

# Osteoblast behaviour on *in situ* photopolymerizable three-dimensional scaffolds based on D, L-lactide, $\epsilon$ -caprolactone and trimethylene carbonate

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Polymer networks formed by photocrosslinking of multifunctional oligomers have great potential as injectable and *in situ* forming materials for bone tissue engineering. Porous scaffolds varying in polyester type and crosslinking density were prepared from methacrylate-endcapped oligomers based on D,L-lactide,  $\epsilon$ -caprolactone and trimethylene carbonate: LA/CL-hexanediol, LA/CL-dipentaerythritol and LA/TMC-HXD. The biocompatibility and bone formation were related with the degradation time and mechanical properties.

The viability of fibroblasts was evaluated after incubation with extraction medium by MTT-assay. All scaffolds showed a good biocompatibility. Rat bone marrow cells were cultured on the scaffolds for 21 days and were able to attach and differentiate on the scaffolds. The cells expressed high alkaline phosphatase activity, have formed a mineralized extracellular matrix and secreted osteocalcin. TEM of the polymer interface revealed osteoblasts which secreted an extracellular matrix containing matrix vesicles loaded with apatite crystals.

LA/TMC-HXD, LA/CL-HXD and LA/CL-DPENT had a 50% mass loss at 3,5 months respectively 6 and 7, 5 months. The mechanical properties improve with increasing the branching of the precursor methacrylates (by replacing HXD by DPENT) but do not depend on their chemical composition. Hence, scaffolds with high elastic properties and variable degradation time can be obtained, which are promising for bone tissue engineering.

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## Introduction

Tissue engineering is emerging as a technique that could potentially be used in the future to develop synthetic bone graft replacement materials for the treatment of large bone defects.

In the last decade, bone formation on 3-D scaffolds based on a diversity of polymers ranging from biopolymers (hyaluronic acid, chitosan, collagen) to synthetic polymers was often studied. Among the synthetic scaffolds, polyesters of D,L-lactide, glycolide (poly(D,L-lactide-co-glycolide) PLGA),  $\epsilon$ -caprolactone and trimethylene carbonate were intensely studied as they are approved by the Food and

Drug Administration. However, these scaffolds are often pre-casted and compression molded [1–7].

The increasing popularity of arthroscopic procedures and the requirement to bridge irregular bone defects resulted in great interest in fixation materials (both ceramic and polymeric-based) that are injectable, *in situ* forming and biodegradable [8]. An (injectable, *in situ* forming) 3-D scaffold should have the following characteristics: (1) biocompatible, (2) biodegradable to promote new tissue formation and vascularization, (3) suitable surface chemistry to allow cell attachment and differentiation, (4) mechanical properties that match those of the tissues at the site of implantation, (5) highly

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porous with an interconnected pore network and (6) contain osteoinductive factors [1, 8]. To achieve the above mentioned characteristics, moldable polymers can be mixed with calcium phosphate cements and pore forming particulates (e.g. gelatin), injected and subsequently crosslinked *in situ*.

To achieve *in situ* polymerisation of the polymers, photo-initiated crosslinking is interesting as it is usually rapid, effective and well-controlled, and can be carried out at low temperatures [9]. Depending on the monomer (s) used, the degradation time as well as the mechanical properties can be varied. The construct should provide structural stability during the healing process without absorbing all of the mechanical stresses, which would result in stress shielding of the natural tissue and its eventual resorption [10].

Although some of the mechanical properties (e.g. hardness of the construct) are correlated with the cements, mechanical properties as compression resistance and tensile strength are related to the polymers and may be important for example to prevent fractures in the time before the implants are replaced by host bone [8]

Some studies concerning injectable, photopolymerizable scaffolds are described yet. These materials are based on polyethylene glycol, lactic acid, poly(anhydrides), poly(propylene fumarate),  $\epsilon$ -caprolactone and trimethylene carbonate but are varying in photoinitiator system, catalysator, light inducing system, . . . [8, 9, 11–13].

In our group, a variety of *in situ*, photopolymerizable 3-D scaffolds were synthesized with variables of polymer type (polyethylene glycol, lactide, glycolide,  $\epsilon$ -caprolactone, trimethylene carbonate . . .), copolymer ratio and initiator systems [14–16].

In the present study, *in situ* photopolymerizable three-dimensional scaffolds were prepared from methacrylate-endcapped oligomers. The scaffolds varied in polyester composition (D, L-lactide,  $\epsilon$ -caprolactone and trimethylene carbonate) and crosslinking density (by replacing 1,6-hexanediol into dipentaerythritol): LA/CL-HXD (P1) respectively LA/CL-DPENT (P2) and LA/TMC-HXD (P3). The biocompatibility and the behaviour of bone marrow derived osteoblastic cells on the scaffolds were compared. These results were correlated with the mechanical properties and degradation time of the polymers.

