GELATIN & HYDROXYAPATITE CELL DELIVERY SYSTEMS IN BONE TISSUE ENGINEERING

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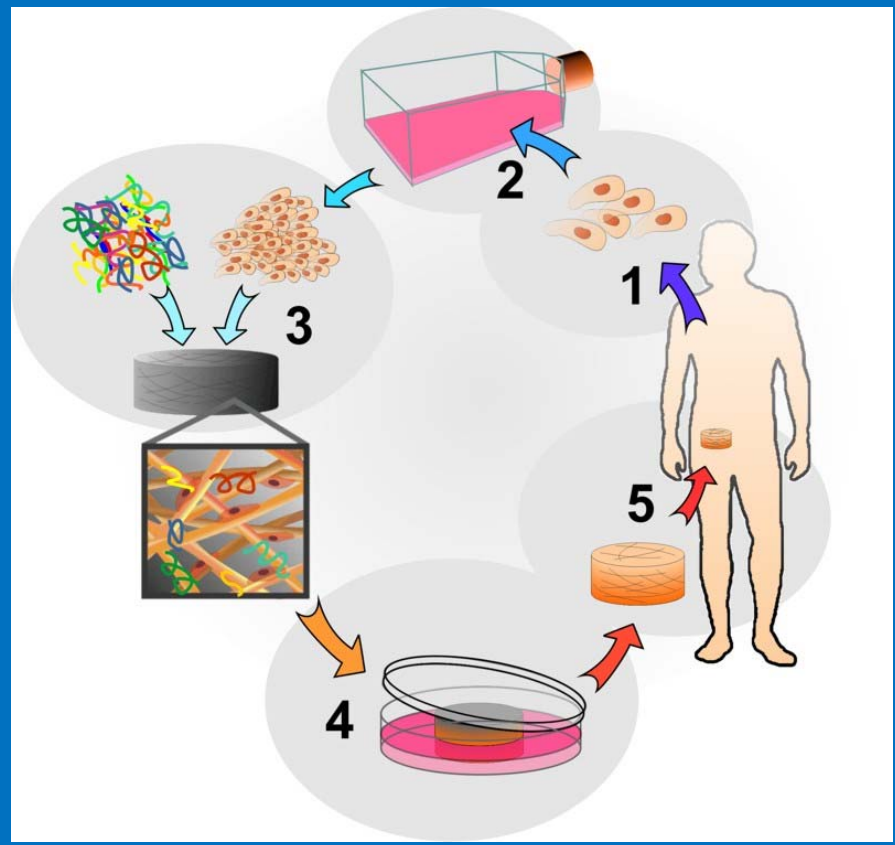
Tissue engineering

Creating living tissue in the lab to replace damaged or lost body parts by mixing:

- Cells
- Biomaterial:

  natural or synthetic
- Growth factors



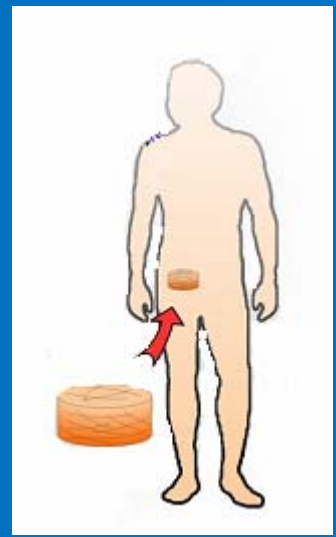
Bone defects

- By trauma
- By congenital disorder
- Resection due to tumor removal

→ **Mostly irregular bone defects**

↓

Cell seeded scaffold not ideal, to fit accurately in the defect.



→ ***In situ* polymerizable filling material**

In situ polymerizable material

- A synthetic polymer paste / liquid,
  - that can be pressed / injected in the defect
  - by photo- or chemical cross-linking, can be hardened in the exact shape of the defect,
  - that degrades over time.

