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Degradation of oligo(lactone) branches linked to poly(methacrylate) networks

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SUMMARY: Partially resorbable composite materials were developed for bone applications and were prepared by crosslinking copolymerization of oligo(lactone) macromonomers with selected comonomers in the presence of hydroxy apatite. The composites were incubated in aqueous solution of different pH and electrolyte content over a period up to 300 days. The percentage of the released hydroxycarboxylic acid from the oligo(lactone) branches was determined by the titration or by high performance liquid chromatography (HPLC). A catalytic influence of *cholesterol esterase* (CE) on the hydrolysis of the materials was observed. Determination of CE activity in the presence of the different composite materials showed that the half-life time of CE was the lowest in the presence of the material undergoing the fastest degradation. After degradation the composite materials have preserved their coherence and have shown higher glass transition temperatures in the dried state than the materials before degradation.

Introduction

Poly(L-lactide) has received much attention as a biodegradable and bioresorbable implant material for application in bone surgery. However, in clinical studies of the implant the degradation of highly crystalline poly(L-lactide) was observed to be incomplete and resulted in the generation of hydrolytically stable microcrystals which were associated with foreign body reactions1). In other work the hydrolytic degradation of crosslinked polymer systems the network chains of which are composed of oligo(lactide), were described as yielding a complete disintegration for the materials2,3).

More recent work has reported on the development of partially degradable, crosslinked polymers which contained oligo(lactone) segments and preserved their coherence even after long degradation times up to 300 days4). The network chains of these latter materials were

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formed from hydrolytically stable methacrylate units and the degradable oligo(lactone) branches were covalently linked to the network chains.

Bisphenol-A(bis(2-hydroxypropyl)methacrylate) (BisGMA) has been proven to be a suitable initiator for the oligomerization of lactide, glycolide, δ-valerolactone and ε-caprolactone[4,5,6)] (Fig. 1).

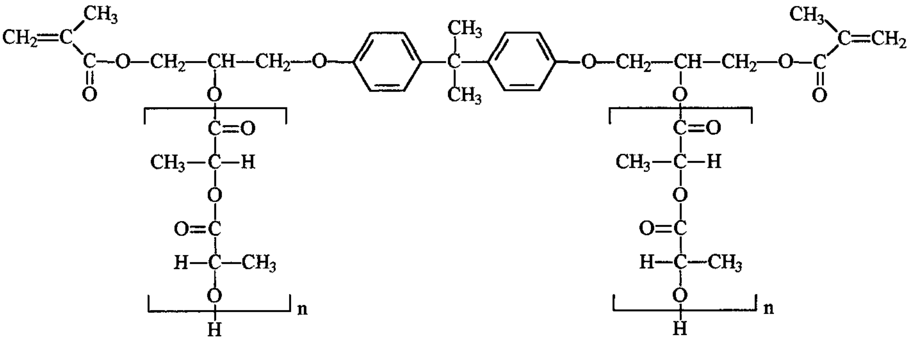

Fig. 1: Macromonomer obtained by ring opening oligomerization of lactide, initiated by BisGMA

BisGMA derived macromonomers were copolymerized with 2-hydroxyethyl methacrylate (HEMA), tri(ethylene glycol) dimethacrylate (TEGDMA) and tetrahydrofurfuryl methacrylate (THFM), respectively, in the presence of hydroxy apatite. The rate of the release of lactic acid from the composites during their incubation in buffered solution (pH 7.4) was found to decrease in the series of composites in accordance with the following order: HEMA > THFM > TEGDMA [4,7)].

The degradation rate was also dependent on the type of the oligo(lactone) branches and decreased in the following order: oligo(L-lactide-co-glycolide) > oligo(D,L-lactide) > oligo(L-lactide).

The current study will report on the influence of different incubation media including the presence of the enzyme *cholesterol esterase* (CE) and the presence of δ-valerolactone units in the oligo(lactone) branches with respect to the degradation behaviour of the composites. In addition, the effect of the composites and their related degradation products on the enzyme activity was studied using methods described elsewhere [8,9)].

