<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Fabrication, mechanical and in vivo performance of polycaprolactone/tricalcium phosphate composite scaffolds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>Lohfeld, Stefan; Cahill, Senan; Barron, Valerie; McHugh, Peter</td>
</tr>
<tr>
<td><strong>Publication Date</strong></td>
<td>2012</td>
</tr>
<tr>
<td><strong>Link to publisher's version</strong></td>
<td><a href="http://dx.doi.org/10.1016/j.actbio.2012.05.018">http://dx.doi.org/10.1016/j.actbio.2012.05.018</a></td>
</tr>
<tr>
<td><strong>Item record</strong></td>
<td><a href="http://hdl.handle.net/10379/3666">http://hdl.handle.net/10379/3666</a></td>
</tr>
<tr>
<td><strong>DOI</strong></td>
<td><a href="http://dx.doi.org/DOI">http://dx.doi.org/DOI</a> 10.1016/j.actbio.2012.05.018</td>
</tr>
</tbody>
</table>

Downloaded 2019-11-03T04:35:31Z

Some rights reserved. For more information, please see the item record link above.
Fabrication, mechanical and in vivo performance of polycaprolactone/tricalciumphosphate composite scaffolds

Stefan Lohfeld*, Senan Cahill¹,², Valerie Barron¹, Peter McHugh¹,², Lutz Dürselen³, Ludwika Kreja³, Christine Bausewein³, Anita Ignatius³

¹ National Centre for Biomedical Engineering Science, National University of Ireland Galway, Galway, Ireland
² Mechanical and Biomedical Engineering, College of Engineering and Informatics, National University of Ireland Galway, Galway, Ireland
³ Institute of Orthopaedic Research and Biomechanics Centre of Musculoskeletal Research, University of Ulm, Ulm, Germany

Abstract

In this paper, the use of the Selective Laser Sintering (SLS) process for the generation of bone tissue engineering scaffolds from PCL and PCL/TCP is explored. Different scaffold designs are generated and are assessed from the point of view of manufacturability, porosity and mechanical performance. Large scaffold specimens are generated, with a preferred design, and are assessed through an in vivo study in a critical size bone defect in the sheep tibia with subsequent microscopic, histological and mechanical evaluation. Further explorations are performed to generate scaffolds with increasing TCP contents.

Scaffold fabrication from PCL and PCL/TCP mixtures with up to 50 mass-% TCP is shown to be possible. With increasing macroporosity the stiffness of the scaffolds is seen to drop, however, the stiffness can be increased by minor geometrical changes, such as the addition of a cage around the scaffold. In the animal study the selected scaffold for implantation did not perform as well as the TCP control in terms of new bone formation and the resulting mechanical performance of the defect area. A possible cause for this is presented.

Keywords


* Corresponding author: Fax: +353 91 49 4596, e-mail: Stefan.Lohfeld@nuigalway.ie

DOI: 10.1016/j.actbio.2012.05.018
1 Introduction

Interest in tissue engineering has grown over the last decade since it offers an alternative approach with great potential for reconstruction or replacement of damaged bone tissues [1-5]. Three of the main elements required to engineer tissue are (i) a scaffold, (ii) cells and (iii) a dynamic environment in which the cell-scaffold construct are conditioned [6-8]. In bone tissue engineering, current techniques employ the use of porous, 3D, biodegradable, biocompatible, and bioresorbable scaffolds, which act as temporary platforms for initial cell attachment and subsequent tissue formation. Biomimetic scaffolds with similar microarchitectures, mechanical and biological properties as native tissue are of significant interest. Some groups established libraries for scaffold or unit cell designs which can be used to build scaffolds with certain mechanical and physical properties to satisfy the needs of the implantation site [2, 9-12]. Limitations of conventional fabrication techniques in producing the desired scaffold design can be overcome by Rapid Manufacturing (RM) techniques, which allow the manufacturing of complex 3D shapes with various pore designs from CAD files [13-16]. One RM technique is selective laser sintering (SLS), which employs a CO₂ laser to melt polymeric powders and form scaffold geometries without the use of any toxic chemicals, blowing agents or support structures [17]. The SLS process was utilised in combinations with above mentioned design libraries to produce scaffolds for cardiac tissue engineering that fulfil certain mechanical needs and efforts have been made to reduce the amount of powder needed even when only small parts are being manufactured [18] for those machines that can facilitate large models and therefore normally use large amounts of powder.

Only a limited number of materials, including composites, have successfully been evaluated thus far using SLS; however, from recent results laser sintering of selected biocompatible materials for tissue engineering has proven promising. Biomaterials considered for tissue engineering scaffold fabricated via SLS include polycaprolactone (PCL) [12, 19-24], polyetheretherketone (PEEK) [17], polylactide acid (PLA) [25] and poly(lactic-co-glycolide) (PLG) [26]. As these materials may not stimulate sufficient bone ingrowth on their own, bioactive components can be added to enhance bone ingrowth, cell attachment, etc. Furthermore, the stiffness of the scaffold may be enhanced with second phase particles, in particular when the scaffold is likely to be loaded after implantation. Hence, scaffolds with optimal properties for bone tissue engineering may consist of two or more phases after the final manufacturing step. Hydroxyapatite (HA), for instance, is known for its bioactive behaviour and osteoconductivity [27] and has been blended with PCL, PE, PEEK, and polyvinylalcohol (PVA) and processed via laser sintering [17, 23, 28, 29]. Wiria et al. [23] reported on the in vitro cell ingrowth of PCL/HA scaffolds fabricated via SLS, however, even though the success of the addition of a bioactive phase is anticipated, the in vivo performance of such composite scaffolds has not been extensively reported. Reports on in vivo testing of PCL based scaffolds fabricated by other rapid prototyping techniques are limited to small animal models [30-36].