Examples:

Modified:

- Lactide caprolactone
- Hydrogels

Cell source

Bone marrow punction

- Density centrifugation
- Cell attachement differences

Haematopoietic population

Mesenchymal population

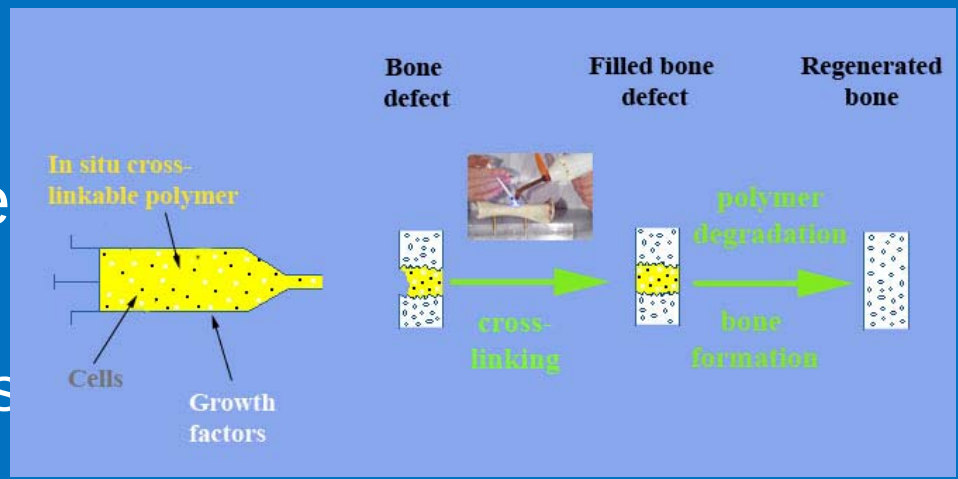
Osteogenic differentiation additives

- Osteoprogenitor cells
- Osteoblasts

Bone tissue engineering

Creating living bone tissue in the lab to replace damaged or lost bone parts by mixing:

- In situ cross-linkable biomaterial
- Osteogenic differentiate bone marrow cells
- Osteogenic growth factors (optional)



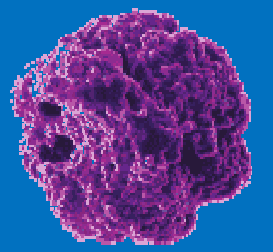
Cell mixing

- Mixing cells with the polymer paste/liquid can be harmfull for the cells:
  - ➔ Seeding cells onto a macroporous microcarrier system.
    - ➔ Protecting cells
    - ➔ Anchorage dependent cells prefer a substrate to adhere

Cell delivery systems

- CultiSpher-S

  = gelatine microcarrier



Ø 130 – 380 µm

Percell Biolytica

Hydroxyapatite

Ø 4 – 5,5 mm

VITO

CultiSpher-S

- = Commercially available macroporeus gelatine microcarrier system.
- Average pore size: 20 µm
- Literature:

  Chondroprogenitor cells (Biomaterials 27, 2970-2979, 2006)

  Keratinocytes (Cell Transplantation 15, 435-443, 2006)

  (Burns 33, 726-735, 2007)

  Nerve cells (Plast. Reconstr. Surg. 120, 2007)

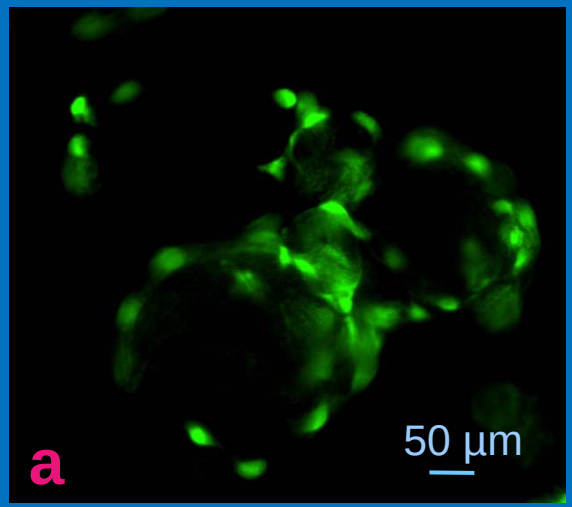
  Stem cells (J. Biotechnology 132, 227-236, 2007)

  (Biomacromolecules 8, 825-832, 2007)

CultiSpher-S

- Our experiences with CultiSpher:
  - Osteoblastic cell lines: MC_3T_3-E1
    UMR-106
  - Bone marrow cells → proliferation
    → osteogenic differentiation
  - Mouse embryonic stem cells
    → undifferentiated proliferation
    → osteogenic differentiation

