Development of a solvent-free polylactide/calcium carbonate composite for selective laser sintering of bone tissue engineering scaffolds

Christoph Gayer\textsuperscript{a, b}, Jessica Ritter\textsuperscript{b}, Martin Bullemer\textsuperscript{c,1}, Stefanie Grom\textsuperscript{d}, Lucas Jauer\textsuperscript{a}, Wilhelm Meiners\textsuperscript{a,2}, Andreas Pfister\textsuperscript{e}, Frank Reinauer\textsuperscript{d}, Marijan Vučak\textsuperscript{e}, Konrad Wissenbach\textsuperscript{f}, Horst Fischer\textsuperscript{b}, Reinhart Poprawe\textsuperscript{f}, Johannes Henrich Schleifenbaum\textsuperscript{g}

\textsuperscript{a} Fraunhofer Institute for Laser Technology ILT, Steinbachstrasse 15, 52074 Aachen, Germany
\textsuperscript{b} Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany
\textsuperscript{c} EOS GmbH, Robert-Stirling-Ring 1, 82152 Krailling/Munich, Germany
\textsuperscript{d} Karl Leibinger Medizintechnik GmbH & Co. KG, Kolbinger Straße 10, 78570 Mühlheim/Donau, Germany
\textsuperscript{e} SCHAEFER KALK GmbH & Co. KG, Louise-Seher-Strasse 6, 65582 Diez, Germany
\textsuperscript{f} RWTH Aachen University - Chair for Laser Technology ILT, Steinbachstrasse 15, 52074 Aachen, Germany
\textsuperscript{g} RWTH Aachen University - Digital Additive Production DAP, Steinbachstrasse 15, 52074 Aachen, Germany

\textbf{A R T I C L E I N F O}

Keywords:
- Polylactic acid (PLA)
- Additive manufacturing
- Laser powder bed fusion
- Selective laser melting
- Biodegradable polymer implant

\textbf{A B S T R A C T}

Since large bone defects cannot be healed by the body itself, continuous effort is put into the development of 3D scaffolds for bone tissue engineering. One method to fabricate such scaffolds is selective laser sintering (SLS). However, there is a lack of solvent-free prepared microparticles suitable for SLS. Hence, the aim of this study was to develop a solvent-free polylactide/calcium carbonate composite powder with tailored material properties for SLS. Four composite powders with a composition of approximately 75\% polylactide (PLLA as well as PDLLA) and 25\% calcium carbonate (calcite) were prepared by a milling process based on GMP standards. Four different grades of polylactide were chosen to cover a broad inherent viscosity range of 1.0–3.6 dl/g. The composite material with the lowest inherent viscosity (1.0 dl/g) showed the best processability by SLS. This was caused by the small polymer particle diameter (50\,\mu m) and the small zero-shear melt viscosity (400\,Pa\,s), which led to fast sintering. The SLS process parameters were developed to achieve low micro-porosity (approx. 2\%) and low polymer degradation (no measurable decrease of the inherent viscosity). A biaxial bending strength of up to 75\,MPa was achieved. Cell culture assays indicated good viability of MG-63 osteoblast-like cells on the SLS specimens. Finally, the manufacture of 3D scaffolds with interconnected pore structure was demonstrated. After proving the biocompatibility of the material, the developed scaffolds could have great potential to be used as patient-specific bone replacement implants.

1. Introduction

The powder-based additive manufacturing technique selective laser sintering (SLS) is widely used in biomedical engineering, especially for the fabrication of implantable medical devices and bone tissue engineering scaffolds \cite{1–3}. SLS enables the manufacture of patient-specific implants with complex interconnected pore structures to promote bone ingrowth \cite{4}. The technique is already used in practice to treat bone defects with titanium and polyetherketoneketone (PEKK) implants \cite{5–7}. Despite being a good solution, titanium and PEKK implants have the disadvantage that they permanently remain in the patient’s body, which could cause late complications \cite{8}. Biodegradable implants, which can be resorbed by the human body, are a promising alternative. Currently, researchers try to qualify biodegradable materials for the SLS

\textsuperscript{1} Present address: AMCM GmbH, Petersbrunner Strasse 1b, 82319 Starnberg, Germany.
\textsuperscript{2} Present address: TRUMPF GmbH + Co. KG, Campus-Boulevard 79, 52074 Aachen, Germany.

https://doi.org/10.1016/j.msec.2019.03.101
Received 24 November 2018; Received in revised form 26 February 2019; Accepted 26 March 2019
Available online 27 March 2019
0928-4931/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).
process. Biodegradable polymers (e.g., polylactide (PLA) [9], poly(lactide-co-glycolide) (PLGA) [10], poly(3-hydroxybutyrate) (PHB) [11], and polycaprolactone [12–15]) are closely investigated. Biodegradable polymers have been in clinical use for decades [19] so that a near-term bench-to-bedside transition of SLS implants might be feasible. These polymers are, for example, applicable in the low load-bearing parts of the cranio-maxillofacial area [20] and have the advantage that their degradation behavior is well understood [21]. Among the biodegradable polymers, polylactide (PLA) has the highest mechanical strength (i.e., tensile strength of approx. 50–70 MPa, tensile modulus of 3000–4000 MPa, flexural strength of 100 MPa, and flexural modulus of 4000–5000 MPa [22, p. 141] [23]). Thus, PLA is of particular interest for bone tissue engineering. However, it should be noted that the high strength comes at the expense of a low elongation at break of 2–10%, which shows that PLA is quite brittle [22, p. 141].

While clinical translation of polycaprolactone implants made with SLS is on its way [24,25], in vivo studies with SLS scaffolds based on PLA are scarce and limited to small animal models [3,26–29]. We hypothesize that translational research into PLA implants made with SLS can be accelerated through the development of suitable, solvent-free PLA powders.

