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Application of microcomputed tomography for quantitative analysis of dental root canal obturations*

Zastosowanie mikrotomografii komputerowej do ilościowej analizy wypełnień kanałów korzeniowych w zębach

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: The aim of the study was to apply microcomputed tomography to quantitative evaluation of voids and to test any specific location of voids in tooth's root canal obturations.

Materials and Methods: Twenty root canals were prepared and obturated with gutta-percha and Tubli-Seal sealer using the thermoplastic compaction method (System B + Obtura II). Roots were scanned and three-dimensional visualization was obtained. The volume and Feret's diameter of I-voids (at the filling/dentine interface) and S-voids (surrounded by filling material) were measured.

Results: The results revealed that none of the scanned root canal fillings were void-free. For I-voids, the volume fraction was significantly larger, but their number was lower ($P = 0.0007$), than for S-voids. Both types of voids occurred in characteristic regions ($P < 0.001$). I-voids occurred mainly in the apical third, while S-voids in the coronal third of the canal filling.

Conclusions: Within the limitations of this study, our results indicate that microtomography, with proposed semi-automatic algorithm, is a useful tools for three-dimensional quantitative evaluation of dental root canal fillings. In canals filled with thermoplastic gutta-percha and Tubli-Seal, voids at the interface between the filling and canal dentine deserve special attention due to of their periapical location, which might promote apical microleakage. Further studies might help to elucidate the clinical relevance of these results.

Key words: **Canal filling, Microcomputed tomography, Root canal obturation, Three-dimensional evaluation**

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Introduction

Successful endodontic therapy depends on effective dental root canal instrumentation and a final three-dimensional (3D) filling of the root canal system that provides hermetic sealing of the available spaces [21]. The main goal of canal obturation is to eliminate leakage pathways from both coronal and apical directions and check multiplication of remaining intracanal microorganisms [26]. However, it has been reported that a complete seal of the root canal system is almost impossible using currently accepted materials and obturation methods and using a combination of gutta-percha with canal sealers [5,7,15]. The ideal canal obturating material should adapt well to the prepared canal walls and its irregularities as a homogenous mass without dimensional changes. Shrinkage and dissolution of sealer may result in the voids created within the filling or between the dentine and the canal filling [18,22].

A variety of *ex vivo* methods have been applied to assess the sealing ability of root canal filling materials. They include mainly leakage methodologies, such as dye penetration, bacteria leakage test, fluid filtration and a glucose penetration model [13,21,26,30]. Also investigations employing electron microscopy have provided significant data for evaluating the quality of canal obturation [4,5,15]. Recently, a novel method of evaluating root filling quality was proposed [6,14,25]. Microcomputed tomography (microCT) is non-destructive, and it provides 3D reproducible data, the quality of which is determined mainly by the settings of a microCT scanner.

The literature to date contains few studies focusing on microCT for imaging of obturated dental root canals. They demonstrate good correlation between the microCT results and conventional histological sections of prepared teeth [14,32]. Zaslansky et al. [32] have compared various 2D and 3D imaging techniques in an attempt to assess differences in observed cross-sectioned areas of root canal fillings. In the studies of Hammad et al. [10,11] a segmentation based on a gray-level intensity histogram thresholding was applied to extract the filling components (sealer, gutta-percha cones, voids) and the percentage of voids and gaps volume in canals obturated with different materials was measured. Somma et al. [27] proposed a manual procedure to detect voids either in the interior or at the interface of the filling material.

In the present paper, an algorithm described in detail by Petryniak et al. [24] was used to analyze the quality of root fillings with respect to two factors. The first aim of this study was to apply microCT to calculate the volume and diameter of voids. Second, the distribution of the voids in the volume of canal obturation was investigated to determine if the voids occur in any specific locations.

Materials and methods

Twenty extracted single-rooted mandibular premolars with a single, straight root canal and fully formed apices were selected. The teeth were free of root caries, cracks and artificial alterations. Each tooth was placed in 5.25% sodium hypochlorite for 24 h for surface disinfection, and then stored in distilled water until further processing.

The teeth were decoronated to achieve a length of 14 mm and the canals were accessed. After pulp removal, a size-10 K-file (Poldent, Warsaw, Poland) was inserted up to the apical foramen and then withdrawn 1 mm, determining the working length. Orifice enlargement was achieved with Gates-Glidden drills (Poldent). The root canals were manually shaped with the step-back technique using size 15 up to 40 S-files (Poldent) to working length, then stepping back 1 mm with the three subsequent instruments and they were irrigated with 2 mL 2% sodium hypochlorite solution. After instrumentation, the canals were filled with 5 mL 17% ethylenediaminetetraacetic acid (EDTA) solution for 5 min, then flushed again with 2% sodium hypochlorite and saline solution, then dried with paper points.