Preparation of composites

Macromonomers were synthesized as previously described [5,6)] by reacting BisGMA with L-lactide (1:10 mol/mol), BisGMA with a mixture of L-lactide and glycolide (1:7:3 mol/mol) as well as from BisGMA, δ-valerolactone and L-lactide by a step reaction of the lactone and lactide (1:2:10 mol/mol and 1:4:10 mol/mol). These macromonomers were copolymerized with either TEGDMA or HEMA (7:3 g/g) as diluent monomer in the presence of 45 wt.% hydroxy apatite (HAp) (Cerasiv ®, average particle diameter 120 µm). The redox system dibenzoylperoxide (DBPO) (0.3 - 0.4 wt% with respect to the whole monomer mixture) / N,N-dimethyl-p-toluidine (equimolar quantity with respect to DBPO) was used as the initiator, and the reaction was carried out at room temperature. After a 10 to 15 min setting time the samples were stored at 37 °C for 24 h. Following this, the specimens were immediately used for degradation studies.

Monitoring of degradation

Sample specimens were prepared in moulds of 2 x 10 x 15 mm (6 mm diameter and 12 mm height for enzyme experiments) and incubated in one of three types of buffer solution (pH 7.4: $Na_2HPO_4 \cdot 2\,H_2O$ / citric acid, $NaHCO_3$ / Na_2CO_3; pH 7.0: $Na_2HPO_4 \cdot 2\,H_2O$ / $NaCl$/ KH_2PO_4 / KCl (PBS)) and in Ringer solution, respectively, at 37 °C. During the incubation period the hydroxyacids released from the samples were potentiometrically titrated with 0,05 N KOH and the solutions of the incubation vials were replenished. The HPLC identification of degradation products was carried out for specimens retrieved at different intervals over 300 days of incubation. 20 µl aliquots of the retrieved incubation solution was filtered through a PTFE filter (20 µm pore size) and injected into the HPLC (Kontron, Germany, and Waters Milford, USA, respectively) units. The columns consisted of Ultra Sep ESFS and 8 MBC 1810 units from Waters.

Eluent solutions of H_3PO_4 (20 mM/L) and CH_3COONH_4/CH_3OH were used for isolation of products in the enzyme studies. The flow rate was act at 5 mL/min for all studies.

CE (no. C 9750, ex beef pancreas, Genzyme Ltd.) solution was prepared by dissolving the CE powder in 0.05 M phosphate buffer (PBS), pH 7.0 (1 unit/ml) which was then stored frozen at -40 °C until required [9)]. The required CE per mL was calculated based on the specifications of the esterase as they were obtained from the supplier (800 - 1600 units/g protein) and the actual activity was determined as described in [8)].

Determination of thermal and mechanical properties

The thermal properties of the composites and copolymers were determined by DSC using a Perkin Elmer DSC 7 device. The heating rate was 10 K/min. The mechanical properties of the composites were studied by dynamic mechanical analysis using a Perkin Elmer DMA-7 thermal analysis system. The system was operated in the dual cantilever mode (22 x 12 x 2 mm samples) at a frequency of 1 Hz and at a heating rate of 2 K/min over the temperature range of -20 °C to 120 °C.

To determine Vickers hardness, 12 samples were prepared and divided into two groups. The control group was incubated in phosphate buffer solution (PBS) and the test group was incubated in PBS with CE solution. The solutions were replenished each day with 100 μl PBS (control group) and 100 μl CE solution (test group).

At each time point (0, 15 and 30 days), samples were removed and surface micro-hardness (Vickers hardness) was measured using the miniload micro-hardness-tester "Leitz-Wetzlar". The samples were subjected to a mechanical load of 300 pounds for a period of 30 seconds. Two diagonal measurements were taken to the nearest 0.7 μm for each indentation. 30 indentations were made on each sample, 6 samples were analyzed per group and 180 measurements were taken for each group. Statistical significance was assessed through analysis of variance.

Results and discussion

The degradation course in buffer and in Ringer solution

The dependence of the degradation behaviour of the composites on the composition of the oligo(lactone) branches linked to the poly(methacrylate) network is illustrated in Fig. 2 where the released acid is given as the percentage of degradable monomer units of the oligo(lactone).

