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Additive Manufacturing of Bioactive

Poly(trimethylene carbonate)/ β -tricalcium phosphate

Composites for Bone Regeneration

*Kasper E.G. Dienel, Bas van Bochove and Jukka V. Seppälä**

Department of Chemical and Metallurgical Engineering, Aalto University, Espoo, Finland

Keywords: bioactive composite, β -tricalcium phosphate, poly(trimethylene carbonate), additive manufacturing, stereolithography, bone regeneration

ABSTRACT

Implants of bioresorbable materials combined with osteoconductive calcium phosphate ceramics show promising results to replace and repair damaged bone tissue. Here we present additive manufacturing of patient-specific porous scaffolds of poly(trimethylene carbonate) (PTMC) including high amounts of β -tricalcium phosphate (β -TCP). Tensile testing of composite networks showed that addition of β -tricalcium phosphate reinforces the composites significantly. 3D structures containing up to 60wt% β -TCP could be built by stereolithography. By lowering the content to 51wt%, manufacturing of a large-sized patient-specific prototype was possible at high resolution. Closer examination revealed that the created scaffolds contained more β -TCP on the surface of the builds. Stereolithography therefore provides a manufacturing technique where the

bioactive agent is directly available for creating an enhanced microenvironment for cell growth. The biocompatibility and bioresorption of PTMC coupled with the osteoconductivity of β -TCP are an important candidate to consider in additive manufacturing of bone regeneration implants.

1. INTRODUCTION

There is a major clinical need to replace and regenerate bone tissue due to trauma or disease. For instance, in craniomaxillofacial surgery there is a demand for patient specific implants that replace the bone until new tissue is generated. Standard procedures in bone regeneration including autografts and allografts encounter problems regarding e.g. donor-site morbidity and long rehabilitation times ¹. Synthetic bone substitutes have the potential of solving these problems. By using bioresorbable materials, an implant can be engineered to replace the damaged tissue as long as needed. Eventually, the implant slowly degrades while being replaced by bone tissue. Additive manufacturing (AM) offers an engineering route to achieve individualized implants based on imaging data. In AM a computer-controlled device unifies material, thereby forming a three-dimensional object defined by a computer-aided design (CAD) model.

In bone grafting, utilization of synthetic biomaterials in composites with bioceramic β -tricalcium phosphate (β -TCP) show promising results due to their osteoconductive properties. Calcium phosphates are a major component of natural bone, whereas β -TCP is a phase pure synthetic, resorbable and osteoconductive ceramic that is widely used ¹ and available as commercially approved products (e.g. chronOS®). Polymer composites containing 60wt% β -TCP have demonstrated to have similar osteogenic activity as pure β -TCP ². However, preparing structures by additive manufacturing with such high TCP content is a challenge ³.

Thermoplastic polyesters are commonly utilized in biomedical applications and bone tissue engineering scaffolds, including different additive manufacturing techniques. For example porous poly(D,L-lactic-co-glycolic acid) composite scaffolds, including 25wt% β -TCP, have been built exploiting AM by fused deposition modelling and tested in a rabbit femoral defect model ⁴. In another study ⁵ enhanced new bone formation in calvarial defects in rabbits was shown using polycaprolactone/poly(lactic-co-glycolic acid) blends containing 20wt% β -TCP. The scaffolds were created using a solid freeform fabrication AM technique. In both of these examples the calcium phosphate content was relatively low. A higher content can be achieved as ceramics can also be sintered, but such additive manufacturing often leads to brittle structures ⁶. Therefore, there is a need to manufacture composite scaffolds with a high bioactive agent content while maintaining good mechanical properties and high quality of the prepared AM structures.

Stereolithography (SLA) is a VAT photopolymerization additive manufacturing technique that allows addition of considerable amounts of β -TCP as is shown for example in this study. In SLA photocrosslinkable resins are cured in a layer-by-layer manner. The resolution of SLA is superior compared to most other rapid prototyping techniques. Traditionally, two forms of SLA can be distinguished: laser-based SLA, and digital light processing SLA (DLP SLA)^{7,8}. In laser-based SLA the resins are illuminated by a computer controlled laser beam. In DLP SLA, the resins are illuminated from below by a UV or blue light projector. The build platform moves up, out of the resin. The here presented structures are prepared using DLP SLA.

