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Non-destructive three-dimensional evaluation of a polymer sponge by micro-tomography using synchrotron radiation

Bert Müller [a,c,*], Felix Beckmann [b], Marius Huser [c], Fabrice Maspero [a,d], Gábor Székely [c], Kurt Ruffieux [d], Philipp Thurner [e], Erich Wintermantel [a]

[a] *Biokompatible Werkstoffe und Bauweisen, Eidgenössische Technische Hochschule Zürich, Wagistrasse 23, CH-8952 Schlieren, Switzerland*
[b] *Hamburger Synchrotronstrahlungslabor HASYLAB am Deutschen Elektronen-Synchrotron DESY, Notkestrasse 85, D-22607 Hamburg, Germany*
[c] *Institut für Bildverarbeitung, Eidgenössische Technische Hochschule Zürich, Gloriastrasse 35, CH-8092 Zürich, Switzerland*
[d] *Degradable Solutions AG, Wagistrasse 23, CH-9852 Schlieren, Switzerland*
[e] *Eidgenössische Materialprüfungs- und Forschungsanstalt, Überlandstrasse 129, CH-8600 Dübendorf, Switzerland*

Abstract

X-ray micro-tomography, a non-destructive technique is used to uncover the complex 3-D micro-architecture of a degradable polymer sponge designed for bone augmentation. The measurements performed at HASYLAB at DESY are based on a synchrotron radiation source resulting in a spatial resolution of about 5.4 μm. In the present communication we report the quantitative analysis of the porosity and of the pore architecture. First, we elucidate that synchrotron radiation at the photon energy of 9 keV has an appropriate cross section for this low-weight material. Modifications in sponge micro-architecture during measurement are not detected. Second, the treatment of the data, an amount of 2.5 Gbyte to generate binary data is described. We compare the 3-D with the 2-D analysis in a quantitative manner. The obtained values for the mean distance to material within the sponge calculated from 2-D and 3-D data of the whole tomogram differ significantly: 12.5 μm for 3-D and 17.6 μm for 2-D analysis. If the pores exhibit a spherical shape as frequently found, the derived mean pore diameter, however, is overestimated only by 6% in the 2-D image analysis with respect to the 3-D evaluation. This approach can be applied to different porous biomaterials and composites even in a hydrated state close to physiological conditions, where any surface preparation artifact is avoided.

Keywords: Biodegradable scaffold; X-ray microtomography; Synchrotron radiation; Three-dimensional image analysis; Porosity

1. Introduction

Bone forms the skeleton and stabilizes the human being mechanically. It is characterized by remodeling. This process implies transport by cell migration and cell supply with nutrients. Consequently, the degradable material that is intended to fill a larger bone defect should be porous to allow cell migration and supply. The size and the architecture of the pores have to be adjusted to optimize the transport processes. Usually the mean pore diameter is used to characterize the scaffold. Typical minimal values for the pore diameter, which enable cell migration, given in the literature are between 40 and 200 μm [1–3]. It becomes obvious, that only a 3-D method for the structural characterization with a spatial resolution better than 10 μm in all of the three directions is suitable to characterize and finally optimize degradable scaffolds for bone augmentation.

A promising material, which is degradable under physiological conditions, is the PLGA co-polymer made of poly-lactic acid (PLA) and poly-glycolic acid (PGA). Varying the ratio between PLA and PGA, the degradation kinetics can be adjusted. Since the mechanical bulk-properties of PLGA are limited and change during degradation, porous structures have to be optimized not only with respect to biological responds but also with respect to mechanical stability. Therefore, different ways are developed to produce porous degradable scaffolds [4–11]. Here, we do not discuss the advantages and disadvantages of the different fabrication processes but it should be noted that the sponges realized have a different amount of porosity, have a

* Corresponding author. Tel.: +41-1-633-6190; fax: +41-1-632-1199
E-mail address: bmueller@vision.ee.ethz.ch (B. Müller).

