Fabrication of bone substitute material by Rapid Prototyping

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Abstract:
Bone tissue engineering has gained much attention in recent years. A key requirement in this field is the development of scaffold structures, on which cells adhere. This can be done by fabricating scaffolds by direct procedures like 3D-printing or by indirect procedures like casting.
With the 3D-printing process different structures were build up by using hydroxyapatite powder (HA) and a special binder material. Afterwards the printed 3D structures were sintered.
For the casting process molds have been made of different resins by stereolithography and other processes using polymers and waxes. These structures were filled by a suspension of HA. By heating the resulting polymer/ceramic composite to a specific temperature it is possible to combust the polymer or wax. By further heating the remaining body, the HA is sintered.
Compared to the 3D printing a better resolution can be obtained here. But there are restrictions regarding the ratio of polymer and the HA ceramic during the heating process which means a limitation for the level of porosity.

Introduction
In the field of biomedical implants attempts to design suitable systems have been made from different directions. One current research field focuses on tissue engineering. The research group Fortepro is developing a processes for fabricating bone substitute material by using rapid prototyping techniques. These implants are designed to replace bone defects at the

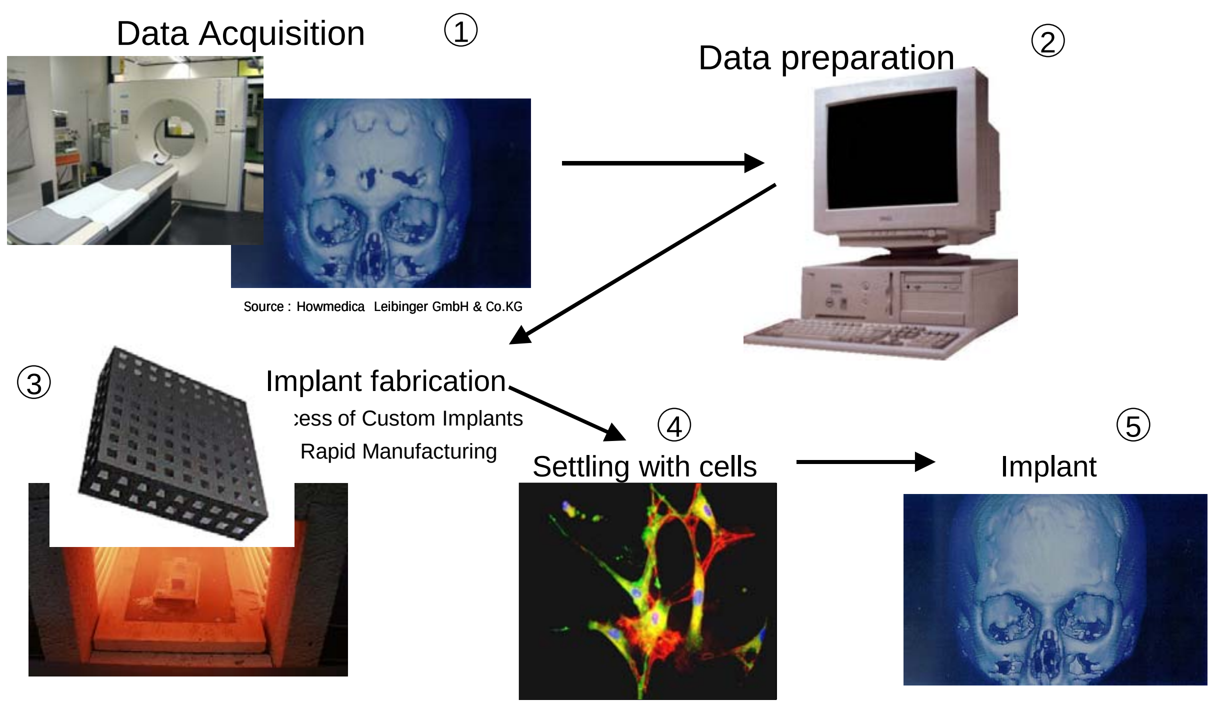


Figure 1. Principle process sequence

head and musculoskeletal system. They are made of hydroxyapatite (HA) and will be replaced by endogenous bone material after implantation by building new tissue. Figure 1 shows the complete process sequence. In a first step data from the defect is collected with computer tomography (CT). Then the three-dimensional geometry of the implant is designed with a special software and converted in a standard triangulated language (STL) file. With different RP processes the implant geometry is produced and later prepared with cells. Afterwards the prepared scaffold can be implanted.

