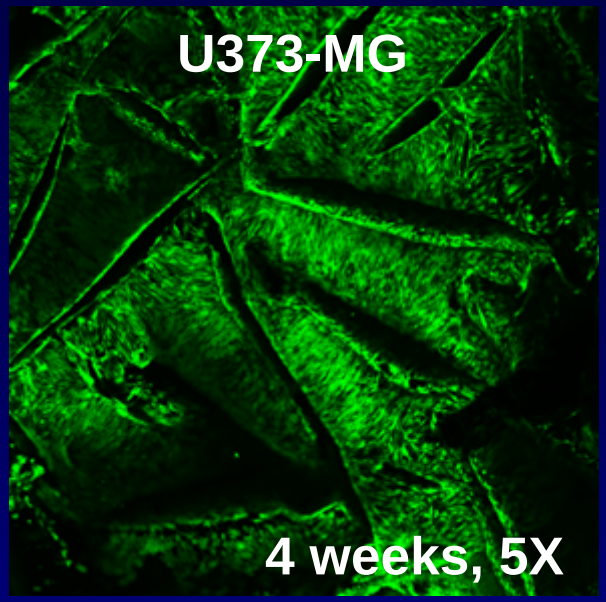
µ C T

116 → 12 µm (µCT)
top → bottom

330 → 20 µm (µCT)
top → bottom







HUVEC: cell attachment + spread-out cell morphology +
cell clusters after 1 week. Cell density ↗ with ↗ incubation time.
MG-63: confluent cell layers after 2 weeks.
CAL-72: cells adhered and spread within 3 days.
Incubation time ↗ → similar to HUVEC.
Fibroblasts, epithelial cells, glial cells: adhesion + proliferation

INTERESTING MATERIAL FOR THE CULTURING OF
A LARGE VARIETY OF HUMAN CELLS

3. In situ crosslinkable thermo-responsive hydrogels for biomedical applications

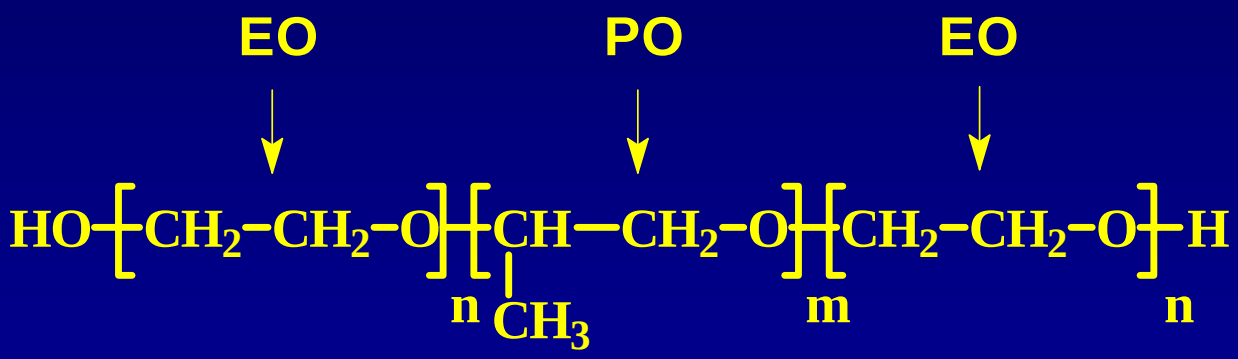
I. Swennen, V. Vermeerch, E. Schacht (PBM-UGhent)

M. Cornelissen, E. Lippens (Cell culture U-Ghent)

F. Gasthuys, L. Vlaeminck, G. Vertenten (Vet. Sci. U-Ghent)

Pluronic® F127

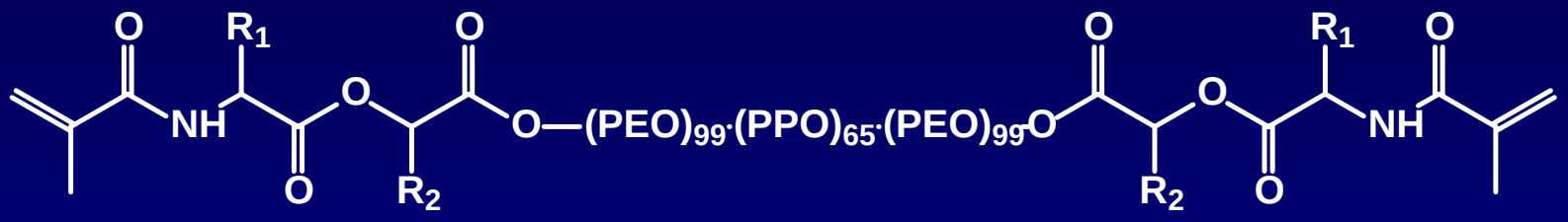
- Pluronic®, important hydrophobic associating amphiphilic ABA block-copolymer of ethylene oxide and propylene oxide