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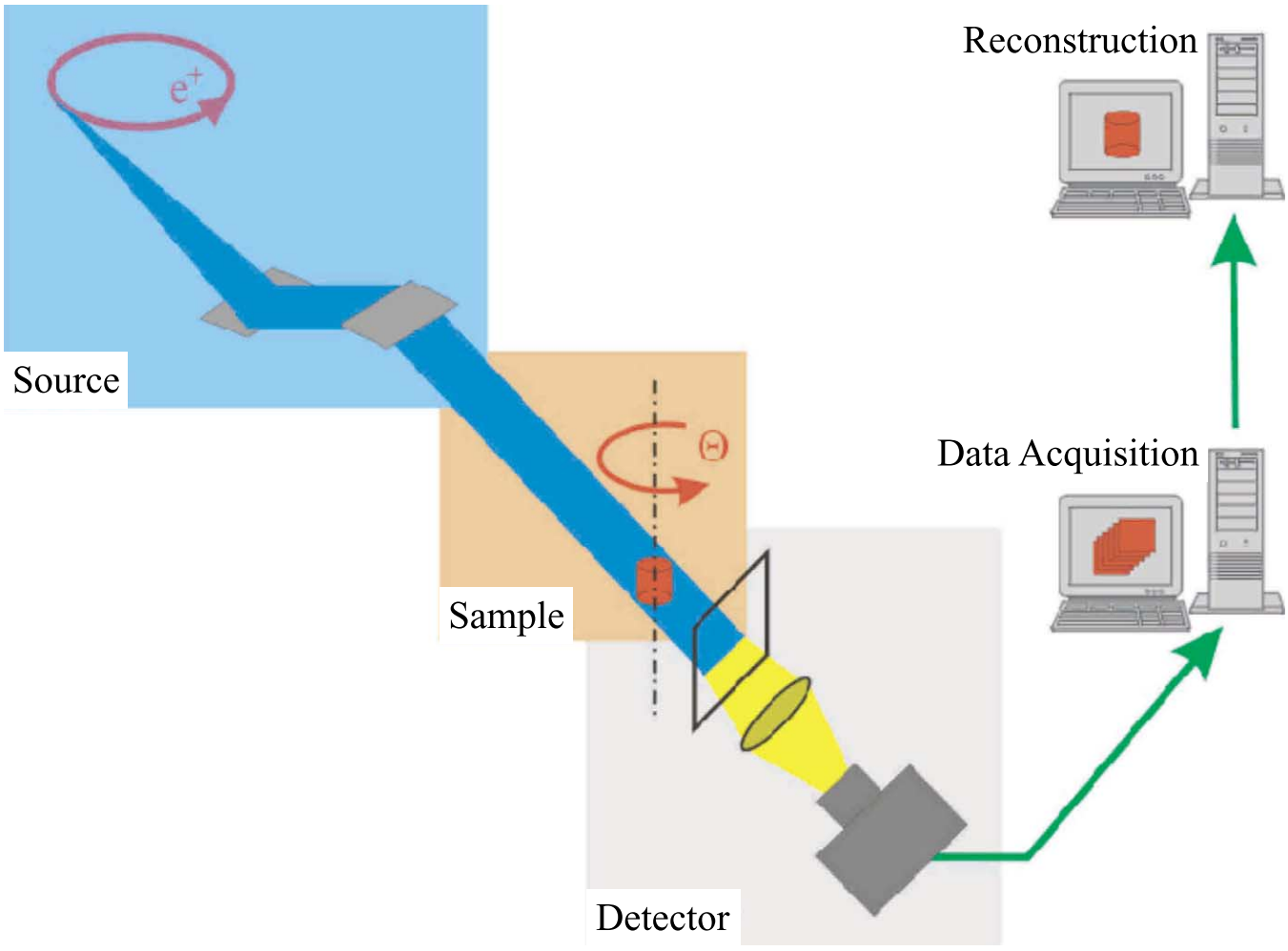