A key requirement is the development of scaffold structures on which cells can adhere. The aim of the institute “Feingerätebau und Mikrotechnik” is to fabricate these scaffolds with controlled internal porosity.

Requirements on the implants

This study aims at the creation of a porous artificial extracellular matrix or scaffold to accommodate cells and guide their growth and tissue regeneration in three-dimensions (3D). These scaffolds should be created individually for each patient, with interconnected and controlled porosity and pore distribution. Furthermore the mechanical strength not only depends on the 3D structure of these scaffolds but also on the used process parameters for example the temperature profile during the sintering process.
An overview of the requirements on the 3-D structures are given in Figure 2.

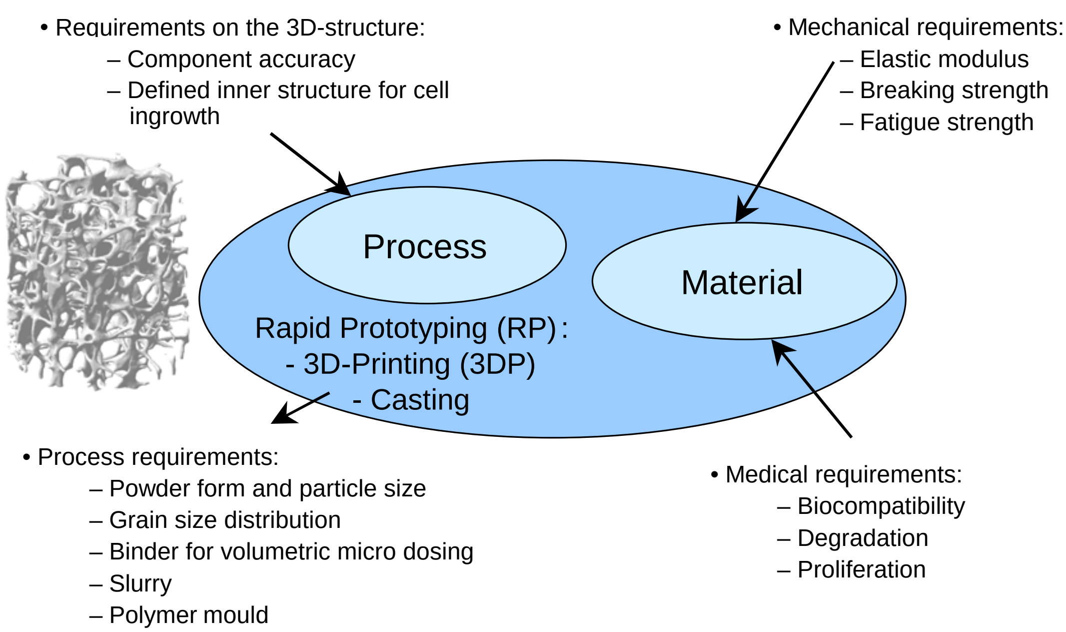


Figure 2. Requirements of the process

Part accuracy:

The process allows to generate patient individual implants. There is no need of post processing the implant by the medicines at the later surgery. Moreover, it is necessary for cell growth and flow transport of nutrients and metabolic waste that the defined inner structure and porosity can be produced consistent. The desired parts should have interconnecting pores with a size of about 400 to 600 µm for cell proliferation and the porosity should be around 50%.

Mechanical requirements:

Desired is an elastic modulus, breaking strength and fatigue strength with valves similar to those of natural bones. Our research group wants to develop an implant which can be handled by the medicines without exhaustive care and will serve for implantation in regions without high loading eg. the head region.