In the present study, poly-ε-caprolactone (PCL) and the SLS process were chosen as the scaffold matrix material and manufacturing process of interest, respectively. Scaffolds fabricated from PCL and from PCL combined with Tricalciumphosphate (TCP) were considered. The objectives of the study were: 1) to evaluate the mechanical performance of scaffolds with a range of microarchitectural designs and compositions, and 2) based on the results of this study and the desire to promote bone ingrowth, to select a scaffold for in vivo investigation in an ovine model, and to compare its performance to that of a reference material.
2 Materials and methods

2.1 Scaffolds

Materials

Poly-ε-caprolactone powder (CAPA6506, Solvay, UK) with a molecular weight of 65,000 and a particle size of 600 µm was cryogenically ground and classified into three powder fractions: i) < 50 µm, ii) 50-110 µm and iii) > 110 µm. The fraction > 110 µm appeared too coarse for SLS applications and was of no further interest. β-tricalcium phosphate (Fluka, particle size 3-5 µm) was blended with PCL powder (50-110 µm) to obtain a composite powder with 10 mass-% TCP for extensive investigation of the performance of a PCL/TCP scaffold. Further TCP was blended with PCL (< 50 µm) at ratios with 10, 20, 30, 40, and 50 mass-% TCP.

Scaffold Design and Fabrication

A Sinterstation 2500plus (DTM, USA) was utilised to fabricate the scaffolds. The software “Sinter v3.3” (3D Systems) was used to prepare the builds. Details of the process are given in Lohfeld et al. [37]. To achieve a minimal strut size, the “outline scan” function was used as described in [37]. The SLS process has certain size limitations, as discussed in [37]. The laser systems on commercially available machines like the one used in this study have a focus point diameter optimised for fast processing of the cross sections (large diameter) while providing a sufficient resolution. As such, the system may not be optimal for processing small structures like scaffold for tissue engineering. Furthermore, heat conduction in the powder bed lead to growth on sintered structures. The temperature control for small parts needs to be precise to prevent excessive growth, as 1 °C temperature can already make a visible difference. The temperature sensors of the machine may not provide the desired precision. After removing the scaffolds from the powder bed, trapped powder was removed using pressurised air.

Mechanical properties are an important feature of the manufactured scaffolds, which depend on the design and on the sintering parameters. The latter were optimised to fabricate scaffolds struts that are stable, but thin to achieve a high porosity. However, for a given scaffold geometry, the optimum parameters depended on the design, e.g. the closer the struts are to each other, the lower the laser power level that can be used in order to prevent strut thickening through heat conduction in the powder bed.

Pro/Engineer (PTC, USA) was used to design the scaffolds and to generate STL files (Stereolithography file format, suffix .STL). The design of the scaffold was based on the capabilities of the Sinterstation and on requirements for bone tissue engineering scaffolds. Similar designs are known for scaffolds manufactured via Fused Deposition Modelling [38]. The laser spot diameter of the Sinterstation was 410 µm, hence this was considered the minimal feature size for the scaffolds, i.e. the strut width cannot be smaller than 410 µm. In fact, using PCL material, heat conduction within the powder bed during sintering led to strut widths of at least 450 µm for struts parallel to the x axis of the machine and 700-800 µm for struts parallel to the y axis of the machine [37]. When struts were directed at any angle between these two axes, the strut thickness also had a value between these two extremes.

Based on the above, all struts were designed to have a width of 500 µm, and the layer thickness, i.e. the height of the struts, was also set to 500 µm.

Scaffolds were developed in several phases. In Phase 1, scaffold specimens for compression testing were generated. During this phase the variation of the microstructure (i.e. changing strut distance and direction), the incorporation of a cage and the ways to incorporate
TCP (i.e. immersion in TCP, immersion and subsequent heating, and sintering of a PCL/TCP powder mixture) were investigated.

The scaffolds manufactured during this phase had cylindrical shapes with ~8 mm diameter and ~5 mm height. For the microarchitectural design, four scaffold types were considered (labelled A, B, C and D, see Figure 1). The strut direction for scaffold type A (Figure 1a) was alternated by 90° from layer to layer and a strut centreline distance of 1 mm was applied. The top and bottom layers had additional struts to prevent tilting under compression. The porosity of this CAD design was 42 %. Pore size was defined as the diameter of a channel through the scaffold when looking in the axial direction (Figure 1a, inset). For scaffold type A this diameter was 500 µm. To increase the porosity for scaffold B (Figure 1b), the strut centreline distance was increased to 1.6 mm and the strut direction was changed by 60° from layer to layer to achieve a pore size of 560 µm. The designed porosity of scaffold type B was 68 % (Figure 1b, inset). For scaffold type C (Figure 1c) further increasing the strut centreline distance to 2 mm allowed for a porosity of 78 % and the generation of large and small pore sizes when changing the strut direction by 30° from layer to layer (Figure 1c, inset). Scaffold type D is the same as type C, only that the strut centreline distance was increased to 2.5 mm. This results in a slightly higher porosity of 80 %.

Table 1 summarises the various scaffold designs used for mechanical evaluation.

