The degradation properties of co-continuous calcium phosphate polyester composites: insights with synchrotron micro-computer tomography

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This study investigates the *in vitro* degradation properties of composites consisting of a porous tricalcium phosphate (TCP) foam filled with degradable poly(DL-lactic acid) (PDLLA) via either *in situ* polymerization or infiltration. The motivation was to develop a material for bone repair that would be initially mechanically strong and would develop porosity during degradation of one of the components. A thorough analysis of the physical *in vitro* degradation properties has been conducted and reported by the same authors elsewhere. Synchrotron micro-computer tomography analysis (conducted at ID19, ESRF, Grenoble, France) allowed detailed insights to be gained into the process of the composites' degradation, which was discovered to be strongly influenced by the manufacturing method. The polymer phase of *in situ*-polymerized TCP–PDLLA degraded as a bulk sample, with faster degradation in the centre of the sample as a whole. In contrast, the polymer phase of infiltrated TCP–PDLLA degraded as individual polymer spheres with faster degradation in the centre of each sphere.

Keywords: poly(lactic) acid; calcium phosphate; composite; degradation; *in vitro* test

1. INTRODUCTION

Porous calcium phosphates, such as hydroxyapatite and tricalcium phosphate (TCP), are very suitable materials for bone repair because of their biocompatibility and osteoconductivity (Galois *et al.* 2002; Daculsi *et al.* 2003; Horch *et al.* 2006). Pore sizes of at least 100 µm and preferably more than 300 µm allow vascularization and proliferation of the newly generated bone (Hutmacher *et al.* 2007). The main drawback of these materials is their limitation to non-major load-bearing application, which arises from their brittle nature and high porosity. Degradable polyesters are of sufficient mechanical stability and can hence be found in orthopaedic applications, for example in fracture fixation and bone replacement (An *et al.* 2000). Upon exposure to the aqueous environment in the human body, polyesters such as poly(DL-lactic acid) (PDLLA) degrade into oligomers that can be metabolized via natural pathways. This is an advantage over metal implants, for which further surgery to remove the implant is necessary (Paivarinta *et al.* 1993). The combination of polymers with calcium

phosphate has been widely studied since this concept offers improved mechanical properties, while maintaining the favourable biocompatibility and osteoconductivity of calcium phosphates (Bonfield *et al.* 1981; Suchanek & Yoshimura 1998). These composites often consist of a degradable polyester matrix to which calcium phosphate fibres or particles are added (Agrawal & Athanasiou 1997; Suchanek & Yoshimura 1998; Imai *et al.* 1999; Heidemann *et al.* 2001; Ignatius *et al.* 2001; Ara *et al.* 2002; Kikuchi *et al.* 2002; Niemela *et al.* 2004a; Niemela 2005). An additional effect of these composites is that, because of the addition of the calcium phosphates, the degradation is delayed (Heidemann *et al.* 2001; Niemela *et al.* 2004a,b) and the pH of the surrounding solution remains stable for longer periods (Agrawal & Athanasiou 1997; Heidemann *et al.* 2001). A more recent development is the study of co-continuous composites consisting of a porous calcium phosphate matrix filled with a reinforcing polymer phase. The motivation for such composites is to create a material that is initially non-porous and mechanically stable and will develop a controlled porosity as the polymer degrades. The remaining calcium phosphate provides an optimized pore architecture for bone ingrowth. This is thought to be a key advantage over composites with dispersed calcium phosphate particles. A possible application is in bone

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Figure 1. Schematic of possible ways of polymer degradation within the composite: (a) bulk degradation; (b) degradation of individual spheres. Black, ceramic; grey, polymer; white, degraded polymer.

repair, where these composites would provide both the required initial mechanical strength and a porous calcium phosphate with optimized pore size and interconnectivity for bone ingrowth, as a result of the degradation of the polymer phase (Farrar *et al.* 2007). In the literature to date, only the manufacture, mechanical properties and bioactivity of these new interpenetrating polymer–ceramic composites have been described (Yunos *et al.* 2008). Reported manufacturing methods included *in situ* polymerization using enzymes (Aizawa *et al.* 2004; Sugiyama *et al.* 2007), microwaves or thermal energy (Pezzotti *et al.* 2002; Jin *et al.* 2006) and vacuum-infiltration of the porous ceramic matrix with the polymer (Miao *et al.* 2005, 2007; Huang & Miao 2007). The mechanical properties were greatly improved over the unfilled ceramic (Nakahira *et al.* 2002; Pezzotti *et al.* 2002; Zhang & Zhang 2002; Li *et al.* 2003; Sharma & Pezzotti 2003; Miao *et al.* 2005, 2007; Jin *et al.* 2006; Huang & Miao 2007). However, to our knowledge, no reports on the degradation properties of these composites have been published.

In this study, the degradation properties of two types of TCP–PDLLA composites are described in detail. Composites were produced either by *in situ* polymerization of D,L-lactide within the porous TCP matrix or by infiltration of molten poly-D,L-lactide granules into the porous TCP. A detailed *in vitro* degradation study was conducted, which monitored the changes in mass, molecular weight, water gain and the pH of the degradation medium. Synchrotron micro-computer tomography (micro-CT) analysis (conducted at ID19, ESRF, Grenoble, France) allowed detailed insights into changes in the sample structure upon degradation.

The widely accepted heterogeneous degradation model of pure polyesters by Li *et al.* (1990*a,b,c*) describes the accelerated degradation in the centre of a sample above a certain thickness. This effect is because of the accumulation of acidic oligomers that are entrapped within the sample and can only be dissolved from near the surface (Li *et al.* 1990*a,b,c*). Typical features of heterogeneous degradation include bimodal molecular weight distribution, more than one glass transition region, visual observation of a less degraded shell and an empty core or a decrease in pH towards the centre of the sample (Li *et al.* 1990*a*;

Li & Vert 1994; Grizzi *et al.* 1995). There is no uniform conclusion in the literature concerning the minimum sample size required for heterogeneous degradation. Some argue that, in thin films or microbeads with a thickness less than 200–300 μm , the degradation products can leave the bulk as soon as they become soluble and do not accelerate the degradation in the centre of the sample because of the proximity of the sample edge (Grizzi *et al.* 1995; Schliecker *et al.* 2003). Others observed heterogeneous degradation even for microspheres and films as small as 10 μm (Park 1994, 1995; Lu *et al.* 1999; Fu *et al.* 2000). A previous study showed that co-continuous TCP–PDLLA composites degraded heterogeneously, but only limited visual evidence could be gained with a small desktop micro-CT (Ehrenfried 2009). Based on these studies, this paper now aims to give a more detailed insight into the degradation properties and to answer the question: *Does the polymer phase within co-continuous composites degrade as one single polymer bulk or rather as an accumulation of individually degrading spheres?* (figure 1).

