Image Based Analysis of Bone Graft Samples made by 3D Printing Using Conventional and Synchrotron-Radiation-Based Micro-Computed Tomography

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Abstract. Rapid Prototyping and especially the 3D printing, allows generating complex porous ceramic scaffolds directly from powders. Furthermore, these technologies allow manufacturing patient-specific implants of centimeter size with an internal pore network to mimic bony structures including vascularization. The non-destructive analysis of the internal structure of such 3D printed scaffolds provides important information. We used computed-microtomography as investigation method. Conventional and Synchrotron radiation based methods were tested and compared.

1. Introduction

The treatment of congenitally, traumatically or surgically caused bone defects is a major problem in reconstructive surgery [1]. Today autogenous materials are still used to fill larger cavities. These autologous implants need to be extracted and it is often difficult to find the suitable extraction site. Therefore, an urgent need exists for synthetic bone replacement with reasonable in-growth behavior and enhanced mechanical stability.

Rapid Prototyping (RP) technology provides a variety of generative fabrication techniques, which can produce real parts directly from computer data. In the last years RP-technologies have been used for medical purposes including the fabrication of anatomical models in oral and maxillofacial surgery. Such models play a crucial role in preoperative planning [2].

In RP-processes, models are built layer-by-layer based on a 3-dimensional (3D) dataset. Prior to fabrication the 3D dataset is sliced into a stack of layers to be processed by the RP machine. Depending on the used RP process, various materials can be used for fabrication.

For the production of ceramic parts, including bone grafts, 3D printing (3DP) is well suited. In 3DP, powder or granular material is selectively glued together layer by layer using ink jet printer technology [3]. Figure 1 shows an image of a Hydroxyapatite granulate, suitable for 3D printing.

Overall, RP-technology allows the fabrication of physical models with nearly no limitation in outer shape. Even more important, a great variety of internal structures can be realized. The data for the clinical morphology can be derived from computed tomography (CT) data, while the internal structure has to guarantee the cell in-growth and nutrition supply.

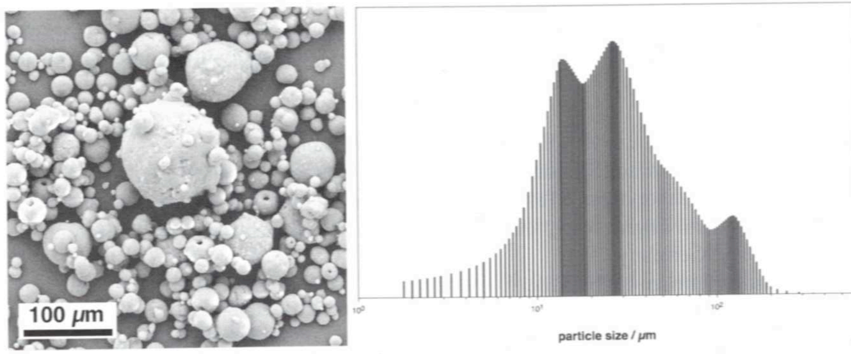

Fig. 1. Hydroxyapatite granulate for use in a 3 D printer and the corresponding particle size distribution. The multi modale size distribution of the grnaulate can easily be seen.

To optimize mechanical stability of 3DP scaffolds, the so-called green bodies undergo post processing, i.e. sintering or infiltration. It should be noted that Hydroxyapatite (HA) is a well-known synthetic biomaterial often used for bone replacement [4]. It provides a similar stoichiometry like the mineral phase of human bone. Beside the chemical and biological properties of the used building material, the internal structure of the scaffold is a key feature for a good bone graft. Interconnected pores having diameters between 100 and 500 µm are the prerequisite for the vascularization of an implanted scaffold [5]. Consequently, the internal structure of the scaffolds is one of the most important criteria for quality control as well as for design optimization.

The aim of this study is to analyze the internal structure of bone grafts fabricated by 3D printing using computer-micro-tomography. A comparison between conventional X-ray based and Synchrotron radiation based µCT is made and the limitation of the methods are discussed.

2. Materials and Methods

Scaffold Fabrication. Spray-dried Hydroxyapatite granules (HA) and a water based polymeric binder solution were chosen for this study [SPIE Paper]. These materials are specially conditioned for the use in a 3D printer. In this study we used simplified test geometries, which were constructed and prepared for 3D printing using the software tool Magics (Materialise, Belgium). The scaffolds had a diameter of 5 mm and a height of 9 mm and they posses internal channels with a diameter of about 1mm.

