ABSTRACT
Additive manufacturing is an innovative manufacturing technique that can build complex porous scaffolds. One promising additive manufacturing technology is 3D printing (3DP) which can be used to build individual scaffolds out of ceramics for bone tissue engineering. However, 3D printed ceramic scaffolds have rather bad mechanical properties and are therefore in focus for improvement. The aim of this study was to improve the mechanical properties of 3DP scaffolds through infiltration with biopolymers. The hypothesis is that through infiltrating the ceramic scaffold, micropores are filled with the polymer leading to a composite with higher compressive strength. As a ceramic, hydroxyapatite powder was used to generate porous scaffolds with a 3D printing machine. The 3D printed scaffolds were sintered and infiltrated with two different biopolymers. Mechanical tests show an improvement on compressive strength. Infiltrated scaffolds have the potential to be used for the treatment of bone defects in load bearing regions.

KEY WORDS
Scaffolds, 3D printing, biopolymers, infiltration

1. INTRODUCTION
There are many applications were calciumphosphates are used as biomaterials [1]. The regeneration of damaged bone tissue is one of these applications in which calciumphosphate scaffolds can be used as a feasible treatment. Three-dimensional printing can be used to manufacture such implants with a defined outer shape based on CT data of the patient. Moreover, 3D printing allows to generate scaffolds with a complex internal structure similar to bone structures [2] and a high porosity between 50 % and 90 % [3]. Today calciumphosphates are approved as promising materials [4] to build scaffolds with the required properties. Scaffolds can be generated from hydroxyapatite (HA) or tricalciumphosphate (TCP) by 3DP. Both calciumphosphates offer different degrees of osteoclastic resorbability and solubility [5]. The printed and sintered calciumphosphate scaffolds can be used for guided bone tissue engineering with patient cells, seeded onto the scaffolds for a better compatibility in the organism.

3D printed scaffold feature relatively bad mechanical properties and cannot be used for the treatment of bone defects in load bearing region. Due to the high porosity of these sintered scaffolds, infiltration with biopolymers is a possibility to achieve better mechanical properties [6-8]. The aim of this study is to improve the mechanical properties of the 3D printed HA scaffolds by the infiltration with different biodegradable polymers.

2. MATERIALS AND METHODS
2.1 3D Printing Process
3D printing is an additive manufacturing technique that allows building physical scaffolds layer by layer. 3DP is a powder based process which starts with a stack of 2D bitmaps obtained from a sliced 3D dataset (CAD). The three dimensional printer uses these two dimensional bitmap files, that represent the printing matrices, to build a 1:1 physical model of the CAD data. The three dimensional part is created by layering and connecting successive cross sections of material with a printhead. After printing one layer, the working platform descents by the thickness of one layer and a new layer of powder is applied. The printing process reiterates until the whole part is finished. The printhead dispenses a certain amount of binder to the underlying powder which generates the pattern of the structure. The binder is used to glue the loose particles together and create the desired structure.
The scaffolds are surrounded by unbound powder which supports the printed object. After completion, the 3D printed green scaffolds are removed from the building platform. All unbound powder from the internal structure of the green scaffold is manually removed by airflow. A detailed description of the 3D printing process was published previously [9]. The calciumphosphate granulate HA SP 19 with an average particle size of 50 µm was obtained from BioCer Entwicklungs-GmbH (Bayreuth, Germany) and used for 3D printing of the scaffolds. As a binder, a water based solution with 20 wt% dextrin, 2.5 wt% sugar and 0.1 wt% citric acid was used. The 3D printed specimens were sintered for 2 hours at 1275° C in an electrically heated chamber furnace (Nabertherm, Germany) in atmosphere. The organic binder is removed by pyrolysis during the sintering process. Significant sintering shrinkage occurs by up to 30 % [10]. The sintered scaffolds obtained their final properties after the sintering process.

2.2 Scaffold design

Cylindrical scaffolds for compression testing were designed with 10 ± 0.5 mm diameter and 20 ± 1 mm height after sintering. All cylinders have a complex internal structure in means of horizontal and vertical cavities with a diameter of 500 µm after sintering. This internal structure provides adequate space for osteoblasts and vascular tissue in-growth as well as nutrient supply and waste removal of cells seeded on scaffolds.