CultiSpher-S

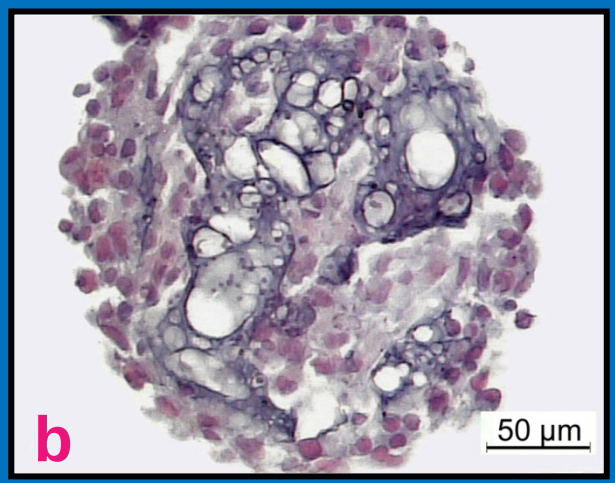


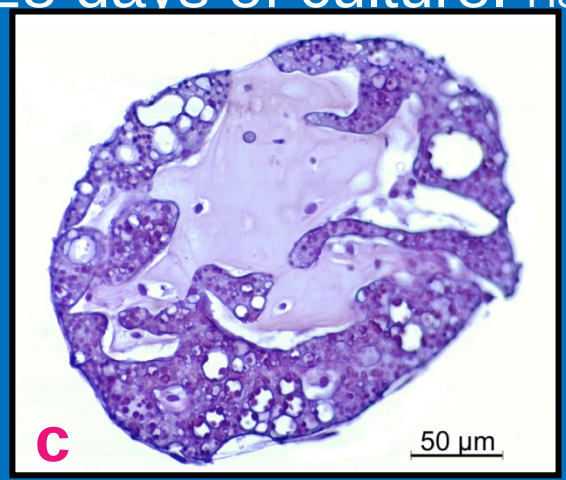
Rat bone marrow cells seeded on CultiSpher-S carriers:

a. 2 days of culture. Calcein AM fluorescent staining

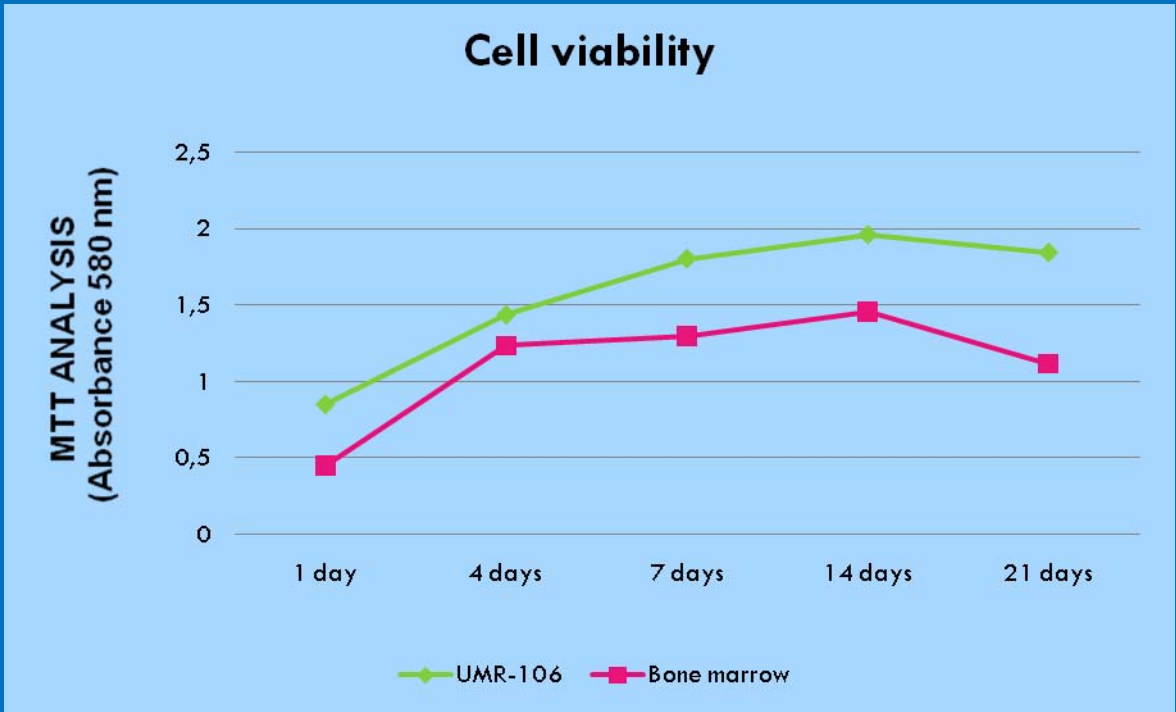
b. 28 days of culture. Trichrome Masson staining

