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Novel linear intercept method for characterizing micropores and grains in calcium phosphate bone substitutes

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ABSTRACT

The micropore and grain thicknesses of calcium phosphate (CaP) bone substitutes are believed to have strong influence on in vivo biological responses. The thickness measurement is challenging due to complex morphologies of the elongated CaP micropores and grains. This study aimed to develop and verify a novel linear intercept (NLI) algorithm based on SEM images to calculate thicknesses accurately for elongated geometrical features. The standard linear intercept (SLI) algorithm overestimates the thickness of micropores and grains wherever the defined lines are not perpendicular to such geometrical features. The NLI was developed by integrating the standard algorithm with a distance transform map to exclude the overestimation errors. Besides the accurate thickness calculation, the NLI measures the orientation angle of architectural features as an indication for isotropy level. The NLI-based thicknesses of five groups of β-TCP scaffolds were compared and verified with those of a refined centerline (RC) algorithm. The RC algorithm was accurate yet human assisted and very time intensive. Compared with RC algorithm, the NLI-based results were 2 ± 13 and $4 \pm 9\%$ (Mean ± SD) lower for average micropore and grain thicknesses, respectively. The NLI results were also compared with two standard and commonly used algorithms. The SLI and maximal-fitted-circle (MFC) algorithms resulted in 72 ± 25 and $91 \pm 47\%$ overestimation errors, respectively.

1. Introduction

Ceramic bone substitutes are increasingly used as alternatives for autografts and allografts [1–3]. Most of the ceramic bone substitutes are classified as resorbable bone grafts that are replaced gradually by new bone after implantation [3–5]. Among ceramic bone substitutes, calcium phosphate scaffolds, with a variable range of resorption rates, serve as a vehicle to promote the repair process in critical-size bone defects [3–5].

Bone substitutes are synthesized to be micro-structured with a large percentage of connected pores to enhance cell migration and eventually the bone deposition [6–12]. The macro- and microscale architectural parameters (e.g., macro- and microporosity, pore and interconnection sizes and grain thickness) are hypothesized to play a key role in controlling biological responses [8,10,12–29]. Nevertheless, a clear understanding of interactions between the architectural parameters and biological responses is yet to be achieved [5,10,26]. In fact, the complex nature of biological responses and their mutual dependence challenge the reductionist paradigm of science. Hence, the dominant paradigm in the biomaterial community focuses on the interaction between in vivo biological responses and the architectural parameters of bone substitutes [8,10,12–28].

Among microscale architectural features of bone substitutes, micropores (<10 μm) were found to be very influential on biological responses [12,21–25,27–32]. Specifically, bone substitutes with higher microporosity resulted in enhanced degradation rate [12,21], in vivo resorption [26], cell activation [12,27–29,33] and new bone deposition [12,21,24,25,27,28,32]. Moreover, micropore thickness (i.e., size) was very effective in controlling the cell proliferation and osteogenic differentiation [28,30–32]. Besides, new bone deposition was found within micropores of CaP scaffolds [22,23,26,34,35]. On the other hand, grains were believed to play a key role in regulating the resorption process [26]. Grains also were effective on the cell proliferation and osteogenic differentiation [30,31].

Architectural analysis of micropores and grains is of great of interest to many biomaterials scientists who study the bone regeneration and scaffold resorption [26]. Open micropores (open to the scaffold periphery) can be characterized using various techniques, such as mercury intrusion porosimetry (MIP), gas pyconometry, gas adsorption, water immersion densitometry and scanning electron microscope (SEM) [9, 21,22,36,37]. The SEM technique also enables evaluation of the closed pores [9,36]. Besides, the grain characterization can be done only by using the SEM or similar high-resolution scanning techniques.

Sophisticated image-processing algorithms have been used in the literature to characterize the elongated architectural features (e.g.,

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bone trabeculae). Such algorithms follow two main approaches (Fig. 1), the centerline-based approach [38–41] and the maximal-fitted-circle approach [42].

In the centerline-based approach (Fig. 1a), the average thickness of each architectural feature is calculated along their centerline pixels (i.e., skeletons and medial axes). The centerline pixels of the features can be extracted using various algorithms such as ridge point [39,43], ultimate thinning [44,45] and Voronoi diagram algorithms [45]. A thickness value is assigned to each centerline pixel which equals twice the distance transform (DT) value (i.e., radius of the largest circle centered at the pixel). As shown schematically in Fig. 1b, extracting an accurate centerline along the architectural features is very challenging, such that any convexities at boundaries may generate a redundant branch in centerlines. The redundant branches lead to significant errors in thickness calculations.

Maximal-fitted-circle (MFC) approach assigns a thickness value to every pixel in the features, not only pixels at centerlines (Fig. 1c). The thickness value at any desired pixel equals the diameter of the largest inscribable and covering (covers the desired pixel) circle. As shown in Fig. 1d, this approach overestimates the thickness values for pixels at narrow cross sections (i.e., necks), when they can be covered partially by large circles centered at neighboring cross sections. Moreover, this algorithm is a volume-weighted averaging algorithm that downgrades the weight of the thinner yet biologically important cross sections (necks) [26].