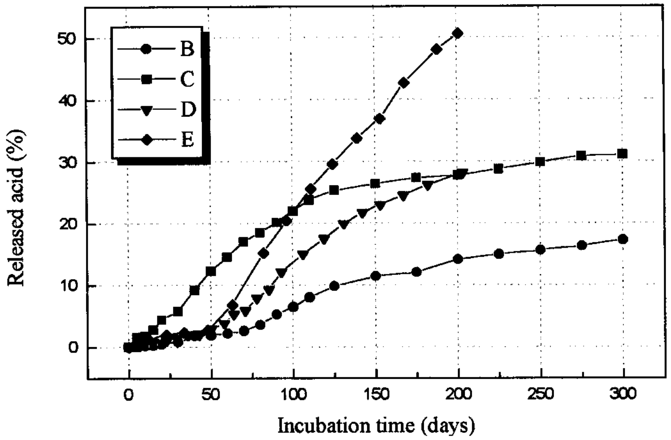

Fig. 2: *In vitro* degradation of copolymer composites from BisGMA endcapped macromonomers -●- BisGMA:L-lactide (1:10 mol/mol) (B), -■- BisGMA:L-lactide: glycolide (1:7:3 mol/mol) (C), -▼- BisGMA:L-lactide:δ-valerolactone (1:10:2 mol/mol) (D), -◆- BisGMA:L-lactide:δ-valerolactone (1:10:4 mol/mol) (E), all copolymerized with TEGDMA (7:3 wt/wt) in the presence of 45 wt% HAp, stored in $Na_2HPO_4 \cdot 2H_2O$ / citric acid buffer solution, at 37°C

The initial degradation rate of the composite C containing glycolide is higher than that of the other composites as was be expected [4,7].

The composites D and E, containing the cooligo(lactone) branches with L-lactide and δ-valerolactone degrade more rapidly and in a higher degree than the composite B containing branches only from L-lactide (Fig. 2) in spite of the hydrophobic character of the δ-valerolactone units. The formation of crystalline domains of the oligo(L-lactide) branches at the crosslinked polymer matrix of the composites may be a factor of influence on the degradation behaviour found. Melting peaks were observed in the DSC thermograms of the composites B (ΔH_m=2,5 J/g) and D (ΔH_m=2,1 J/g) as well as their crosslinked polymer matrices B' (ΔH_m=6,5 J/g) and D' (ΔH_m4,2 J/g) with a maximum of the melting peak at around 90°C. The intensity of these peaks in the thermograms of composite E and its polymer matrix was too small for a quantitative analysis. A forthcoming paper dealing with the dependence of the crystallization behaviour of the macromonomers on the sequence length of their lactone units as well as the crystallization behaviour of copolymer matrix and composites from these macromonomers is in preparation.

This series of increasing crystallinity of the composites E<D<B corresponds to their decreasing degradation rate. Therefore, the uptake of buffer solution by the composites D and E attains faster its maximum during their incubation (Fig. 3). These effects are assumed to enable the faster release of degradation products.

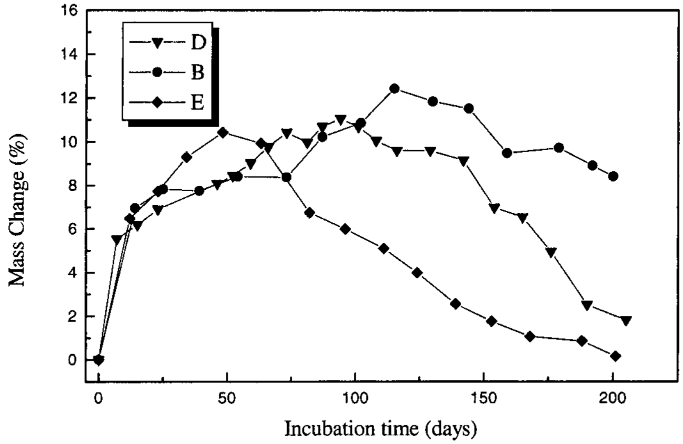

Fig. 3: *In vitro* degradation of copolymer composites from BisGMA endcapped macromonomers -●- BisGMA:L-lactide (1:10 mol/mol) (B) -▼- BisGMA:L-lactide:δ-valerolactone (1:10:2 mol/mol) (D), -◆- BisGMA:L-lactide:δ-valerolactone (1:10:4 mol/mol) (E), all copolymerized with TEGDMA (7:3 wt/wt) in the presence of 45 wt% HAp, stored in $Na_2HPO_4 \cdot 2H_2O$ / citric acid buffer solution, at 37 °C