c. 28 days of culture. H&E staining



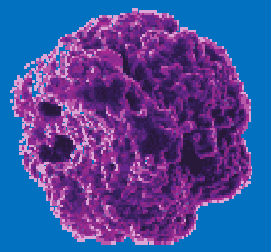


CultiSpher-S



Cell delivery systems

- CultiSpher-S
  = gelatine microcarrier



Ø 130 – 380 µm

Percell Biolytica

Hydroxyapatite

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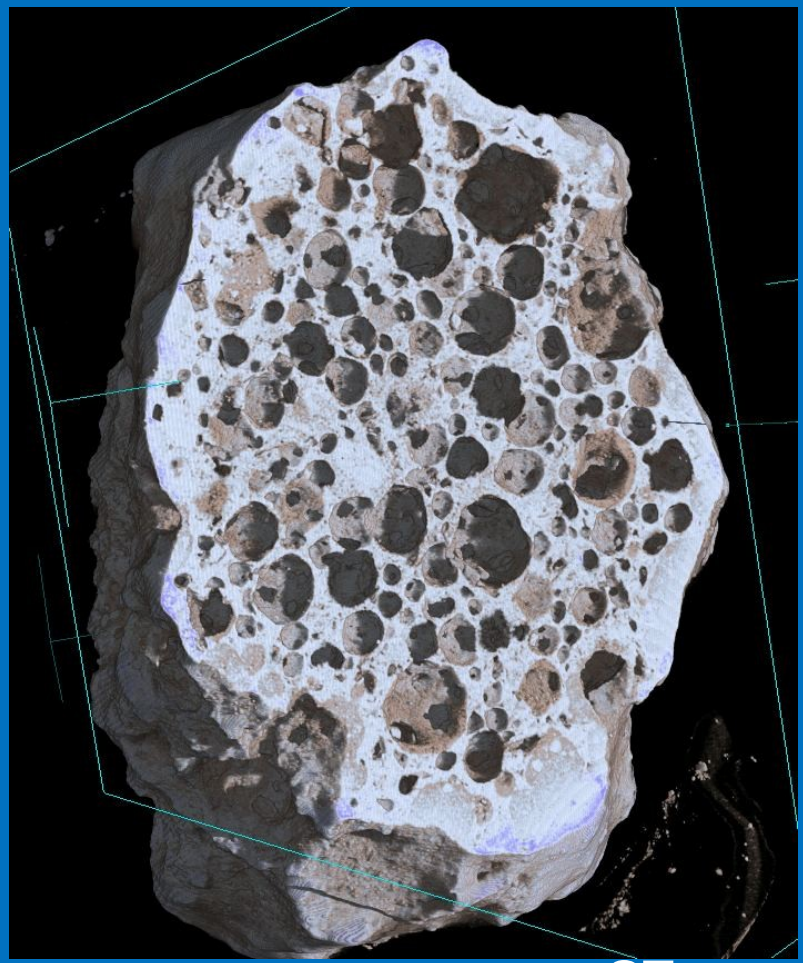
Hydroxyapatite