## Materials and methods

### Materials

$\alpha$ -MEM supplemented with nucleotides ( $\alpha$ -MEM DNA/RNA) (Cat No. 22571-020), fetal bovine serum (FBS heat inactivated, E.C. approved), L-glutamine, penicillin-streptomycin (10000 U/ml-10000  $\mu$ g/ml) and Fungizone<sup>®</sup> were purchased from Gibco BRL (Life Technologies, Merelbeke, Belgium). L-ascorbic acid 2-phosphate,  $\beta$ -glycerophosphate, dexamethasone (Cat No. D-2915), 1,25 dihydroxyvitamin  $D_3$  (Cat No. D-1530-10UG), tetracycline (T-7660) and MTT (thiazolyl blue tetrazolium (M-5655) were supplemented from Sigma (Sigma-Aldrich NV/SA, Bornem, Belgium).

Tissue culture dishes were purchased from Greiner (Frickenhausen, Wommel, Belgium). Gelatin was from Gelatines Rousselot<sup>®</sup>.

P-nitrophenylphosphate, p-nitrophenol and glycine were purchased from ICN Biomedicals, Inc (Asse-Relegem, Belgium). UltraClear was obtained from J.T. Baker (Klinipath, Geel, Belgium). The rat osteocalcin EIA kit BT 490 was purchased from Biomedical Technologies, Inc. (Sanbio, Uden, The Netherlands).

## Scaffold preparation

### Synthesis of methacrylate-endcapped oligomers

Methacrylate-endcapped oligomers were prepared by esterification of the corresponding hydroxyl-terminated oligomers with methacryloyl chloride in the presence of triethylamine at room temperature.

Poly(D,L-lactide-co- $\epsilon$ -caprolactone)-HXD (LA/CL-HXD) (P1) respectively poly(D,L-lactide-co- $\epsilon$ -caprolactone)-DPENT (LA/CL-DPENT) (P2) and poly(D,L-lactide-co-trimethylencarbonate-HXD (LA/TMC-HXD) (P3) were synthesized as described below.

Linear and star-shaped, telechelic poly(D,L-lactide-co- $\epsilon$ -caprolactone) respectively poly(D,L-lactide-co-trimethylene carbonate) with hydroxyl groups at the termini was synthesized by the ring-opening polymerization of D,L-lactide (LA) and  $\epsilon$ -caprolactone (CL) respectively D,L-lactide and trimethylene carbonate (TMC) in the presence of 1,6-hexanediol (HXD) as an initiator and zinc acetate as a catalyst. A variable was synthesized by replacing HXD into dipentaerythritol (DPENT). Polymerization was performed in bulk at 140 °C for 48 h (on a 10–20 g scale). A glass tube was silanized with dichlorodimethylsilane, dried in an oven, and cooled in a dessicator. A typical experimental procedure was as follows: the glass tube was charged with predetermined amounts of comonomers (LA/CL(TMC) molar ratio 50/50), the initiator (monomer/initiator molar ratio 20/1), and the catalyst (1 mol% per total amount of hydroxyl groups derived from the alcohol initiator). The target number average molecular weight was 2700  $g\text{mol}^{-1}$ . The tube was degassed three times, sealed, and placed in a constant-temperature oven. Polymerization was terminated by cooling the tube in a refrigerator.

In the second step, the corresponding oligomers with hydroxyl groups at the ends of the polymer chains were functionalised using excess of methacryloyl chloride (2 folds excess to the total number of hydroxyl groups derived from the oligomer) in the presence of triethylamine (1.5 molar excess to the amount of methacryloyl chloride) in methylene chloride (2:1 volume ratio) to give the corresponding methacrylate-endcapped oligomer. The esterification proceeded at room temperature for 24 h. The triethylamine hydrochloride produced from the reaction was removed by filtration and the remaining solution was concentrated and stored in a freezer for one hour, and again a white solid was filtrated. Finally, the remaining solution was dialysed (Spectra/Por<sup>®</sup> Membrane, MWCO: 1000) in

dry acetone for 2 days. The yield was in the range 50–80%.

Oligomers based on TMC were dissolved in dichloromethane (10 wt%) and mixed with 15% of triacetin (plasticizer). The solvent was evaporated and the polymer was vacuum-dried at room temperature for 24 h.

### Scaffold preparation

Scaffolds based on D, L-lactide,  $\epsilon$ -caprolactone and trimethylene carbonate with apparent porosity of 70 were prepared with gelatin as porosigen (particle size 250–355  $\mu\text{m}$ ), respectively LA/CL-HXD (P1), LA/CL-DPENT (P2) and LA/TMC-HXD (P3).

The methacrylate-encapped copolymers can be converted into a solid, 3-D polymer network by visible-light irradiation in the presence of D,L-camphorquinone (CQ)/ethyl 4-dimethylaminobenzoate (EDMAB) initiator system. By adding leachable particulates (gelatin), a porous 3-D network can be obtained [11, 14, 15, 17, 18]. The porosigens (gelatin) was sieved with ASTM E11-70 standard testing sieves with openings of 250 and 355  $\mu\text{m}$ , placed on the Retsch AS 200 sieved shaker until particulates with a defined particle size (250–355  $\mu\text{m}$ ) were obtained.