With higher strut separations the scaffold’s compressive stiffness was found to deteriorate. To address this, a porous cage around the scaffold was introduced which adds paths to support axial compressive forces on the scaffold. The cages were designed individually for the scaffolds type B, C, and D, corresponding to their strut layout to allow radial fluid flow through the scaffold. The type C caged scaffold is shown in Figure 1d.

Three batches of scaffolds were manufactured from pure PCL. One batch was left as produced, and two batches were subsequently immersed in TCP powder. One of these two batches of TCP powder covered scaffolds was heated to approximately 50 °C to soften the PCL and make the TCP particles stick to the surface. Excess TCP was removed by pressurised air following the immersion/immersion+heating procedures. Though immersion in TCP powder had the advantage that the TCP particles sit on the surface of the scaffold, the disadvantage of this procedure was that the amount of TCP in the scaffold remained unknown. Hence, in preparation for the generation of the material to be used in vivo, a further step was taken. A defined amount of TCP was mixed with PCL powder prior to sintering. Using this mixture, which consisted of 90 mass-% PCL and 10 mass-% TCP (97:3 vol-%), a fourth batch of scaffolds was produced. During the sintering process only the PCL was sintered, while the TCP particles were bound to the solidified PCL structure.

In Phase 2, large scaffolds from a composite powder with 90 mass-% PCL and 10 mass-% TCP were manufactured for the in vivo study (Figure 2a). The powder mixture was preferred over the immersion method because it allowed for the quantification of the TCP content. With an outer diameter of 20 mm, a height of 20 mm and a bore of 7.5 mm diameter these scaffolds were designed to replace a section of the sheep’s long bone (tibia). The strut pattern was the $0°/60°/120°$ design of the type B1 scaffolds which proved to have a high porosity while preserving good mechanical properties (see Results section below). It was found that with increasing scaffold diameter, the cage became less important as more contact points within the scaffold provided paths for the compressive load through the scaffold. Hence, the large scaffolds for the in vivo study did not contain a cage. This allowed for an improved fluid flow through the scaffold in radial direction.

In addition to these two phases, in Phase 3 further research was performed to explore the possibility of increasing the TCP percentage in the SLS process. Trials performed prior to the current study produced very brittle scaffolds from PCL particles of a size of 50-100 µm mixed with TCP amounts of 20 mass-% and higher. This was considered to be due to the size
mismatch of the two phases, where TCP settled on the PCL particles and effectively separate them, allowing only poor fusion during sintering. Therefore, for the present investigation, the PCL powder fraction with a particle size of <50 µm was mixed with TCP powder at ratios of 90:10, 80:20, 70:30, 60:40, and 50:50 mass-% (ratios in vol-%: 96:4, 92:8, 88:12, 82:18, and 75:25). One batch of scaffolds was manufactured using solely the PCL powder fraction mentioned. These scaffolds, however, were not part of the mechanical tests because of their purpose to mainly investigate laser sintering feasibility of high TCP content composites and because of their late production during the study period.

**Mechanical Testing**

To compute the effective E-modulus, compression tests of small scaffold specimens manufactured in Phase 1 (~8 mm x ~5 mm cylinders) were carried out on a materials testing machine (Z2.5, Zwick, Ulm, Germany) with a 1 kN load cell. Eight samples per scaffold type were tested at a compression rate of 1 mm/min.

For the large PCL/TCP scaffolds from Phase 2 with a diameter of 20 mm, the compression tests were performed on a Zwick Z010 material testing machine (Zwick, Ulm, Germany). The samples were preloaded with 0.5 N and the sample height under preload was registered. Subsequently, a compression rate of 5 mm/min was applied. Sample heights under preload and during compression were measured using a high precision digital displacement transducer. To ensure loading perpendicular to the surface of the scaffold, a metal ball was placed between the loading cell and the plate in contact with scaffold.

**Microscopy**

SEM investigations were performed using a Hitachi S-4700 SEM (Hitachi, UK), while μCT (micro scale computed tomography) scans were conducted on a Skyscan 1072 μCT-scanner (Aarstseelaar, Belgium). μCT scans of PCL scaffolds were used for porosity calculations of the manufactured scaffolds in order to get the overall porosity of the scaffolds and compare this to the calculated porosity of the CAD models. For this purpose, the scans were imported into MIMICS (Materialise, Belgium). Two layers usually from the centre of each scaffold were selected and an STL 3D model was generated using the quality setting “medium”. With better quality settings or more scaffold layers the file size of the STL models became too large to handle. The volume of the 3D model was compared against that of a cylinder that enclosed the 3D model, i.e. being of the same overall diameter and height. To reveal the microporosity of the sintered struts of the scaffolds, single struts from each scaffold were selected and an STL 3D model was generated using the quality setting “optimal” (Figure 3a). These models were then imported into the software 3-matic (Materialise, Belgium) and cropped to a width of 0.5 mm, which is the designed width, and a length of 5 mm (Figure 3b and c). The frame to crop the models was placed towards the centre of the struts. The volume of the strut within the volume of interest with dimensions of 5 mm x 0.5 mm x strut model height was used to calculate the microporosity.

