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In vitro hydrolysis and magnesium release of poly(D,L-lactide-co-glycolide)-based composites containing bioresorbable glasses and magnesium hydroxide

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ABSTRACT: Magnesium is important for both bone growth and cartilage formation. However, the postoperative intake of antibiotics such as quinolones may cause a reduction in magnesium levels in tissue. The addition of magnesium to scaffolds may therefore be beneficial for the regeneration of osteochondral defects. In this study, porous composite scaffolds were produced by gas foaming of poly(D,L-lactide-co-glycolide) (PLGA) rods with magnesium-containing bioresorbable glasses and magnesium hydroxide as fillers. The *in vitro* hydrolytical degradation of the composite scaffolds in Tris buffer was followed over a 10-week period. Mg^{2+} was released in a controlled manner from the scaffolds with varying release profiles between the different materials. Higher glass content resulted in a reduced mass loss compared to scaffolds with lower glass content. As a result of the foaming method, the scaffolds shrank initially, without evidence that the addition of hydrophilic fillers would decrease the initial shrinkage. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42646.

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INTRODUCTION

Poly(α-hydroxy acids) such as poly(D,L-lactide-co-glycolide) (PLGA) are biocompatible and biodegradable polymers, which can beneficially be used for the regeneration of bone tissue.[1,2] PLGA is hydrolytically unstable and the absorption of water into the polymer matrix causes scaffold degradation and gives space for tissue growth. The PLGA chains degrade in aqueous environments by random chain scission into acidic water-soluble low-molecular-weight fragments.[3,4] Bone growth is negatively impacted by acidic pH[5–7] and it has been suggested that it may be advantageous to reduce the acidity of PLGA scaffolds for bone regeneration.[8] It has even been suggested that increasing the pH from physiological values improves new bone formation.[9]

Composite materials of biodegradable aliphatic polyesters containing bioresorbable glasses generally exhibit improved mechanical properties compared to their constituents, and bioresorbable glasses also have a neutralizing effect on the acidity which is caused by the polyester degradation products.[2,10,11] Composites of biodegradable polymers containing bioresorbable glasses have been widely studied and they effectively combine the flexibility and degradation properties of polymers and the strength and potential bioactivity of the inorganic glass phase.[12,13]

Magnesium deficiency has been shown to negatively impact bone tissue and bone growth in animal models by increasing the osteoclast number,[14] reducing the bone mineral content and the volume of bone,[15,16] and inducing osteoporosis.[17,18] There is also clinical evidence of negative effects of magnesium deficiency on bone tissue.[18,19] In addition, magnesium deficiency has been proven to have a negative effect on bone tissue around osseointegrated implants.[20,21]

It has been found that the magnesium levels in tissue are decreased by certain antibiotics, quinolones, which potentially contributes to the formation of cartilage defects.[22] This is of special concern because quinolones are used in orthopedic surgery to reduce the risk of infection.[23] Supplementary magnesium reduces the quinolone-induced damage to chondrocytes.[22] The bioavailability of quinolones can be reduced by coadministration with magnesium, which may encourage the use of different administration routes for quinolones and magnesium.[24]

Magnesium and its alloys have been found to be suitable for the production of orthopedic implants.[25] Mesoporous magnesium silicate has enhanced the efficiency of new bone formation in bone defects in rabbits[26] and magnesium-containing alloys have been found to favor bone growth also in rats[27] and guinea

pigs.[28] Magnesium ions enhance the proliferation of human bone marrow stromal cells and support mineralization of the extracellular matrix.[29] Biomimetic scaffolds with added magnesium ions have been shown to favor osteochondral tissue regeneration in a clinical trial[30] and the beneficial effects of magnesium to chondrocyte proliferation and cartilage formation have been proven in several studies as well.[31–33] Pure magnesium corrodes to $Mg(OH)_2$ in aqueous environments.[34] The addition of $Mg(OH)_2$ to PLGA has been shown to neutralize the acidic environment inside the scaffold.[35] In several studies, it has been proven that magnesium-doped bioresorbable glasses are biocompatible, but because no undoped control groups have been used, the potential osteogenic effects of magnesium release from the glasses remain unproven.[36]

Shrinkage in physiological conditions is a typical but generally unwanted characteristic for several different types of polymeric structures and scaffolds used in biomedical applications. It has been reported for polylactic acid fibres,[37] microparticles,[38] and nanofibrous[39] and gas-foamed[40] scaffolds. Also foams produced by particulate leaching have been observed to shrink.[41,42] Shrinkage of polymeric scaffolds occurs when stretched amorphous polymer chains are able to relax when the internal energy of the polymer matrix increases, e.g., through an increase in the temperature or a decrease in the strength of intermolecular interactions.[43] Shrinkage of tissue regenerating scaffolds may result in displacement of the scaffold, which can possibly lead to a detrimental outcome of the regeneration process. Shrinkage also affects the pore structure of the scaffold, possibly obstructing cell infiltration, e.g., during *in vitro* cell culturing. In order to prevent these negative consequences, it would be desirable to inhibit the dimensional shrinkage of scaffolds under *in vitro* or physiological conditions.

The aim of this study was to analyze the *in vitro* degradation properties of porous scaffolds produced from PLGA in combination with magnesium-containing fillers. Mg^{2+} release from the scaffolds to the surrounding solution was studied because of its potential beneficial effects for tissue growth *in vivo*. The dimensional stability of the scaffolds was evaluated. Water absorption, weight loss, pH of the surrounding medium, and changes in molecular weight were also measured.

MATERIALS AND METHODS

Materials

Medical grade D-lactide and glycolide monomers were obtained from Corbion (Purac, Gorinchem, the Netherlands). L-lactide monomer (>99%) was purchased from Futerro (Escanaffles, Spain). Tin(II) 2-ethylhexanoate (approx. 95%) and 1-decanol (99%, distilled prior to use) were purchased from Sigma-Aldrich. Low molecular weight polylactic acid (PLA) polymerized by polycondensation was supplied by the Laboratory of Polymer Technology, Åbo Akademi, Finland. Magnesium hydroxide (≥99.0%) was purchased from Sigma-Aldrich (Helsinki, Finland). Bioresorbable glasses 13–93 and NC–5 were supplied by BonAlive Biomaterials Ltd (Turku, Finland), and their composition is shown in Table I.

