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Inner strut morphology is the key parameter in producing highly porous and mechanically stable poly(-caprolactone) scaffolds via selective laser sintering / Tortorici, Martina; Gayer, Christoph; Torchio, Alessandro; Cho, Simone; Henrich Schleifenbaum, Johannes; Petersen, Ansgar. - In: MATERIALS SCIENCE AND ENGINEERING. C, BIOMIMETIC MATERIALS, SENSORS AND SYSTEMS. - ISSN 0928-4931. - ELETTRONICO. - 123:(2021), pp. 1-12. [10.1016/j.msec.2021.111986]

Availability:

This version is available at: 11583/2875253 since: 2021-03-23T12:55:26Z

Publisher:

Elsevier

Published

DOI:10.1016/j.msec.2021.111986

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<http://dx.doi.org/10.1016/j.msec.2021.111986>

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1 **Inner strut morphology is the key parameter in producing highly porous and**
2 **mechanically stable poly(ϵ -caprolactone) scaffolds via selective laser sintering**

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20
21 **Abstract**

22 Selective laser sintering (SLS) is an established method to produce dimensionally accurate
23 scaffolds for tissue engineering (TE) applications, especially in bone. In this context, the FDA-
24 approved, biodegradable polymer poly(ϵ -caprolactone) (PCL) has been suggested as a
25 suitable scaffold material. However, PCL scaffold mechanical stability – an attribute of
26 particular importance in the field of bone TE – was not considered as a primary target for SLS
27 process parameters optimization so far. Here, we investigated the influence of SLS process
28 parameters on the sintered scaffolds with the aim of producing highly porous (> 70% porosity)
29 PCL scaffolds with sub-mm geometrical features for bone TE. Specifically, we studied the
30 influence of laser power, beam compensation and laser beam diameter on the dimensional
31 accuracy and mechanical stiffness of the produced PCL scaffolds. We found that the ratio
32 between the diameter of the molten cross-section within scaffold struts and the outer strut
33 diameter (including partially sintered particles) depended on the SLS process parameters. By
34 maximizing this ratio, the mechanical stability could be optimized. The comparison with *in silico*
35 predictions of scaffold mechanical stiffness revealed that the diameter of the molten cross-
36 section within struts and not the strut diameter controlled the mechanical behaviour of the
37 scaffold. These observations should be considered when evaluating the quality of the sintering

38 process based on dimensional accuracy, especially for features < 1 mm. Based on these
39 findings, we suggested an approach to evaluate the sintering outcome and to define SLS
40 process parameters that enable the production of highly porous scaffolds that are both
41 dimensionally accurate and mechanically stable. Moreover, the cytocompatibility of PCL
42 scaffolds was evaluated by elution tests with primary human mesenchymal stromal cells. No
43 evidence of cytotoxicity was found in any of the investigated scaffolds, confirming the suitability
44 of SLS as production technique of PCL scaffolds for bone TE over a wide range of SLS process
45 parameters.

46

47 **Keywords**

48 Selective laser sintering; poly(ϵ -caprolactone); tissue engineering; bone; scaffold;
49 cytocompatibility.

50

51 **1. Introduction**

52 Selective laser sintering (SLS) is an additive manufacturing (AM) technique that uses a CO₂
53 laser beam to fuse particles and build three-dimensional (3D) objects in a layer-by-layer
54 fashion [1]. Due to its features, such as the possibility to reproduce complex 3D shapes without
55 the need of an additional support material [1,2] nor the use of potentially toxic binders [2], the
56 technique has gained attention for scaffold production in the field of tissue engineering (TE),
57 whose aim is the restoration, maintenance or improvement of living tissue functions [3].
58 Moreover, many suitable TE materials can be processed by SLS [4,5], including poly(ϵ -
59 caprolactone) (PCL), which is an FDA-approved biodegradable polymer [6]. Pure PCL
60 scaffolds produced by SLS have already been suggested to be used in bone TE [7,8], cartilage
61 TE [9] and cardiac TE [10].

62

63

64 Prior to device fabrication, suitable SLS process parameters, such as laser power, scan speed,
65 scan spacing, layer thickness, laser beam diameter, and part bed temperature (i.e.
66 temperature in the build chamber) have to be identified [2]. The effects of these parameters on
67 the sintering outcome are interdependent, as well as material dependent, making it challenging
68 to define the ideal sintering conditions for a specific application. Tan and colleagues, for
69 example, investigated the feasibility of sintering for a number of biocompatible polymers,
70 including PCL [11]. In their study, the effects of laser power, scan speed, and part bed
71 temperature variations on melting behaviour were analysed and the authors suggested 2-3 W,
72 3810 mm/s, and 40°C, respectively, to be the optimal parameters for PCL (Table 1). In further
73 studies, a set of scaffolds produced with the recommended optimal parameters were tested
74 for cytotoxicity and mechanical behaviour [12]. Partee and colleagues studied variations of

75 laser power, scan speed, scan spacing, part bed temperature and delay in new powder
 76 deposition with the aim of obtaining fully dense parts, which were not only dimensionally
 77 accurate, but also easily removed from the surrounding unsintered powder [13]. As a result,
 78 they proposed two sets of parameters, one for solid parts and one for porous parts (Table 1).
 79 Objects produced with the suggested SLS parameters were mechanically characterized in a
 80 subsequent study [14]. Lohfeld and colleagues aimed at producing PCL scaffolds with 500 μm
 81 strut diameter and tried different scan modalities, as well as different laser power, part bed
 82 temperature and number of consecutive scans [15]. The best dimensional accuracy was
 83 achieved by having a single scan, with the laser scanning first the outline of the object, then
 84 filling the core with a half-strut-thickness offset. Values of the other parameters are listed in
 85 Table 1.

86

87 *Table 1: SLS process parameters for PCL as reported in literature.*

LASER POWER (W)	SCAN SPEED (mm/s)	SCAN SPACING (μm)	PART BED TEMPERATURE ($^{\circ}\text{C}$)	OTHERS	REFERENCE
2-3	3810	N.A.	40	N.A.	[11]
3-4	5080	N.A.	40	N.A.	[16]
4.1	1079.5	152.4	46	Delay in new powder deposition: 0 s (solid parts) 8 s (porous parts)	[13]
4	N.A.	N.A.	38	Number of consecutive scans: 1	[15]

88 N.A. = Not available

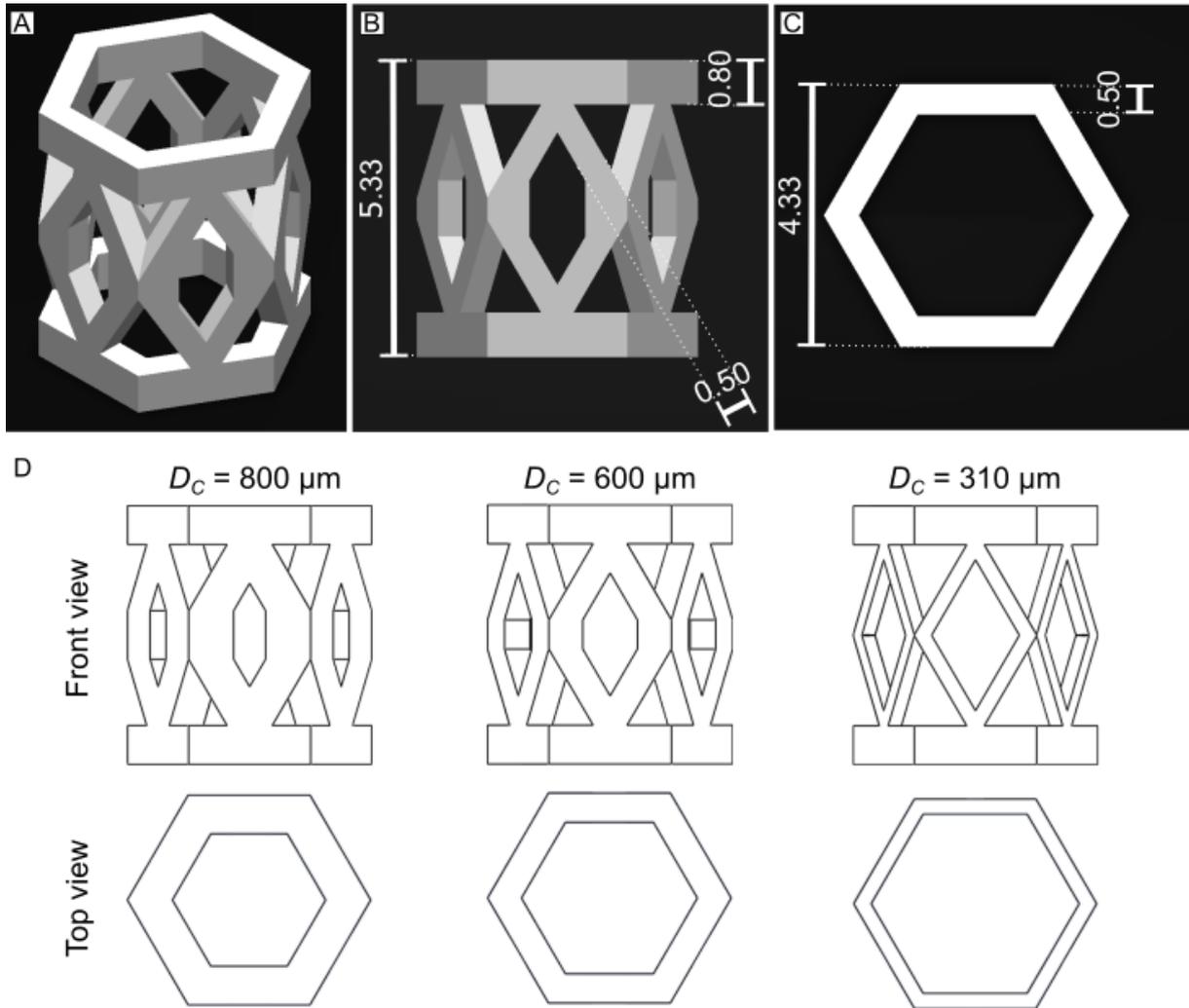
89

90 In these studies, however, only dimensional accuracy was considered as the primary factor in
 91 defining the most suitable SLS process parameters. The mechanical properties of the sintered
 92 objects were measured only after the identification of SLS process parameters. Therefore,
 93 these studies do not provide insights into how the SLS process parameters, individually and in
 94 combination, influence the mechanical behaviour of sintered objects. However, when
 95 employing SLS to produce TE scaffolds, the scaffold mechanical properties are of particular
 96 importance. This is the case, for example, for bone TE, where scaffolds should be completely