All prepared root canals were obturated with gutta-percha and Tubli-Seal (a zinc oxide-eugenol-based sealer) (Kerr, Salerno, Italy) using the thermoplastic compaction method. The apical part of the canal was sealed to working length with a size 40 gutta-percha cone (VDW, Munich, Germany). Downpacking was performed with a System B plugger (Sybron Endo, Orange, CA, USA), preheating the insert tip to 200°C. Backfilling was done by injecting heated gutta-percha into the canal using an Obtura II unit (Obtura Spartan, Fenton, MO, USA), according to the manufacturer's instructions. Complete root canal fillings were confirmed radiographically and access openings were sealed with glass ionomer cement (Ketac Cem Radiopaque, 3M ESPE, Seefeld, Germany). Subsequently, the roots were stored at 37°C and 100% humidity for 7 days to allow the sealers to set completely.

MicroCT evaluation

An X-tek Benchtop CT160Xi (Nikon Metrology, Tring, UK) micro-CT scanner was used to scan the teeth. The X-ray tube was operating at 110 kV and 50 μA, the samples were scanned with a rotational step of 0.2° and a rotational angle of 360°, resulting in an isotropic voxel resolution of 10.6μm. A 0.25 copper filter was used to suppress beam-hardening artifacts, and a ring artifact-minimizing algorithm was applied to the data. ImageJ was used for 3D volumetric visualization (Fig. 1) of microCT data.

Custom-written software was developed to detect objects of interest in 3D images: dentine, root filling material and voids. Firstly, the filling material was segmented based on the histogram of gray-level intensities. In all cases, the histogram

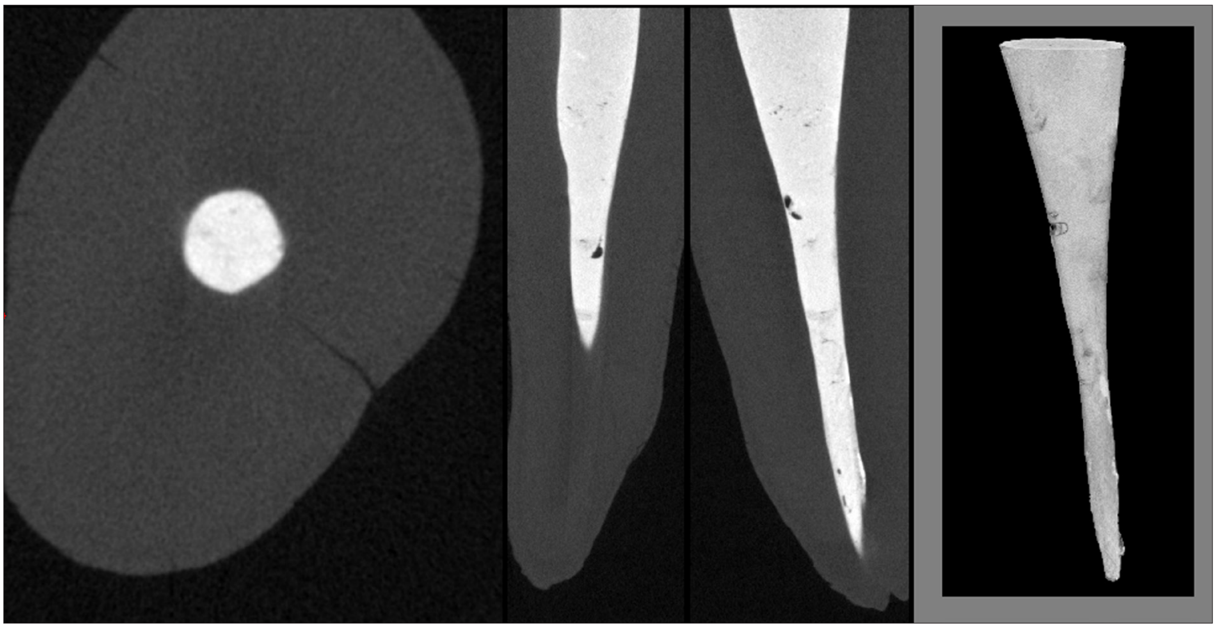

Fig. 1. Three mutually perpendicular cross-sections of the reconstructed 3D image of a tooth and a 3D rendering of a root with an obturated root canal. Voids in the filling material are clusters of dark pixels surrounded by bright pixels. Z-coordinate points along an axis of a tooth and is used to calculate the distance of the voids from the apical tip of the gutta-percha

