Hydrolytic Degradation of Composites of Poly(L-lactide-co-ε-Caprolactone) 70/30 and β-Tricalcium Phosphate

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Abstract

There is an increasing need for synthetic bone substitute materials that decrease the need for allografts and autografts. In this study, composites of β-TCP and a biodegradable poly(L-lactide-co-ε-caprolactone) were manufactured using extrusion to form biodegradable composites with high β-TCP contents for osteoconductivity. The hydrolytic degradation of the composites containing 0, 10, 20, 35 and 50% of β-TCP was studied *in vitro* for 52 weeks. During the study, it was observed that β-TCP did not have an effect on the degradation rate of the polymer matrix. However, the crystallinity of the materials increased throughout the test series and changes in Tgs were also observed as the comonomer ratio of the polymer matrix changed as the degradation proceeded. The results show that the materials have desirable degradation properties and, thus, possess great potential as bioabsorbable and osteoconductive bone filling materials.

Introduction

Due to the problems associated with autografts and allografts, there is an obvious need in orthopaedics for synthetic bone substitute materials that are easy to handle in surgical conditions and do not carry the risk of infection or suffer from limited availability (1). An optimal material that is biodegradable, bioactive, and easy to handle has not been found as yet. The currently available polymers or ceramics alone do not provide a sufficient solution to this problem. Numerous studies have been published where the properties of biodegradable polymers and bioceramics have been combined to form composites (2-5). Such composites have promising properties for use in the fabrication of scaffolds for bone tissue engineering applications (6,7).

In order to overcome the problems of autografts and allografts, β-tricalcium phosphate (β-TCP), a bioresorbable ceramic, is used as a bone graft substitute due to its osteoconductive properties (1,8). This material is clinically used, for example, as granules and blocks (9). β-TCP has the same Ca/P ratio as the amorphous inorganic phase of bone (7) and does not dissolve as quickly as α-TCP (9). According to Aunoble *et al.* (10), 60 wt-% of β-TCP in poly-L-lactide is as active as plain β-TCP with regard to osteogenesis. This fact supports the idea that composites combine the advantageous properties of biodegradable polymers and bioceramics.

The mechanical and hydrolytic degradation properties of poly-ε-caprolactone and poly-L-lactide are very different as homopolymers. Both polymers have proven biocompatibility and are Food and Drug Administration (FDA) approved for a wide range of applications in the biomedical field (11). Via the copolymerization of the monomers, lactide (D, L or DL), and ε-caprolactone, interesting properties can be

achieved that combine the elasticity, good processability and drug releasing properties of poly-ε-caprolactone as well as mechanical properties and faster degradability via the hydrolysis of polylactides (11-18).

Many groups have studied the composites of poly-ε-caprolactone or polylactide and various bioceramics (19-25). However, the reported research results of *in vitro* testing have usually concentrated on relatively short periods of time, often less than a month (22,26) or less than 26 weeks (4,21). Reports of the studies performed on copolymers of lactide and ε-caprolactone can be found, but the majority of them concentrate on copolymers of D,L-lactide and ε-caprolactone or different comonomer ratios than the one reported in this study.

In this study, various ratios of a filler, β-tricalcium phosphate, and a bioabsorbable polymer, poly(L-lactide-co-ε-caprolactone) with the comonomer ratio of 70/30, were extrusion compounded in order to create a material that is biodegradable, osteoconductive and easy to handle. The properties as well as the potential of the composites for use as bone graft substitutes were examined in a long term *in vitro* test lasting for 52 weeks. Despite the fact that some of the composites had high β-TCP content, they showed good processability and handling properties as well as promising degradation behaviour. Special attention will be paid in the report to the effect of various β-TCP contents on the degradation of the composites.

Materials and Methods

Materials

Medical grade poly(L-lactide-co-ε-caprolactone) with the comonomer ratio of 70/30 and initial M_w of 229 000 g/mol was purchased from Purac Biomaterials (the

Netherlands), and β-tricalcium phosphate (granule size < 38 μm) was purchased from Plasma Biotal Ltd (United Kingdom). Sörensen buffer solution was prepared according to the standard ISO 15814 (27). The chemicals used for the buffer solution (Na_2HPO_4 and KH_2PO_4) were purchased from J.T. Baker (the Netherlands). For the phosphate dissolution testing, Tris buffer solution was used and it was prepared using Tris Base Ultra Pure ($C_4H_{11}NO_3$) (ICN Biomedicals, USA) and Trizma HCl ($C_4H_{11}NO_3 \times HCl$) (Sigma-Aldrich, Germany). Other chemicals used for the determination of phosphate release from the composites were ammoniumheptamolybdate (Merck, Germany), L(+)-ascorbic acid (Merck, Germany), acetic acid (J.T. Baker, the Netherlands), sodium acetate (Merck, Germany) and sulphuric acid (J.T. Baker, the Netherlands).

Processing

Dried (72 h in vacuum at room temperature) polymer and β-TCP powder were processed into rod-shaped billets with a diameter of approximately 2.5 mm with a co-rotating custom-built intermeshing twin-screw extruder (L/D ratio = 22.5) in a nitrogen atmosphere. Temperature range in the extruder was 25-130 °C. PLCL copolymer and β-TCP were delivered in the process with separate gravimetric screw feeders and the mixing of the components took place in the extruder. Total feed rate of the components was 200g/h in all of the cases. A haul-off unit was used to guide the extrudate from the die and the diameter of the billets was fine-tuned adjusting the speed of the haul-off unit. Four different composites and a plain copolymer were processed. The β-TCP-contents of the composites were 10, 20, 35 and 50 wt-% and the composites are denoted as PLCL + 10% TCP, PLCL + 20% TCP, PLCL + 35% TCP, and PLCL + 50% TCP, respectively. Pellet shaped samples (length approximately 2.5 mm) were cut from the

billets. Before degradation studies were carried out, the samples were packed and sterilized using gamma irradiation (minimum dose 25 kGy).

In vitro procedure

Degradation tests were conducted at 37°C *in vitro* following the standard ISO 15814 (27). First, weighed test samples were placed in brown glass bottles with 20 ml Sörensen buffer solution. A test sample consisted of 15 pellets weighing approximately 300 mg in total and five parallel test samples were tested at each time point. Then the bottles were placed in a shaking bath at 37°C. The pH of the buffer solution was measured periodically with a calibrated pH meter and the buffer solutions were changed every two weeks in the beginning of the test series and once a week as the degradation accelerated. Test samples were withdrawn at predetermined time points of 2, 4, 6, 8, 10, 12, 16, 20, 26, 39 and 52 weeks.

Methods of analysis

Residual monomer

The determination of residual L-lactide and ε-caprolactone monomer contents was performed by Ramboll Analytics Oy (Lahti, Finland). The ε-caprolactone and L-lactide contents were measured after chloroform extraction of the samples using gas chromatography (DC8000, CE Instruments, Rodano, Italy) and an FI-detector after chloroform dilution. The measuring resolution was 0.02%. The monomer contents of the processed samples were analyzed from three time points in the processing batch. Two parallel samples were taken from the beginning, middle and the end of the processing batch. Additionally, the monomer content of the raw material was measured.

Molecular weights

The molecular weights (number average, M_n, and weight average, M_w, molecular weights) and polydispersity values of the copolymer were determined at room temperature by size exclusion chromatography (SEC) (Waters Associates system equipped with a Waters 717plus autosampler, a Waters 510 HPLC solvent pump, four linear PL gel columns (10^4, 10^5, 10^3 and 100 Å) connected in series, and a Waters 2414 differential refractometer). Chloroform (Riedel-de Haën AG, stabilized with 1% ethanol) was used as solvent and eluent. The samples were filtered through a 0.5 μm Millex SR filter. The injected volume was 200 μl and the flow rate was 1.0 ml/min. Monodisperse polystyrene standards were used for primary calibration. Two parallel samples were analyzed at each time point.