The amount of materials available for SLA is relatively limited especially regarding biopolymers.

⁹ Examples of biomedical polymers in SLA research include poly(ϵ -caprolactone) ¹⁰, poly(D,L-lactic acid) ¹¹, poly(trimethylene carbonate) ¹², polyurethane ¹³, poly(propylene fumarate) ¹⁴ and hydrogels such as poly(ethylene glycol-co-depsipeptide) ¹⁵ or gelatin methacrylate ¹⁶. Ronca *et*

*al.*¹¹ have successfully manufactured composite structures of methacrylated poly(D,L-lactic acid) and 20wt% nano-hydroxyapatite by stereolithography. With poly(trimethylene carbonate) contents of 47wt% nano-hydroxyapatite in a composite scaffold manufactured by SLA have been reported³.

Poly(trimethylene carbonate) (PTMC) is biocompatible, bioresorbable and surface eroding polymer¹⁷. PTMC can be functionalized with methacrylate ends groups to enable photocrosslinking in stereolithography. As PTMC networks degrade slowly by enzyme mediated surface erosion, the mechanical properties are maintained well over time^{18,19}. The degradation products of PTMC have been shown to be better resorbed and tolerated than those of e.g. polylactides¹⁹. Due to extensive mass loss related to bulk erosion of many lactides, an inflammatory reaction, associated with the clearance of a burst of polymer fragments have been observed²⁰. Composites of high molecular weight PTMC with biphasic calcium phosphate have shown promising results for orbital floor reconstruction²¹. Geven *et al.*¹² later manufactured patient specific orbital floors by stereolithography using photocrosslinkable low molecular weight PTMC. The rubber-like material properties of PTMC compensate well for the brittleness of the ceramic calcium phosphate component. Scaffolds fabricated with PTMC containing nano-hydroxyapatite have showed encouraging results with bone marrow stem cells *in vitro* and improved bone generation *in vivo* in calvarial defect model on rabbits²².

In this paper resorbable and bioactive PTMC/ β -TCP composite scaffolds for bone regeneration were successfully manufactured by stereolithography. A different composition of calcium phosphate, β -TCP, with a larger particle size was chosen instead of e.g. nanosized HA. β -TCP is bioresorbable compared to HA that is hardly resorbed. Furthermore, β -TCP has shown improved osteoconductivity compared to HA including mechanical properties closer to that of cancellous

bone^{1,23}. Structures containing up to 51 wt% β -TCP could be built at a high resolution and building in general was successful with as high amounts as 60 wt%. The upscaling and personalization aspects of the technique were proven through printing of a large jaw implant prototype. The focus in this paper is on the material specific structure property correlation and technical aspects regarding manufacturing composite scaffolds. Further work is ongoing to assess the biocompatibility and bone regeneration capacity of the presented scaffolds *in vitro* and *in vivo*. Those results will be presented in a separate publication.

2. MATERIALS AND METHODS

2.1 Materials

Trimethylene carbonate (TMC) was purchased from Huizhou Foryou Medical Devices Co., Ltd (China). Trimethylol propane (TMP, $\geq 98.0\%$), tin(II) 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$, $\sim 95\%$), dichloromethane (DCM, $\geq 99.8\%$), methacrylic anhydride (MAAH, 94%), triethylamine (TEA, $\geq 99.5\%$), hydroquinone ($\geq 99\%$), deuterated chloroform (99.9 %), chloroform (99.0 - 99.4 %) and β -tricalcium phosphate (β -TCP, powder particle size 2-6 μm , surface area $\geq 80 \text{ m}^2/\text{g}$, $\geq 90\%$ phase purity of β -tricalcium phosphate) were acquired from Sigma-Aldrich (Germany). Propylene carbonate ($\geq 99.0\%$) was obtained from Merck Millipore (Germany). Free radical photo initiator Omnirad TPO-L was received from IGM Resins (the Netherlands) and dye Orasol Orange G was obtained from CIBA Specialty Chemicals (Switzerland). All chemicals were used as received.

