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Development of a new calcium phosphate powder-binder system for the 3D printing of patient specific implants

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Abstract A key requirement for three-dimensional printing (3-DP) of medical implants is the availability of printable and biocompatible powder-binder systems. In this study we developed a powder mixture comprising tetracalcium phosphate (TTCP) as reactive component and β-tricalcium phosphate (β-TCP) or calcium sulfate as biodegradable fillers, which can be printed with an aqueous citric acid solution. The potential of this material combination was demonstrated printing various devices with intersecting channels and filigree structures. Two post-processing procedures, a sintering and a polymer infiltration process were established to substantially improve the mechanical properties of the printed devices. Preliminary examinations on relevant application properties including in vitro cytocompatibility testing indicate that the new powder-binder system represents an efficient approach to patient specific ceramic bone substitutes and scaffolds for bone tissue engineering.

Keywords Three-dimensional printing · Rapid prototyping · Calcium phosphate · Ceramic · Bone repair

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Introduction

Calcium phosphate based ceramics are widely used in medicine as bone substitutes, implants, and coatings on dental and orthopaedic prostheses [1–3]. Because of their chemical and structural similarities to the inorganic phase of human bone, hydroxyapatite (HA) and other calcium phosphates like α- or β-tricalcium phosphate (α- or β-TCP) show an excellent biocompatibility. Most of the relevant application properties of these materials including their biological influence on tissues and especially their biodegradation behaviour are determined by their special chemical composition, morphology and surface topology. Therefore, a proper material design offers numerous possibilities for the use of calcium phosphate materials in hard tissue replacement and regeneration.

Currently, in clinical practice the common formulations of calcium phosphate based bone substitutes are granules, pastes, self-hardening cements or porous devices of rather simple geometry [4, 5]. Patient specific ceramic implants exactly matching an individual bone defect are normally not available as commercial products. Especially for the repair of large or complex bone defects, custom-designed implants with a geometry fitted to the defect which has to be treated would be very helpful. The positioning and fixation of such implants in the defect would be much easier and no additional implant modelling or processing before or during the operation would be necessary saving time and costs. Furthermore the development of bone tissue engineering methods requires implantable scaffolds not only with a defined shape and size but with a controlled inter-connective pore architecture.

A group of advanced fabrication techniques that allow the generation of devices with complex shape directly from computer aided design (CAD) files are the solid freeform fabrication or rapid prototyping (RP) techniques [6–10]. Various RP techniques including stereolithography [11], selective laser sintering [12], and three-dimensional printing (3-DP) [13, 14] or plotting [15] have been used to produce ceramic implants based on calcium phosphates or composites of calcium phosphates and organic polymers. The 3-DP technique employing conventional ink jet printing technology [16] represents a simple and versatile method to process a wide variety of powder materials. During fabrication a liquid binder is ejected from a printer head onto a thin layer of powder following the sliced two-dimensional profile of a computer model. The subsequent stacking and printing of powder layers on previously printed layers generates the complete structure of the desired three-dimensional object. The function of the used binder is to join adjacent powder particles of the same and of neighbouring layers.

Concerning HA- or TCP-based implants, a limited number of powder-binder combinations are described in the literature. In a common approach particulate mixtures of a polylactone (e. g. a poly(L-lactide-co-glycolide)-copolymer) and β-TCP are printed with organic solvents like chloroform as binder to create bone repair scaffolds [14]. The use of organic solvents as binders can cause problems because they are able to affect plastic components of the printer head or the supply tubes and the complete removal of the solvent from the created device is a rather difficult procedure. Therefore alternative systems using aqueous binders like aqueous polyacrylic acid solution in combination with HA powder [17] have been developed. In spite of newly developed material combinations there is an urgent need for new biocompatible and easily usable powder-binder systems leading to printed ceramic devices with adequate accuracy and mechanical stability.

It was the aim of the present work to explore the feasibility of using a reactive, HA forming, tetracalcium phosphate (TTCP) as a printing powder for 3-DP in combination with aqueous binder solutions. The potential of this new powder-binder system to fabricate complex devices of different shape and size has been studied and two different post-treatment procedures of printed samples were performed to improve their mechanical stability. Preliminary results on the in vitro cytocompatibility of samples fabricated in this way are reported.