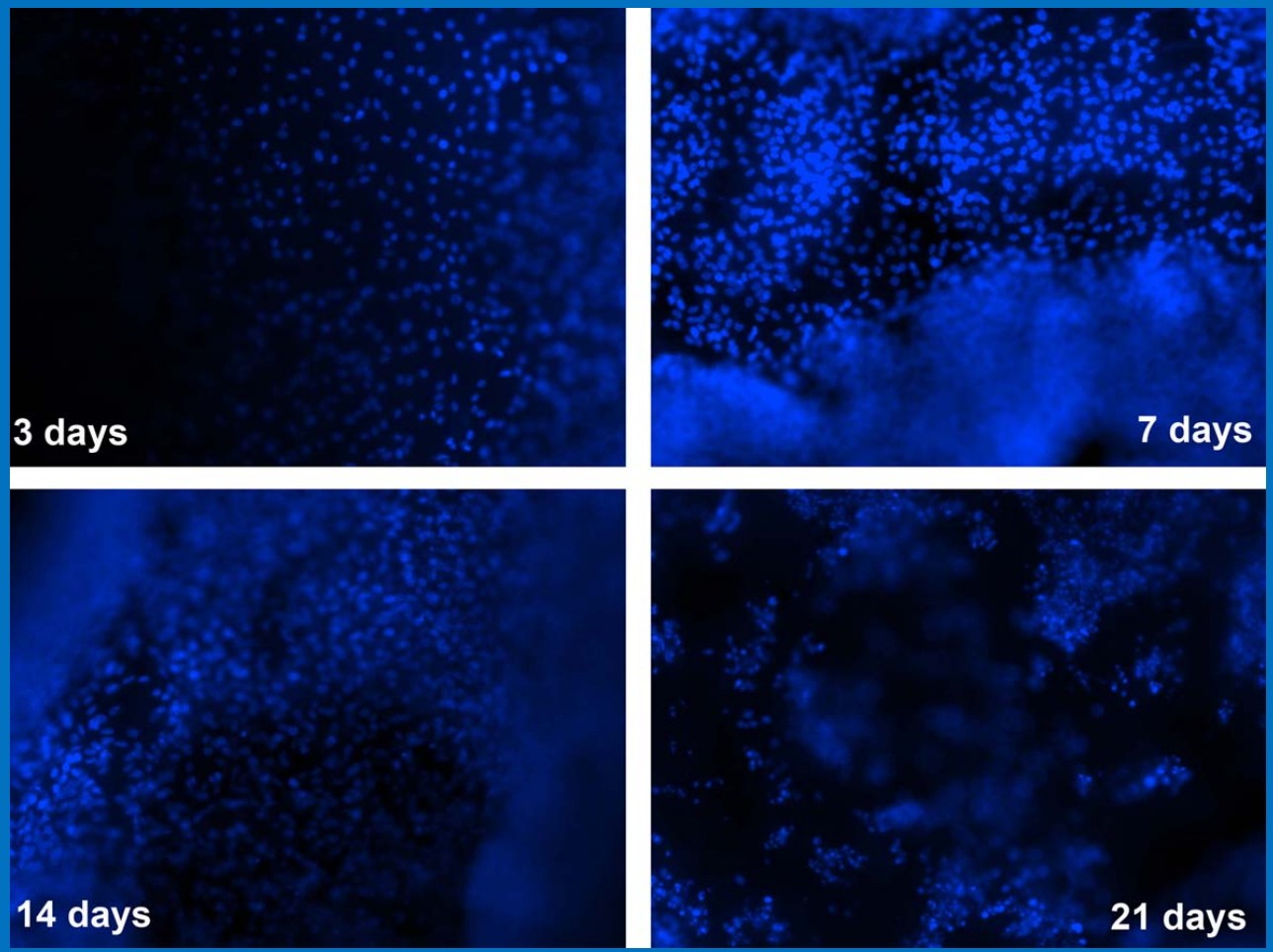


µCT scan

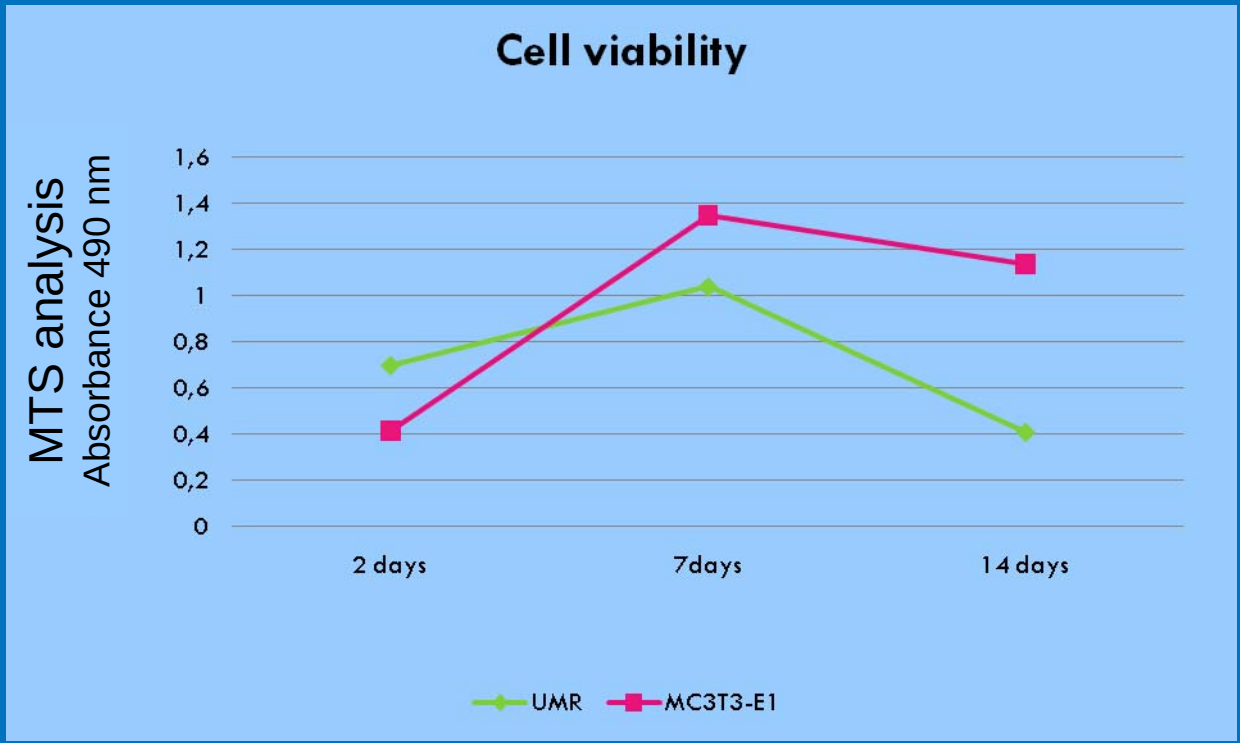
- Prepared by mixing hydroxyapatite powder (portion size 30 - 150 µm) with water and agar, as gel forming agent and some surface active products.
- Average pore size: 100 µm.
- Hydroxyapatite is the major bone matrix component.