97 or at least partially load bearing [17]. Additionally, bone TE scaffolds should have a high and
98 interconnected porosity to allow cell invasion and to promote oxygen and nutrients exchange;
99 however, the fulfilment of the porosity requirement usually results in low mechanical properties
100 [17]. The production of mechanically stable scaffolds with suitable and reproducible porosity is
101 one of the challenges currently faced by bone TE. Mechanically optimized bone TE scaffolds
102 manufactured by AM techniques, such as SLS, have the potential to comply with both
103 mechanical and structural needs.

104 Here, we aim at developing SLS process parameters to produce PCL scaffolds for bone TE
105 that are not only dimensionally accurate, but also mechanically stable. To the best of our
106 knowledge, such a combined optimization has not been performed on PCL scaffolds before.
107 To achieve our aim, we first compared PCL powders with two different molecular weights and
108 two different particle size distributions concerning their behaviour during SLS and we evaluated
109 the effect of the sterilization method on scaffold mechanical properties. Subsequently, we
110 systematically varied laser power (P_L), laser beam compensation (BC) and laser beam
111 diameter (BD). The influence of these parameters on mechanical stability and dimensional
112 accuracy of the resulting highly porous scaffolds (>70% porosity, Figure 1A) was investigated
113 by quantifying the compressive elastic modulus (E), the strut diameter (D_S) and the cross-
114 sectional area of the molten core (A_M) inside of the struts. Importantly, the intended use of the
115 here studied scaffold will be in combination with an external fixator, as bone-like stiffness is
116 challenging to achieve with a purely polymeric approach. Finally, the cytocompatibility of PCL
117 scaffolds produced with six different sets of SLS process parameters was studied *in vitro* by
118 eluate tests with primary human mesenchymal stromal cells (hMSCs). The chosen cell
119 phenotype is of relevance for the intended use of the scaffolds in bone TE, as hMSCs are the
120 progenitor cells that differentiate into the cell phenotype involved in new bone formation, i.e.
121 osteocytes [18].

122



123

124 *Figure 1: CAD model of scaffold for bone TE. A) Isometric view; B) front view; C) top view; D)*
 125 *Examples of CAD with different strut diameter for in silico analysis. Left, middle and right*
 126 *columns show geometries with strut computational diameter (D_C) of 800, 600 and 310 μm ,*
 127 *respectively. All reported values are in mm if not specifically stated otherwise.*

128

129 **2. Materials and Methods**

130

131 **2.1 Scaffold design**

132 A bone TE scaffold with 73.3% porosity and 500 μm strut diameter was designed using
 133 SolidWorks (Dassault Systèmes, France) (Figure 1A, B, and C). The design was intended as
 134 one structural unit of a larger scaffold, which will be composed of a honeycomb of these
 135 individual units. Thus, the scaffold here studied had a hexagonal cross-section.

136

137 **2.2 PCL powders**

138 PCL powder particles with 60,000 and 100,000 g/mol number average molecular weight (\bar{M}_n)
 139 and particle size distribution of 25–50 μm were purchased from Aqtis Medical BV

140 (Netherlands). Additionally, particles with \bar{M}_n of 60,000 g/mol and particle size distribution of
 141 50–80 μm were acquired from the same supplier. For each particle type, at least $N = 3$ scaffolds
 142 were produced by SLS with $P_L = 0.4$ W, $BC = 200$ μm and $BD = 390$ μm and mechanically
 143 characterized as described below.

144

145 2.3 SLS process

146 The bone TE scaffold was produced by SLS using a laboratory SLS machine equipped with
 147 an ionizer to prevent powder agglomeration. P_L , BC and BD were varied as listed in Table 2.
 148 P_L is the power that the laser applies to the material. BC is a geometric parameter: the laser
 149 starts scanning the material with an offset BC from the border of the designed object to
 150 compensate for the width of the melting track. BD is the size of the laser beam that scans the
 151 material. The other sintering parameters were kept constant at the following values: 50 mm/s
 152 scan speed, 67° scan pattern rotation between layers, unidirectional scans, 20 μm hatch
 153 distance, 50 μm layer thickness. For each set of SLS parameters, at least $N = 3$ scaffolds were
 154 produced from PCL particles with \bar{M}_n of 60,000 g/mol and 50–80 μm particles size distribution
 155 and mechanically characterized as described below. After the SLS process, the scaffolds were
 156 treated with compressed air (2 bar for 10 s) to remove loosely attached particles.

157

158 *Table 2: Investigated SLS process parameters: laser power (P_L), laser beam compensation*
 159 *(BC) and laser beam diameter (BD).*

P_L (W)	BC (μm)	BD (μm)
0.30	150	260
0.35	200	390
0.40	205	
0.45	210	
0.50	225	
0.60	230	
0.70		

160

161 2.4 Scaffold sterilization

162 $N = 9$ scaffolds were produced with $P_L = 0.40$ W, $BC = 200$ μm , and $BD = 260$ μm . From these,
 163 $N = 3$ scaffolds were randomly chosen to be sterilized by ethylene oxide (EtOx), $N = 3$ by
 164 gamma (γ) irradiation, and $N = 3$ were kept unsterile as reference. Sterilization was performed
 165 by HA2 Medizintechnik GmbH (Germany). Sterilized and reference scaffolds were
 166 mechanically characterized as described below.

167

168 *2.5 Morphological characterization*

169 Morphological characterization was performed by measuring height and width of the produced
170 scaffolds with a digital calliper (DigitCal, Tesa, Switzerland). The strut diameter (D_S) was
171 measured from light microscope images (SMZ1270, Nikon, Japan) using the software NIS-
172 Elements D 4.30.02. The measurement of D_S was performed on the four struts of each one of
173 the six faces of the scaffolds, as exemplarily shown in Figure 4B.

174 To evaluate the morphology of the strut cross-section, scanning electron microscopy (SEM)
175 was performed using a JCM-6000 machine (Jeol, Japan). For SEM, $N = 1$ scaffolds per type
176 were prepared by embedding in TissueTek® (Sakura Finetek Europe B.V.) and cryocut to $\frac{3}{4}$
177 of their height at -20°C with a Leica CM3050 S cryomicrotome (Leica, Germany) using a slice
178 thickness of $20\ \mu\text{m}$. TissueTek® was removed by repeated washings in deionized water.
179 Scaffolds were dried at room temperature for one day, then gold sputtered for 30 s at 8 Pa
180 pressure and 40 mA electrical current prior to SEM imaging. The molten cross-sectional area
181 (A_M), defined as the region in which particles were completely molten during the SLS process
182 and re-solidified in a compact area, was measured from SEM pictures of individual struts using
183 Fiji (ImageJ, NIH) (**Errore. L'origine riferimento non è stata trovata.**H). For each sample, N
184 = 12 strut cross-sections were measured. The molten cross-sectional diameter (D_M)
185 corresponding to A_M was calculated as the diameter of a circular cross-section using equation
186 (1):

187

188
$$D_M = 2\sqrt{\frac{A_M}{\pi}} \cdot (1)$$

189

190 Results are reported as average \pm standard deviation.

191

192 *2.6 Mechanical characterization*

193 Scaffolds were mechanically tested in monoaxial compression using a BOSE Test Bench (LM1
194 TestBench, TA Instrument ElectroForce System Group, USA) equipped with a 222.5 N load
195 cell. During the test, applied displacement and measured reaction force were recorded and
196 subsequently used to calculate the stress-strain curves. The compressive elastic modulus (E)
197 was obtained by a linear fit of the elastic region of the stress-strain curves. At least $N = 3$
198 scaffolds per type were tested.

199 Results are reported as average \pm standard deviation.

200

201 *2.7 Molecular weight characterization*

202 The molecular weight distribution of raw powders and of sterilized and reference scaffolds was
203 measured by a size exclusion chromatography instrument (Agilent Technologies 1200 Series,

204 USA) equipped with a refractive index detector, according to Boffito et al. [19]. Briefly, materials
205 were dissolved at 2 mg/ml concentration in a solution of N,N-dimethylformamide (Chromasolv
206 HPCL grade, CarloErba Reagents, Italy) added with lithium bromide (Sigma Aldrich, Italy) at
207 0.1% w/v and filtered using a 0.45 µm poly(tetrafluoroethylene) syringe filter (Lab Logistic
208 Group GmbH, USA). Then, the analysis was performed at 55°C with a flow rate of 0.5 ml/min
209 through two Waters Styragel columns (HR1 and HR4). Finally, number average molecular
210 weight (\bar{M}_n), weight average molecular weight (\bar{M}_w) and dispersity index ($DI = \bar{M}_w/\bar{M}_n$) were
211 estimated using the Agilent ChemStation Software and a calibration curve based on
212 poly(methyl methacrylate) (PMMA) standards (\bar{M}_n ranging from 4,000 to 200,000 g/mol).

