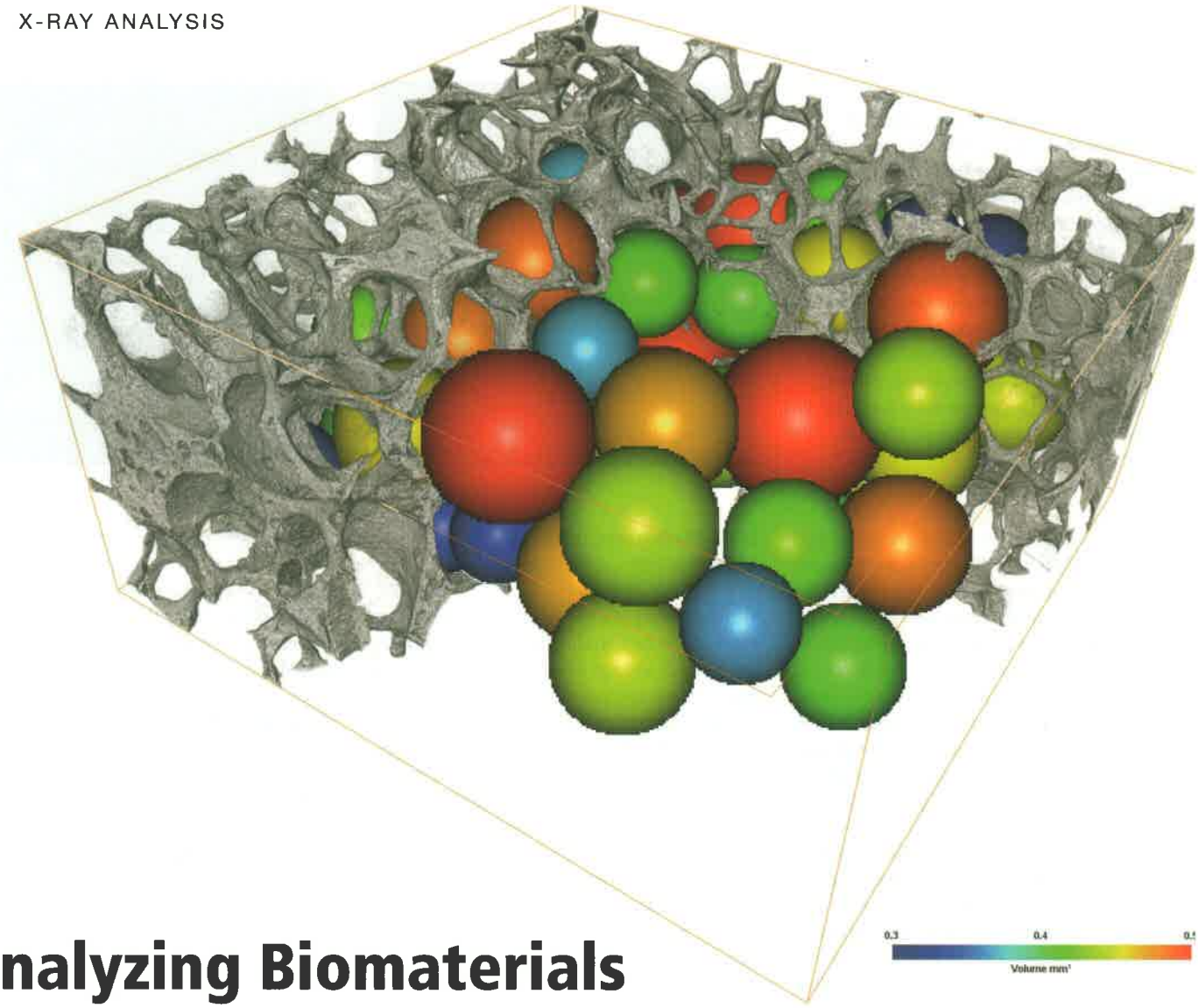

Analyzing Biomaterials by nanoCT

Characterizing Ceramic Biomaterials for Bone TE Applications

3D biomaterials are known to function as alternative bone grafts. In the area of Tissue Engineering (TE) 3D matrices are often used in combination with mesenchymal stem cells to build up a functional tissue graft. In this approach the selection of a matrix is a crucial factor. For bone TE applications the matrix should fulfill the following geometrical properties. It should be: (a) 3D and highly porous with an interconnected architecture, (b) mechanically robust to withstand forces in the area of transplantation and (c) equipped with a large and suitable surface for cell attachment, proliferation and differentiation.