Materials and methods

Materials

All chemicals and solvents were reagent grade and used as received. TTCP was prepared by a solid state reaction according to [18]. A homogenized mixture of equimolar amounts of calcium carbonate (Fluka, Switzerland) and calcium hydrogenphosphate (Riedel-de-Haen, Germany) was heated in a furnace at 1400 °C for 6 h followed by quenching the reaction mixture to room temperature in a desiccator. XRD analysis confirmed the formation of nearly pure TTCP containing only traces of calcium oxide and α-tricalcium phosphate.

The crosslinkable macromer dianhydro-D-glucitol [bis(dilactoylmethacrylate)] (DLM-1) was synthesized by ring-opening oligomerization of L-lactide in the presence of dianhydro-D-glucitol and stannous octanoate as catalyst followed by esterification of the terminal hydroxyl groups of the formed oligolactide with methacryloyl chloride in dichloromethane as described earlier [19].

Microporosity estimation

The microporosities were calculated according to [20] using printed cubic specimens of 10 mm of edge length. The apparent volume (V_a) was determined from the dimensions and the weight of the printed specimens and the bulk (''true'') volume (V_t) was determined measuring the density of weighted specimen by a gas pycnometer (Ultrapycnometer 1000, Quantachrome, USA). The general formula p (porosity) = $(V_a - V_t)/V_a$ was used to calculate the microporosity of the printed specimen. For each composition five specimens were tested.

Compression strength

A tensile testing machine (Instron 4467, USA) equipped with a 30 kN maximum load cell was used for the measurement of the compression strength. Six cylindrical samples of each configuration, 10 mm in diameter and 10 mm in height, were tested.

Weight loss and shrinkage

Weight loss and shrinkage were determined by measuring the dimensions with a micrometer and exact weighing of specimens, resp., before and after sintering. Samples were run in triplicate.

X-ray diffraction

X-ray diffraction patterns were recorded on a D 5000 powder diffractometer (Siemens, Germany) with graphite-monochromatized copper Kα radiation. Data were collected from 2 $\Theta = 8 - 60°$ in a continuous step mode with a step size of 0.01° and a normalized count time of 8 s. The phase composition was checked by means of JCPDS.

Cell culture

MC3T3-E1 cells (DSMZ No. ACC 210, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were cultured in alpha medium (Biochrom, Germany) with addition of 2 mM N-Acetyl-alanyl-glutamine, 50 U/ml penicillin, 0.05 mg/ml streptomycin, 10% fetal calf serum at 37 °C under 5% CO_2 atmosphere. Cell suspensions were obtained after trypsination by standard protocol. Printed sample slices of β-TCP/TTCP of about 12 mm in diameter and 4 mm in height were separately placed in a 24 well cell culture plate, disinfected with ethanol (70%) for 1 h and washed three times with phosphate buffer saline (PBS) and once with culture medium. The samples were seeded with 1 ml cell suspension of 50,000 cells/ml. Further cultivation was performed in osteogenesis inducing medium, slightly modified from Winter et al. [21] (culture medium supplemented with 0.1 µM dexamethason, 10 mM β-glycerophosphate) that was renewed every 2 days. Wells without samples were seeded and cultured with MC3T3-E1 cells in an analogous manner serving as controls.

Alkaline phosphatase (AP) staining

TCP/TTCP specimen were sampled at 7, 14 and 21 days, respectively, washed with PBS and cells were fixed on the samples with ethanol (70%) for 10 min. Cells were washed with AP-buffer (0.1 M Tris/HCl pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$) and incubated in AP-staining solution (0.0188% (w/v) of BCIP, 0.0375% (w/v) of NBT in AP-buffer) at room temperature for 30 min. The reactions were stopped by washing with PBS. Controls were handled in an adequate manner. The specimen were placed onto microscope slides, covered with coverslips and evaluated by means of bright field light microscopy in the reflective mode (Axiotech, Carl Zeiss Jena, Germany). Control cells were evaluated in the wells by means of bright field or phase contrast microscopy in the transmission mode (Axiovert 25, Carl Zeiss Jena, Germany). Photomicrographs were recorded using a CCD fluor microscope imager MP 5000 (Intas Göttingen, Germany). Imaging was supported by Image Pro Plus software (Media Cybernetics, Silver Spring, USA).

