3D-printed poly- ϵ -caprolactone- CaCO_3 -biocomposite-scaffolds for hard tissue regeneration

R. Neumann^{1*}, J. Neunzehn¹, C. Hinüber², T. Flath³, F. P. Schulze³, H-P. Wiesmann¹

¹Technische Universität Dresden, Institute for Materials Science, Dept. Biomaterials, Budapester Straße 27, 01069 Dresden, Germany

²Leibniz-Institute of Polymer Research Dresden, Dept. Processing, Hohe Str. 6, 01069 Dresden, Germany

³University of Applied Sciences Leipzig Dept. of Mechanical and Energy Engineering, Karl- Liebknecht-Straße 134, 04277 Leipzig, Germany

Received 5 April 2018; accepted in revised form 12 July 2018

Abstract. Adopting the beneficial chemical composition of mineral bone grafts and the interesting biomedical properties of the approved polycaprolactone, versatile manufacturing processes offering a near-net-shape fabrication and cost-effective scalability, has been used to fabricate highly porous polymer-ceramic biocomposite scaffolds with different amounts of the inorganic component CaCO_3 by molded casting and fused deposition modelling. The mechanical properties and surface characteristics were evaluated after several steps of degradation by means of compression tests and scanning electron microscopy, respectively. Calcium release has been determined over a period of 4 weeks and the calcium phosphate phase formation on the surface was observed and validated by energy dispersive x-ray spectroscopy. The established production path and the use of the material combination polycaprolactone and calcium carbonate has enormous potential to manufacture individual and application-oriented open-porous scaffolds for hard tissue replacement.

Keywords: biocomposites, mechanical properties, biodegradable polymers, 3D-printing, tissue engineering

1. Introduction

The need for surgical reconstruction or replacement, often the result of a trauma, pathological degeneration or congenital deformity of tissue, is rising. In case of large bone defects, the substitution with artificial bone grafts has become a common task in skeletal orthopedic and surgical reconstruction. The current treatment options, e.g. using donor tissue or artificial grafts, are fairly successful. However, they do not provide optimal therapies due to suboptimal characteristics of the artificial grafts and issues of limited supply or rejection of autologous/allogenic tissue. To address the rather challenging demands of clinical practice, rapidly developing concepts in the field of tissue engineering try to establish biological substitutes that restore, maintain or even improve the functions of the tissue.

As bone tissue consists of about 65% inorganic matter (mostly calcium), ceramic bone substitution materials, exhibiting the desired chemical and surface structure, have been widely considered. To process such materials, additive manufacturing techniques are interesting as they offer to fabricate tissue scaffolds while maintaining the advantageous chemical compositions of ceramic bone substitution materials [1, 2]. Furthermore, three-dimensional scaffolds produced by additive manufacturing feature a defined open and interconnected porosity supporting vascularization and tissue ingrowth.

In additive manufacturing of tissue engineering scaffolds, mainly polymers are utilized. Their macromolecular structure and adjustability allows using light-based techniques such as stereolithography (SLA)

*Corresponding author, e-mail: ard.neumann@googlemail.com
© BME-PT

and melt based techniques such as fused deposition modelling (FDM) or the powder based selective laser sintering (SLS). While SLA requires chemically altered curable functionalized polymers, SLS requires advanced equipment more often found in industrial application. With FDM (often referred to as 3D-printing) thermoplastic pellets or filaments can be processed on basic machines and therefore it is the most commonly used laboratory scale method to produce polymer scaffolds.

Many types of biodegradable thermoplastic polymers such as polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL) and their copolymers have been used extensively in regenerative studies already [2–4]. Because of the fact, that the mechanical performance of polymers like PCL compared to the bone tissue [5, 6] appears too weak and flexible to satisfy the mechanical requirements for the hard-tissue engineering in earlier years, it seemed more attractive for long-term implants and controlled release applications. Later, PCL has been synthesized and structurally modified or copolymerized in order to improve degradation properties [7–9] and was mentioned as a potential candidate polymer for bone tissue engineering [10–12].

Especially the creation of polymer-based composite materials, mimicking the composite nature of real bone with inorganic fillers such as hydroxyapatite (HA), carbonated apatite or calcium carbonate (CaCO_3) represents an alternative choice to try and overcome the mechanical limitations but mainly introduces mineral components promoting the bone regeneration [5, 13–15, 20–29]. Hydroxyapatite has received attention for its strong similarity to bone mineral in its structure and composition and for its biocompatible and osteoinductive properties; faster bone regeneration and the ability to directly bond to the regenerated bone without intermediate connective tissue has been reported. Therefore, it is widely used as a bone graft substitute for the hard tissue repair in clinical applications, like orthopedic surgery and dentistry [17–19]. However, some results suggested that better osteoconductivity would be achieved, if synthetic HA not only resembles bone minerals in composition, but also in size. Other reports on the ceramic/PCL composites demonstrated increased degradation rates with the addition of HA, tricalcium phosphate (TCP), their biphasic characteristic and the feasibility of the HA/PCL composites in drug-delivery systems. Like PLA, PCL undergoes

hydrolytic degradation. Thus, the introduction of a hydrophilic component, e.g. a hydrophilic ceramic, is reported to be effective in improving the hydrophobic properties and the degradation rate of PCL.

Calcium carbonate (CaCO_3) is one of the most abundant materials on earth and a fundamental building block in nature (e.g. in limestone, in snake skin, pearls, shells of sea organisms, egg shells). Particularly, nano-sized CaCO_3 has received a lot of attention because of its wide range of potential applications and has been used for various polymer composites. Chan *et al.* [26] found that the impact strength of polypropylene (PP) can be increased from 55 to 133 J/m by the addition of 9.2 vol% of the surfactant-treated 44 nm sized CaCO_3 particles. Wang *et al.* [27] reported that the mechanical properties of PP, especially the ductility, were effectively improved because of the incorporation of CaCO_3 particles. Maeda and coworkers [28, 29] demonstrated that bone substitutes made of PLA and calcium carbonate had a much higher hydroxycarbonate apatite (HCA)-forming ability in the simulated body fluid (SBF), than the pure polymer or the hydroxyapatite/polymer composites. The rapid formation of HCA on the surface was attributed to the large amount of calcium carbonate, which has the ability to effectively enhance the supersaturation of HCA because of fast dissolution of the used calcium carbonate phase vaterite. HCA is considered to be a novel biomaterial, as it exhibits very similar structure and chemical composition to the apatite in living bone [28]. HCA shows effective compatibility in cell attachment, proliferation and differentiation [28]. Thus, the aim of this study is the incorporation of calcium carbonate in different concentrations into highly porous polycaprolactone scaffolds in order to enhance the osseointegration, to adapt the degradation rate and to improve the mechanical characteristics of this material combination. To reproduce scaffolds with defined porosity, 3D printing by a type of FDM technique was chosen allowing for laboratory scaled processing of composite pellets.

2. Experimental section

Polycaprolactone (PCL, $M_n \sim 45\,000$) was purchased from Sigma-Aldrich (Taufkirchen, Germany); the calcium carbonate (CaCO_3) in form of calcite powder and hydroxyapatite as mineral powder was obtained from VWR International GmbH, Darmstadt, Germany.