Medical requirements:

Biocompatibility is necessary for the implantation. Furthermore the scaffold structures should allow the infiltration of stem cells. This is necessary to replace the biodegradable artificial bone substitute material by autogenous material. Literature shows that the hydoxyapatite ceramics have been well established because of their high biocompatibility qualities. The degradation of the implant also depends on the used material and is so far not clarified completely.

Process requirements:

For the different processes the used materials have big influence on the results. For the 3D printing process the particle size and a spherical particle form are very important. Furthermore it is necessary to prevent the particles from agglomeration. A lot of printing experiments were done to investigate the influence of different HA and binder combinations. On the other hand the quality of the filling of the negative molds depends strongly on the prepared slurry. Different proportion of HA ceramic, deflocculant, solvents and water have big influence on the results. Moreover the slurry and the temperature profile can be matched to the different materials of the molds.

Processes 3D-printing

This process creates parts by a layered printing process with adhesive bonding, using HA powder as a base material. After loading the STL-file, which defines the geometry and the interconnecting pores, the software of the Rapid Prototyping machine slices the three-dimensional data into two-dimensional pictures according to the cross-sectional area of the object. Each layer of powder is selectively joined where the scaffold is to be formed by ink-jet printing. This process is repeated layer by layer until the green body of the scaffold is complete. Afterwards the powder which is not joined together is removed and the green body is sintered.

This printing process requires a special powder. The resolution depends on the form and particle size and the grain size distribution. The major problem is to choose an appropriate binder for micro dosing. The used hydroxyapatite powder is spray dried with particle sizes between 100–200µm diameter. The binder, which joins the powder particles, was also specially developed for this process. Each powder layer is about 200µm thick and finished with a counter rotating roll, which sleeks the HA. The micro dosing of the binder is realized with a piezoelectric drop on demand system or an electromagnetic valve. Therewith it became possible to dose drops with a diameter of 60-80µm.

The temperature profile for sintering only depends on the ceramic. Starting at room temperature the temperature is increased to 1250 °C at 100 °C/hour. This temperature is hold for about 1 hour min and then the ceramic is cooled down to room temperature with about 100 °C/hour.

Results 3D-printing

The first printed bodies have been fabricated without internal structure. These tests proved, that the green bodies can be sintered and led to an overview of the quality of the printing results by using different powders and binders. On scanning electron microscope pictures, (Figure 3) the sinter-necks between the agglomerates can be observed as well as the sintered powder-particles in the agglomerate. These results prove the feasibility of the process.

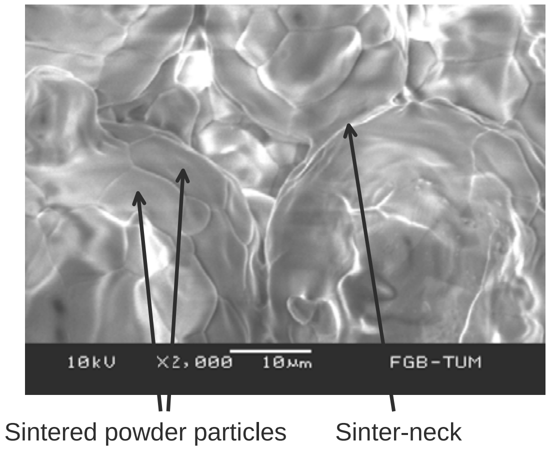


Figure 3. Sintered necks between the spray dried particles

Figure 4 (left) shows a printing result of a binder/ceramic combination where the printed plates were bended up at the edges and the edges themselves were damaged. The right side of this figure shows the results of a very good ceramic binder combination but with bad process parameters (speed, droplet size, amount of binder etc.).