2. EXPERIMENTAL SECTION

2.1. Materials

2.1.1. TCP–PDLLA composite manufacture. Porous TCP (Synhapor- β -TCP, Dytech, Sheffield, UK) with an average pore size of 500 μm and 75 per cent porosity was purchased. The material was highly interconnected with interconnecting pore sizes ranging from less than 10 μm to 500 μm as characterized with micro-CT. Describing the characterization of the initial TCP in detail is beyond the scope of this paper. Yet, in figure 2*a,b*, micro-CT images of the as-made composites show that the polymer reached all pores, demonstrating the interconnectedness of the TCP.

The *in situ* polymerization of D,L-lactide monomer (Purasorb DL, Purac, Gorinchem, The Netherlands) was conducted with the methods previously described (Ehrenfried 2009). Briefly, the TCP matrix was filled with molten monomer by the application of a vacuum. The polymerization reaction was initiated using a catalyst-initiator solution of tin(II)chloride dehydrate (99.995%, Aldrich) in diethylene glycol (Aldrich) that was added to the D,L-lactide monomer

Figure 2. (a,c) Comparison of *in situ*-polymerized and (b,d) infiltrated TCP–PDLLA composites as made (top row) and after 1.25 days of degradation in PBS at 42°C (bottom row). Circles in the image of as-made infiltrated composites (b) indicate cracks in the composite that were present in the ceramic matrix initially. (a–d) Bar, 200 μm .

under a nitrogen atmosphere. Polymerization was carried out at 180°C for 1 day followed by one week at 90°C.

The infiltrated samples were produced by surrounding the porous TCP with ample PDLLA granules (Purasorb PDL20, Purac) within a heatable cylindrical cavity. The composite was created by slowly pressing the melted polymer into the ceramic matrix followed by cooling of the sample at a constant pressure of 15 MPa. Samples were cut with a diamond blade Struers Accutom-5, ± 0.1 mm) and were stored in a desiccator at room temperature.

2.2. Methods

2.2.1. Degradation. Degradation studies of $2 \times 5 \times 5$ mm samples were conducted in 0.01 M phosphate-buffered saline solution (PBS, Sigma Aldrich) following the method used by Hurrell *et al.* (2003). A ratio of 6 mg of sample to 1 ml of buffer was used. The samples were degraded at a temperature of 42°C, which allows faster degradation of the PDLLA without changes in the degradation mechanisms (Ehrenfried *et al.* 2007). Infiltrated composites were dissolved for up to 105 days, *in situ*-polymerized composites were degraded for up to 80 days. Three samples were investigated per time point to ensure reproducibility of the results.

2.2.2. Physical properties. Throughout the degradation study, sample mass and water absorption were

monitored. The percentage mass loss (m_{loss}) and water absorption (w_{abs}) were calculated from the original mass (m_0), the dry mass after degradation (m_d) and the wet mass (m_w) as displayed in equations (2.1) and (2.2),

$$m_{\text{loss}} = \frac{m_d - m_0}{m_0} \times 100 \quad (2.1)$$

and

$$w_{\text{abs}} = \frac{m_w - m_d}{m_d} \times 100. \quad (2.2)$$

The molecular weight was characterized by gel-permeation chromatography (GPC). Composite samples were separated with a scalpel into ‘edge’ and ‘centre’ regions, which then allowed the calculation of a molecular weight ratio. The molecular weight ratio was obtained by dividing the average molecular weight of the edge by the average molecular weight of the centre of the samples. Samples (in duplicate) were dissolved in chloroform and filtered through 0.45 μm nylon filters before analysis. Calibration was carried out against polystyrene calibrants (Polymerlabs). Further details on the methods can be found in Ehrenfried (2009).

2.2.3. Synchrotron micro-CT. Synchrotron micro-CT was carried out on the ID19 beamline at the European Synchrotron Research Facilities (ESRF) in Grenoble,

France, with an energy of 30 keV, a 2 mm aluminium filter and a 1 mm silicon filter. The sample–detector distance was 400 mm, thus allowing phase-contrast tomography. The resolution was 5.05 μm per pixel. Samples were rotated through 180° with a rotation step of 0.12° and an exposure time of 0.7 s. Reconstruction was done with algorithms provided by the ESRF. The total scan time was 30 min. The samples investigated at the ESRF were, in contrast to those of the long-term degradation study, not exposed to 42°C throughout their degradation time owing to logistical constraints. The degradation of these samples was started in the laboratories in Cambridge at 42°C, but samples needed to be sent to the ESRF in advance of the scheduled beam time, where they were left at room temperature for at least 4 days. It must also be noted that the initial molecular weight of the batch of the *in situ*-polymerized samples used in the micro-CT study was slightly below the average of all *in situ*-polymerized batches used in the degradation study. This is because of the nature of the *in situ* polymerization process.

Synchrotron micro-CT was preferred over electron microscopy, which requires drying and cutting of the samples before analysis. These processes destroy important features of the polymer, for example causing highly swollen regions to collapse. Tomography also allowed non-destructive imaging of the entire sample, enabling comparison of the edge and the centre of the sample. Desktop micro-CT was attempted, but did not give high-quality images. This was because, at the voltages required to penetrate the TCP, the contrast between the polymer and the air was marginal.