In order to prepare the biocomposite samples used in this study, two types of manufacturing methods, i.e. molded casting and fused deposition modelling, were applied to manufacture different sample types. To produce a composite mixture, PCL was molten (at 80 °C) and different amounts of 20, 33 and 50 wt% CaCO₃ (C20, C33, C50) and 20 wt% hydroxyapatite (H20) were added and carefully homogenized in a heated beaker. After cooling the biocomposite was cut into small pellets for further processing.

2.1. Preparation of 3D composite scaffolds

Fused deposition modelling (FDM)

The porous three-dimensional scaffolds were fabricated by 3D-printing using a Bioscaffolder® (SYSENG/Germany). The composite pellets were molten at 74 °C (with the exception of the case group C50: this mixture was molten at 76 °C) and extruded through a needle with an inner diameter of 0.33 mm in a meandering pattern to produce three different geometries with the same internal layered grid structure. For the processing parameters, the air pressure and extruder screw rotation speed was kept constant throughout the case groups while slight variation in the feed rate occurred (Table 1). The line spacing in x-y levels was set to 0.55 mm and the level spacing in z was set to 0.26 mm. From layer to layer the

Table 1. FDM 3D-printing process parameters for different composite case groups.

Case group	Extruder temperature	Feed rate	Extruder screw rotation speed	Air pressure
P0	74 °C	190 mm/min	25 rpm	5 bar
C20	74 °C	180 mm/min	25 rpm	5 bar
C33	74 °C	160 mm/min	25 rpm	5 bar
C50	76 °C	170 mm/min	25 rpm	5 bar
H20	74 °C	160 mm/min	25 rpm	5 bar

Figure 1. Technical drawing of the different 3D-printed scaffold types: a) the line spacing and the level spacing in the inner scaffold structure and different scaffold types for b) degradation tests, c) release tests and SEM-investigation and d) for mechanical compression tests.

Table 2. 3D-printed composite scaffolds – geometry and application.

Process	Geometry	Size	Experimental application
Molded casting	cylinder	$h = 20 \text{ mm}$ $d = 10 \text{ mm}$	compression test
FDM	cylinder	$h = 20 \text{ mm}$ $d = 10 \text{ mm}$	compression test
FDM	cuboid flat cuboids	$8 \times 8 \times 4 \text{ mm}$ $8 \times 8 \times 1 \text{ mm}$	Ca release, degradation

strand orientation was rotated to 90° (Table 2 and Figure 1).

2.2. Molded casting

To obtain bulk samples without any porosity, cylindrical specimen (Table 2) were fabricated using an aluminum two-piece casting mold with 16 cavities. The measurements of the cavities were analogous to the cylindrical 3D-printed specimen ($h = 20 \text{ mm}$, $d = 10 \text{ mm}$). A defined mass of the pelletized biocomposite (3.5 g) was filled in each cavity and heated up to 80 °C on a heating plate in order to reach melting of PCL. A compaction step was performed in the melt to get rid of air cavities and the mold subsequently was kept at 100 °C for 12 h (Heraeus ST 6200). After cooling the aluminum mold to room temperature, the cylindrical specimens were removed and the remaining edges deburred. The specimens were rinsed in 70% ethanol and dried in the flue.

2.3. Determination of porosity

The fabricated 3D-printed scaffolds have an open circumferential surface with accessible pores. The cuboidal scaffolds with the geometry $8 \times 8 \times 4 \text{ mm}$ ($l \times w \times h$) were used representatively for the porosity calculation. The porosity of all the specimens was calculated from Equation (1):

$$P = 1 - \frac{V_{\text{scaffold strands}}}{V_{\text{cuboid}}} \quad (1)$$

where P : scaffold porosity, $V_{\text{scaffold strands}}$: volume of all the strands in the scaffold, V_{cuboid} : volume of the used calculation geometry $8 \times 8 \times 4$ mm ($l \times w \times h$).

2.4. Sterilization by gamma irradiation

As a standard sterilization procedure, according to ISO 11137-2:2006, a certified dosage of 25 ± 2 kGy in air was used in these experiments (Gamma Service Produktbestrahlung GmbH, Radeberg, Germany) to sterilize all specimens, being exposed to hydrolytic degradation (P0, C20-C50). To evaluate the influence of gamma irradiation on the mechanical properties of pure PCL and bio composites, 3D-printed cylindrical specimens of the group P0 and C33 were exemplarily selected. Eight irradiated specimens of each group and eight non-irradiated controls were tested.

2.5. Degradation

For the degradation studies 3D-printed flat cuboids (see Table 2 and Figure 1b) of each condition were selected, each specimen was weighed before degradation, 24 specimens in each case group (P0, C20–C50 and H20). Degradation was investigated after the exposure to I) phosphate buffered saline (PBS) and under catalyzed conditions using II) lipase containing PBS for a period of 28 days. Lipase from *Pseudomonas cepacia* (Grade 62309-100mg, Sigma Aldrich) was dissolved in PBS at a concentration of 0.5 mg/ml. The specimens were placed in 1 ml degradation solution each and were kept at 37°C and 5% CO_2 . PBS and PBS-lipase was changed twice a week. The pH value, the mass loss (to within 0.001 g) and the mechanical properties were determined after 1, 2, 4, 8, 12, 16, 20 and 28 days ($n = 3$). All specimens were tested in triplicates. For analytical purposes the specimens were rinsed in ddH₂O and dried for 24 hours at 37°C .

2.6. Analytical methods

Compression Tests

In order to evaluate the effect of the included inorganic component, the influence of the sterilization procedure and the degradation on the mechanical properties compression tests according to ISO standard DIN EN 604 were carried out at a rate of 10 mm/min by using an INSTRON 5566 testing machine. The Young's modulus was evaluated as the

slope of the initial linear region of the stress-strain curve (secant method). The mean value and the standard deviation were calculated for six specimens of the case groups P0, C20, C33 and C50.

The compressive Young's modulus E_c is calculated as the slope in the linear-elastic range within the stress-compression-diagram following Hooke's law, see Equation (2):

$$E_c = \frac{\sigma}{\epsilon} = \frac{\sigma_2 - \sigma_1}{\epsilon_2 - \epsilon_1} = \frac{\Delta\sigma}{\Delta\epsilon} \quad (2)$$

The compression strength σ is derived from the compression force F_c (*max force before failure*) calculating the base area of the cylindrical specimen A_0 , see Equation (3):

$$\sigma = \frac{F_c}{A_0} = \frac{F_c}{\pi r^2} \quad (3)$$

The nominal Compressive Strain at Break ϵ is calculated from the deformation length lb at break compared to the original height h of the specimen, as shown by Equation (4):

$$\epsilon b = \frac{\Delta lb}{h} \cdot 100\% = \frac{h - lb}{h} \cdot 100\% \quad (4)$$

2.7. pH measurement

In order to control the pH value as indicator for acidic degradation products, 800 μl of the PBS-lipase solution was taken from each specimen prior to each solution exchange. The pH value was determined using a seven multi S80-K (Mettler-Toledo, USA).

2.8. Scanning electron microscopy (SEM)

SEM imaging (XL 30 ESEM-FEG, Philips Deutschland GmbH, Hamburg, Germany and 982 Gemini, Zeiss GmbH, Germany) was used to visualize the surface and the cross-section obtained by cryo-fracture in liquid nitrogen of the biocomposite scaffolds before and after exposure to degradation. Samples were mounted on SEM sample holders and sputter coated with a thin gold/palladium layer.