F127: n = 99, m = 65

Molecular weight approx.: 12,600

Covalent hydrogel: crosslinking

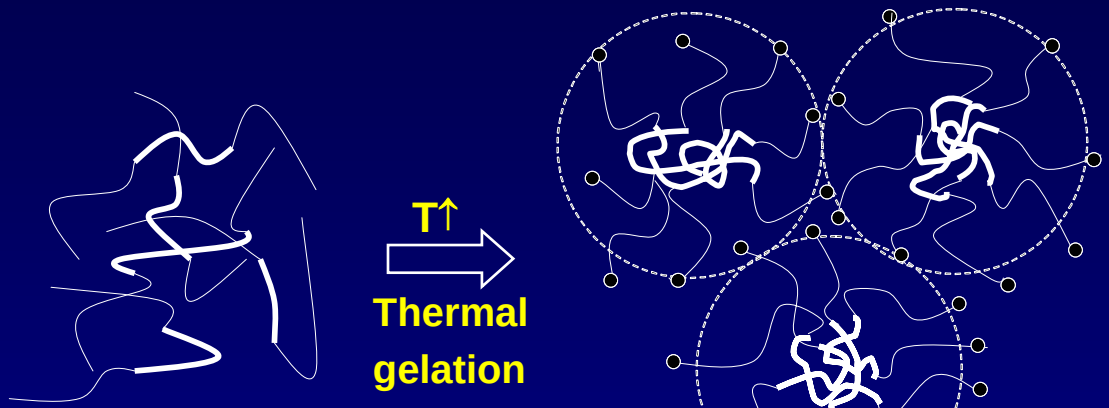


free radical polymerization

photo-initiator irgacure 2959

3D crosslinked hydrogel network

Concept



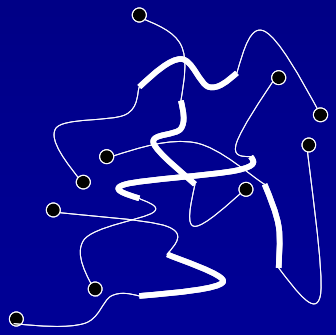
T↑
Thermal
gelation

less suitable
for biomedical
applications

Chemical