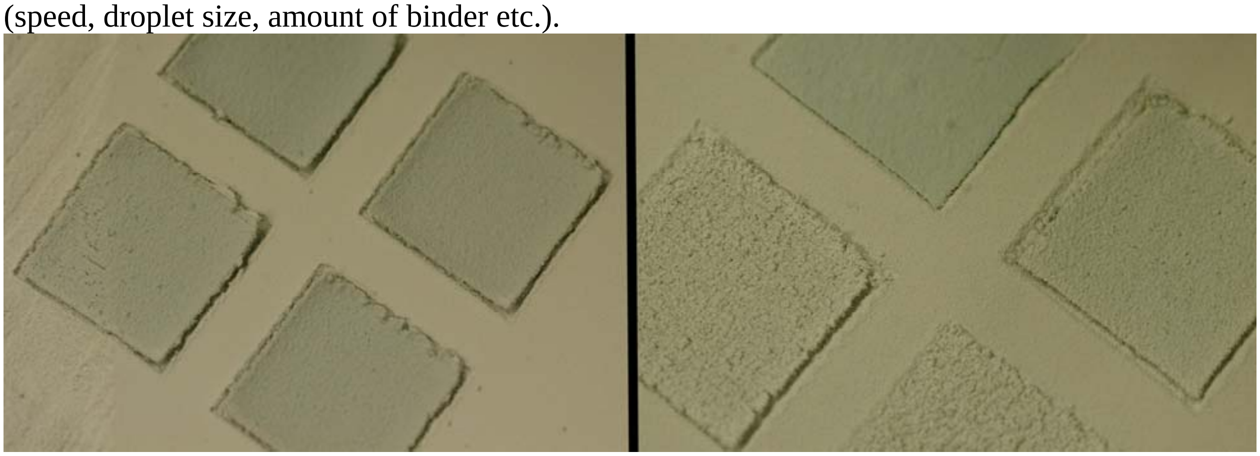


Figure 4. Printed HA ceramic plates with different process parameters

The next step was the production of scaffolds with defined inner structures in three dimensions. These parts are demonstrated in Figure 5. The size is 10mm x 10mm x 6mm which fits perfectly in cell seeding equipment and is made of rectangular beams with a cross section of about 0.5mm x 0.5mm. The strength of the printed parts (green bodies) is low but after sintering at 1250°C they are solid enough for handling. The powder-binder interaction influences the strength of the part and the behaviour of the wetted powder. The theoretical achievable strength and the strength of the printed scaffolds is still under investigation.

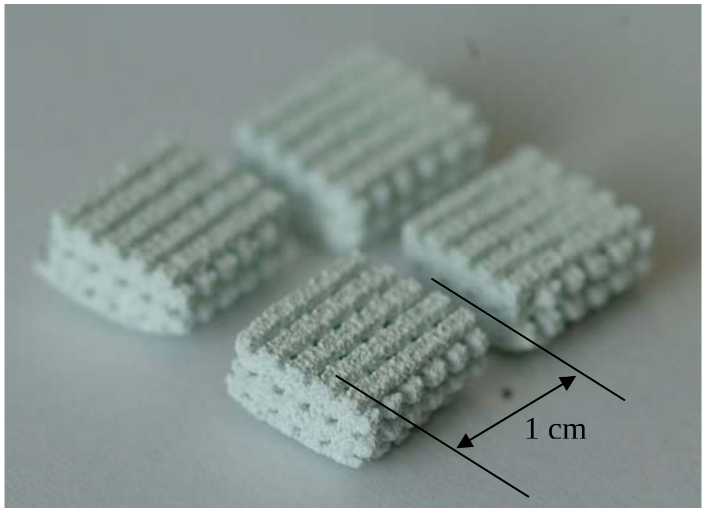


Figure 5. 3D-printed ceramic parts with internal porosity

The scanning electron microscope pictures (Figure 6) shows the cross section of a printed and sintered scaffold. The surface remains very rough and spherical ceramic particles are visible. At the fractured surface of the ceramic micro porosity is remaining.