2.9. Energy dispersive X-ray spectroscopy (EDX)

EDX, as an additional measurement of the SEM 982 Gemini (10 keV), was used to examine the mineralized CaP layer, formed during degradation in PBS-lipase. The calcium/phosphate ratio was determined for C33 after 2, 4 and 8 days of degradation.

2.10. Calcium-release

To investigate the calcium release from the different samples, three samples of each case group were incubated in 2.5 ml of PBS (0,1 M, Biochrom AG) and NaCl (0,9% physiological solution, Braun). After 2, 4, 6, 10, 14 and 20 days 50 μ l of the medium was removed and the calcium content was determined by means of a Ca-fluorescent (analyticon) in double determination. In order to display the total amount of calcium released, the individual measurement results were cumulated over the entire period.

2.11. Statistical analysis

The compression tests were conducted in triplicates per composition. Results are presented as mean $\pm$ standard deviation. The one way analysis of variance (ANOVA) and the t-test was applied for statistical analysis. *P* values <0.05 were considered significant and indicated by a horizontal bar.

3. Results

3.1. Preparation of 3D composite scaffolds and the determination of porosity

The cylindrical specimens, fabricated for mechanical testing, exhibit the following dimension (Figure 2a): 10 $\times$ 20 mm, containing 77 layers.

The cuboid-like specimens were chosen for the calcium release determination, the degradation tests, SEM-investigation and exhibit the following dimension:

8 $\times$ 8 $\times$ 4 mm, consisting of 16 layers (Figure 2b) and 8 $\times$ 8 $\times$ 1 mm with 5 layers (Figure 2c), respectively.

The strand dimension and the line spacing during processing was similar for all printed specimens in order to obtain an analogous porosity (Table 3). The determined porosity (shown in Table 3) demonstrates different values for the investigated case groups. The porosity seems to increase slightly with the increasing amount of inorganic component as a consequence of the lowered PCL amount (effective volume: with the same processing parameters the decreased PCL amount yields to down-sized strand diameter from 0.35 for neat PCL to 0.33 for C50).

3.2. Mineral distribution after 3D-printing (SEM-investigation)

The distribution of the CaCO₃-particles in the composite scaffolds was investigated by means of SEM-imaging. The presence and homogenous distribution of the CaCO₃-particles within the PCL matrix is evident for all the case groups. The visible particle density is clearly increasing with the growing amount of CaCO₃ (Figure 3). Up to contents of 33wt % CaCO₃, the particle distribution is homogenous and no agglomeration or enrichment in the fringe area is observed.

The specimens of the case group C50 (mineral content 50%) obviously show differences in the inclusion of the mineral particles into the polymer matrix.

Table 3. Detailed dimensional parameters of the scaffolds. The inner nozzle diameter and the line spacing were preset by the BioScaffolder. The strand diameter and the line spacing were measured on the resulting scaffolds during the test. The scaffold porosity is calculated by Equation (1).

Case group	Inner nozzle diameter [mm]	Strand diameter $\pm$ SD ($N_{sd} = 15$) [mm]	Line spacing ($N_{ls} = 10$) [mm]	Line spacing $\pm$ SD [mm]	Scaffold porosity [1]
PCL	0.33	0.347 $\pm$ 0.0135	0.55	0.557 $\pm$ 0.0166	36%
C20	0.33	0.338 $\pm$ 0.0065	0.55	0.554 $\pm$ 0.0118	38%
C33	0.33	0.353 $\pm$ 0.0063	0.55	0.540 $\pm$ 0.0121	38%
C50	0.33	0.330 $\pm$ 0.0100	0.55	0.552 $\pm$ 0.0159	41%

Figure 2. 3D-printed scaffolds in different shapes for further investigations: a) the mechanical compression tests; b) the release tests and the SEM-investigation, c) the degradation tests.

Figure 3. SEM-images (material contrast) of the 3D-printed composite scaffolds with different amounts of CaCO_3 . a) pure PCL (0%), b) C20 (20%), c) C33 (33%) and d) C50 (50%). SEM images of the magnified cross-sections for e) C33 and f) C50 in the material contrast mode (scale bar a–d: 200 μm ; e and f: 20 μm).

In detail, it can be observed that mineral agglomerates have formed and small voids appear between the mineral particles and the polymer matrix. In contrast to the other case groups (for example C33 (Figure 3e), the surface of the calcium carbonate particles is not completely covered by PCL (Figure 3f). The particles of calcium carbonate range from 1 to 10 μm and are of cubic shape. HAP particles were of cubic shape as well, but in general slightly smaller ($<3 \mu\text{m}$).

3.3. Mechanical properties

In this study, the cylindrical samples were evaluated by means of uniaxial compression tests. Both the pure bulk material and the biocomposite-scaffolds (P0, C20–C50) have been investigated. Both of the stress-strain curves (Figure 4) show a decrease in the stress yield point (beginning of plastic deformation) with increasing calcium carbonate content. This can be seen in the decreasing plateaus after linear elastic

deformation. The determined compression modulus is increasing with higher amounts of the mineral component for the non-porous specimens compared to plain PCL as known for composite or dispersion strengthening. This effect was also demonstrated in 3D printed specimen already [5]. However, for the porous specimens no significant change in Young's/compression modulus has been determined for the varying CaCO_3 amounts (Figure 5). Here, the structural influence is dominant in affecting the mechanics only and the mineral filler amount seems irrelevant, at least before the exposure to degradation. With increasing mineral amount, an increased degradation rate can be expected, and thus, the mechanical stability of the specimens with high filler content is estimated to be lower, compared to the low or no mineral component within the PCL matrix, shown in Figure 5 after 16 days for P0 vs. C33.

In addition to this investigation the influence of a gamma sterilization procedure (Figure 6) as well as the influence of the storage in a degradation solution (PBS-Lipase-solution) (Figure 6) concerning the Young's/compression modulus was investigated. For this test, the 3D-printed cylindrical samples from the case groups PCL and C33 were used.

As shown in Figure 6, a slight increase in the compression modulus as a result of the radiation-induced

Figure 6. Graphical representation of the effect of the different influencing factors (gamma sterilization, degradation by incubation in PBS-Lipase-solution after 16 days) on the mechanical properties of the 3D-printed scaffolds of the case groups P0 and C33. P values < 0.05 were considered significant and indicated by a horizontal bar.

cross-linking of PCL could be observed for P0. This effect was reported in irradiation-sterilized PCL before [16]. However, in case of C33 with the calcium carbonate concentration of 33 wt% the effect is not obvious. The cross-linking of PCL seems to be overlaid by chain scission, most likely due to the carbonate radicals, formed during the radiation.

Finally, the change of the mechanical properties of the FDM cylinders during the incubation in PCL-Lipase solution over 16 days was investigated. In Figure 6, both case groups showed similar trends: with

Figure 4. The stress-strain-curves of the mechanical compression test for the different sample bodies of different compositions a) bulk/molded cast, b) FDM 3D-printed.