Three-dimensional printing procedure

A commercial Z 402 printer (Z Corp., USA) was used in all printing experiments. Samples of different shape and size were designed using a conventional CAD software. The designs were transferred to the 3-DP software where the model was sliced by a slicing algorithm. The resulting 2D sliced layers were built layer by layer in the printing process. The layer thickness of the powder (particle size < 100 µm) at the piston plate of the printer was about 100 µm. Two powder mixtures containing 30 wt% of TTCP and 70 wt% of β-TCP (PM-1), and calcium sulfate dihydrate (PM-2), resp., were used for printing. A solution of 25 wt% of citric acid (Fluka) in distilled water was employed as binder unless otherwise stated. After finishing the construction, unbound powder was brushed away from the fabricated object.

Cuboid-like samples with cylindrical channels of 2 mm diameter in various axes were printed. For testing the mechanical and biological properties, cylindrical samples 10 mm in diameter and 10 mm in height and for the measurement of the microporosity cubes of 10 mm in edge length were fabricated.

Post-treatment of printed objects

Two different methods (A and B) were used for the post-treatment of printed objects. In method A objects were sintered at 1200 and 1400 °C, resp., (powder mixture PM-1), and 1000 °C (PM-2) for different times using heating rates between 100 and 400 °C/h (details see Table 2).

In method B the printed objects were infiltrated with a liquid mixture of macromer DLM-1 and of 2-hydroxyethyl methacrylate in 9:1 weight ratio containing dibenzoyl peroxide (4 wt% related to the total mass of the mixture) as initiator. The infiltrated objects were cured for 1 h at 110 °C.

Results and discussion

Development of suitable powder-binder systems

TTCP reacts rather slowly with water at ambient temperature forming HA and calcium hydroxide [22]. The reaction can be represented by

$$3\ Ca_4(PO_4)_2O + 3H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2\ Ca(OH)_2 \quad (1)$$

For the use of this reaction in the 3-DP process, the reaction rate has to be accelerated by a suitable additive. From the literature it is known that the hydrolysis rate of TTCP is increased with decreasing pH value [23]. Therefore we studied the hardening reaction of TTCP powder at room temperature in the presence of aqueous binders containing different additives and additive concentrations. Various acids (phosphoric acid, sulfuric acid, lactic acid, citric acid) and sodium hydrogenphosphate were tested as binder additives. As it is demonstrated in Fig. 1, shortest hardening times have been obtained with citric acid using concentrations in a range between 20 and 40 wt% and with lactic acid of concentrations between 30 and 40 wt%. For the following investigations aqueous citric acid solution was chosen as binder.

Based on the hydrolysis reaction of TTCP in water (Eq. 1), it is assumed that the treatment of TTCP with citric acid leads to the formation of HA and calcium citrate. XRD analysis of the reaction product between TTCP and an aqueous citric acid solution showed a higher amorphous content in the reaction mixture compared to the untreated TTCP whereas the reflexes of calcium oxide are disappeared (Fig. 2, TTCP + CA).

Because HA is a highly biocompatible but very slowly resorbable biomaterial, we studied the possibility of a partial substitution of TTCP powder by β-TCP or calcium sulfate dihydrate which are known to show higher resorption rates. Powder mixtures containing TTCP and different amounts of β-TCP and calcium sulfate dihydrate, resp. were treated with an aqueous solution of citric acid (30 wt%) as binder and the resulting products were examined with regard to complete hardening and shape stability. It was found that up to 80 wt% of TTCP can be substituted by β-TCP or calcium sulfate dihydrate without noticeably influencing the curing reaction and the shape stability of the resulting products.

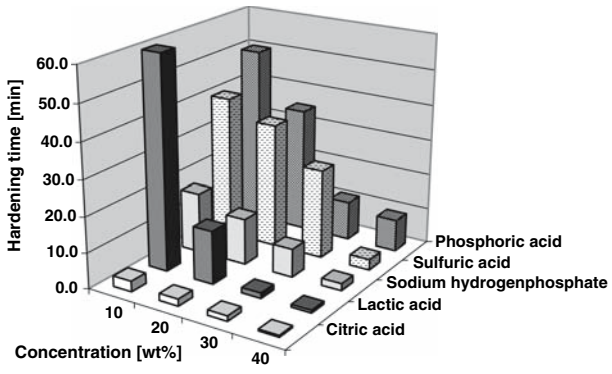

Fig. 1 Influence of different additives and additive concentrations in aqueous solution on the hardening of TTCP