3. RESULTS

3.1. Water uptake, mass loss and molecular weight changes

The degradation properties of TCP–PDLLA composites were studied in detail as reported elsewhere (Ehrenfried 2009). Briefly, the mass loss and water gain were much faster in *in situ*-polymerized composites than in infiltrated composites (figure 3*a,b*). Mass loss started after 20 days for *in situ* composites and after only 80 days for infiltrated composites. The molecular weight also decreased faster in the *in situ*-polymerized composites (figure 3*c*). Since the overall understanding of the composite degradation process is based on the results of both the degradation and the ESRF study, the physical degradation properties of both studies must be comparable. Figure 3*a,b* compares water absorption and mass loss data for *in situ*-polymerized and infiltrated TCP–PDLLA composites from the ESRF study (one sample per time point only) with the degradation study (performed in triplicate). Both water absorption and water gain data were in good agreement. The molecular weight decrease was similar in both studies, with a slightly faster rate in the degradation study for both types of composite (figure 3*c*). This is thought to be due to the shorter exposure to 42°C of the ESRF samples. From these results, it was

Figure 3. (a) Water absorption and mass loss of infiltrated and (b) *in situ*-polymerized TCP–PDLLA composites. Crosses, water absorption (deg. study); open diamonds, mass loss (deg. study); filled triangles, water absorption (ESRF); filled diamonds, mass loss (ESRF). Samples were investigated during a long-term degradation study (triplicates) and with synchrotron micro-CT at the ESRF (one sample per time point). Molecular weight changes for both *in situ*-polymerized and infiltrated composites are shown in (c). Open squares, infiltrated TCP–PDLLA (ESRF); filled squares, infiltrated TCP–PDLLA (deg. study); grey diamonds, *in situ* TCP–PDLLA (ESRF); black diamonds, *in situ* TCP–PDLLA (deg. study).

concluded that comparison and combined analysis of both sets of data are reasonable.

Synchrotron micro-CT gave very detailed insights into the degradation of TCP–PDLLA composites. First, for both manufacturing methods, the as-made samples and composites were compared after immersion for 1.25 days in PBS at 42°C. Then later degradation time points are presented, first for the *in situ*-polymerized, then for the infiltrated samples. The water absorption and mass loss of the samples shown in the micro-CT images are listed in table 1. Since only one sample was scanned per time point, no standard deviations are given in table 1. However, the degradation parameters reported here show good agreement with the separate study performed in triplicate.

Table 1. Comparison of mass loss and water absorption of the samples investigated with micro-CT and shown in figures 4 and 5. Only one sample was investigated per time point. However, the degradation parameters reported here show good agreement with the separate study performed in triplicate.

	<i>in situ</i> -polymerized TCP–PDLLA					infiltrated TCP–PDLLA				
deg. time (days)	0	1.25	5	14	25	0	1.25	10	35	50
mass loss (wt%)	0	0.7	1.6	1.8	8.7	0	0.3	0.5	0.4	0.6
water absorption (wt%)	0	5.3	8.2	44.3	68.4	0	2.4	3.5	40.7	43.3

3.2. Tomographic images of as-made composites and initial water uptake

Figure 2*a,b* shows the composites in the as-made state. Both samples were cut with a diamond blade, resulting in a straight edge. The *in situ*-polymerized sample has only very few remaining air bubbles, whereas in the infiltrated composite many air bubbles remain. Quantitative micro-CT image analysis measured a remaining porosity of 0.3 per cent of the total sample volume for *in situ*-polymerized composites, while infiltrated composites had a remaining porosity of 4.0 per cent.

Several small cracks are present in the TCP matrix of the infiltrated composites (circles in figure 2*b*). In addition to these visual differences, the molecular weight was greater in the infiltrated composites (M_{n0} (*in situ*) = 15 100 dalton, M_{n0} (infiltrated) = 84 500 dalton).

The initial water absorption study showed no visual changes in the sample structure of the infiltrated TCP–PDLLA composites during the first 1.25 days of degradation (figure 2*d*). In contrast, in the *in situ*-polymerized samples, after 1.25 days, the first swelling of the polymer phase was observed (figure 2*c*). Water absorption at this time point was 2.4 per cent for infiltrated and 5.3 per cent for *in situ*-polymerized composites.

3.3. Tomographic images of degradation

3.3.1. In situ-polymerized TCP–PDLLA. Figure 4 shows micro-CT images of *in situ*-polymerized TCP–PDLLA composites after various degradation times in PBS. All samples were scanned while immersed in PBS.

After 5 days, further swelling and thin cracks in the ceramic matrix can be observed (figure 4*a*). Water has reached the centre of the sample, as can be seen in partly filled air bubbles in the centre of the sample (figure 4*b*).

After 14 days, a range of new features are visible. Significant swelling of the edge of the sample to distances up to 800 μm (figure 4*c*) is observed. Very small pores (less than 50 μm ; referred to as ‘foaminess’) are observed around the edge of the sample (for example as indicated by the top arrow in figure 4*d*). Small individual pores of size 50–100 μm are seen in the centre of the swollen polymer spheres at the edge of the sample (for example as indicated by the middle arrow in figure 4*d*). Finally, large individual pores of size 100–200 μm are formed in proximity to the polymer–ceramic interface at the edge of the sample (as indicated by the bottom arrow in figure 4*d*).

After 25 days, the edge of the composite was composed of extremely swollen and foamy polymer spheres with more than 1000 μm swelling from the original edge (figure 4*e,g*). The foaminess of the polymer was only present near the sample edge (figure 4*g,h*), and not in the centre (figure 4*e,f*). Small polymer spheres, even if in close proximity to the edge, had developed only very limited porosity. Only polymer spheres with sizes smaller than or equal to around 300 μm had no or very limited porosity.

3.3.2. Infiltrated TCP–PDLLA. Figure 5 shows micro-CT images of infiltrated TCP–PDLLA composites after various degradation times in PBS. All samples were scanned while immersed in PBS.

After degradation for 10 days in PBS, water absorption into the pores was observed, but no swelling of the polymer phase was notable (figure 5*a*). Cracks in the ceramic matrix that were presumably caused during the infiltration process opened up (highlighted by circles).

After 35 days of degradation, micro-CT images show slight swelling of the polymer phase around the edge (less than 200 μm ; figure 5*b*). Foaminess is seen in the centre of very big polymer spheres (around 2000 μm diameter; figure 5*c*). Cracks in the ceramic matrix opened up further (circles in figure 5*b*).

After 50 days of degradation, there is still only slight swelling of the polymer phase at the edge of the sample (less than 200 μm ; figure 5*d*). Large pores can be seen in the centre of the big polymer spheres (greater than 600 μm ; figure 5*e*). Foaminess is seen at the edges of big polymer spheres and in smaller polymer spheres (figure 5*f*). However, no difference in the amount of foaminess is observed between the centre and the edges of the sample. Further cracking of the ceramic matrix is also seen (figure 5*d*).