Hydroxy ethyl methacrylate (HEMA) (15 wt%) was added to the katalysator (0,6 wt% CQ and 0,7 wt% EDMAB) until dissolved. The HEMA/katalysator solution followed by the porosigen is added to the viscous prepolymer and mixed thoroughly. The viscous paste is then brought into cylindrical Teflon molds (diameter 5 mm, height 1 mm) and the prepolymer is photopolymerized for 80 s at each side with the dental lamp curing unit (500 mW/cm<sup>2</sup>) (3M Unitek™, Ortholux™ XT) (3M Dental Products). After polymerization, the scaffolds are removed from the mold and immersed in distilled water (37 °C, bench shaker, 55 rpm) for a period of 3 days (to leach out the porosigen). The scaffolds were dried under reduced pressure in a vacuum oven (3 days, room temperature) and sterilized by ethylene oxide (12 h, 60 °C, 48 h aerated) [14, 15].

### Scaffold characterization

#### Porosity

The density of the solid polymeric material (measured by pycnometry in triplicate) and gelatin is 1.13 g/cm<sup>3</sup> respectively 0.97 g/cm<sup>3</sup> [18]. The porosities of the porous scaffolds were calculated from the theoretical apparent densities of the scaffolds using the following equation described by Mikos *et al.*  $\varepsilon = [w_s/d_s]/[(1 - w_s)/d_p + w_s/d_s]$  where  $\varepsilon$  is the theoretical prediction of an apparent porosity,  $w_s$  is the porogen weight fraction,  $d_s$  and  $d_p$  is the density of respectively the porogen and the polymer [19].

#### Degradation

The degradation tests of crosslinked polymer networks (without HEMA) (diameter 5 mm, height 1 mm) were

performed in 20 ml of phosphate buffer at 37 °C in a Julabo SW 21 incubator. The buffer was exchanged after one week for the first time and later every three weeks. At one week and later approximately every three weeks the samples (three for each time point) were removed to undergo gravimetric analysis [16].

### Mechanical testing

Mechanical properties of the porous scaffolds were evaluated in the compression test using the Hounsfield machine with a cell load of 1 kN operating at a crosshead speed of 1 mm/min. For the compression test cylindrical samples were prepared according to the standard specification ISO 5833 keeping the aspect ratio height/diameter of 2/1. The compressive modulus was defined by the slope of the initial linear section of the stress-strain curve. The samples were stored and tested in air at room temperature and three specimens were measured for each batch [16].

### Biocompatibility

Three scaffolds (diameter 5 mm, height 1 mm) of each type (P1, P2 and P3) were extracted at 37 °C (bench shaker) in 1 ml culture medium according to 1,65 cm<sup>2</sup>/ml (contact surface/extraction medium) for respectively 24 h and 8 days according to the ISO 10993-1 guidelines. Primary embryonic chicken fibroblasts were seeded in 96-well tissue culture plate at a density of 25000 cells/well in 200  $\mu\text{l}$  of Hanks' medium. After 24 h the medium was withdrawn and 200  $\mu\text{l}$  extraction medium (corresponding to 1,65 cm<sup>2</sup>/ml, 0,82 and 0,41 cm<sup>2</sup>/ml) was then added and incubated for 2 days. The number of viable cells was determined by MTT-assay. 0,5 mg/ml MTT containing medium was added to the cells and incubated. After 4 h, the MTT containing solution was withdrawn and 200  $\mu\text{l}$  of 1% Triton X-100 in isopropanol/0,4 N HCl was added. The formazan was dissolved by shaking at 37 °C for 30 min. The absorbance was measured at 580 nm (Universal Microplate Reader EL 800, BIO-TEK instruments Inc.) and the viability was calculated as percentage of the control.

### Bone formation

#### Isolation and culture of bone marrow derived cells

Bone marrow cells were obtained from the tibiae and femora of young (6 weeks old) adult male Wistar rats. Skin, soft connective tissue and periosteum were removed. Tibiae and femora were washed and the diaphyses were cut free of epiphyseal cartilage. The bone marrow was flushed out repeatedly with MEM Alpha medium (containing 10 vol% fetal bovine serum, 0,5 vol% penicillin-streptomycin, 1 vol% Fungizone®), supplemented with 100  $\mu\text{M}$  L-ascorbic acid 2-phosphate and 10 nM dexamethasone (complete MEM Alpha) and centrifuged (10 min, 1000 rpm) [20]. The cell pellet was resuspended and seeded in T75 tissue culture dishes. After 24 h, the medium was changed to remove nonadherent cells. The cells were

cultured until confluence (5% CO<sub>2</sub>/95% air, 37 °C). After 7 days of primary culture, the cells were detached using trypsin/EDTA, concentrated by centrifugation at 1000 rpm for 10 min and resuspended in complete MEM Alpha medium.

### *Cell seeding and growth on porous scaffolds*

Before cell seeding, the scaffolds were immersed in serum-free MEM Alpha medium in Eppendorf tubes. Air was removed from their pores by generating vacuum with a 20 ml syringe equipped with an 18-gauge needle. The scaffolds were left in medium on a bench shaker (37 °C, 55 rpm). After 24 h, the scaffolds were placed into 96-well tissue culture dishes (for suspension culture).