213

214 *2.8 In silico analysis of scaffold elastic modulus as function of strut diameter*

215 The theoretical relationship between elastic modulus and strut diameter was assessed by
216 computational analysis. A variation of scaffold strut diameter in dependency of SLS process
217 parameters was experimentally observed. To reproduce these experimentally measured strut
218 size variations, six additional CADs were prepared in SolidWorks (Dassault Systèmes,
219 France). The CADs had the same architecture as the produced scaffold, but their strut diameter
220 was 310, 400, 500, 600, 700 and 800 µm (Figure 1D). The CADs had square cross-sections
221 to be consistent with the original CAD design, but, as they were used to give a computational
222 representation of the experimental irregular cross-section of the struts, the cross-sectional
223 thickness of the CADs will be named computational diameter (D_C). The six scaffold geometries
224 with varying strut diameter were not fabricated by SLS, but they were evaluated in compression
225 by finite element (FE) analysis using Abaqus (Dassault Systèmes, France). Scaffold
226 geometries were meshed with a tetrahedral mesh with seed size of 0.15 mm and assigned
227 elastic material properties of the bulk material with compressive modulus of 70 MPa and
228 Poisson's ratio of 0.3. The *in silico* scaffold material properties were derived by running FE
229 analyses with different material values and selecting the set of values that best matched the
230 experimental data. An encastre boundary condition was imposed to the bottom face of the
231 geometry and an axial 3% compressive displacement was applied to the top face, simulating
232 the compressive test experimentally performed. This range of compressive displacement was
233 expected to be within the region of elastic behaviour of the scaffolds. The reaction force (RF)
234 of each CAD was calculated and used to evaluate the computational elastic modulus (E_C) with
235 equation (2):

236

$$237 \quad E_C = \frac{\sigma}{\varepsilon} = \frac{RF}{A_C} \cdot \frac{L_C}{\Delta L_C}, \quad (2)$$

238

239 where σ is the stress, ε is the strain, A_C is the CAD hexagonal cross-section, L_C is the CAD
240 height and ΔL_C is the applied displacement.

241

242 *2.9 Cytocompatibility test*

243 Six scaffold prototypes (“A” to “F”, Table 4) were tested for cytocompatibility of the eluate with
244 primary human mesenchymal stromal cells (hMSCs).

245 Cells were obtained by density gradient separation from the bone marrow of patients
246 undergoing total hip replacements. The use of primary human bone marrow mesenchymal
247 stromal cells was approved by the ethics committee of the Charité–Universitätsmedizin Berlin,
248 and donors' written informed consent was given. Cell culture was performed in expansion
249 medium composed of Dulbecco's modified Eagle's medium (DMEM, Sigma, 1000 mg/l
250 glucose), 10% foetal bovine serum (FBS, Biochrom AG), 1% penicillin/streptomycin (P/S,
251 Biochrom AG), 1% L-glutamine (glutaMAX, Invitrogen). Cells were trypsinized (PAA
252 Laboratories GmbH) at approximately 80% confluency and experiments were carried out with
253 cells in passages 3 or 4.

254 Batches of conditioned medium were prepared by placement of the prototypes in 3 ml of
255 expansion medium and by incubation at 37°C and 5% CO₂ for at least 24 hours before
256 exposure to cells. Specifically, cells of the day 3 and 6 time points were exposed to medium
257 conditioned for 24 hours and 3 days, respectively. At each experimental time point, a maximum
258 of 1200 µl (specifically, 200 µl/well) of conditioned medium was taken from each batch to be
259 used in cell culture. The same volume that was removed was subsequently refilled with fresh
260 expansion medium.

261 Cells were seeded in 48-well plates with a confluency of 30%. At day 0, i.e. 24 hours after
262 seeding, and 3, hMSCs were exposed to 200 µl of conditioned medium. Cells cultured in 200 µl
263 of expansion medium were used as controls. Medium evaporation was reduced by surrounding
264 the cell-seeded area with wells filled with 600 µl of phosphate buffered saline (PBS, Gibco, Life
265 Technologies Limited), which were refilled at day 3. At day 0, 3 and 6, AlamarBlue (#DAL 1100,
266 Invitrogen) and Cyquant (#C7026, Thermo Fisher Scientific) assays were performed to assess
267 cellular metabolic activity and number, respectively. For each tested condition, 3 wells/time
268 point were seeded, with the exception of the day 0 time point, which consisted of 6 control
269 wells. Four independent repetitions of the experiment were performed. To reduce the risk of
270 introducing systematic errors by pipetting order or by sample position in the well plate (e.g.
271 edge effects due to non-uniform environmental conditions at the border of the cell-seeded
272 area), the position of the wells conditioned with the six media and of the control wells was
273 randomly varied at each repetition. Results of the AlamarBlue and Cyquant assays were
274 evaluated with an Infinite M200 Pro plate reader (Tecan).

275 During the result analysis of each experimental repetition, outliers in the raw data were
276 identified within the 3 wells of each condition. The average of the two wells having the highest
277 fluorescence intensity values was taken as reference and the intensity of the third well was

278 calculated as percentage of the reference. If the resulting percentage was lower than 50%, the
279 third well was considered an outlier and removed from the data set. Similarly, outlier values
280 between the experimental repetitions were identified by calculating the percentage of the
281 repetition with the lowest average value in respect to the average of the other three repetitions.
282 Also in this case, a percentage lower than 50% was considered an outlier.
283 Statistical significance was tested between the control and each investigated condition by
284 means of a two-sided Mann-Whitney-U test performed in Origin 2019b (OriginLab
285 Corporation). Values of $p < 0.05$ were considered as statistically significant, while values of
286 $p > 0.05$ were considered as non-significant.
287 Values were normalized to day 0 and are given as average \pm standard deviation of fold change.

288

289 **3. Results and Discussion**

290

291 *3.1 Influence of PCL powders on scaffold mechanical properties*

292 Size exclusion chromatography (SEC) was performed on PCL powders to study their
293 molecular characteristics and to serve as a reference for the subsequent evaluation of how the
294 SLS process would influence the properties of PCL. The number average molecular weight
295 and dispersity index resulting from the SEC analysis are shown in Table 3.

296

297 *Table 3: Number average molecular weight and dispersity index of the different raw materials*
 298 *and powder-based scaffolds investigated in this study. All scaffolds were produced from the*
 299 *PCL powder with nominal molecular weight of 60,000 g/mol and particle size distribution of*
 300 *50–80 μm .*

SAMPLE TYPE	PARTICLES SIZE DISTRIBUTIO N (μm)	NUMBER AVERAGE MOLECULAR WEIGHT (g/mol)	DISPERSITY INDEX
Powder – low molecular weight	25–50	59,800	1.85
Powder – high molecular weight	25–50	95,500	1.84
Powder – low molecular weight and larger particle diameter	50–80	57,600	1.81
Scaffold - Unsterile	50–80	55,000	1.80
Scaffold - EtOx	50–80	57,700	1.80
Scaffold - Gamma	50–80	37,700	2.20

301
 302 Mechanical testing showed that higher molecular weight PCL particles yielded scaffolds with
 303 lower E . In fact, SLS of lower (60,000 g/mol) and higher (100,000 g/mol) molecular weight
 304 particles, but equal particles size of 25–50 μm , resulted in scaffolds with $E_{S,60} = 11.3 \pm 0.5$ MPa
 305 and $E_{S,100} = 5.6 \pm 1.2$ MPa, respectively. The elastic moduli values were normalized to the
 306 elastic modulus of the scaffold produced from particles having molecular weight of 60,000
 307 g/mol and size of 25–50 μm to better highlight the differences between groups (Figure 2A). In
 308 general, semi-crystalline polymers, such as PCL, tend to be more ductile and stronger at high
 309 molecular weights, as the polymeric chains with low molecular weight tend to collect in between
 310 crystalline regions, thereby lowering the number of “tie molecules” [20]. Thus, semi-crystalline
 311 polymers with low molecular weight tend to be brittle and have low strength, while those with
 312 high molecular weight tend to have a higher toughness [20]. Therefore, it could be expected
 313 to obtain higher E scaffolds using a higher molecular weight polymer, as it was shown for PCL
 314 scaffolds produced by 3D-printing and tested in compression [21]. However, the higher the
 315 molecular weight of PCL is, the higher is the viscosity of the molten polymer [22].
 316 Consequently, the lower E measured for the higher molecular weight scaffolds might be
 317 explained by a lower sintering rate resulting from the higher melt viscosity [23,24]. Moreover,
 318 the crystallinity of PCL was shown to decrease with increasing molecular weight, contributing

319 to the production of specimens with lower E [25]. In the scaffolds produced from the PCL
320 powder with the higher molecular weight (Figure 2F), more loosely attached powder particles
321 were observed compared to the scaffolds produced from the powder with lower molecular
322 weight (Figure 2E and G). This might be ascribed to the lower crystallinity of the high molecular
323 weight PCL, which could lead to more necking next to the scanning path of the laser.
324 The deposition of an evenly distributed layer of powder platform is an important premise for a
325 robust and reproducible SLS process. In this context, powder behaviour during deposition is
326 described by its flowability, defined as the powder ability of flowing in a desired manner in a
327 specific piece of equipment [26]. Powder flow during the SLS process was remarkably different
328 for the two powders with the same molecular weight of 60,000 g/mol but different particle size.
329 Bigger particles of 50–80 μm were homogeneously spread by the roller, which was
330 additionally equipped with an ionizer to avoid particle aggregation (Figure 2B), while smaller
331 particles of 25–50 μm formed aggregates despite the presence of the ionizer (Figure 2D).
332 During the movement of the roller from the powder reservoir to the building platform and back,
333 such aggregates dropped randomly onto the deposited powder layer, thereby impairing a
334 uniform powder distribution. If a powder aggregate would be on the laser path during the
335 sintering process, the locally increased layer thickness would cause this specific object area
336 to have different properties from the neighbouring ones. Thus, the reproducibility of the
337 technique would be impaired. These observations are consistent with literature, where it was
338 reported that powders consisting of particles with diameter smaller than 45 μm have
339 unfavourable flow properties due to high electrostatic forces [2]. Nevertheless, scaffolds were
340 produced successfully with the two particle sizes and their mechanical characterization
341 revealed a comparable elastic modulus of $E= 11$ MPa (Figure 2A). Because of the identical
342 mechanical stiffness of the resulting scaffolds and the favourable flowability, the PCL powder
343 with 60,000 g/mol molecular weight and 50–80 μm particles size was selected to produce the
344 scaffolds for all subsequent experiments.