Mass loss and water absorption

After the test samples were withdrawn from the shaking bath, they were rinsed twice with distilled water and the surfaces of the pellets were carefully wiped with tissue paper. The test samples were weighed immediately after wiping. The test samples were dried for at least three days at ambient conditions and for one week in vacuum. After vacuum drying, the test samples were weighed again to obtain the dry masses. Dried test samples were stored in a desiccator for further analysis.

The mass loss was calculated as the difference between the mass of the initial test sample and the mass of the dried test sample divided by the initial mass of the test sample. The water absorption was calculated as the difference between the mass of the wet test sample and the mass of the dried test sample divided by the mass of the dried test sample.

Thermal properties

Thermal analysis was performed using DSC Q1000 differential scanning calorimeter (TA Instruments, Delaware, USA). To ensure similar thermal histories of the samples, they were heated twice and cooled rapidly in between. The heating rate was 20 °C/min, the cooling rate 50 °C/min, and the temperature range was -60 °C to +200 °C. Nitrogen was used as the sweeping gas. The results were analyzed using Universal Analysis Software. Second heating cycle was used for the analysis of glass transition temperatures (T_g) and first heating cycle for the analysis of melting temperatures (T_m) and enthalpies (ΔH_f). Five parallel samples were tested for each material and time point. Averages and standard deviations were then calculated.

Ceramic content and dissolution

The β-TCP content of the test samples was measured using thermogravimetric analysis (TGA Q500 (TA Instruments, Delaware, USA)). Approximately 20 mg of a sample was used and the samples were heated at a rate of 20°C/min up to 700°C. Five parallel samples were tested at each time point, and the results were then analyzed using Universal Analysis Software.

The dissolution of phosphate ions from the composites was measured using a method described in ISO 6878 (28). In this method, the orthophosphate ions that have been dissolved in the buffer solution form an ammonium phosphomolybdate complex in acidic solution. This complex is then reduced by ascorbic acid into a blue complex. The absorbance of the complex was measured using a Unicam UV 500 spectometer (ThermoSpectronic, Cambridge, United Kingdom) at the wavelength of 700 nm. Five parallel samples of each composite weighing about 250-300 mg were tested once a week up to 48 weeks. Plain PLCL was used as a negative control and plain β-TCP

samples weighing about 160 mg were tested as positive controls. Concentrations of the released phosphate ions were calculated with the help of a standard curve prepared with known concentrations of phosphate ions.

Molecular structure

The proton spectra of the samples were measured using a Varian Mercury 300 MHz NMR Spectrometer (Varian Associates Inc., Palo Alto, California, USA) at room temperature. Tetramethylsilane (TMS) was used as an internal standard, and chemical shifts were measured relative to TMS. The ^{1}H NMR spectra were measured at room temperature in standard 5 mm tubes in deuterochloroform. The data were gathered until the quality of the spectrum was sufficient, and the number of scans was 190-300. The proton NMR spectra of the samples were processed and analyzed using SpinWorks 3.1 software. Phase correction and baseline correction were applied to all spectra. Information on the molecular composition and the comonomer ratio of the copolymer was obtained.

Microstructure of the samples

The microstructure of the composites was observed using scanning electron microscopy (Philips XL-30 SEM equipped with a LaB6 filament with SE and BSE detectors, Philips, the Netherlands) with an acceleration voltage of 12.0 kV. The micrographs were taken both on the surface of the samples and on cryogenically fractured samples that were coated with gold (Edwards S150 Sputter Coater) prior to microstructure examination. An Energy Dispersive X-ray Spectrometer (Edax DX4 and eDXi software) was used to analyze the composition of the surfaces of the samples.

The structure of the materials was also studied using micro-computed tomography (µ-CT), which is an efficient way to do non-destructive analysis of the structure. In this study, µ-CT imaging was employed to analyse pores inside the material. All the imagings were done by MicroXCT-400 (Xradia, Pleasanton, CA, USA) and the same imaging parameters were applied for every sample. 40 kV as source voltage was used and 150 µA as source current. The isometric voxel size was 2.2 µm. No filters were utilized. Reconstruction was done by using Xradia's XMReconstructor software. Images were segmented by manual thresholding using ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, U.S.A). The same software was used for the analysis.

Results and discussion

The effects of processing and sterilization on the materials

Processing using the twin-screw extruder degraded the matrix polymer only slightly. The average weight average molecular weight (M_w) of the raw material was 229 000 g/mol and after processing the M_w was 227 000 g/mol. However, the sterilization using gamma irradiation with the measured dose of 28.7-34.0 kGy, caused significant degradation. The M_w of the plain, processed polymer decreased 14% and the M_n decreased 29% during sterilization. The M_w of the composites decreased 18-29% and M_n 31-43% with a tendency that gamma irradiation caused more degradation to those composites with higher β-TCP contents There is almost linear dependency of the percentual decrease in the molecular weights versus the β-TCP content in the samples, which is shown Figure 1. This may be attributed to the hydrophilicity of the β-TCP which may have caused slight water absorption to the material as it was not stored in totally dry conditions before sterilization. As a result, the composites with higher β-TCP content may have absorbed more water and thus were more degraded during

sterilization. It has also been suggested that gamma irradiation may cause not only chain scission in the polymer chains but also cross-linking for pure poly-ε-caprolactone (29). This would have been seen as a drop in the M_n and an increase in the M_w of the polymer. This kind of effect was not observed in this study. Gamma-irradiation caused a significant decrease both in number average and weight average molecular weights. This effect is well known on biodegradable polymers and is caused by the chain-scission of the polymer chain due to high energy gamma irradiation (30) It has been reported by other authors as well (31,32).

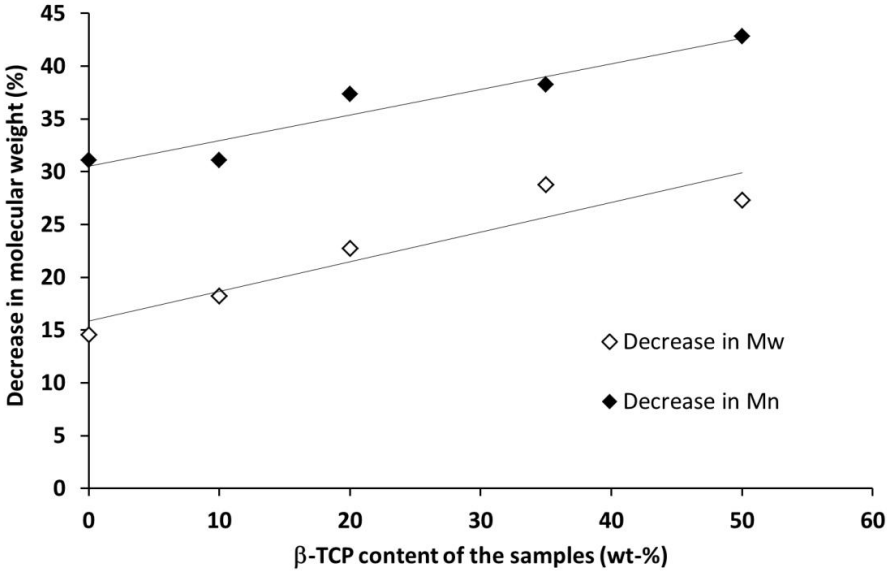

Figure 1. Decrease in weight average molecular weight (M_w) and number average molecular weight (M_n) during the sterilization step.