Polylactides are typically delivered in the form of millimeter-sized granules. However, for the SLS process, micrometer-sized particles are necessary to ensure high detail resolution and good melting behavior. In addition, the particles should have a rounded shape to ensure high flowability without agglomeration. Basically, there are two methods to prepare micrometer-sized PLA particles: solvent evaporation [30–32] or mechanical milling [33,34]. Solvent evaporation has the advantage that the particle size can be well controlled and that the particles are spherical, which ensures high flowability. The disadvantage is that PLA is only soluble in toxic organic solvents like dichloromethane (DCM) or chloroform (CHCl_3), which pose serious health risks [35,36]. Therefore, upscaling and medical approval are complicated. In contrast, mechanical milling works without toxic solvents and can be more easily upscaled. The disadvantage of mechanical milling is that PLA is hard to grind due to inelastic deformation (e.g., flattening instead of the creation of new fracture surfaces) at small particle sizes [37]. Moreover, particles obtained by milling are often irregular shaped, which reduces flowability and packing density [38]. Although both powder production methods have advantages and disadvantages, the toxic solvents pose the most serious obstacle for moving from laboratory to industrial-scale application. For this reason, here we employed an industrial milling process based on GMP standards for the solvent-free production of poly(lactides)/calcium carbonate composite powders [33].

The inorganic biomaterial calcium carbonate (CC) was chosen based on information gained from the literature that shows that CC has a high buffering capacity, which can effectively neutralize the acidic milieu caused by the degradation of polylactides [39,40]. In addition, CC improves the cell reaction in relation to the pure polymer as it is a source of calcium ions that promote bone regeneration [41].

A major technical issue for PLA processing with SLS is the high micro-porosity that can result from incomplete coalescence of powder particles due to high melt viscosity. For example, micro-porosities as high as 46% or 55% are reported in the literature [38,42]. Since a high micro-porosity generally leads to poor mechanical properties, this issue hinders the wider application of laser-sintered PLA. As the melt viscosity strongly increases with polymer chain length or molecular weight, we hypothesize that a careful selection of the inherent viscosity (which is a measure for the molecular weight) can improve processability.

In the literature, a broad inherent viscosity range (0.2–3.0 dl/g) was studied so far. However, the inherent viscosity was not systematically varied in one coherent study. For example, Tan et al. [43] and Leong et al. [44] used poly(l-lactide) (PLLA) with inherent viscosity (IV) of 2.5 dl/g and prepared powder particles by centrifugal grinding. Zhou et al. [9,45] and Duan et al. [46] used PLLA with IV of 1.9 dl/g to prepare powder particles by solvent evaporation using dichloromethane. Xu et al. [47] used PLLA with IV of 2.5–3.0 dl/g and prepared powder particles by mechanical grinding. Shuai et al. used PLLA with IV of 1.5 dl/g, which was already delivered in powder form [48], Antonov et al. [49,50], Bukharova et al. [27], Kanzler et al. [28], Kuznetsova et al. [51], and Grebenik et al. [52] used poly(DL-lactide) (PDLLA) with IV of about 0.2–0.8 dl/g and prepared powder particles mainly by rotary-milling or by using pestle and mortar. In previous studies, we prepared PDLLA/β-tricalcium phosphate (50/50) composite particles by ball milling [53,54]. The inherent viscosity of the PDLLA after the milling process was 1.3 dl/g [54]. Here, we used different PLA grades to investigate the extent to which the inherent viscosity influences how well a polylactide/calcium carbonate composite can be prepared by mechanical milling and how well it can be processed with SLS. The goal was to develop a composite powder with improved processability in order to achieve low micro-porosity and high mechanical strength.

2. Materials and methods

2.1. Raw materials

Four medical grade polylactides were used: three semi-crystalline polyl-lactides with inherent viscosity (IV) of 1.1, 1.9, and 3.9 dl/g as well as an amorphous poly(DL-lactide) with IV of 2.0 dl/g (Evronik Nutrition & Care GmbH, Darmstadt, Germany). Lower inherent viscosities than 1 dl/g were not chosen due to the steep decrease of the mechanical properties of PLA for IV < 1 dl/g [55]. The PDLLA contained 50% D-lactide monomers and 50% l-lactide monomers. The PLLA contained 100% l-lactide monomers. The mass average molecular weight $M_a$ ranged from 100,000 to 460,000 g/mol as determined by size exclusion chromatography in chloroform with polysytrene standards. The thermal properties as determined by differential scanning calorimetry (DSC) are summarized in Table 1.

Specially designed biomedical grade precipitated calcium carbonate (Schaefer Kalk GmbH & Co. KG, Diez, Germany) was used as filler material. The precipitated calcium carbonate was supplied in the form of fine powder (spherulite-shaped with calcite crystal structure and mean particle diameter of 12 μm). In contrast, the PLA was supplied in the form of granules with large particle diameter (approx. 1 mm) not suitable for the SLS process. Hence, the raw materials had to be ground.

Table 1

| Material   | IV (dl/g) | $T_g$ (°C) | $T_m$ (°C) | $ΔH_m$ (J/g) |
|------------|-----------|------------|------------|--------------|
| PLLA-1.1   | 1.1       | 53         | 187        | 87           |
| PLLA-1.9   | 1.9       | 57         | 188        | 76           |
| PLLA-3.9   | 3.9       | 61         | 192        | 71           |
| PDLLA-2.0  | 2.0       | 57         | Amorphous  | Amorphous    |

2.2. Preparation and characterization of PLA/CC composite powders

2.2.1. Powder preparation

For powder preparation, a mixture of PLA and CC was dry processed in an impact mill (NH-1, NARA Machinary, Tokyo 143, Japan) at ambient temperature. Inductively coupled plasma mass spectrometry (ICP-MS) verified that no metallic abrasion occurred during milling. After the milling process, each composite powder was sieved with test sieves of 20 μm and 250 μm mesh size. By containing the...
fraction > 20 μm and < 250 μm, composite powders with good flowability (Hausner ratio < 1.25) and small particle size were obtained. Four PLA/CC composite powders were prepared that way (Table 2).

The CC content was determined by thermogravimetric analysis (Section 2.2.5) and amounted to 22–27 wt%. This filler content was chosen based on preliminary SLS experiments and represents a compromise between medical demand (preferably high filler content) and processability by SLS (preferably low filler content). The moisture content of the powders was 0.7–0.8% as determined by Karl Fischer titration (Section 2.2.3). After preparation, the composite powders were stored in a refrigerator at 5°C to prevent aging.