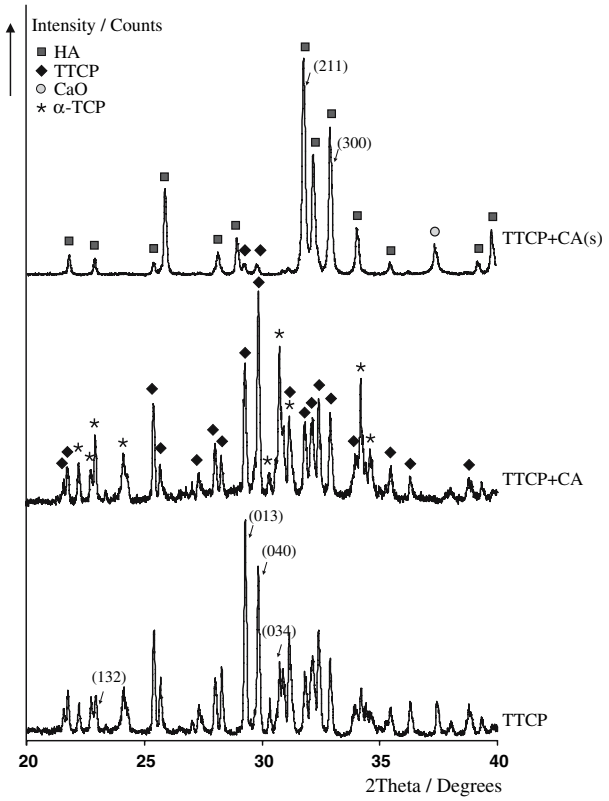

Fig. 2 XRD pattern of TTCP, TTCP treated with 30 wt% aqueous citric acid (TTCP + CA), and TTCP treated with aqueous citric acid and sintered at 1200°C for 6 h (TTCP + CA(s)). Prominent diffraction reflexes are labelled

Three-dimensional printing experiments

Based on the curing experiments described above both the mixtures PM-1 and PM-2 (Table 1) were used as powder components. For both powder mixtures a

Table 1 Composition of used powder mixtures for 3-DP and selected properties (porosity, compression strength) of printed samples

Powder mixture	Powder composition	Porosity (%)	Compression strength (MPa)
PM-1	30 wt% TTCP 70 wt% β-TCP	38.0	0.70 ± 0.06
PM-2	30 wt% TTCP 70 wt% $CaSO_4$ x 2 H_2O	38.5	0.59 ± 0.07

Table 2 Sintering conditions and resulting compression strengths of printed samples

Sample	Sintering temperature (°C)	Heating rate (°C/h)	Sintering time (h)	Compression strength (MPa)
PM-1a	1200	400	6	1.3 ± 0.1
PM-1b	1400	400	6	3.9 ± 0.2
PM-1c	1400	200	6	3.9 ± 0.1
PM-1d	1400	100	6	4.3 ± 0.3
PM-1e	1400	100	24	3.7 ± 0.4
PM-2a	1000	400	6	0.1 ± 0.01

solution of 25 wt% of citric acid in water was employed as binder. Higher concentrations of the binder led to unsatisfying results because nozzles of the printer head were plugged during the printing process by the formation of citric acid crystals.

Specimens of different shape and size have been printed to study the usability of the developed powder-binder systems in the 3-DP process. In a first run, compact cylindrical specimen, 10 mm in height and 10 mm in diameter, with sufficient mechanical stability could be successfully printed from both powder-binder systems. In a next set of experiments cuboid specimens with open channels of diameters between 1 and 2 mm in either x-, y-, or z-direction were produced. Channels with diameters smaller than 1 mm can also be printed but with decreasing pore diameter it becomes more and more difficult to remove unbound powder material out of the channels. The distance between two channels has been varied to study the accuracy and feature resolution of the process. Bundles of open channels with distances between neighbouring channels down to 500 μm could be generated maintaining accurate contours (Fig. 3). Subsequently, a specimen was printed with channels of 2 mm diameter penetrating through the cuboid orthogonally in x-, y-, and z-direction to mimic a regular three-dimensional interconnecting structure (Fig. 4). A dimensionally stable scaffold with a thoroughly open channel system could be successfully produced. In all cases printed objects were strong enough to be manually handled without damaging the integrity of the devices.