3.3.3. Molecular weight. Molecular weight and glass transition measurements of the sample bulk indicated heterogeneous polymer degradation for both types of composites, as shown by the formation of two main peaks in the molecular weight distribution curve and two glass transition regions (reported in detail in Ehrenfried (2009)). The micro-CT images suggest that the heterogeneous degradation process for infiltrated and *in situ*-polymerized composites may not be the same. Therefore, an analysis of the molecular weight of the centre and the edge of the composites after degradation was performed for both the ESRF and the degradation study. The aim was to determine whether

Figure 4. Micro-CT images of *in situ*-polymerized composites after degradation. (a) Five days (magnified section: b), (c) 14 days* (magnified section: d), (e) 25 days* (central slice, magnified sections: f and g; h: slice close to the edge). (a, c, e, h) Bar, 200 μm (not given for the magnifications). In samples highlighted with asterisks (*), the greyscale range was adjusted to increase the contrast between polymer and PBS.

the heterogeneous degradation of the co-continuous composites used in this study remained the same as observed for pure polymer samples with faster degradation in the centre of the bulk sample (Li *et al.* 1990a,b,c) or if different mechanisms were in place.

3.3.4. Molecular weight ratios (edge/centre). Figure 6 displays the average molecular weight ratio over degradation time. The molecular weight ratio was obtained by dividing the molecular weight of the edge by the molecular weight of the centre of the samples. The

Figure 5. Micro-CT images of infiltrated TCP–PDLLA composites after degradation for (a) 10 days, (b) 35 days* (magnified section: c), (d) 50 days* (magnified sections: e, f). (a, b, d) Bar, 200 μm (not given for the magnifications). In samples highlighted with asterisks (*), the greyscale range was adjusted to increase the contrast between polymer and PBS.

measurements were performed on samples from both the ESRF and the main degradation study. This way of plotting the data gives an indication of when the degradation is faster in the edge ($\text{Mn}_{\text{edge}}/\text{Mn}_{\text{centre}} < 1$) and when it is faster in the centre ($\text{Mn}_{\text{edge}}/\text{Mn}_{\text{centre}} > 1$). Figure 6 shows very clear trends for *in situ*-polymerized samples

with a faster degradation in the centre after only 10 days. After around 30 days their average molecular weight was three times higher in the edge than in the centre. For infiltrated TCP–PDLLA composites, the average molecular weights of the edge and the centre remained almost constant.

Figure 6. Ratio of molecular weight (Mn_{edge}/Mn_{centre}) for TCP composites degraded in PBS at 42°C for various times. Samples were from the main degradation study and the additional degradation for synchrotron measurements. Values less than 1 indicate a faster degradation in the edge, whereas values greater than 1 indicate a faster degradation in the centre. Filled diamonds, *in situ* TCP–PDLLA (deg. study); filled squares, infiltrated TCP–PDLLA (deg. study); open diamonds, *in situ* TCP–PDLLA (ESRF); open squares, infiltrated TCP–PDLLA (ESRF).

3.3.5. Polydispersity index. The polydispersity index (PDI) is calculated by dividing the weight average molecular weight (Mw) by the number average molecular weight (Mn) ($PDI = Mw/Mn$). This is a measure of the broadness of the molecular weight distribution, since Mw is influenced by the high-molecular-weight species and Mn is influenced by the low-molecular-weight species. Figure 7 shows the PDI as a function of degradation time for *in situ*-polymerized and infiltrated TCP–PDLLA composites from both the degradation study and the ESRF experiment and both the centre and the edge of the sample. It is clear that the PDI of the edge and the centre of a given sample were about the same. *In situ*-polymerized samples maintained a PDI of 2–2.4 (figure 7, diamond symbols), while the PDI of infiltrated samples increased during degradation to over 9 (figure 7, square symbols).

4. DISCUSSION

In §4.1, we provide an overview of the differences in physical properties of *in situ*-polymerized and infiltrated TCP–PDLLA composites, which were discussed in detail in Ehrenfried (2009). Then, in §4.2, we give an overview of the features revealed by synchrotron micro-CT. We explain how these features may have formed and how they relate to the physical properties. Finally, in §4.3, we explain how the physical and structural observations may be linked to the manufacturing methods and initial properties of the composites.

4.1. Influence of manufacturing method on physical degradation properties

The lower number of air bubbles remaining in the *in situ* composites was presumably due to the application of a vacuum during the manufacturing process, which allowed a very good penetration of the TCP matrix with the monomer and hence the polymer (Ehrenfried 2009).

Figure 7. Polydispersity index ($PDI = Mw/Mn$) for TCP–PDLLA samples after degradation. The open symbol represents the edge of the sample; the closed symbol the centre of the sample. Lighter colours refer to samples investigated at the ESRF; dark symbols refer to samples from the degradation study. Grey filled triangle, *in situ* as made; grey open diamond, *in situ*, ESRF, edge; black open diamond, *in situ*, deg. study, edge; grey open square, infiltrated, ESRF, edge; black open square, infiltrated, deg. study, edge; black filled triangle, infiltrated as made; grey filled diamond, *in situ*, ESRF, centre; black filled diamond, *in situ*, deg. study, centre; grey filled square, infiltrated, ESRF, centre; black filled square, infiltrated, deg. study, centre.

As a result of their lower initial molecular weight, more endgroups are present in *in situ*-polymerized composites and their hydrophilicity is increased (Schliecker *et al.* 2003). Hence, they absorbed water more quickly and degraded faster than infiltrated composites, as measured by monitoring water gain, mass loss and decrease in molecular weight (figure 3; Park 1994; Ehrenfried 2009).

Molecular weight distribution curves of the bulk material showed asymmetrical broadening and skewing of the peaks, which points towards the presence of populations of polymer chains of high and low molecular weight (Ehrenfried 2009). The presence of two glass transition temperatures was consistent with this measurement (Ehrenfried 2009). Both of these results suggest that areas with different degrees of degradation are present within the polymer phase (Park 1994). The study presented here reports the distinct measurements of the respective molecular weights of edge and centre, which allowed the calculation of a molecular weight ratio between these regions (figure 6).