2.2 Synthesis and characterization of PTMC macromer

The synthesis of poly(trimethylene carbonate) is visualized in Figure 1a. Three-armed poly(trimethylene carbonate) (PTMC) was synthesized by ring opening polymerization of TMC with TMP (0.01 mol/mol TMP/TMC) as initiator targeting for an oligomer with a molar mass of

10 000 g/mol. Using $\text{Sn}(\text{Oct})_2$ (0.0002mol/mol $\text{Sn}(\text{Oct})_2/\text{TMC}$) as catalyst the polymerization was carried out at 130°C for 3 days under nitrogen atmosphere. The prepared hydroxyl terminated oligomer was dissolved in DCM (2ml/g monomer) and functionalized with MAAH (6 mol/mol MAAH/oligomer) at room temperature for 5 days in the presence of TEA (6mol/mol TEA/oligomer) and hydroquinone (0.1wt%). Finally, the methacrylate functionalized oligomer (macromer) was precipitated in cold ethanol and dried in vacuum at 40°C for 5 days. Polymerization and functionalization were followed with ^1H -NMR spectroscopy (Brüker AVANCE III 400 MHz) to determine the number average molar mass (M_n), monomer conversion and degree of functionalization. For ^1H -NMR measurements samples were dissolved in deuterated chloroform at room temperature. Size-exclusion chromatography (SEC) was performed using a Waters SEC system to analyze the M_n and polydispersity index (PDI) by comparing to a linear narrow molecular weight polystyrene standard calibration. The Waters SEC system consisted of a Waters 717plus autosampler, a Waters 510HPLC pump and a Waters 2414 refractive index detector including columns for molecular weight separation.

2.3 Manufacturing of networks and printed scaffolds

The workflow for manufacturing networks and printed scaffolds can be seen in Figure 1b. Resins were prepared by dissolving the methacrylated PTMC macromer in propylene carbonate at 70°C. β -tricalcium phosphate powder was mixed into the composite resins (PTMC 20-60) during heating. Photoinitiator TPO-L (5wt% relative to macromer) and Orasol Orange dye were finally mixed into the resin at room temperature. All resins and their compositions are described in Table 1.

a. Synthesis of PTMC macromer

b. Manufacturing of networks and printed scaffolds

Figure 1. (a) Synthesis of three-armed poly(trimethylene carbonate) and subsequent functionalization in order to obtain photocrosslinkable macromers. (b) The workflow for manufacturing networks and printed scaffolds from photocrosslinkable resins.

Table 1. Resin compositions used within the study. Values given as weight percentage of the total resin.

		PTMC0 ^a	PTMC20 ^b	PTMC40 ^b	PTMC50 ^b	PTMC60 ^c
Intended β -TCP content in networks/scaffolds		0wt%	20wt%	40wt%	50wt%	60wt%
Polymer	PTMC macromer	49	39	25	21	21
Bioactive agent	β -tricalcium phosphate	-	10	17	21	31
Diluent	Propylene carbonate	49	49	57	57	46
Photoinitiator	TPO-L	2	2	1	1	2
Dye	Orasol Orange G	0.06	0.05	0.03	0.03	0.03
Total		100	100	100	100	100

^a resin used only for scaffolds, ^b resin used both for networks and scaffolds, ^c resin used only for networks

To prepare photocrosslinked flat networks the mixed resins were crosslinked in an in-house built crosslinking box ($\lambda=395\text{-}405\text{nm}$, intensity $\approx 1\text{mW}/\text{cm}^2$) for 30min under nitrogen atmosphere. Samples were then postcured in a Dentsply Triad 2000 light curing system for 6min/side. To remove the propylene carbonate diluent from the crosslinked networks, the samples were extracted in a 60/40 (V/V) mixture of propylene carbonate and ethanol. The extraction liquid was changed daily while lowering the propylene carbonate with 10 V-% units each day until only extracting with ethanol. The slow extraction allows slow shrinkage of the networks and thorough removal of the diluent. Networks were finally dried in vacuum at 40°C until constant weight.