Fig. 1. Scheme of the experimental set-up for synchrotron-radiation-based X-ray μCT. The X-ray source produces a monochromatic beam with the photon energy adjusted to the sample including its size and composition. The sample is mounted on a rotation stage to be precisely rotated by at least 180°. The projections are recorded by the detection unit and, finally, the sample is reconstructed.

certain amount of closed porosity not accessible for the cells and yield various pore geometries. Classical porosity measurements are based on Hg-porosity, a method which works quite well for diameters below 10 μm. Pore diameters larger than about 10 μm are in general quantified by image analysis from histological cuts. A fully 3-D analysis is impossible because the spatial resolution in the third direction is lost and preparation artifacts frequently occur especially for composites. Note the sponge can be regarded as a composite of material and air. A promising alternative is tomography based on X-rays or visible light. Using light the sample has to be semitransparent often not fulfilled for scaffolds of centimeter size. The spatial resolution for classical X-ray tomography based on bremsstrahlung sources is limited to about 15 μm, not enough to quantify the pore architecture and forecast cell migration properties. Therefore, synchrotron-radiation-based micro-tomography [12] should be best suited to characterize the micro-architecture of scaffolds for bone augmentation quantitatively.

2. Synchrotron-radiation-based micro-tomography

Computed X-ray microtomography (μCT) is known to be a unique technique for the non-invasive, non-destructive 3-D characterization of materials in medicine, material science, and biology. A representative experimental setup for synchrotron-radiation-based μCT is schematically shown in Fig. 1. The synchrotron radiation — a kind of X-rays — originates from the accelerated motion of charged particles having a velocity close to the speed of light in particle accelerators or storage rings. For example, at beamline BW 2 at HASYLAB (DESY) positrons with a kinetic energy of 4.5 GeV, are accelerated by a series of magnets (56 pole wiggler), which give rise to a broad white band X-ray