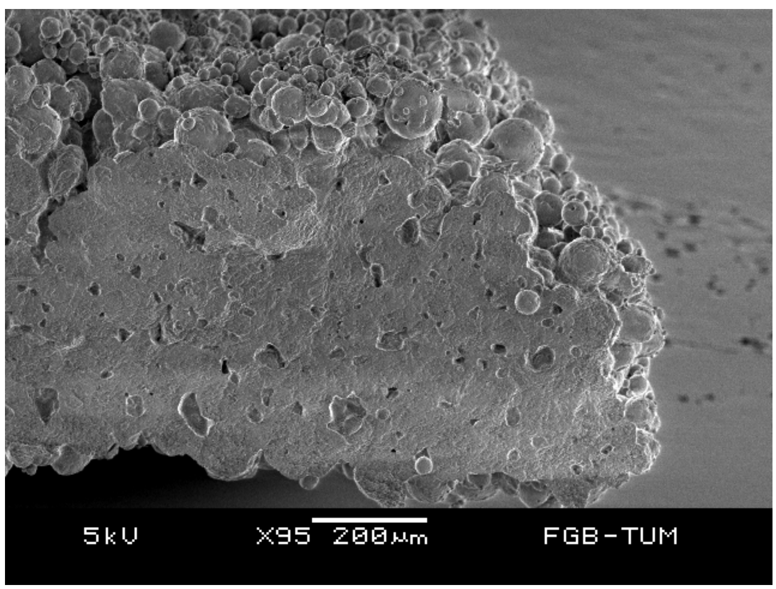


Figure 6. Micro porosity at the fractured surface of a printed HA-ceramic

Processes Casting

After presenting the results of the 3D printing process now the second RP process is described. Figure 7 shows the flow chart of the implant manufacturing process by casting. On the left side the process of the hydroxyapatite suspension preparation can be seen. This is done by the Friedrich Bauer Institute which is highly experienced in ceramic materials.

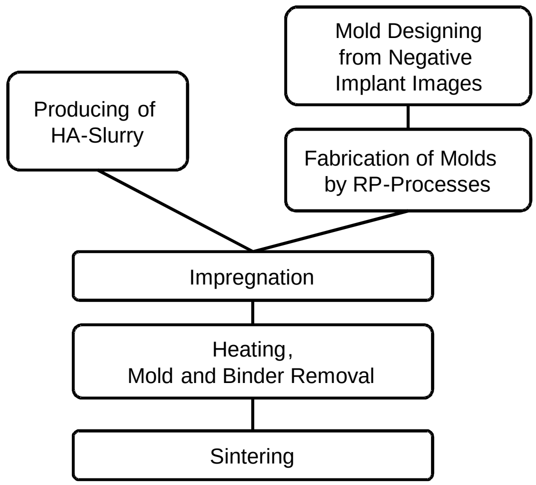


Figure 7. Flow chart of the manufacturing process

In this chapter the designing of molds from negative images of implants and the fabrication of the molds by RP processes is described. At the beginning of the project we focused on the stereolithography as the highest accuracy for getting the desired internal structure of the molds were expected. But also, the 3D Wax printing process is under investigation.
In the next step the prepared slurry is filled into the mold (Impregnation of the mold). Followed by a thermal process to remove the binder components from the suspension and to combust the mold. By rising the temperature up to 1250° Celsius the HA ceramic is sintered. In this cage the sinter profile has to be adjusted not only to the ceramic but also to the material of the mold. The heating for combusting the molds has to be done very slowly with holding the temperature at a special point. After the combustion of the mold is completed, the heating process for sintering is comparable with the temperature profile described for the 3D printing process. The critical points are the correct impregnation (filling of the molds) and the prevention of cracks in the ceramic during the first heating process, when the mold causes stress because of its thermal expansion.

Figure 8 demonstrates the feasibility of this process. The mold was fabricated by stereolithography and filled by a standard procedure of slip casting. The geometry of the parts is similar to the geometry used in the 3D printing process. The rough surface due to the layer by layer fabrication on the mold (left) can also be observed at the sintered scaffold (right). But the cracks in the structure led to a failure of the whole scaffold.