**2.2 In vivo functionality test**

In the in vivo functionality test, the performance of PCL/TCP scaffolds from Phase 2 as described above were compared against a reference material (Figure 2b), which was a β-tricalciumphosphate implant (β-TCP; Biovision, Illmenau, Germany), which was manufactured by cold pressing of β-TCP granules into the cylindrical shape (diameter: 20 mm, height: 20 mm), thermal sintering and then milling of the inner bore (diameter: 10 mm). Total porosity was 55-60 % with a micropore size of 5 µm and a macropore size up to 1 mm.
Animal study

The animal experiment was conducted following national regulations for the care and use of laboratory animals and approved by German Government (Regierungspräsidium Tübingen, no 898). 16 female mountain sheep with an age of 2 – 6 years and weighing 60 kg (41 – 83 kg) were randomly distributed to the PCL/TCP (n = 8) and the β-TCP groups (reference, n = 8). The surgery was performed under general isoflurane anaesthesia and premedication with 0,04 mg/kg Atropin (Atropinsulfat, Braun 0,5 mg®, Braun Melsungen, Germany), 1,2 mg/kg Xylacin (Rompun® 2%, Bayer, Germany) and thiopental (Trapanal® 2,5g Byk Gulden, Germany). A critical size defect of 20 mm of full thickness in the middle of the diaphysis of the right tibia was created with an oscillating saw (Synthes, Oberdorf, Switzerland). The defect was stabilized by a six-hole interlocking titanium plate (Litos, Hamburg, Germany) at the medial side of the tibia. Hollow cylindrical shaped PCL/TCP implants and β-TCP implants (Figure 2) were placed into the defect. Immediately post surgery, the animals received an additional external fibreglass-cast to prevent fractures and screw breakage during the complete implantation period. The fluorochrome markers calcein green (10 mg/kg, Calcein-Grün, Synopharm, Germany) and tetracycline (25 mg/kg, Tetracyclinhydrochlorid, Caelo, Caesar und Loretz GmbH, Hilden, Germany) were injected intravenously after 4 weeks and after 8 weeks respectively to label newly formed bone in the defect. The animals were sacrificed after an implantation period of 14 weeks. The operated and the intact contra-lateral tibiae were removed.

Conventional x-rays were performed post-surgery and in week 2, 4, 6, 8 and 10. Additional x-rays of the explanted tibiae were performed post mortem in week 14. Newly formed bone and callus formation in the defect area characterised qualitatively and the tibiae were controlled for possible bone fracture and screw breakage.

Mechanical Testing

A non-destructive mechanical four-point bending test was performed on explanted tibial specimens to evaluate the bending stiffness of the defect area in comparison to the intact contra-lateral tibiae (Z010, Zwick, Ulm, Germany). Load was applied by two load supports (distance 40 mm) at a deflection rate of 6 mm/min (intact tibiae: 2 mm/min) up to a maximum force of 40 N. The bones were incrementally rotated 30° in longitudinal axis. This protocol was repeated to determine bending stiffness in 12 loading directions.

Microscopy, Tomography and Histology

The apparent bone mineral density of the central part of the tibia diaphysis including the defect zone was evaluated with a peripheral quantitative computed tomography scanner (pQ-CT) (XCT 960A, Stratec, Pforzheim, Germany). Five measurements were performed in five sagittal planes through each defect. The bone specimens were also imaged with a μCT scanner (Fan Beam μ-Scope, Stratec, Pforzheim, Germany) at 70 μm spatial resolution. The data were analysed with an image analysis software (VGStudioMax, 1.0, Heidelberg, Germany) to calculate the volume of newly formed bone in the defect. The evaluation of the pQ- and μCT-data was only performed in the PCL/TCP group and in the defect that was left empty. It was not possible to differentiate the defects filled with a β-TCP implant because this material has the same density and grey values as newly formed bone.

For undecalcified histology the bone specimens were embedded in methacrylate. 100 μm slices were prepared in the longitudinal direction of the tibiae. The surface was stained by Paragon. Conventional light microscopy (Axiophot, Zeiss, Oberkochen, Germany) was used to evaluate the formation of new tissue in the defect zone. The relative distribution of
remaining material, bone, soft tissue and cartilage was measured. A point counting method was used with an eyepiece reticle at 50-fold magnification. Two sections were evaluated for each bone defect. The slides were also inspected under fluorescent microscopy (Axiophot, Zeiss, Oberkochen, Germany) to examine the infiltration of the defect with newly formed bone that was formed after 4, 8 and 14 weeks.

Statistics

The data from the animal study were presented as median, minimum and maximum values. To determine significances between the PCL/TCP and the β-TCP group the Wilcoxon-Test for unpaired samples was used. The level of significance was set at p < 0.05.

3 Results

3.1 Manufacturing

For Phase 1, scaffolds were successfully manufactured from pure PCL (batches 1-3) and a PCL/TCP powder mixture (batch 4). Batches 2 and 3 were immersed in TCP powder after production. Batch 3 was taken one step further and was heated, while immersed in the TCP powder, to approx. 50 °C. SEM investigations proved that even after removing excessive TCP from the immersed PCL scaffolds using pressurised air, a substantial amount of TCP particles could be found on the struts of the scaffolds (Figure 4). The exact amount was seen to vary for each scaffold but this was not investigated in detail. Due to the nature of the powder based manufacturing process, partially molten particles stick to the struts, giving them a rough surface. μCT scans were used to generate models of a number of the scaffolds and of single struts of a PCL scaffold. An investigation of these models regarding porosity revealed porosities as listed in Table 1. While for most scaffolds the calculated and actually measured porosities were similar (scaffolds B1, C1, C2, D2), there was a discrepancy for others (A, B2). Focussing on the B and C type scaffolds, where caged and uncaged versions were fabricated, the effect of the cage was not the same for both types. In B there was a considerable difference between the expected and actual porosity change; it was much greater than expected (69% vs. 51%). For type C, the change was closer to what was expected (77% vs. 70%)

The microporosity was calculated from selected struts as shown in Figure 3a, which were then cropped as described above. The struts were selected from several layers of each scaffold. Calculated microporosities are given in Table 2. No significant differences were observed between struts from scaffolds of different designs, apart from scaffold D2, where the microporosity is about 10 % higher than in the other scaffolds.