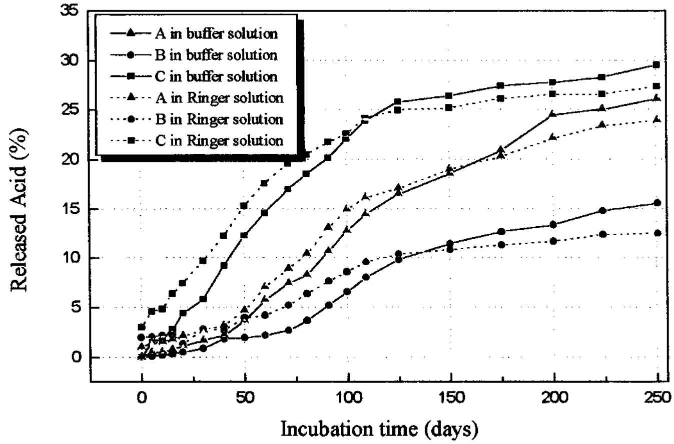

Fig. 4: *In vitro* degradation of copolymer composites from BisGMA endcapped macromonomers -▲- BisGMA:L-lactide (1:10 mol/mol) (A) with HEMA (7:3 wt/wt), -■- BisGMA:L-lactide: glycolide (1:7:3 mol/mol) (C) and -●- BisGMA:L-lactide (1:10 mol/mol) (B) with TEGDMA (7:3 wt/wt) in the presence of 45 wt% HAp, stored in $Na_2HPO_4 \cdot 2H_2O$ / citric acid buffer solution

Data in Fig. 4 show that the rate of hydrolytic degradation of the oligo(lactide) branches as measured by acid release is dependent on the buffering of the incubation solution. The initial degradation rate of the three composites A, B and C is higher in Ringer solution than in buffer solution. This observation can be explained by the fact that acidic conditions generate a catalytic effect. However, near 100 to 150 days incubation the rate of degradation slows down and the samples incubated in the buffered system start to show a higher release of acid than those in the Ringer solution. This result can be explained on the basis of the swelling behaviour for the samples (Fig. 5).

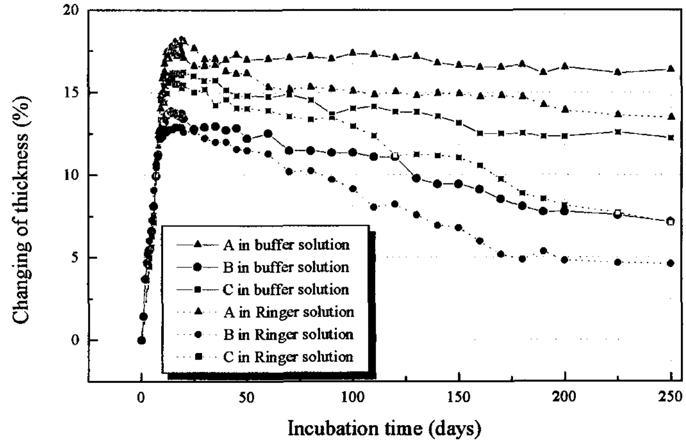

Fig. 5: *In vitro* degradation of copolymer composites from BisGMA endcapped macromonomers -▲- BisGMA:L-lactide (1:10 mol/mol) (A) with HEMA (7:3 wt/wt), -■- BisGMA:L-lactide:glycolide (1:7:3 mol/mol) (C) and -●- BisGMA:L-lactide (1:10 mol/mol) (B) with TEGDMA (7:3 wt/wt) in the presence of 45 wt% HAp, stored in $Na_2HPO_4 \cdot 2H_2O$ / citric acid buffer solution and Ringer solution

After fast swelling of the samples up to a maximum degree, it follows a period where the thickness of the samples decreases faster in Ringer solution than in the buffer solution. The faster release of acid from the samples in Ringer solution during this period is obviously connected with a stronger deswelling of the samples for osmotic reasons. This effect is assumed to cause the retarded release of the degradation products in Ringer solution in the later stage of degradation (> 100 days incubation time) (Fig.4).

The degradation behaviour of the composites did not exhibit any differences in the two types of buffer solution each with pH 7.4: $Na_2HPO_4 \cdot 2H_2O$/citric acid and $NaHCO_3/Na_2CO_3$, respectively.

After degradation of various composites in phosphate buffer solution for different times, the samples which retained in all cases their coherence, were dried in vacuum over P_2O_5 and then analyzed by DMA.

Tab. 1 T_g and storage modulus E′ at 25°C of macromonomer/TEGDMA/HAp composites.

