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Observations of mineralised tissues of teeth in X-ray micro-computed tomography

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Background: *The one of the most recent imaging technology is X-ray microtomography which allows non-invasive three-dimensional visualisation of structures. It also offers the opportunity to conduct a comprehensive quantitative analysis of the tested objects such as measuring the shares of the various phases, determining the material density and distribution of the size of pores and particles. The aim of the paper was to present an overview on the applicability and relevance of X-ray microtomography in the study of mineralised tissues of the teeth.*
Materials and methods: *The article is based on the most recent and significant literature and own observations.*
Results: *The use of X-ray microtomography in dentistry has recently increased and includes, inter alia, the assessment of the density of minerals in enamel and dentin, the detection of demineralisation in an artificially and a naturally induced caries, the automatic measurement of the depth of cavities in dentin, the measurement of the amount of removed dentin in preparation of carious lesions by various methods, the assessment of microleakage around fillings and fissure sealants, cortical bone density measurement, evaluation of root canal morphology, comparison of the accuracy of root canal working and filling by various methods.*
Conclusions: *X-ray microtomography offers within the analysis of mineralised tissues — complex structures of bone, teeth and biomedical materials, turn out to be indispensable since it opens new opportunities for cognitive and implementation research.* (Folia Morphol 2017; 76, 2: 143–148)

Key words: micro-computed tomography, mineralised tissues, teeth

INTRODUCTION

One of the most recent imaging technologies created on the basis of computed tomography (CT) is X-ray microtomography (micro-CT, μ-CT), which allows non-invasive visualisation of structures in three-dimensional (3D). The main purpose of the CT scan is usually a non-destructive qualitative analysis of the structure, the detail or sample. X-ray microtomography also offers the opportunity to conduct a comprehensive quantitative analysis of the tested objects such as measuring the shares of the various phases, determining the material density (densitometry in case of bone tissue) and distribution of the size of pores and particles. This is a method used for *ex vivo* imaging in high contrast resolution of the internal structure of the examined objects. The mode of action

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is similar to a CT scan. The main difference lies in the smaller size of focal spot X-ray tube, which provides higher resolution images. Different is also the movement of the object by so-called *angular step*, when X-ray source and detector remain in the same position during the measurement. A projection is made at each angular position. Based on all collected projections, a reconstruction of the examined object is performed. Micro-CT is also a non-destructive method — there is a possibility of repeated testing without destroying or mechanical procession of the sample. These features make obtaining images at much higher resolution possible, which reaches submicron scale [8, 15, 19]. However, micro-CT is not used for human *in vivo* studies because of high mutagenic potential of X-ray used in the present technique [14, 29]. Therefore, research studies using micro-CT are carried out *in vitro* or on animal model [8, 13, 14, 24, 29, 30], and the only limitation then may be the size of the sample.

The use of X-ray microtomography in dentistry has recently increased and includes, inter alia, the assessment of the density of minerals in enamel and dentin, the detection of demineralisation in an artificially and a naturally induced caries, the automatic measurement of the depth of cavities in dentin, the measurement of the amount of removed dentin in preparation of carious lesions using various methods, the assessment of microleakage around fillings and fissure sealants, cortical bone density measurement, evaluation of root canal morphology, comparison of the accuracy of root canal working and filling using various methods [8, 19]. However, the studies using micro-CT in dentistry are mainly conducted using previously extracted teeth or animal model.

The aim of the paper was to present an overview on the applicability and relevance of X-ray microtomography in the study of mineralised tissues of the teeth. The article is based on the most recent and significant literature and on own examples.

MORPHOLOGY AND MINERALISATION OF HARD TEETH TISSUES IN MICRO-CT

Micro-CT is an effective and non-destructive technique for measuring the thickness of the enamel, which can be used, inter alia, in anthropological research and evolutionism of man, as well as in anthropological research on the history of the development of the human species. The analysis of mineralised tissues is important — including tooth tissues. The extraordinary durability of the enamel allows determining by characteristics, inter alia, the age, suffered illnesses, eating habits, lifestyle, and makes comparing individual species among themselves possible [11, 21]. In addition to the thickness of the enamel, X-ray micro-CT system can also generate the image of tooth structures accurately and reliably: the thickness and surface of the enamel, dentin and pulp chamber [11], and root canal system on different levels (Fig. 1). Furthermore, by using imaging software, 3D reconstructions of the test area can be produced, which also provides inter alia, the volume data of enamel and dentin [11, 15]. The accuracy of the enamel thickness measurements using the micro-CT is comparable with measurements made by using the direct method (physical sections) and micro-CT sections differing by only 3–5% [11]. X-ray microtomography is applied to the qualitative and quantitative analysis if the test sample has a sufficiently large variation of absorption of X-ray radiation by its individual attenuation coefficient, and therefore for the highly mineralised tooth areas, reliable identification of different tissues using a micro-CT can be difficult. Moreover, the areas of very thin enamel (less than 100 microns) can be difficult to identify [21]. Computed X-ray microtomography facilitates performing a full morphometric analysis and thus provides a full set of quantitative data describing the tested structure [15]. Morphometric analysis consists in determining the geometrical characteristics of structures such as phase shares, porosity, particle size, pore size and their statistical distributions. Micro-CT can be also used to study the dentin structure (Fig. 2) [4]. The degree of