### 2.2.2. Powder morphology and particle size distribution

The morphology of the powder particles was analyzed using scanning electron microscopy (SEM, LEO 1455 EP, Carl Zeiss, Oberkochen, Germany) with a voltage of 10 kV. The powder was sputter-coated with gold/palladium (Au/Pd) to make it conductive. The volumetric powder particle size distribution was obtained from laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). The powder was dispersed under dry conditions (RODOS, Sympatec, Clausthal-Zellerfeld, Germany).

### 2.2.3. Moisture content

The moisture content of the composite powders was determined by coulometric Karl Fischer titration. For this, 250 mg of each composite powder was heated at 150°C for 10 min.

### 2.2.4. Inherent viscosity

The inherent viscosity (IV) was measured at 25°C, 0.1% w/v in chloroform (CHCl₃), with a size 0c Ubbelohde glass capillary viscometer (St-Analytik type 541 03, Mainz, Germany). Samples were dissolved in CHCl₃ (EMSURE® ACS, ISO, Reag PhEur, Merck) with stirring for about 12 h and subsequently filtered with PTFE filters with 1.0 μm pore size.

### 2.2.5. Thermal stability

The thermal stability of the composite powders was analyzed using thermogravimetric analysis (TGA, STA6000/8000, PerkinElmer, Waltham, USA). The powder samples (20–30 mg) were heated under nitrogen atmosphere (N₂ flow of 20 ml/min) from 40 to 1000°C at a heating rate of 20°C/min. The CC content was determined from the decomposition step at 600–1000°C, where CaCO₃ decomposes into CaO and CO₂. According to the molar masses, the CC content can be obtained by multiplying the weight loss (which is due to evaporating CO₂) by 2.274. The decomposition peak temperature T<sub>d</sub> was determined from the peak of the DTG curve (first derivative of the TGA curve). The DTG curve was smoothed using a Savitzky–Golay filter with polynomial order two and by taking into account 200 data points (approx. 25 s time span).

#### 2.2.6. Melt rheology

A rotational rheometer (ARES, TA Instruments, New Castle, USA) was used to determine the complex viscosity η<sup>c</sup> (hereinafter referred to as melt viscosity) of the composite melts. Oscillatory tests were performed by applying a time dependent strain of |γ(t)| = γ<sub>0</sub> sin(ωt), where γ<sub>0</sub> and ω are the amplitude and the angular frequency of the oscillation, respectively. The absolute value of the melt viscosity is given by |η<sup>c</sup>(ω)| = [G″(ω)² + G′(ω)²]¹/² / ω, where G″(ω) and G′(ω) are the storage and loss modulus, respectively. The quotient G″/G′ = tan(δ) is called loss factor and determines whether the samples show a gel (tan(δ) < 1) or liquid character (tan(δ) > 1) [56]. A parallel-plate measuring system with plate diameter of 25 mm and gap width of 0.4–1.0 mm was used, ensuring that the gap size was much larger than the CC particle size of 12 μm. The measurements were performed in a convention oven under nitrogen (N₂) atmosphere at 190°C. Prior to the measurements, the powders were vacuum-dried overnight at 50°C to eliminate the possible influence of different moisture contents on the measurement. After drying, which resulted in a residual moisture content of about 0.3–0.5%, the powder was directly loaded between the plates where it was equilibrated at 190°C for about 2 min before starting the measurement. To determine the linear viscoelastic range, amplitude sweeps were performed at an angular frequency of ω = 10 rad/s. Moreover, frequency sweeps were carried out from 100 to 0.04 rad/s (starting at 100 rad/s) to determine the zero-shear viscosity η₀ (i.e., the viscosity at ω → 0). In addition, the zero-shear viscosity was determined by time sweeps at 0.1 rad/s with the goal to avoid degradation affecting the measured viscosity values. An amplitude of γ<sub>0</sub> = 0.5% was used for the frequency and time sweeps. This amplitude corresponds to a maximal angular displacement of the rheometer plate that is given by θ = H/tan(R), where H is the gap width and R is the plate radius, and hence θ = 1 mm × 0.005/12.5 mm = 0.4 mrad.

### 2.3. Manufacturing of test specimens by selective laser sintering

#### 2.3.1. Experimental setup

A CO₂ laser (FEHA 600 SM-E, FEHA LaserTec, Bitterfeld-Wolfen, Germany) with maximum power of 600 W and power stability of ±3% was operated in continuous wave mode at a wavelength of 10.6 μm. The output power was set to 40 W, which corresponds to the power threshold. The effective power, which was used for laser sintering, was adjusted to 0.30–0.70 W by a water-cooled manual power attenuator (C-MA-WJ, ULO Optics, Stevenage, UK). The Gaussian laser beam (M² < 1.25) was deflected into the powder bed using a galvanometer scan system (hurrySCAN 25, SCANLAB, Munich, Germany) with a 300 mm F-Theta lens, resulting in a laser beam diameter of approximately 360 μm (1/e²). The powder was deposited by a contra-rotating roller. Specimens were built onto a polyamide (PA 6) substrate without preheating. The specimens were built in air (i.e., no inert gas was used) and the powder was not dried before processing. The laboratory setup was used to perform single-layer experiments as well as to manufacture rectangular and circular specimens for basic research (Sections 2.3.2, 2.3.3, and 2.3.4).