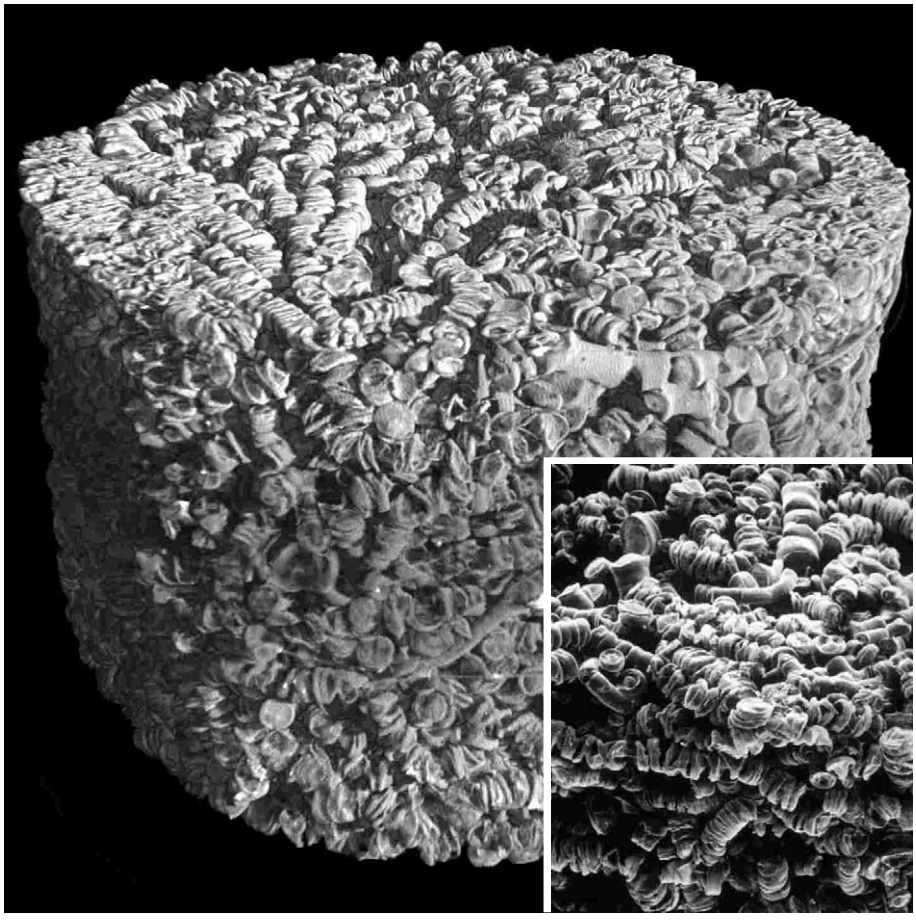

Fig. 2. μCT and SEM images (inset) of the outer shape of a PLGA on almost identical scale. The scaffold has a diameter of 5.0 mm and a height of 3.6 mm. The images demonstrate the complex structure of the porous sample.

beam of high intensity. The fixed exit double crystal monochromator, Si (111), selects an energy range of about 10^{-4} between 8.0 and 24.5 keV from the white spectrum. This way the X-ray source becomes tunable, and the energy can be adjusted to produce the maximal projected absorption of 2 [13]. The sagittally bent second crystal is used to reduce the beam divergence and to provide an approximately horizontal parallel beam 10.0 mm wide and 3.5 mm high.

Synchrotron radiation μCT is based on the precise rotation of the sample with respect to source and detector. For the present study we have chosen 720 projections within an angular range of 180°. Reference images without sample have been recorded to eliminate intensity inhomogeneities and variations of the X-ray beam. Repositioning of the sample with 1 μm precision is necessary. The projections of the parallel beam are detected using a fluorescent screen made of a $CdWO_4$ single crystal 200 μm thick. The optical images are recorded by the use of a video-camera lens with a focal length of 25 mm in front of the CCD chip, 1536×1024 pixel each 9×9 μm^2 (Kodak KAF 1600) with 14 bit digitalization at a frequency of 1.25 MHz (KX 2, Apogee Instruments).

To demonstrate the power of μCT a porous PLGA scaffold of cylindrical shape (diameter 5 mm, height 3.6 mm) has been fabricated according to the procedure given in [11]. The measurements were performed with a monochromatic X-ray beam having the photon energy of 9 keV. The spatial resolution has been experimentally determined to be 5.4 μm on the basis of the modulation transfer function [14,15]. The 3-D structure was finally reconstructed from 720 2-D projections using the BKFIL-procedure [16].

3. Results and discussion

The total porosity of a material is given by the ratio of the pore volume to the total volume of a representative sample. Therefore, the total porosity of a cylindrically shaped sample is simply obtained by the measurement of its diameter, height, and mass. Only the density of the compact material has to be known. This method termed