Hydroxyapatite

DAPI staining of MC_3T_3-E1 cell nuclei



Hydroxyapatite



In situ hardening biomaterial

1. Lactide caprolactone polymer paste

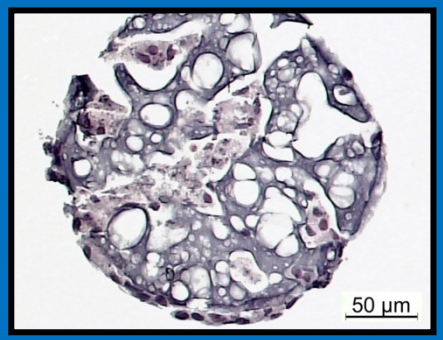
- = Methacrylate-endcapped poly-D,L-lactide-co-caprolactone
- Cross-linking: illumination with blue light.

2. Hydrogel liquid

- = Pluronic ALA-L
- Cross-linking: illumination with UV light.

In situ hardening biomaterial

1. Lactide caprolactone polymer paste



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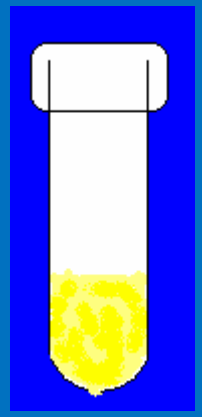
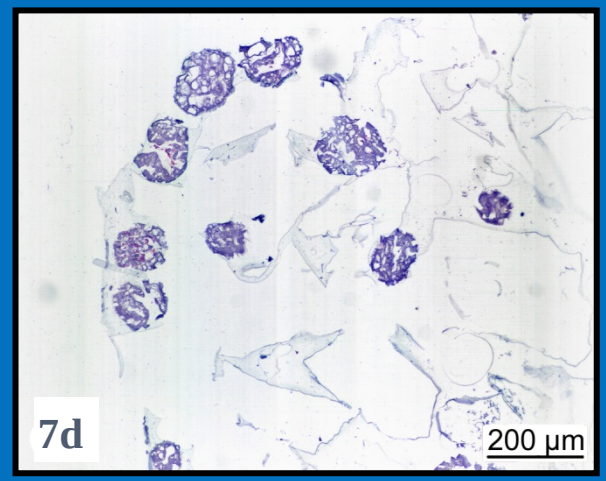


Cross-linking
(500mW/cm²)



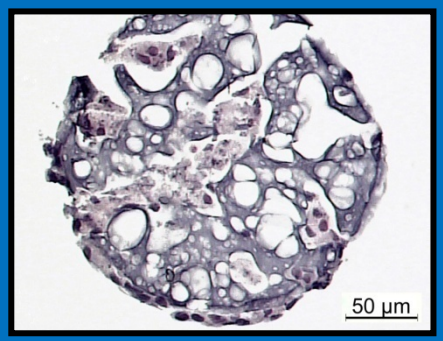
Polymer: methacrylate-endcappedpoly-D,L-lactide-co-caprolactone

