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A novel method for segmenting and aligning the pre- and post-implantation scaffolds of resorbable calcium-phosphate bone substitutes

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ABSTRACT

Micro-computed tomography (microCT) is commonly used to characterize the three-dimensional structure of bone graft scaffolds before and after implantation in order to assess changes occurring during implantation. The accurate processing of the microCT datasets of explanted β-tricalcium phosphate (β-TCP) scaffolds poses significant challenges because of (a) the overlap in the grey values distribution of ceramic remnants, bone, and soft tissue, and of (b) the resorption of the bone substitute during the implantation. To address those challenges, this article introduces and rigorously validates a new processing technique to accurately distinguish these three phases found in the explanted β-TCP scaffolds. Specifically, the microCT datasets obtained before and after implantation of β-TCP scaffolds were aligned in 3D, and the characteristic grey value distributions of the three phases were extracted, thus allowing for (i) the accurate differentiation between these three phases (ceramic remnants, bone, soft tissue), and additionally for (ii) the localization of the defect site in the post-implantation microCT dataset. Using the similarity matrix, a 94 ± 1% agreement was found between algorithmic results and the visual assessment of 556,800 pixels. Moreover, the comparison of the segmentation results of the same microCT and histology section further confirmed the validity of the present segmentation algorithm. This new technique could lead to a more common use of microCT in analyzing the complex 3D processes and to a better understanding of the biological processes occurring after the implantation of ceramic bone graft substitutes.

Statement of Significance

Calcium-phosphate scaffolds are being increasingly used to repair critical bone defects. Methods for the accurate characterization of the repair process are still lacking. The present study introduced and validated a novel image-processing technique, using micro-computed tomography (mCT) datasets, to investigate material phases present in biopsies. Specifically, the new method combined mCT datasets from the scaffold before and after implantation to access the characteristic data of the ceramic for more accurate analysis of bone biopsies, and as such to better understand the interactions of the scaffold design and the bone repair process.

1. Introduction

Bone substitutes made of synthetic materials are a viable alternative to autografts and allografts [1,2]. Among these materials, calcium phosphates (CaP) are particularly interesting due to their chemical and physical similarity to bone minerals [3,4]. Next to their biocompatibility, CaP bone graft substitutes have other desirable properties, including bioactivity and osteoconductivity [4–8]. The CaP materials, such as hydroxyapatite (HA) or β-tricalcium

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phosphate (β-TCP), are routinely used for filling traumatic and pathological bone defects [9–11]. Compared to HA, β-TCP is gradually resorbed and replaced by neo-bone [12,13]. The quest continues for the optimum bone scaffold design, in terms of material and pore geometry, and for controlled interactions with the host bone [14,15]. Besides the *in vitro* studies, animal studies have played a significant role in this quest [8,16–21].

Characterizing the three-dimensional macro-porous bone substitute scaffold before and after implantation requires the use of accurate methods, and microcomputed tomography (microCT) imaging modality is commonly employed [17,22–27]. Compared to the post-implantation characterization [8,17,24–26], the pre-implantation characterization of β-TCP scaffolds, using the microCT technique, has been more accurate and therefore successful [22,23]. Methods to distinguish and isolate the three phases (bone, ceramic, soft tissue) of the explanted ceramic bone substitute samples still do not exist. Furthermore, the spatial and temporal evolution in the mineralization levels, in the form of the newly deposited bone, and of the partially localized resorption of the β-TCP implant further increases the difficulty in establishing precise and accurate methods for differentiating the grey-intensity levels of the three phases [7,8,17,24]. Specifically, applying the local segmentation technique to the post-implantation microCT dataset requires the use of accurate techniques and new development. In the commonly used global thresholding segmentation technique, two global threshold values must be defined, one separating the grey levels of fibrous tissue and bone, and the second one separating the grey levels of bone and ceramic remnants. Specifically, global thresholding techniques, for example Otsu's method, were applied in several *in vivo* studies to distinguish the bone from the ceramic [7,17,24,26,28,29]. These methods often led to inaccurate results. Gauthier et al. [24] reported a significant disagreement between the bone volume fraction measured using microCT and that using scanning electron microscopy. Van Lenthe et al. [17] reported some segmentation errors, and used, for example, dilation-based refinement, in particular for bone being in contact with the scaffold. To take into account the inaccuracy resulting from the global thresholding technique, Komlev et al. [28] classified the microCT data into four phases: bone, scaffold remnants, soft tissue and an uncertain phase. The latter could be bone or partially resorbed scaffold.