345

346 *3.2 Influence of sterilization on mechanical properties*

347 Sterilization is particularly relevant when planning the production of TE devices [17].
348 Importantly, the chosen sterilization method should not significantly alter the properties that
349 were engineered for the specific device. For this reason, we tested two different sterilization
350 methods, namely ethylene oxide (EtOx) and gamma (γ) irradiation, and we evaluated their
351 influence on scaffold elastic modulus and PCL molecular weight. By performing these
352 sterilization tests at the first stages of scaffold development, we ensured that the subsequent

353 fine-tuning of scaffold properties could be maintained throughout the device processing until
354 its actual application.

355 Mechanical properties of unsterile, EtOx- and γ -sterilized scaffolds were found to be similar,
356 with values of $E_S = 20.9 \pm 1.9$, $E_{S,EtOx} = 18.1 \pm 1.2$, and $E_{S,\gamma} = 18.6 \pm 3.0$ MPa, respectively.
357 Elastic moduli values were normalized to the elastic modulus of the unsterile scaffold to better
358 highlight differences between groups (Figure 2C). However, SEC analysis revealed a large
359 35% decrease in molecular weight for γ -sterilized scaffolds (Table 3), with a reduction in
360 number average molecular weight from $\bar{M}_{n_{pow}} = 57,600$ g/mol (raw powder) to
361 $\bar{M}_{n_{S,\gamma}} = 37,700$ g/mol (γ -sterilized scaffold). The observed variation in molecular weight was
362 significant, as the usual error of SEC analysis is approximately 10% [27]. At the same time,
363 the dispersity index increased from 1.80 to 2.20 (Table 3). On the contrary, the number average
364 molecular weight of EtOx sterilized scaffolds was $\bar{M}_{n_{S,EtOx}} = 57,700$ g/mol, similar to both the
365 raw powder ($\bar{M}_{n_{pow}} = 57,600$ g/mol) and the scaffold before sterilization ($\bar{M}_{n_S} = 55,000$ g/mol)
366 (Table 3). Raw powder, unsterile and EtOx sterilized scaffolds had also equal dispersity index
367 of 1.80 (Table 3).

368 Various effects of γ irradiation on PCL have been reported in literature. For example, a
369 reduction in molecular weight and in tensile stiffness and strength was observed in PCL
370 electrospun membranes [28]. In another study, γ irradiation of PCL films resulted in larger
371 tensile yield points and lower number average but higher weight average molecular weight,
372 indicating both crosslinking and chain scissions [29]. Furthermore, no remarkable differences
373 in tensile mechanical behaviour was measured in γ -sterilized PCL electrospun membranes,
374 but at the same time there were indications of chain scission [30]. Taken together, these
375 studies suggest a controversial relationship between γ -irradiation and PCL mechanical
376 properties. However, a clear impact on the polymer molecular weight can be concluded, in
377 agreement with our findings. Although there was no obvious immediate effect on the
378 compressive elastic modulus, the reduction in molecular weight caused by γ -sterilization might
379 have an impact on PCL degradation speed, and thereby on long-term scaffold stability. For
380 example, PCL films were produced with different ratios of high and low molecular weight
381 polymer and the films having a higher amount of low molecular weight chains were shown to
382 have a greater *in vitro* weight loss after 21 weeks [31]. Furthermore, PCL undergoes a two-
383 stage degradation *in vivo*, in which each phase is determined by the molecular weight [32].
384 First, long polymeric chains are cleaved by hydrolysis [6]. Second, PCL fragments smaller than
385 3,000 g/mol undergo intracellular uptake [33] and are eventually excreted through faeces and
386 urine [32]. The *in vitro* degradation behaviour of the PCL scaffolds was not investigated in this
387 work. Previous studies showed that specimens with an even lower molecular weight showed
388 no significant degradation over a timeframe of up to three months. Specifically, electrospun

389 PCL membranes with number average molecular weight of 15,000 g/mol showed no changes
390 in molar mass after 100 days of immersion in phosphate buffered saline (PBS) [34]. In another
391 case, PCL films with an initial number average molecular weight of 17,000 g/mol were
392 degraded in PBS and simulated body fluid (SBF) for 90 days, resulting in a maximum mass
393 loss of 3% and a reduction in molecular weight of 13% in PBS [35]. The *in vivo* degradation of
394 the scaffolds is expected to be in the range of 2 to 4 years depending on the initial molecular
395 weight [6]. For a translational approach, the material degradation should be evaluated at the
396 specific site of scaffold implantation to gain insights into PCL degradation in the according
397 environment.

398 Considering the performed observations, EtOx was selected here as the preferred sterilization
399 method, since it altered neither the molecular weight of the polymer nor the mechanical
400 properties of the scaffold.

401

402 *3.3 Influence of SLS parameters on scaffold properties*

403 After identifying which PCL powder was most suitable for the SLS process and evaluating the
404 least material-altering sterilization method, a systematic SLS parameter variation was
405 performed to investigate effects on the mechanical and morphological properties of the
406 produced scaffolds. An identical scaffold design (Figure 1A) was produced with different SLS
407 parameters (Table 2). The elastic modulus (E) and strut diameter (D_s) in dependency of laser
408 power (P_L), beam compensation (BC) and beam diameter (BD) variations are shown in Figure
409 3.

410 Depending on the parameter set, the stiffness of PCL scaffolds varied by a factor of 18.9 from
411 values as low as $E = 1.76 \pm 0.05$ MPa to as high as $E = 33.33 \pm 2.27$ MPa. Moreover, the
412 resulting strut diameter D_s ranged from approximately 400 to 800 μm , whereas the designed
413 strut diameter was 500 μm in all cases (Figure 1A, B, and C).

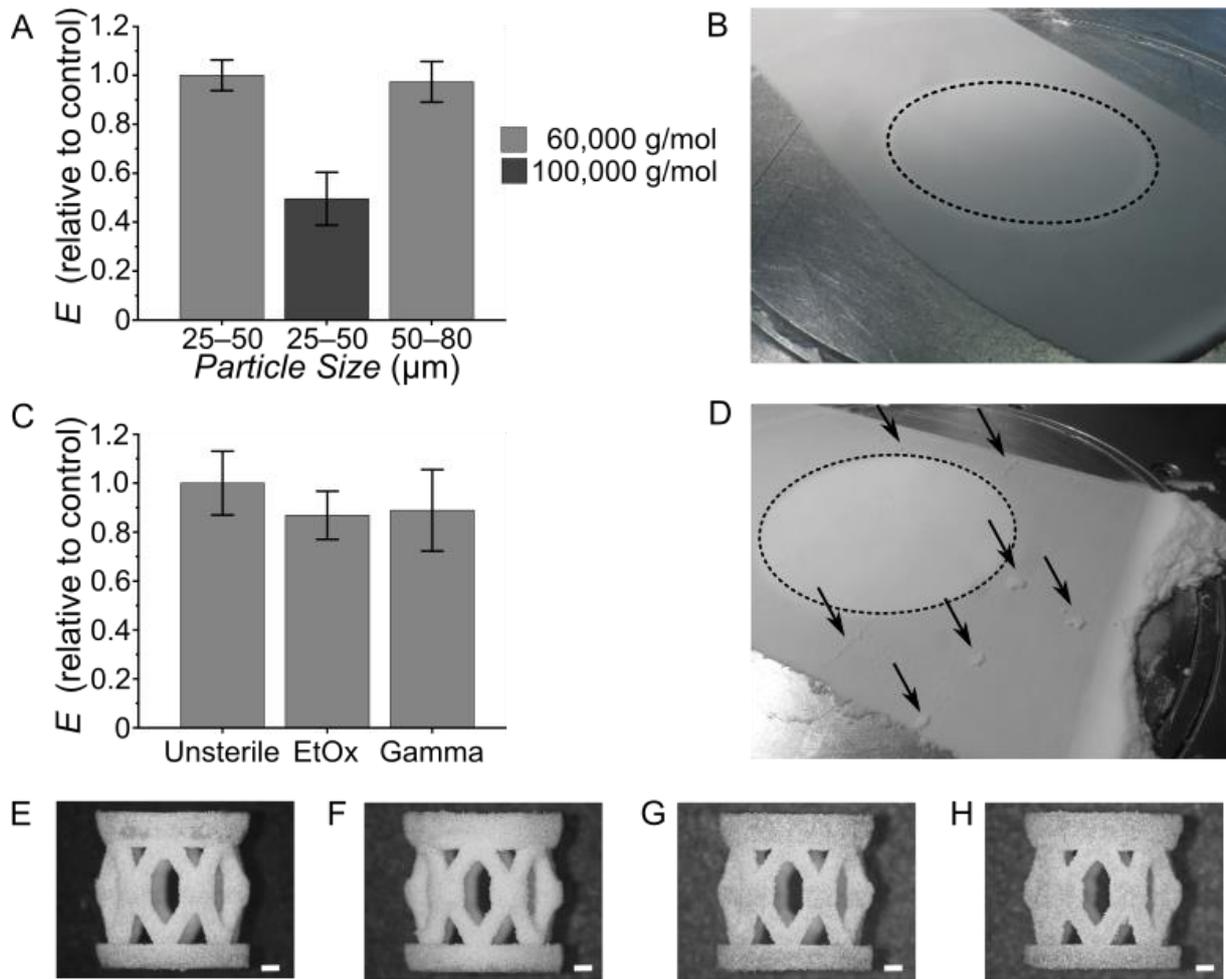
414 As discussed above, SEC analysis of sintered material showed no PCL degradation due to the
415 SLS process (Table 3), as opposed to the circa 20% reduction in post-sintering molecular
416 weight reported in literature [14]. This different material behaviour might be ascribed to gentler
417 temperature-time cycles employed in the present study, as a low scan speed (50 mm/s) and
418 no preheating were used here (c.f. Table 1).