katalyst: D,L-camphorquinone

Solvent: Triacetin

In situ hardening biomaterial

2. Hydrogel liquid



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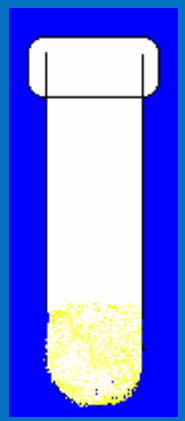
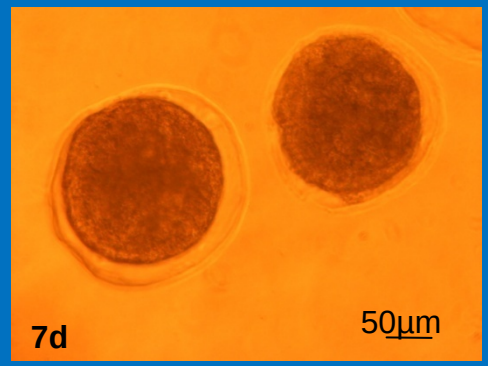
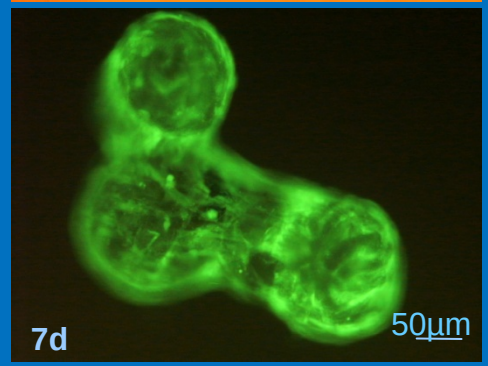


UV Cross-linking



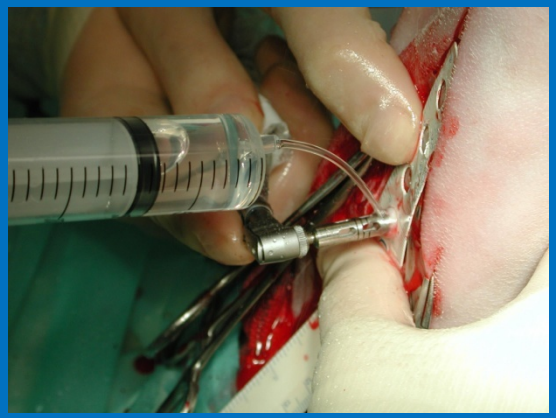


Pluronic ALA-L 15 (w/v)%

Polymer: Pluronic ALA-L

UV katalyst: Irgacure

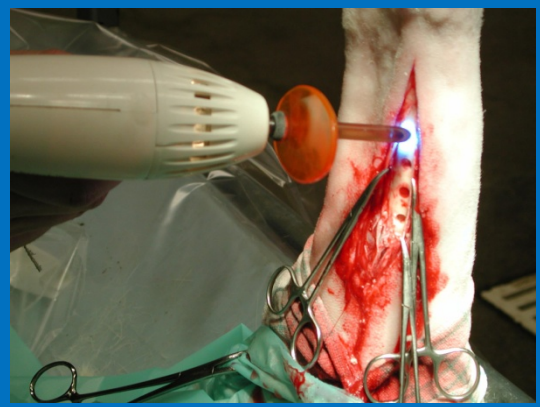
In vivo test: implantation



6 mm ø holes were drilled wit
← a trephine burr in the media
diaphyseal cortex of a tibia.