Table I. The Composition (wt %) of Bioresorbable Glasses 13–93 and NC–5

Oxide	13-93	NC-5
Na_2O	6	17
SiO_2	53	62
CaO	20	13
P_2O_5	4	2
MgO	5	4
B_2O_3	0	2
K_2O	12	0

Polymerization

PLGA with a D-lactide-to-L-lactide ratio of 1 : 1 and a lactide-to-glycolide molar ratio of 7 : 3 was synthesized in inert atmosphere by ring-opening polymerization. Briefly, 100 g L-lactide (0.694 mol), 100 g D-lactide (0.694 mol), and 69 g glycolide (0.594 mol) that were freshly obtained and had been stored at −18°C were weighed in a round bottle. The bottle was heated at 120°C until all monomers were molten after which 803 mg tin(II) 2-ethylhexanoate (1.98 mmol) and 192 mg 1-decanol (1.21 mmol) were added. The temperature was then raised to 150°C for 5 h. After cooling to room temperature, the product was dissolved in altogether 2 L dichloromethane and precipitated in a sixfold amount of heavily stirred ethanol to remove unreacted monomers and other possible impurities. The polymer was dried in a vacuum oven (40°C, <50 mbar) for approximately 1 week until no residual solvent could be observed in ^{1}H-NMR. The dried polymer was manually cut to granules with a diameter of approximately 5 mm.

Extrusion

The bioresorbable glasses 13–93 and NC–5 were ground from fibers into particles of up to 50 μm in size with a Philips Mini-Mill ball mill. PLGA and PLA granules as well as the glass and the $Mg(OH)_2$ particles were dried in a vacuum oven (40°C, <50 mbar) overnight before extrusion. The blends containing PLGA and either PLA, 13–93, NC–5, or $Mg(OH)_2$ were extruded with a counter-rotating twin-screw extruder (Rheocord System 40, Haake Buchler) into rods. The temperature profile during extrusion was 75, 85, and 95°C and the die temperature was 95°C. The screw speed was 120 rpm.

Fabrication of Porous Scaffolds

The extruded rods, with a diameter generally between 4.0–5.5 mm, were cut into approximately 17-mm-long pieces and placed into cylindrical PTFE molds with a diameter of 1.5 times the diameter of the rod. The molds were placed in an autoclave and the foaming was performed at room temperature. A CO_2 pressure of 55 bar was applied on the rods for 22 h after which the excess pressure was quickly released during a time span of approximately 8 s. The molds with the expanded rods were placed in an oven (80°C, 45 s) after which the rods were kept inside of their molds for an hour at ambient temperature and pressure. The rods were stored in a desiccator until used in the hydrolysis experiments.

The degree of expansion was calculated according to eq. (1):

$$Volumetric\ expansion\,(\%) = \left[\left(V_{expanded} - V_{initial}\right)/V_{initial}\right] \times 100\% \quad (1)$$

Table II. The Theoretical and Measured Weight % of the Fillers and the Measured Weight % of PLGA

Filler	Theoretical filler content	Measured filler content	Measured PLGA content
13-93	10	11.5	88.5
13-93	20	20.9	79.1
13-93	35	35.0	65.0
NC-5	10	10.7	89.3
NC-5	20	20.3	79.7
NC-5	35	36.3	63.7
$Mg(OH)_2$	10	11.7	88.3
$Mg(OH)_2$	20	19.4	80.6
$Mg(OH)_2$	35	34.6	65.4
PLA	20	18.7	81.3

where $V_{expanded}$ is the volume of the sample after expansion and $V_{initial}$ is the volume of the scaffold before expansion.

The amount of bioresorbable glass in the glass composites was determined gravimetrically, based on the residual weight of a rod after burning it in open fire in a glass vial. The $Mg(OH)_2$ content was determined in a similar manner by burning, but corrected with the change in weight caused by the oxidation of Mg from $Mg(OH)_2$ to MgO. The amount of low-molecular-weight PLA was determined by ^1H NMR analysis.

In Vitro Hydrolysis

For *in vitro* tests, the foamed rods were cut into approximately 5-mm-long scaffolds which were measured to the nearest 0.01 mm using a caliper and weighed with an accuracy of 0.1 mg. The diameter of the foamed scaffolds varied approximately between 6 and 11 mm and the weight varied between 12 and 53 mg. The weight of the scaffolds was highly dependent on the filler content and on the volumetric expansion in the foaming process. The degradation tests of the porous scaffolds were carried out in 0.1 M tris(hydroxymethyl)aminomethane solution (Tris buffer) made from ultrapure (Millipore) water adjusted to pH 7.42 with hydrochloric acid. The scaffolds were immersed in syringes containing Tris buffer so that for each 3.5 mg of sample, 1 mL of buffer solution was added. The syringes were then stored in an incubating orbital shaker (Unimax 1010, Heidolph) at 37°C for predefined time periods (4 h, 1 d, 3 d, 7 d, 14 d, 21 d, 35 d, 49 d, and 70 d). At weekly intervals, the buffer solution was replaced with fresh solution. At the predefined time points, three samples of each scaffold type were removed from the syringes, dried superficially with moisture-absorbent paper, and characterized. The results shown in this article represent average values for the three parallel samples.

Scaffold Characterization

After removing the scaffolds from the Tris buffer, their dimensions and weight were measured. The scaffolds were subsequently freeze dried for 48 h. The dried scaffolds were weighed again and the molecular weight was determined for each scaffold.

The changes in diameter and length of the scaffolds were calculated according to eq. (2):

$$Change\ in\ dimension\ (\%) = (D/D_0) \times 100\% \quad (2)$$

where D is the dimension in wet state after immersion and D_0 is the dimension before immersion in Tris buffer.

The weight loss of the scaffolds was calculated according to eq. (3):

$$Weight\ loss\ (\%) = (W/W_0) \times 100\% \quad (3)$$

where W is the weight of the dried scaffold after immersion in Tris buffer and W_0 is the weight of the scaffold before immersion.

Water absorption was calculated according to eq. (4):

$$Water\ absorption\ (\%) = \left[\left(m_{wet} - m_{dry}\right)/m_{dry}\right] \times 100\% \quad (4)$$

where m_{wet} is the mass of the sample in wet state after immersion in Tris buffer and m_{dry} is the mass of the sample after drying.

The determination of the molecular weight of the polymers was performed using gel permeation chromatography (GPC) with an LC-10ATVP HPLC-pump (Shimadzu Corporation), an AM GPC Gel 10 μm Linear colon (American Polymer Standards), and a Sedex 85 light scattering detector (Sedere). The GPC measurements were carried out at 40°C at a flow rate of 1 mL min^{-1} with tetrahydrofuran as solvent and a sample concentration of 1 mg mL^{-1}. Polystyrene standards from Polymer Standard Service were used for calibration. The samples were filtered with 0.22 μm PTFE filters before analysis.