419 Part bed preheating has been suggested in literature to minimise not only powder thermal
420 expansion due to the laser, but also shrinkage of semi-crystalline polymers, such as PCL,
421 during recrystallization in the cooling phase, thereby avoiding the sintered piece to curl and be
422 deformed during production [2]. Even though the part bed was at room temperature, all
423 scaffolds in the present study were successfully produced without deformation. However, it
424 was necessary to use a substrate material as base plate, as it is known from laser powder bed
425 fusion of metals. Moreover, the absence of part bed heating might have contributed to the

426 here-observed absence of polymer degradation through a shorter exposure of the material to
427 high temperatures. However, the transferability of the findings to larger scaffolds might be
428 difficult as curling effects are a matter of part size.

429 Systematic parameter variations showed that the same mechanical properties of the sintered
430 scaffolds could be achieved with multiple sets of SLS parameters (Figure 3). Even though a
431 certain scattering of the data was observed, there was a dominating dependency between the
432 scaffold elastic modulus E and the strut diameter D_S . In fact, different parameter sets resulted
433 in scaffolds with comparable E only if D_S was similar. Therefore, we concluded that the SLS
434 parameters influenced the mechanical properties of scaffolds mostly to the extent to which
435 they influenced the strut diameter D_S .

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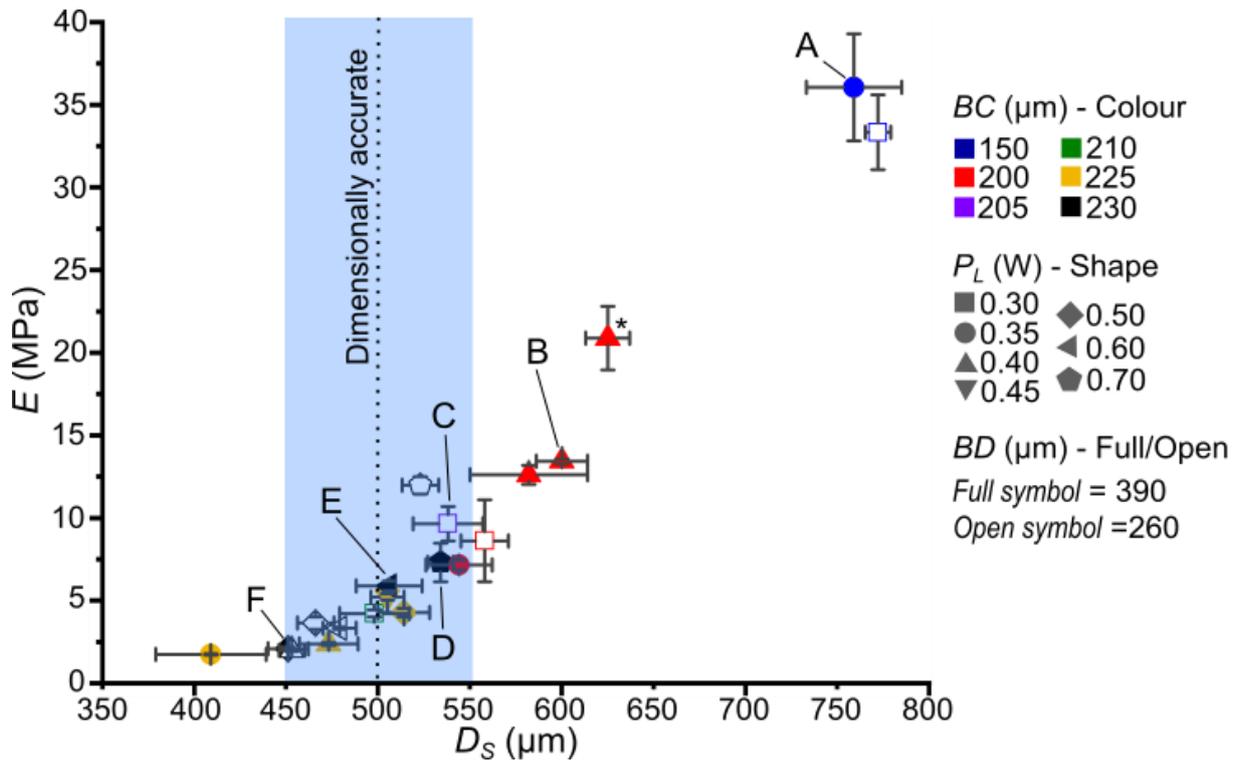
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Figure 2: Influence of PCL powder and sterilization method on scaffold elastic modulus and SLS process. A) Elastic modulus ratio of scaffolds produced with 60,000 and 100,000 g/mol molecular weight particles and with particle size of 25–50 and 50–80 μm . Values were normalized to the elastic modulus of the control, which were the scaffolds produced from PCL particles with molecular weight of 60,000 g/mol and size of 25–50 μm ; B) deposition of powder with particle size of 50–80 μm and 60,000 g/mol molecular weight. The black dashed circle marks the building platform (diameter 70 mm); C) elastic modulus ratio of unsterile, EtOx- and γ -sterilized scaffolds. Values were normalized to the elastic modulus of the control, which were the unsterile scaffolds; D) deposition of powder with particles size of 25–50 μm and 60,000 g/mol molecular weight. The black dashed circle marks the building platform (diameter 70 mm). Arrows indicate powder aggregates; E), F) and G) representative pictures of scaffolds produced with “25–50 μm and 60,000 g/mol”, “25–50 μm and 100,000 g/mol” and “50–80 μm and 60,000 g/mol” particles, respectively; H) representative picture of unsterile scaffold from the batch that underwent sterilization. Scale bars are 620 μm .



452

453 *Figure 3: Influence of laser power (P_L), beam compensation (BC) and beam diameter (BD)*
 454 *variations on scaffold elastic modulus (E) and strut diameter (D_s). Colours, shapes and*
 455 *full/empty contours indicate different BC, P_L and BD, respectively. The dotted line shows the*
 456 *CAD strut diameter of 500 μm . The blue shaded area is the diameter range that was*
 457 *considered as sufficiently accurate. Prototypes “A” to “F” are marked in the plot and were*
 458 *further investigated. The asterisk marks the scaffold that was used as unsterile control in the*
 459 *sterilization test.*

460 **3.4 Influence of SLS parameters on scaffold morphology**

461 To better understand the relationship between strut diameter and mechanical properties, six
 462 prototypes (“A” to “F”, see Table 4) were selected from the many parameter combinations
 463 shown in Figure 3 for a more detailed analysis. Selection criteria were based on

- 464 • high elastic modulus despite low dimensional accuracy (prototypes “A” and “B”, Figure
 465 4A and B, respectively);
- 466 • high dimensional accuracy despite low elastic modulus (prototypes “D”, “E”, and “F”,
 467 Figure 4D, E, and F, respectively);
- 468 • good compromise between dimensional accuracy and elastic modulus (prototype “C”,
 469 Figure 4C).