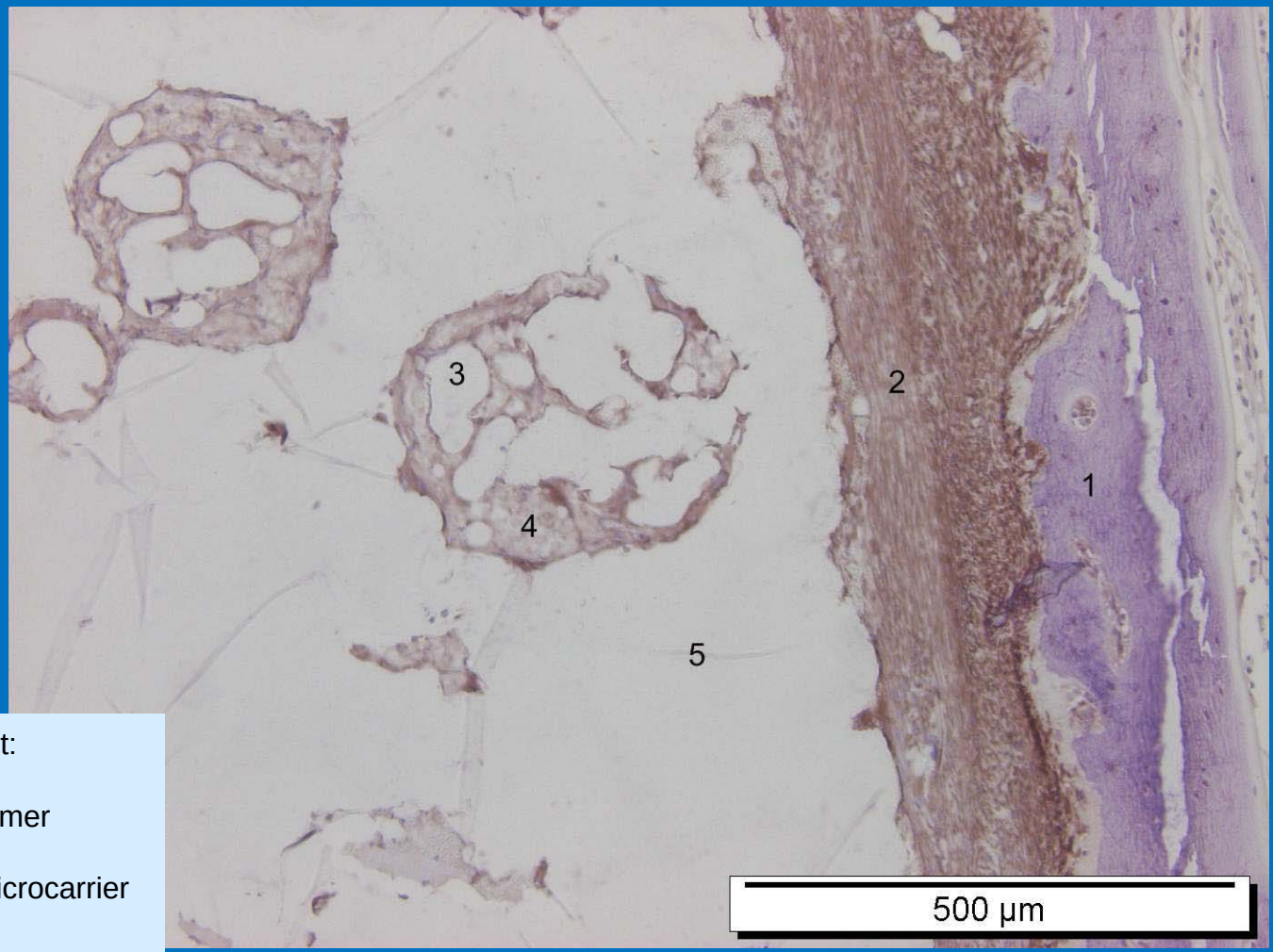
3 holes were filled with *in situ*
polymerizable methacrylate
polymer. →

Cross-linking of the polymer in the defect with blue



← After UV cross-linking

In vivo test: histology

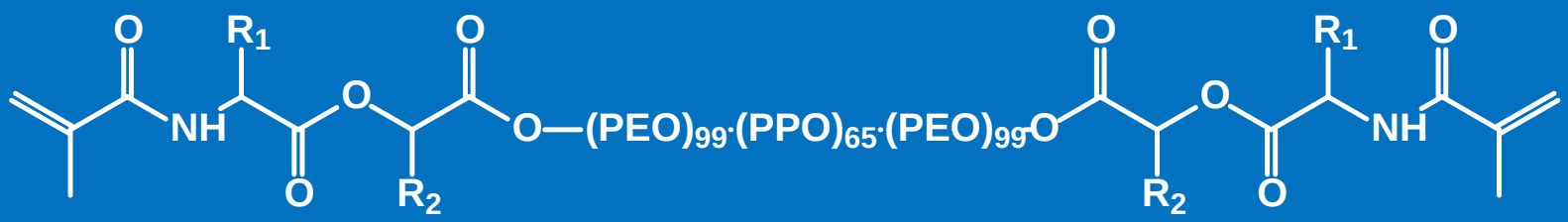


Photomicrograph of a tibial defect:
1: Newly formed trabecular bone
2: Capsule of tissue around polymer
3: Microcarrier
4: Tissue and cells around the microcarrier
5: Polymer

Conclusions

- Osteoblasts colonize the 2 types of macroporeus carrier systems.
- These cell loaded carriers can be mixed with different types of in situ polymerizable filling materials.
- *In vivo* tests with CultiSpher in goat tibiae revealed that the cells remain viable in the methacrylate polymer.
- Future in vivo testing will be done with CultiSpher & hydroxyapatite cell delivery systems encapsulated in Pluronic ALA-L.

Covalent hydrogel: crosslinking



free radical polymerization

photo-initiator irgacure 2959

3D crosslinked hydrogel network