Using the same resins, high-resolution gyroid scaffolds were built using an Envisiontec Perfactory III Mini SXGA+ digital light processing stereolithography device. The scaffolds were designed to have an 83% porous cylindrical ($d=10\text{mm}$, $h=10\text{mm}$) structure with an interconnected gyroid pore architecture and a pore size of 1.3mm . To achieve this the designs were scaled before printing to account for the isotropic shrinkage of the structure during extraction. Structures were printed by exposing each layer for 12s at an intensity of $700\text{mW}/\text{dm}^2$ and wavelength of 400-550nm. The layer thickness used, $50\mu\text{m}$, was reached by optimizing the dye content in each resin using a working curve. Similarly, a porous large human mandibular defect prototype was manufactured according to the same method described above for porous gyroid scaffolds. The jaw CAD files were modified from “Human Jaw 3D Scanned” by scsuvizlab licensed under CC BY 3.0 ²⁴. The porous gyroid structures were rendered by combining the imaging data with a designed pore architecture as described before by e.g. van Bochove et al. ⁸. Further, solid disc cylinder specimen ($d=15\text{mm}$, $h=10\text{mm}$) were manufactured by DLP SLA for water contact angle and atomic force microscopy measurements. Propylene carbonate was removed from the printed structures similarly as for crosslinked networks described above.

2.4 Network and printed scaffold characterization

The PTMC macromer, crosslinked networks and printed scaffolds were characterized by thermogravimetric analysis (TGA) using TA Instruments Q500 equipment. Samples were heated at a rate of $20^\circ\text{C}/\text{min}$ to 600°C while the mass change was monitored. Differential scanning calorimetry (DSC) measurements were carried out with a TA Instruments Q2000 device to analyze the PTMC macromer, networks and scaffolds. Samples were heated to 100°C at a rate of $10^\circ\text{C}/\text{min}$ followed by cooling to -90°C at $200^\circ\text{C}/\text{min}$. After equilibration at -90°C for 5min a second heating

cycle was performed to 100°C at a rate of 10°C/min. The glass transition temperatures (T_g) of the samples were determined from the second heating cycle.

The mechanical properties of the networks were investigated by tensile testing according to ASTM D882-02 using an Instron 4204 Universal Tensile Tester equipped with a 1kN load cell. Samples were cut from crosslinked networks with a thickness of 1.2-1.4mm. The used samples ($n=3$) were 50mm long and 5mm wide. Grip to grip separation was initially 35mm and a crosshead separation rate of 50mm/min was used. Based on the results the stress at yield (σ_{yield}), strain at break (ϵ_{break}) and tensile modulus (E) were determined. If no clear yield point was observed the stress at yield was determined from the intersection of the tangents between the elastic and the plastic region. Toughness was calculated from the area under the stress-strain curve.

The dimensions of the extracted and dried printed scaffolds were measured. Based on these values the actual porosities of the scaffolds were calculated using the densities of the materials included in the composites while accounting for the experimental values from TGA regarding β -TCP content. To examine the surface topology of the 3D scaffolds a Hitachi TM-1000 Scanning Electron Microscope (SEM) was utilized without metal coating the samples. The wettability of solid discs manufactured by stereolithography was measured by KSV Instruments CAM 200 Contact Angle Meter.

3. RESULTS AND DISCUSSION

3.1 PTMC macromer synthesis

The ring opening polymerization (ROP) of the three-armed PTMC hydroxyl terminated oligomer followed by the functionalization, is visualized in Figure 1a. Monomer conversion in ROP as determined by $^1\text{H-NMR}$ was calculated to be <97%, while the number average molar mass (M_n)

was determined to be 8600g/mol. For the methacrylation, the degree of functionalization was 76%. The M_n based on SEC measurement was 11 800g/mol confirming that the molecular weight is in the range of the targeted value 10 000g/mol. However, as the retention times were compared to a linear polystyrene standard, while the PTMC macromer is three-armed, the M_n based on NMR calculations is likely more accurate. The PDI was determined to be 3.5 based on SEC measurements. The T_g of the PTMC macromer was -22°C and the decomposition temperature was 290°C as determined by DSC and TGA. Both are in line with previously reported values ^{3,25} for PTMC with a molecular weight in the range of 10 000g/mol. All of the characterization values presented above confirm a successful synthesis of the PTMC macromer.

