Materials Characterization 118 (2016) 454–467

Contents lists available at ScienceDirect

Materials Characterization

journal homepage: www.elsevier.com/locate/matchar

Individual pore and interconnection size analysis of macroporous ceramic scaffolds using high-resolution X-ray tomography

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ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form 7 June 2016
Accepted 23 June 2016
Available online 25 June 2016

Keywords:
Calcium phosphates
Isolating pores
Interconnection
Subvoxelization
μCT limitations

ABSTRACT

The pore interconnection size of β-tricalcium phosphate scaffolds plays an essential role in the bone repair process. Although, the μCT technique is widely used in the biomaterial community, it is rarely used to measure the interconnection size because of the lack of algorithms. In addition, discrete nature of the μCT introduces large systematic errors due to the convex geometry of interconnections. We proposed, verified and validated a novel pore-level algorithm to accurately characterize the individual pores and interconnections. Specifically, pores and interconnections were isolated, labeled, and individually analyzed with high accuracy. The technique was verified thoroughly by visually inspecting and verifying over 3474 properties of randomly selected pores. This extensive verification process has passed a one-percent accuracy criterion. Scanning errors inherent in the discretization, which lead to both dummy and significantly overestimated interconnections, have been examined using computer-based simulations and additional high-resolution scanning. Then accurate correction charts were developed and used to reduce the scanning errors. Only after the corrections, both the μCT and SEM-based results converged, and the novel algorithm was validated. Material scientists with access to all geometrical properties of individual pores and interconnections, using the novel algorithm, will have a more-detailed and accurate description of the substitute architecture and a potentially deeper understanding of the link between the geometric and biological interaction.

1. Introduction

Resorbable bone substitutes such as beta-tricalcium phosphate (β-TCP) are increasingly being used to promote the bone repair in critical size defects [1,2] caused by trauma, osteoporosis, cancer, infection or bone cyst [1,3]. The substitute should be highly porous, interconnected and osteoconductive to promote the bone repair [3–5]. The interactions of substitute architecture and in vivo biological response continue to be debated significantly in the biomaterials community [6].

Numerous studies focused on the interactions between scaffold's architecture and biological responses [5–14]. Based on an extensive review of the literature, Karageorgiou and Kaplan [5] proposed a minimum required macro-porosity larger than 50%, a minimum pore size of the substitute larger than 100 μm, and a minimum interconnection size between the neighboring pores larger than 50 μm. Other architectural features such as pore connectivity number (i.e., number of interconnections to pores ratio in whole scaffold) [15,16], and the accessible porosity parameters [16] are also considered to be relevant for the biological process.

Different techniques are successfully used to study the substitute porosity [17,18]. Only a few techniques such as mercury intrusion porosimetry (MIP), scanning electron microscope (SEM) and micro-computed tomography (μCT) can be used to characterize additional architectural features such as pore and interconnection sizes [17–19]. The SEM and MIP are destructive methods [17,18]. The SEM analysis uses 2D projections [18]. The MIP is insensitive to large or closed pores [17,18]. The μCT is a 3D non-destructive technique, and it has therefore received significant attention [6,11,17–33].

In the μCT technique, thousands of X-ray projections of the scaffold are acquired. The projections are then used to determine attenuation in small 3D volumes, referred to as voxels [17,19]. Accurate image processing algorithms are then required to segment this set of voxels of differing gray levels into the solid (material) volume fraction and the porous space. Of great importance is that the transitions of the solid to the void are not clearly defined in the μCT images. These transitions are smooth rather than sharp gray-value transitions, resulting in fuzzy interfaces. Recently, the fuzzy distance transform (FDT) algorithms have emerged in order to enhance the accuracy of segmenting such transitions [20,22,23,34]. The smaller the pore-to-voxel size ratio

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http://dx.doi.org/10.1016/j.matchar.2016.06.030
1044-5803/

(PVSR), the fuzzier the transitions [34]. In addition to fuzzy-based segmentation algorithms, post-acquisition subvoxelization algorithms have been shown to be useful [21,35]. Specifically, the resolution of μCT data is increased by dividing an original voxel into eight subvoxels.

Precise characterization (i.e., unique size, coordinates, volume and surface) of individual pores or interconnections is also challenging and requires dividing the pore space into single and isolated pores [24–30,36,37]. Different approaches such as the watershed algorithm [38–43], the Voronoi diagram [44,45], shape-based algorithms [46,47] and contour-based algorithms [48,49] were used to isolate objects in image processing. The watershed algorithm has been used recently to isolate pores in 3D data sets [11,24–30]. This algorithm identifies a unique seed or marker in each pore that is then used or grown to isolate the space of isolated pores [11,24,29]. Accurately determining the markers is the crucial step in pore isolating algorithms [29]. Inaccurate markers lead to incorrectly isolated pores, and accordingly unfitted/unmatched interconnections, which eventually leads to erroneous characterization results. Therefore, a special attention is required for verifying the implemented isolating algorithms. In addition to the verification, the algorithm accuracy should be carefully examined, for example by comparing the μCT-based results of the algorithm, and SEM-based results. This accordingly allows developing a reliable pore level characterization techniques.