Figure 5. The results of the mechanical compression test for the different sample bodies a) printed scaffolds, b) bulk material) of different compositions without any additional treatment. P values < 0.05 were considered significant and indicated by a horizontal bar.

increasing time of exposure to the aqueous solution the compression modulus as indicator for mechanical strength is decreasing. After 16 days of incubation the compression modulus decreased significantly more for C33 compared to P0 as the result of the CaCO_3 incorporation (CaCO_3 accelerates the degradation). In course of time the resulting pores from the released mineral particles yield to significant by rapid degradation.

3.4. Degradation study, mass loss and pH-value buffering

For the degradation studies, the flat-printed FDM scaffolds of all the case groups were incubated in a solution of lipase and phosphate buffer in order to simulate enzymatic degradation. The mass loss of the scaffolds and the pH-value in the medium were measured and the degraded samples were analyzed by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). Figure 7 displays the mass loss of the tested scaffolds over a period of 28 days.

In contrast to pure PCL samples (P0), the mass loss of the biocomposites indicate that the samples with lower additive contents show an accelerated degradation, but with the increasing calcium carbonate contents (see C50) the degradation rate decreases. It is noticeable that the scaffolds of the case group H20 (with hydroxyapatite) have lost the least weight over the test period of 28 days of enzymatic triggered degradation.

During the degradation study, the acidification of the media was investigated starting after 24 hours of immersion in the Lipase/PBS solution. Depending on the material composition, the measured pH-values show clear differences (Figure 8).

Figure 7. Mass loss of the flat-printed FDM scaffolds during degradation in PBS and lipase (case groups with different amounts of 20, 33 and 50 wt% CaCO_3 (C20, C33, C50) and 20 wt% hydroxyapatite (H20)).

Figure 8. pH values during the degradation of the flat-printed FDM scaffolds in PBS and lipase. Measurements were started after 1 day of incubation, day 0 control = pH 7,4.

After control solutions at day 0 (pH 7,4) the acidification was immediately measurable after 24 hours. The highest acidification (pH value <7) was measured in the samples with the pure PCL scaffolds. With increasing CaCO_3 content the pH-values in the media increased to a neutral range. While C20 still fluctuates between 5 and 6, pH values up to 7.4 (neutral range) were measured in the media of the samples with C33 and C50. The scaffolds with 20% hydroxyapatite (H20) show pH-level around 4.5.

Furthermore, the degraded samples were characterized by SEM-imaging. Figure 9 shows thin, clearly degraded strands with decayed structures on the right side for Figure 9b, 9d, 9f, 9h, 9j) after 20 days of incubation. It can be observed that the enzymatic triggered degradation does not proceed uniformly or in bulk, but also acts locally or superficially. The scaffolds outer upper strand layers seem to degrade much quicker than the strands beneath. Furthermore cross contacts of strands degraded slower compared to free hanging strands. All in all a surface erosion can be observed which seems to act stronger on the outer parts of the scaffold compared to layers beneath and inner parts as well as layer cross contact points.

Another conspicuous feature is the formation of a crystalline layer on the scaffolds C20, C33 and C50, which seems to take place in addition to degradation. This phenomenon showed up just after 4 days (Figure 9 left side). In the material contrast this layer appears lighter, indicating a higher atomic number. No layer formation could be observed on the scaffolds with hydroxyapatite (H20) and on those without any mineral additive (P0).

Figure 9. The SEM images after 4 (left column) and 20 days (right column) of incubation in PBS-lipase of the different investigated case groups a), b) P0, c), d) C20, e), f) C33, g), h) C50 and i), j) H20. No mineral formation was detected for the control P0 (a, b) and the samples including HAP (H20 (i, j)) (the case groups with different amounts of 20, 33 and 50 wt% CaCO_3 (C20, C33, C50) and 20 wt% hydroxyapatite (H20)). Scale bar left side: 100 μm ; right side: 200 μm .

3.5. Energy dispersive X-ray spectroscopy

During the degradation tests, a mineral layer has been formed on the scaffolds of the case groups C20, C33 and C50, which was also analyzed by means of SEM and EDX. The exemplary analyzed sample is an FDM scaffold with 33% calcium carbonate (C33) with CaP layer formation, shown in Figure 10.

The rose-shaped structure and the platelets are less than 1 μm (Figure 10a and 10b). As can be seen in Figure 10c and 10d, the layer is bright in the material contrast, indicating higher atomic numbers than the polymer matrix. The calcium carbonate particles in the PCL matrix are also recognizable as light cuboids even after long degradation times underneath the apatite layer (Figure 10d). The layer has been intentionally broken at some points during the sample preparation in order to show the covered, degrading bio-composite. Figures 10c and d show how the mineral layer deposits on the surface of the underlying degraded polymer strand.

The result of energy dispersive X-ray spectroscopy is shown in Table 4. In the measured areas calcium, phosphate, oxygen and carbon were detected. Thereby the element composition of the layer remains constant over the degradation time. The calcium/phosphate ratio ranges between 1.53 and 1.66.

Table 4. Results of the EDX-investigations of the newly formed mineral layer on the samples of the case group C33 after 2, 4 and 8 days. Information in each case in atomic%: (C = carbon, O = oxygen, Ca = calcium, P = phosphorus).

Time [days]	C [%]	O [%]	Ca [%]	P [%]	Ca/P
2	33.63	42.83	14.47	8.73	1.66
4	33.69	42.70	13.99	9.17	1.53
8	30.07	44.41	15.26	9.72	1.57

3.6. Calcium-release

Calcium release of the flat-printed scaffolds of all the case groups was determined. The samples were incubated in phosphate buffer (PBS) and in physiological saline solution (NaCl) and the calcium concentration was measured after defined intervals, respectively. Figure 11a shows the calcium release of all case groups in the physiological saline solution (0.9% NaCl). During this process the tendency towards plate formation of the measured release values is shown. Furthermore, it can be observed that the calcium release from the scaffolds increases with the increasing calcium carbonate content (C20 to C50). The samples of the H20 case group with hydroxylapatite remain at low level, below 2 mmol/l.

Figure 10. Topography (a and b) and material contrast (c and d) clearly show biomaterialized overall coating for C33 after 20 days in PBS-lipase.

Figure 11. Graphical representation of the calcium release of the flat-printed scaffold in a) 0,9% NaCl-solution and b) 0.1 M PBS.

Figure 11b shows the calcium release of all the case groups in the phosphate buffer solution (0.1 M PBS). It is striking that the release of all tested specimens is far below, compared to the release in the phosphate-free saline solution (Figure 11a). The case groups again show a rising calcium release with the increasing calcium carbonate content analogous to the medium saline solution. However, it seems to reach its release limit earlier. Same trend is obvious for H20; the calcium release is very low in PBS. The H20 precipitation group with hydroxyapatite does not show any considerable calcium release and remains at the level of P0 without any mineral content.

4. Discussion

The scaffolds of different geometries fabricated in this work can be produced reliably and reproducibly by means of fused deposition modelling. Depending on the mineral content within the specially prepared pastes, the process parameters such as the depositing temperature had to be changed. The fabricated scaffolds differ slightly in their pore size due to the mineral content. The scaffolds of pure PCL exhibit the lowest porosity of 36%, the porosity of C50 was the highest with 41%, while the porosity value of all the other case groups range between 37 and 38%. With increasing amount of the inorganic component the lowered PCL matrix amount (less material at the same injection volume due to a higher spec. density of the mineral (2.73 vs. 1.21 g/cm³)) leads to differences in viscosity and to lowered material volume during injection.