The scaffolds were seeded with 435000 rat bone marrow cells/90  $\mu$ l/scaffold (2.2  $\cdot$  10<sup>6</sup> cells/cm<sup>2</sup>) and incubated for 4 h. 160  $\mu$ l medium was added to each well and the seeded scaffolds were further incubated overnight to allow for cell attachment.

After 24 h, scaffolds were evaluated for their seeding efficiency by replacing the medium by MTT containing medium (0,5 mg/ml). After 4 h incubation, the MTT containing solution was withdrawn and 0,5 ml of 1% Triton X-100 in isopropanol/0,4 N HCl was added. The formazan was dissolved by shaking at 37 °C for 30 min. The absorbance of 300  $\mu$ l solution was measured at 580 nm (Universal Microplate Reader EL 800, BIO-TEK instruments Inc.) and the viability was calculated as percentage of the control (tissue culture dish). Each scaffold was tested in triplicate.

Scaffolds for continuous growth were placed on a needle (3 scaffolds/needle) in a 5 ml spinner flask. 3 ml complete MEM Alpha medium supplemented with 10 mM  $\beta$ -glycerophosphate was added and the cell/scaffold constructs were cultured for 21 days on a bench shaker at a constant rate of 55 rpm (5% CO<sub>2</sub>/95% air, 37 °C). Each scaffold was tested in 3 independent experiments in triplicate.

### *Alkaline phosphatase activity*

Cell/scaffold constructs were lysed into 0,5 ml of a 0,1% Triton X-100 containing Tris HCl buffer, homogenized by two freeze-and-thaw cycles and sonicated on ice for 3  $\times$  10 s (40 mA) (Vibra Cell™ SONICS (ANALIS)). 50  $\mu$ l samples were added to 50  $\mu$ l p-nitrophenylphosphate (4,34 mM) in glycine buffer (pH 10,3) and incubated (37 °C, 30 min) on a bench shaker. 50  $\mu$ l NaOH (1 M) was added, the absorbance was measured at 405 nm and the enzyme activity was calculated according to p-nitrophenol standards. Alkaline phosphatase activity was expressed as mM p-nitrophenol/scaffold.

Scaffolds of 3 independent experiments in duplicate were analyzed.

### *Osteocalcin*

48 h prior to assaying (21 days), each scaffold was placed into 3 ml serum-free osteogenic medium con-

taining 10<sup>-9</sup> M 1,25-dihydroxyvitamin D<sub>3</sub> in 12 well tissue culture dishes. After 48 h, the medium was harvested and frozen (-20 °C). At the time of analysis, the medium was thawed and osteocalcin was measured at 450 nm by an enzyme immuno assay according to the company's instructions.

### *Histology*

4 h prior to fixation, 10  $\mu$ g tetracycline/ml was added to the osteogenic medium. Scaffolds were rinsed with Ringer solution, fixed with 4% phosphate (10 mM) buffered formaldehyde (pH 6,9) (4 °C, 24 h), dehydrated in a graded alcohol series (UltraClear is used instead of toluene because toluene dissolves the polymer) and embedded in paraffin. 5–7  $\mu$ m sections were made and stained with hematoxylin & eosin, Masson's Trichrome, von Kossa and mounted with Canada balsam. Tetracycline incorporation, indicative for calcium depositions, was evaluated in unstained sections by fluorescence microscopy.

### *Transmission electron microscopy*

Scaffolds were fixed (4 °C, 1 h) in 1% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2), washed in the same buffer, postfixed with osmium tetroxide, dehydrated in graded concentration of alcohol and embedded in epoxy-resin. Thin sections (60 nm) were cut with a diamond knife, mounted on copper grids, stained with uranyl acetate and lead citrate and examined using a JEOL 1200 EX II transmission electron microscope operating at 80 KeV.

## **Results and discussion**

### *Biocompatibility*

Cytotoxicity assays are particularly aimed at establishing the possible toxic effects of leachables released from medical polymers during extraction. For our crosslinked scaffolds, the cytotoxicity could be the result from leachables deriving from the initiator system, non-crosslinked polymer, plasticizer triacetin, . . .

The viability of primary fibroblasts in the presence of extraction media of the scaffolds was determined by MTT-assay (Fig. 1). Scaffolds extracted for 24 h showed a good biocompatibility. At the highest surface ratio (1,65 cm<sup>2</sup>/ml), the viability of the cells was minimal 80% for all the three types of scaffolds. Scaffolds extracted for 8 days also had a minor cytotoxic response, with no specific differences between the scaffold composition. At a surface ratio of 1,65 cm<sup>2</sup>/ml, viability reaches 56 to 63%. The viability of cells in contact with diluted extraction media (0,82 cm<sup>2</sup>/ml) reaches 75 to 95%.