For the raw material, the residual monomer contents were 0.08 wt-% for the L-lactide monomer and below detection limit (<0.02 wt%) for the ε-caprolactone monomer. No increase was seen in the content of either monomer during the processing event which indicates that the polymer did not decrease during processing and thus no monomers were generated during processing. Because there were no differences in the monomer

contents of any of the manufactured materials, it can be assumed that the monomers did not cause differences in the hydrolytic degradation behaviour of the studied composites. (32).

In vitro degradation of the materials

Molecular structure

^{1}H NMR spectra were measured from five samples (PLCL raw material, PLCL 26 weeks *in vitro*, PLCL 52 weeks *in vitro*, PLCL + 50% TCP 26 weeks *in vitro* and PLCL + 50% TCP 52 weeks *in vitro*). The ^{1}H NMR spectrum of plain PLCL raw material and the assignment of the signals is presented in Figure 2. The signal of the -CH group proton of the lactide comonomer appeared at δ 5.16 as a multiplet. The signals of methyl protons of lactide and the aliphatic protons of caprolactone units (δ 0.5-1.8) were overlapping with each other and accurate integration of the peaks was not possible, and they were not included in the calculations. The most informative signals were the signals of the α-oxy methylene protons of the ε-caprolactone comonomer that appeared at δ 4.05-4.13 and were clearly split into two signals according to the position in the polymer chain. The triplet at δ 4.13 indicated the CH_2 group in the ε-caprolactone fragment bonded to an L-lactide unit and the broader multiplet at δ 4.05 indicates the α-oxy methylene group bonded to another ε-caprolactone unit. (33, 34) Additionally, the signal at δ 2.3-2.4 that corresponds to the signal of the methylene proton of the ε-caprolactone that is bonded to the carbonyl group was split the same way. The triplet at δ 2.4 indicates a group bonded to a L-lactide group and the broader multiplet at δ 2.3 corresponds to a group that is bonded to another ε-caprolactone group (33, 34).

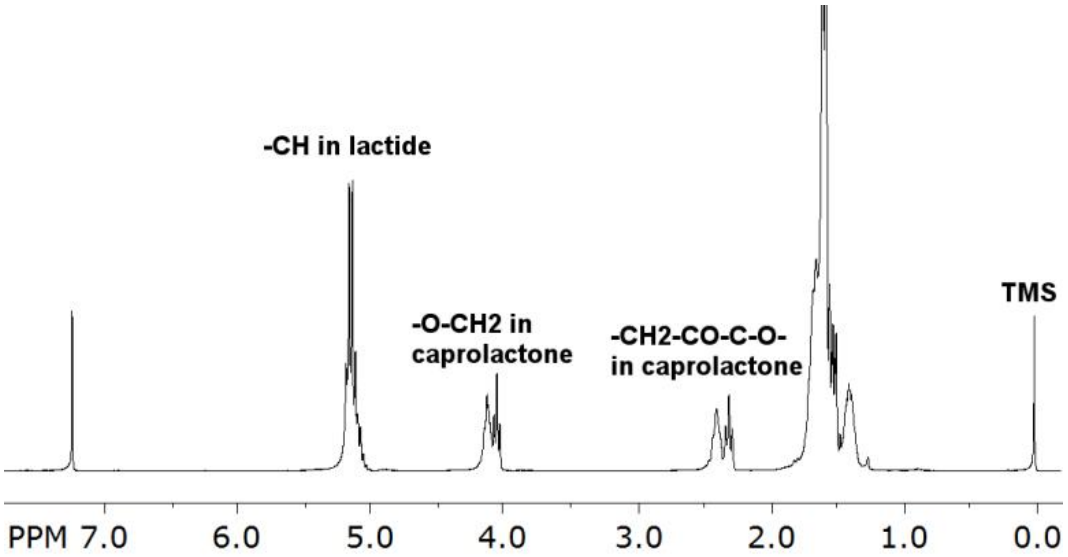

Figure 2. ^{1}H NMR spectrum of the raw material poly(L-lactide-co-ε-caprolactone).

The NMR results showed that the ratio of integrals for these two signals, and therefore the comonomer ratio, changed as the hydrolysis proceeded. The ratio of lactide to caprolactone (LA/CL) was increased from the initial proportion of the raw material 68/32 to 80/20 for the plain copolymer at 52 weeks and 75/25 for the composite containing 50% β-TCP at 52 weeks of hydrolysis. The comonomer ratios are presented in Table 1. The results of NMR measurements also demonstrated that the presence of β-TCP in the composite slowed down the change in the comonomer ratio. The change in the comonomer ratio of a copolymer of L-lactide and ε-caprolactone has also been observed by Jeong *et al.* (33). They suggested that the amorphous regions of the copolymer contain more caprolactone and are more easily attacked by water as water diffuses first into the amorphous regions of the polymer. Additionally, they found that the degradation and increase in the comonomer ratio LA/CL were slightly accelerated *in vivo*. The results of our studies indicate that although β-TCP does not affect the

degradation of the copolymer in a way that could be seen in the SEC measurements, it has an effect on which ester bonds the breaking of the polymer chain preferably occurs.

Table 1. Results of the ^{1}H NMR analysis.

Sample	Comonomer ratio (LA/CL)	Average sequence lengths		
		$\tilde{n}_{LA}$	$\tilde{n}_{CL}$	R
PLCL raw material	68/32	12.2	5.7	0.26
PLCL 26 wk hydrolysis	73/27	16.8	6.3	0.22
PLCL 52 wk hydrolysis	80/20	28.9	7.1	0.18
PLCL + 50% TCP 26 wk	69/31	14.1	6.3	0.23
PLCL + 50% TCP 52 wk	75/25	20.6	6.8	0.20

Because the properties of copolymers do not only depend on the comonomer composition but also on the distribution of the comonomers in the polymer chains, analysis of the microstructure of the polymer was also needed (35, 34). According to the text by Herbert (35), the number average sequence lengths of the comonomers can be calculated using Equations 1 and 2

$$\tilde{n}_{LA} = \frac{2(LA)}{(LA-CL)} \qquad (1)$$

$$\tilde{n}_{CL} = \frac{2(CL)}{(LA-CL)} \qquad (2)$$

where (LA) and (CL) are the molar fractions of the L-lactide and ε-caprolactone comonomers in the copolymer and (LA-CL) is the average dyad relative fraction, which can be calculated from the ^{1}H NMR data of the copolymer. The calculation is well explained in an article by Fernández (34). Additionally the randomness factor, R, can be calculated using the Equation 3

$$R = \frac{(LA-CL)}{2(LA)(CL)} \qquad (3)$$

The randomness factor is 1 for a random copolymer and 0 for a block copolymer (34). The results of the average sequence length calculations and the randomness factor are presented in Table 1.

First of all, the results show that the copolymer is rather blocky, having R values between 0.18-0.26, whereas the totally random copolymer would show the R value of 1. Randomness of the copolymers of lactides and ε-caprolactone are greatly affected by the polymerization conditions and depends on the polymerization temperature and time (13,15,30).

The randomness of the studied copolymer samples is decreased as the degradation proceeds. The average sequence lengths of the comonomers show that L-lactide tends to form long blocks. The notable increase in the average sequence length of the L-lactide comonomer as the degradation proceeds shows that the shorter blocks of the L-lactide are removed from the polymer chains as the copolymer is hydrolytically degraded. There is also a slight increase in the ε-caprolactone sequence length as the degradation proceeds, which indicates that the sequence length is not much affected by the hydrolytic degradation although the ε-caprolactone comonomer fraction in the copolymer is decreased.

The decrease of the randomness factor also supports the conclusion that the random parts of the copolymer are removed from the structure and more blocky structures remain as the degradation proceeds. In conclusion, the ^{1}H NMR analysis shows that the copolymer is blocky having some random parts in the structure but the random parts are removed as the hydrolytic degradation proceeds and the blocky characteristic of the

copolymer is increased. The bonds between the L-lactide and ε-caprolactone comonomers are most susceptible to hydrolytic degradation in this studied copolymer.