A modified laser sintering machine (Formiga P 110, EOS, Munich, Germany) was used to manufacture a patient-specific implant demonstrator (Section 2.3.5). The modified Formiga P 110 featured a small build envelope of 60 × 75 × 120 mm (instead of the usual 200 × 250 × 330 mm). This downscaled build envelope was specially designed to enable experiments with small powder quantities (<200 g). Thus, it is particularly suited for material development and medical application. The laser power was adjusted to 2.2 W, which corresponds to the lowest possible emission of the laser (power threshold). The CO₂ laser beam was focused with a 440 mm F-Theta lens to a spot diameter of approximately 450 μm (1/e²). The powder

| Material composition (wt %) | Particle size (sieve analysis) | IV (dl/g) before milling | IV (dl/g) after milling | T<sub>d</sub> (°C) | Moisture content (%) |
|-----------------------------|------------------------------|--------------------------|------------------------|-----------------|----------------------|
| PLLA-1.0/CC                 | > 20 μm, < 250 μm            | 1.1                      | 1.0                    | 307             | 0.7                  |
| PLLA-1.9/CC                 | > 20 μm, < 250 μm            | 1.9                      | 1.9                    | 296             | 0.8                  |
| PLLA-3.6/CC                 | > 20 μm, < 250 μm            | 3.9                      | 3.6                    | 292             | 0.8                  |
| PDLLA-1.9/CC                | > 20 μm, < 250 μm            | 2.0                      | 1.9                    | 284             | 0.8                  |

Table 2: Properties of the polylactide (PLA)/calcium carbonate (CC) composites. The CC content and the decomposition peak temperature T<sub>d</sub> were determined by thermogravimetric analysis (mean of double determination) with heating rate of 20°C/min. The moisture content was determined by Karl Fischer titration. The PLLA-1.0-based material had the lowest inherent viscosity and the highest thermal stability.
was deposited by a recoating blade and preheated to a temperature of 40 °C that was below the PLA's glass transition temperature of 53 °C to prevent possible powder aging. As for the laboratory setup, the specimens were built in air onto a PA 6 substrate and the powder was not dried before processing.

2.3.2. Single-layer experiments

To evaluate the melting behavior of the different composite materials, single-layer experiments were conducted. For this, a powder layer with thickness > 1 mm was manually compacted by using a scraper. Subsequently, the scanning laser beam exposed an area of 6 x 6 mm. The scanning was unidirectional. Identical process parameters were used for each composite to study the influence of the material properties on the melting behavior under the scanning laser beam. The surface morphology of the single layers was studied by light microscopy (SMZ1270, Nikon, Tokyo, Japan). Different from the description in Section 2.3.1, a smaller laser beam diameter of 250 μm was used for the single-layer experiments. This was done to highlight the different processability of the composites as a smaller laser beam diameter leads to higher intensities and thus higher thermal load.

2.3.3. Rectangular specimens for analysis of micro-porosity and cell viability

Rectangular specimens (6 x 6 x 4 mm) were manufactured from the PLLA-1.0-based composite material as this one indicated the best processability in the single-layer experiment. The scan vectors were oriented in parallel to the specimen’s edges and were rotated by 90° from layer to layer. By using different laser powers (P_l = 0.3–0.5 W), the influence of the laser power on the surface morphology and micro-porosity was investigated (Section 3.6.2). In addition, the specimens were used for cell viability assays (Section 3.6.3).

2.3.4. Circular specimens for mechanical strength evaluation

Circular specimens (10 mm diameter and 1 mm thickness) were manufactured from the PLLA-1.0-based composite material with different laser powers (P_l = 0.35–0.65 W) to study the influence of the laser power on the biaxial bending strength and the process-related degradation. Five specimens were manufactured for each laser power setting. The remaining process parameters (hatch distance, scan speed, layer thickness, and laser beam diameter) were kept constant. In this way, the influence of the laser power was studied for a constant interaction time between laser radiation and powder material. The specimens were built flat; that is, the build height was 1 mm. This is the preferred orientation to obtain short processing times and high bending strength because of the favorable orientation of the layers relative to the applied force [57].

2.3.5. Patient-specific implant demonstrator

Two patient-specific implant demonstrators were manufactured with the modified Formiga P 110 system described in Section 2.3.1. The demonstrators were designed to fit skull defects of about 44 mm diameter and 11 mm thickness. The pore structure was created using the Within generative design software (Autodesk, San Rafael, USA). The pore structure was trimmed to fit the curved surface of the skull and had a designed porosity of about 72% with about 1 mm strut size. Support structures were necessary to prevent the parts from being wiped away by the recoating blade.

2.4. Micro-porosity analysis

The rectangular specimens (Section 2.3.3) were cold-embedded in polyester resin and polished with aluminum oxide. The cross-sections were prepared in parallel to the build direction. The micro-porosity was measured by light microscopy of the polished cross-sections.

2.5. Cell viability assay

Cell viability of MG-63 osteoblast-like cells was investigated using Promega Cell Titer Blue Assays. The cells were seeded on the surface of the rectangular SLS specimens (Section 2.3.3) with a density of 20,000 cells/cm² and cultivated for 10 days at 37 °C and 5% CO₂. Six specimens (n = 6) were tested for each laser power setting (P_l = 0.3, 0.4, and 0.5 W). Tissue culture plastic was used as control. The supernatants were removed and analyzed according to the Cell Titer Blue protocol. The fluorescence of resorufin was measured by using a fluorometer (SpectraMax M2, Molecular Device, California, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey’s pairwise test between the four groups. A value of p < 0.05 was considered to be statistically significant and p < 0.01 highly significant. Statistical analysis was performed with OriginPro 2019 software.

2.6. Mechanical strength testing

The ball on three balls (B3B) test was used to determine the biaxial bending strength of the circular SLS specimens (Section 2.3.4). Five specimens (n = 5) were tested for each laser power setting. The data were analyzed by following the approach of Börger et al. [58,59] as described in [60]. It should be noted that we did not measure the Poisson’s ratio of the PLA/CC composite material but assumed a conservative value of ν = 0.30 (e.g., ν = 0.36 is given in the literature for pure PLA [61] and would yield approx. 4% larger strength values). After mechanical strength testing, the fractured surfaces were imaged by SEM.

3. Results

3.1. Powder morphology and particle size distribution

Fig. 1 shows the morphology of the four PLA/CC composite powders. Most of the powder particles had a favored rounded potato-like shape. Concerning the filler material, intact CC spherulites (diameter 12 μm) as well as their fragments (< 1 μm) were observed. The CC partly covered the surface of the PLA particles.

It can be seen from Figs. 1 and 2 that there were significant particle size differences between different grades of PLA. For example, the PLLA-1.0-based material had the smallest particle size while the PDLLA-1.9-based material had the largest particle size (Fig. 2). The reasons for this might be that the impact resistance of PLLA decreases with decreasing inherent viscosity and that amorphous PDLLA is softer than semi-crystalline PLLA [62]. Further aspects regarding grindability are discussed in Section 4.