In Phase 2, the scaffolds for the in vivo study were manufactured based on the process settings found for batch 4 above.

For the investigations on high TCP content scaffolds in Phase 3, composite scaffolds with up to 50 mass-% were successfully produced using the PCL powder fraction of < 50 μm. During the manufacturing process the powder flow was poor when low TCP contents were used, causing a rough and porous powder bed and holes in the sintered struts (Figure 5). This PCL powder fraction seemed unsuitable for zero or low TCP content scaffold production. With increasing TCP amounts the powder flow improved and good, although still porous, scaffold struts were achieved at higher TCP amounts ≥ 20 %. Laser power settings had to be adjusted for each mixture, and ranged between 5 and 9 W, with the higher settings used when more TCP was present.
3.2 Mechanical properties of the scaffolds

Compressive mechanical tests were performed to determine the effective moduli. The effective modulus is defined as the effective nominal stress (which is the force divided by the cross-sectional area of a cylinder that encloses the scaffold) divided by the nominal compressive strain. Effective moduli for a range of scaffold designs and compositions are shown in Figure 6.

**PCL scaffolds and composite scaffolds fabricated from PCL and immersed in TCP**

The Phase 1 scaffolds type B1 and C1 with porosities of 68% and 78%, respectively, had lower effective moduli than scaffold type A (porosity 42%). For type B1 the effective moduli approximately ranged from 1.0 to 1.4 MPa, for type C1 from 0.06 and 1.02 MPa, and for type A between 2.0 and 3.0 MPa. Type B2 scaffolds with a cage had moduli between 6.6 and 8.7 MPa, whereas the increase caused by the cage was not as significant for the type C2 scaffold with effective moduli between 0.07 and 0.45 MPa. Actually, from Figure 6, the modulus was seen to decrease for the immersed and heated scaffold of type C2.

**Large scaffolds**

The 20 mm scaffolds for sheep tibia implantation, which were fabricated in Phase 2 directly from a PCL/TCP powder mixture with 10 mass-% TCP, had an effective modulus of 6.0 MPa, despite the fact that this scaffold did not have a cage to support the stiffness (Figure 6).

3.3 Animal study

One animal of the PCL/TCP-group died of aspiratory pneumonia 1 day post-surgery. All other animals completed the study without any complications. The x-rays showed the formation of mineralized callus mainly by external bridging in the β-TCP group in 5 of 8 animals (Figure 7a). A weaker mineralized callus formation was seen in the PCL/TCP group (2 of 7 animals, Figure 7b).

All operated tibiae revealed a significantly lower bending stiffness compared to the intact tibia from the contra-lateral leg indicating that the healing was not fully completed in all groups. In comparison to the PCL/TCP group (1.2 ± 2.5 Nm²) the reference group (β-TCP; 7.9 ± 7 Nm²) showed a higher bending stiffness (Figure 8). The intact bones had a six times higher stiffness (46 ± 3.6 Nm²) compared to the reference group (β-TCP).

There seemed to be less bone volume in the defect area in the PCL/TCP group (median 7 %) than in the defect that was filled with the reference material (β-TCP) (Figure 9). However, a proper evaluation of the volume of newly formed bone in the reference group (β-TCP) was not possible because bone and the remaining material had very similar grey levels in the µ-CT. The mineral density in the defect area was 70 mg/cm³ on average for the PCL/TCP implants. This was a significantly lower mineral density compared to the intact contra-lateral tibiae (median 1388 mg/cm³). It was not possible to measure the mineral density in the defects of the reference group (β-TCP), because bone and the material had the same density.

The qualitative histological evaluation showed a moderate inflammatory reaction with macrophages and foreign body cells in the PCL/TCP group. Hardly any inflammatory reaction was found in the reference group (β-TCP, Figure 10). Cartilage was found in three defects of the PCL/TCP group and in five defects of the reference group (β-TCP). Six sheep of the PCL/TCP group showed bone formation in the medullary cavity and seven sheep in the reference group (β-TCP). The space between the cortical osteotomy margins was not completely bridged in any of the animals. The lateral space between the cortical margins of
the osteotomy contained the most mineralized bone in the β-TCP group. Along the osteotomy margins both resorption and new bone formation could be observed and moderate remodelling was seen in the adjoining cortical bone (Figure 10). The gap between the osteotomized cortices was filled with scaffold material and connective tissue and with newly formed bone in all sheep. In the quantitative histological evaluation the relative amount of newly formed bone, cartilage and soft tissue varied between the different groups. Most newly formed bone was found in the reference group (β-TCP; median 39 %). The relative amount of newly formed bone was significant lower in the PCL/TCP group compared to the reference group (β-TCP) (median 11 %). There were no significant differences in the relative amount of soft tissue between the groups (Figure 11). There was significant more residual implant material found in the PCL-TCP group (median 34 %) compared to the reference group (β-TCP; median 14 %) after 14 weeks. Under fluorescent light it was apparent that only very low amounts of new bone had formed after 4 weeks in all groups. There were large amounts of new bone formed after 8 weeks in the medial defect area in the lateral defect area of the reference group (β-TCP) and very low amounts of new formed bone in the PCL/TCP group (Figure 12).