The release of magnesium ions from the scaffolds was studied using an inductively coupled plasma optical emission spectrometer (ICP-OES) instrument (Optima 5300, PerkinElmer). The bestowed buffer solution was diluted with ultrapure water in a 1 : 1 ratio and 4 drops of nitric acid per sample were added. The samples were stored in closed vials in a refrigerator prior to analysis.

Statistical Analysis

Analysis of variance (ANOVA) was performed using general linear models with SAS 9.2 software (SAS Institute Inc.). Two-tailed linear models with an alpha-level of 0.05 were used. Assumptions of linear regressions were studied by observing normality of error distribution. Differences were considered significant at p values <0.05.

RESULTS AND DISCUSSION

Scaffold Fabrication and Characterization

The compositions of the studied composites are shown in Table II. In this study, the theoretical (aimed) composition values are used in the text for clarity, but the measured values were used in the statistical analysis.

The filler particles were uniformly distributed in the scaffold matrix, as is shown in the scanning electron microscope (SEM) images in Figure 1. The size distribution of the 13–93 and NC–5 glass particles was broad and especially the smaller glass particles were well-embedded into the matrix and pore walls. The shape of the pores varied from clearly elongated to almost

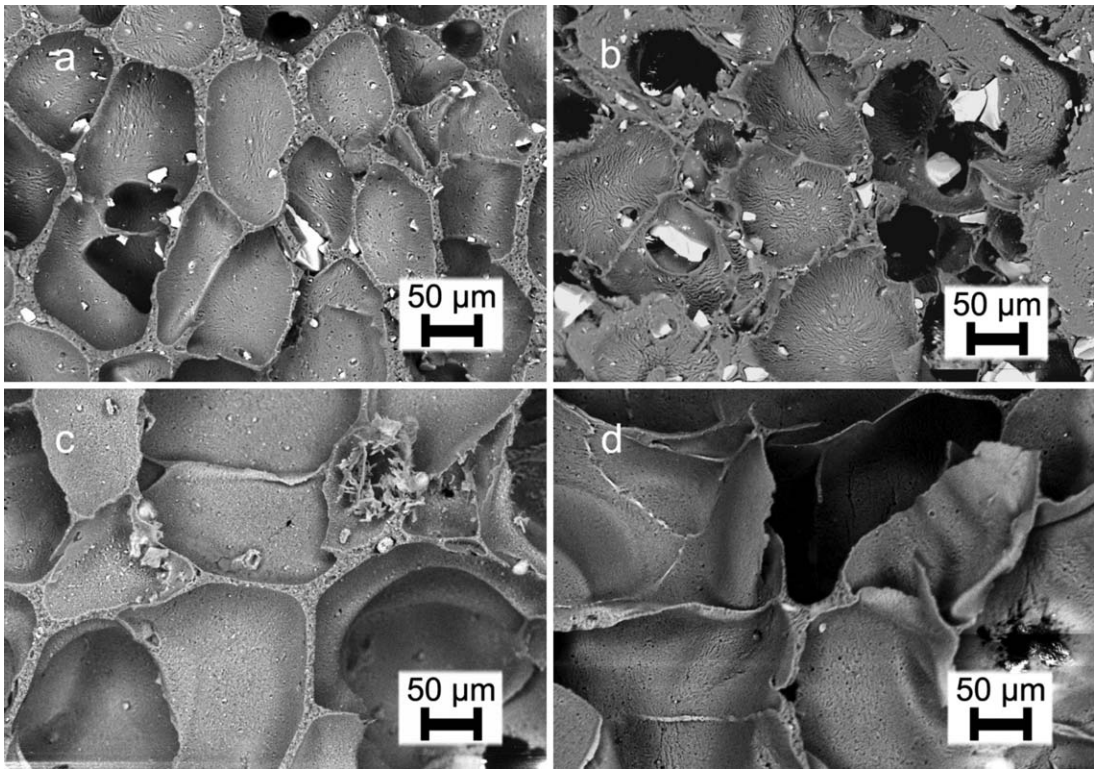

Figure 1. SEM images with 250× magnification of cross-sections of (a) 13–93 20%, (b) NC–5 20%, (c) $Mg(OH)_2$ 20%, and (d) PLA 20% scaffolds after 1 day of immersion in Tris buffer.

circular. The initial pore structure was predominantly closed and the pore diameter was mainly between 50 and 300 μm.

The average degrees of expansion of the prepared composites are shown in Figure 2. The volumetric expansion of the scaffolds in the foaming process varied from 460% to 2216%. The expansion in length was between 190% and 360% for most materials, whereas the diameter expansion was limited by the PTFE molds and was typically between 70% and 100%. A higher filler content (bioresorbable glasses or $Mg(OH)_2$) significantly decreased the degree of expansion ($p < 0.0001$, $n = 51$). The high expansion of PLA 20% scaffolds is explained by the fact that the filler (i.e., the low molecular weight PLA) is similar to the main matrix material (PLGA) and is in itself also expandable by gas foaming. Expansion of pure PLGA rods is comparable to the expansion of PLA 20% rods (approximately 2000% for similar samples).

Dimensional Change

The diameter of the scaffolds decreased significantly when they were immersed in Tris buffer at 37°C (Figure 3). The initial shrinkage for most materials was approximately 30%, with the range being from 22% to 44%. After the initial shrinkage, all materials started to expand. At the last time point (49 days), when the dimensions could reliably be measured, scaffolds containing bioresorbable glass and PLA were close to their original diameter or had exceeded it. At the same time point, the $Mg(OH)_2$-containing scaffolds had not regained their original diameter. The poor recovery of the original dimensions of the $Mg(OH)_2$-containing scaffolds may be linked to their considerable weight loss. Similar to the decrease in diameter, a significant reduction in length was observed for all samples when they were immersed in Tris solution (data not shown). For all 13–93- and NC–5-containing scaffolds, the maximum shrinkage in length was approximately 40%. At 49 days, they had regained their length to a large extent. Composite scaffolds with a filler content of $Mg(OH)_2$ 20% shrank noticeably less than those containing $Mg(OH)_2$ 10% and 35% and they also regained their dimensions to a much greater degree. No obvious explanation

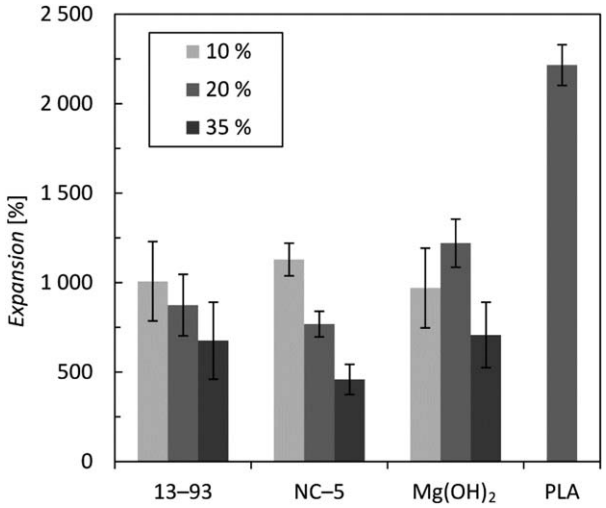

Figure 2. The average expansion percentages of the composite materials with different filler concentrations in the gas foaming process.

