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Three-dimensional arrangement of β-tricalcium phosphate granules evaluated by microcomputed tomography and fractal analysis

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ABSTRACT

The macrophysical properties of granular biomaterials used to fill bone defects have rarely been considered. Granules of a given biomaterial occupy three-dimensional (3-D) space when packed together and create a macroporosity suitable for the invasion of vascular and bone cells. Granules of β-tricalcium phosphate were prepared using polyurethane foam technology and increasing the amount of material powder in the slurry (10, 11, 15, 18, 21 and 25 g). After sintering, granules of 1000–2000 μm were prepared by sieving. They were analyzed morphologically by scanning electron microscopy and placed in polyethylene test tubes to produce 3-D scaffolds. Microcomputed tomography (microCT) was used to image the scaffolds and to determine porosity and fractal dimension in three dimensions. Two-dimensional sections of the microCT models were binarized and used to compute classical morphometric parameters describing porosity (interconnectivity index, strut analysis and star volumes) and fractal dimensions. In addition, two newly important fractal parameters (lacunarity and succolarity) were measured. Compression analysis of the stacks of granules was done. Porosity decreased as the amount of material in the slurry increased but non-linear relationships were observed between microarchitectural parameters describing the pores and porosity. Lacunarity increased in the series of granules but succolarity (reflecting the penetration of a fluid) was maximal in the 15–18 g groups and decreased noticeably in the 25 g group. The 3-D arrangement of biomaterial granules studied by these new fractal techniques allows the optimal formulation to be derived based on the lowest amount of material, suitable mechanical resistance during crushing and the creation of large interconnected pores.

1. Introduction

Several types of biomaterials have been proposed that can be used to fill bone defects in implant dentistry, orthopaedics or plastic surgeries. The use of morcellized bone particles or synthetic biomaterial granules has been recognized for decades, to provide templates for osteoconduction [1,2]. Synthetic biomaterials such as ceramics offer the possibility of being produced in large amounts and of being prepared with a micro-and/or macroporosity [3,4]. The geometry of the grafted material is considered as a critical parameter to favor bone formation [5]. This has been particularly studied for designing porous scaffolds because an interconnected porosity is required to allow the migration of fluids and cells through the three-dimensional (3-D) microarchitecture [6–8]. However; little is known about the 3-D disposition of the biomaterial granules implanted in a grafted site. β-tricalcium phosphate (β-TCP) is a well-known ceramic with bioactive properties [9,10]. When prepared by the polyurethane-foam technique, the shape of β-TCP granules can be controlled by scanning electron microscopy (SEM) or X-ray microcomputed tomography (microCT) [11]. The spatial disposition of β-TCP granules according to their shape has never been studied. It is essential to develop granules with the best adapted shape that would favor the invasion of vascular growth bringing bone cells and allowing the diffusion of nutrients in the very center of the grafted area. MicroCT allows a quantitative evaluation of the material volume and porosity in three dimensions but the method cannot provide a good description of pore interconnectivity and geometry. Several algorithms have been described to evaluate the interconnectivity of marrow spaces in bone tissue (i.e. the porosity of trabecular bone) on two-dimensional (2-D) sections [12,13]. The fractal dimension can evaluate the complexity of the material to fill the reference space and is helpful in the study of porous objects such as bone

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[14,15] or materials [16,17]. Recently, new fractal parameters have been described (lacunarity and succolarity) to evaluate the heterogeneity and connectivity of the drainage patterns within an object [18–20]. Lacunarity is a concept introduced by Mandelbrot to describe the nature of gaps, voids or pores within texture images [21]. Succolarity is another concept proposed by Mandelbrot and represents the degree of percolation of an image (i.e., how a fluid can flow through an image) [21]. In the present study, we have used granules of β-TCP with different shapes by varying the amount of the slurry in the polyurethane foam. Granules were placed in test tubes and allowed to settle. The 3-D arrangement of the granules was evaluated by microCT, and 2-D sections were used to compute fractal dimensions, lacunarity and succolarity in order to better characterize the porosity created between the granules.

2. Materials and methods

2.1. Preparation of β-TCP granules

Granules of β-TCP were prepared by the polyurethane foam technology as previously described [11,22]. Different volume fractions were investigated: 10, 11, 15, 18, 21 and 25 g of β-TCP powder were mixed with distilled water to produce six types of slurries. They were used to impregnate 1 g cubes of polyurethane foam under vacuum. The blocks impregnated with the slurries were then dried in an oven and heated at 800 °C to completely burn the organic polymer foam. Then the 3-D scaffolds of β-TCP were sintered at 1200 °C; granules were obtained by crushing the blocks in an alumina mortar and the 1000–2000 μm fraction was collected by sieving. This size of granule is the most commonly used for dental and implant surgery. The frequency distribution of the granules' size (measured by SEM) was always found to follow a log-normal distribution as expected (data not shown). This method provided a series of granules that differed in shape, according to the amount of β-TCP in the slurry. The shape of the granules was controlled by SEM before use.