3.2. Moisture content

The moisture content of the composite materials was 0.7–0.8% (Table 2).

3.3. Inherent viscosity

The IV of the PLA was determined before and after the milling process. A decrease of the IV of 0–9% was measured after the milling process (Table 2).

3.4. Thermal stability

The decomposition peak temperature T_d differed by up to 23 °C between the composite materials (Table 2 and Fig. 3). The PLLA-1.0-based composite showed the highest thermal decomposition peak temperature of 307 °C compared to 284 °C for the PDLLA-1.9-based composite. A second small decomposition stage was observed around...
Fig. 1. Scanning electron microscope images of the poly(lactide) (PLA)/calcium carbonate (CC) composite powder particles [63]. The PLLA-1.0 with the lowest inherent viscosity of 1 dl/g could be ground to the smallest particle size. Some intact CC spherulites are marked by white circles to show that they were present in each composite.
415 °C. Calcium carbonate decomposed at a peak temperature of 760 °C. The CC content was 22–27 wt%.

3.5. Melt rheology

The amplitude sweeps (Fig. 4a and b) showed that the PLLA-1.0-based and the PLLA-1.9-based composite had a liquid character ($G'' > G'$), whereas the PLLA-3.6-based composite had a gel character ($G'' < G'$) and the PDLLA-1.9-based composite was at the gel point ($G'' \approx G'$). The loss factor $\tan(\delta) = G''/G'$ is given for $\gamma_A = 0.5\%$ [marked by dashed lines in Fig. 4a and b] and the PLLA-1.0-based composite had the highest loss factor ($\tan(\delta) = 9$). The amplitude sweeps showed that the linear viscoelastic (LVE) range, where the $G'$- and $G''$-curves remained on a constant plateau value (< 10% deviation tolerated), only extended to a limiting amplitude $\gamma_L$ of approximately 0.1%.

For the frequency and time sweeps, an amplitude of $\gamma_A = 0.5\%$ (i.e., slightly outside the LVE range) was chosen to obtain reasonable signal...
intensities also for the very liquid PLLA-1.0-based composite in order to avoid questionable results such as the dip of $G'$ for small $\gamma_A$ in Fig. 4a. In the frequency sweeps, a degradation-related viscosity drop was observed for all composites at low frequencies (i.e., at long measurement times) (Fig. 4c). The drop occurred earliest for the PDLLA-1.9-based composite due to its comparatively low thermal stability. In the time sweeps, all composites showed a fast decrease of the melt viscosity due to thermal degradation (Fig. 4d). The measurement signal of the PLLA-1.0-based material showed some fluctuations, which might be due to the low melt viscosity (100–400 Pa·s) that was at the limit of the measuring range of the rheometer. This might also explain the increase of the melt viscosity for the lowest frequencies in Fig. 4c. In summary, the composites covered a broad melt viscosity range (400–57,000 Pa·s at 0.1 rad/s) and the PLLA-1.0-based composite had the lowest melt viscosity.

3.6. Analysis of SLS test specimens

3.6.1. Single-layer experiment

The light microscope images of the single-layer surfaces show differences between the composites with regard to melting behavior and discoloration (Fig. 5). Whereas the PLLA-1.0-based composite showed continuous melt tracks, the other composites predominantly formed isolated melt drops. Aside from the melting behavior, all composites except the PLLA-1.0-based material showed a good melting behavior (Fig. 6a).

In conclusion, the PLLA-1.0-based material offered the best SLS processability under the examined process conditions, and thus was selected for further investigation.

3.6.2. Micro-porosity analysis

With increasing laser power, the surface texture of the multi-layer specimens (6 × 6 × 4 mm) changed from rough and porous to smooth and dense (Fig. 7a and b). The formation of a flat surface coincided with a yellow discoloration. For low laser power (0.30–0.40 W), the polished cross-section revealed lack of fusion in the form of many irregular shaped voids (Fig. 7c). As the laser power was increased, the number of irregular shaped voids decreased resulting in a lower micro-porosity $\phi$. The best processing conditions were observed at 0.40 W resulting in the lowest micro-porosity of approximately 2%. While lower laser powers led to lack of fusion, higher laser powers caused yellowing and increased gas pore formation.

A close-up of the polished cross-section of the 0.40 W specimen gives information about the CC distribution in the PLA matrix (Fig. 7d).
Fig. 5. Light microscope images of single-layer surfaces after laser exposure. Identical process parameters (i.e., the same laser power, hatch distance, scan speed, and laser beam diameter) were used for all composite materials. The scan tracks were oriented horizontally and the exposure started at the top [schematically visualized in (g)]. The dashed lines indicate a continuous melt track or melt droplets. The PLLA-1.0-based composite showed the best melting behavior because it formed continuous melt tracks instead of melt droplets and did not discolor. Thus, it was selected for further investigation.
Fig. 6. Top view photograph of layer number three during the build of multi-layer specimens (6 × 6 × 4 mm) with different laser power settings. For the PLLA-1.0-based material, the build could be completed to full height. In contrast, for the PLLA-1.9-based material, the build had to be aborted after layer number three due to lack of mechanical stability. The arrow depicts the direction of powder deposition.

Fig. 7. Analysis of rectangular SLS specimens (6 × 6 × 4 mm) manufactured from the PLLA-1.0-based composite: (a) light microscope (LM) images of the top surfaces as a function of laser power $P_L$, (b) SEM images of these top surfaces, (c) LM images of polished cross-sections parallel to the build direction (indicated by arrow) including the micro-porosity $\phi$ determined from these cross-sections, and (d) LM close-up (left) and SEM close-up (right) of the polished cross-section for $P_L = 0.40$ W.
5.0 W) had no adverse effect on cell activity.

3.6.5. Patient-specific implant demonstrator

Two complex cranial implant demonstrators, which perfectly fit the designed skull defects, were successfully manufactured from the PLLA-1.0/CC (77/23) composite material (Fig. 10) [63]. The contour-adapted pore structure was designed using Autodesk’s Within software. The build time for the implants (44 mm diameter and 11 mm thickness) including support structures (not shown) was about 2 h each.