Molecular weights

The degradation of the composites was tested in Sörensen buffer solution, *in vitro* at 37°C and pH 7.4. The decrease of the molecular weights (both M_w and M_n) of the polymer matrix was rapid for the plain copolymer and for all the composites and obeys the first order kinetics with the degradation rate constants of $1.4 \cdot 10^{-3}$-$1.7 \cdot 10^{-3}$ 1/h. Although there were small differences in the molecular weights of the polymer matrix at the beginning of the hydrolysis test series, the degradation proceeded in all the composites following the same trend. There were no significant differences in the degradation behaviour during the 52-week test series between the different composites. The same kind of result was recently reported by Daculsi et al. (31) who studied composites of poly(96L/4D)lactide and β-TCP with β-TCP contents of 0, 10, and 24 wt-%. In our study, the M_n of all the composites had already decreased approximately 96% of the initial value of the raw material after 20 weeks of hydrolysis at 37°C in pH 7.4. After 52 weeks, the decrease was 99%. The M_n values of the samples as a function of time *in vitro* up to 20 weeks are presented in Figure 3. The rapid decrease of molecular weights is typically caused by random scission of the hydrolytically labile ester bonds of the polymer backbone. This happens first in the amorphous parts of the polymer. (36)

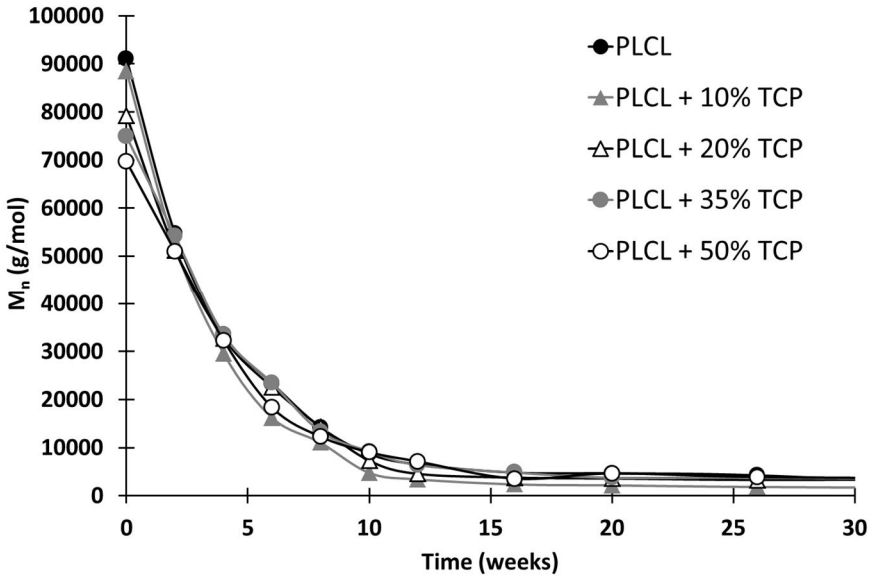

Figure 3. The number average molecular weight (M_n) of the composites of poly(L-lactide-co-e-caprolactone) (PLCL) 70/30 and β-tricalcium phosphate (β-TCP) as a function of time *in vitro* (n=2).

The SEC distribution plots showed bimodality after 20 weeks for all the composites and the plain copolymer except for the samples with 10 wt-% of initial β-TCP content that showed emerging bimodality at the time point of 39 weeks. An example of the emerging bimodality is shown in Figure 4, where 20, 26 and 39-week SEC results of PLCL + TCP 20% are shown.

Bimodality in the SEC distribution curve can be explained with the blocky structure of the copolymer, which is pronounced as the hydrolysis proceeds. The ^{1}H NMR analysis of the plain copolymer and PLCL + 50% TCP showed that the copolymer is rather blocky and the random parts are degrading first. This might cause an increase in a certain part of the SEC distribution curve as the blocky parts consisting mainly of L-lactide monomers remain in the copolymer and the amount of other parts in the copolymer is decreased.

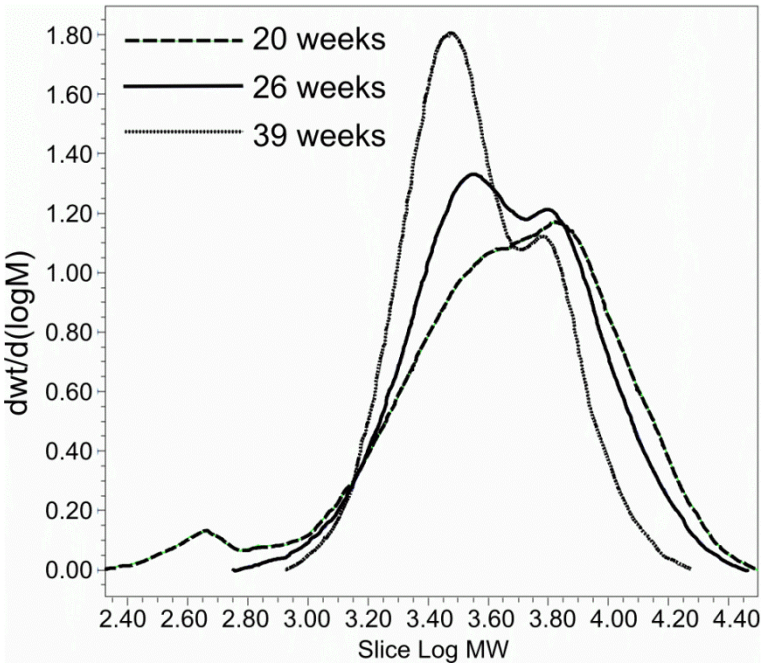

Figure 4. An example of the development of the bimodality in the size exclusion chromatography curves. The samples were poly(L-lactide-co-ε-caprolactone) (PLCL) + 20% tricalcium phosphate (TCP) at 20, 26 and 39 weeks.

Bimodality is often explained by the autocatalytic effect in the inner parts of the polymer (31,36-38), but it is not likely in this case because of the porous structure of the composites induced by the β-TCP granules in the structure, which enables the short chain degradation products to escape from the inner parts on the samples and thus do not catalyse the degradation reaction.

The polydispersity of the polymer matrix changed as the hydrolytic degradation proceeded (Figure 5). These changes are typical in the hydrolysis of biodegradable polymers. First, the molecular weight distribution was narrowed because chain scission reduced the number of long polymer chains. After reaching the lowest values at the 8-week time point, the molecular weight distribution widened because of the introduction of short polymer chains resulting from the chain scission. PD reached the highest value

at the time point of 12 weeks (1.8-2.7) and started to decrease again as the short molecular chains were short enough to be dissolved into the surrounding fluid.

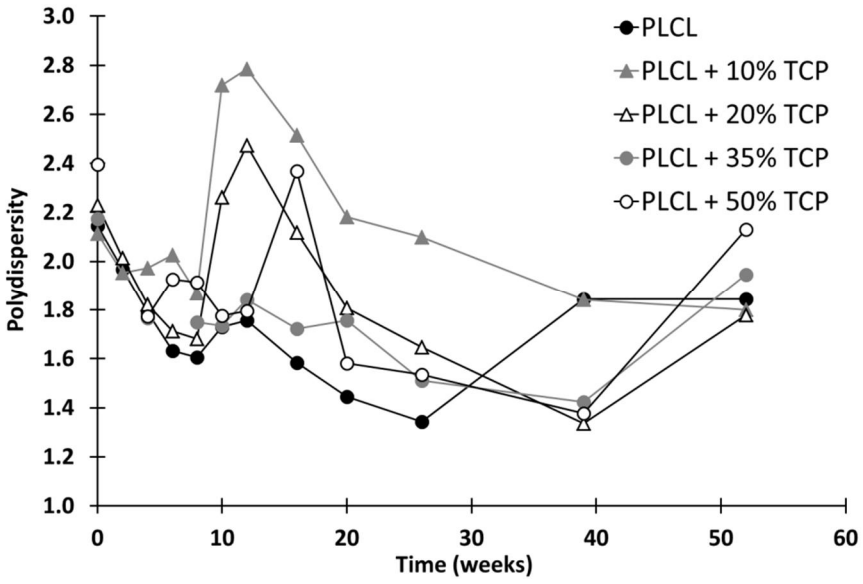

Figure 5. Development of the polydispersity of the poly(L-lactide-co-ε-caprolactone) (PLCL) during hydrolytic degradation of composites of PLCL and β-tricalcium phosphate (TCP).