modification

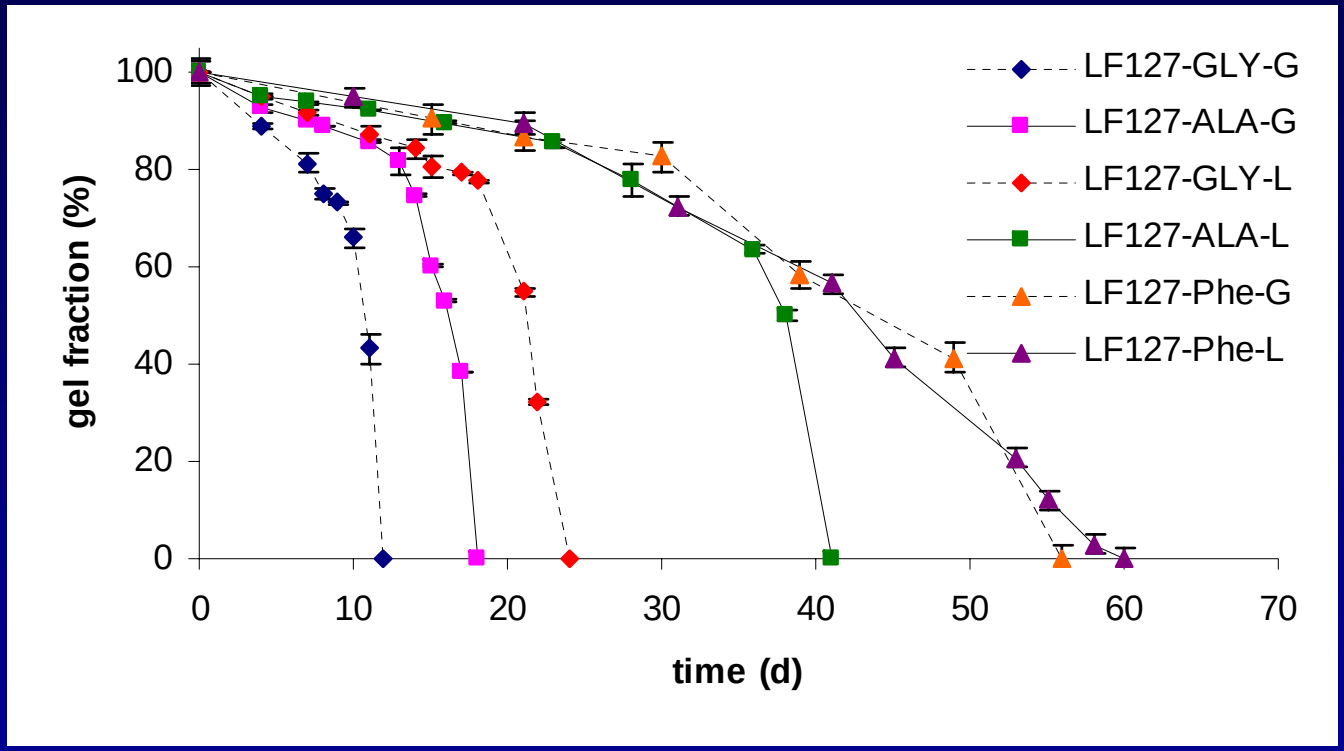
T↑

Chemical crosslinking



THERMO-RESPONSIVE
DEGRADABLE HYDROGEL

In Vitro Degradation



Mass loss 30w/w% hydrogels, incubation in PBS-buffer, (37°C)

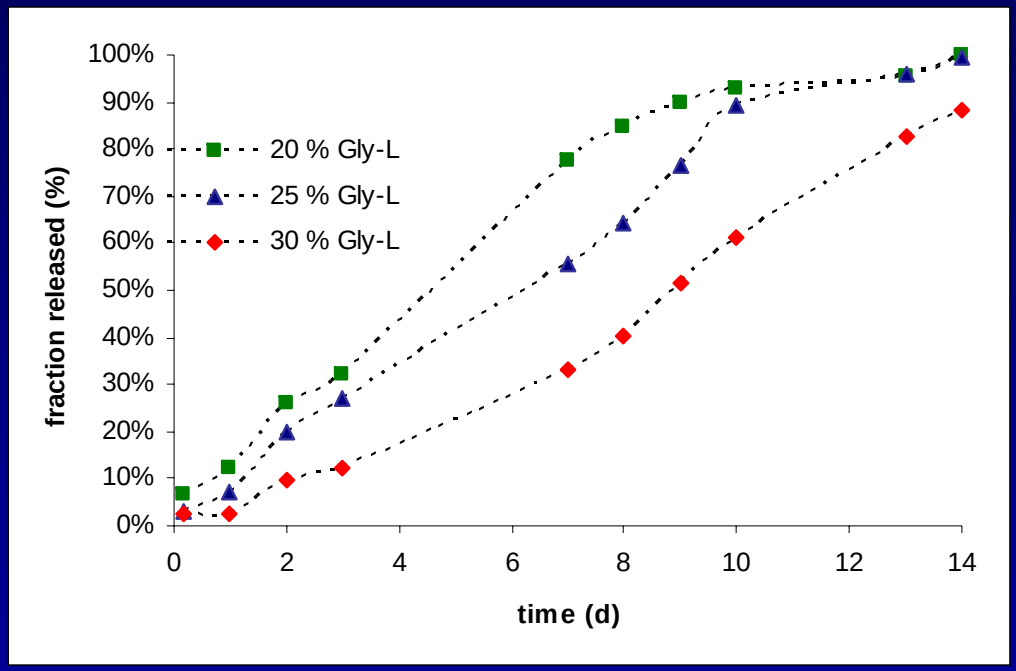
Degradation rate inversely related to the hydrophobicity of the -R_1 and –R_2 side groups of the depsipeptide