To measure the grains in SEM images, many line-fraction standard approaches such as linear intercept method have been proposed to calculate the average sizes (ASTM E112). In standard linear intercept (SLI) approach, a set of grid lines are defined and the average thickness of grains equals the length of the drawn lines divided by the number of interceptions of grain boundaries (ASTM E112). The SLI algorithm is fast and easy to be implemented. This algorithm was used also for characterizing micropores of CaP scaffolds using SEM images [24,25,37]. Nevertheless, the standard approaches are not developed for, nor adapted to, morphologically complex micropores and grains in CaP scaffolds with snakelike configurations [24–26,30,31,37,46,28].

The first objective of this study is to develop and verify a novel linear intercept algorithm to accurately characterize micropores and grains in CaP scaffolds. The novel linear intercept (NLI) algorithm is hypothesized to be very fast, robust, and easy for implementing by other research groups. The NLI algorithm is developed based on exclusion of overestimating lines crossing micropores and grains. Furthermore, this algorithm enables measuring the orientations of the microscale features as an indication for isotropy in manufacturing.

The second objective is to demonstrate the application of the NLI algorithm via studying five groups of β-TCP scaffolds with different microporous structures [26,46]. Specifically, group mg, Mg, mG, MG [26], and A [46] were fabrication and implanted in sheep model to study the correlations between biological responses and the scaffolds architecture. The semispherical macropores of such CaP scaffolds were characterized earlier using μCT-based algorithms [26,43,47]. However, for pore interconnections, accurate sizes just recently were achieved by isolating algorithm [47].

2. Material and methods

2.1. Scaffold fabrication and preparation

Five β-TCP scaffolds with different microporous architectures (mean microporosity and mean micropore and grain thicknesses) were selected to verify the consistency of the improvements by the developed algorithm.

As described in an earlier study [26], four groups of microporous β-TCP scaffolds were produced with different microporosity levels and

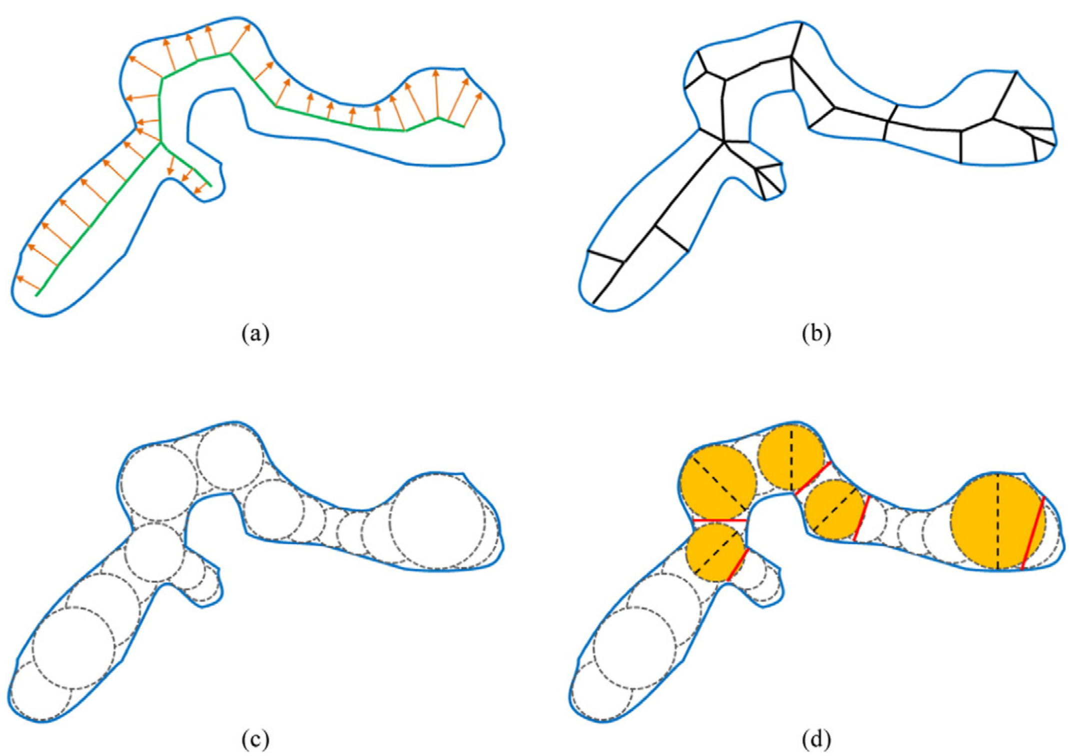

Fig. 1. A schematic snakelike architectural feature in CaP scaffolds measured (thickness) by two different image-processing algorithms. (a) Centerline-based algorithm. A specific thickness value is assigned to each centerline pixel (one per each cross-section). The thickness value is the diameter of the largest inscribable circle which is centered at the centerline pixel. (b) Redundant branches are present in all skeletonization algorithms. Each convexity on the features boundary leads to a redundant branch. (c) Maximal-fitted-circles (MFC) algorithm. One thickness value is assigned to each pixel (not only centerline pixels). Thickness value equals the diameter of the largest covering circle which might be centered at other cross sections. (d) Overestimating nature of the MFC algorithm. The real thicknesses at five sections are indicated by red lines. The estimated thicknesses are indicated by dashed diameter of the orange covering circles.