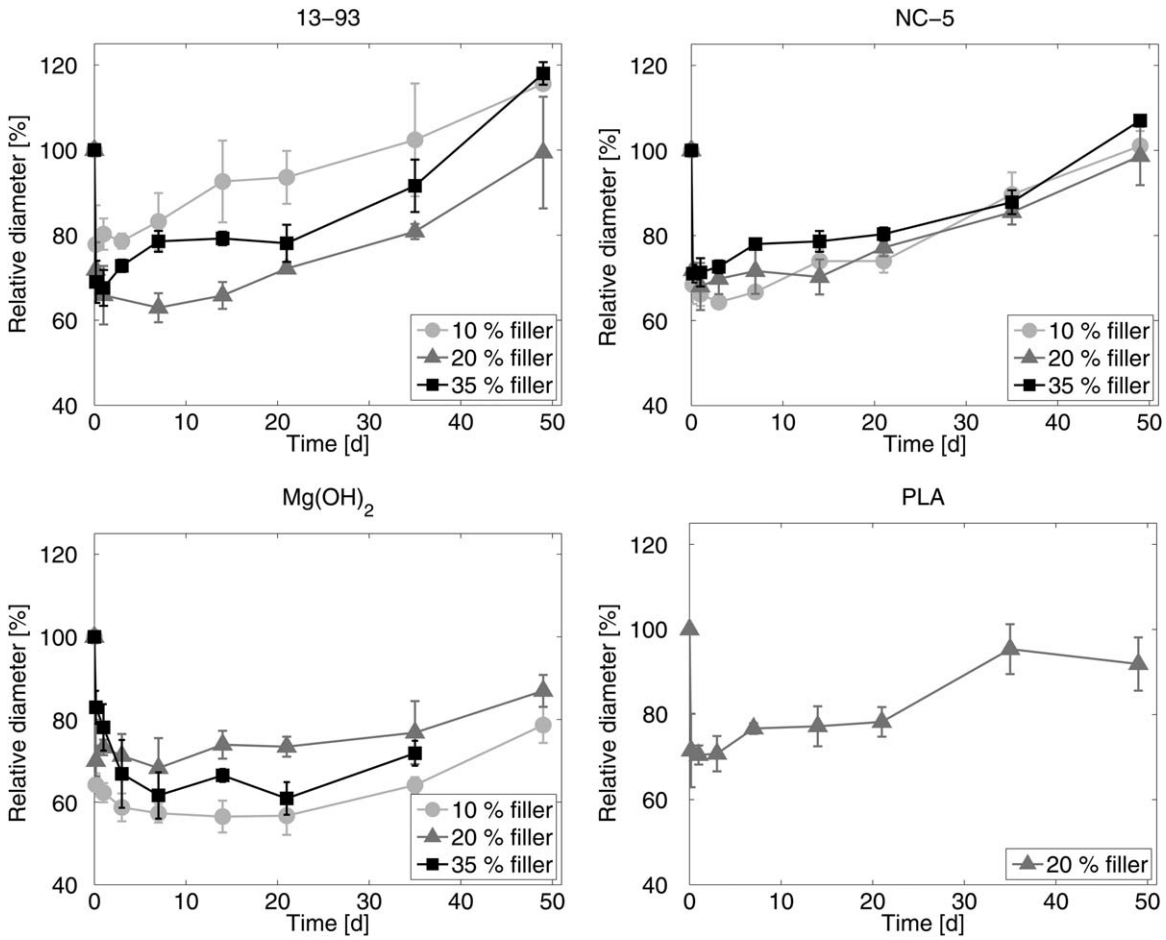

Figure 3. Change of normalized diameter of porous PLGA-based scaffolds with different amounts of magnesium-containing fillers.

to this phenomenon can be identified, as the expansion in the length dimension of the 20% scaffolds was similar to the 10% scaffolds and significantly greater than for the 35% scaffolds. The least initial length shrinkage was observed for PLA 20% scaffolds which showed a maximum shrinkage of 21%. At 49 days also they had regained their original length.

Regarding both diameter and length measurements, at 49 days many scaffolds were already very soft and the reliability of the measurements was worse than for scaffolds which had been immersed for shorter times in Tris buffer. The diameter and the length of the $Mg(OH)_2$ 35% scaffolds could not be measured at 49 days because all three parallel samples fell apart when removing them from the Tris buffer.

Gas foaming is a suitable method for the production of various types of porous scaffolds because it does not involve the use of solvents and the foaming process can be performed at ambient temperature. However, in the foaming process, the polymer chains become stretched. The initial shrinkage of the scaffolds seems to be caused by the stress relaxation of stretched polymer chains when they are exposed to elevated temperatures.[37] For the PLGA-based scaffolds used in this experiment, physiological temperatures were enough to induce the shrinkage. The initial shrinkage was not dependent on the expansion of the scaffolds when the type and amount of filler were controlled ($p = 0.82$, $n = 30$). PLA 20% scaffolds, which had the highest expansion percentage of all scaffolds by a wide margin, shrank less than all other types of scaffolds.

The volumetric changes for PLGA-based scaffolds with hydrophilic fillers are in line with gas-foamed scaffolds of pure PLGA having a similar physical structure tested earlier at our laboratory (results not shown here). It is evident that the addition of hydrophilic fillers did not significantly improve the dimensional stability of PLGA-based scaffolds. This may be attributable to the fact that the relaxation forces of the polymer chains appear higher than the countering forces induced by the water absorption of the scaffolds.

The shrinkage of porous scaffolds may at least partially be prevented by increasing the crystallinity of the polymer matrix[37,44] even though this could to some extent prevent expansion of the scaffolds during the processing and it also affects the rate of degradation.