contained three well-separated modes corresponding to the background (air), dentine and filling material. Segmentation, depending on two parameters (ILOW and IHIGH), consisted of histogram thresholding followed by region growing [2]. The threshold ILOW was selected automatically at the minimum between the modes corresponding to the dentine and the filling material. After thresholding, black color was assigned to the background, or voids voxels, and white was assigned to the filling material. To correct for the partial volume effect, a region-growing procedure was applied to the thresholded image. For this purpose, clusters of black voxels merged with white voxels characterized by original gray-level intensities lower than the threshold IHIGH (Fig. 2).

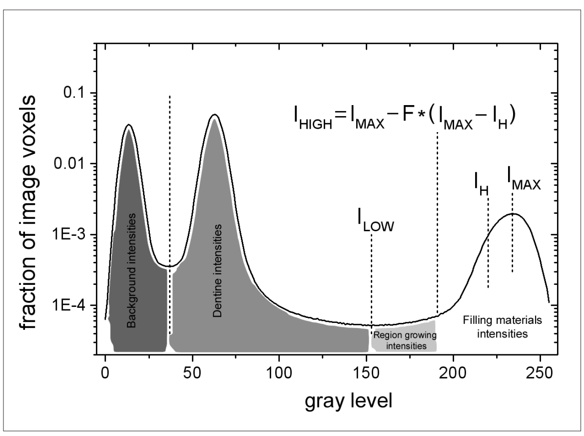

Fig. 2. Histogram of gray-level intensities of a microCT image of a root with an obturated root canal. IH is the intensity corresponding to the half-width of the filling materials intensities peak. The user-adjusted parameter F was equal to 3 in all calculations

After segmentation, voids entirely surrounded by filling material (S-voids) were detected with a hole-filling algorithm [2]. Then, concavities present in the interface of the dentine and root canal filling (I-voids) were detected, based on a notion of a convex hull. Finally concavities corresponding to I-voids were selected interactively from the 3D images.

The volume, coordinates of the centre of mass and maximal Feret's diameter were calculated for each void. These are standard parameters used to qualitatively characterize compact objects in 3D images. The parameters were calculated with a BoneJ plugin for ImageJ [3,12]. The x-y reference plane was parallel to the reference plane was parallel to the microCT slices. The z-axis pointed approximately in the direction of the root axis. The z-coordinate was used to calculate the distance of the voids from the tip of the gutta-percha. The total volume (V) of the root canal filling material, the volume (VS) of the S-voids and the VI of the I-voids were also measured. The volume fraction of the S-voids is equal to the ratio of VS to the sum of V, VS and VI. The volume fraction of the I-voids is defined analogously. Next, for every slice M, the number of S-void voxels NS(M) and the number of I-void voxels NI(M) present in the slice were calculated. The relative volume of S-voids in the Mth slice is equal to the ratio of NS(M) to the sum of V, VS and VI. The relative volume of I-voids is defined in a similar manner.

Statistical analysis

Mean and standard deviations were calculated for all measured parameters. The difference between distributions of the same parameters, determined for S- and I-voids, was tested with the two-tailed Mann-Whitney test. The two-tailed Wilcoxon test was used to quantify the significance of the difference between the volume fractions (or number) of S- and I-voids. Uniformity of the distribution of voids within the volume of the canal filling was tested with the chi-square test. Before applying the test, the relative volumes were calculated in layers of 1-mm thickness and the chi-square test was applied to the resultant data. The p-value of <0.001 was considered statistically significant.

Results

The mean, standard deviation and range of all measured parameters are shown in Table 1. The volume fraction of I-voids was about five times as large as the volume fraction of S-voids, and this difference was statistically significant (p= 0.0007). In contrast, the average number of I-voids in the obturation was almost five times as small as the average number of S-voids, and this difference was also statistically significant (p= 0.0007). Distributions of the volumes and Feret's diameters of S-voids and I-voids are shown in Fig. 3. Both these parameters were significantly larger for I-voids (p< 0.0001). Only about 4% (58 out of 1505) of the S-voids have a volume larger than 1000 voxels. In contrast, the volume of more than 34% (128 out of 319) of the I-voids was larger than 1000 voxels. Feret's diameter was larger than 30 voxels for only about 2.5% of the S-voids and about 40% of the I-voids.