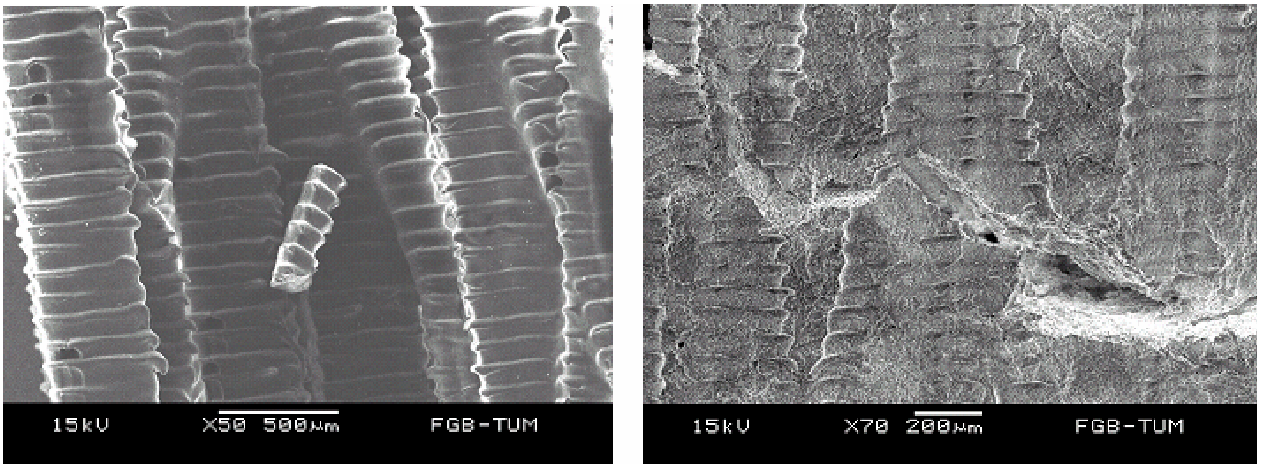


Figure 8: ESEM micrographs of the RP mold and the resulting scaffold

The interconnecting pores size for cell proliferation and the maximum wall thickness for degradation are limiting the design of the implants. To avoid cracks due to thermal stress, the ratio between resin and ceramic had to be decreased. This was realized by inserting hollow structures in the mold which are not filled by the ceramic slurry. This way, the stress on the ceramic during combusting the molds can be reduced. But still, there was a high reject due to defects. Therefore a new design of the parts and a better filling process were established.

Filling process

Figure 9 shows a micro computed tomography (µ-CT) of an impregnated mold prepared at the beginning of the project. The structure in dark-grey is the shot of the mold. The white color presents the ceramic. The origin of the cracks during the drying of the ceramic can be traced very well. The insufficient filling of the mold made it necessary to improve this process. By adding different tensides to the slurry and by investigating different filling methods the characteristics of the slurry have been changed.

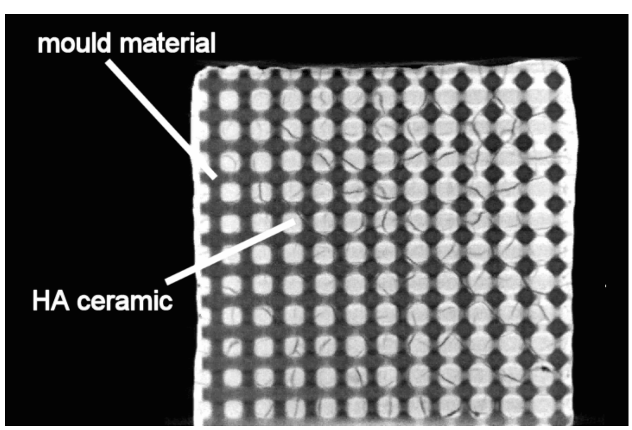


Figure 9. Mikro CT of an impregnated mold

The vacuum-, the centrifuge- and the pressure/vacuum methods have been investigated. The pressure/vacuum method (see Figure 10) solved most of the problems during the impregnation of the molds. A small chamber is filled with the HA slurry and the mold. By exceeding pressure on one side and low pressure on the other side of the chamber the small cavities could be reasonable filled without any air bubbles. By using low pressure it became possible to dry the slurry during the filling process. This way the swelling of the resin molds by the water-based slurry can be reduced.