4. Discussion

The PLLA-1.0-based composite showed the best laser sintering processability of the tested materials (Fig. 5). This can be ascribed to its material properties that were best suited for SLS. With reference to the Frenkel–Eshelby model, the coalescence occurring by viscous flow for two identical spherical polymer particles can be described by $x/\alpha = (\sigma a/(\mu a t))^1/2$, where $x$, $\alpha$, $\sigma$, $\mu$, and $\eta$ are the sintering neck radius, particle radius, surface tension, sintering time, and melt viscosity, respectively [65,66]. In order to obtain parts with high mechanical strength, it should be aimed at complete coalescence (i.e., $x/\alpha \to 1$). In addition, the processing time should be preferably small to ensure high productivity. As a result, high surface tension, low melt viscosity, and small particle radius are preferred. As no external shear forces are applied to the melt during SLS processing, it is assumed that the melting behavior is essentially determined by the zero-shear viscosity [67,68]. This indicates that the low zero-shear viscosity (approx. 400 PAs, see Fig. 4d) and the small particle size (53 μm, see Fig. 2) of the PLLA-1.0-based composite are the key properties regarding its good laser sintering processability. Possible differences in the surface tension, which were not studied here, are expected to have a lower impact on processability compared to particle size and melt viscosity.

The key feature of the PLLA-1.0-based composite is its low inherent viscosity, which is closely related to molecular weight (i.e., low inherent viscosity means low molecular weight). The IV determines the zero-shear viscosity as well as the particle size that can be achieved by grinding. Low inherent viscosity improved grindability (important for powder production) and melting behavior (important for SLS). Lower inherent viscosities than 1 dl/g might further improve processability but typically result in a steep decrease of the mechanical properties as the polymer chains lose their entanglement [55]. Hence, the IV of 1 dl/g can be regarded as lower reasonable limit when one seeks for medical devices with high strength. Inherent viscosities > 1 dl/g are well worth studying since higher molecular weights provide higher potential strength [62]. In particular, IVs between 1 and 1.9 dl/g (corresponding to zero-shear composite melt viscosities between 400 and 13,000 PAs), which were not covered in this study, should be investigated. In summary, a trade-off between high inherent viscosity (high potential strength) and low inherent viscosity (good processability) needs to be found. The ultimate goal is to identify an inherent viscosity range (i.e., molecular weight range) that gives the best results (high-strength specimens) under common SLS processing conditions.

Concerning grindability, hard and brittle materials are typically better suited for micronization by milling than soft and tough materials [69]. For PLLA, it is known that the mechanical strength, the elongation at break, and the impact strength decrease with decreasing molecular weight [62]. This could explain why the PLLA with the lowest inherent viscosity could be ground to the smallest particle size (Fig. 2). In addition, the high enthalpy of fusion of the PLLA-1.1 (87 J/g, see Table 1) probably improved the grindability, since it is known from the literature that the impact strength as well as the fracture toughness of PLLA decrease with increasing enthalpy of fusion or crystallinity [70,71]. Notably, tough fracture behavior was observed in impact testing for enthalpies of fusion of 40–80 J/g in contrast to brittle behavior for $\Delta H_m > 80$ J/g [70]. With respect to the amorphous PDLLA, it must be noted that it is generally softer than PLLA [62] and that it gradually softens at temperatures above $T_g \approx 57^\circ C$, which complicates grinding.

Concerning melting behavior, it is known that the zero-shear
viscosity $\eta_0$ of PLA decreases strongly with decreasing mass average molecular weight $M_w$ according to $\eta_0 \propto M_w^{-3.4}$ [72,73]. This is the reason for the low melt viscosity of the PLLA-1.0-based composite (Fig. 4c and d). In summary, the low inherent viscosity led to small melt viscosity (approx. 400 Pa·s) and particle size (approx. 50 μm), which was favorable for the laser sintering process. Besides the melt viscosity, the loss factor tan(δ) measured by the amplitude sweeps (Fig. 4a and b) might also serve as a measure of processability. A high loss factor means that the melt is more viscous than elastic (i.e., it exhibits flow when in the steady-state), which is assumed advantageous for the formation of a smooth molten film.

The Frenkel–Eshelby model describes the coalescence of pure
polymer particles. Here, we also need to take into account the CC filler material. The SEM images (Fig. 1) indicate that the CC spherulites (12 μm) were partly crushed into fragments (< 12 μm) during milling. Notably, the surface of the PLLA-1.0 particles seemed to be less covered by CC fragments than the surface of the other PLA composite particles. This was probably due to the better grindability of the PLLA-1.1. The better grindability implied that the PLLA-1.1 broke into pieces more easily than the other PLA grades so that less force was transferred to the calcium carbonate during grinding. As a result, more calcium carbonate remained intact and were not crushed into finer calcium carbonate particles that would have covered the PLA’s surface. With regard to laser sintering processability, the less CC-covered surface of the PLLA-1.1 was more advantageous with respect to laser sintering processability [54,74].

A further property that contributed to the good processability of the PLLA-1.0-based composite was its comparatively high thermal stability. Although in general the thermal stability increases with higher molecular weight [75], the PLLA-1.0-based composite, despite having the lowest molecular weight, showed the highest decomposition peak temperature of 307 °C (Table 2 and Fig. 3). The reason for this might be a combination of its comparatively low moisture content, low filler content, and large CC particle size as larger particles are less reactive [76]. The comparatively high decomposition temperature could explain why no discoloration was observed in the single-layer experiment (Fig. 5). A further possible explanation why the PLLA-1.0-based composite did not yellow in the single-layer experiment might be the comparatively fast coalescence according to the Frenkel–Eshelby model. In consequence of the fast coalescence, thermal bridges were rapidly formed between adjacent particles. Hence, the locally absorbed laser energy could be dissipated faster into the surrounding material. This might have led to lower processing temperatures compared to the other materials so that no temperature-induced discoloration occurred.