3.2 Characterization of networks

The goal within study was to manufacture composites by stereolithography with as high amounts of bioactive β -TCP as possible, in order to evaluate the optimal ceramic/polymer ratio. In the composites the crosslinked PTMC with its rubber-like properties compensates for the brittleness of the ceramic component. By starting with creating crosslinked composite networks it could be assessed how high amounts can theoretically be manufactured before reaching brittle structures. A set of resins was prepared that are described in Table 1. Networks were created by crosslinking the resins under UV-light. After properly extracting the non-reactive diluent and drying, the networks were characterized. TGA experiments confirmed that the networks contain the desired amount of β -TCP (Table 2). DSC tests showed the amorphous nature of crosslinked PTMC. No melting peaks were visible and the glass transition temperatures varying from -11 to -18°C, are in line with previously reported values for methacrylated PTMC networks ²⁶.

Table 2. Thermogravimetric analysis, differential scanning calorimetry and tensile testing results for networks and printed structures. Values for tensile modulus, stress at yield, strain at break and toughness are given as averages (n=3) including standard deviations.

	Networks						SLA builds	
	β -TCP content by TGA	Glass transition, T_g (°C)	Tensile modulus, E (MPa)	Stress at yield, σ_{yield} (MPa)	Strain at break, ϵ_{break} (%)	Toughness (J/m ²)	β -TCP content by TGA	Glass transition, T_g (°C)
PTMC20	19%	-17.9	5±0.5	0.8±0.05	- ^a	286±86	32%	-12.0
PTMC40	40%	-11.2	52±4	1.9±0.2	- ^a	353±110	51%	-11.0
PTMC50	52%	-12.3	231±47	3.0±0.05	84±31	264±110	60%	-10.9
PTMC60	62%	-14.9	353±115	4.0±0.07	10±3	38±13	- ^a	- ^b

^a did not break, but slipped ^b scaffolds were not manufactured of this composition

The results from the tensile testing can be seen in Figure 2 and Table 2. The modulus for neat networks is around 3-4MPa²⁶. Results showed a tensile modulus of 5, 50 and 230MPa for crosslinked networks containing 20, 40 and 50wt-% respectively. With networks containing 60wt-% β -TCP a modulus of 350MPa was measured. This significant increase in modulus shows that the composites are reinforced by the addition of β -TCP as the stiffness increases with increasing β -TCP content. A neat PTMC network will elongate to several times its initial length²⁶. In the experimental data presented in Figure 2 the elongation at break could not be determined for 20 and 40wt% samples as those slipped of the grips before breaking during the measurement. However, a significant elongation was still achieved showing the rubber-like nature of these networks. For 50wt% samples the average strain at break was 84% while the same value for 60wt% networks was 10%. Toughness values (Table 2) are comparatively similar for 20-50wt% networks, but a drop in toughness can be seen at 60wt% as the rubber-like properties decrease. Composites

containing 50wt% show tough rubber-like behavior, while 60wt% composites exhibit less ductility but are not completely brittle. Consequently, aiming for higher than 60wt% will likely lead to brittle structures. Therefore, to ensure suitable mechanical properties and maximum bioactivity² the aim should be to prepare 3D structures containing approximately 50wt% β -TCP.

Figure 2. (a) Representative stress-strain curves, (b) average tensile modulus (n=3) and (c) average toughness (n=3) of composite networks prepared with varying amounts of β -TCP including standard deviations.

3.3 Characterization of printed scaffolds

Preparing composite structures containing 50-60wt% β -TCP with stereolithography (SLA) is not straightforward. Guillaume *et al.*³ have previously reported successful building of structures by DLP SLA including 46.5wt% nano-hydroxyapatite. A higher load of nHA was also attempted, but as the resins were non-homogeneous manufacturing was not possible. In order to build using SLA the viscosity of the prepared resins need to be low enough to flow and spread properly during the printing, therefore, ensuring accurate printing of each layer⁸. As the β -TCP content in the resin is increased the viscosity increases and more diluent needs to be added to ensure a low enough

viscosity. By doing so, the polymer content that keeps the structure together by crosslinking, decreases. Therefore, building with high contents has its limits when the polymer content in the resin becomes too low or the viscosity of the resin is too high. By optimizing the resin recipe, surprisingly large amount of bioactive agent can still be added.