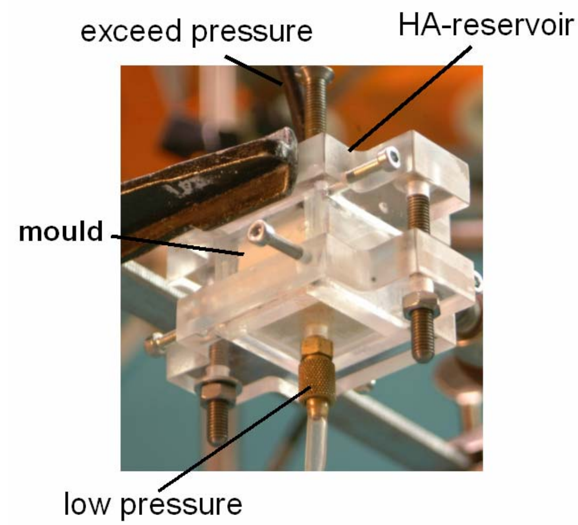


Figure 10. The exceed pressure / vacuum filling station

The used materials for the molds are standard resins for stereolithography and standard wax of 3D printing respectively. Therefore one major question is the influence of the toxic vapor on the ceramic parts during combustion the mold material. This is done by technical analysis like EDX or by analyzing the cell proliferation on the material.
EDX analysis of sintered ceramics produced with different stereolithography materials and waxes have been curried out (Figure 11).

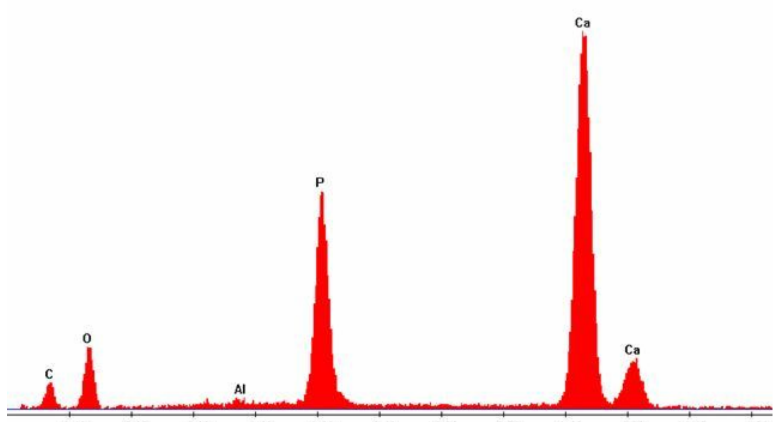


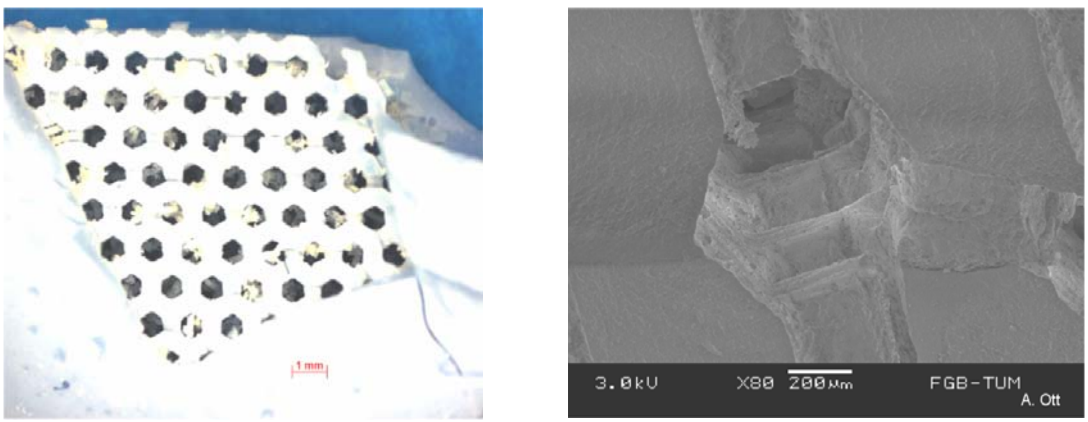
Figure 11. EDX spectrum of a molded ceramic scaffold

No toxic residua could be found at the surface of the ceramic parts. First experiments with cell proliferation on the surface of the sintered parts show good results. Next investigations with stem cells have to prove if the chosen wall and pore size are adequate for cell proliferation on the sintered implants.