Based on computer tomography scan data of a human cranium, a computer based 3D model of a corresponding horizontal cranial segment was generated and imported to the printer software. Printing of this cranial segment with all its filigree structures was successfully performed (Fig. 5) using both the developed powder-binder systems.

Microporosity analysis of printed samples without any post-processing gave values of about 38 % (Table 1) for both the used powder-binder systems.

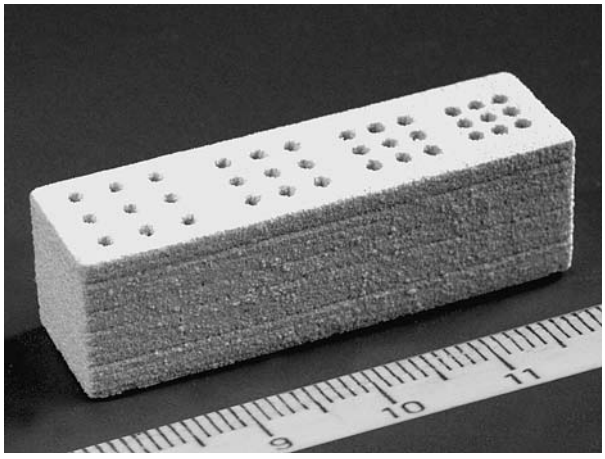

Fig. 3 3-D printed cuboid specimen with channels in one direction and variation of channel diameter and channel distance

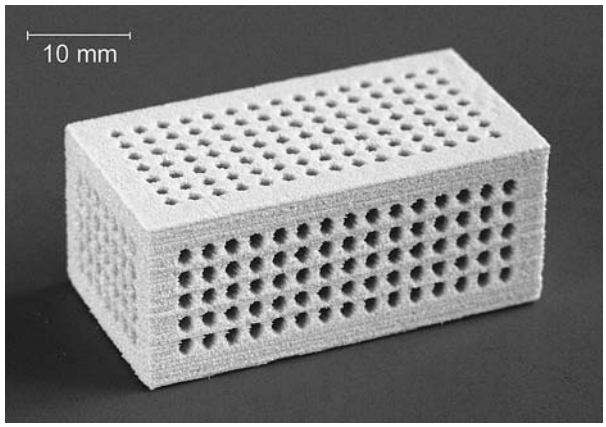

Fig. 4 3-D printed cuboid specimen with channels in x-, y-, and z-direction and orthogonal design

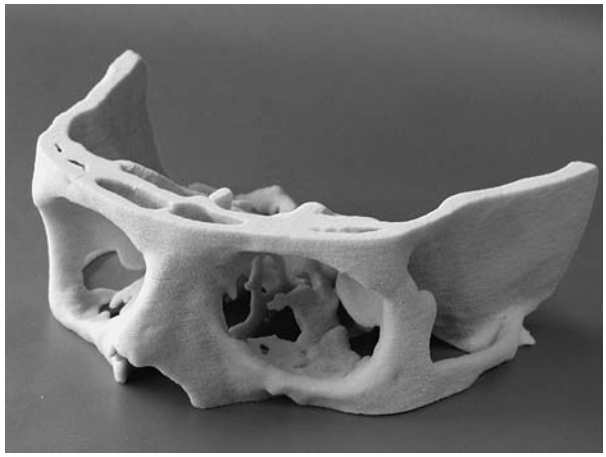

Fig. 5 3-D printed cranial segment

The similar results for the two materials indicate that the porosity is strongly influenced by the particle size of the printing powders.

The relatively high microporosity seems to be one reason for the low compression strengths of the printed samples which were for all samples without post-processing below 1 MPa (see Table 1). Based on these results, a suitable post-processing of the printed devices was necessary to enhance their mechanical strength.

Post-processing of printed devices

Two different techniques for the post-processing of printed objects have been used in this study. As a common method to enhance the mechanical stability of ceramic materials sintering of the printed samples was performed (method A). In a model experiment, pure TTCP was treated with an aqueous citric acid solution and the formed product was sintered at 1200 °C for 6 h. As expected, in the corresponding X-ray diffraction pattern, calcium oxide was detected along with HA (Fig. 2, TTCP + CA(s)). Based on these result, the occurrence of HA as main component, TTCP, and calcium oxide is assumed in devices which are printed from TTCP/citric acid mixtures and subsequently sintered.