4 Discussion

In overall terms for this study, it has been shown that it is possible to fabricate scaffolds from PCL and PCL/TCP mixtures. With increasing porosity the stiffness of the scaffolds was seen to drop, however, geometrical changes significantly added to the stiffness of the small scale scaffolds (diameter 8 mm). Tricalciumphosphate was added as a second phase and composite scaffolds were successfully fabricated with various PCL/TCP ratios. In the animal study the selected scaffold for implantation did not perform as well as the TCP control, which may be due to the relatively low TCP content used in the scaffolds for the animal study.

As regards to the macroscale porosity of the scaffolds, discrepancies between CAD models and fabricated models were observed in a number of cases (in particular scaffold types A and B2). Also, the effect of including the cage on porosity was significantly different to what was expected for type B. Focussing on the discrepancies for the type B scaffolds, these can be attributed to accentuated growth of the sintered areas by surrounding heat in the powder bed. This is magnified the more strut overlaps there are in the structure, which is much more the case for type B in comparison to types C and D. Without the cage, porosities of CAD and fabricated scaffolds were quite similar, except for design A, where the fabricated model had a significantly higher porosity than the CAD model. The reason for this remains unclear, but may be due to overall more porous sintering results caused by temperature differences in the powder bed.

Concerning the microporosity analysis, the higher microporosity of scaffold type D2 in comparison to all other scaffolds could be due to the large gap between neighbouring struts, which minimised the influence from the sintering of adjacent struts. A difference in the microporosity between the lower and higher layers of the scaffolds is obvious for all scaffolds (Table 2), where the struts in the upper region of the scaffolds have a lower porosity. This can be attributed to the increasing temperature in the build chamber of the machine and in the powder bed during the build.

In terms of mechanical properties, the small scaffolds B1 and C1 appeared less stiff than type A because their strut layers have less contact points with the adjacent layers due to the higher inter-strut distance and hence the reduced number of struts per layer. The effective modulus is based on the cross sectional area of the scaffold as if it is solid. In fact, the load is transferred through the scaffold only at the contact points of the scaffold struts, which are
fewer with increasing inter-strut distance. Overall, the addition of a cage around the scaffold added to the effective modulus, as it provides a continuous loading path from top to bottom of the scaffold and takes a significant amount of the load. For scaffold type C2, the cage did not add significantly to the stiffness, because it could not properly take up the load, which was due to its porous design which did not allow for a strong continuous load carrying path in comparison to the cage for scaffold type B2 for example. In Figure 6 it can be seen that the TCP immersion tends to result in a slightly higher effective modulus, further increased by heating of the scaffold before the excess TCP is removed. The TCP particles settle in the porous struts, filling the pores, and thereby contributing to the support of the compressive load. The heating of the immersed scaffolds improved the binding of the TCP particles to the PCL, so during the removal of excess material more TCP adhered to the structure, generating a stiffer scaffold.

Even without a cage, the large scaffolds had an effective modulus comparable to that of the small scaffolds with cage. A possible explanation of this is that in case of the large scaffolds the TCP was incorporated into the structure. This could have added to the stiffness of the scaffold. More likely, however, is that the sintering was better when manufacturing the large scaffolds. These specimens were manufactured in a different batch than the small scaffolds, and sintering results were not 100% consistent due to powder aging, temperature readings, etc., and considering the low temperature PCL was sintered at, a temperature difference as little as 1°C can have a significant influence. The PCL preheat temperatures for sintering (set for the system used to approximately around 40°C) are at the lower range of operating temperatures for the SLS system used. It may be that a pure PCL scaffold from the same batch would have had the same modulus. Unfortunately it is not possible to produce scaffolds with different material compositions within one build with the current layout of the SLS machine.

In summary, the mechanical tests proved that the addition of a cage around the scaffolds significantly improved the stiffness especially of small scaffolds. The stiffness is very sensitive to the strut layout and scaffold size due to the number of overlaps. Bigger scaffolds, as the one used for the in vivo tests, appear stiffer even without the cage. The size limitations of the SLS process as used for this research indicated better usability for larger scaffolds, e.g. for long bone applications. Higher porosities, if required, are only possible by reducing the number of struts, which would make the scaffolds less stiff, unless a cage is added, which in turn again reduces the porosity. However, as the diameter of the scaffold increases, this effect will become negligible. Other methods of improving the stiffness are the introduction of pillars within the scaffold, which depending on their diameter, may have a smaller effect on the overall porosity.

Within Phase 1 it was found that type B was the best scaffold design, as it provided high porosity combined with good stiffness. The stiffness was significantly improved when adding the cage. For these small scaffolds in load bearing environments scaffold type B2 is superior over all other scaffolds investigated. When compared with the scaffolds of Phase 2, the compression tests showed that the effect of the cage is compensated when the scaffold size increases. In Phase 2, scaffold B1 had a similar stiffness to scaffold type B2 in Phase 1. Although not being investigated, it is anticipated that the cage’s effect on the stiffness is less dominant for large scaffolds as manufactured in Phase 2 of the research, simply because the load is distributed over more contact points within the scaffold already. Without the cage the radial flow through the scaffold is improved. Hence, for large scaffolds, the cage is not needed.

In Phase 3 of the research it was shown that the production of PCL scaffolds with up to 50 mass-% TCP via selective laser sintering is possible, however, the sizes of PCL and
TCP particles should not differ too much. Small TCP particles will settle on the PCL particle surface, impeding proper sintering.