The rheological measurements showed that all composites were prone to thermal degradation that led to a viscosity decrease as a function of measurement time (Fig. 4d). Since no initial viscosity plateau was observed in the time sweeps, the thermal stability time at 190 °C was even shorter than 5 min (2 min equilibration time + 3 min measurement time until the second data point). As a result, the data in Fig. 4 must be interpreted with caution as it is superimposed by the degradation-related viscosity decrease. This also explains the viscosity decrease for low frequencies in Fig. 4e and the differences between the measured viscosity values in Fig. 4c and d.

Thermal degradation was also observed in the SLS experiments. If the laser power was too high, a yellow discoloration occurred (Figs. 7 and 9), which coincided with a decrease of the inherent viscosity and increased gas pore formation (CO and CO₂ are known degradation products of PLA [77]). The size of the gas pores of 20–120 μm was of the order of the powder particle diameter. Since water can also cause gas pores as well as hydrolytic degradation, drying of the composite material as well as a dry inert gas atmosphere might be beneficial. For example, the material could be dried before processing and/or during processing by using preheating temperatures of up to 100 °C. However, it should be noted that drying and preheating temperatures higher than $T_d \approx 53–61$ °C might limit powder reusability due to molecular weight loss, and thus might be uneconomic. In general, a moisture content of < 100–250 ppm is recommended for PLA processing [78]. Here, we processed the materials having a moisture content of 7000–8000 ppm. Despite this very high moisture content, it was possible to manufacture specimens without measurable decrease of the inherent viscosity (Fig. 9a).

With regard to SLS processing, the lowest micro-porosity of 2% (Fig. 7c) as well as the highest biaxial bending strength of 75 MPa (Fig. 9a) was achieved right at the transition from partial to full coalescence (Fig. 9b) and just before the onset of degradation. In conclusion, there is an optimum energy input that maximizes the mechanical strength so that the process parameters must be carefully adapted if...
high mechanical strength is desired. It should be noted that the B3B test was designed to test the biaxial bending strength of brittle materials [58,59]. Since PLLA is usually hard and brittle [79] and as the PLLA/CC specimens showed a brittle behavior, the B3B test was applicable in this study. Five specimens were tested per laser power setting and the standard deviation was < 10% on average.

The highest activity of MG-63 osteoblast-like cells occurred on the 0.3 W specimens that had the roughest surface of the investigated specimens (Figs. 7b and 8). It can be assumed that the rough surface had a positive influence on cell behavior. Therefore, further experiments concerning cell attachment and proliferation have to be done. Cell viability was also proved on the specimens with denser surface texture as well as on the discolored specimens. This might indicate that the process-related degradation/discoloration products do not impair cell viability. Although process-related degradation/discoloration should be avoided at all events, this finding gives a safety buffer in the case of inhomogeneous or imperfect processing conditions.

5. Conclusions

In conclusion, we developed a polylactide/calcium carbonate composite powder whose properties (e.g., small particle size and low melt viscosity) were particularly suited for selective laser sintering. An inherent viscosity of approximately 1 dl/g proved beneficial with regard to grindability and melting behavior. A careful adaption of the SLS process parameters enabled the manufacture of test specimens without measurable decrease of the inherent viscosity and with high strength (up to 75 MPa) due to low micro-porosity (approx. 2%). Moreover, the SLS test specimens showed good cell compatibility with MG-63 osteoblast-like cells. Finally, the newly developed composite powder was used to manufacture patient-specific implant demonstrators with interconnected pore structure on a modified EOS Formiga P 110 additive manufacturing system. In the future, such structures could be used as medical devices to promote the healing of critical size bone defects in craniomaxillofacial surgery. In addition, the scaffolds could be seeded with cells in the context of bone tissue engineering. The next steps towards these goals will include further in vitro and in vivo experiments to evaluate the biocompatibility and degradation behavior of the developed materials and scaffolds.

Acknowledgments

This work was supported by the Federal Ministry of Education and Research (BMBF) within the project ActiveBone [grant numbers 13N12129–32]. The authors would like to thank Monika Jobmann and Marlies Walter of the Fraunhofer Institute for Applied Polymer Research IAP for the rheological measurements. We thank Rainer Haas, Thomas Schmidt, and Luc Ubaghs of the Leibniz Institute for Interactive Materials DWI for polymer analytics. We thank Evonik Industries AG for providing the materials. We thank Herbert Horn-Solle, Maris Preußer, and Basim Uweis for taking the SEM images as well as Alexander Esser for preparing the polished cross-sections. Many thanks go to Michaela Krämer who performed the TGA and the inherent viscosity determination. We thank Rainer Neumeyer for taking the photos of the implant demonstrators.

Declaration of interest

Marjan Vučak is employed by Schaefer Kalk GmbH & Co. KG. Schaefer Kalk produces calcium carbonate. Martin Bullemner and Andreas Pfister are employed by EOS GmbH. EOS produces laser sintering machines. Stefanie Grom and Frank Reinauer are employed by Karl Leibinger Medizintechnik GmbH & Co. KG (KLM). KLM produces biorsorbable polymer implants. Christoph Gayer conducted contractual research for Schaefer Kalk, EOS, and KLM within a joint research project.