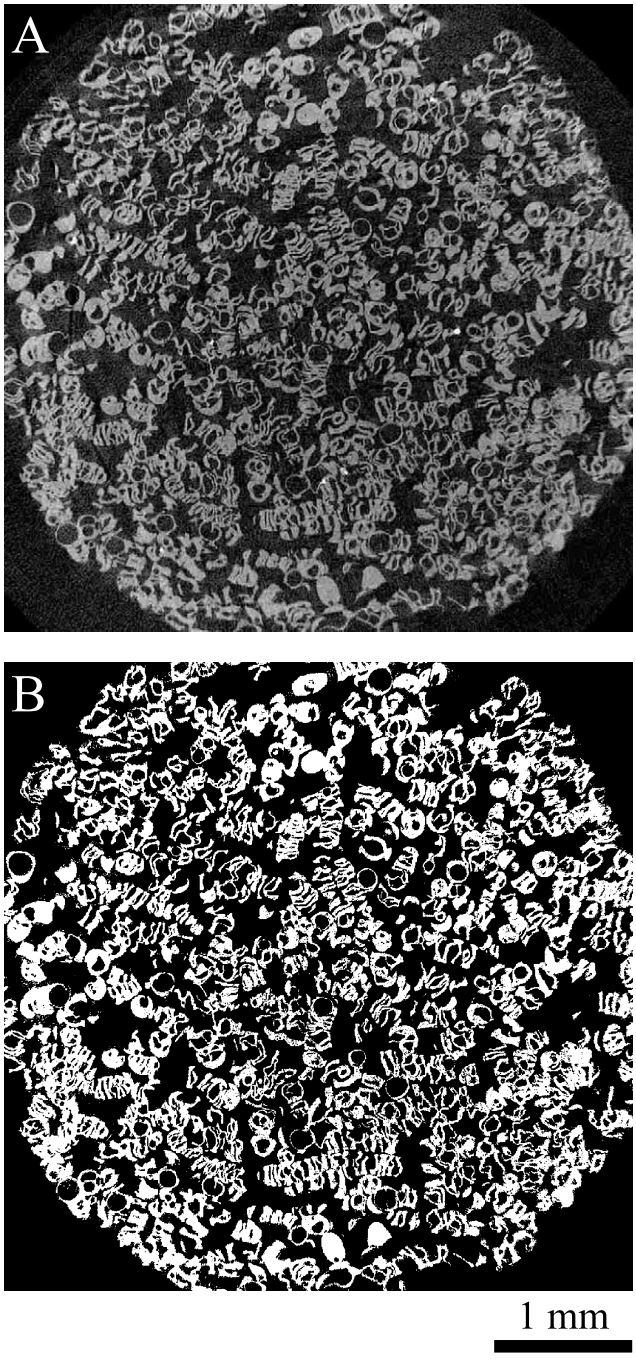

Fig. 3. Slice of the tomogram obtained from the PLGA scaffold with a voxel size of 3.8 μm at a photon energy of 9 keV. (A) raw data (8 bit), (B) binary data after thresholding and filtering.

gravimetry belongs to the integral techniques. The distinction between open (interconnected) and closed porosity is impossible. A common technique to analyze integral parameters of porosity of solid state materials is Hg-porosimetry. The incompressibility of mercury allows the determination of the pore sizes distribution. The applicability of Hg-porosimetry, however, is limited to pore sizes below ~ 10 μm for interconnected pores [11]. Thin walls of closed pores can be destroyed during

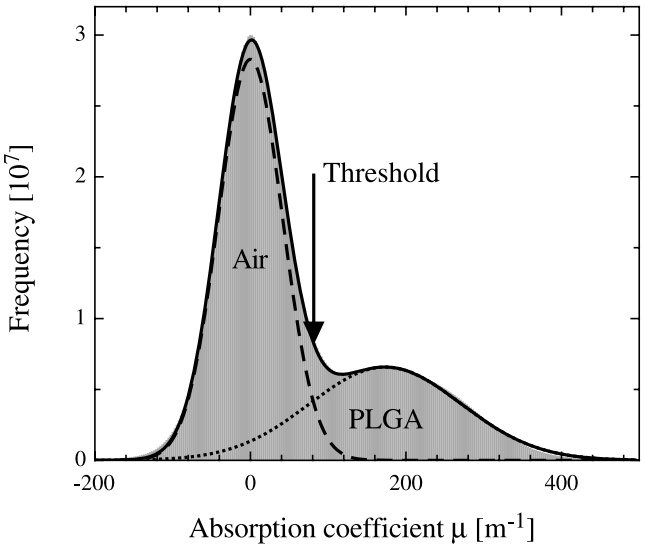

Fig. 4. Histogram of the absorption used to choose the threshold between air and polymer. Air and polymer gives rise to a Gaussian broadening as a result of partial volume effects and scattering at internal and external interfaces as well as within the detection unit.

measurement. Hence, Hg-porosimetry produces only a rough estimate.