This study had accordingly three objectives. The first objective was to develop, implement and verify an algorithm to isolate pores and their corresponding interconnections in morphologically complex geometries of the calcium phosphate (CaP) bone substitute. The new μCT-based and pore-level algorithm is to define, localize, isolate, label, and quantify accurately each pore and interconnection in the pore space. Accordingly, the algorithm identifies a unique marker of each pore to enable accurate counting, visualization, and localization of each pore. The verification process was based on the 3D inspections and visualization of a large number of randomly-selected individual pores.

The second objective was to define a criterion and a correction chart to compensate and reduce the scanning errors inherent in the μCT technique. The digitalization error, resulting from converting the continuous scaffold geometry into the discrete geometry, has often been ignored in the material community. As per example, the μCT process cannot detect thin ceramic walls between neighboring pores, and therefore the process systemically introduces erroneous interconnections [50]. Another example is that the convex pore geometry systematically leads to significantly higher interconnection sizes [50]. Because of the systematic nature of the errors, using a combined approach of computer-based simulation and higher spatial scanning resolution, charts could be developed to correct for the systematic discretization error.

The third objective was to investigate the effect of varying the μCT spatial resolutions to the pore and interconnection sizes. Specifically, the errors inherent in the μCT process were examined using both higher resolution data and a computer-based subvoxelization algorithm. To demonstrate the utility of the novel algorithm, two classes of β-TCP bone substitutes of high percentage porosity and complex geometry were characterized and studied.

2. Material and methods

2.1. Bone substitute fabrication and preparation

The calcium-phosphate emulsion method was used to fabricate the two experimental groups [15]. The β-TCP cylindrical scaffolds had a macro-porosity close to 55% [15], a diameter of 8 mm, and a length of 13 mm. Briefly, in two prepared batches of calcium phosphate paste with emulsifier, small oil droplets were dispersed via stirring approach. The concentration of the emulsifier controlled the size of the oil droplets and finally the average pore size when the oil disappeared. The prepared pastes were hardened and then sintered at 1250 °C to get the pure β-TCP porous scaffolds [15]. The details of fabrication process are given in an earlier study [15]. In this study, four samples were randomly selected from each group for the architectural characterization. The characterization results were used to verify the herein described pore-level algorithm.

2.2. Image acquisition

All eight β-TCP scaffolds were scanned using a μCT 40, Scanco Medical AG scanner (Bassersdorf, Switzerland) at 30 μm isotropic voxel size [15]. The whole cylinder was selected as region of interest (ROI). To study the sensitivity of characterization results to variations of scanning resolution, one sample in group II was scanned at three different resolutions to obtain 30, 15 and 7.5 μm isotropic voxel sizes [21]. The selected sample was scanned using a SKYSCAN1172 μCT scanner (Desktop X-ray Microtomography, Aartselaar, Belgium). The two ends of the cylindrical scaffolds were also scanned using scanning electron microscopy (SEM; Cambridge S360). The ends were specifically coated with carbon before scanning. The average pixel size in captured SEM images was 2.3 μm.

2.3. Subvoxelization process

To improve the accuracy of size calculation as well as porosity reconstruction, a post-acquisition subvoxelization process was applied to the μCT data of all eight scaffolds with 30-μm voxel size. Thus, the scanning resolutions were increased artificially with a 15 μm isotropic voxel size. The subvoxelization process helps identifying more accurately the solid-porosity interfaces (fuzzy zones). The details of the subvoxelization algorithm are given in earlier studies [21,35].

2.4. Fuzzy distance transform

Fuzzy distance transform (FDT) was used for later described size measurements of porosity in the μCT data. A fuzzy thresholding method was applied to segment the porous and solid spaces [20,22,23,34,51]. In more details, two gray-level threshold values were determined for each set of μCT data, based on the gray level histogram and visual inspection of the solid-porosity interfaces by image processing experts [20]. Using the threshold values, each voxel was assigned a fuzzy membership value (μ, $\mu \in [0,1]$) [20]. Afterward, the fuzzy Euclidean distance of porosity voxels to the solid space was calculated using the μ in porosity voxels to generate the FDT map. The details of the FDT algorithm were described in earlier studies [20,22,34,51].

2.5. Isolating connected pores

A 3D marker-based algorithm was developed and implemented through an in-house code using Matlab (The MathWorks, Inc.) and Python (www.python.org) software. The isolating algorithm comprised three main steps, “idle markers elimination”, “region growing” and “merging”.

Firstly, all voxels at local maxima of FDT map were found and then labeled exclusively as markers or seeds for their associated pores. Secondly, the idle markers (i.e., redundant local maxima) in each pore were eliminated using the “idle markers elimination” algorithm. Each idle marker leads to an over-segmentation error. In this context, the over-segmentation error refers to a pore, which is divided into two or more pores. Conversely, the under-segmentation error refers to two or more pores that are recognized as one pore. Thirdly, the correct markers remaining after elimination of idle markers were used as seeds for the “region growing” algorithm. In this step, pores were isolated and defined through an iterative approach using the pore space FDT map. For a 2D example, the region growing from correct markers is illustrated schematically in Fig. 1. For the three connected pores, the correct markers were located at local maxima of FDT map (Fig. 1a) and labeled as M_1, M_2 and M_3 in Fig. 1b. As shown in Fig. 1d, using the “region