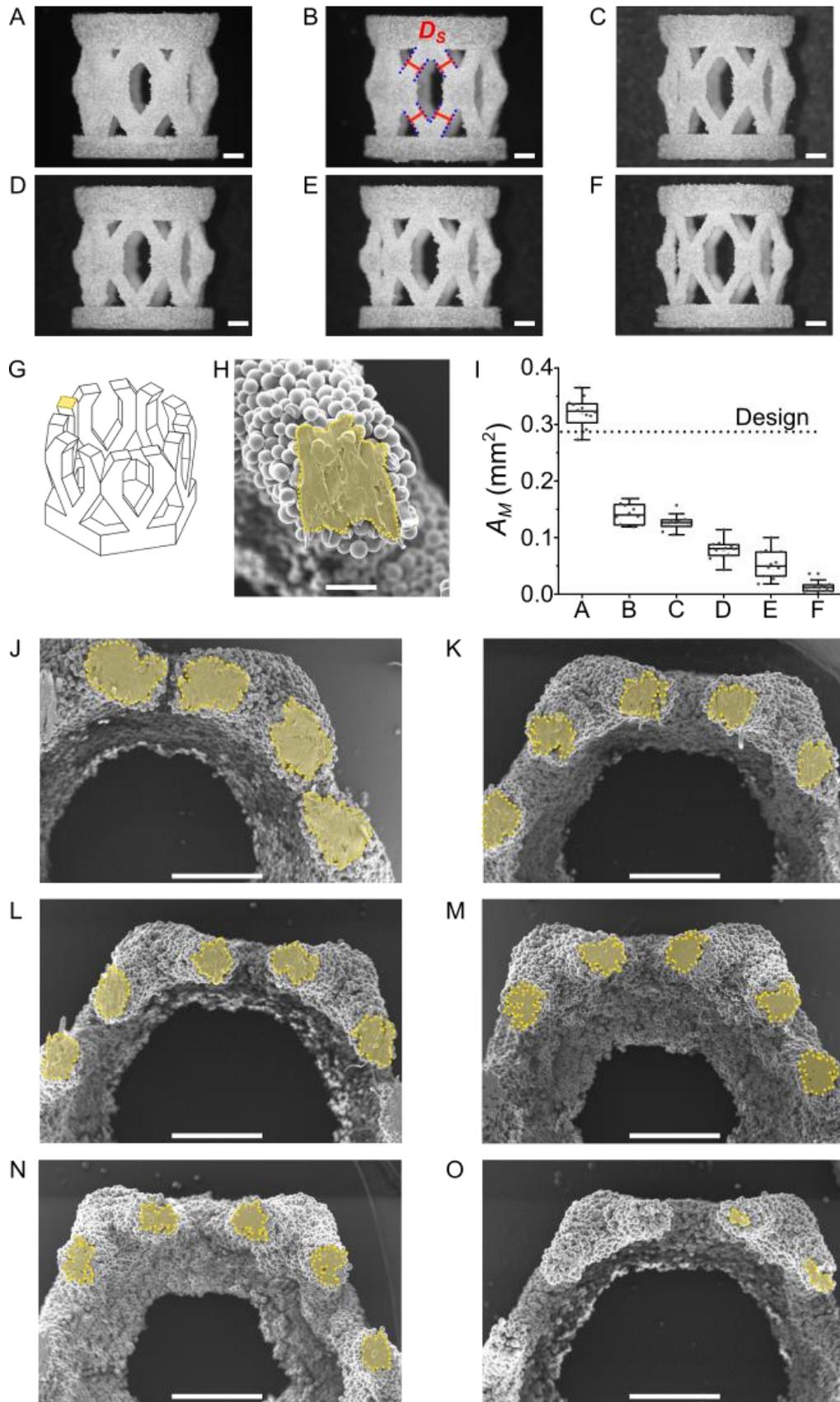
470

471 *Table 4: SLS parameters and resulting properties of prototypes that were selected for*
 472 *morphological analysis.*

Type	Parameter	Prototype A	Prototype B	Prototype C	Prototype D	Prototype E	Prototype F
Laser sintering parameters	P_L (W)	0.35	0.40	0.30	0.70	0.60	0.40
	BC (μm)	150	200	205	230	230	230
	BD (μm)	390	260	390	390	390	260
Scaffold properties	D_S (μm)	759	600	538	534	506	453
	E (MPa)	36	13	10	7	6	2

473
 474 The selected scaffolds were representative prototypes of different SLS parameter sets as
 475 shown in Table 4. Moreover, the selected prototypes covered well the observed broad range
 476 of strut diameter and mechanical stiffness. The strut molten cross-sectional area A_M , defined
 477 as the region in which the material was fully molten during the SLS process and individual
 478 particles were not distinguishable anymore, was evaluated from the SEM images of individual
 479 struts (Figure 4H). To do so, the selected prototypes were cryocut to $\frac{3}{4}$ of their height, as
 480 schematically shown in Figure 4G, and imaged by SEM. The quantification of A_M (Figure 4I)
 481 revealed that the molten cross-sectional area greatly varied depending on the SLS parameters,
 482 ranging from $A_M = 0.01 \pm 0.01$ to 0.32 ± 0.03 mm² (Figure 4J to O), although all scaffolds were
 483 produced from the same CAD with designed cross-sectional area A_M of 0.29 mm² (Figure 4G).
 484 Interestingly, for dimensionally accurate scaffolds, i.e. scaffolds whose D_S was close to the
 485 design value of 500 μm , a fully molten cross-section was not necessarily found within every
 486 strut. This effect was particularly clear in scaffolds with small D_S , such as prototype “F” (Figure
 487 4O) and pointed to a certain degree of variability within the same printing process (intra-
 488 process variability). Variability was also observed between different printing processes (inter-
 489 process variability), where scaffolds produced with the same SLS parameters, but at different
 490 times, showed slightly different properties (see upright red triangles with full shape in Figure
 491 3). The maximum recorded inter-process variations of E and D_S were 40% and 7%,
 492 respectively. Such inter- and intra-process variability has been discussed in literature as a
 493 characteristic feature of laser sintering and melting production techniques and it has been
 494 described for their application to different materials, such as semi-crystalline polymers [36] and
 495 metals [37]. The origins of the variability are numerous, but they can be ascribed both to
 496 material-dependent [36] and to process-dependent [36,37] aspects. A detailed discussion on
 497 SLS variability sources, however, is beyond the scope of this work. Despite the SLS intrinsic
 498 variability, relevant observations could be performed on the analysed scaffolds. In fact,
 499 quantification of the molten cross-sectional area showed that scaffolds whose D_S was close to
 500 500 μm generally had A_M ranging from 0.01 ± 0.01 to 0.13 ± 0.01 mm², i.e. 97% to 55% lower
 501 than the design value of 0.29 mm², respectively (Figure 4I).

502 The strut molten cross-sectional diameter D_M was calculated from A_M using equation (1). Even
503 though the original CAD design had a square cross-section (Figure 1), the calculation of D_M
504 as the diameter of a circular cross-section was more appropriate for the observed shape of the
505 molten cross-sectional area (Figure 4J to O). Evaluating the strut diameter from light
506 microscope images is common practice in literature [13,15] but it clearly over-estimates the
507 dimension of the fully molten cross-section represented here by D_M .
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Figure 4: Results of morphological evaluation. A) to F) light microscope pictures. Image labels correspond to prototype names. Scale bars are 800 μm . An example of strut diameter (D_s) measurement is shown in B); G) CAD representation of the cut scaffold. The design reference

513 A_M is highlighted; H) representative SEM image used for A_M quantification. The dotted line
514 marks the A_M of the strut. The scale bar is 200 μm ; I) quantification of A_M of analysed
515 prototypes. The dotted line indicates the reference A_M of the design with value of 0.29 mm^2 ; J)
516 to O) overview SEM images of prototypes “A” to “F”, respectively. The dotted lines mark the
517 A_M of individual struts. Scale bars are 1 mm.

518

519 As the scaffold architecture with inclined and crossing struts leads to a complex deformation
520 behaviour under compression, a straightforward prediction of how the scaffold stiffness would
521 depend on strut diameter was not possible. Thus, the principle relationship between elastic
522 modulus and strut diameter was derived using an *in silico* approach (i.e. via computational
523 analysis) for a later comparison with the experimental results. It is worth mentioning that the *in*
524 *silico* method provided insights into how the scaffold would behave mechanically if all powder
525 was molten into a compact material, representing the ideal situation where D_M equals D_S . The
526 experimentally observed variation of strut diameter resulting from the different SLS parameter
527 sets was represented by six CADs having the same principle geometry as the produced
528 scaffold, but different strut diameters ranging from 310 to 800 μm (exemplarily shown in Figure
529 1D), as motivated by the experimentally observed strut diameters (400–800 μm). These
530 geometries were used to study the theoretical dependency between elastic modulus and strut
531 diameter by finite element (FE) analysis. Each CAD underwent a FE evaluation in
532 compression, from which the computed elastic modulus (E_C) was calculated using equation
533 (2). The relationship between elastic modulus E_C and strut computational diameter D_C was
534 found to follow a quadratic polynomial expressed by equation (3) (Figure 5A).

535

$$536 \quad E_C = 7.2 \cdot 10^{-5} D_C^2 \quad (3)$$

537

538 Where the factor $7.2 \cdot 10^{-5}$ had units of $\text{MPa}/\mu\text{m}^2$. Equation (3) was subsequently used as fit-
539 function for the experimentally derived data. To do so, a pre-factor K was introduced to correct
540 for the unknown elastic modulus of the sintered PCL material, which was an ad-hoc
541 assumption in the *in silico* approach.

542 The *in silico*-derived fit function with a pre-factor $K = 1.05$, as stated in equation (4), matched
543 well the experimental data points when expressing scaffold elastic modulus as function of the
544 molten cross-sectional diameter $E(D_M)$ with $R^2 = 0.9997$ (Figure 5B).

545

$$546 \quad E = K \cdot 7.2 \cdot 10^{-5} \cdot D_M^2 \quad \text{with } K = 1.05(4)$$

547

548 On the contrary, there was a strong disagreement between the *in silico*-predicted dependency
 549 and the analysed dependency between scaffold elastic modulus and the outer strut diameter
 550 $E(D_S)$ indicated by a low value $R^2 = 0.32$. The data could be fitted through the polynomic
 551 function shown in equation (5), but it was not in agreement with the theoretical requirement
 552 that an elastic modulus $E = 0$ would be reached at zero strut diameter. In contrast, $E = 0$ was
 553 already reached at $D_S \approx 400 \mu\text{m}$ (precisely at $D_S = 428 \mu\text{m}$) according to equation (5) (see also
 554 Figure 5B). This roughly corresponds to the resolution limit of the SLS process, which depends
 555 on laser beam diameter and heat conduction.