The viscosity increased with the mineral content. This effect can be explained by the matrix-additive-interaction, which increases with higher amounts of the additive and smaller particle size, due to higher specific surface. These interactions limit the movement of molecule chains and therefore increase the viscosity [30–33].

The viscosity of the different basic materials increase with rising mineral content. Due to the high concentrated mineral, the case group C50 shows a very high viscosity with the result, that the strands were more stretched, the strand diameters were smaller and the gaps between the strands grew slightly larger. In addition, the examined mineral composites in the melt may be considered non-Newtonian liquids due to the polymer content. The shear stresses in the nozzle orient the molecular chains [shear-thinning]. After leaving the nozzle the strand widens again as the polymer coils [entropy maximization]. Depending on the calcium content, the orientation in the nozzle is probably already prevented.

In general, in spite of minor differences it was possible to obtain comparable porosity during the scaffold fabrication by means of FDM through all the groups. The pellets from the custom-made pastes of PCL and CaCO₃ were produced as the basic material for the fabrication of different test specimens.

This special process method is simple to use and enables to fabricate reproducible and application-adapted sterilizable specimens of high quality up to a calcium carbonate concentration of 50 wt%. In the case of the groups C20 and C33 a homogeneous distribution of the mineral phase additive with closing contact to the polymer matrix was detected as can be seen in SEM imaging of strand cross sections.

4.1. Mechanical properties

Scaffolds for biomedical applications may be used in load-bearing components. Therefore, one requirement is an equal or comparable mechanical stability of the graft material, compared to the surrounding tissue, because a significantly higher stiffness can lead to stress shielding and bone resorption [34–36]. The hard tissues of the human body are based on more or less mineralized organic matrices. To investigate the mechanical properties of the different bulk

materials and the porous FDM-scaffolds, a uniaxial compression test was carried out.

By conducting these measurements, errors during the 3D printing process could be demonstrated. It is also possible to estimate, in which body parts the material of polycaprolactone and calcium carbonate could be applied to substitute the defect tissue.

The first considerable result of this mechanical experiment was that no brittle fracture of the tested material compositions was observed. All the investigated materials, both the bulk materials and the FDM-scaffolds, showed plastic deformation behavior in all the tested case groups. In the experiment range up to about 50% compression it came to a lateral bulging and buckling, but not to a split of the cylindrical specimens. For the mechanical biocompatibility this is a desirable result. A plastic deformation of the material is interpretable as a kind of alert signal for a failure in the implant, because sudden brittle fractures with material spalling could damage the surrounding tissue. It is assumed that the polymer matrix of polycaprolactone is responsible for this plastic behavior, since mineral components show a rather brittle behavior. The examination of the cast cylindrical bulk specimens (see Figure 5) shows that in contrast to pure PCL an increase in the compression modulus with the increasing calcium carbonate content is detectable. At the same time, the plateau for plastic deformation (yield point) was reached at lower stresses already when higher contents of calcium carbonate were added to the composite (see curves Figure 4b). The modulus of pure PCL as a bulk material in this study is measured with about 400 MPa. In the literature values of 300 MPa are mentioned. In this context it is important to note that in this study relatively short chained PCL (45 000 kD) is used, which is characterized by a higher proportion of the crystalline material, what justifies the higher values in this study [32, 33, 37, 38]. The modulus of the bulk materials increases up to 600 MPa at a concentration of 50% calcium carbonate. With varying content of mineral particles it seems to be possible to influence stiffness (Young's Modulus) as well as yield (Plateau) behavior of the composite.

The cylindrical 3D-printed scaffolds did show a similar behavior for the yield plateau (Figure 4a), but not a comparable increase of the compression modulus (Figure 5a). On the contrary, only a slight increase of the modulus was recognizable by the specimens of the case group C20. As described before,

the printed scaffolds with the calcium carbonate contents of 50% showed inhomogenities, concerning the mineral distribution and the contact between mineral particles and polymer matrix. The values of the FDM-scaffolds were measured in the range of about 150 MPa, 60% less than the values of the bulk materials.

A special feature is represented in the case group H20 (3D printed, PCL + 20 % hydroxyapatite) with values up to 270 MPa. This impact may be caused by the smaller particle size of the mineral phase. Evidently the investigation of various particle sizes of the different mineral phases is promising good results in the continuing leading investigations.

The mechanical properties of a composite material are highly influenced by its matrix-matrix, matrix-additive, and additive-additive interactions. Additives with high specific surfaces have an increased amount of bonds between them and the matrix, which results in higher mechanical properties of the material. This phenomenon is increased by smaller particle size and irregular particle shape. Also the particle (size) distribution plays an important role [30, 39, 40].

It was also shown that both, the performed gamma sterilization and the enzymatically catalyzed degradation had an effect on the mechanical properties of the scaffolds. A slight increase of the modulus of the pure PCL-cylinders and the ones with different levels of mineral additives can be explained by the properties of the matrix material PCL. Its crystallinity increases with the decrease of the polymer chains. This correlation explains the impact of the gamma sterilization. The high-energy gamma radiation can have two effects on the polymer. On the one hand, it can break the polymer chains, lowering the molar mass. The shorter chains can be orientated easier and therefore increase the crystallinity and the mechanical properties. On the other hand, crosslinking of the polymer chains can occur (depending on the radiation energy >25 kGy) and raise the molecular mass. The crosslinked chains cannot reorient into crystalline regions and will decrease the mechanical properties of the material. Also the crosslinked chains will increase the degradation time [32, 33, 41, 42]. Viana *et al.* [43] showed that the high-energy gamma radiation has minor effects on calcium-carbonate. A polymer chain cleavage could be caused by degradation and by gamma sterilization [44]. Although the bulk modulus of human cortical bone is about 70 times higher than the measured values of the bulk

materials, it was shown, that it is possible to adapt the mechanical properties by the content of the mineral additive and its particle size. In addition, it is as well possible to print scaffolds with a larger strand diameter to improve the mechanical stability. Olubamiji *et al.* [38] showed, that there is an inverse linear relationship between the porosity and the mechanical properties of PCL-scaffolds.

4.2. Degradation

Resorbable biomaterials are subjected to different mechanisms of degradation, which are of great importance for wound healing. In this study, the hydrolytic degradation of the scaffolds was catalyzed enzymatically by the use of lipase in PBS as degradation medium. This method is widely described in the literature [15] and allows to investigate PCL degradation without changing the pH-value by the use of acids and alkalis [45], representing the complete decomposition and the mass reduction of the polymer as the last physiological degradation step. Lipase attacks the carbonyl group and oxygen in lipids. This chemical bond is also destroyed during hydrolytic degradation of PCL. Therefore, lipase does not change the degradation mechanism but increases the degradation rate. While in-vivo it can take up to three years to the complete degradation of PCL [41], it is not unusual to see a mass loss of PCL by *in-vitro* degradation with lipase within 24 hours. During the degradation the crystallinity of the PCL increases while the molecular weight remains approximately the same [46].