Weight Loss

The degradation pattern of the scaffolds is important for the regeneration of bone because the degrading scaffold provides space for new tissue formation. Weight loss differed significantly between $Mg(OH)_2$-containing scaffolds and the other scaffolds

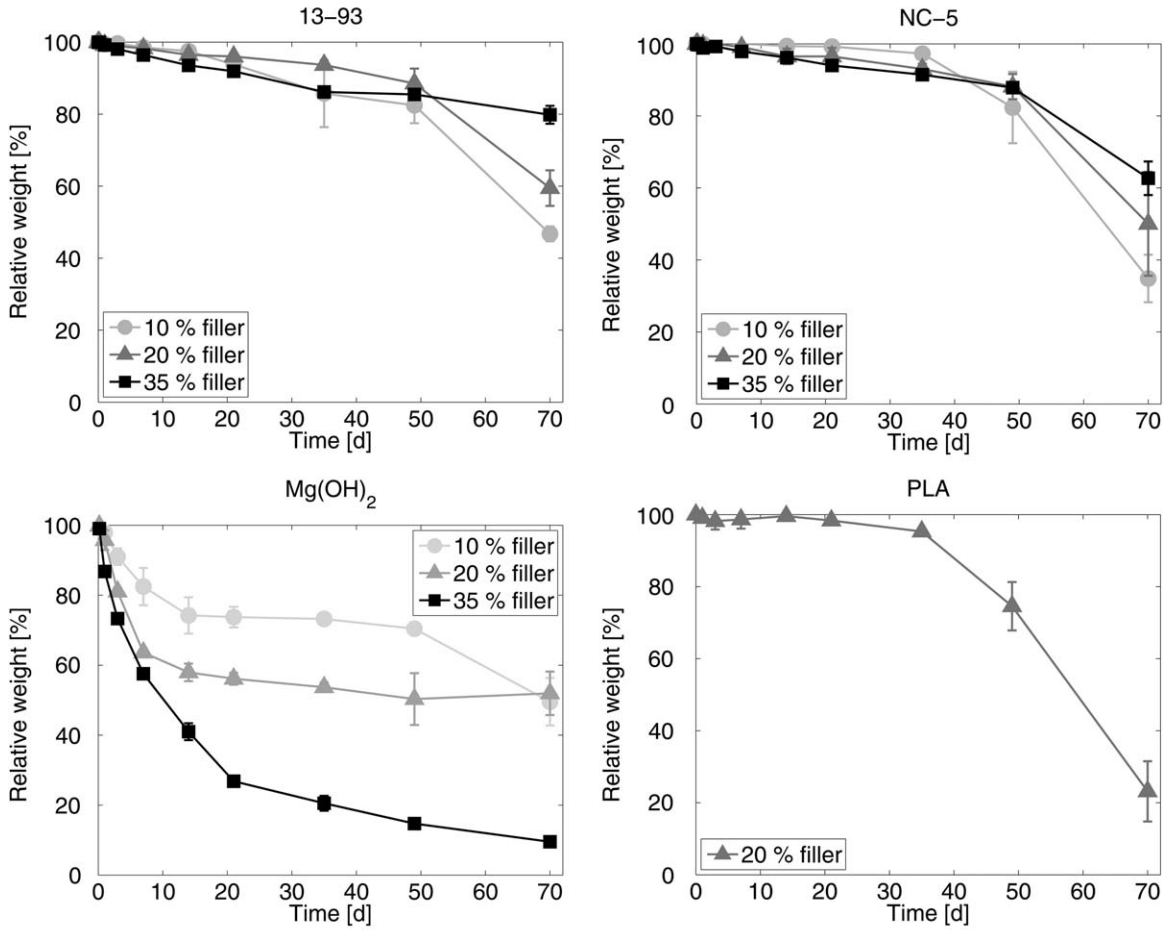

Figure 4. Change of normalized weight of porous PLGA-based scaffolds with different amounts of magnesium-containing fillers.

(Figure 4). The weight loss for $Mg(OH)_2$-containing scaffolds was very high during the first 2–3 weeks of immersion in Tris buffer, with a 25% loss for $Mg(OH)_2$ 10% scaffolds and a 73% loss for $Mg(OH)_2$ 35% scaffolds after 21 days. This is partly attributed to the quick dissolution of the $Mg(OH)_2$ particles, as shown below in the analysis of the Mg^{2+} release into the Tris buffer. However, as the early weight loss for the $Mg(OH)_2$-containing scaffolds was considerably higher than the total amount of $Mg(OH)_2$ in the scaffolds, it is evident that also the weight of the PLGA matrix decreased early during the immersion. A reason for this pattern may be that the rapid dissolution of $Mg(OH)_2$ resulted in a significant pH increase in the buffer solution (results not shown) which could increase the rate of degradation through alkaline hydrolysis. A similar degradation pattern has been observed in films consisting of poly(D,L-lactide) and 30% MgO.[45] The early weight reduction may also be attributable to the effect of an increased surface area because of dissolved $Mg(OH)_2$ particles originating from within the matrix. When the surface area increases, more of the soluble fragments may leach out into the solution. By replacing $Mg(OH)_2$ partly or fully by, e.g., magnesium chloride ($MgCl_2$), it may be possible to release similar amounts of magnesium and reduce the early increase in alkalinity, which would reduce the early degrading impact on the matrix.

The weight loss of the bioresorbable glass- and PLA-containing scaffolds followed a pattern reported earlier.[46] Weight loss was initially small until 35 days. At 49 days and 70 days, PLA 20% showed the most significant reduction in weight. Of 13–93- and NC–5-containing scaffolds, those with more glass had initially (during the first 21 days) a higher weight loss, but showed after that a slower reduction in weight than the scaffolds with a smaller amount of glass. The higher initial weight loss of scaffolds with a higher amount of glass has been attributed to the early leaching of glass from the scaffolds.[40,47] The reason for the slower weight loss later during the hydrolysis seems to be the subdued autocatalytic effect because of the higher pH induced by the neutralizing effect caused by the glasses. Scaffolds which contained the more rapidly resorbing 13–93 glass exhibited a smaller weight loss than NC–5-containing scaffolds, even though the more quickly dissolving 13–93 would intuitively elicit a higher weight loss. The bulk of the weight loss is, however, tied to the degradation of the polymer during the 70 day period, and as 13–93 is more quickly dissolving than NC–5, it also has more capacity to neutralize the medium and in that way possibly reduce the rate of polymer degradation.