Results of the casting process

The desired channel and wall size of less than 500µm can easily be achieved by using the SLS technology. But due to the thermal expansion and the exited stress it is necessary to keep a ratio of polymer to ceramic of less than 50%. Using a honeycomb structure with hollow blocks, allows the reduction of the polymer ratio dramatically. Therefore the filled mold is more suitable for the

Figure 12. Ceramic parts with internal porosity



thermal process. Moreover sharp edges can be reduced which is expected to be better for cell proliferation and the filling process.

In contrast with the rough surface of the 3D printing scaffolds the results by casting are very smooth. Figure 13 shows a ESEM micrograph of the scaffold with a very dense structure and nearly no micro-porosity. On the left side, the single layers of the molds are also visible in the scaffold.

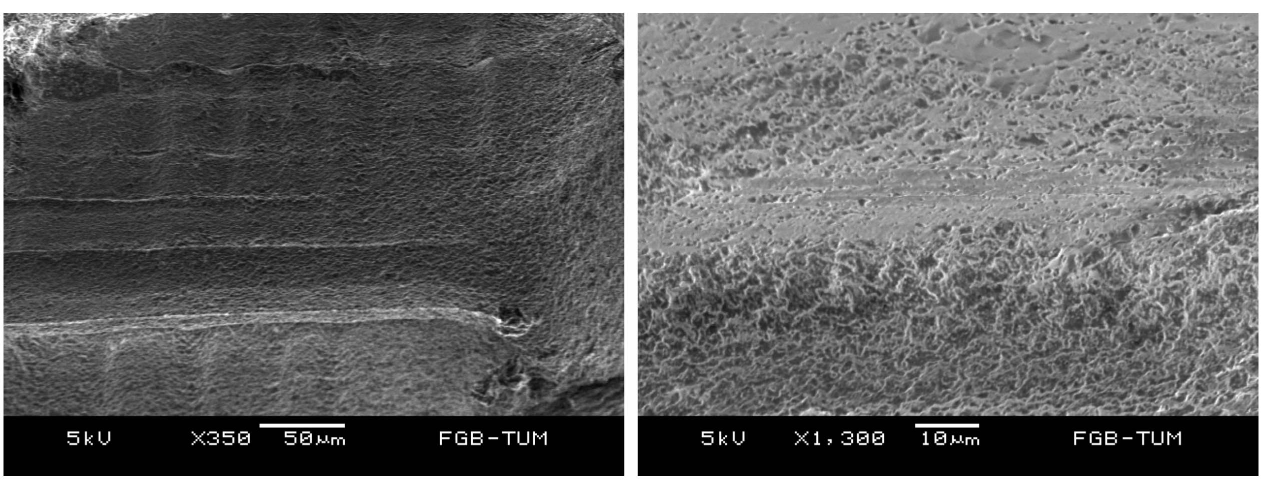


Figure 13. High-magnification SEM-micrograph of scaffold surface

Conclusion

Both RP processes are suitable for producing scaffolds for tissue engineering. First investigations proofed the possibility of cell proliferation on the surface of the structures. Further steps will include the habitation of the scaffold structures with stem cells and investigate the degradation of the ceramic and bone morphogenic protein induced osteogenesis.

Acknowledgement:

We thank the “Bayrische Forschungsstiftung” for founding the project Fortepro. Furthermore our industrial partners Alphaform Ag, Forschungszentrum Caesar, KL Technik and Siemens and the involved institutes: Chirurgische Klinik und Poliklinik, München; Forwiss, Passau; Friedrich Bauer Institut, Bayreuth; Hightech Forschungszentrum, München; Institut für Umformtechnik, München; Zentralinstitut für Medizintechnik, München

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