A 3D printing test setup [3] was used for scaffold fabrication. The flexibility of this 3D printing setup makes it possible to investigate both modified process techniques and different material combinations. Here, the 3D printer was used with a spatial resolution of 106 dpi and a slice thickness of 200 µm.

The 3D printed green bodies were freed from unglued granulate using an air blower. In this state the scaffolds are fragile and have to undergo a post processing procedure to obtain reasonably stable scaffolds. For final solidification the green bodies were sintered in air at 1250 °C for 3 hours.

X-Ray-Computed-Micro-Tomography. Micro focus Computed Tomography scans were done using an desktop µCT from Skyscan (model 1072).The voxel size of the reconstructed models (Feldkamp cone beam reconstruction) was 10.5 µm.

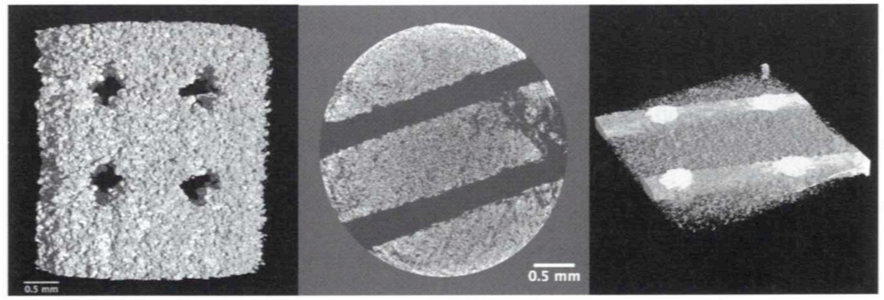

Fig. 2. 3D-visualisation of a µCT scan of a HA Scaffold (left image). The middle and right images illustrate segmentations of the scaffolds internal structure in 2D (middle) and 3D (right).

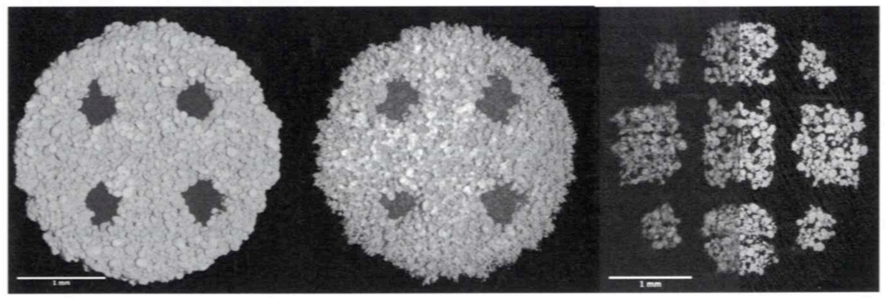

Fig. 3. 3D Visualisation of a sample scaffold based on SRµCT (left) and conventional µCT data (middle). The right image shows the resolution difference between both experiments using a slice image. For better illustration the image shows slices representing the same area of the scaffold.

Synchrotron Based Computed Micro Tomography. Synchrotron based computed-micro-tomography (SRµCT) was performed at the beamline BW 2 at HASYLAB (DESY, Hamburg, Germany) in absorption contrast mode [6] using the photon energy of 24 keV. The voxel length corresponds to 4.3 µm. The spatial resolution was determined using the modulated transfer function of a gold edge to

6.4 µm [7]. 3D data were reconstructed out of 720 projections by the filtered back-projection algorithm [8].

Image Analysis. For image analysis the slice images were cropped using the software AnalySIS (Soft imaging Systems, Münster, Germany). Line profiles for binarization were calculated using the same software package. The distance transforms for quantitative 3D porosity characterization were obtained by the means of the Gigatools software [9].

Results and Discussion

Qualitative Results. Figure 2 shows the visualization of a 3D-printed and sintered HA bone graft. The dataset was gathered using conventional X-ray µ-CT. The middle image of figure 2 shows a selected slice of the dataset while the left and right image represent 3D reconstructions of the dataset. The internal channel network as well as a structural defect of the scaffolds interior can easily be seen in the 2D representation. Furthermore the image allows a qualitative estimation of the scaffolds porosity. This is important to evaluate the influence of the size distribution of the granulates used for 3D printing. A bimodal or multimodal distribution of the granules is desirable to optimize the stability and the porosity of the 3D printed scaffolds.