It has been shown that the addition of a slightly alkaline component to PLGA decreases the rate of degradation of the

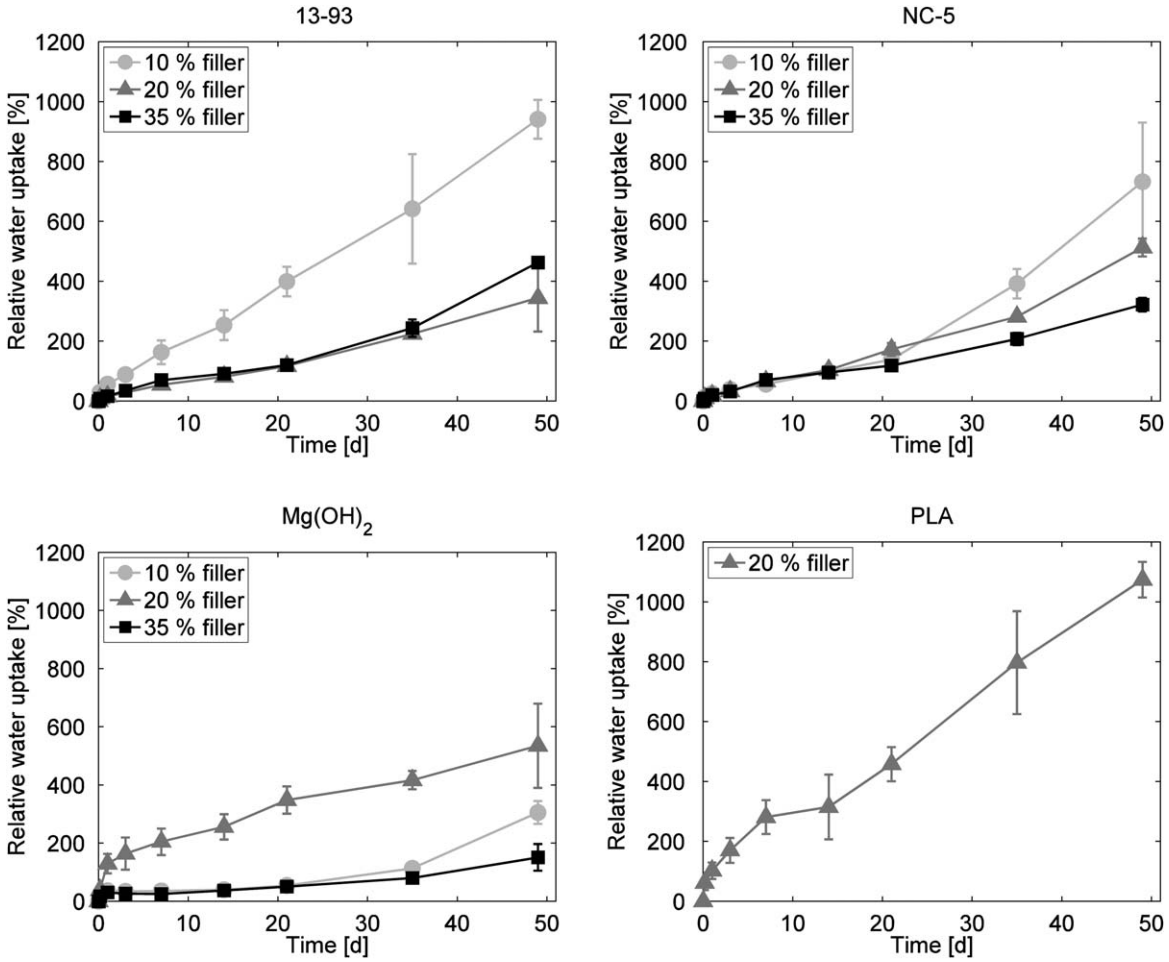

Figure 5. Change of normalized water uptake of porous PLGA-based scaffolds with different amounts of magnesium-containing fillers.

polymers, whereas the addition of acidic components accelerates the degradation process.[48] The dissolution process of bioresorbable glasses creates a slightly basic environment, which as expected slows down the degradation rate of aliphatic polyesters.[10] A drop in pH of 0.2 units has been shown not to affect bone healing negatively,[49] but greater changes significantly reduce osteoblast activity and affect bone growth negatively.[5–7] For bone regeneration, a pH-neutralizing filler in quickly degrading PLGA-based scaffolds may be a favorable solution.

Water Absorption

Water absorption, as shown in Figure 5, increased over time for all materials but did not show significant differences with regard to the amount of added filler ($p = 0.15$, $n = 259$). The highest rate of water absorption was observed for PLA 20% scaffolds, with relatively steadily increasing amount of absorbed water throughout the measurable 7-week period, with a maximum of 1074% at 49 days. The 13–93 10% scaffolds had an almost equally high water uptake, with a maximum of 941% at 49 days. Throughout the study, the water absorption of $Mg(OH)_2$ 10% and 35% scaffolds was at very low levels compared to the other materials. This correlates with the fact that their dimensions did not recover from their initial shrinkage as much as the other materials. The water absorption of the $Mg(OH)_2$ 20% scaffolds was initially very high but supposedly because of the considerable weight loss the water uptake increased relatively little over time.

The differences in the water uptake were partly a result of different porosities of the scaffolds. The scaffolds which contained 13–93 or NC–5 were initially denser than especially the PLA 20% scaffolds. In this work, we have not differentiated the water uptake in pores versus water uptake in the bulk matrix. This contributes to effect that the water uptake levels for PLA 20% appear higher than for the other scaffolds.

Mg^{2+} Release

Figure 6 shows the release of Mg^{2+} from the scaffolds into the Tris buffer. The highest rates of Mg^{2+} release were recorded during the first 1–2 weeks of immersion after which the rate leveled for most scaffolds. As the amount of magnesium in 13–93 and NC–5 is only a small fraction of the total mass of the glass, the Mg^{2+} release for 13–93- and NC–5-containing scaffolds was approximately two orders of magnitude smaller than for scaffolds with similar amounts of $Mg(OH)_2$. The fact that 13–93 contains more magnesium coupled with its higher resorption rate compared to NC–5 are the reasons for the higher Mg^{2+} release rate from 13–93 than from NC–5.