An improvement in isolating the different phases after implantation was recently achieved using new segmentation algorithms. Specifically, Jones et al. [30] applied contour-tracking segmentation [31] and watershed algorithms to explanted HA implants, and reported a more accurate segmentation compared to global thresholding. Still, they reported disagreement between the algorithmic results and visual assessment. Polak et al. [25] advantageously combined atlas registration and statistical thresholding to distinguish bone from ceramic in the explanted samples [25], and reported accurate results. However, this algorithm only applies to scaffolds made of orthogonal rods and the controlled geometry of slow-resorbing ceramic implants. To our knowledge, an accurate method for segmenting the three phases of the *in vivo* resorbable porous bone CaP scaffolds does not yet exist.

Pre- and post-implantation scaffolds' datasets were aligned to find the thresholding value to segment the three phases. Komlev et al. [28] who studied Si-TCP scaffolds, used a volume registration method based on 3D correlation and affine linear registration. Their technique is computationally demanding for application to 3D data sets of complete samples. In addition, the Si-TCP material exhibits slow resorption, which means that the pre-implantation scaffold geometry was not greatly changed during implantation [8,17]. The alignment of the pre- and post-implantation structures of geometrically non-uniform and resorbing β-TCP scaffolds poses a great challenge, in particular because of the lack of efficient and accurate techniques for coupling the alignment and the accurate segmentation of the microCT post-implantation.

The present manuscript had two related objectives. The first objective was to develop and implement a novel segmentation method to isolate the three phases present in the explanted ceramic bone substitute scaffolds, i.e. bone, ceramic remnants, and soft tissue. For that purpose, a contour-based method combined with a compaction algorithm specifically treating the fuzzy grey-intensity transitions often found between phases were used to yield more accurate results than those of currently existing global thresholding methods. The second objective was to improve the accuracy of the segmentation results even more by aligning pre- and post-implantation microCT datasets, hence allowing the extraction of the grey intensity value distribution of each phase. These distributions, once extracted, were subsequently applied to refine the contour-based segmentation method to achieve even more accurate results. Once the post-implantation datasets are accurately segmented in three phases (bone, ceramic remnant, soft tissue), the changes and the biological interaction during the scaffold implantation can be studied more accurately.

2. Materials and methods

2.1. Scaffold fabrication, in vivo model, and microCT scanning

Pure β-TCP cylindrical scaffolds of constant porosity were fabricated using the calcium-phosphate emulsion method [32]. Briefly, the calcium-phosphate cement paste (CPC) was mixed with paraffin oil and an emulsifier to obtain a metastable dispersion of oil droplets in the CPC paste. Once set, the oil-filled CPC was incubated at 60 °C in a phosphate-buffered solution (0.15 M, pH = 7.4) for 48 h, cleaned in petroleum ether, and sintered at 1250 °C for 1 h. The resulting blocks were lathed to obtain cylinders (Ø = 8 mm, L = 13 mm). The samples contained 54% macroporosity (pore diameter greater than 50 μm) and 21% microporosity (pore diameter smaller than 50 μm) [32]. The macropores of the sample used in this study had a smooth spherical appearance, with an average pore size close to 1200 μm, as shown in Fig. 1d of Bohner et al. [32]. The β-TCP scaffolds were implanted in the metaphyseal or epiphyseal regions of the sheep for 6, 12 and 24 weeks [8]. Specifically, the scaffolds were implanted in 13-mm deep bone defects/cavities created using an 8-mm diameter drill (KaVo INTrASurg 500 s, KaVo Dental AG Biberach, Germany). More details are presented in von Doernberg et al. [8]. Only one randomly selected scaffold, explanted at the 6-week time point was used to demonstrate the utility of the novel processing method presented in this study. The animals were sacrificed, in accordance with the local Ethics Committee and veterinary authorities (application number 176/2003), and bone blocks containing the scaffolds were cut to retrieve the β-TCP *in vivo* scaffolds. The β-TCP scaffolds were microCT-scanned before implantation and after retrieval, using a commercial desktop microCT scanner (μCT 40, Scanco Medical AG, Bassersdorf, Switzerland) [21–23] at 30-μm isotropic resolution. The pre-implantation dataset had undergone geometrical and morphometric analyses in recent studies [22,23]. As described by von Doernberg et al. [32], following the microCT scanning, the explanted scaffold was fixed in 40% ethanol, dehydrated in a series of graded ethanol baths (40–100%) and then defatted using xylene. The scaffold was then infiltrated with polymethylmethacrylate at 4 °C, which was then left to polymerize at room temperature. Once polymerized, the scaffold was then sectioned perpendicular to its longitudinal axis using a precision saw (Leica SP 1600, Leica Microsystems, Nussloch, Germany). Finally, the sections were then stained with toluidine blue dye commonly used in histology staining.