For *in situ*-polymerized samples, the molecular weight ratio increased with degradation time. After 20 days, the molecular weight (both Mn and Mw) of the centre was up to three times smaller than the edge (figure 6). This leads to the assumption that the composite degraded as a bulk polymer with accelerated degradation in the centre (bulk degradation model, figure 1a). The PDI in both the edge and the centre of *in situ*-polymerized samples showed only slight, irregular variations between 2.0 and 2.4. This indicates that, within both the edge and the centre, the molecular weight decreased fairly uniformly with time, albeit at different rates.

For infiltrated TCP–PDLLA composites, separate investigation of the edge and the centre showed that, within the time frame investigated, the degradation in the edge and in the centre took place at a very similar rate. The molecular weight ratio remained around 1

throughout the investigated time (figure 6). Hence, the differences in molecular weight distribution curves were not as pronounced as for the *in situ*-polymerized samples. Interestingly, in contrast to the *in situ*-polymerized samples, the PDI of infiltrated composites increased from 2 to over 9 during the time frame observed (figure 7). This indicates that, within the respective regions of the sample (edge, centre), there is a wide spread of molecular weights present which points towards the ‘individual sphere degradation’ process depicted in figure 1b.

4.2. Overview of the most important features in micro-CT

4.2.1. Cracks in the ceramic matrix. Infiltrated samples showed wide cracks only after 10 days of degradation when only 3.5 per cent water had been absorbed (figure 5a). The damage to the ceramic matrix increased with degradation time and hence water absorption and polymer swelling (figure 5b,d). During the infiltration process, the molten polymer was pushed into the ceramic matrix. This may have damaged the ceramic considerably, even though not all cracks were necessarily visible with micro-CT (e.g. hairline cracks). In the *in situ* polymerization process, less force was applied on the ceramic, resulting in few or no cracks in the ceramic of both the as-made and the degraded material. As the polymer phase swelled in the infiltrated samples, pre-existing hairline cracks in the ceramic phase opened and became visible.

4.2.2. Water absorption and swelling. In *in situ*-polymerized samples, the first bulging of the polymer spheres at the edge of the sample was observed after 1.25 days, when only 5 per cent water absorption was measured (figure 2c). This bulging strongly increased with degradation to more than 1000 μm after 25 days. However, in the centre of this sample, no swelling was observed, and the ceramic was almost intact (figure 4). This might hinder excessive water absorption of the bulk polymer. The early water absorption and the excessive swelling seen in *in situ*-polymerized samples are presumably a result of their lower initial molecular weight because in infiltrated samples only slight swelling of the polymer phase was observed. In the infiltrated samples, this slight swelling was sufficient to open up the cracks in the ceramic matrix, which are apparently caused during the infiltration process.

4.2.3. Foaminess. The term ‘foaminess’ describes an accumulation of pores within the polymer phase. It is possible to theorize that foaminess is caused in two different ways. The very small pores along the polymer edge of *in situ*-polymerized samples after 14 days may be caused by the first dissolution of oligomers (figure 4d). An increase in T_g was observed for *in situ*-polymerized samples after 7, 10 and 20 days of degradation (Ehrenfried 2009), which may be linked to the dissolution of short oligomers or monomers (Park 1994; Schliecker *et al.* 2003). The formation of a porous surface of degrading polyester microspheres was also reported in the literature (Witt & Kissel 2001). However, this mechanism cannot account

for the majority of the incidences in the bulk of the materials and when little or no material loss has occurred.

Foaminess can also be explained by another mechanism which is very plausible for foaminess in the bulk, which was observed in *in situ* samples after 25 days and in infiltrated samples after 35 and 50 days of degradation (figures 4e and 5b,d). This mechanism may also explain the foaminess along the edge of the sample (*in situ*-polymerized samples days 10 and 14). In this second mechanism, foaminess is possible even before the onset of considerable mass loss, which was the case for infiltrated composites after 35 and 50 days. It is assumed that highly degraded soluble oligomers are enclosed in the bulk polymer of higher average molecular weight. Such highly degraded oligomers can first be found in near-edge regions (earlier hydration) and in the centre of big polymer spheres where accumulated acidic degradation products accelerate the degradation. The oligomers are dissolved by the PBS present in the sample, forming a droplet of highly concentrated oligomer solution. This creates an osmotic pressure between the external solution and the droplet. This pressure drives further solution into the sample, thus further increasing the size of the droplet and causing the polymer to swell. The formation and swelling of internal droplets in polymer have also been observed for dissolvable calcium phosphate particles in polymeric bone cement (Rahman 2004) and impurities in rubber (Fedors 1979). It has also been reported that the water uptake to PDLLA was reduced when the samples were immersed in a solution of pH 3.7 in comparison with one of pH 7.4 (Vert *et al.* 1991). This was attributed to a reduced osmotic pressure at a lower pH.

Summarizing, it is proposed that foaminess is formed when both low-molecular-weight groups and sufficient solution are available. In the case of *in situ*-polymerized composites degraded for 25 days, foaminess can be observed only in proximity to the sample edge, but not in the centre. This indicates that additional solvent could not access the centre, probably because of the largely intact ceramic matrix that resists the osmotic pressure. The limited diffusion of water into and oligomers out of the sample is thought to be the reason why *in situ*-polymerized composites degrade as ‘bulk polymer’: this was also observed from the molecular weight ratio of the edge to the centre, which was greater than 1 after degradation (figure 6). Infiltrated composites, however, appeared to degrade as ‘individual polymer spheres’, uniformly throughout the sample. Foaminess was observed in both the edge and the centre of the bulk sample because water could easily access the more open sample structure, perhaps facilitated by cracks in the ceramic. Measurements of molecular weight ratio (approx. 1, figure 6) and PDI (which increased for both the centre and the edge over degradation time, figure 7) also point towards this interpretation.

4.2.4. Individual large pores within the polymer spheres. Large pores were observed in the centre of big polymer spheres throughout some samples, in particular the infiltrated composites degraded for 50 days (figure 5d). These pores are thought to be a

super-sized version of the droplets of oligomer solution, previously described as foaminess. In the case of *in situ*-polymerized samples, such pores were observed only in close proximity to the edge, where, in addition to low-molecular weight groups, sufficient water supply was available (figure 4e).