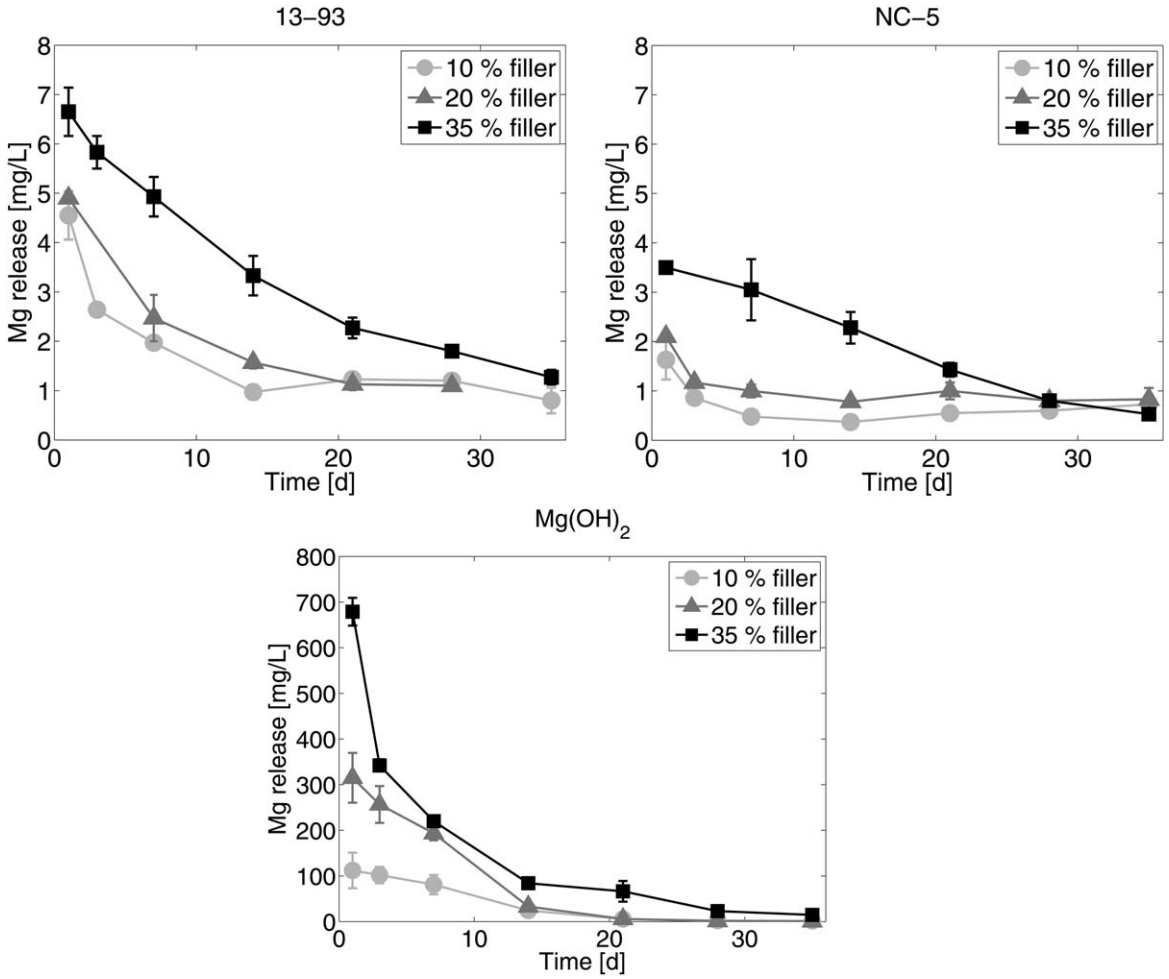

Figure 6. Normalized 7 day Mg^{2+} release rate (mg L^{-1}) into Tris buffer of porous PLGA-based scaffolds with different amounts of magnesium-containing fillers. Note different *Y*-axis scale for $Mg(OH)_2$.

With respect to antibiotics which cause magnesium deficiency in tissue, the rate of Mg^{2+} release may have the highest importance during the first weeks after the operation of the implant into the body. The tissue ingrowth into the scaffold may benefit from increased magnesium levels over longer periods of time. Janning *et al.*[50] demonstrated an enhanced bone growth using slowly dissolving nonporous cylinders of $Mg(OH)_2$ in a rabbit model. The effect is attributed either to the local magnesium

Table III. The Weight Average Molecular Weights (M_w) of the PLGA in the Composites Before and After Extrusion Processing (g mol^{-1}). Polydispersity indices (PDI) Before and After Extrusion are Also Shown

Filler	M_w before	M_w after	PDI before	PDI after
13-93 10%	107,000	58,000	1.95	1.99
13-93 20%	112,000	74,000	1.86	1.88
13-93 35%	112,000	65,000	1.86	1.91
NC-5 10%	107,000	74,000	1.95	1.93
NC-5 20%	107,000	79,000	1.95	1.73
NC-5 35%	112,000	69,000	1.86	1.84
$Mg(OH)_2$ 10%	107,000	71,000	1.95	1.69
$Mg(OH)_2$ 20%	107,000	51,000	1.95	1.72
$Mg(OH)_2$ 35%	112,000	84,000	1.86	1.61
PLA 20%	107,000	46,000	1.95	3.77

PDI = M_w/M_n, where M_n is the number average molecular weight. The values for PLA 20% reflect a mixture of PLGA and low molecular weight PLA.