Mass loss, water absorption and β-TCP content of the materials

The mass loss of the tested materials as a function of time *in vitro* is presented in Figure 6 along with the water absorption results. The mass loss of the plain copolymer was fastest and the mass loss of the composite containing 50% of β-TCP was slowest. This is because the polymer degrades and disappears from the composites and β-TCP dissolves slower than the polymer phase. This result is also supported by the phosphate dissolution test series, where very slow dissolution of β-TCP was observed. The results are shown in Figure 7 as cumulative dissolution of the phosphate ions. The maximum values of 1000-2500 μg of β-TCP at 52 weeks correspond to less than 10 wt-% of the total amount of β-TCP in the samples.

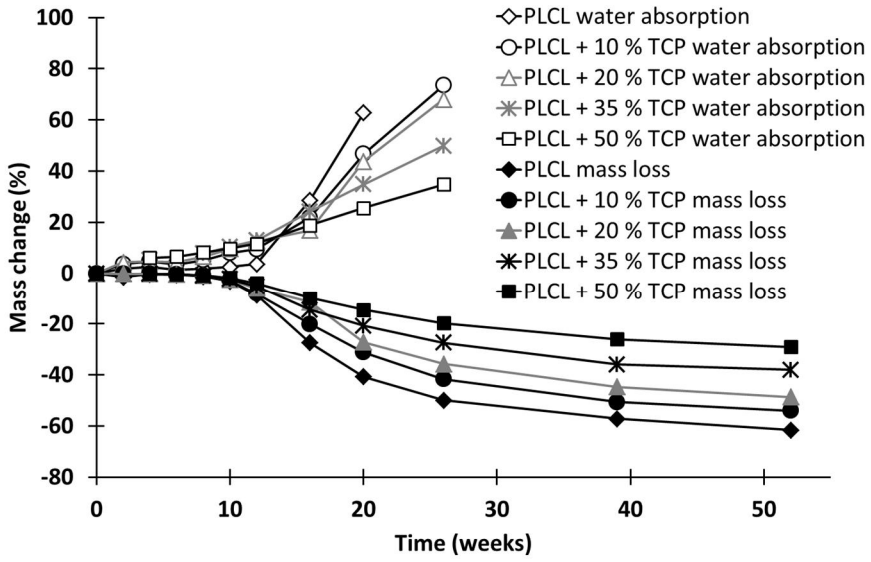

Figure 6. Mass loss and water absorption of the composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and β-tricalcium phosphate (TCP) seen as gained weight as a function of time *in vitro*.

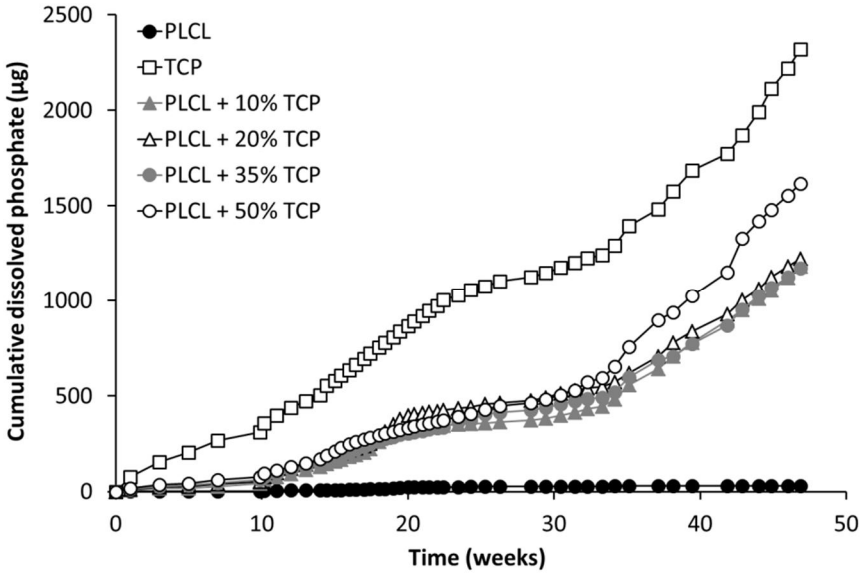

Figure 7. Dissolution of β-tricalcium phosphate (TCP) from the composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and TCP. Plain PLCL was used as a negative and plain TCP as a positive control.

From the β-TCP content analysis, it can be seen that the increase in the β-TCP-content of the test samples started at the same time as the mass loss of the samples (i.e. after 10 weeks in hydrolysis) (Figure 8). At this time point, the M_w of the polymer had decreased to a level of 12000-15000 g/mol and the M_n to a level of 4000-9000 g/mol, which apparently enabled the start of mass loss. In earlier studies, (37) the mass loss of pure poly-ε-caprolactone had begun when the M_w had decreased to a level of 5000 g/mol and for pure poly-DL-lactide to a level of 15 000 g/mol.

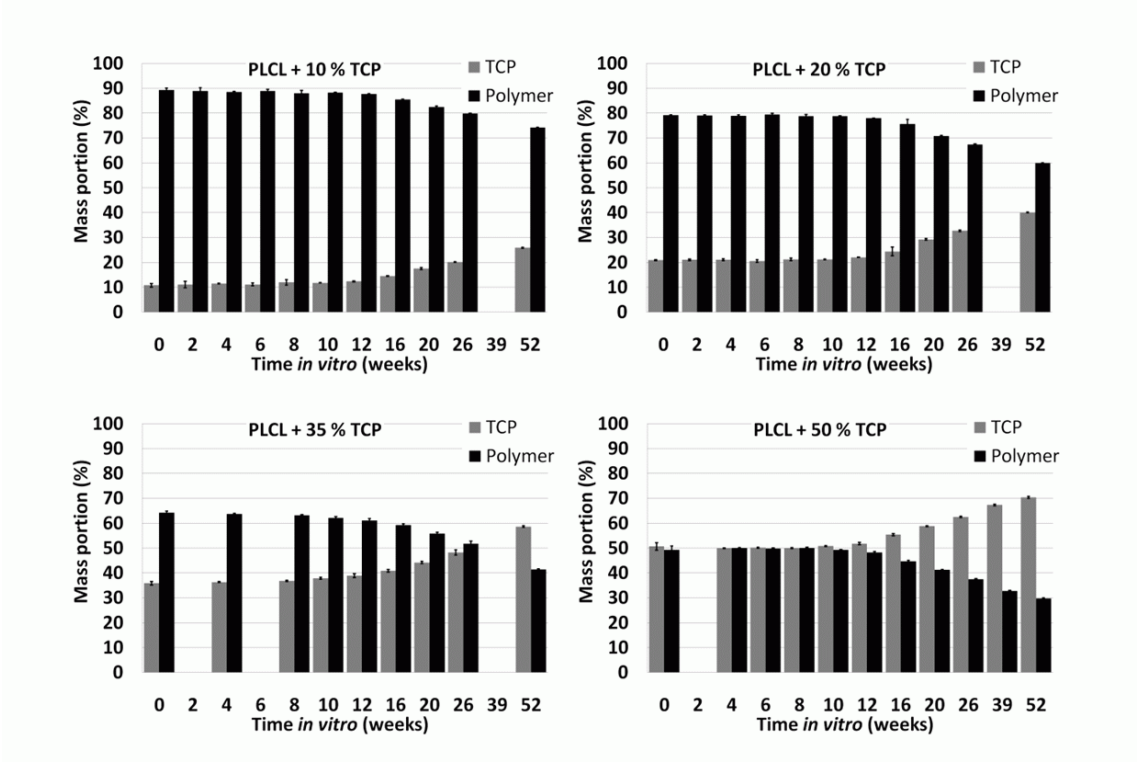

Figure 8. β-tricalcium phosphate (TCP)-contents of the composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and TCP as a function of time *in vitro*. Results shown as averages with standard deviations (n=5).