The measurement of the mass reduction shows that the scaffolds of all the case groups decompose by the chosen experimental modification evenly within 20 to 30 days. It should be noted at this point, that the mass loss measurement shows two processes at once. The first is the degradation of the PCL matrix and the second one is the buildup of an apatite layer which will be discussed later on.

By recording the pH-values (Figure 8) it is possible to measure the acidification of the surrounding medium by the degradation of polycaprolactone. It is known that the degradation products of polyesters such as PCL are acidic [47].

The case group P0 (PCL without any mineral additive) generated pH values below 4 during the degradation period. It is possible to buffer this acidification by means of adding calcium carbonate. From the level of 33% calcium carbonate the pH-values

rise in the medium during the decomposition of the scaffolds (C33, C50) to the neutral range. The buffering of the scaffolds with 20% hydroxyapatite (H20) is lower and remains at the level of the scaffolds with 10% calcium carbonate (C10). Due to these buffering effects, the auto-catalyzed degradation by acidification from PCL may be prevented as well. This may be an explanation why P0 is losing its mass the fastest although the other case groups should have bigger surfaces due to mineral particles. But in the composites also mass is build up due to apatite formation.

The higher buffering effect is an interesting advantage of calcium carbonate, compared to hydroxyapatite as an additive material to PCL. In particular, the contact of polymer biomaterials with bone tissue repeatedly causes complications, associated with the acidification and the pH-value instability of polyester materials [45, 48, 49].

The SEM-investigation of the degraded scaffolds from pure PCL and combined with HAP (H20) showed surface erosion acting mainly on the outer parts of the scaffolds, as well as the decreases in the strand diameters, depending on the degradation time. The scaffolds with added calcium carbonate showed in addition to the normal decomposition phenomena a mineral layer of the apatite-typical plate- and needle-type crystals on the material surface.

The calcium/phosphorus atomic ratio of the new built layers, measured by EDX, is in hydroxyapatite similar range of 1.6 [50].

Kokubo and Takadama [51] defined the bioactivity of a bone substitute material as the ability to build up a fixed connection, wherein the first step after the implantation of the material into the body is the formation of an apatite-like layer on its surface. Furthermore, they state that this in vivo behavior could be reached by incubating the material in a simulated body fluid [51].

It is shown in this study that an apatite layer could be built by the use of a calcium-free medium during the enzymatic degradation of the 3D printed scaffolds with calcium carbonate as a mineral additive. That represents the bioactivity and suitability of the FDM-biocomposite scaffolds as a bone substitute material.

4.3. Calcium-release

To confirm the evidence of bioactivity of the biocomposite scaffolds as shown in the degradation test,

a calcium release test was performed. The scaffolds of all the case groups were incubated for 20 days in the phosphate buffer and physiological saline solution. In both media the calcium release of the scaffolds with 20% calcium carbonate was higher than from the scaffolds with 20% hydroxyapatite. The scaffolds of the other case groups (C20–C50) showed the increasing calcium release with the increasing calcium carbonate content in both media. In the saline solution the calcium release of all the case groups was significantly higher than in the phosphate buffer, what might be caused by the phosphate induced mineral formation on the scaffold surfaces which does not occur in a phosphate-free medium.

Furthermore, the results show that the mineral particles are tightly bound within the PCL matrix and do not diffuse during the degradation. Thus it can be assumed that the calcium release first purely emanate from the surface of the scaffolds.

5. Conclusions

The study can be considered as a first step in the direction of a new hard tissue replacement material. By means of various experiments the first base of data was collected and the basics for further more detailed biocompatibility studies of 3D printed biocomposite scaffolds of PCL and CaCO_3 are given. The established production path has enormous potential and it is possible to manufacture open-porous scaffolds for the individual and application-oriented hard tissue replacement. The mechanical behavior of the scaffolds can be controlled by the amount of added calcium carbonate to the PCL. The advantages of calcium carbonates over calcium phosphates as additive minerals to PCL were demonstrated. Thus, a significant buffer effect of the pH value was detected by the use of calcium carbonate, which can compensate the acidification by degradation products of the polymer. Furthermore, the bioactivity of the used composite scaffolds was shown by a clear mineral formation *in vitro*. It was shown that the chosen approach could be a promising innovation in the field of hard tissue replacement. From the tested case groups we believe that a PCL composite with around 33% of calcium carbonate serves best in terms of processability, desired buffering effect as well as *in-vitro* apatite formation. Finally, the full potential of the approach has to be exploited in ongoing studies, and more extensive comparative trials must be made to show that the benefits of calcium carbonate outweigh calcium

phosphate. The possibility of modifying the suitability of the investigated biocomposites for bone replacement is the aim of further investigations. Here, different scaffold geometries as well as the use of different calcium carbonate phases and their effects concerning mechanical properties, degradation and biocompatibility have to be investigated.

References

- [1] Chia H. N., Wu B. M.: Recent advances in 3D printing of biomaterials. *Journal of Biological Engineering*, **9**, 4/1–4/14 (2015).
<https://doi.org/10.1186/s13036-015-0001-4>
- [2] Do A-V., Khorsand B., Geary S. M., Salem A. K.: 3D printing of scaffolds for tissue regeneration applications. *Advanced Healthcare Materials*, **4**, 1742–1762 (2015).
<https://doi.org/10.1002/adhm.201500168>
- [3] Doppalapudi S., Jain A., Khan W., Domb A. J.: Biodegradable polymers – An overview. *Polymers for Advanced Technologies*, **25**, 427–435 (2014).
<https://doi.org/10.1002/pat.3305>
- [4] Wu G-H., Hsu S-H.: Review: Polymeric-based 3D printing for tissue engineering. *Journal of Medical and Biological Engineering*, **35**, 285–292 (2015).
<https://doi.org/10.1007/s40846-015-0038-3>
- [5] Heo S-J., Kim S-E., Wei J., Hyun Y-T., Yun H-S., Kim D-H., Shin J. W., Shin J-W.: Fabrication and characterization of novel nano- and micro-HA/PCL composite scaffolds using a modified rapid prototyping process. *Journal of Biomedical Materials Research*, **89**, 108–116 (2009).
<https://doi.org/10.1002/jbm.a.31726>
- [6] Olah L., Borbas L.: Properties of calcium carbonate-containing composite scaffolds. *Acta of Bioengineering and Biomechanics*, **10**, 61–66 (2008).
- [7] Griffith L. G.: Polymeric biomaterials. *Acta Materialia*, **48**, 263–277 (2000).
[https://doi.org/10.1016/S1359-6454\(99\)00299-2](https://doi.org/10.1016/S1359-6454(99)00299-2)
- [8] Huang M-H., Li S., Hutmacher D. W., Schantz J-T., Vacanti C-A., Braud C., Vert M.: Degradation and cell culture studies on block copolymers prepared by ring opening polymerization of ϵ -caprolactone in the presence of poly(ethylene glycol). *Journal of Biomedical Materials Research*, **69**, 417–427 (2004).
<https://doi.org/10.1002/jbm.a.30008>
- [9] Zhu Z., Xiong C., Zhang L., Deng X.: Synthesis and characterization of poly(ϵ -caprolactone)-poly(ethylene glycol) block copolymer. *Journal of Polymer Science Part A: Polymer Chemistry*, **35**, 709–714 (1997).
[https://doi.org/10.1002/\(SICI\)1099-0518\(199703\)35:4<709::AID-POLA14>3.0.CO;2-R](https://doi.org/10.1002/(SICI)1099-0518(199703)35:4<709::AID-POLA14>3.0.CO;2-R)
- [10] Williams J. M., Adewunmi A., Schek R. M., Flanagan C. L., Krebsbach P. H., Feinberg S. E., Hollister S. J., Das S.: Bone tissue engineering using polycaprolactone scaffolds fabricated *via* selective laser sintering. *Biomaterials*, **26**, 4817–4827 (2005).
<https://doi.org/10.1016/j.biomaterials.2004.11.057>