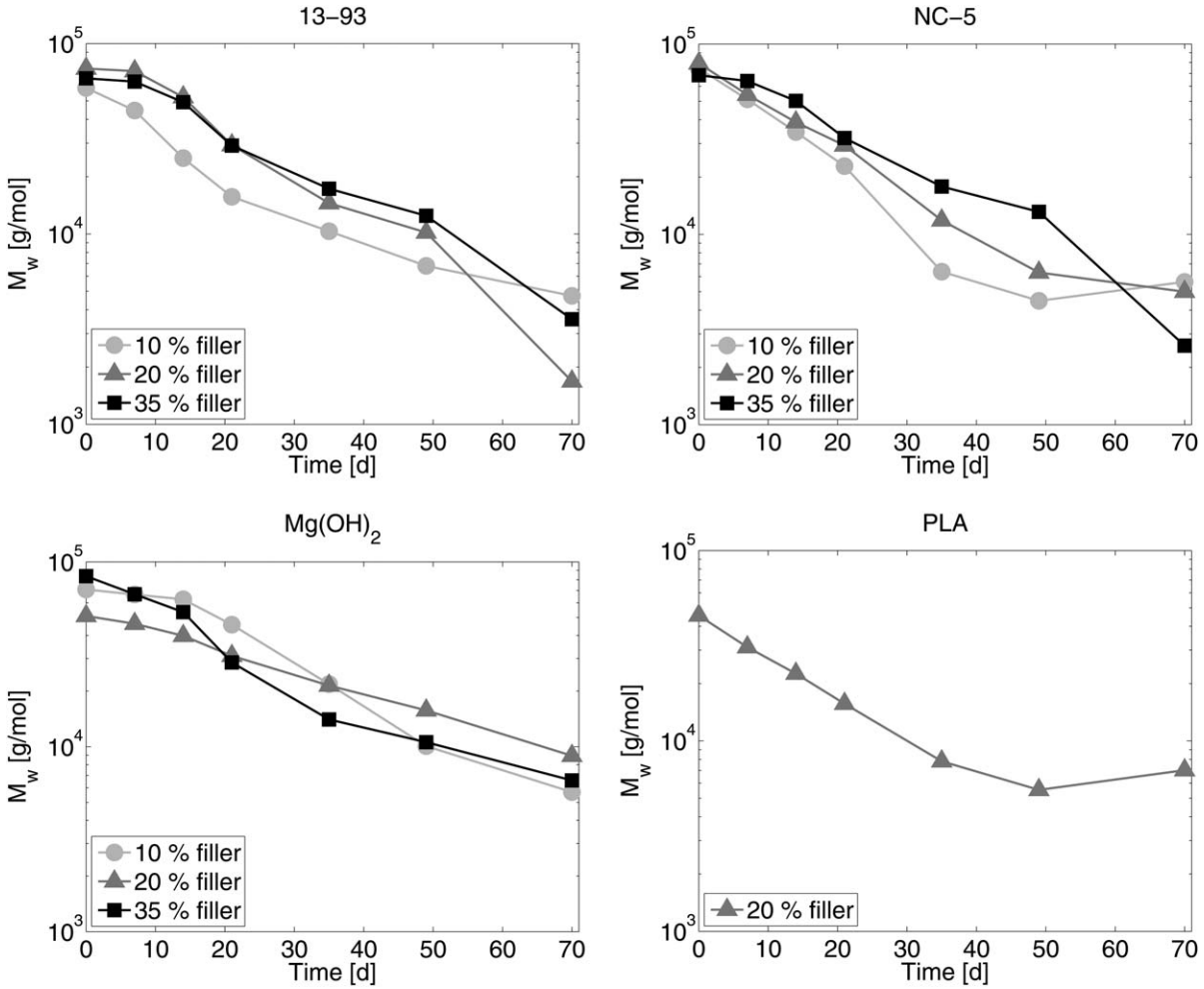

Figure 7. Change of weight average molecular weight (M_w) of porous PLGA-based scaffolds with different amounts of magnesium-containing fillers. The PLA 20% scaffolds have a lower initial M_w because of the low molecular weight PLA used in the blend.

concentration or to the local alkalosis. In that study, the release of magnesium into tissue was not directly measured. In another study, porous scaffolds, which were made of alloys containing 90% magnesium, were inserted in rabbit knees.[51] Three months after implantation, the scaffolds had largely degraded, and no significant harm was observed in the neighboring tissues. Actually, magnesium alloys have been shown to induce bone cell activation and increase bone mass around implants.[28] When compact magnesium scaffolds were immersed in cell culture medium in a previous study,[52] the release of magnesium from uncoated magnesium scaffolds into the medium during the first 7 days was approximately 110 mg L^{-1}, which is approximately equal to the release from $Mg(OH)_2$ 10% scaffolds during the first 7 days of immersion in this study. However, results from *in vitro* corrosion tests of magnesium alloys have been shown to correlate poorly with results from *in vivo* studies with the same materials.[53]

Feyerabend *et al.*[31] showed that $Mg(OH)_2$ release has a beneficial effect on chondrocyte proliferation, with an optimal concentration of magnesium at 10 mM, which corresponds to 243 mg L^{-1}. Magnesium levels equal to or higher than 15 mM were found to negatively affect chondrocytes. Yoshizawa *et al.*[29] showed osteogenic activity of bone marrow stromal cells to be at optimal levels at a magnesium concentration of 10 mM. In that study, the proliferation of the cells was slightly increased at a concentration of 10 mM compared to the base concentration of 0.8 mM, but the proliferation rate was low at a concentration of 100 mM. The deposition of extracellular matrix was enhanced at magnesium concentrations of 5 and 10 mM, and the protein expression which represented osteogenic activity was highest at 10 mM. *In vivo* tests with $Mg(OH)_2$ scaffolds show that even apparently high magnesium levels may improve bone formation.[50] The number of quinolone-treated chondrocytes decreased less when they were cultured in magnesium-containing medium as compared to culturing in a magnesium-free medium, and the effect was more pronounced when the amount of magnesium was tripled from the base amount of approximately 50–60 mg L^{-1},[22] where the tripled amount equaled roughly 6.8 mM.

On the basis of the studies mentioned above, a magnesium concentration of 10 mM seems to be favorable for osteogenesis and chondrocyte proliferation. This level is similar to the concentration in the Tris buffer of $Mg(OH)_2$ 20% and 35% scaffolds during the first week of immersion in this study. The concentration of magnesium is supposedly higher inside the scaffolds than in the Tris buffer, and even the lower concentrations of magnesium

ions released from the 13–93 and NC–5 scaffolds may therefore be of biological significance. The results of the above mentioned studies and this study are, however, not directly comparable, as neither the scaffold structure nor the quality and quantity of the immersion medium are standardized. Moreover, one cannot fully compare *in vitro* and *in vivo* conditions.

Molecular Weight

The weight average molecular weights (M_w) of the composites before and after extrusion processing are shown in Table III. M_w decreased considerably, by 26–52%, during the melt extrusion. The M_w of PLA 20% was notably low and the polydispersity index (PDI) was high after the extrusion because of the added low M_w PLA to PLGA during the extrusion.

The changes in M_w during the immersion in Tris buffer for 70 days are shown in Figure 7. For 13–93- and NC–5-containing scaffolds, the early degradation was slower for scaffolds with more glass, but toward the end of the immersion, their rate of degradation seemed to increase rapidly. The M_w of the $Mg(OH)_2$-containing scaffolds decreased less than the M_w of the glass-containing scaffolds, even though the $Mg(OH)_2$-containing scaffolds showed a more rapid weight loss. This implies that the early weight loss of the $Mg(OH)_2$-containing scaffolds was in addition to the $Mg(OH)_2$ dissolution mainly caused by the dissolution of fragments of the polymer matrix.

CONCLUSIONS

Mg^{2+} was released in a continuous and controlled manner from the scaffolds for an initial time span of at least 35 days. The release from all magnesium-containing scaffolds peaked in the beginning of the immersion in Tris buffer, which correlates with the time when postoperative antibiotics may reduce magnesium levels in tissue and affect tissue regeneration negatively. Comparisons to studies in the literature (see discussion and references above) indicate that the amount of released Mg^{2+} from the $Mg(OH)_2$ 20% and 35% scaffolds may be sufficient to elicit biological responses.

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