Phe-L ~ Phe-G < Ala-L < Gly-L < Ala-G < Gly-G

Drug release : release of BSA

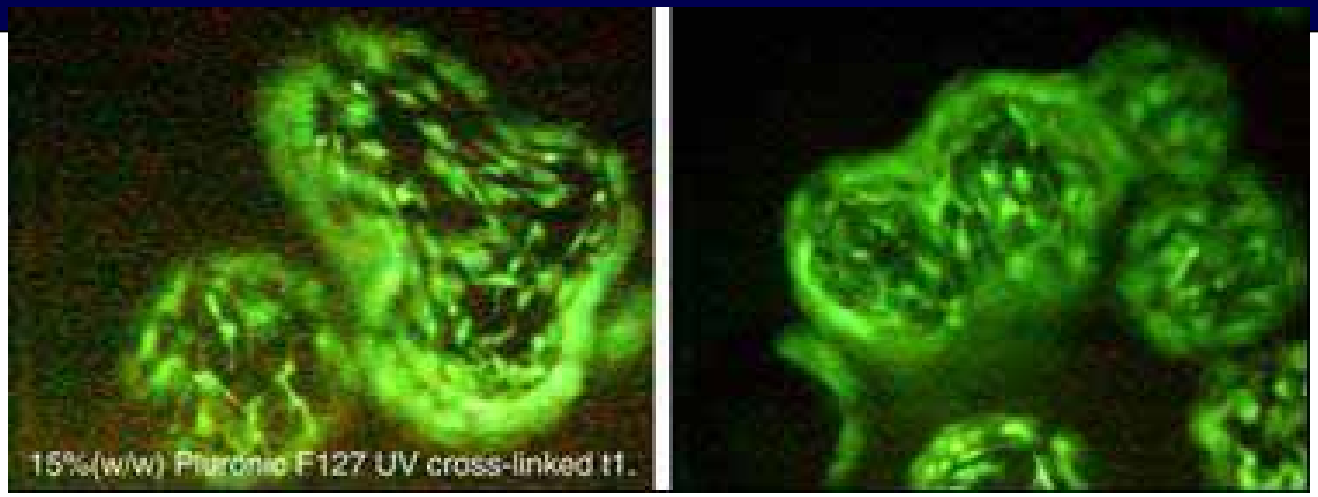
Influence of polymer concentration

Bovine Serum Albumin (MM = 66.4 kDa): Model for monoclonal antibodies, growth factors



± zero order release; rate propotional to the polymer concentration

Use of X-linked hydrogels for cell immobilisation



Goat bone marrow cells cultured on Cultisphere-S microcarriers for 29 days
Carrier-seeded microcarriers incorporated in F127-Ala-L (10%, 15%)
Fluorescente (PI & Calcein AM) staining after 1 day

Microscopy of Haematoxylin & Eosin stained carriers after 12, 14 resp. 21 days in X-F127-Ala-L hydrogels, show viable cells

Acknowledgements

1. Porous scaffolds based on biodegradable polyesters

T. Gorski, J. Mendez (PBM-U-Ghent), J. San Roman (U-Madrid)
M. Cornelissen (Histology, U-Ghent)
F. Gasthuys, G. Vertenten (Vet. Sci., U-Ghent)
A. Bakker, F. Luyten (KULeuven)

2. Cryogenic prepared porous gelatine scaffolds with controlled pore morphology

S. Van Vlierberghe, P. Dubruel, P. Jacobs, V. Cnudde (U-Ghent)
R. Unger, J. Kirckpatrick (U-Mainz)

3. Biodegradable thermoresponsive hydrogels

I. Swennen (PBM), M. Cornelissen, E. Lippens (Histology, U-Ghent)
E. Adriaens, J.P. Remon (Pharmacy, U-Ghent)
M. Hornof, A. Urti (Drug Discovery & Techn.,U-Helsinki)

SPONSORS

- University of Ghent
- Fund for Scientific Research- Flandern (FWO)
- Flemish Institute Promotion of Sci. &Techn. Research in Industry (IWT)
- Belgian Ministry of Scientific Programming: (IUAP/PAI-V-03)
- A. Von Humboldt Foundation, Germany
- TEKES programme Finland

THANK YOU !!!