- [11] Ciardelli G., Chiono V., Vozzi G., Pracella M., Ahluwalia A., Barbani N., Cristallini C., Giusti P.: Blends of poly(ϵ -caprolactone) and polysaccharides in tissue engineering applications. *Biomacromolecules*, **6**, 1961–1976 (2005).
<https://doi.org/10.1021/bm0500805>
- [12] Ma Q., Wang Y., Zheng H., Ning C., Deng C.: Preparation of novel bioactive PCL bone tissue engineering scaffold (in Chinese). *Journal of Biomedical Engineering*, **26**, 550–554 (2009).
- [13] Rezwan K., Chen Q. Z., Blaker J. J., Boccaccini A. R.: Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials*, **27**, 3413–3431 (2006).
<https://doi.org/10.1016/j.biomaterials.2006.01.039>
- [14] de Santis R., Gloria A., Russo T., D'Amora U., D'Antò V., Bollino F., Catauro M., Mollica F., Rengo S., Ambrosio L.: Advanced composites for hard-tissue engineering based on PCL/organic–inorganic hybrid fillers: From the design of 2D substrates to 3D rapid prototyped scaffolds. *Polymer Composites*, **34**, 1413–1417 (2013).
<https://doi.org/10.1002/pc.22446>
- [15] Bianco A., del Gaudio C., Baiguera S., Armentano I., Bertarelli C., Dottori M., Bultrini G., Lucotti A., Kenny J. M., Folini M.: Microstructure and cytocompatibility of electrospun nanocomposites based on poly(ϵ -caprolactone) and carbon nanostructures. *International Journal of Artificial Organs*, **33**, 271–282 (2010).
<https://doi.org/10.1177/039139881003300502>
- [16] Filipczak K., Wozniak M., Ulanski P., Olah L., Przybytniak G., Olkowski R. M., Lewandowska-Szumiel M., Rosiak J. M.: Poly(ϵ -caprolactone) biomaterial sterilized by e-beam irradiation. *Macromolecular Bioscience*, **6**, 261–273 (2006).
<https://doi.org/10.1002/mabi.200500215>
- [17] Koshino T., Murase T., Takagi T., Saito T.: New bone formation around porous hydroxyapatite wedge implanted in opening wedge high tibial osteotomy in patients with osteoarthritis. *Biomaterials*, **22**, 1579–1582 (2001).
[https://doi.org/10.1016/S0142-9612\(00\)00318-5](https://doi.org/10.1016/S0142-9612(00)00318-5)
- [18] Porter A. E., Patel N., Skepper J. N., Best S. M., Bonfield W.: Effect of sintered silicate-substituted hydroxyapatite on remodelling processes at the bone–implant interface. *Biomaterials*, **25**, 3303–3314 (2004).
<https://doi.org/10.1016/j.biomaterials.2003.10.006>
- [19] Woodard J. R., Hildore A. J., Lan S. K., Park C. J., Morgan A. W., Eurell J. A. C., Clark S. G., Wheeler M. B., Jamison R. D., Wagoner Johnson A. J.: The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity. *Biomaterials*, **28**, 45–54 (2007).
<https://doi.org/10.1016/j.biomaterials.2006.08.021>
- [20] Gay S., Arostegui S., Lemaitre J.: Preparation and characterization of dense nanohydroxyapatite/PLLA composites. *Materials Science and Engineering: C*, **29**, 172–177 (2009).
<https://doi.org/10.1016/j.msec.2008.06.005>
- [21] Kim H-W., Knowles J. C., Kim H-E.: Development of hydroxyapatite bone scaffold for controlled drug release via poly(ϵ -caprolactone) and hydroxyapatite hybrid coatings. *Journal of Biomedical Materials Research Part B, Applied Biomaterials*, **70**, 240–249 (2004).
<https://doi.org/10.1002/jbm.b.30038>
- [22] Kim H-W., Knowles J. C., Kim H-E.: Effect of biphasic calcium phosphates on drug release and biological and mechanical properties of poly(ϵ -caprolactone) composite membranes. *Journal of Biomedical Materials Research Part A*, **70**, 467–479 (2004).
<https://doi.org/10.1002/jbm.a.30100>
- [23] Kikuchi M., Koyama Y., Yamada T., Imamura Y., Okada T., Shirahama N., Akita K., Takakuda K., Tanaka J.: Development of guided bone regeneration membrane composed of β -tricalcium phosphate and poly(L-lactide-co-glycolide-co- ϵ -caprolactone) composites. *Biomaterials*, **25**, 5979–5986 (2004).
<https://doi.org/10.1016/j.biomaterials.2004.02.001>
- [24] Ural E.: Poly(D,L-lactide/epsilon-caprolactone)/hydroxyapatite composites. *Biomaterials*, **2**, 2147–2154 (2000).
- [25] Liao S. S., Cui F. Z., Zhang W., Feng Q. L.: Hierarchically biomimetic bone scaffold materials: Nano-HA/collagen/PLA composite. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, **69**, 158–165 (2004).
<https://doi.org/10.1002/jbm.b.20035>
- [26] Chan C-M., Wu J., Li J-X., Cheung Y-K.: Polypropylene/calcium carbonate nanocomposites. *Polymer*, **43**, 2981–2992 (2002).
[https://doi.org/10.1016/S0032-3861\(02\)00120-9](https://doi.org/10.1016/S0032-3861(02)00120-9)
- [27] Wang G., Chen X. Y., Huang R., Zhang L.: Nano- CaCO_3 /polypropylene composites made with ultrahigh-speed mixer. *Journal of Materials Science Letters*, **21**, 985–986 (2002).
<https://doi.org/10.1023/A:1016044204168>
- [28] Kasuga T., Maeda H., Kato K., Nogami M., Hata K-I., Ueda M.: Preparation of poly(lactic acid) composites containing calcium carbonate (vaterite). *Biomaterials*, **24**, 3247–3253 (2003).
[https://doi.org/10.1016/S0142-9612\(03\)00190-X](https://doi.org/10.1016/S0142-9612(03)00190-X)
- [29] Maeda H., Kasuga T., Nogami M., Ueda M.: Preparation of bonelike apatite composite for tissue engineering scaffold. *Science and Technology of Advanced Materials*, **6**, 48–53 (2005).
<https://doi.org/10.1016/j.stam.2004.07.003>
- [30] Beun S., Bailly C., Dabin A., Vreven J., Devaux J., Leloup G.: Rheological properties of experimental bis-GMA/TEGDMA flowable resin composites with various macrofiller/microfiller ratio. *Dental Materials*, **25**, 198–205 (2009).
<https://doi.org/10.1016/j.dental.2008.06.001>