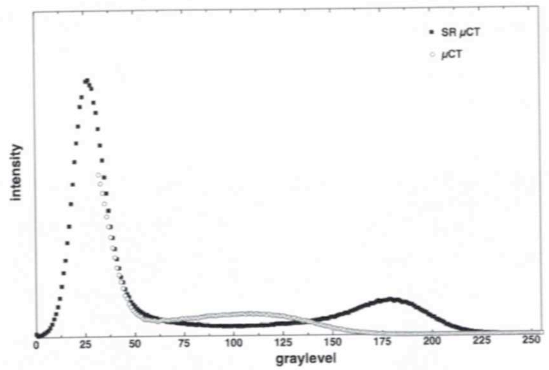

Fig 4. Histograms of one selected slice image. The circles represent the conventional µCT-scan while the filled squares are derived from the SRµCT dataset.

In summary conventional X-ray µCT is a versatile tool for nondestructive qualitative sample investigation, which allows the easy detection of structural failures in the µm scale caused by the 3D printing process or during the final sintering step.

Figure 3 and 4 on the other hand demonstrate the limits of conventional X-ray µCT for a quantitative porosity analysis. The left and middle image are 3D reconstructions of the same scaffold using synchrotron µCT (left) and conventional X-ray µCT (right) respectively. The enhanced contrast and resolution of the synchrotron based dataset resolves the single granules of the HA

building material down to their internal structures, which lie in an size range below 1 µm. The right image in figure 3 illustrates a direct comparison between the synchrotron and an X-ray based µCT scan of the same scaffold. Here the synchrotron data provide a much better density contrast and a higher detail resolution. The corresponding histograms are depicted in figure 4. In case of X-ray µCT a reliable separation of between material and air is not possible whereas in case of synchrotron µCT the two peaks for air and material is difficult but possible [10].

Quantitative Results. In order to quantify the effect of sintering on the internal scaffold structure, information about the porosity of the scaffolds is needed. To achieve the mean pore diameter the ratio of volume to surface has to be calculated. Since the extraction of the surface is hardly detectable for complex scaffolds like the ones used for this study, we calculated 3D distance transform, an alternative approach to gather porosity information. In this method the minimal distance of each voxel to material is determined [9].

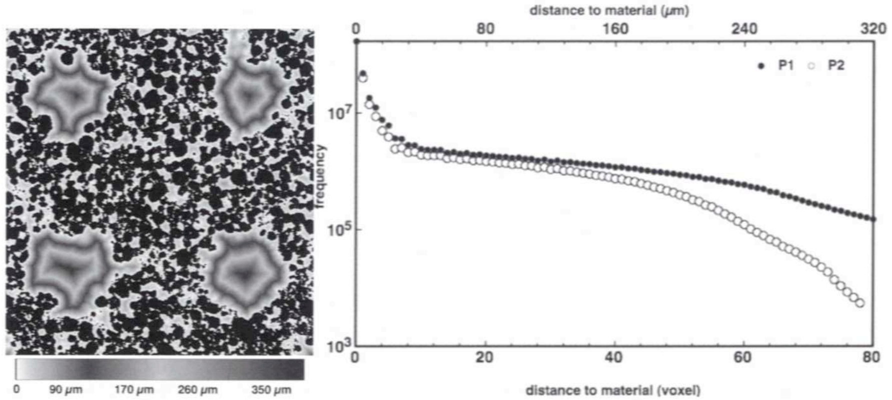

Fig. 5. 3D Distance transform map of a selected region of a sintered bone graft made by 3D printing. The right figure shows the distance to material distribution of the scaffold in before and after sintering.

Figure 5 shows the 3D distance map (left) of a representative slice and the distribution function (right). This demonstrates the effect of sintering on the internal structures. A significant difference between both samples is visible for distances above about 30 voxels (120 µm). The total porosity of both scaffolds is reduced from 52% to 49% during sintering.

Conclusion

In conclusion, image based analysis of the internals scaffold structure of 3D printed-printed bone grafts is a powerful too both in qualitative and quantitative means. Conventional X-ray based µCT can be used for the analysis in scale down to 100 µm or for the qualitative estimation of the porosity. So Conventional µCT is ideal for every days quality analysis. Here the experimental effort of synchrotron-based measurements is overdone. For more sophisticated tasks like a quantitative interpretation of the results, synchrotron based experiments allow investigations on the µm scale. These results are important for the optimization of the scaffolds internal geometry as well as the optimization of the compromise between mechanical stability and porosity.

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