Table 2 gives an overview on the employed sintering conditions and their influence on the compression strength of the printed samples after sintering. In the case of powder mixture PM-1 containing β-TCP as main component, a remarkable increase in the compression strength of printed samples was observed after sintering at 1400 °C for 6 h. Prolonged sintering times and a variation of the heating rate had no or little effect on the mechanical stability.

As expected for powder mixture PM-2, containing 70 wt% of calcium sulfate dihydrate, a dramatic decrease in the compression strength of printed samples was found after sintering due to the liberation of water during the sintering process. The liberation of water results in an increase in the porosity connected with an extensive weight loss and shrinkage of the samples (Table 3). In contrast to these findings, the porosity of samples fabricated from powder mixture PM-1 is only slightly changed during sintering and the extent of shrinkage ranging about 14% is much lower.

Table 3 Porosity, weight loss, and shrinkage of printed and sintered devices

Powder mixture (sintering conditions)	Porosity (%)	Weight loss (wt%)	Shrinkage (%)
PM-1a (1200 °C, 6 h)	36.0	8.1	14.5
PM-2a (1000 °C, 6 h)	50.1	23.8	28.5

A further approach to enhance the mechanical stability of 3D-printed constructs with interconnective porosity consists in their infiltration with molten waxes, polymers or with curable monomers. Unfortunately, most of the common infiltration mixtures used for printed devices are not well suited for medical implants because they may cause cytotoxic reactions. In this study, we used a mixture of the bismethacrylated oligolactide macromer (DLM-1), containing 10 wt% of 2-hydroxyethyl methacrylate as co-monomer for the infiltration of printed samples. DLM-1 (Fig. 6) forms a three-dimensional polymeric network with hydrolyzable ester moieties during radical polymerization. In a recent study [19], it was demonstrated that the formed, rather slowly degradable polymer network possess an excellent in vitro cytocompatibility.

After infiltration with the macromer mixture and curing at 110 °C, wear-resistant samples with remarkably improved mechanical stability have been obtained. In Fig. 7 the influence of post-processing on the compression

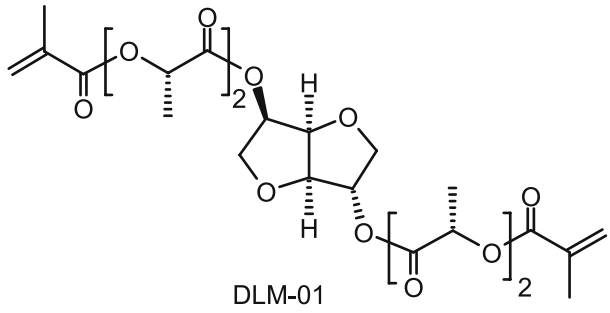

Fig. 6 Chemical structure of the macromer dianhydro-D-glucitol [bis(dilactoylmethacrylate)] (DLM-1) used for infiltration of printed samples

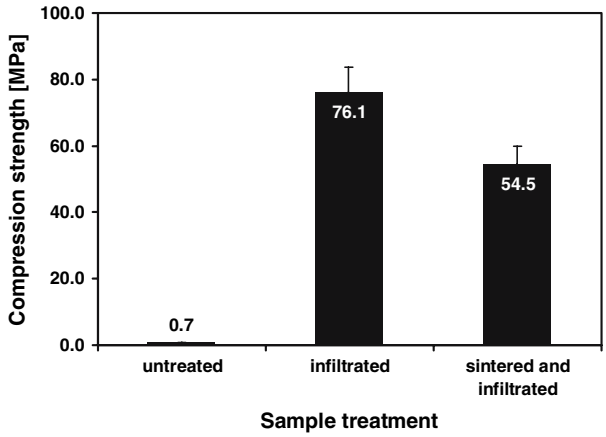

Fig. 7 Influence of post-processing procedures on the compression strength of printed samples

strengths of printed samples is depicted. Compared to untreated samples a 10-fold increase in the compression strength was observed for infiltrated but not sintered samples. The infiltration of sintered samples results in slightly lower values for the compression strength presumably due to their somewhat lower porosity.