556

$$557 \quad E = -2.6 - 4.1 \cdot 10^{-2} \cdot D_S + 1.1 \cdot 10^{-4} \cdot D_S^2 \quad (5)$$

558

559 D_M was consistently smaller than D_S (Figure 5C) and none of the analysed prototypes fulfilled
 560 the ideal sintering case, where D_M would be equal to D_S . However, prototypes showed an
 561 asymptotic trend to a line that represented a situation where D_S was larger than D_M by a
 562 constant value of $100 \mu\text{m}$ (grey dotted line in Figure 5C), as described by equation (6):

563

$$564 \quad D_S = e^{6.1+3.9 \cdot 10^{-4} \cdot D_M + 7.8 \cdot 10^{-7} \cdot D_M^2} \quad (6)$$

565

566 For strut diameter $\gg 550 \mu\text{m}$, this might be a constant factor representing a layer of partially
 567 sintered particles with a thickness of $50 \mu\text{m}$ (≈ 1 particle diameter) surrounding the molten
 568 cross-section. However, this layer was found to be over-proportionally thick for strut diameters
 569 $< 550 \mu\text{m}$, as the curve deviated strongly towards larger values of D_S for decreasing D_M ,
 570 approaching $D_S = 428 \mu\text{m}$ for $D_M = 0$ (black fit curve in Figure 5C).

571 To better illustrate how the layer thickness of partially sintered particles increases with
 572 decreasing diameter of the molten cross-section, the ratio D_M/D_S was calculated and plotted
 573 as function of D_M (Figure 5D). In this case, six additional data points were extrapolated by
 574 assuming an offset of $100 \mu\text{m}$ (i.e. two times the thickness of the layer of partially sintered
 575 particles) between D_S and D_M for diameters $\gg 550 \mu\text{m}$, as derived from the observations
 576 reported for Figure 5C. Data point extrapolation was performed to better represent the shape
 577 of the curve D_M/D_S for large values of D_M . Data points were fitted well by the function expressed
 578 in equation (7), which assumed that the curve would asymptotically approach the value of 1
 579 for D_M approaching an infinite value. Moreover, equation (7) considered that $D_M/D_S \approx 0$ if $D_M =$
 580 $0 \mu\text{m}$.

581

$$582 \quad \frac{D_M}{D_S} = \frac{D_M}{D_M + 100} - \frac{0.165}{1 + 9.7 \cdot 10^{-9} \cdot D_M^3} \quad (7)$$

583

584 Generally, the measure of the molten cross-sectional diameter D_M of the sintered scaffolds
585 better explained the observed differences in mechanical stiffness amongst scaffolds with
586 similar strut diameter D_S (see prototypes “C”, “D”, and “E” in Figure 5B). Moreover, D_M better
587 reflected the theoretical relationship between the scaffold mechanical properties and
588 dimensions compared to D_S . A similar observation was reported for the production of lattice
589 structures by selective laser melting from an aluminium alloy, where computational and
590 experimental results matched only if the computational model reproduced the elliptic strut
591 cross-section resulting from the production process, as opposed to the circular cross-section
592 of the CAD [38].

593 Additionally, we observed that the difference between D_M and D_S increased for small strut
594 diameters, while it tended towards a constant value for large strut diameters (Figure 5C). The
595 effect could be clearly visualized by observing the asymptotic behaviour of the data in Figure
596 5C and by expressing the ratio D_M/D_S in function of D_M in Figure 5D. The value $D_M/D_S = 0.8$
597 (molten cross-sectional diameter = 80% of strut diameter) corresponding to $D_M = 590 \mu\text{m}$ was
598 defined to mark the transition between the steep decline of D_M/D_S for $D_M < 550 \mu\text{m}$ and the
599 approximation towards $D_M/D_S = 1$ for values $D_M > 550 \mu\text{m}$. A low value of D_M/D_S indicates that
600 an over proportional amount of powder is only partially sintered and does not contribute in an
601 optimal way to the load-bearing function of the scaffold. This might be a consequence of a
602 reduced heat conduction into the depth of the underlying sintered material, as there is only
603 very little fully fused and compact material beneath the top layer. This compact material,
604 however, can be expected to have a higher heat conductivity than the powder or partially
605 sintered particles and thus would dissipate the heat more effectively. If little compact material
606 is available, more heat might be conducted laterally into the powder bed to partially sinter
607 particles without full melting. This would lead to the comparably large D_S for small D_M observed
608 here.

609 Our observations indicated that the presence of a molten core with sufficiently large cross-
610 section was the key factor in the production of mechanically stable scaffolds by SLS. Therefore,
611 we suggest optimizing SLS parameters to produce mechanically stable scaffolds by evaluating
612 the strut molten cross-sectional diameter, rather than the strut diameter. We observe that the
613 partially sintered particles attached to the molten cross-section have a low contribution to the
614 mechanical competence of the sintered structure as $E(D_M)$ but not $E(D_S)$ is in agreement with
615 the principle dependency $E(D_C)$ predicted by computational analysis. When evaluating the strut
616 diameter D_S only, one might in fact produce scaffolds that have the exact dimensions of the
617 input CAD file, but fail under even moderate mechanical loading as they might be composed
618 of partially sintered particles only. This finding is particularly relevant when producing scaffolds
619 with small strut diameter, high porosity and high mechanical requirements, as it is generally
620 the case in bone TE. For such applications the ratio between the diameter of the molten cross-
621 section and the diameter of the strut should not deviate strongly from the ideal value of
622 $D_M/D_S = 1$. We here recommend $0.8 < D_M/D_S \leq 1$ for the material type (PCL) and particle size
623 (50–80 μm) used in this study.

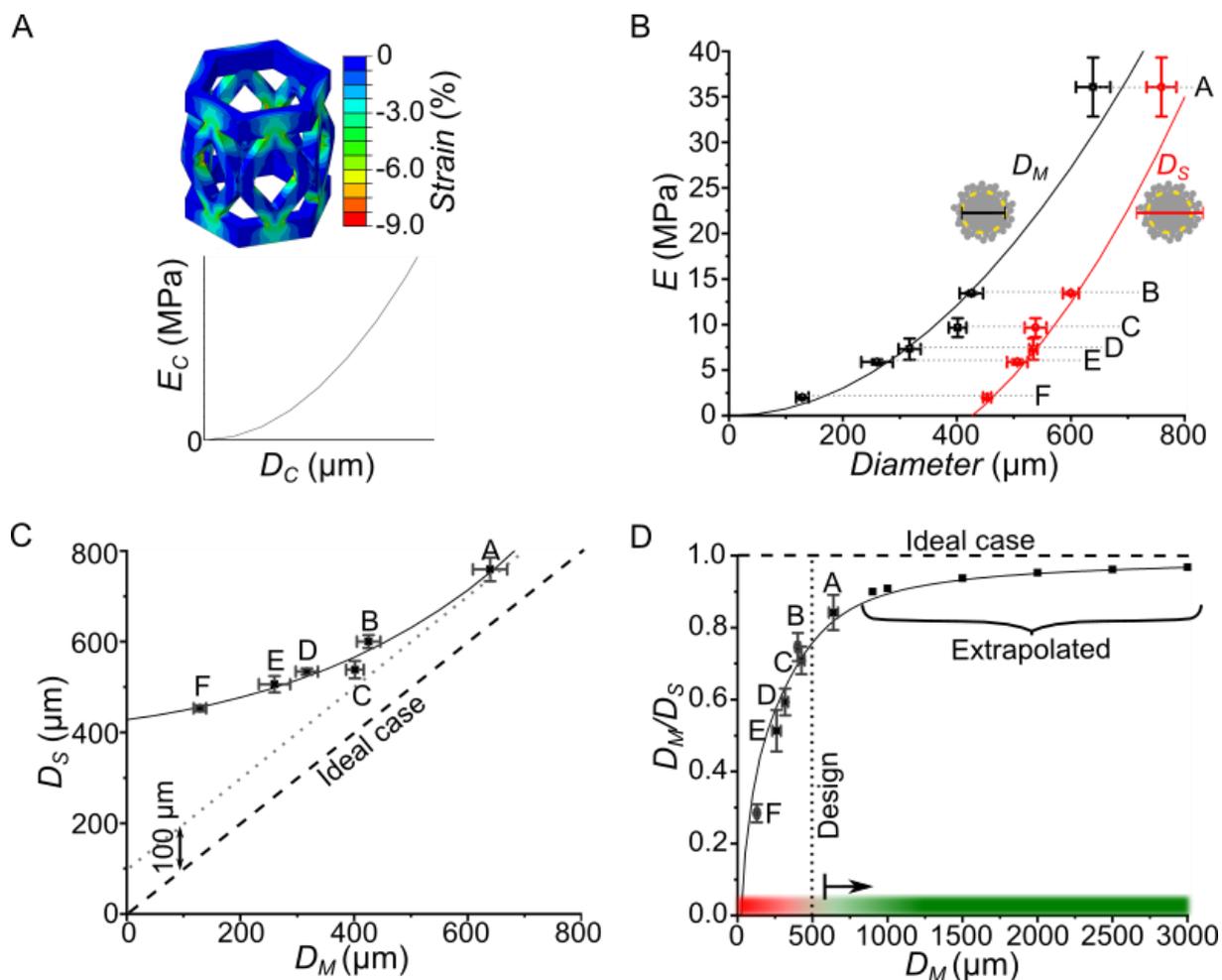
624 The ratio $D_M/D_S = 0.8$ was found to be reached at a molten cross-sectional diameter of
625 $D_M = 590 \mu\text{m}$ and a strut diameter of $D_S = 740 \mu\text{m}$, corresponding to 9.1 and 11.4-times the
626 mean powder particle diameter of 65 μm , respectively (powder particle size distribution: 50–
627 80 μm). The production of struts with a diameter above this value is predicted to be a reliable
628 way to obtain scaffolds with high mechanical stiffness, as it can be deduced by equation (4)
629 and Figure 5B. If the appropriate sterilization method is applied (ethylene oxide), mechanically
630 competent and sterile scaffolds can be produced, for which the dispersity index predicts no
631 significant alterations of the polymer during the process (see Table 3).