Keywords

Biomaterials, Bone Tissue Engineering, Computed Tomography, nanoCT, Pore Size, Interconnectivity

Additionally, the porosity and pore size of the matrix play an important role for vascularization and bone integration. However, optimal pore sizes and porosities for the regeneration of bone tissue have not yet been reported. For bone TE applications biomaterials with pore sizes between 10–2250 µm and porosities between 30–90% have been implemented, resulting in varying degrees of tissue formation and material in-growth [2]. Aim of this study is not to identify the optimal matrix for bone TE, but to show the range of possibilities presented by nanoCT technique. Furthermore, the results shall draw the attention to the point that there might be huge intra-batch variations due to production restrictions. In order to guarantee constant matrix quality and consequently minimize animal experiments, matrices may be analyzed in advance and non-suitable discarded.

Methods

Four ceramic biomaterials, Sponceram-Al, Osseolive, CerasorbM and 45S5-Bioglass, have been characterized and evaluated by nanoCT technique. Prior to this study all biomaterials have been identified as biocompatible. Moreover, a rather rough surface has been identified for all biomaterials by scanning electron microscopy (SEM).

Biomaterial	Producer	Chemical composition	Fabrication
Sponceram-Al	Zellwerk GmbH	Zirconium dioxide ceramic with aluminium oxide coating	Sintering
Osseolive	Curasan AG	Calcium alkali orthophosphate glass ceramic	Slip-cast technique
CerasorbM	Curasan AG	ß-tricalciumphosphate ceramic	Slip-cast technique
45S5-Bioglass	University Erlangen-Nuremberg, Department of Materials Science and Engineering	Silicate glass ceramic	Sintering

Table 1: NanoCT scanned biomaterials

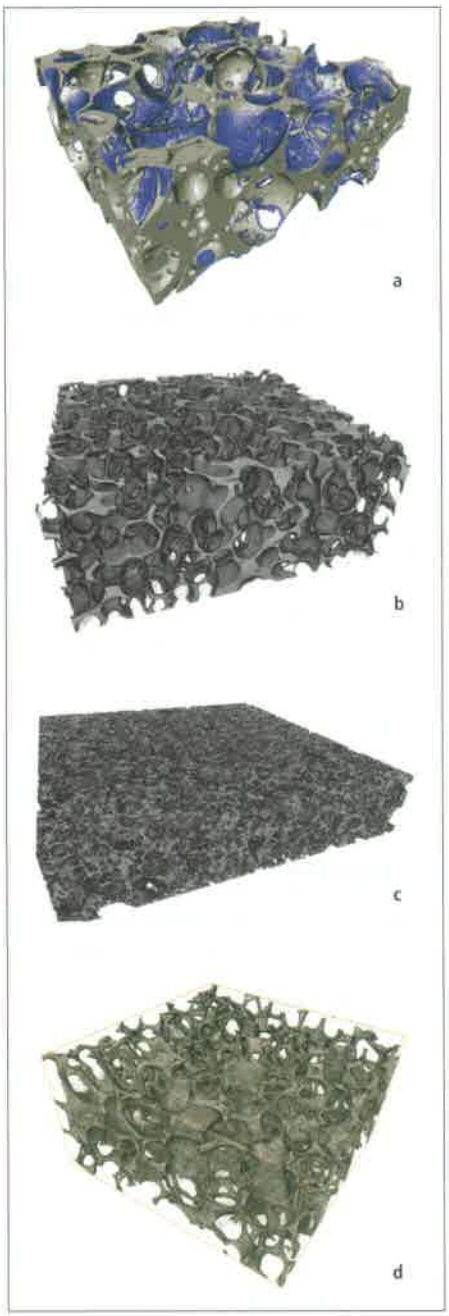

Fig. 1: NanoCT scans:
(a) Sponceram-Al, aluminium oxide coating is svhown in blue,
(b) Osseolive,
(c) CerasorbM,
(d) 45S5-Bioglass

High resolution nanofocus computed tomography (nanoCT) analyses allow a non-invasive and non-destructive visualization of detailed 3D architecture of biomaterials. Different methods of data processing can help to classify the following quantitative parameters [3]: Surface area, total volume, void space volume, matrix porosity, pore wall thickness, interconnectivity, pore diameter distribution, average pore diameter, average pore wall thickness.