To determine whether the voids occur in any specific regions of the obturation, the relative volume of voids was plotted against the distance from the tip of gutta-percha (Fig. 4). The I-voids were present mainly in the apical third, while the S-voids occurred in the coronal third of the canal filling. Results of the chi-square test further support the hypothesis about the specific location of voids (p< 0.001 for both S-voids and I-voids), that is, there are regions in which voids occur preferentially.

Discussion

Recently, X-ray microCT has appeared to be a promising research tool in endodontics [6,20,25]. It has been successfully applied to visualize the complexity of root morphology [8,23,28], to investigate efficacy of canals instrumentation [10,17,23] and to evaluate quality of canal filling in many aspects [11,14,15]. MicroCT technique enables a precise 3D reconstruction of the complex shape of the root canal filling and allows demonstrating many details not detectable by 2D radiographs.

In this paper authors revealed findings of the first in Poland research demonstrating application of microCT in endodontic treatment. The present report focused on mi-

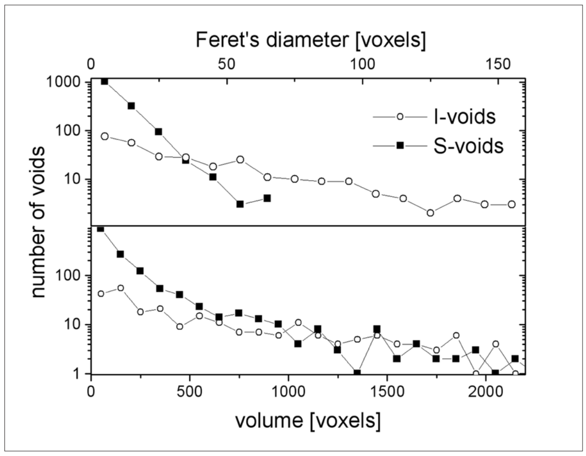

Fig. 3. Distributions of Feret's diameters (top panel) and volumes (bottom panel) of voids

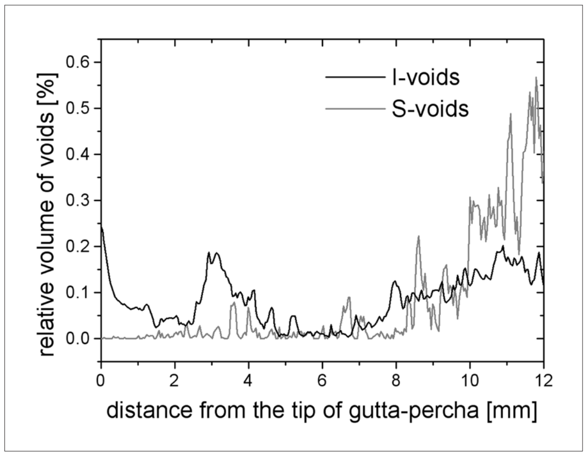

Fig. 4. Relative volume of voids plotted vs. distance from the tip of gutta-percha. For the definition of z-coordinate see caption of Fig. 1

Table 1. Mean, standard deviation and range of all parameters characterizing voids in the root canals filled with gutta-percha and Tubli-Seal

	S-voids		I-voids	
Parameter	Mean (standard deviation)	Range	Mean (standard deviation)	Range
Volume fraction (%)	0.14 (0.2)	0.01 - 0.77	0.62 (0.54)	0.12 - 1.75
Number	75 (109)	7 - 422	16 (6)	7 - 28
Volume (voxels)	222 (636)	3 - 16927	4482 (19093)	7 - 198458
Feret's diameter (voxels)	9.3 (8.5)	1 - 88	48 (66)	1 - 522

croCT evaluation of the quality of the canals filled with thermally softened gutta-percha with zinc-oxide-eugenol sealer. These filling materials were chosen because the microCT X-ray beam used in this study is not monochromatic: beam-hardening artifacts can distort the reconstructed image, even if dedicated filters are applied. These artifacts are especially intrusive for marked differences in contrast of materials present in the field of view. Thus, to suppress these artifacts, filling materials were chosen to minimize the difference between the radiopacities of the gutta-percha and the sealer. For these reasons, we were unable to quantify the volumetric relationship between gutta-percha and the sealer in the bulk of the obturation, although these data are clinically significant because risk of leakage increases with increasing volume of the sealer [15,32]. Note that with the microCT system used in the present study, it is not possible to accurately quantify the volume of the voids, the gutta-percha and the sealer at the same time. Low contrast between the gutta-percha and the sealer is necessary to reliably analyze microCT images, otherwise artifacts arising at the interface between the various filling compartments can be falsely identified as voids. On the other hand, high contrast is necessary to distinguish between gutta-percha and sealer, but in this case, the volume of voids cannot be quantified accurately. This problem can be solved if monochromatic microCT systems are used to capture 3D images of the obturated root canals.