2.3 Infiltration with biopolymers

The infiltration process adds the visco-elastic properties of the polymer to the rigid properties of the hydroxyapatite scaffolds. Different biodegradable polymers have been chosen for infiltration. The biopolymers used in this study were poly-ε-caprolactone (PCL) obtained from Sigma Aldrich Co., St. Louis, MO, USA, as well as poly-d-l-lactide-co-glycolide (PDLLGA) (RESOMER RG 858 S) obtained from Boehringer Ingelheim Pharma GmbH & Co. KG, Germany. PDLLGA has a molar ratio of 83:17 to 87:13 / d,l-lactide : glycolide. The solvent Trichlormethan/Chloroform ≥ 99 % (Carl Roth) was used as solvent. The biodegradable polymers were dissolved in the solvent. For the infiltration processing, the scaffolds were inserted into a glass tube. Afterwards every glass tube was filled with a 5wt% polymer solution and the scaffolds were infiltrated for 15 minutes. The samples were then dried for 24 hours in air.

2.4 Mechanical Characterization

Compression testing was performed on 3D printed HA scaffolds infiltrated with PDLLGA, PCL as well as uninfiltred specimens as seen in figure 1. The compressive strength of the scaffolds was determined in accordance to standard DIN EN ISO 604 with a uniaxial testing system Zwick/Roell Z0.5 from Zwick GmbH & Co. KG, Ulm, Germany. At least five specimens were tested at a speed of 2 mm/min.

Figure 1. Test setup for the compressive testing.

3. RESULTS

The organic solution has successfully infiltrated the micro pores of the scaffolds, leaving the interconnected macro pores (channels) open. Figure 2 shows scanning electron microscopy (SEM) images of the microscopic structure of an uninfiltred sample and an infiltrated sample with PCL, respectively.

It was found that the uninfiltred samples had an average maximum compressive strength of 1.6 ± 0.1 MPa. The results of the scaffolds infiltrated with PCL show an average maximum compressive strength 2.3 ± 0.3 MPa.
This is an improvement compared to the uninfiltrated samples of over 50% in load acceptance. The samples with the polymer PDLLGA show an even higher mechanical load acceptance. The average maximum compressive strength is 3.1 ± 0.3 MPa. The load acceptance is about 95% and 29% higher compared to the uninfiltrated and the PCL samples, respectively. Figure 3 shows an exemplary force-strain diagram of the samples, infiltrated with PCL and a load area of 75 mm².

Each sample has different breaking characteristics. Due to the polymers, the infiltrated samples keep their integrity better than the uninfiltrated samples. Figure 4 shows a comparison of the different samples after the compression test. These results show that the fabrication of HA scaffolds with a 3DP lead to higher compressive strength than the fabrication of HA/TCP scaffolds (average pore size: 500 μm) by a polyurethane (PU) foam replica method infiltrated with PLGA and a compressive strength up to 660 KPa [6]. Gburek et al. have shown comparative results of compressive strength with a similar approach of 3D printing and sintering TCP scaffolds [11]. In a review, a comparison of structural and mechanical parameters concerning various 3DP calcium phosphate scaffolds has been represented [12]. The results show that the compressive stresses of these scaffolds are in the range of the values of native bone tissue. According to a literature review, the compressive strength for cancellous bone is in the range of 2-12 MPa. However, the values are still far below that of cortical bone, which is in the range of 100-230 MPa [13].

3. CONCLUSION

Samples made from hydroxyapatite were generated with a 3D printing machine and post-processed through sintering and infiltration with biopolymers. The mechanical tests showed significantly higher values in compressive strength for the infiltrated samples than the uninfiltrated reference samples, as seen in figure 5. Due to the infiltration process, the compressive stresses of these scaffolds are in the range of cancellous bone tissue. To further improve the mechanical properties of hydroxyapatite ceramic based implants, the infiltration process can be optimized. The infiltration process, used in this paper, was realized by gravity and capillary forces alone. Furthermore, it is believed that repeated polymer infiltration could also lead to an improvement of mechanical strength. Scaffolds that are coated with biopolymers could also be enriched with drugs, signal molecules, growth factors, or other substances to further improve biological, chemical, physical or mechanical properties.
REFERENCES