2.2. MicroCT

The Skyscan 1172 X-ray microcomputed tomograph (Bruker microCT, Kontich, Belgium) was used in the cone beam acquisition mode. Granules of the different grades were placed in polyethylene test tubes (5 mm in diameter, 4 cm^3 of granules in each tube). The tubes were gently agitated to allow granules to settle (Fig. 1). For each series, three test tubes were prepared and microtomographed at 80 kV, 100 μA with a 0.5 mm aluminum filter. The pixel size was fixed at 4.95 μm and a 0.25° rotation angle was applied at each step. 2-D cross-sections of the stacks of granules were reconstructed from the series of projections images. They were used to prepare 3-D models of the granule arrangements by using the software provided by Bruker microCT (CtVol for surface rendering and CtVox for volume rendering). The fractional amount of material (Mat.V/V) and the porosity (Po.V/V = 100 – Mat.V/V; in %) were determined by the CtAn software. The fractal dimension (D_{3D}) of the granules arrangement was determined in three dimensions by the Kolmogorov "cubic box counting" method, which is an extrapolation of the 2-D method (see below) to the 3-D space. The side of the cube ranged from 2 to 100 pixels.

For determining lacunarity and succolarity, 2-D sections were re-sliced from the 3-D models: two sagittal sections (separated by 200 μm) were obtained, and two others sections were done in orthogonal planes. So, for each test tube, four sections were obtained and binarized.

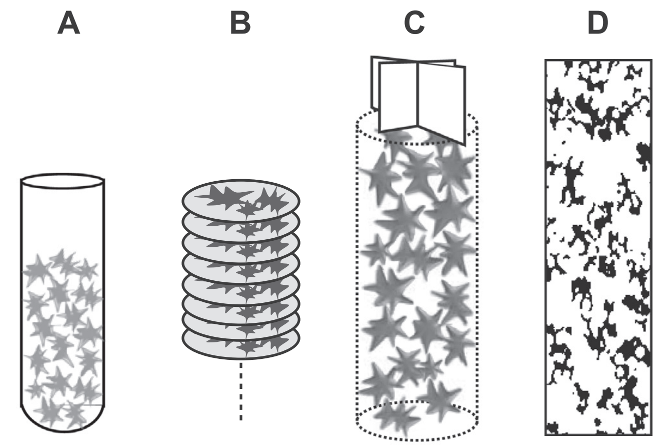

Fig. 1. Methods used to prepare the images suitable for 2-D and 3-D analysis. (A) Granules of β-TCP are placed in a test tube and are allowed to settle. (B) The test tube is analyzed by microCT. (C) The 3-D model is reconstructed and re-sliced in two orthogonal directions to provide the 2-D images suitable for analysis after binarization (D).

2.3. Image analysis on 2-D sections

Binarized images were transferred to a Leica Q550 image analyzer to determine classical parameters describing both the materials and the porosity between the granules. The interconnectivity index of the porosity (ICI) was determined by skeletonization of the spaces between the granules, as previously reported [23]. The star volumes were determined by the grid algorithm (this method is sometimes referred as the MIL, maximum intercept length) [13,24]. The star volume was determined on the pores V^*_{Pore} (a high star volume indicates a highly fragmented material or the presence of larges pores). The star volume of the granules (V^*_{Mat}) was also determined. The Euler–Poincaré number (E) was measured by counting the number of enclosed medullar cavities and connected trabeculae [25]. Assessment of the granules' shape was described by "strut analysis" [26]. The granules were skeletonized; the number of nodes and free ends of the skeleton were computed and the node-to-free-end ratio was determined (N/F) [27]. A compact granule has a high number of nodes and a low number of free ends. A granule with numerous branches has a high number of free ends and a low number of nodes.

The Kolmogorov fractal dimension (D_K) was measured by the box-counting method and the Minkowski–Bouligand (D_{MB}) dilatation method as previously described [14]. Briefly D_K was computed by over imposing grids of square boxes (with ε pixels as side length) on the granules border and intersecting boxes were counted. The total number of boxes required to completely fill the granules boundaries reflected the perimeter with a scale ratio of ε. This step was repeated with ε varying from 2 to 100 pixels and a log–log graph (i.e. log [N] against log [ε]) was used to determined D_K from the slope of the regression line. D_{MB} was obtained by using a series of dilatations of the granule's boundary and was repeated from 10 to 10 times; after each new dilatation φ, the granule border thickened and the surface area of the dilated image A was measured. D_{KM} was determined on the log–log graph from the slope of φ/A φ.

For computing lacunarity and succolarity, the 2-D images were transferred to a lab-made software written in Matlab (Math Works, Natick, Ma; release 7.10). Lacunarity (δ) was measured by the