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

- Viscous crosslinkable prepolymer
- Calciumphosphate cement
- Bioactive components
- Plastic precursor material

Injection in a bone defect → Cross-linking → Filled bone defect → Repaired bone defect
Curable polymers: methacrylates of polyesters

methacrylate endgroup

hydrolyzable ester bond

monomers

glycolide
D,L-lactide
trimethylene carbonate
ε-caprolactone
derivatization of the hydroxyl endgroups into methacrylate esters

\[ \text{HO-}L_{n}\text{-Cl}_{m}\text{-H XD-}L_{n}\text{-Cl}_{m}\text{-OH} + 2\text{ vinyl C l} \rightarrow \text{TEA} \]

\[
\begin{align*}
\text{CH}_2\text{Cl}_2 & \quad 24 \text{ h} \\
\text{room temp.} & \\
\end{align*}
\]

\[ \rightarrow \text{O-O-LA-CL-H XD-LA-CL-O-C O} + 2\text{HCl} \cdot \text{TEA} \]
in situ photopolymerization - formation of the 3-D polymer network
Hydrolytic degradation of the 3-D polymer network

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 cells after 20 hours

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%
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

Gelatin Structural Chain

Glycine Proline Y Glycine X Hydropoline

thermoreversible gelation transition temp 30-35°C
Chemical modification of Gelatin

Synthesis of gelatin methacrylamide

gelatin

$\text{CH}_2\text{C} = \text{C} - \text{O} - \text{O} - \text{C} = \text{CH}_2$

methacrylic anhydride

gelatin methacrylamide
Hydrogel Preparation

Crosslinking of gelatin methacrylamide

gelatin methacrylamide \rightarrow \text{UV-light + watersoluble photo-initiator} \rightarrow \text{watersoluble redox-initiator} \rightarrow \text{high energy irradiation} \rightarrow \text{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 (⇒ pore gradient)
Influence of gelatin concentration

5 w/v% ↔ 15 w/v%

SEM

147 ± 41 (µm)

70 ± 24 (µm)

µCT

160 (µm)

105 (µm)
Effect of T gradient:

10°C ↔ 30°C

116 → 12 µm (µCT)  top → bottom

330 → 20 µm (µCT)  top → bottom
foreskin fibroblasts

4 weeks, 5X

HELA

4 weeks, 5X

U373-MG

4 weeks, 5X

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

\[
\text{HO-}\left[\text{CH}_2-\text{CH}_2-\text{O}\right]_n\left[\text{CH}_3-\text{CH}_2-\text{O}\right]_m\left[\text{CH}_2-\text{CH}_2-\text{O}\right]_n\text{H}
\]

F127: \( n = 99, \ m = 65 \)
Molecular weight approx.: 12,600
Covalent hydrogel: crosslinking

free radical polymerization

photo-initiator irgacure 2959

3D crosslinked hydrogel network
Thermal gelation less suitable for biomedical applications.

Chemical modification

T↑ Thermal gelation

Chemical crosslinking

T↑

THERMO RESPONSIVE DEGRADABLE HYDROGEL
In Vitro Degradation

Degradation rate inversely related to the hydrophobicity of the \(-R_1\) and \(-R_2\) side groups of the depsipeptide

Phe-L \sim Phe-G < Ala-L < Gly-L < Ala-G < Gly-G

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