Imaging techniques such as Scanning Electron Microscopy (SEM) easily provide the surface morphology of the sample with high lateral spatial resolution. The 3-D micro-architecture of porous materials, however, is only destructively accessible (micro-section, 3-D histology) as a result of the high cross section of electrons. The histological images have a high lateral resolution (~ 10 nm) but the resolution in the third dimension is hardly better than 100 μm. Furthermore, the images occasionally involve artifacts due to the surface preparation, especially for fragile composites. The same applies to methods based on light such as Confocal Laser Scanning Microscopy (CLSM) if non-transparent samples are investigated. The cross section of X-rays, however, is appropriate to non-destructively uncover the 3-D structure of porous materials. By the use of a synchrotron radiation source a resolution of a few micrometers is routinely accessible as shown by the porous PLGA scaffold represented in Fig. 2. The SEM image on identical scale, integrated as inset, obviously provides the same morphology than μCT. Therefore, significant modifications of the morphology of the polymer scaffold due to the intense X-rays are excluded.

Although the spatial resolution is reasonable (5.4 μm), it gives rise to a significant amount of partial volume effects. Furthermore, the scattering at the external and internal interfaces and within the X-ray detector results in a considerable background noise as shown in Fig. 3A by a representative tomographic slice. Quantitatively, the noise is illustrated in the histogram of Fig. 4. The two peaks in the absorption spectrum belong to air and material, respectively. Both peaks exhibit a Gaussian