Macromonomer composition	Incubation time [d]	T_g [°C]	E′ (25°C) [MPa]
BisGMA:VL:LLA= 1:2:6 mol/mol	0	61	1360
	60	70	1194
	120	77	1178
	180	92	862
BisGMA:VL:LLA= 1:2:10 mol/mol	0	70	1654
	60	69	1073
	120	76	1300
	180	101	915
BisGMA:LLA= 1:10 mol/mol	0	74	2400
	204	77	4200
BisGMA:GLY:LLA= 1:3:7 mol/mol	0	77	1400
	204	82	1600

Tab. 1 shows the glass transition temperature T_g (temperature at the maximum of the loss factor) and the storage modulus E' at 25 °C for the non-incubated samples and degraded specimens in the dry state. Degradation of the oligo(lactone) branches results in all cases in higher T_g because the plasticizing effect of these branches on the poly(methacrylate) network 7) is diminished. E' was observed to decrease in the cases where the composites contain oligo(δ-valerolactone-co-L-lactide) branches, whereas an increase of E' corresponding to that of T_g was established after degradation of composites containing oligo(L-lactide) and oligo(L-lactide-co-glycolide) branches. During the synthesis of δ-valerolactone containing macromonomers transesterfication reactions were observed resulting in methacryloylated hydroxyl endgroups of the lactone 10). The incorporation of such macromonomers into the network yields degradable network chains. Their hydrolytic cleavage should result in the decrease in E' as observed.

Influence of CE on the degradation course

The percentage of acid released during incubation with CE treatment was shown to be higher by a factor of about 1.3 (Fig. 6).

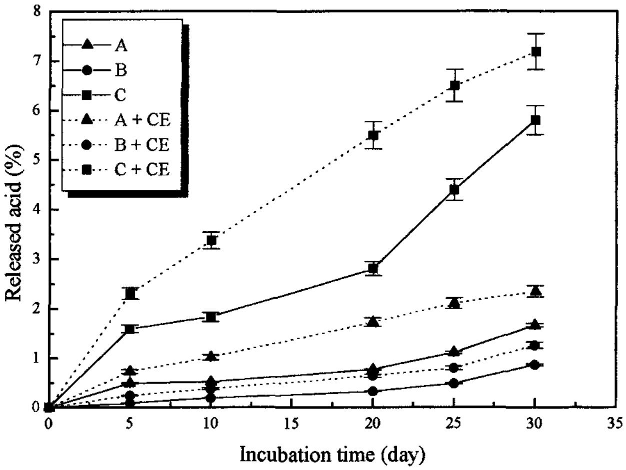

Fig. 6: *In vitro* degradation of copolymer composites from BisGMA endcapped macromonomers -▲- BisGMA:L-lactide (1:10 mol/mol) (A) with HEMA (7:3 wt/wt), -■- BisGMA:L-lactide: glycolide (1:7:3 mol/mol) (C) and -●- BisGMA:L-lactide (1:10 mol/mol) (B) with TEGDMA (7:3 wt/wt) in the presence of 45 wt% HAp, stored in $Na_2HPO_4 \cdot 2H_2O$ / citric acid buffer solution, with and without CE

Hence, this result illustrates that CE can catalyze the hydrolysis of the composites. As previous illustrated in Fig. 2 and 4, the degradation rate of composites A and B is still very low out to day 20 of incubation. However the quantity of released acid from composite C shows very high values from the beginning of incubation.

The influence of CE on a composite was characterized by determination of its Vickers micro-hardness values before and after incubation in buffer solution (Tab. 2). While there was a significantly greater decrease in the hardness of the material in the presence of CE, overall, the change was small.

Tab. 2 Vickers micro-hardness (H_v) under hydrated conditions of composites from BisGMA endcapped macromonomers BisGMA:L-lactide (1:10 mol/mol) copolymerized with TEGDMA (7:3 wt/wt)

	H_v (N/mm^2)		
	before incubation	after 15 days	after 30 days
Control group	241.3 ± 0.3	240.4 ± 0.5	239.5 ± 0.2
Test group	241.3 ± 0.3	238.3 ± 0.6	237.1 ± 0.5

This study also considered the influence of reactants and reaction products on the acitvity of the enzyme. Fig. 7 shows that the activity of CE decreases faster in buffer solution containing composite samples than without the sample.