4.2.5. Buffering. Both *in situ*-polymerized and infiltrated samples showed reduced foaminess and porosity of the polymer phase in proximity to the TCP matrix and around the sample edge. For example, in infiltrated composites after 50 days of degradation, large pores formed only within polymer spheres with a diameter exceeding 600 μm (figure 5e). In *in situ*-polymerized composites after 25 days foaminess was observed in large polymer spheres (figure 4e). However, polymer spheres less than 300 μm developed only very limited porosity. This suggests that the degradation was enhanced in the centre of large polymer spheres, where acidic oligomers accumulated. On the edge of polymer spheres, the degradation was decreased either because of the buffering effect of the ceramic or, on the very edge of the sample, because oligomers could dissolve. The better neutralization of pH in the centre of smaller polymer spheres has previously been observed in a degradation study of polylactic-co-glycolic acid microspheres (Fu *et al.* 2000).

4.3. Influence of manufacturing method on physical and structural observations

Both physical properties and micro-CT analysis revealed several differences in the degradation of TCP–PDLLA composites, depending on their manufacturing method. The degradation processes, as observed from physical and structural evidence, can be summarized for *in situ*-polymerized and infiltrated TCP–PDLLA composites as follows.

4.3.1. *In situ*-polymerized TCP–PDLLA. As a result of the manufacturing process, the molecular weight was lower in these composites. Therefore, water was absorbed quickly and the polymer started swelling at early time points in a very pronounced manner, with more than 100 μm polymer swelling observed at the sample edge (figure 4e). Within a short time, some polymer chains were sufficiently degraded to go into solution, which was observed by the early onset of mass loss. The first foaminess around the very edge of the sample may also be a result of this very first mass loss. The *in situ* polymerization process was conducted with the application of a vacuum, which allowed the liquid monomer to penetrate the ceramic matrix without damaging the ceramic structure. The intact ceramic matrix restricted diffusion of water and oligomers across the sample and also prevented significant swelling of the polymer. Therefore, the polymer phase behaved like a single polymer sample, developing faster degradation in the centre, where acidic degradation products accumulated (seen visually and measured with distinct molecular weight measurements). Since foaminess is thought to be a result of oligomer dissolution and further water uptake owing

to the osmotic pressure created, these facts also explain why foaminess was observed only at the edge and not at the centre.

4.3.2. Infiltrated TCP–PDLLA. The composite was created by melting and slowly pressing purchased polymer granules into the ceramic matrix. The polymer granules were produced under arguably more optimum conditions than the polymer in the *in situ* polymerization process; therefore, the molecular weight of these composites was higher. As a result of their higher molecular weight, infiltrated composites had a slower water uptake rate, degraded more slowly and lost mass at later time points. The pressure necessary for infiltration with molten polymer appeared to have damaged the ceramic matrix. This facilitated the diffusion of oligomers out of the sample and water into the sample. Therefore, the polymer spheres behaved as individual spheres rather than as one bulk polymer. This could be seen in micro-CT images, where big pores and foaminess formed throughout the sample. The molecular weight ratio, showing similar changes between the edge and centre of the composite, but an increasing PDI, confirmed these results.

5. CONCLUSIONS

This study showed that the degradation properties of co-continuous polymer–ceramic composites are strongly influenced by the manufacturing method. It presents, for the first time, detailed visual insights into the degradation process of co-continuous composites. As a result of the manufacturing method, *in situ*-polymerized samples had a lower initial molecular weight and absorbed water more rapidly and degraded faster than infiltrated composites. Heterogeneous polymer degradation was observed in both *in situ*-polymerized and infiltrated composites. Distinct molecular weight measurements suggest that *in situ*-polymerized composites degrade as a bulk with faster degradation in the centre of the sample, whereas the polymer phase in infiltrated composites appears to degrade as individual spheres with faster degradation in the centre of each sphere. These results were substantiated with evidence from micro-CT analysis. Micro-CT gave visual evidence for faster water absorption and mass loss of *in situ*-polymerized samples. Micro-CT also confirmed that *in situ*-polymerized composites degrade as a bulk with faster degradation in the centre of the sample, whereas, in infiltrated composites, the polymer spheres appear to degrade individually with faster degradation in the centre of each sphere.

The investigations revealed that both manufacturing methods produce samples with some unfavourable properties: significant swelling and fast degradation of *in situ*-polymerized composites and cracking of the ceramic matrix of infiltrated composites. These properties challenge the suitability of the concept of interpenetrating polymer–ceramics for bone-replacement applications, if PDLLA is used as the filler. Further optimization of the polymer phase is therefore required in order to maintain the porous ceramic

structure and to serve as a scaffold for bone ingrowth after the degradation of the polymer. A surface-degrading polyester could be obtained by irradiating the composite with an electron beam, which decreases the molecular weight at the surface, resulting in its faster degradation (Leonard *et al.* 2009). Reduced swelling of the polymer phase can be achieved by the use of PLLA with the addition of lauric acid (PLLA-la; Renouf-Glauser *et al.* 2005, 2006). Infiltrated TCP-PLLA-la composites indeed have shown some promising initial results with respect to their water absorption and swelling behaviour (Ehrenfried 2009).