- [31] Lee J-H., Um C-M., Lee I-B.: Rheological properties of resin composites according to variations in monomer and filler composition. *Dental Materials*, **22**, 515–526 (2006).
<https://doi.org/10.1016/j.dental.2005.05.008>
- [32] Kósa C., Sedlačík M., Fiedlerová A., Chmela Š., Borská K., Mosnáček J.: Photochemically cross-linked poly(ϵ -caprolactone) with accelerated hydrolytic degradation. *European Polymer Journal*, **68**, 601–608 (2015).
<https://doi.org/10.1016/j.eurpolymj.2015.03.041>
- [33] Yeh C-C., Chen C-N., Li Y-T., Chang C-W., Cheng M-Y., Chang H-I.: The effect of polymer molecular weight and UV radiation on physical properties and bioactivities of PCL films. *Cellular Polymers*, **30**, 261–276 (2011).
- [34] Arabnejad S., Johnston B., Tanzer M., Pasini D.: Fully porous 3D printed titanium femoral stem to reduce stress-shielding following total hip arthroplasty. *Journal of Orthopaedic Research*, **35**, 1774–1783 (2017).
<https://doi.org/10.1002/jor.23445>
- [35] Hutmacher D. W.: Scaffolds in tissue engineering bone and cartilage. *Biomaterials*, **21**, 2529–2543 (2000).
[https://doi.org/10.1016/S0142-9612\(00\)00121-6](https://doi.org/10.1016/S0142-9612(00)00121-6)
- [36] Abedalwafa M., Wang F., Wang L., Li C.: Biodegradable poly-epsilon-caprolactone (PCL) for tissue engineering applications: A review. *Reviews on Advanced Materials Science*, **34**, 123–140 (2013).
- [37] Eshraghi S., Das S.: Mechanical and microstructural properties of polycaprolactone scaffolds with one-dimensional, two-dimensional, and three-dimensional orthogonally oriented porous architectures produced by selective laser sintering. *Acta Biomaterialia*, **6**, 2467–2476 (2010).
<https://doi.org/10.1016/j.actbio.2010.02.002>
- [38] Olubamiji A. D., Izadifar Z., Si J. L., Cooper D. M. L., Eames B. F., Chen D. X. B.: Modulating mechanical behaviour of 3D-printed cartilage-mimetic PCL scaffolds: Influence of molecular weight and pore geometry. *Biofabrication*, **8**, 025020/1–025020/18 (2016).
<https://doi.org/10.1088/1758-5090/8/2/025020>
- [39] Zuiderduin W. C. J., Westzaan C., Huétink J., Gaymans R. J.: Toughening of polypropylene with calcium carbonate particles. *Polymer*, **44**, 261–275 (2003).
[https://doi.org/10.1016/S0032-3861\(02\)00769-3](https://doi.org/10.1016/S0032-3861(02)00769-3)
- [40] Bartczak Z., Argon A. S., Cohen R. E., Weinberg M.: Toughness mechanism in semi-crystalline polymer blends: II. High-density polyethylene toughened with calcium carbonate filler particles. *Polymer*, **40**, 2347–2365 (1999).
[https://doi.org/10.1016/S0032-3861\(98\)00444-3](https://doi.org/10.1016/S0032-3861(98)00444-3)
- [41] Cottam E., Hukins D. W. L., Lee K., Hewitt C., Jenkins M. J.: Effect of sterilisation by gamma irradiation on the ability of polycaprolactone (PCL) to act as a scaffold material. *Medical Engineering and Physics*, **31**, 221–226 (2009).
<https://doi.org/10.1016/j.medengphy.2008.07.005>
- [42] Darwis D., Mitomo H., Enjoji T., Yoshii F., Makuuchi K.: Heat resistance of radiation crosslinked poly(ϵ -caprolactone). *Journal of Applied Polymer Science*, **68**, 581–588 (1998).
[https://doi.org/10.1002/\(SICI\)1097-4628\(19980425\)68:4<581::AID-APP9>3.0.CO;2-I](https://doi.org/10.1002/(SICI)1097-4628(19980425)68:4<581::AID-APP9>3.0.CO;2-I)
- [43] Viana P. S., Orlandi M. O., Pavarina A. C., Machado A. L., Vergani C. E.: Chemical composition and morphology study of bovine enamel submitted to different sterilization methods. *Clinical Oral Investigations*, **22**, 733–744 (2018).
<https://doi.org/10.1007/s00784-017-2148-5>
- [44] Spenlehauer G., Vert M., Benoit J. P., Boddaert A.: *In vitro* and *in vivo* degradation of poly(D,L lactide/glycolide) type microspheres made by solvent evaporation method. *Biomaterials*, **10**, 557–563 (1989).
[https://doi.org/10.1016/0142-9612\(89\)90063-X](https://doi.org/10.1016/0142-9612(89)90063-X)
- [45] Ara M., Watanabe M., Imai Y.: Effect of blending calcium compounds on hydrolytic degradation of poly(DL-lactic acid-co-glycolic acid). *Biomaterials*, **23**, 2479–2483 (2002).
[https://doi.org/10.1016/S0142-9612\(01\)00382-9](https://doi.org/10.1016/S0142-9612(01)00382-9)
- [46] Ferreira J., Gloria A., Cometa S., Coelho J. F. J., Domingos M.: Effect of *in vitro* enzymatic degradation on 3D printed poly(epsilon-caprolactone) scaffolds: Morphological, chemical and mechanical properties. *Journal of Applied Biomaterials & Functional Materials*, **15**, E185–E195 (2017).
<https://doi.org/10.5301/jabfm.5000363>
- [47] Sung H-J., Meredith C., Johnson C., Galis Z. S.: The effect of scaffold degradation rate on three-dimensional cell growth and angiogenesis. *Biomaterials*, **25**, 5735–5742 (2004).
<https://doi.org/10.1016/j.biomaterials.2004.01.066>
- [48] Linhart W., Peters F., Lehmann W., Schwarz K., Schilling A. F., Amling M., Rueger J. M., Eppe M.: Biologically and chemically optimized composites of carbonated apatite and polyglycolide as bone substitution materials. *Journal of Biomedical Materials Research*, **54**, 162–171 (2001).
[https://doi.org/10.1002/1097-4636\(200102\)54:2<162::AID-JBM2>3.0.CO;2-3](https://doi.org/10.1002/1097-4636(200102)54:2<162::AID-JBM2>3.0.CO;2-3)
- [49] Schiller C., Rasche C., Wehmöller M., Beckmann F., Eufinger H., Eppe M., Weihe S.: Geometrically structured implants for cranial reconstruction made of biodegradable polyesters and calcium phosphate/calcium carbonate. *Biomaterials*, **25**, 1239–1247 (2004).
<https://doi.org/10.1016/j.biomaterials.2003.08.047>
- [50] Williams D.: *Essential biomaterials science*. Cambridge University Press, Cambridge (2014).
- [51] Kokubo T., Takadama H.: How useful is SBF in predicting *in vivo* bone bioactivity? *Biomaterials*, **27**, 2907–2915 (2006).
<https://doi.org/10.1016/j.biomaterials.2006.01.017>