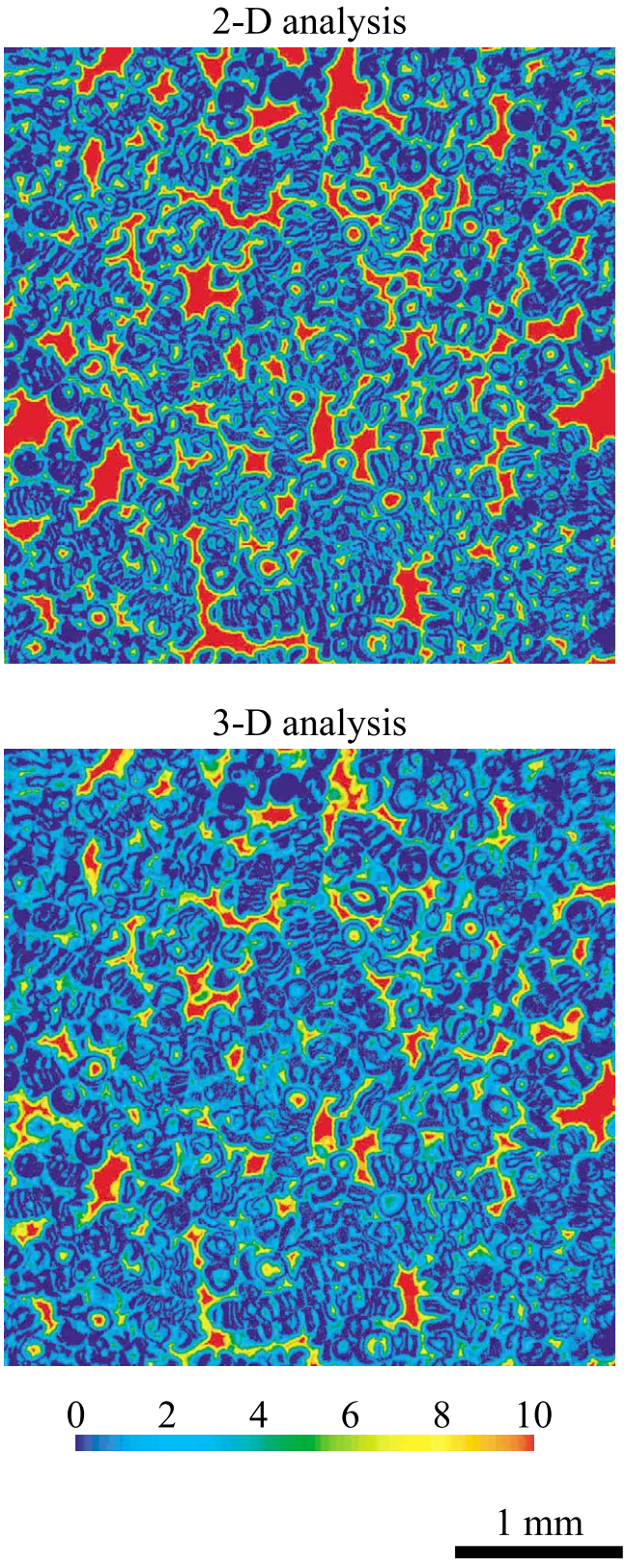

Fig. 5. The comparison of the 2-D and 3-D distance map of a part of one representative slice demonstrating the larger distance to material for the 2-D than for the 3-D analysis by the red-colored areas.

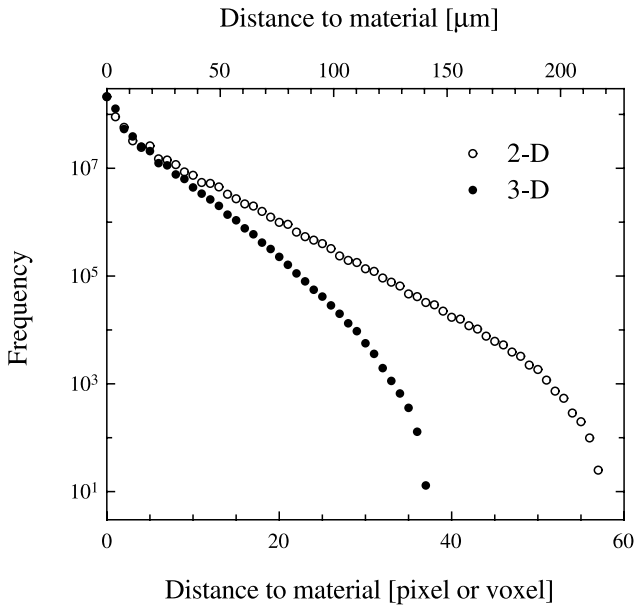

Fig. 6. The distance distribution of the porous PLGA scaffold from 2-D and 3-D analysis. The values are rounded to obtain equidistant distributions for 2-D and 3-D analysis, which can be directly compared.

shape. Since the value for air is set to zero, negative values occur. First, we have chosen a threshold to generate binary images suppressing the visualization of any partial volume effect. Here, the threshold is set to the crossing point of the two Gaussians determined from the combined fit on the basis of the Levenberg–Marquardt algorithm using the ProFit computer code. However, a significant amount of small particles still exists within the 'air'. The same applies to 'small pores' within the polymer. Therefore, we have removed the objects smaller than 100 voxel that are not connected to material. Not connected is defined as at least one voxel distance from the object to the material. Subsequently, the tomogram is inverted and the same algorithm is applied to the pores. Although this filtering procedure belongs to empirical approaches, it seems to be reasonable. Integral values such as total porosity are not affected. The result of the filtering is given in Fig. 3B by a representative slice. These binary data are used to evaluate the pore architecture in a quantitative manner.

It is often desired to extract the surface of the scaffold. The ratio of volume to surface gives the mean pore diameter. For a complex structure, i.e. our scaffold, however, the surface is hardly detectable as discussed and a definition of the surface is rather arbitrary. A better approach to characterize the pore architecture seems to be the distance mapping. Here, the minimal distance of each voxel to material is determined. This value is the color label of the distance map given in Fig. 5. Dark blue means 'zero' or material. Identical labeling for 2-D and 3-D analysis is obvious. For the labeling of pores, however, the 2-D analysis gives only an upper

limit, because material can be present above and below the pixel in a distance closer than in the 2-D plane. Consequently, the mean value for the distance transform is considerably larger for the 2-D analysis than for the 3-D analysis as shown quantitatively in Fig. 6. In the case of 2-D analysis, a mean distance to material of 17.6 μm is derived, whereas for the 3-D analysis the mean distance to material corresponds to 12.5 μm. Hence, we conclude a fully 3-D method is necessary to characterize the pore architecture quantitatively by the distance transform.