Under the applied photopolymerization conditions (with HEMA as a co-crosslinker), the polymer networks have sol contents of 13%, 3% and 25% for P1, P2 and P3 respectively (for detailed description see reference [16]). The high sol fraction of 25%, as observed for P3 (LA/TMC-HXD) is mostly due to the presence of triacetin (glycerol triacetate), approved by the FDA

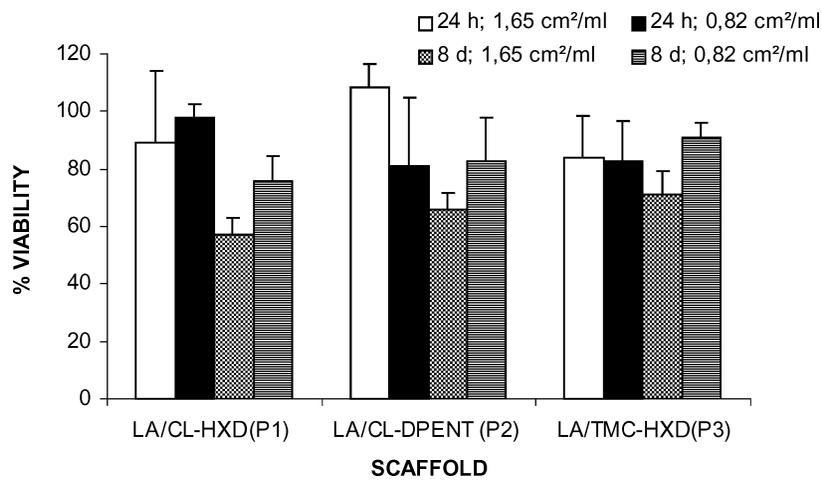


Figure 1 MTT assay. LA/CL-HXD (P1), LA/CL-DPENT (P2) and LA/TMC-HXD (P3) scaffolds were extracted for respectively 24 h and 8 days. The toxicity of the extraction medium was tested on primary fibroblasts. Results are expressed as the percentage of viable cells calculated according to the control. Mean and SD,  $n = 3$ .

and known to have a low toxicity. The very low sol content of P2 is the result of the use of DPENT, a more efficient initiator compared to HXD [16].

Rat bone marrow cells cultured on the scaffolds for up to 21 days remained viable. Analysis by TEM showed cells with euchromatic nuclei and the presence of a high number of mitochondria with intact, undisrupted cristae (Fig. 4(b')). This is in accordance with data described by Burdick *et al.* showing that the degradation products of *in situ* forming lactic acid materials had no detrimental effect on the function of the cells [12, 13, 21].

## Bone formation

### Cell adhesion

The rat bone marrow cells were able to attach to the polymer scaffolds. Seeding efficiencies for the three scaffolds were comparable. After 24 h of incubation,  $40,46 \pm 13,85\%$  of the total number of cells adhered to the scaffolds.

Comparable seeding efficiencies were described by others on PLGA scaffolds [22].

Although cell adhesion on scaffolds based on CL, LA, ... is described, the surface chemistry is rather hydrophobic [3]. The hydrophobicity of the different poly-

mers was characterized by water contact angle measurements. By replacing CL by TMC, a decrease in the contact angle was achieved, which leads to the conclusion that copolymers based on TMC are less hydrophobic than the CL copolymers with the same chemical composition. Furthermore, the addition of the hydrophilic monomer HEMA significantly decreases the hydrophobic properties of the polymer networks resulting in comparable seeding efficiencies for the three types of scaffolds [16].

### Bone formation

All scaffolds seeded with rat bone marrow cells were cultured dynamically for 21 days. The alkaline phosphatase activity and osteocalcin secretion were evaluated as markers representative for differentiated osteoblast-like cells present on the scaffold. Cellular infiltration was studied by histological analysis. The ultrastructure at the polymer/bone interface was examined by TEM.

Rat bone marrow derived cells were able to attach and differentiate on these 3-D scaffolds. After three weeks, the cells present on the scaffolds expressed high ALP activity and had high osteocalcin secretion (Fig. 2), independent of the scaffold type.

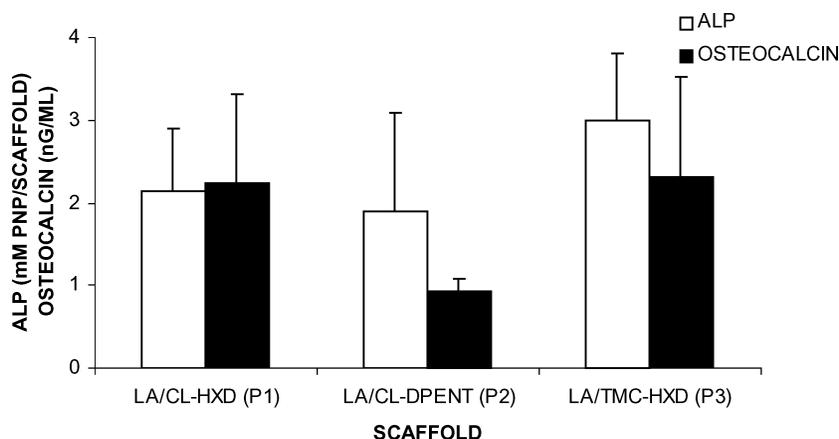


Figure 2 Differentiation of rat bone marrow cells cultured for 21 days on scaffolds prepared from respectively LA/CL-HXD (P1), LA/CL-DPENT (P2) and LA/TMC-HXD (P3). ALP activity and osteocalcin was expressed as respectively mM p-nitrofenol/scaffold and ng/ml. Mean and SD,  $n = 3$ .