632 It has to be mentioned that the threshold diameter of $D_S = 740 \mu\text{m}$ defined in this study cannot
633 be generalized to all SLS applications, as the outcome of the sintering process depends on
634 material type, particle size distribution and shape, sintered object geometry, and SLS
635 parameters [36]. While the principle presented here is expected to be generally valid, the
636 minimum feature size needs to be determined experimentally for each specific application.
637 Altogether, the understanding acquired here will help to improve the production of scaffolds for
638 bone TE by SLS, especially when high porosity, small feature size and optimized mechanical
639 properties are targeted.

640 As an optimal SLS process is strongly material- and design-dependent, we do not intend to
641 offer a final set of optimal parameters for PCL scaffold production, but rather suggest a
642 methodology to find such parameters when employing new materials. As limitations of the
643 approach proposed here, the need for preliminary trial-and-error sintering tests and the
644 destructive method, i.e. SEM imaging, employed to assess the molten cross-section within

645 individual struts should be mentioned. However, samples could be non-invasively evaluated
646 by imaging techniques such as micro-computed tomography.

647 A further limitation of this work is that the scaffolds were tested in the range of elastic
648 deformation only. This did not allow to evaluate their ultimate strength, which is a commonly
649 measured parameter in the characterization of PCL and PCL composite scaffolds produced by
650 SLS (e.g. in [39]). However, the elastic behaviour of the here-investigated scaffold prototypes
651 was regarded most relevant to evaluate the sintering quality in dependency of PCL powders
652 and SLS process parameters. The ultimate strength could be more significantly influenced by
653 the chosen architectural features and local imperfections which were not the primary focus of
654 this work. However, the ultimate strength of the scaffolds should be evaluated prior to
655 implantation *in vivo*.

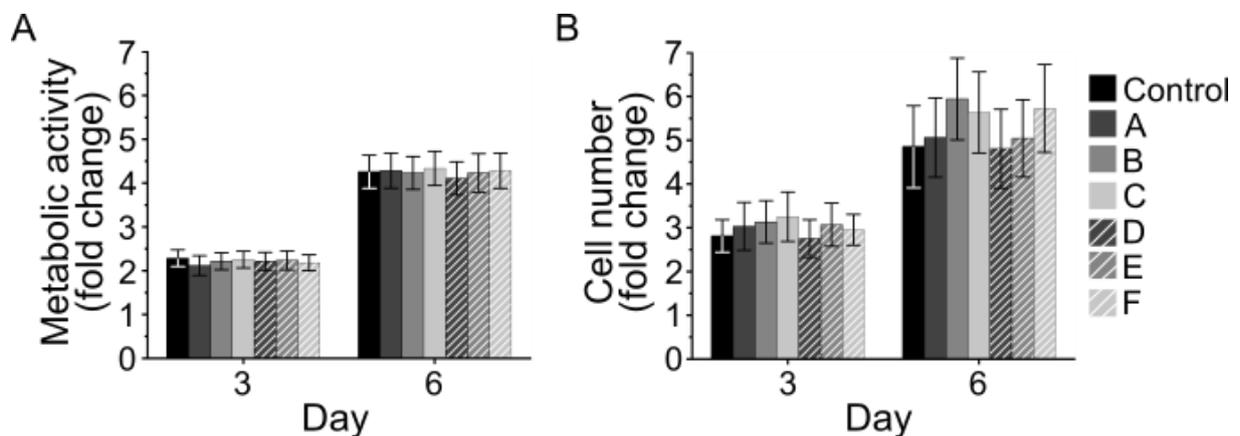


656
 657 Figure 5: Comparison of prototype properties. A) Top: representative strain distribution in the
 658 scaffold as obtained from the FE analysis of the design with $D_C = 500 \mu\text{m}$. Bottom: in silico-
 659 derived relationship between computational elastic modulus (E_C) and computational diameter
 660 (D_C); B) experimental elastic modulus (E) as function of molten cross-sectional diameter (D_M ,
 661 black) and strut diameter (D_S , red). Black and red solid lines represent the polynomial fit of D_M
 662 and D_S , respectively; C) D_S as function of D_M . The dashed black line represents the ideal case,
 663 i.e. $D_S = D_M$. The solid black line shows the asymptotic behaviour of the data, where the
 664 asymptote is the grey dotted line; D) Ratio D_M/D_S as function of D_M . Six additional points were
 665 extrapolated to better show the asymptotic behaviour of the data (solid black line) at large
 666 diameters. The black dashed line indicates the D_M/D_S ratio in the ideal case where D_M equals
 667 D_S . The black dotted line shows the CAD strut diameter. The colour bar highlights areas of
 668 favourable (green) and unfavourable (red) SLS-dependent mechanical stability and the arrow
 669 indicates the minimum recommended strut diameter, corresponding to $D_M/D_S = 0.8$.

670 3.5 Influence of SLS parameters on scaffold cytocompatibility

671 Finally, the six prototypes “A” to “F” (see Table 4) were tested for cytocompatibility by
 672 evaluating cellular metabolic activity and cell proliferation over 3 and 6 days of *in vitro* culture
 673 with human primary mesenchymal stromal cells (hMSCs).

674 For all tested conditions, cellular metabolic activity increased approximately 2.2- and 4.2-fold
675 at day 3 and 6, respectively, when compared to day 0 (Figure 6A). The rise in metabolic activity
676 could be ascribed to the higher number of cells resulting from cellular proliferation over the 6
677 days of *in vitro* cell culture (Figure 6B). At day 3, cell number increased approximately 3-fold
678 for all tested conditions. Data points were more scattered at day 6, with a minimum increase
679 of 4.8 ± 0.9 -fold and a maximum increase of 5.9 ± 0.9 -fold measured for prototypes “D” and
680 “B”, respectively, while cell number increased by 4.9 ± 0.9 -fold in controls. In summary, no
681 impairment of total cell metabolic activity or cell number was observed when exposing hMSCs
682 to medium conditioned by the investigated prototypes in comparison to unconditioned control.
683 This indicates that, even after processing with SLS with a wide range of process parameters,
684 PCL remains cytocompatible.
685 The cyto- and biocompatibility of PCL have long been known [6] and the successful *in vitro*
686 culture of various cell phenotypes in PCL scaffolds has been reported for scaffolds produced
687 by SLS [10] and by other production techniques, such as fused deposition modelling [40].
688 Moreover, the cytocompatibility of the eluate of PCL scaffolds produced by SLS has been
689 previously shown over 3 days of *in vitro* culture with osteoblasts [12], in accordance with the
690 results reported here. To the best of our knowledge, the dependency of the cytocompatibility
691 of PCL scaffolds produced by SLS on the SLS process parameters was not discussed before.
692 Our results indicate that PCL is cytocompatible when the polymer undergoes SLS over a broad
693 range of SLS process parameters.



694
695 *Figure 6: Cytocompatibility of prototypes. A) Cellular metabolic activity; B) Cellular proliferation.*
696 *Results are normalized to day 0 and given as fold change. The legend to read both plots is on*
697 *the right side of the figure. Statistical analyses revealed no significant differences in metabolic*
698 *activity nor in cell number between the control and the six investigated conditions at any time*
699 *point.*

700 **4. Conclusions**

701 Taken together, our data suggest that aiming at dimensional accuracy is not sufficient to
702 determine the ideal SLS parameters for the fabrication of PCL scaffolds for bone TE. We
703 propose to optimize SLS parameters by evaluating both dimensional accuracy and mechanical
704 properties. When considering both factors, we found that multiple sets of SLS parameters were
705 suitable to produce mechanically stable scaffolds. The mechanical stiffness of the scaffold
706 could not be deduced from the outer strut diameter (measured by light microscopy), which is
707 the parameter commonly used for the optimization of process parameters. Rather, the cross-
708 sectional area of fully molten material within the struts (measured by SEM of cryo-cut cross-
709 sections), excluding partially sintered particles, correctly predicted the mechanical stiffness of
710 the scaffolds. Thus, process optimization should aim at maximizing the ratio between molten
711 cross-sectional diameter and strut diameter. This is especially important when producing
712 scaffolds with thin struts, as it is generally the case when a high porosity and a small feature
713 size is required. Dry ice blasting might be used to remove loosely attached particles from the
714 scaffold struts more efficiently compared to air-blasting applied here. In this way, the ratio
715 between molten cross-sectional diameter and strut diameter might be further increased.
716 We identified a critical minimal value of 590 μm for the diameter of the molten cross-section
717 corresponding to 740 μm outer strut diameter, i.e. including the layer of partially sintered
718 particles, for the specific PCL powder and the SLS machine used here. Below this value, a
719 sufficiently large molten cross-sectional area, and thereby scaffold mechanical stability, could
720 not be guaranteed.
721 Independently of mechanical properties, prototypes produced with six different sets of SLS
722 process parameters were found to be fully cytocompatible over 6 days of *in vitro* cell culture
723 with primary human mesenchymal stromal cells, confirming the general suitability of SLS as
724 production technique of PCL scaffolds for bone TE.

725

726 **Acknowledgments**

727 The authors acknowledge funding by the German Federal Ministry of Education and Research
728 (BMBF) via grant numbers 13XP5048D and 13XP5048C. We would like to thank the BIH
729 Center for Regenerative Therapies (BCRT) Core Unit "Cell Harvesting" for providing human
730 mesenchymal stromal cells for this study. Contribution for the PhD student was made possible
731 by DFG funding through the Berlin-Brandenburg School for Regenerative Therapies GSC 203.
732 We thank Prof. Gianluca Ciardelli and Dr. Monica Boffito (Politecnico di Torino) for their support
733 in performing the SEC analysis, Hans Leemhuis (Matricel GmbH) and Andreas Höss (Innotere
734 GmbH) for the helpful discussions and Alessandro Mecconi for reviewing the language.

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