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REFERENCES

- Agrawal, C. M. & Athanasiou, K. A. 1997 Technique to control pH in vicinity of biodegrading PLA-PGA implants. *J. Biomed. Mater. Res.* **38**, 105–114. (doi:10.1002/(SICI)1097-4636(199722)38:2<105::AID-JBM4>3.0.CO;2-U)
- Aizawa, M., Ito, M., Takeoka, Y., Rikukawa, M. & Okada, I. 2004 Fabrication of porous tricalcium phosphate ceramics from calcium-phosphate fibers for a matrix of biodegradable ceramics/polymer hybrids. *Phosphorus Res. Bull.* **17**, 213–214.
- An, Y. H., Woolf, S. K. & Friedman, R. J. 2000 Pre-clinical *in vivo* evaluation of orthopaedic bioabsorbable devices. *Biomaterials* **21**, 2635–2652. (doi:10.1016/S0142-9612(00)00132-0)
- Ara, M., Watanabe, M. & Imai, Y. 2002 Effect of blending calcium compounds on hydrolytic degradation of poly (DL-lactic acid-co-glycolic acid). *Biomaterials* **23**, 2479–2483. (doi:10.1016/S0142-9612(01)00382-9)
- Bonfield, W., Grynpas, M. D. & Tully, A. E. 1981 Hydroxyapatite reinforced polyethylene—a mechanically compatible implant material for bone replacement. *Biomaterials* **2**, 185–186. (doi:10.1016/0142-9612(81)90050-8)
- Daculsi, G., Laboux, O., Malard, O. & Weiss, P. 2003 Current state of the art of biphasic calcium phosphate bioceramics. *J. Mater. Sci.-Mater. Med.* **14**, 195–200. (doi:10.1023/A:1022842404495)
- Ehrenfried, L. M. 2009 Co-continuous degradable polyester-calcium-phosphate composite for bone-replacement applications. PhD thesis, University of Cambridge, Cambridge, UK.
- Ehrenfried, L. M., Farrar, D., Morsley, D. & Cameron, R. E. 2007 Accelerated degradation by increased temperature to study degradable polymer-bioceramic composites. In *Proc. 21st ESB Conference, European Society of Biomaterials, Brighton, UK, 10–13 September 2007*.
- Farrar, D. F., Brown, M., Hall, M., Brunelle, J. E., Cotton, N. J., Berube, R. & Lipchitz, J. 2007 *Tissue repair and replacement*. US 2007/0129810 A1.
- Fedors, R. F. 1979 Osmotic effects in water absorption by polymers. *Polymer* **21**, 207–212. (doi:10.1016/0032-3861(80)90062-2)
- Fu, K., Pack, D. W., Klivanov, A. M. & Langer, R. 2000 Visual evidence of acidic environment within degrading poly(lactic-co-glycolic acid) (PLGA) microspheres. *Pharm. Res.* **17**, 100–106. (doi:10.1023/A:1007582911958)
- Galois, L., Mainard, D. & Delagoutte, J. P. 2002 Beta-tricalcium phosphate ceramic as a bone substitute in orthopaedic surgery. *Int. Orthop.* **26**, 109–115.
- Grizzi, I., Garreau, H., Li, S. & Vert, M. 1995 Hydrolytic degradation of devices based on poly (DL-lactic acid) size-dependence. *Biomaterials* **16**, 305–311. (doi:10.1016/0142-9612(95)93258-F)
- Heidemann, W., Jeschkeit, S., Ruffieux, K., Fischer, J. H., Wagner, M., Kruger, G., Wintermantel, E. & Gerlach, K. L. 2001 Degradation of poly(D,L)lactide implants with or without addition of calcium phosphates *in vivo*. *Biomaterials* **22**, 2371–2381. (doi:10.1016/S0142-9612(00)00424-5)
- Horch, H. H., Sader, R., Pautke, C., Neff, A., Deppe, H. & Kolk, A. 2006 Synthetic, pure-phase betatricalcium phosphate granules (Cerasorb (R)) regeneration in the ceramic for bone reconstructive surgery of the jaws. *Int. J. Oral Maxillofac. Surg.* **35**, 708–713. (doi:10.1016/j.ijom.2006.03.017)
- Huang, X. & Miao, X. 2007 Novel porous hydroxyapatite prepared by combining H₂O₂ foaming with PU sponge and modified with PLGA and bioactive glass. *J. Biomater. Appl.* **21**, 351–375.
- Hurrell, S., Milroy, G. E. & Cameron, R. E. 2003 The degradation of polyglycolide in water and deuterium oxide. Part 1: the effect of reaction rate. *Polymer* **44**, 1421–1424. (doi:10.1016/S0032-3861(02)00883-2)
- Hutmacher, D. W., Schantz, J. T., Lam, C. X. F., Tan, K. C. & Lim, T. C. 2007 State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J. Tissue Eng. Regen. Med.* **1**, 245–260. (doi:10.1002/term.24)
- Ignatius, A. A., Augat, P. & Claes, L. E. 2001 Degradation behaviour of composite pins made of tricalcium phosphate and poly (D,L-lactide). *J. Biomater. Sci. Polym. Ed.* **12**, 185–194. (doi:10.1163/156856201750180915)
- Imai, Y., Nagai, M. & Watanabe, M. 1999 Degradation of composite materials composed of tricalcium phosphate and a new type of block polyester containing a poly (L-lactic acid) segment. *J. Biomater. Sci. Polym. Ed.* **10**, 421–432. (doi:10.1163/156856299X00180)
- Jin, H. H., Min, S. H., Hwang, K. H., Park, I. M., Park, H. C. & Yoon, S. Y. 2006 Synthesis of biodegradable beta-TCP/PLGA composites using microwave energy. *Mater. Sci. Forum* **510–511**, 758–761. (doi:10.4028/www.scientific.net/MSF.510-511.758)
- Kikuchi, M., Koyama, Y., Takakuda, K., Miyairi, H., Shirahama, N. & Tanaka, J. 2002 *In vitro* change in mechanical strength of beta-tricalcium phosphate/copolymerized PLA composites and their application for guided bone regeneration. *J. Biomed. Mater. Res.* **62**, 265–272. (doi:10.1002/jbm.10248)
- Leonard, D. J., Pick, L. T., Farrar, D. F., Dickson, G. R., Orr, J. F. & Buchanan, F. J. 2009 The modification of PLA and PLGA using electron-beam radiation. *J. Biomed. Mater. Res. Part A* **89A**, 567–574. (doi:10.1002/jbm.a.31998)
- Li, S. M. & Vert, M. 1994 Morphological changes resulting from the hydrolytic degradation of stereocopolymers derived from L-lactides and DL-lactides. *Macromolecules* **27**, 3107–3110. (doi:10.1021/ma00089a031)
- Li, S. M., Garreau, H. & Vert, M. 1990a Structure–property relationships in the case of the degradation of massive aliphatic poly-(a-hydroxy acids) in aqueous media. Part