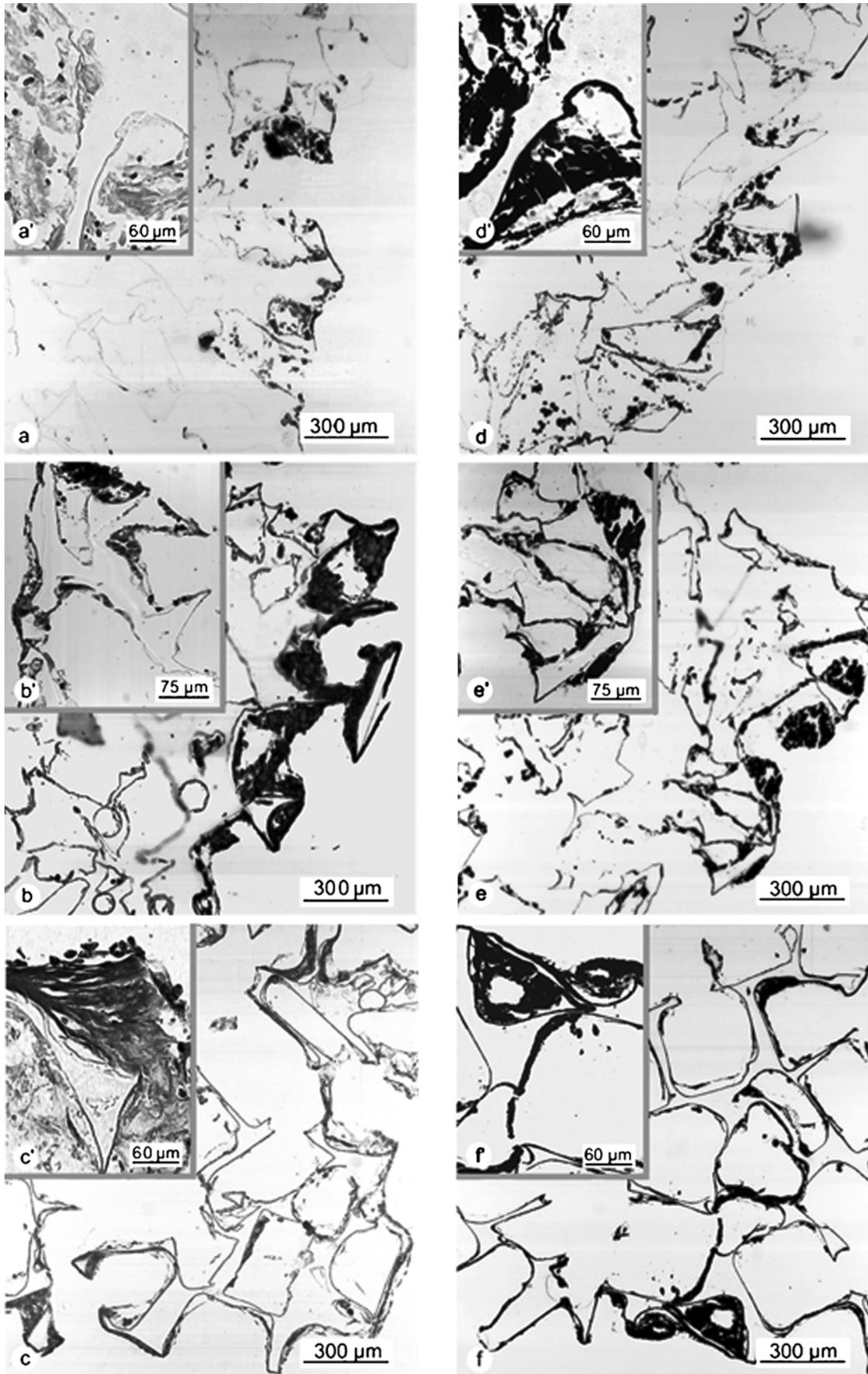


Figure 3 Cross-sections of scaffolds prepared from respectively LA/CL-HXD (P1) (a, a', d, d'), LA/CL-DPENT (P2) (b, b', e, e') and LA/TMC-HXD (P3) (c, c', f, f'). TM stain (a, a', b', c'). Von Kossa stain (d, e, f, d', e', f'). H&E stain (b, c).