Preliminary results on cytocompatibility

MC3T3-E1-cells grew on the TCP/TTCP scaffolds as adherent cells. During the time course of the experiment the number of cells as well as the intensity of the AP staining were increasing. AP positive cells on TCP/TTCP scaffolds were more evenly distributed than clones of AP positive cells in the control cultures on tissue culture polystyrene. In controls AP positive cells differentiated under formation of larger spots consisting of several intensively stained cells within a cell layer containing also a high portion of AP-negative cells (Fig. 8a). These clonal differences were observed on TCP/TTCP too, but the cell layers were not completely confluent and the MC3T3-E1 cells showed an altered morphology (Fig. 8b). Particularly the cells possessed finer extensions suggesting that the cell adhesion might be influenced by this surface.

Experiments were done to measure the AP-activity of MC3T3-E1 cells grown on TCP/TTCP in comparison with controls by a kinetic method. Results are not shown, because absorption of AP-activity from the fetal calf serum on TCP/TTCP produced too high background levels and cell lysis from the surfaces possibly was accompanied with the solubilization of phosphate interfering the measurements. Nevertheless, protein corrected AP-activity on TCP/TTCP scaffolds were less than 10% of the controls at 14 and 21 days, results that have to be ensured yet. However, adherent growth of MC3T3-E1 cells and development of AP-activity over 3 weeks showed the usability of this material in bone repair.

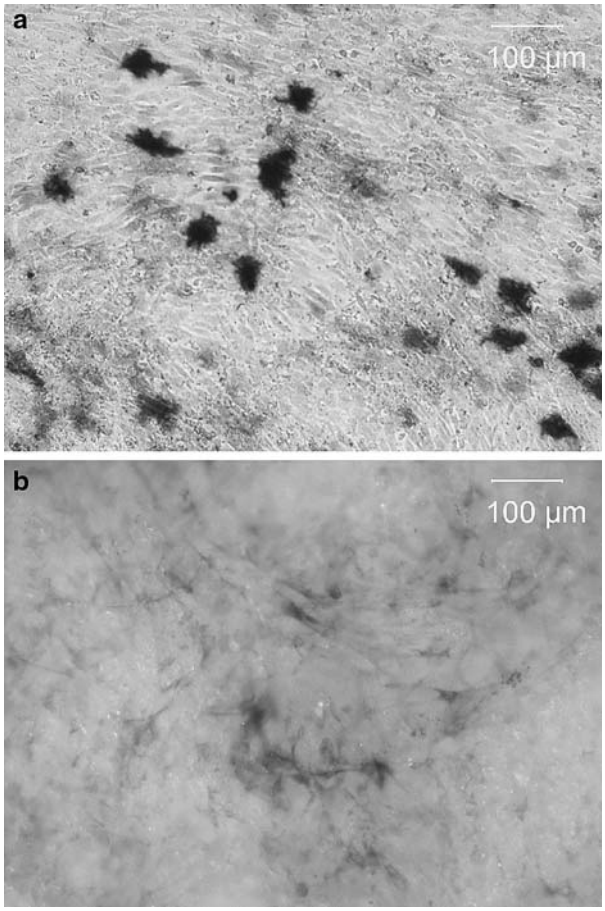

Fig. 8 LM-micrographs of MC3T3-E1 cells stained for endogenous AP at 21 days of culture in osteogenesis inducing medium (**a**) on tissue culture polystyrene; phase contrast, transmission mode, (**b**) on TCP/TTCP scaffolds; bright field, reflective mode

Conclusions

3-DP is a versatile and simple rapid engineering technology offering an unique potential to generate patient specific bone implants and tissue engineering scaffolds. A key role in this technology plays the design of a suitable powder-binder system fulfilling the requirements of the fabrication technology as well as the demands for biocompatibility and often also biodegradability. Calcium phosphates similar to bone mineral have been known as highly biocompatible materials for many years and their adaptation to 3-DP technology seems to be a promising matter of research in this area. In this work we developed a TTCP based powder mixture for 3-DP which can be used in combination with biodegradable fillers like β-tricalcium phosphate or calcium sulfate. Conventional 3-DP equipment can be used to print this material with an aqueous citric acid solution as binder. No organic solvents are needed in the printing process. The reaction of TTCP with citric acid results under the used conditions in the formation of HA and calcium citrate as found by XRD analysis of products in corresponding model reactions.

It could be demonstrated that objects with complex internal architecture and shape are available using the described powder-binder systems. The mechanical properties of the printed devices can be improved by a conventional sintering process. In this case the shrinkage of the sintered devices has to be taken into account. An alternative post-processing procedure consists in a simple infiltration of the printed devices

with a biocompatible and slowly degradable macromer followed by curing the penetrated devices. Objects with high compression strengths are obtained in this process without sintering.