The SLA machine used in this study is a digital light processing stereolithography device in which liquid resins are photo-crosslinked in a layer-by-layer manner. The crosslinked/cured structure builds on a moving build platform that rises up from the resin in a stepwise routine between the light exposures. DLP SLA projects the pixel pattern of each layer during a certain exposure time. During the exposure time the prepared resin needs to cure a layer that is slightly higher than the layer thickness used. If the cure depth is too high also the previous layer will be exposed to the light image of the ongoing layer resulting in over curing. The cure depth (C_d) is affected by the energy of the light (E), the critical energy of curing (E_c) and the effective light penetration depth (D_p)⁹:

$$C_d = D_p \ln \frac{E}{E_c} \quad (1)$$

By adding a dye to the resin the light penetration depth can be decreased. The resin compositions used in this study can be seen in Table 1. A working curve was made for each resin on the SLA to calculate how much dye is needed to reach the layer thickness of 50 μ m that was used in this study. In Figure 3 the working curve of the neat and 40wt% resins can be seen where the thickness of the cured layer is shown as a function of the dye concentration in the resin. It is clear that an increase in dye results in a decrease of the light penetration. Furthermore, the incorporation of a solid white powder in the resin clearly affects the light penetration. This is evident as the amount

of dye needed to obtain the correct layer thickness in a composite resin is considerably decreased as compared to the amount needed in a neat resin. From Table 1 it can be seen that the dye amount in the resins decreased with increasing amounts of β -TCP. A similar decrease of dye concentration with increasing amounts of solid white powder was reported previously for PTMC resins containing nano-hydroxyapatite ^{3,12,22} It appears that the solid β -TCP and nano-hydroxyapatite powders scatter the light significantly, acting similarly as the dye in the resin.

Figure 3. Examples of working curves for a neat resin and a 40wt% β -TCP resin. The cured layer thickness was achieved by illuminating resin for 12s with the SLA device.

Designed porous composite structures from PTMC containing β -TCP were successfully built by SLA using the optimized resin compositions. The manufactured scaffolds including dimensions and porosities can be seen in Figure 4. Scaffolds containing up to 51wt% β -TCP can be built at very high resolution. Structures including 60wt% β -TCP were also built, but as can be seen, a high resolution could not be reached. Nevertheless, the quality of builds with as high content of bioactive agent as 51wt-% can be compared with the quality of neat scaffolds as can be seen in Figure 5 showing magnified pictures of a neat PTMC scaffolds (without β -TCP) and PTMC/ β -TCP composite scaffolds containing 31 and 51wt% β -TCP. The dimensions of the built scaffolds

showed minor deviations compared to the design. For example the built 51wt-% β -TCP scaffolds measured an average diameter of 10.4mm and an average height of 10.4mm compared to the design of 10.0mm. The average porosity was calculated to be 88% which is higher than the designed 83%. For scaffolds with lower β -TCP content the dimensions were smaller than the designed. The difference in obtained dimensions can be explained by differences in shrinkage during the removal of the diluent in a solvent exchange with ethanol (a non-solvent for PTMC) after the DLP SLA fabrication. This phenomenon has been described previously for DLP SLA fabricated structures of PTMC and PDLA²⁶⁻²⁸. As this shrinkage is isotropic, it can be taken into account during the design of the structure. In our work we scaled the structures to account for the shrinkage, similarly to Melchels *et al.*²⁸. In addition, shrinkage due to diluent removal has a few benefits: when building at the highest resolutions of the equipment, shrinkage will result in structures with smaller features than technically possible based solely on resolution, and in our specific case shrinkage may be beneficial in obtaining a structure with the β -TCP directly on the surface (see below). Furthermore, diluent removal results in networks with better mechanical properties after extraction as compared to networks prepared without non-reactive diluent²⁹. It has been hypothesized that this is the result of the polymer chains being disentangled due to the presence of solvent³⁰. The obtained networks have fewer chain junctions and inter-chain entanglements. Consequently, the polymer chains are less firmly embedded in the network structure and deform more non-affinely.

Figure 4. Representative pictures of and dimensions of the CAD model, neat scaffolds (0wt% β -TCP) (n=3) and PTMC/ β -TCP composite scaffolds containing, 32wt% (n=3), 51wt% (n=3) and 60wt% (n=1) β -TCP. Note the difference in manufacturing quality as the bioactive agent content increases to 60wt%.