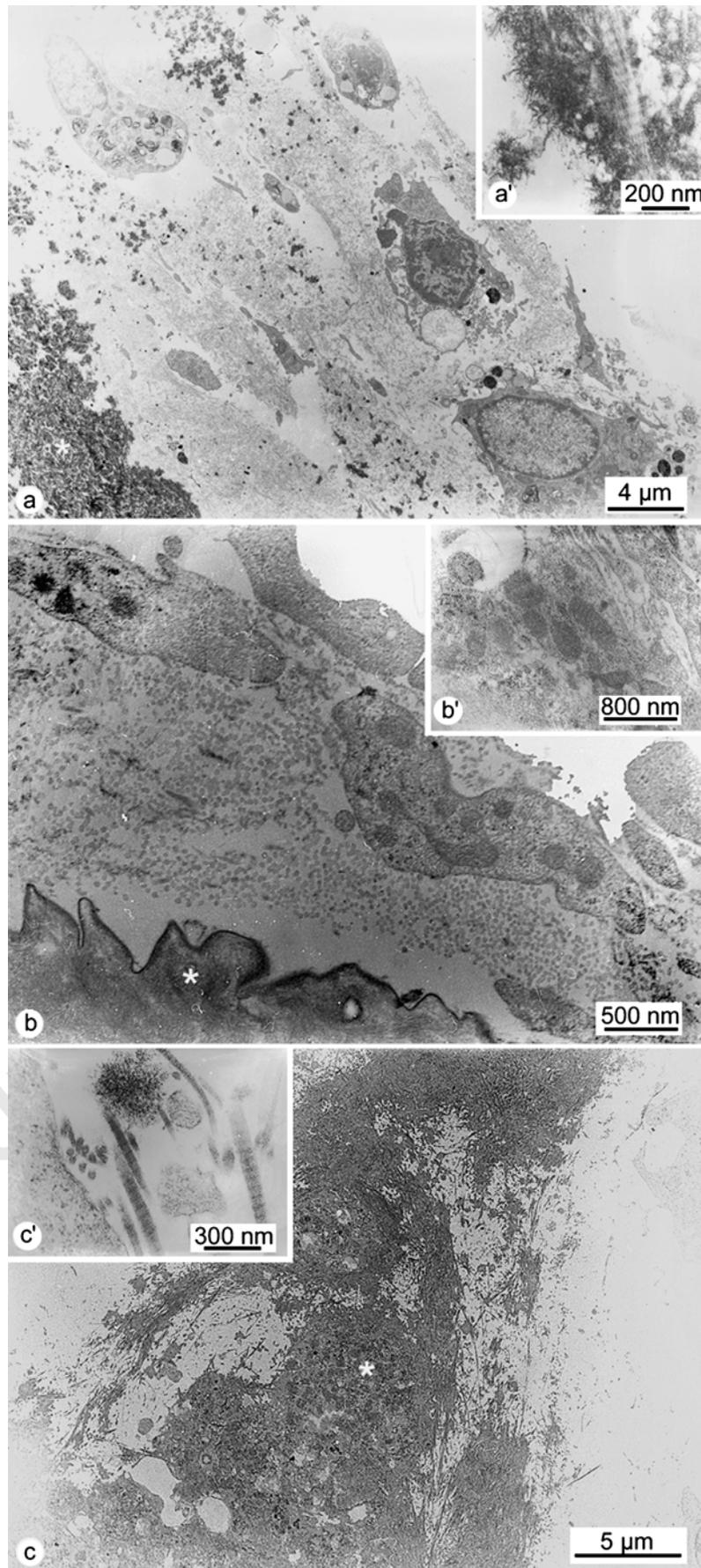


Figure 4 TEM of scaffold prepared from respectively LA/CL-HXD (a, a'), LA/CL-DPENT (b, b') and LA/TMC-HXD (c, c'). a, b, c: Bone/polymer interface. a', c': Extracellular matrix formation (collagen I fibers) and apatite crystals. b' : mitochondria (crystae). Asterisk: polymer scaffold.

Cross-sections showed bone formation following the contours of the polymer walls for all the three scaffolds as is shown in Fig. 3. Masson's Trichrome (Fig. 3 (a), (a'), (b'), (c')) and H&E (Fig. 3 (b), (c)) staining showed a typical morphology of cuboidal osteoblasts which secreted an extracellular matrix. Calcium phosphate deposits are present in the ECM as is demonstrated by von Kossa staining (Fig. 3 (d), (d'), (e), (e'), (f), (f')). Hence, the cells were able to mineralize the extracellular matrix.

Transmission electron micrographs at the polymer/bone interface for the three types of scaffolds showed osteoblast-like cells which secreted an abundant collagen I containing extracellular matrix (P1, P2 and P3 is shown in respectively Fig. 4a, b and c). Among the collagen I fibers, matrix vesicles loaded with bone apatite crystals can be observed (Fig. 4 (a'), (c')). The cells, cultured for 21 days on the three different scaffolds had a good viability, as seen by the mitochondrial structure (crystae) (Fig. 4 (b')). Dying cells (seen as cells beginning to lose their shape and break down) were rarely observed.

Bone formation as demonstrated by ALP activity, osteocalcin secretion and histological analysis was also described by others in scaffolds based on pure caprolactone, PLGA, . . . [3, 22], but was not yet described for *in situ* forming scaffolds based on LA, CL and TMC.

### Degradation and mechanical properties

Ideally, the polymer scaffold should degrade at a rate equal to the rate of tissue ingrowth, allowing for maintenance of the scaffold structure and mechanical support during the early stages of tissue formation.