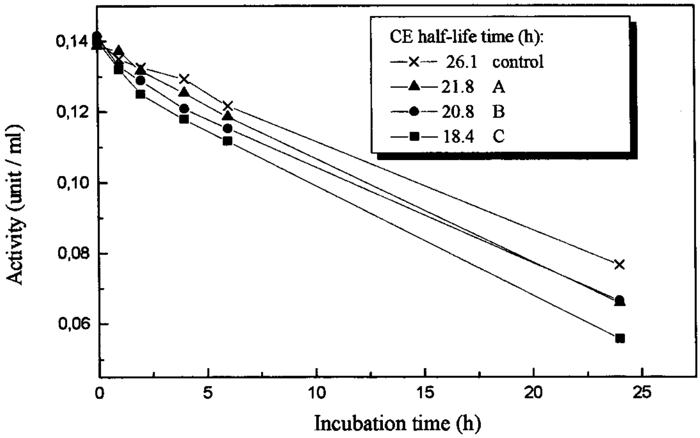

Fig. 7: Activity of CE influenced by copolymer composites from BisGMA endcapped macromonomers -▲- BisGMA:L-lactide (1:10 mol/mol) (A) with HEMA (7:3 wt/wt), -■- BisGMA:L-lactide: glycolide (1:7:3 mol/mol) (C) and -●- BisGMA:L-lactide (1:10 mol/mol) (B) with TEGDMA (7:3 wt/wt) in the presence of 45 wt% HAp, stored in buffer solution ($Na_2HPO4 \cdot H_2O$ / NaCl / KH_2PO_4 / KCl)

Therefore, the half-life time of CE with the composites A, B and C was found to be lower than without composite. There was no significant difference in the half-lifes for composites

A and B (22 and 21 h , respectively), however, the half-life of CE with composite C is significantly shorter (≈ 18 h). Hence there would appear to be a correlation with the high degradation rate of composite C (Fig. 6). However, the half-lifes of CE with the composites A and B do not reflect the differences between the degradation rate of these composites. It can be assumed that also the influence of the content of non-converted monomers on the enzyme activity has to be considered. Higher conversions of monomers were observed for composite A containing HEMA as comonomer in comparison with composite B containing TEGMA[4].

Conclusions

The degradation rate and the degree of degradation for composite composed of a polymer matrix containing oligo(lactone) branches linked to a poly(methacrylate) network has been shown to accommodate a wide range of values by variation of these polymer components. Acceleration of the degradation does not only occur by incorporation of glycolide units into the oligo(lactone) branches or by use of the hydrophilic HEMA comonomer, but also by incorporation of δ-valerolactone units which lower the tendency of the branches to form crystalline domains.

The influence of CE and other relevant enzymes on the degradation of the composites *in vitro* must be considered in order to anticipate higher degradation rates under *in vivo* conditions.

Acknowledgement

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References

1) E. J. Bergsma, F. R. Rozema, R. R. M. Bos, W. C. de Bruijn: *Oral Maxillofac. Surg.* **51**, 666, **(1993)**

2) Y.-K. Han, P. G. Edelman, S. J. Huang, *J. Macromol. Sci., Chem.* **A 25,** 847 (1988)

3) I. Barakat, P.Dubois,R. Jerome, P. Teyssie, E. Goethals, *J. Polym. Sci., Polym. Chem. Ed.* **32**, 2099 (1994)

4) B. Sandner, S. Steurich, S. Wartewig, *Makromol. Symp.* **103**, 149 (1996)

5) B. Sandner, S. Steurich, U. Gopp, *Polymer* **38**, 2515 (1997)

6) U. Gopp, B. Sandner, M. Schöch, K. Schlothauer, H. Pasch, R. Ghahary, *Macromol. Symp.* **130,** 113 (1998)

7) B. Sandner, S. Steurich, A. Bernstein, S. Wartewig, K. W. M. Davy, M. R. Anseau, *Polymers in Medicine and Surgery,* Glasgow 1996, Conference Paper p. 209 - 215

8) J. P. Santerre, F. G. B. Wang, R. S. Labow, *J. Biomat. Mater.* Res. **36,** 407 (1997)

9) Labow, R. S., Duguay, D., Santerre, J. P., *J. Biomater. Sci.,* Polym. Ed. **6,** 169 (1994)

10) U. Gopp, B. Sandner to be published