Five different samples of each biomaterial have been scanned using a nanoCT system phoenix nanotom (GE). All samples have been measured with 90 kV and 90 µA. For quantitative analysis of the data one mm³ of each sample has been analyzed with Avizo (VSG).

Results

NanoCT images of the different biomaterials indicate varying porosities and interconnectivities (fig. 1). These results can be supported by analyzing the raw data using Avizo. CerasorbM e.g. shows an average porosity of only 28%, where as 45S5-Bioglass has an average porosity of 89% (fig. 3a). Osseolive shows the highest variability in interconnected porosity; it ranges from 17–75%. The difference in interconnectivity can also clearly be seen in 2D cross-sections. Exemplarily comparing 45S5-Bioglass and Osseolive, it can be noticed that 45S5-Bioglass shows only small parts of closed porosity. On the contrary, Osseolive shows larger areas of closed porosity (fig. 2, red arrows). The calculated average closed porosity of 45S5-Bioglass is 0.12%, of Osseolive 1.68% respectively (fig. 3a). Furthermore, the pore wall thickness of each biomaterial can be identified in 2D cross-sections. It is obvious, that Osseolive has thicker pore walls than 45S5-Bioglass. Generally, thicker pore walls result in a higher stability, depending on the biomaterials composition. The interconnected surface area per mm³ of 45S5-Bioglass, Osseolive and Sponceram-Al is about 5 mm² (fig. 3b). Worth mentioning is the extremely high surface area of CerasorbM with about 11 mm², but on the contrary it exhibits with about 30% the lowest porosity. The high surface area results from very small pores (average pore diameter of 190 µm) and less void space volume, compared to the other matrices. With Avizo pore volumes of 45S5-Bioglass have exemplarily been calculated and illustrated (see lead picture). Distribution of the pore diameters (calculated from the volume) within the five matrices is demonstrated in figure 4. The pore diameters range between 690–1090 µm, with an average pore diameter of 926 µm. The average pore diameters within sample one to five range from 907–936 µm. In summary, it can be stated that the pore variability within one matrix is relatively high; pore diameters differ in about 400 µm. However, the variability within the five samples is very low.

Conclusion

3D biomaterials are widely used in the field of bone TE. Nevertheless, the variations in e.g. pore size, porosity and surface area, within one batch of matrices have rarely been studied. Constant production quality is necessary to work under good manufacturing practice conditions and to guarantee a stable quality of tissue engineered transplants.

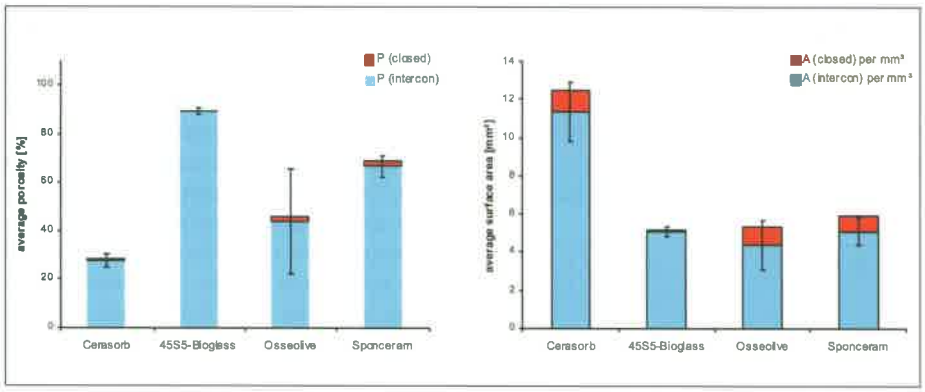

Fig. 3: Average data for closed and interconnected (a) porosity, (b) surface area. The standard deviation is given for interconnected porosity and surface area respectively.