The results revealed that none of the scanned root canal fillings were void-free. These outcomes corroborate studies by other authors [11,14,27]. In most cases, unfilled spaces were found as the boundary voids (I-voids), which formed between the material and the canal wall. They occupied more space in the canal as their mean volume (expressed both in percentage and voxels) and Feret's diameters were significantly higher than those of the S-voids. On the contrary, the S-voids, situated inside the filling, were much more compact, although their number was fivefold higher.

Furthermore, it follows from our analysis that both types of voids arise at specific locations. The distribution of unfilled spaces is important from clinical standpoint since it might be responsible for apical and coronal leakage after treatment. The interfacial adaptation of the filling material to canal dentine, especially in the apical third, was incomplete. Similar results were reported in other studies, which used different obturation methods and imaging techniques [1,4,9].

The presence of the largest I-voids in the apical part of canal obturation may be explained in several ways. In our study, the thermoplasticized filling method was used. Several studies have confirmed that warmed gutta-percha is more adaptable to the irregularities in the canal walls, permitting a better seal than does traditional cold condensation [1,4,15,29,30]. However, it has been suggested that using the thermal method might have a negative effect on the apical seal, at least initially, when the gutta-percha cools [30]. Moreover, high temperatures may cause dimensional changes in the eugenol-containing sealer [22]. It has been found that the combination of sealer contraction and air entrapment during compaction promotes apical void formation [7,19]. Some physical properties of Tubli-Seal, particularly high viscosity, flow, porosity and solubility might also have a deleterious effect on the apical seal [7,16]. According to Mutal and Gani [19], structural defects, like pores and vacuoles, observed in Tubli-Seal were larger and more frequent than in other studied sealers. However, Hammad et al. [11] demonstrated that application of gutta-percha with Tubli-Seal induced fewer voids than GuttaFlow sealer and resin-based sealer.

Interestingly, in the present study, voids occurred in many canals between the apical and middle segments of the obturation, most likely as a result of changing the filling technique from System B to Obtura. Moreover, at this level, canals often become oval-shaped and more difficult to fill [18,29]. The aforementioned voids were not noticeable in conventional radiographs. Similar observations were made by Anbu et al. [1], who used spiral computed tomography.

The numerous, small S-voids in the pericoronal part of the gutta-percha decrease the obturation homogeneity, which may promote microleakage from the oral environment. The cause of the filling porosity might be inadequate condensation of the injected warm gutta-percha within in the widest part of the canal. The importance of protection from coronal leakage was emphasized by Zakizedeh et al. [31] in a microCT study of intra-orifice barriers.

In this paper we have shown that quantitative calculation of canal obturation volume can be successfully performed without destruction of the teeth. Nevertheless, according to Zaslansky et al. [32], results obtained by microCT are underestimated as a consequence of the dehydration and moderate dimensional changes that accompany the prolonged scanning and reconstruction time. Underestimation of internal root size, evaluated after instrumentation, has been reported in other studies [17]. Moreover, reconstruction image artifacts due to extreme contrast between filling materials is a disadvantage of microCT technique [32]. Thus, further research is necessary to fully demonstrate the potential of microCT method in canal filling evaluation. In particular, to improve the quality of root canal filling evaluation procedures, based on microCT examination, a standard methodology must be developed, including imaging protocols and image processing methods.

Concluding, within the limitations of this study, our results indicate that microCT, with proposed semi-automatic algorithm, is an effective tool for endodontic research. MicroCT can be successfully used in a study of dental fillings; especially it is suitable for 3D quantitative evaluation of root canal fillings. Thus, in canals filled with thermoplastic gutta-percha and Tubli-Seal, voids at the interface between the filling and canal dentine deserve special attention because of their periapical location, which might promote apical microleakage. Further studies might help to elucidate the clinical relevance of these results.

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