Based on the known biocompatibility of the used components, patient specific implants or scaffolds fabricated by this technology represent promising biomaterials for bone reconstruction even in load-bearing areas dominated by compressive stresses.

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References

1. S. V. DOROZHKIN and M. EPPLE, *Angew. Chem. Intern. Ed.* **17** (2002) 3130
2. W. G. Billotte, in ''Biomaterials: Principles and Applications'' edited by J. B. Park and J. D. Bronzino (CRC Press, Boca Raton, 2003) p. 21
3. A. Krajewski A. and RAVAGLIOLI in ''Integrated Biomaterials Science'' edited by R. Barbucci (Kluwer Academic/Plenum Publishers, New York, 2002) p. 189
4. J. O. HOLLINGER, J. BREKKE, E. GRUSKIN and D. LEE, *Clin. Orthop. Relat. Res.* **324** (1996) 55
5. M. Wang in ''Biomaterials and tissue engineering'', edited by D. Shi (Springer, Berlin, 2004) p. 1
6. W. SUN and P. LAL, *Comput. Methods Program Biomed.* **67** (2002) 85
7. K. F. LEONG, C. M. CHEAH and C. K. CHUA, *Biomaterials* **24** (2003) 2363
8. E. SACHLOS and J. T. CZERNUSZKA, *Eur. Cells Mater.* **5** (2003) 29
9. V. L. TSANG and S. N. BHATIA, *Adv. Drug Deliv. Rev.* **56** (2004) 1635
10. D. W. HUTMACHER, M. SITTINGER and M. V. RISBUD, *Trends Biotechnol.* **22** (2004) 354
11. V. K. POPOV, A. V. EVSEEV, A. L. IVANOV, V. V. ROGINSKI, A. I. VOLOZHIN and S. M. HOWDLE, *J. Mater. Sci.: Mater Med.* **15** (2004) 123
12. K. H. TAN, C. K. CHUA, K. F. LEONG, C. M. CHEAH, P. CHEANG, M. S. ABU BAKAR and S. W. CHA, *Biomaterials* **24** (2003) 3115
13. J. K. SHERWOOD, S. L. RILEY, R. PALAZZOLO, S. C. BROWN, D. C. MONKHOUSE, M. COATES, L. G. GRIFFITH, L. K. LANDEEN and A. RATCLIFFE, *Biomaterials* **23** (2002) 4739
14. T. DUTTA ROY, J. L. SIMON, J. L. RICCI, E. D. REKOW, V. P. THOMPSON and J. R. PARSONS, *J. Biomed. Mater. Res.* **66A** (2003) 283
15. T. H. ANG, F. S. A. SULTANA, D. W. HUTMACHER, Y. S. WONG, J. Y. H. FUH, X. M. MO, H. A. T. LOH, E. BURDET and S. H. TEOH, *Mater. Sci. Eng.* **C20** (2002) 35
16. E. SACHS, J. S. HAGGERTY, M. J. CIMA and P. A. WILLIAMS, 5. US Patent No. 5204055, 1993
17. T. DUTTA ROY, J. L. SIMON, J. L. RICCI, E. D. REKOW, V. P. THOMPSON and J. R. PARSONS, *J. Biomed. Mater. Res.* **67A** (2003) 1228
18. K. CIESLA and R. RUDNICKI, *Pol. J. Chem.* **61** (1987) 719
19. S. VOGT, Y. LARCHER, B. BEER, I. WILKE and M. SCHNABELRAUCH, *Eur. Cells Mater.* **4** (2002) 30
20. C. X. F. LAM, X. M. MO, S. H. TEOH and D. W. HUTMACHER, *Mater. Sci. Eng.* **C20** (2002) 49
21. A. WINTER, S. BREIT, D. PARSCH, K. BENZ, E. STECK, H. HAUNER, R. M. WEBER, V. EWERBECK and W. RICHTER, *Arthritis Rheum.* **48** (2003) 418
22. R. I. MARTIN and P. W. BROWN, *Adv. Cement Res.* **5** (1993) 119
23. L. C. CHOW, M. MARKOVIC, S. A. FRUKHTBEYN and S. TAKAGI, *Biomaterials* **26** (2005) 393