References

[1] B. Duan, M. Wang, Selective laser sintering and its application in biomedical engineering, MRS Bull. 36 (2011) 998–1005.
[2] A. Mazzoli, Selective laser sintering in biomedical engineering, Medical & biological engineering & computing 51 (2013) 245–256.
[3] A. Yousef, S.J. Hollister, P.D. Dalton, Additive manufacturing of polymer melts for implantable medical devices and scaffolds, Biofabrication 9 (2017) 12002.
[4] N. Taniguchi, S. Fujiyabashi, M. Takemoto, K. Sasaki, B. Otsuki, T. Nakamura, T. Matsushita, T. Koko, S. Matsuda, Effect of pore size on bone ingrowth into porous titanium implants fabricated with additive manufacturing, Mater. Sci. Eng. C Mater. Sci. Biol. Appl. 59 (2016) 690–701.
[5] M. Salmi, J. Tuomi, K.-S. Palohelio, R. Björkrand, M. Palohelio, J. Salo, R. Konttio, K. Mesimäki, A.A. Mikitie, Patient-specific reconstruction in 3D modeling and DMLS additive manufacturing, Rapid Prototyp. J. 18 (2012) 209–214.
[6] A.L. Jardini, M.A. Larroa, M.F. Macedo, L.C. Lambert, C.-A.C. Zavaglia, R.M. Filho, D.R. Calderoni, E. Ghizoni, P. Kharmandayan, Improvement in cranioangioplasty, Procedia CIRP 49 (2016) 203–208.
[7] FDA 2013 Oxford performance materials Ostefab patient-specific cranial device—S10 k summary (K121818), https://www.accessdata.fda.gov/cdrh_docs/pdf12/K121818.pdf, Accessed date: 7 April 2019.
[8] T. Mikami, K. Miyata, K. Komatsu, K. Yamashita, M. Waniabuchi, N. Mikuni, Exposure of titanium implants after cranioangioplasty, Interdisciplinary Neurosurgery 8 (2017) 64–67.
[9] W.Y. Zhou, S.H. Lee, M. Wang, W.L. Cheung, W.Y. Ip, Selective laser sintering of porous tissue engineering scaffolds from polylactide–carbonated hydroxyapatite nanocomposite micropheres, Journal of materials science. Materials in medicine 19 (2008) 2535–2540.
[10] C. Shuai, B. Yang, S. Peng, Z. Li, Development of composite porous scaffolds based on polylactide–glycolide–nano-hydroxyapatite via selective laser sintering, Int. J. Adv. Manuf. Technol. 69 (2013) 51–57.
[11] S. Saska, L.C. Pires, M.A. Cominotte, L.S. Mendes, M.A. Maia, J.V.L. da Silva, S.J.L. Ribeiro, J.A. Girioli, Three-dimensional printing and in vitro evaluation of poly(3-hydroxybutyrate) scaffolds functionalized with osteogenic growth peptide for tissue engineering, Mater. Sci. Eng. C Mater. Biol. Appl. 89 (2018) 265–272.
[12] J.M. Williams, A. Adviswumi, R.M. Schoeck, C.L. Flanagan, P.H. Krebsbach, S.E. Feinberg, S.J. Hollister, S. Das, Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering, Biomaterials 26 (2005) 4817–4827.
[13] C.-H. Chen, M.-Y. Lee, V.-H. Shyu, Y.-C. Chen, C.-Y. Chen, J.-P. Chen, Surface modification of polycaprolactone scaffolds fabricated via selective laser sintering for cartilage tissue engineering, Mater. Sci. Eng. C Mater. Biol. Appl. 40 (2014) 389–397.
[14] S. van Baal, T. Denmet, Y.C. Chai, G. Pyka, P. Dubreuil, J.-P. Kruth, J. Schroten, In vitro cell-biological performance and structural characterization of selective laser sintered and plasma surface functionalized polycaprolactone scaffolds for bone regeneration, Mater. Sci. Eng. C Mater. Biol. Appl. 33 (2013) 3404–3412.
[15] S. Eosoly, N.E. Vrana, S. Lohfeld, M. Hindie, L. Looney, Interaction of cell culture with composition effects on the mechanical properties of polycaprolactone-hydroxyapatite scaffolds fabricated via selective laser sintering (SLS), Mater. Sci. Eng. C 32 (2012) 2250–2257.
[16] C. Gao, B. Yang, H. Hu, J. Liu, C. Shuai, S. Peng, Enhanced sintering ability of biphasic calcium phosphate by polymers used for bone scaffold fabrication, Mater. Sci. Eng. C Mater. Biol. Appl. 33 (2013) 3802–3810.
[17] V. Manakari, G. Parande, M. Gupta, Selective laser melting of magnesium and magnesium alloy powders, Mater. Sci. Eng. C 32 (2012) 73–77.
[18] P. Wen, L. Jauer, M. Voshage, Y. Chen, R. Poprawe, J.H. Schleifenbaum, Densification behavior of pure Zn metal parts produced by selective laser melting for manufacturing biodegradable implants, J. Mater. Process. Technol. 258 (2018) 128–137.
[19] J.C. Middleton, A.J. Tipton, Synthetic biodegradable polymers as orthopedic devices, Biomaterials 21 (2000) 2335–2346.
[20] D.J. Meera, M.R. Knoll, J.D. Holmes, D.M. Clark, Fixation of Le Fort I osteotomies with poly–lactic acid mesh and ultrasonic-welding—a new technique, Journal of oral and maxillofacial surgery official journal of the American Association of Oral and Maxillofacial Surgeons 70 (2012) 1139–1144.
[21] A. Göpüerich, Mechanisms of polymer degradation and erosion, Biomaterials 17 (1996) 103–114.
[22] R. Auras, L.-T. Lim, S.E.M. Selke, H. Tsuji, Poly(lactic Acid), John Wiley & Sons, Inc, Hoboken, NJ, USA, 2010.
[23] I. Engelberg, J. Kohn, Physico-mechanical properties of degradable polymers used in medical applications: a comparative study, Biomaterials 12 (1991) 292–304.
[24] D.A. Zopf, S.J. Hollister, M.E. Nelson, R.G. Ohye, G.E. Green, Bioreosorbable airway splint created with a three-dimensional printer, N. Engl. J. Med. 363 (2011) 2042–2045.
[25] R.J. Morrison, S.J. Hollister, M.F. Niedner, M.G. Mahani, A.H. Park, D.K. Mehta, R.G. Ohye, G.E. Green, Mitigation of tracheobronchomalacia with 3D-printed personalized medical devices in pediatric patients, Sci. Transl. Med. 7 (2015) 283ra64.
[26] B. Smetts, M. Barbeck, H. Hanken, H. Fischer, M. Lindner, M. Heiland, M. Wolte, S. Ghanati, A. Kolk, Selective laser-melted fully biodegradable scaffold composed of poly(l-lactide) and β-tricalcium phosphate with potential as a biodegradable implant for complex maxillofacial reconstruction, Journal of Biomedical Materials Research Part B Applied Biomaterials 105 (2017) 1216–1223.
[27] T.B. Bukharova, E.N. Antonov, V.K. Popov, T.K. Fathkhudinov, A.V. Popova,