Above 3D scaffolds containing 32, 51 and 60wt% β -TCP are described. Interestingly these scaffolds were built using resins containing 20, 40 and 50wt% respectively. Thermogravimetric analysis indicated β -TCP contents of around 10wt% higher for built scaffolds compared to the amounts present in the resins. For networks this phenomenon does not occur, indicating that the

differences originate from the crosslinking method. The explanation for this is most likely that the resin was not fully crosslinked in interfaces of the curing layer during printing. While the uncrosslinked resin dissolved during extraction the β -TCP remained in the structure. Similar differences between experimental and theoretical values have been reported for SLA builds including PTMC and nHA ³.

To achieve optimal mechanical properties and a shorter degradation profile, PTMC with a higher molecular weight would be preferred. Even so, such polymers have revealed to be challenging to print. ⁸ Alternatively, in order to lower the viscosity of the resin a polymer with a low molecular weight could be utilized, as the viscosity is increased when the molecular weight rises ²⁶. However, crosslinked networks manufactured from lower molecular weight tend to be brittle ²⁵. The molecular weight 10 000g/mol for this study was chosen as it is the lowest molecular weight that shows rubber-like mechanical properties, while it is relatively straightforward to use in SLA.

A common problem when manufacturing composites by melt processing thermoplasts is that a polymer skin layer is formed on the surface. Therefore, the polymer needs to start degrading for the bioactive agent to be available at the surface. The problem can be solved by coating the thermoplastic composite with a bioactive agent rich solution, but this requires an extra processing step. Pictures and SEM imaging of the samples printed by DLP SLA, however, show an accumulation of β -TCP on the surface of the scaffolds as can be seen in Figure 5. When the surface of the scaffolds is cut, a clear accumulation of β -TCP can be seen on the surface compared to inside the structure (Figure 5d). The importance of this surface enrichment of the bioactive agent has previously been described by Guillaume *et al.* ²² and can be seen as an advantage of manufacturing composites by SLA compared to methods using melt processing. Guillaume *et al.* ²² suggests that the surface enrichment, of in their case needle-shaped nano hydroxyapatite

nanoparticles, could be due to regaining of entropy as the flexible polymer chains push particles to the surface in order to not stretch around them. This sounds like a plausible mechanism also in our case considering round β -TCP particles. This theory is strengthened by the fact that a solvent is used during the printing, and therefore the crosslinked polymer is swollen, consequently allowing more movement as the network slowly shrinks.

As the bioactive agent of the composite is readily available on the surface it can directly be utilized for bone formation. Furthermore, the β -TCP on the surface creates a micro scaled topographical roughness of the surface that may be beneficial for attachment of osteogenic cells³¹. SEM pictures in Figure 5 confirm a surface roughness and β -TCP enrichment of the manufactured 3D composite scaffolds. Round β -TCP particles are clearly visible on the surface of the scaffold with a particle size of 1-10 μ m. In contrast the surface of the neat PTMC scaffold is smooth. Looking at the cut surface in with SEM (Figure 5), the concentration difference of β -TCP between the surface and bulk is evident. Wettability results (Table 2) of 3D printed discs showed that the hydrophilicity increases with rising β -TCP content. The water contact angles for a neat PTMC scaffold were $74\pm6^\circ$ and decreased to $43\pm2^\circ$ with the addition of 32wt% β -TCP. For discs containing 51 and 60 wt% β -TCP a water contact angle could not be measured as no drop was formed on the surface. Even though the hydrophilicity likely still has increased this is probably due to the markedly increased surface roughness of the samples.

Figure 5. (a) SEM pictures of a neat printed scaffold with 0wt% β -TCP showing a smooth surface. (b)- (c) SEM of similar printed scaffold containing 32wt% and 51wt% β -TCP where a clear microscaled topographical roughness can be seen formed by the round β -TCP particles. (d) SEM pictures of a cut scaffold where a clear accumulation of β -TCP on the surface of the scaffold can be seen compared to bulk as can be seen where the surface has been cut open.