- 1: poly (DL-lactic acid). *J. Mater. Sci. Mater. Med.* **1**, 123–130. (doi:10.1007/BF00700871)
- Li, S. M., Garreau, H. & Vert, M. 1990*b* Structure–property relationships in the case of the degradation of massive poly-(α -hydroxy acids) in aqueous media. Part 2: degradation of lactide-glycolide copolymers: PLA 37.5GA25 and PLA75GA25. *J. Mater. Sci. Mater. Med.* **1**, 131–139. (doi:10.1007/BF00700872)
- Li, S. M., Garreau, H. & Vert, M. 1990*c* Structure–property relationships in the case of the degradation of massive poly-(α -hydroxy acids) in aqueous media. Part 3: influence of the morphology of poly(L-lactic acid). *J. Mater. Sci. Mater. Med.* **1**, 198–206. (doi:10.1007/BF00701077)
- Li, S. H., de Wijn, J. R., Layrolle, P. & de Groot, K. 2003 Toughening of HA through interpenetrating organic/inorganic microstructure. *Key Eng. Mater.* **240–242**, 147–150. (doi:10.4028/www.scientific.net/KEM.240-242.147)
- Lu, L. C., Garcia, C. A. & Mikos, A. G. 1999 *In vitro* degradation of thin poly(DL-lactic-co-glycolic acid) films. *J. Biomed. Mater. Res.* **46**, 236–244. (doi:10.1002/(SICI)1097-4636(199908)46:2<236::AID-JBM13>3.0.CO;2-F)
- Miao, X., Lim, W. K., Huang, X. & Chen, Y. 2005 Preparation and characterization of interpenetrating phased TCP/HA/PLGA composites. *Mater. Lett.* **59** (doi:10.1016/j.matlet.2005.07.062)
- Miao, X., Tan, L. P., Tan, L. S. & Huang, X. 2007 Porous calcium phosphate ceramics modified with PLGA-bioactive glass. *Mater. Sci. Eng. C Biomimet. Supramol. Syst.* **27**, 274–279.
- Nakahira, A., Tamai, M., Miki, S. & Pezzotti, G. 2002 Fracture behaviour and biocompatibility evaluation of nylon-infiltrated porous HA. *J. Mater. Sci.* **37**, 4425–4430. (doi:10.1023/A:1020681309572)
- Niemela, T. 2005 Effect of beta-tricalcium phosphate addition on the *in vitro* degradation of self-reinforced poly-L,D-lactide. *Polym. Degrad. Stab.* **89**, 492–500. (doi:10.1016/j.polymdegradstab.2005.02.003)
- Niemela, T., Kellomäki, M. & Tormala, P. 2004*a* *In vitro* degradation of osteoconductive poly-L/DL-lactide beta-TCP composites. In *Proc. 16th Int. Symp. on Ceramics in Medicine, Porto, Portugal, 69 November 2003*. Key Engineering Materials, vol. 254-2, pp. 509–512. Zurich, Switzerland: Trans Tech.
- Niemela, T., Kellomäki, M. & Törmälä, P. 2004*b* Self-reinforced composites of bTCP and bioabsorbable polymer *in vitro*: comparison of two matrix polymers. In *Proc. 7th World Biomaterials Congress 2004, Sydney, Australia, 17–21 May 2004*. Red Hook, NY: Curran Associates.
- Paivarinta, U., Bostman, O., Majola, A., Toivonen, T., Tormala, P. & Rokkanen, P. 1993 Intraosseous cellular-response to biodegradable fracture fixation screws made of polyglycolide or polylactide. *Arch. Orthop. Trauma Surg.* **112**, 71–74. (doi:10.1007/BF00420258)
- Park, T. G. 1994 Degradation of poly(D,L-lactic acid) microspheres: effect of molecular-weight. *J. Controll. Rel.* **30**, 161–173. (doi:10.1016/0168-3659(94)90263-1)
- Park, T. G. 1995 Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* **16**, 1123–1130. (doi:10.1016/0142-9612(95)93575-X)
- Pezzotti, G., Asmus, S. M., Ferroni, L. P. & Miki, S. 2002 *In situ* polymerization into porous ceramics: a novel route to tough biomimetic materials. *J. Mater. Sci. Mater. Med.* **13**, 783–787. (doi:10.1023/A:1016127209117)
- Rahman, F. F. 2004 Composites of poly(ethylmethacrylate)tetrahydrofurfuryl methacrylate with tricalcium phosphate and hydroxyapatite for bone scaffolds. PhD thesis, University of Cambridge, Cambridge, UK.
- Renouf-Glauser, A. C., Rose, J., Farrar, D. & Cameron, R. E. 2005 A degradation study of PLLA containing lauric acid. *Biomaterials* **26**, 2415–2422.
- Renouf-Glauser, A. C., Rose, J., Farrar, D. F. & Cameron, R. E. 2006 Comparison of the hydrolytic degradation and deformation properties of a PLLA-lauric acid based family of biomaterials. *Biomacromolecules* **7**, 612–617. (doi:10.1021/bm050746v)
- Schliecker, G., Schmidt, C., Fuchs, S., Wombacher, R. & Kissel, T. 2003 Hydrolytic degradation of poly(lactide-co-glycolide) films: effect of oligomers on degradation rate and crystallinity. *Int. J. Pharm.* **266**, 39. (doi:10.1016/S0378-5173(03)00379-X)
- Sharma, L. & Pezzotti, G. 2003 Biomimetic hybrids: an advancement for biomaterials. *Key Eng. Mater.* **240–242**, 143–146. (doi:10.4028/www.scientific.net/KEM.240-242.143)
- Suchanek, W. & Yoshimura, M. 1998 Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. *J. Mater. Res.* **13**, 94–117. (doi:10.1557/JMR.1998.0015)
- Sugiyama, N., Kunibu, R., Yoshizawa-Fujita, M., Takeoka, Y., Aizawa, M. & Rikukawa, M. 2007 Ring-opening bulk polymerization of L-lactide in porous hydroxyapatite. *Chem. Lett.* **36**, 1476–1477. (doi:10.1246/cl.2007.1476)
- Vert, M., Li, S. & Garreau, H. 1991 More about the degradation of LA/GA-derived matrices in aqueous media. *J. Controll. Rel.* **16**, 15–26. (doi:10.1016/0168-3659(91)90027-B)
- Witt, C. & Kissel, T. 2001 Morphological characterization of microspheres, films and implants prepared from poly(lactide-co-glycolide) and ABA triblock copolymers: is the erosion controlled by degradation, swelling or diffusion? *Eur. J. Pharm. Biopharm.* **51**, 171–181. (doi:10.1016/S0939-6411(01)00130-8)
- Yunos, M., Bretcanu, O. & Boccaccini, A. R. 2008 Polymer-bioceramic composites for tissue engineering scaffolds. *J. Mater. Sci.* **43**, 4433–4442.
- Zhang, Y. & Zhang, M. Q. 2002 Three-dimensional macroporous calcium phosphate bioceramics with nested chitosan sponges for load-bearing bone implants. *J. Biomed. Mater. Res.* **61**, 1–8. (doi:10.1002/jbm.10176)