The increase in β-TCP content of the composites was very clear and the fact that polymer degraded and was lost from the composites may be beneficial in the desired end application of this kind of composite material. β-TCP is left in the bone cavity and as it is known to be an osteoconductive material (1), it may enhance bone ingrowth and healing.

When only the polymer component in the composites was considered, the mass loss was slightly decelerated by increasing β-TCP content. At the time point of 52 weeks, the mass loss of the polymer component of the composites was 62% for plain PLCL and 57% for the composite containing 50 % β-TCP. The mass losses of the polymer composites containing 10-35% of β-TCP fell between these values.

Water absorption, which indicates the hydrophilicity of the material, of all the tested samples was small during the first ten weeks *in vitro* (Figure 6). It is well known that water diffuses first into the amorphous parts of the polymer and causes degradation. (30). The composites containing β-TCP showed greater water absorption than the pure copolymer in the first 12 weeks of the test series, after which the behaviour was changed. The water absorption of the plain polymer increased rapidly and at 20 weeks the water absorption of the plain polymer was already over 60 wt-%. After this time point, the wet masses of the plain polymer test samples were not measurable anymore. From the 12-week time point on, the water absorption of the composites with high β-TCP contents was less than for the composites with low β-TCP contents. The wet masses of the composites were measurable up to 26 weeks. Since TCP is hydrophilic, the composites with high β-TCP contents absorbed more water in the beginning of the *in vitro* time than pure polymer or the composites with less β-TCP. When the polymer

degradation had proceeded to a certain level i.e. the polymer chain scission had reached the point when mass loss is possible and there were more hydrophilic end groups of the polymer, the water absorption behaviour changed and the composites with no or little β-TCP absorbed more water.

Changes in pH of the buffer solution

The pH-values of the buffer solution in the *in vitro* test series were measured periodically and the buffer solution was changed (20 ml) every two weeks at the beginning of the test series. once a week after 10 weeks, and twice a week after the time point of 16 weeks. The pH mainly retained values between 7.2-7.5 and the pH was very stable in the first weeks of the test series. The results of the pH measurements are presented in Figure 9. A significant decrease in the pH-values was observed from the 10^{th} week to the 19^{th} week of the test series. At the 10-week time point, the M_w had decreased to a level of 12 000 - 15 000 g/mol for all the composites and the plain copolymer and mass loss started. This was seen as the decrease in the pH values because the acidic degradation products of the polymer degradation were released to the hydrolysis medium. Throughout the *in vitro* test series, a tendency of increasing pH-value towards the composites with higher β-TCP contents was observed. This is likely due to the buffering effect of the β-TCP that has also been observed earlier by other authors (39,40).

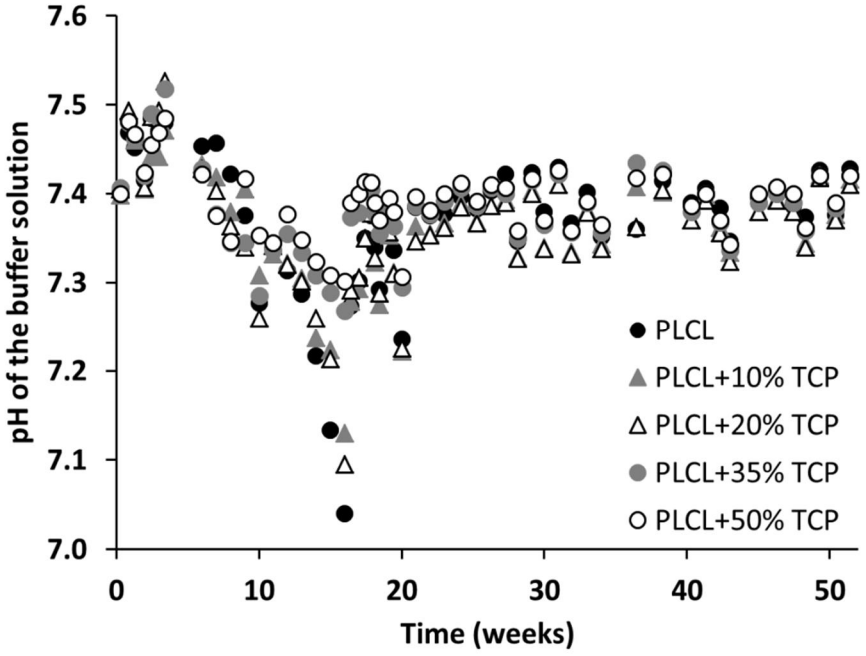

Figure 9. Results of the pH measurements in the *in vitro* degradation test series of composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and β-tricalcium phosphate (TCP).

Bernstein *et al.* (3) reported results where composites of nanosized tricalcium phosphate and polycaprolactone with very high β-TCP contents (85 vol-% and 95 vol-%) showed the same kind of dissolution behaviour as pure β-TCP. They also proposed that β-TCP may enhance the hydrolytic degradation of polycaprolactone due to the increased hydrophilicity. On the other hand, a buffering effect of the dissolution products of β-TCP against the acidic degradation products of the polymer has been proposed (39,40). This would lead to the slower degradation of the polymer component in the composite. These two factors work in opposite ways and, if simultaneous, their overall effect on the degradation may be evened out. In this study, no significant differences in the hydrolytic degradation behaviour of the studied composites compared with the plain poly(L-lactide-co-caprolactone) copolymer were observed.

Thermal properties

T_g, T_m and ΔH_f were measured using a differential scanning calorimeter (DSC) at a temperature range from -60°C to 200°C with a 20°C/min heating rate. The T_gs of the raw material determined during the first and second heating were 15°C and 23°C respectively. This corresponds quite well with the results of Ragaert *et al.* for the same commercial polymer (41). After processing, the T_gs (obtained from the second heating) of all the composites and the pure copolymer were between 21°C and 22°C. DSC analysis revealed changes in the T_g as the hydrolysis proceeded. This can be seen in Figure 10, where the values from the second heating cycle are presented. The T_g started to decrease in the beginning of the *in vitro* test series and reached the lowest values at the 12-week time point (9°C – 14°C). During the same time period, the molecular weights of the samples decreased rapidly and this had a decreasing effect on the T_g. After 12 weeks, the T_gs started to increase and ended up at 34°C for the pure copolymer and at around 30-33°C for the composites at the 52-week time point. The T_gs of the samples were detectable only on the second heating scan from the 16-week time point on. At this point, the degradation of the polymers had proceeded to M_ws of less than 10 000 g/mol. The ^{1}H NMR results showed that the caprolactone monomers disappeared from the copolymer as the hydrolysis went on and, therefore, L-lactide content increased. It is well known that the T_g is dependent on the comonomer composition of the copolymer. In the case of copolymers of lactide and ε-caprolactone, the T_g increases as the lactide content in the copolymer increases (42). The change in the T_g is significant even in small increments in the lactide content.