In the in vivo investigations the PCL/TCP composite scaffold showed inferior behaviour compared to the reference material (β-TCP) in a critical size defect [39] regarding promotion of bone regeneration, scaffold degradation and inflammatory reaction. Several studies showed via FTIR analyses that PCL did not degrade during processing via SLS [23, 40, 41] and hence, the cause for the inflammation remained unclear. Due to the lower level of newly formed bone, PCL/TCP led to less biomechanical strength of the repaired bone, too. However, it was proven that in principle the laser sintered PCL/TCP composite worked in vivo, and this approach has advantages such as freedom of design and porosity control, and a tougher final material behaviour compared to the brittle nature of β-TCP, etc. The tested composite only had 10 mass-% of TCP, which converts into about 3 vol-%, part of which sits within the struts where it was consequently less effective at promoting bone ingrowth unless the PCL already degraded. Higher contents of TCP may enhance bone ingrowth. 50 mass-% of TCP in the compound equals about 25 vol-%, and would provide a higher surface area of TCP to promote bone formation and ingrowth. This may be further investigated in future research. As shown previously, additions like the cage or pillars could improve mechanical properties, if required. TCP particles at the same size or bigger than the PCL particles may also have a beneficial effect on a high TCP compound's capability of being sintered and also on bone ingrowth into the scaffold.

5 Conclusion

In conclusion PCL/TCP composites that have potential for use in long bone can be manufactured via selective laser sintering. This study demonstrated that the current PCL/TCP composite with 10 mass-% had only limited success in promoting bone regeneration in critical size defects with a low regenerative capacity and did not appear to be superior in comparison to the reference material (β-TCP). However, composites with at least up to 50 mass-% TCP are possible. Based on the osteoconductive properties of TCP [42-44] and its enhancement of the mechanical properties of porous polymer constructs [45], it is suggested that changes to the material composition, in particular the successful inclusion of high TCP content in the composite in the final phase of the study, have potential to improve new bone formation for future usage of these materials. Further improvements can potentially be achieved by using TCP particles matching the size of the PCL particles, and an improved cleaning procedure to remove any loose particles.

The microporosity investigations performed during the present research can help in generating accurate computational models (e.g. finite element models) for the simulation of the mechanical performance of laser sintered scaffolds. The idealised CAD models are inappropriate, as it has been shown in Cahill et al. [46], as they do not take the roughness and microporosity into account.

6 Acknowledgments

The authors acknowledge research funding from the European Union through the STEPS FP6 project (contract number FP6-500465, www.stepproject.com)) and also the partners in the STEPS project for their input.
7 References


Figure captions

Figure 1:
Scaffold designs with a) 50 %, b) 68 %, and c) 78 % porosity, d) scaffold c) with cage. The scaffold pore structure is illustrated in the inset images in a), b), and c).

Figure 2:
Implants in hollow cylindrical shape for the in vivo study. a) PCL/TCP composite, b) β-TCP.

Figure 3:
a) Model of a single strut generated from µCT scanning. b) Cropping frame placed on strut. c) Cropped strut used for microporosity calculations.

Figure 4:
SEM image of PCL scaffold immersed in TCP and heated after removing excessive TCP powder using pressurised air.

Figure 5:
PCL/TCP composite scaffolds with 0% to 50% TCP.

Figure 6:
Effective moduli of PCL and PCL/TCP composite scaffolds. Error bars indicate standard deviation.

Figure 7:
X-rays of the operated tibia, one example for each group post mortem. a) β-TCP, b) PCL/TCP. Mineralized external callus could be observed in the β-TCP group and in the PCL/TCP group.

Figure 8:
The bending stiffness in Nm2 measured by the 4-point-bending test of a) β-TCP (n=8) and b) PCL/TCP (n=7). The bones were rotated 30° around the longitudinal axis and the protocol was repeated to determine bending rigidity in 12 loading directions.

Figure 9:
Three-dimensional image of a β-TCP (a) and a PCL/TCP (b) treated defect by the μ-CT scanner at 70 μm spatial resolution. The right side (lateral) shows the external callus formation, at the left side (medial) the plate was located.
**Figure 10:**
a) Macroscopic histological pictures of a longitudinal section through the bone defect, example for group 3 (β-TCP). b) Macroscopic histological pictures of a longitudinal section through the bone defect, example for group 1 (PCL/TCP). c) Direct contact between newly formed bone and remaining material (β-TCP). 100-fold magnification. d) Partial direct contact between new bone and remaining material (PCL/TCP). 100-fold magnification. e) β-TCP with good vascularization (arrows) and without any inflammatory cells in the pores of the implant; f) PCL/TCP surrounded by foreign body giant cells (arrows). S = Scaffold in the defect; M = remaining material; CB = cortical bone; NB = newly formed bone; C = Callus; MC = Medullary cavity.