Mean distance to material is a quantity not common in medicine and biology. More often the mean pore diameter is used to quantify the pore micro-architecture. Therefore, it is desirable to link the mean distance to material with the mean pore diameter. Here, however, the pore shape has to be known. Since there exist a variety of pore shapes within the complex scaffold, we assume one of the simplest cases of spherical pores frequently present (cp. Fig. 3).

In the 2-D case a flat disc with radius R has to be considered to find the mean pore diameter D^{2D}. The mean distance to material for flat discs $R_M^{2D}$is simply:

$$R_M^{2D} = \frac{\int\int (R - r) r \, d\varphi \, dr}{\int\int r \, d\varphi \, dr} = \frac{R}{3} = \frac{D^{2D}}{6} \quad (1)$$

Thus, the mean pore diameter derived for pores of spherical shape from the mean distance to material corresponds to 106 μm. For the 3-D evaluation, spheres with radius R lead to a mean distance to material R_M^{3D}:

$$R_M^{3D} = \frac{\int\int\int (R - r) r^2 \sin\vartheta \, d\vartheta \, d\varphi \, dr}{\int\int\int r^2 \sin\vartheta \, d\vartheta \, d\varphi \, dr} = \frac{R}{4} = \frac{D^{3D}}{8} \quad (2)$$

Here, a factor of 8 is found giving rise to a mean pore diameter D^{3D} of 100 μm. This means that the 2-D analysis overestimates the mean pore diameter only by 6%. The result supports the intuitive opinion in medicine and biology that the error in the determination of the mean pore diameter by application of 2-D techniques is rather small.

In conclusion, synchrotron-radiation-based μCT is an appropriate method to quantify the micro-architecture of porous materials. The optimal cross section adapted to the given sample diameter and to the kind of material and its total porosity can be adjusted by the tunable, monochromatic X-ray source. Consequently, low weight materials such as polymers or organic mater can be visualized with reasonably small artifacts. Nevertheless, a significant amount of noise originating from partial volume effects, scattering at internal and external interfaces as well as within the detection unit has to be removed by empirical procedures. The filtered tomograms, however, can be quantitatively evaluated to extract data relevant for the characterization and optimization of scaffolds in tissue engineering.

References

[1] Mikos AG, Sarakinos G, Lyman MD. Biotech Bioeng 1993;42:716–23.
[2] Mooney DJ, Kaufmann PM, Sano K. Transplant Proc 1994;26:3425–6.
[3] Ishaug SL, et al. J Biomed Mater Res 1997;36:17–28.
[4] Mikos AG, et al. Polymer 1994;35:1068–77.
[5] Agrawal CM, Kennedy ME, Micallef DM. J Biomed Mater Res 1994;28:851–9.
[6] Whang K, Thomas CH, Healy KE. Polymer 1995;36:837–42.
[7] Mooney DJ, et al. Biomaterials 1996;17:1417–22.
[8] Athanasiou KA, Schmitz JP, Agrawal CM. Tissue Eng 1998;4:53–63.
[9] Harris L, Kim B, Mooney D. J Biomed Mater Res 1998;42:396–402.
[10] Nam YS, Park TG. Biomaterials 1999;20:1783–90.
[11] Maspero F, et al. J Biomed Mater Res 2002;61.
[12] Bonse U, Busch F. Prog Biophys Mol Biol 1996;65:133–69.
[13] Grodzins L. Nucl Instrum Method Phys Res 1983;206:541–5.
[14] Schneiders JN, Bushong SC. Med Phys 1978;5:31–3.
[15] Müller B, et al. Nondestructive three-dimensional evaluation of biocompatible materials by microtomography using synchrotron radiation. In: Bonse U, editor. Developments in X-ray Tomography III, Proceedings of SPIE, vol. 4503, 2002:178–88.
[16] Herman GT. Image reconstruction from projections, implementation and applications. Berlin, Hei-delberg, New York: Springer-Verlag, 1979.