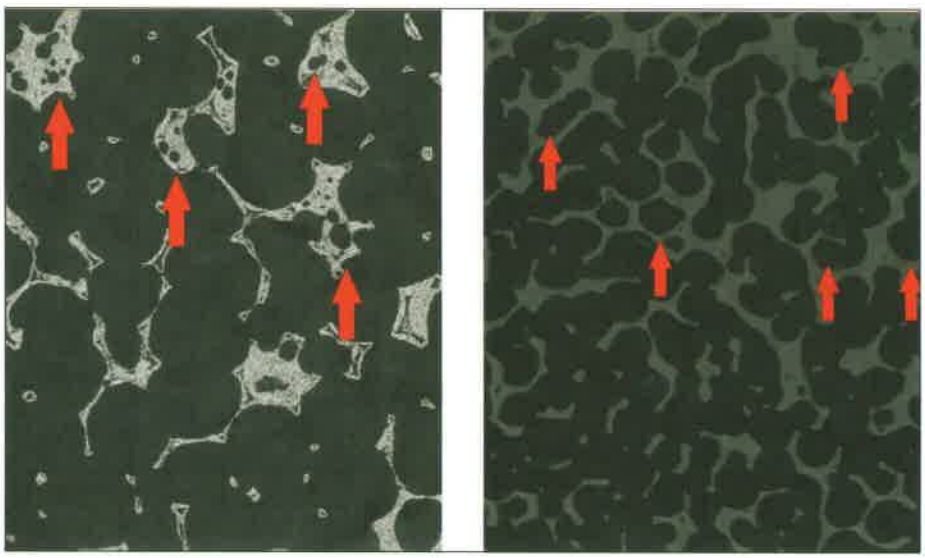

Fig. 2: 2D cross-section of (a) 45S5-Bioglass (b) Osseolive

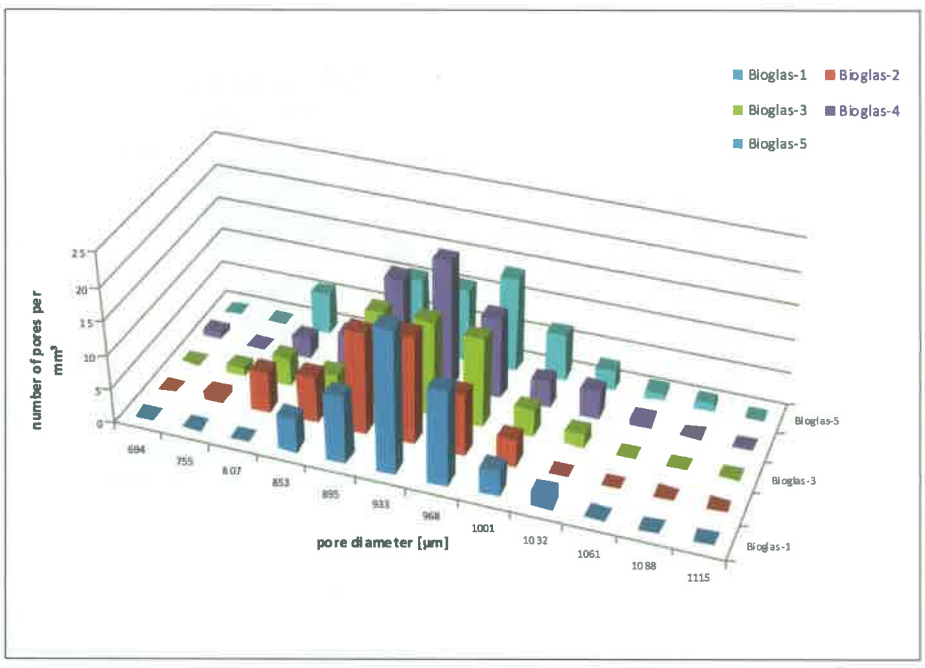

Fig. 4: Pore size distribution within one mm³ of five different samples of 45S5-Bioglass

SEM images give a detailed impression of the biomaterials surface, but only a rare idea of the morphology. On the contrary, nanoCT scans show the 3D architecture of a biomaterial in detail. Moreover, mathematical methods allow the analysis of quantitative data, such as surface area, pore size, porosity and more. Aim of the study was to show the capability of nanoCT technique to analyze matrices for TE applications and to direct the attention of the scientist to the possible effect of intra-batch variations.

We have e.g. observed huge variations in porosity and pore size within the five samples of Osseolive. The consequences of these variations on tissue formation has until today rarely been examined. Therefore, we would like to scan matrices in the future prior to cell culture experiments and analyze the influence of matrix variations on stem cell behavior. Moreover, other research groups have used nano or µCT images to assess e.g. 3D vascularization in bony integration of a biomaterial [4] or identify newly formed bone tissue in an *in vivo* study [5].

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