**Figure 11:**
Relative amount (median, 25 % and 75 % quartiles) of implant material (M), bone (B), soft tissue (ST) and cartilage (C) in the defect in % of β-TCP (n=8) and PCL/TCP (n=7).
* significant difference in comparison to the reference material (β-TCP) (p < 0.05)

**Figure 12:**
Infiltration of newly formed bone into the defect at the proximal-medial, proximal-lateral, distal-medial and the distal-lateral edge of the osteotomy after 4, 8 and 14 weeks.
* significant difference in comparison to the reference material (β-TCP) (p < 0.05)
Tables

Table 1: Scaffold designs for mechanical evaluation and comparison of porosities of CAD models vs. fabricated scaffolds

<table>
<thead>
<tr>
<th>Design</th>
<th>Pattern of struts</th>
<th>Distance between strut centrelines</th>
<th>Cage</th>
<th>Porosity of CAD design</th>
<th>Porosity of fabricated model</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0°/90°</td>
<td>1.0 mm</td>
<td>No</td>
<td>42 %</td>
<td>51 %</td>
</tr>
<tr>
<td>B1</td>
<td>0°/60°/120°</td>
<td>1.6 mm</td>
<td>No</td>
<td>68 %</td>
<td>69 %</td>
</tr>
<tr>
<td>B2</td>
<td>0°/60°/120°</td>
<td>1.6 mm</td>
<td>Yes</td>
<td>59 %</td>
<td>51 %</td>
</tr>
<tr>
<td>C1</td>
<td>0°/30°/60°/90°/120°</td>
<td>2.0 mm</td>
<td>No</td>
<td>78 %</td>
<td>77 %</td>
</tr>
<tr>
<td>C2</td>
<td>0°/30°/60°/90°/120°</td>
<td>2.0 mm</td>
<td>Yes</td>
<td>67 %</td>
<td>70 %</td>
</tr>
<tr>
<td>D1</td>
<td>0°/30°/60°/90°/120°</td>
<td>2.5 mm</td>
<td>No</td>
<td>80 %</td>
<td>-</td>
</tr>
<tr>
<td>D2</td>
<td>0°/30°/60°/90°/120°</td>
<td>2.5 mm</td>
<td>Yes</td>
<td>70 %</td>
<td>69 %</td>
</tr>
</tbody>
</table>

Table 2: Microporosities as calculated from single struts

<table>
<thead>
<tr>
<th>Design</th>
<th>Microporosity</th>
<th>Microporosity in upper struts</th>
<th>Microporosity in lower struts</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>31 %</td>
<td>26 %</td>
<td>38 %</td>
</tr>
<tr>
<td>B1</td>
<td>29 %</td>
<td>19 %</td>
<td>38 %</td>
</tr>
<tr>
<td>B2</td>
<td>31 %</td>
<td>29 %</td>
<td>34 %</td>
</tr>
<tr>
<td>C1</td>
<td>28 %</td>
<td>19 %</td>
<td>36 %</td>
</tr>
<tr>
<td>C2</td>
<td>29 %</td>
<td>23 %</td>
<td>37 %</td>
</tr>
<tr>
<td>D2</td>
<td>40 %</td>
<td>30 %</td>
<td>50 %</td>
</tr>
</tbody>
</table>
Figures

Figure 1: Scaffold designs with a) 50 %, b) 68 %, and c) 78 % porosity, d) scaffold c) with cage. The scaffold pore structure is illustrated in the inset images in a), b) and c).
Figure 2: Implants in hollow cylindrical shape for the in vivo study. a) PCL/TCP composite, b) β-TCP.
Figure 3: a) Model of a single strut generated from µCT scanning. b) Cropping frame placed on strut. c) Cropped strut used for microporosity calculations.
Figure 4: SEM image of PCL scaffold immersed in TCP and heated after removing excessive TCP powder using pressurised air.
Figure 5: PCL/TCP composite scaffolds with 0% to 50% TCP.
Figure 6: Effective moduli of PCL and PCL/TCP composite scaffolds. Error bars indicate standard deviation.
Figure 7: X-rays of the operated tibia, one example for each group post mortem. a) β-TCP, b) PCL/TCP. Mineralized external callus could be observed in the βTCP group and in the PCL/TCP group.
Figure 8: The bending stiffness in Nm² measured by the 4-point-bending test of a) β-TCP (n=8) and b) PCL/TCP (n=7). The bones were rotated 30° around the longitudinal axis and the protocol was repeated to determine bending rigidity in 12 loading directions.
Figure 9: Three-dimensional image of a β-TCP (a) and a PCL/TCP (b) treated defect by the μ-CT scanner at 70 μm spatial resolution. The right side (lateral) shows the external callus formation, at the left side (medial) the plate was located.
Figure 10: a) Macroscopic histological pictures of a longitudinal section through the bone defect, example for group 3 (β-TCP). b) Macroscopic histological pictures of a longitudinal section through the bone defect, example for group 1 (PCL/TCP). c) Direct contact between newly formed bone and remaining material (β-TCP). 100-fold magnification. d) Partial direct contact between new bone and remaining material (PCL/TCP). 100-fold magnification. e) β-TCP with good vascularization (arrows) and without any inflammatory cells in the pores of the implant; f) PCL/TCP surrounded by foreign body giant cells (arrows). S = Scaffold in the defect; M = remaining material; CB = cortical bone; NB = newly formed bone; C = Callus; MC = Medullary cavity.
Figure 11: Relative amount (median, 25 % and 75 % quartiles) of implant material (M), bone (B), soft tissue (ST) and cartilage (C) in the defect in % of β-TCP (n=8) and PCL/TCP (n=7).

* significant difference in comparison to the reference material (β-TCP) (p < 0.05)
Figure 12: Infiltration of newly formed bone into the defect at the proximal-medial, proximal-lateral, distal-medial and the distal-lateral edge of the osteotomy after 4, 8 and 14 weeks.

* significant difference in comparison to the reference material ($\beta$-TCP) ($p < 0.05$)