The degradation behaviour for the three types of polymers is shown in Fig. 5. In general, there was little mass loss at early degradation times. Degradation for all the polymers starts after about 2–3 months. Polymers based on respectively LA/CL-DPENT (P2), LA/CL-HXD (P1) and LA/TMC-HXD (P3) had a 50% mass loss ( $t_{1/2}$ ) of 7,5 months respectively 6 months and 3,5 months. As expected, the more hydrophilic networks (LA/TMC-HXD) degraded faster [16].

To allow cell migration and infiltration as well as the transport of nutrients and cellular waste products, it is important to create scaffolds showing initially a porous

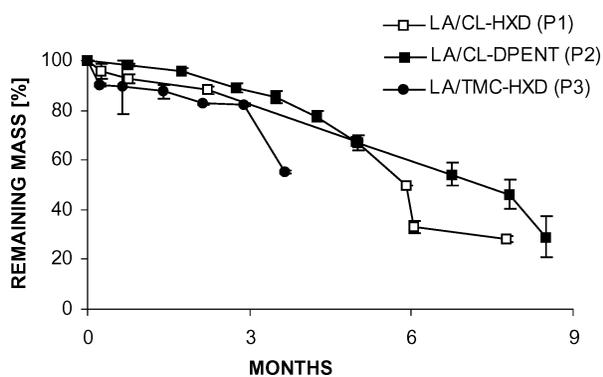


Figure 5 Degradation. Remaining mass (%) versus degradation time for polymer discs based on LA/CL-HXD (P1), LA/CL-DPENT (P2) and LA/TMC-HXD (P3). Mean and SD,  $n = 3$ .

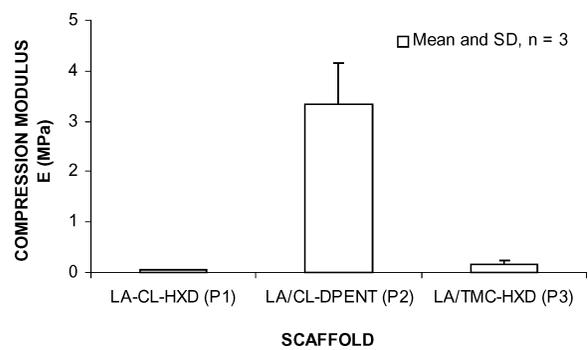


Figure 6 Compression modulus, E (Mpa). The influence of the crosslinking density (LA/CL-HXD (P1) versus LA/CL-DPENT (P2)) and polyester type (LA/CL-HXD (P1) versus LA/TMC-HXD (P3)) on the mechanical properties. Mean and SD,  $n = 3$ .

structure. To obtain porous scaffolds with interconnecting pores, gelatin with particle size of 250–355  $\mu\text{m}$  as a porosigen was used. An initial high porosity (and interconnectivity) however leads to a decrease of the mechanical properties of the implant material.

Fig. 6 shows the influence of crosslinking density and composition (polyester type) on the compressive modulus of the scaffolds obtained after photocrosslinking.

An increased crosslinking density obtained by replacing HXD by DPENT as initiator system resulted in scaffolds with compression moduli in the range of 3 MPa. In general, a lower compressive modulus indicates that the network-crosslinking density is lower.

The chemical composition (by replacing CL by TMC) does not have a significant influence on the compression modulus. Storey *et al.* and Pego *et al.* described that the toughness of a scaffold is imparted by the presence of TMC units [23, 24]. For LA/TMC networks, the tensile modulus decreases with increasing TMC content [7]. For our scaffolds, the addition of HEMA significantly improves the tensile strength and the strain at break yielding in networks with good elastic properties [16].

Our compression moduli (in the range of 3 MPa) of the porous scaffolds are similar to those found for other types of photocurable poly (propylene fumarate), LA and PLGA based scaffolds [12]. In contrary, the compressive modulus of the polymer network based on LA/CL-DPENT is in the range of 92 MPa [16]. This value is also found for other polymer networks [25] and can be compared with the compression modulus of trabecular bone. This discrepancy with our low compressive modulus is due to the high porosity (porosity of 70). Hence, for *in vivo* applications, the ratio between the initial porosity and the mechanical properties should be considered.

For injectable composite materials, the polymer and the calcium phosphate cements should have high tensile properties respectively compression properties as the polymer and the calcium phosphate cement reflect respectively the collagen and HAp fraction in bone.

### Conclusions

This study demonstrated that rat bone marrow cells can attach and differentiate when cultured on 3-D porous

*in situ* photopolymerizable scaffolds based on D, L-lactide,  $\epsilon$ -caprolactone and trimethylene carbonate. In vitro cytotoxicity experiments showed no significant difference in cell viability when cultured in the presence of extraction medium of the scaffolds.

The polymer networks' mechanical properties tend to improve with increasing the branching of the precursor methacrylates but do not depend on their chemical composition.

For *in vivo* applications, the scaffolds obtained with DPENT as an initiator are promising because of their improved elastic properties. Scaffolds based on TMC are interesting as their degradation is faster. Above all, by varying the copolymers and the initiator system, scaffolds can be synthesized combining the hydrophilic properties of TMC with DPENT as an initiator and are promising for bone tissue engineering.

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