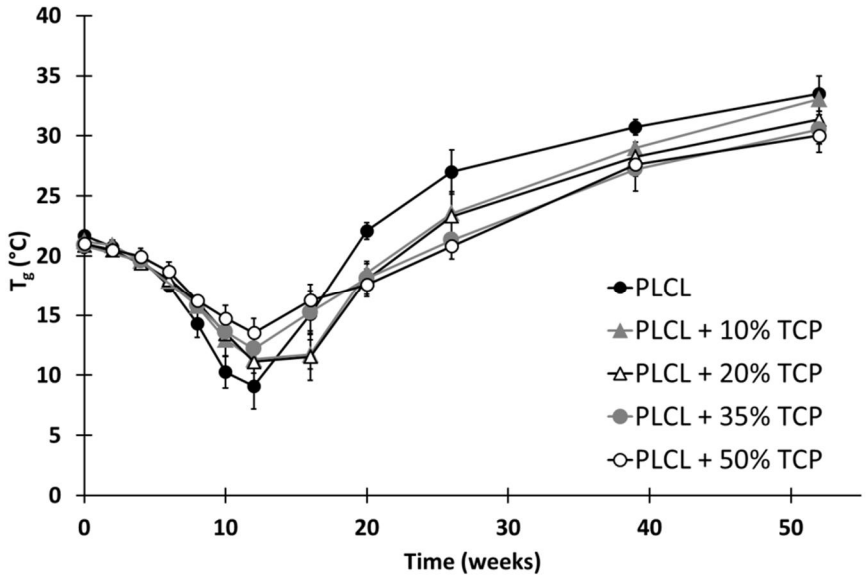

Figure 10. Glass transition temperatures (T_g) of composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and β-tricalcium phosphate (TCP) as a function of time *in vitro*. Results shown as averages with standard deviations (n=5).

T_ms and ΔH_fs were analyzed from the first heating of the samples. This was done because during the second heating the melting peaks did not appear anymore due to the fast cooling in the DSC analysis in which the polymer solidifies to an amorphous structure. The samples showed bimodality in the melting peaks from the 6-week time point on and the bimodality was seen especially clearly in the samples from the 16-week time point on until the 39-week time point. This can be due to two different kinds of crystals in the sample (43). More probable reason for this according to Sarasua *et al.* (43) is the annealing during DSC scan, where imperfect crystals might have time to melt and recrystallize and then melt again in a few degrees higher temperature. It was observed that the melting temperatures increased from the 111-113°C at the 2-week time point to 116-120°C at the 16-week time point. After this, the melting temperatures decreased constantly and they were 107-110°C at the 52-week time point.

Increasing melting enthalpies were recorded throughout the test series (Figure 11). The values were corrected to correspond to the polymer portion of the samples. They indicated increasing crystallinity as the amorphous parts of the polymer are the first to degrade. Also, the fact that shorter polymer chains can more easily rearrange themselves into crystals has an effect on the increasing crystallinity. The increase in crystallinity was largest in the plain copolymer and lowest in the composite containing 50 % of β-TCP. Absolute crystallinity values were not calculated because the theoretical value of 100% crystalline poly(L-lactide-co-ε-caprolactone) 70/30 was not available. It has been reported that the sequence length of 14 is required for lactides to be able to crystallize (43). Additionally, it has also been suggested that in a copolymer of L-lactide and ε-caprolactone, only the L-lactide is able to form crystals. These factors also explain the increasing crystallinity as the L-lactide fraction in the copolymer is increased during *in vitro* degradation.

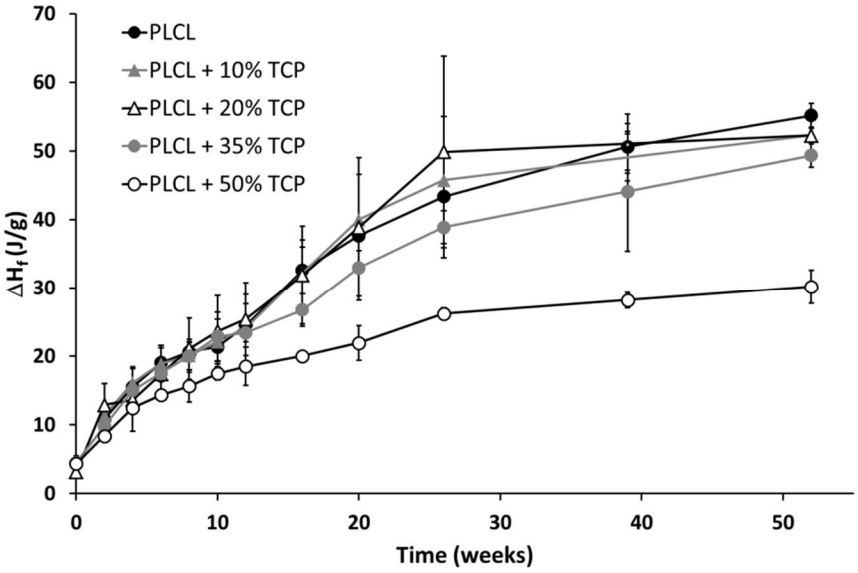

Figure 11. Melting enthalpies (ΔH_f) of the composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and β-tricalcium phosphate (TCP) as a function of time *in vitro*. Results shown as averages with standard deviations (n=5).

Microstucture

SEM micrographs, which were taken both on the surface of the samples and on cryogenically fractured samples, showed some porosity in all the samples. The micrographs of the surface and cross section of composites containing 50% β-TCP are shown in Figure 12. The appearance of the composites changed when the hydrolysis proceeded and the formation of smaller pores was observed. The majority of the pores were however smaller than 100 μm, and according to the literature, this is not enough to enhance bone ingrowth (44).