The presented porous structures are the highest β -TCP containing structures reported for composites manufactured by SLA. Scaffolds including 60wt% osteoconductive ceramic could be printed. By lowering the amount a high resolution and build consistency could be reached. Further, the upscaling capability of the process was shown by manufacturing a personalized porous implant for a large mandibular defect (Figure 6). The large implant was prepared with the PTMC 40 resin and therefore contains up to 51wt% osteoconductive β -TCP. This prototype was built with a process where the parameters such as porosity, pore-size, scaffold architecture as well as possible addition of growth factors can be optimized to create optimal bone regeneration environment. In a real case the injured side can be remodeled by mirroring the healthy side to create an implant.

Figure 6. The engineering route to manufacture a porous individualized implant based on imaging data including a large mandibular defect prototype containing 51wt% β -TCP printed with stereolithography.

Bone is a natural composite and therefore an intuitive approach is to replace and regenerate bone by utilizing a composite. Polymer/calcium phosphate composites have been extensively studied^{32,33} with different compositions and model parameters. From a scaffold material point of view, we can say that the polymer needs to provide a matrix that is biocompatible, has mechanical properties close to that of bone and can accommodate a large volume of the bioactive component. The demand on the bioactive component, in this case calcium phosphates, is to provide optimal bone regeneration. In terms of scaffold architecture important properties are: surface area, porosity, pores size and interconnectivity. One can state that the suitability of a composite is best described by analyzing the constituents. Consequently, to evaluate PTMC/ β -TCP composites in regards of biocompatibility and suitability for bone regeneration applications, we will not compare them to other composites, but instead discuss the properties of the individual components. Photocrosslinked poly(trimethylene carbonate) has been studied *in vitro*^{22,34,35} and *in vivo* in rats¹⁸, rabbits²² and sheep²¹ showing appropriate biocompatibility. Considering that PTMC degrades relatively slow (0.5% mass loss *in vivo* in 36 weeks¹⁸), copolymers of PTMC e.g. with ϵ -caprolactone could be utilized if a faster degradation profile is needed¹⁸. The molecular weight of the PTMC polymer for this study was chosen to be low enough for building composites with SLA, but high enough²⁵ to have adequate mechanical properties. Furthermore, the polymer enables addition of surprisingly high amounts of β -TCP that further enhances the mechanical properties of the composite.

β -tricalcium phosphate is used as granulates for example in reconstructions of large bone defects in humans with positive long-term follow up results³⁶⁻³⁹. However, the metallic meshes used to contain the granules are often sharp and can hurt the soft tissue. Incorporation of large β -TCP amount into a porous individually designed composite scaffold solves this problem by eliminating

the need to use a mesh. This also streamlines the clinical procedure shortening the length of the surgery. These observations based on the existing information about bone regeneration composites further highlights the relevance of the here presented work. Work on similar structures containing lower amounts of a bone inducing component have showed improved bone healing²², indicating the great potential of the obtained structures. If the goal is to maximize the fraction of the osteoconductive component in the composite, based on results on mechanical properties and printability a PTMC composite including around 50% β -TCP is an optimal choice. However, bio-evaluation in form of *in vitro* and *in vivo* studies are required to show the effect of this particular composite. Following successful manufacturing of large implants, such studies have been planned and are currently ongoing.

4. CONCLUSIONS

The goal of the presented research was to utilize additive manufacturing to produce polymer composite scaffolds containing an optimal amount of osteoconductive β -tricalcium phosphate for patients-specific bone grafting implants. Composite networks were created from poly(trimethylene carbonate) and β -tricalcium phosphate. Based on mechanical testing of the networks it could be concluded that addition of β -TCP reinforces the rubber-like PTMC by increasing the tensile modulus. Based on these results composites with a 50/50 PTMC/ β -TCP ratio would be most favorable for creating individualized implants by stereolithography. Resorbable and bioactive PTMC/ β -TCP composite scaffolds for bone regeneration were manufactured by stereolithography containing up to 60wt-% β -TCP. By reducing the bioactive agent content to 51wt% the excellent resolution of DLP SLA could be utilized to generate high-quality builds. SLA also provides a manufacturing method enabling automatic surface enrichment of the bioactive agent on the surface without extra processing steps. The manufactured scaffolds show great potential to be used in bone

grafting due to the biocompatibility and bioresorbability of PTMC and the high amount of osteoconductive β -TCP incorporated in the structure.

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