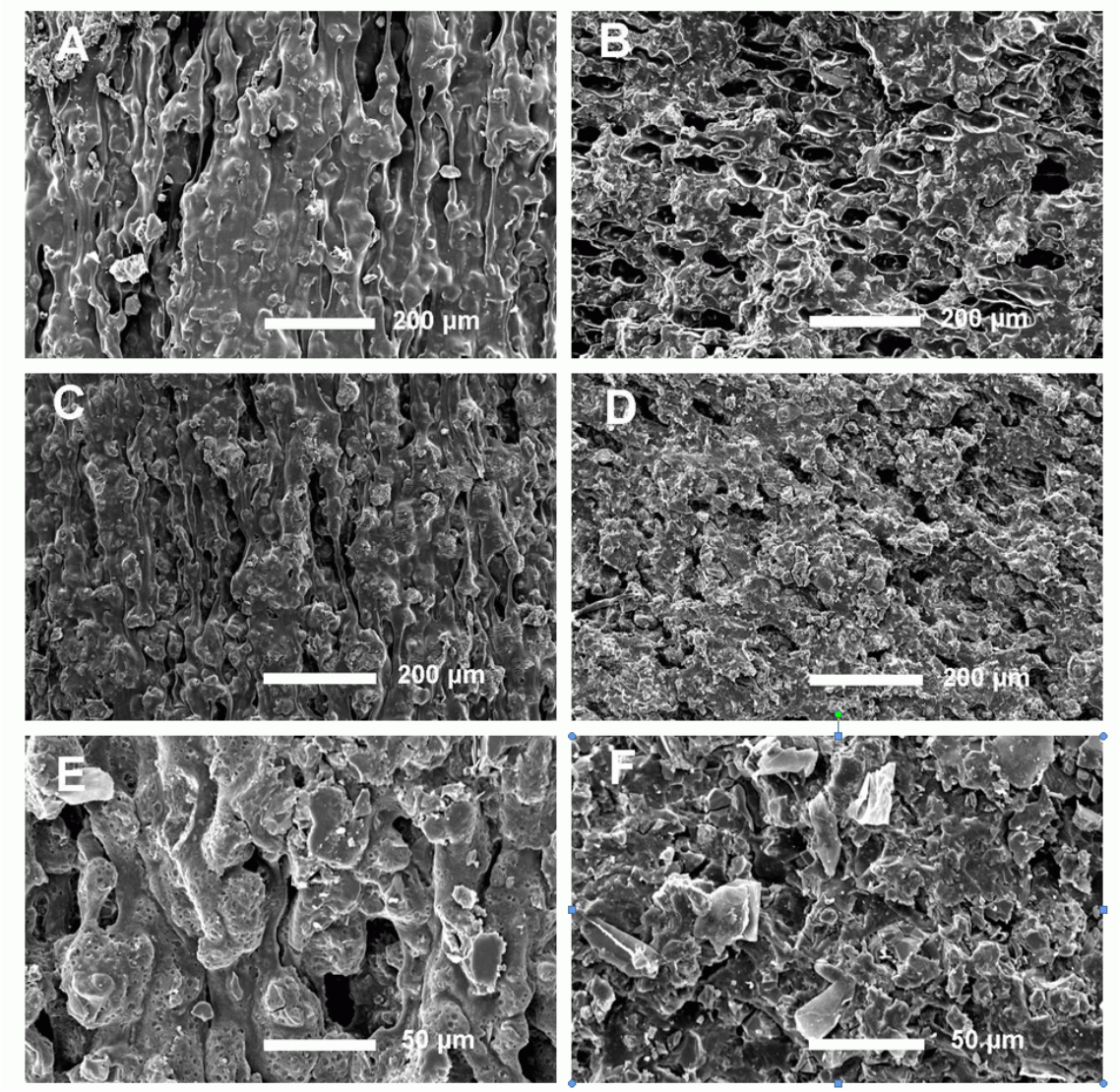

Figure 12. Scanning electron microscopy (SEM) micrographs of the composites of poly(L-lactide-co-ε-caprolactone) (PLCL) and β-tricalcium phosphate (TCP) containing 50 wt-% of TCP. (A) surface at 0 weeks, (B) cross section at 0 weeks, (C) surface at 26 weeks, (D) cross section at 26 weeks, (E) surface at 52 weeks and (F) cross section at 52 weeks (E and F are at higher magnification).

The porosity of the samples containing 50 % of β-TCP (at 0, 26 and 52 weeks) was additionally imaged using µ-CT. Examples of the imaging are shown as Figure 13. The analysis showed surprisingly that the porosity decreased in the samples during

hydrolysis test series. The porosity analysis results are presented as Table 2. Overall, the pore sizes and the porosity values were very small. Additionally, the pores were generally not interconnected. There may however reside an artefact in this analysis, because the samples were imaged dry. If the imaging would have been done wet, right after the samples were withdrawn from the incubator shaker, the results might have been different, because the samples may shrink during drying.

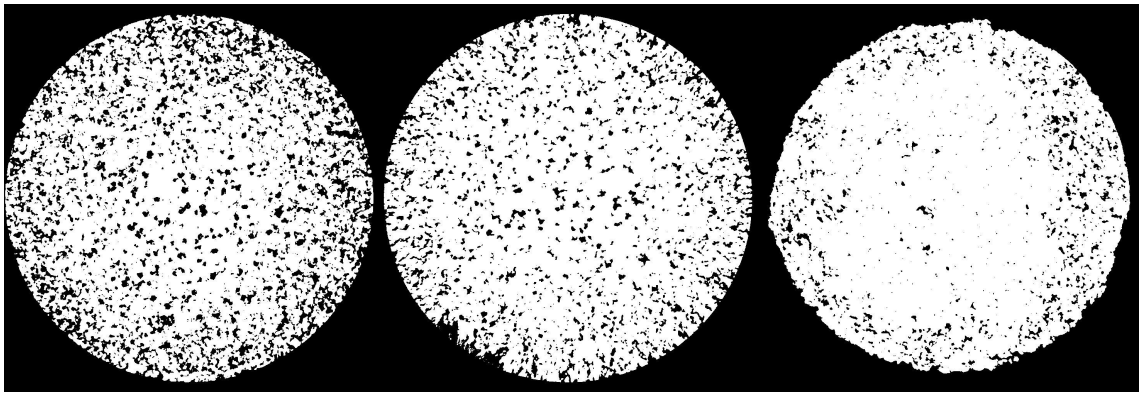

Figure 13. Micro-computed tomography (μ-CT) imaging of samples of poly(L-lactide-co-ε-caprolactone) (PLCL) and β-tricalcium phosphate (TCP) containing 50% of TCP at 0 weeks (left), 26 weeks (middle), and 52 weeks (right).

Table 2. Results of the porosity analysis

Sample name	Porosity (%)	Mean pore size (μm)
PLCL + 50% TCP 0 weeks	10.5	16.10
PLCL + 50% TCP 26 weeks	7.6	13.16
PLCL + 50% TCP 52 weeks	2.6	9.82

The EDS analysis was performed both on the outer surfaces of the samples and the cryogenically fractured surfaces of the samples. It showed that there were no significant differences in the Ca/P ratio of the β-TCP. If β-TCP was dissolved and precipitated again as hydroxyapatite, the Ca/P molar ratio would have increased (it is initially 1.67 for hydroxyapatite and 1.5 for β-TCP) (1). However, this was not seen in the results of the EDS analysis. It should be noted that Sörensen phosphate buffer solution was used in our study, and bioactivity studies are usually performed using simulated body fluid as the buffer solution (26). Similar kinds of composites comprising poly-α-hydroxy acids and β-TCP have been reported to show bioactivity that has been observed as the formation of hydroxyapatite on the surface of the composites when simulated body fluid has been used in *in vitro* studies as the buffer solution (2,3,26).

There were also very small amounts of sodium and potassium present in the samples that had been in hydrolysis for over 26 weeks. Na and K ions are present in the Sörensen phosphate buffer solution and have most likely not been totally washed from the samples during the rinsing of the samples with distilled water before drying.

Conclusions

Composites of poly(L-lactide-co-ε-caprolactone) with an initial comonomer ratio of 70/30 and β-tricalcium phosphate were studied in a long-term *in vitro* test series for up to 52 weeks. The degradation was studied and the effect of β-TCP on the degradation of the composites was evaluated using various analysis methods.

β-TCP had only a slight effect on the degradation properties of the composites studied and the effect on the degradation properties was mainly seen in the water absorption behaviour, as β-TCP dissolution was very slow in comparison with the copolymer

degradation. Mass loss and water absorption behaviour were significantly changed at 12 weeks *in vitro*. At this point, the molecular weight of the polymer had decreased to a level that enables mass loss.

Although the degradation of the tested composites proceeded similarly, there was a slight buffering effect of β-TCP seen throughout the *in vitro* test series in the pH values of the hydrolysis medium. The effect of β-TCP on the degradation was also seen in ^{1}H NMR analysis. It showed that the presence of β-TCP in the composites decelerated the change in the comonomer ratio as the hydrolysis proceeded. Additionally, the copolymer was noticed to have rather blocky structure, where the more amorphous parts degraded first leaving crystalline parts consisting mainly of L-lactide blocks in the structure.

For the end use in bone applications, it is desirable that the polymer degrades relatively quickly, leaving the β-TCP-particles behind to promote bone healing. The results presented here show that the β-TCP content of the tested materials was constantly increased throughout the *in vitro* test series. Additionally, it was shown that even high β-TCP contents can be compounded in the poly(L-lactide-co-ε-caprolactone) 70/30 copolymer without significant effect on the degradation of the polymer. These composite materials show good processability, handling properties, and potential to be used as bone filling materials. In addition, the processing method is easy to scale up to commercial scale.

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