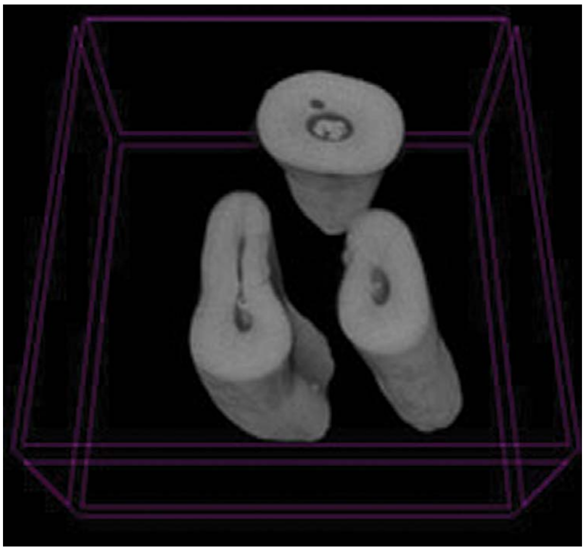

Figure 1. Three-dimensional image of root canal system of human teeth in micro-computed tomography analysis (SkyScan 1172, Bruker).

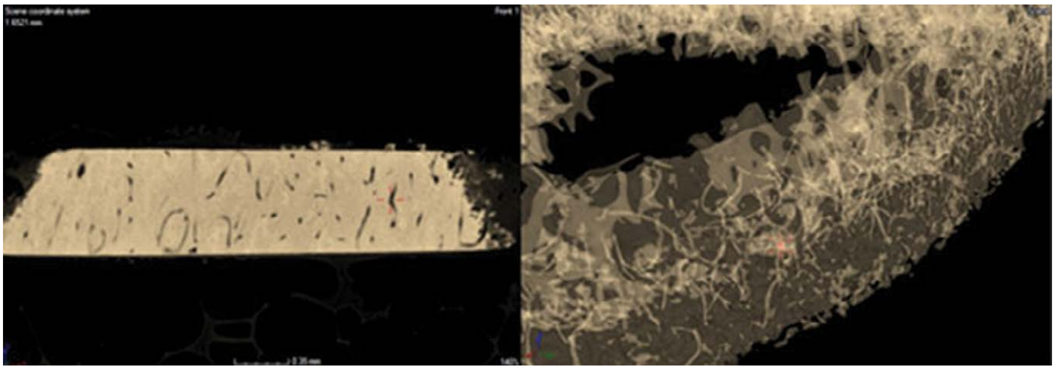

Figure 2. The image of dentinal tubules using micro-computed tomography system 1: 0.035 (v|tome|x m, General Electric-Measurement & Control Solutions, Germany).

variability in the concentration of minerals within the hard tissues of healthy teeth in different individuals, in different teeth, and even within a single tooth is known, as well as the relationship between the degree of mineralisation of tooth enamel and caries susceptibility [28]. Previous studies had shown that specific weight of the deciduous teeth enamel was lower than of the permanent teeth enamel and the level of mineralisation of deciduous teeth enamel had an increasing gradient from the enamel-dentin junction to the outer enamel surface [26, 28]. The most recent research based on the results of microtomography, developed to measure the concentration of minerals in bones and teeth with accuracy higher than 1% and a resolution of 5–30 microns, confirms a large variation in the enamel mineralisation level and the gradient of concentrations between the enamel-dentin border and an outer surface of enamel in healthy milk molars of different persons [3, 10, 30]. Davies et al. [8] had similar observations based on linear attenuation coefficient (LAC), describing higher values of LAC in dentin than in the pulp region. Application of micro-CT was also a very good tool for comparing the mineral content of hard tissues of premolars and enamel pearls [2]. It was found that the mineral content in the superficial and deeper layers of enamel pearls was similar to that observed in the enamel of premolars. In contrast to the dentine mineral content in pearls, which assumed the highest values on the enamel-dentine border, the gradient proceeded contrary to the dentine of premolars.

CARIOUS TISSUES AND CAVITY PREPARATION ASSESSMENT IN MICRO-CT

One of the major applications of micro-CT is the study of the porosity, which is facilitated by a high contrast in density between the solid phase and air. This has been used to measure the demineralisation of enamel and dentin [13, 14, 24, 29]. The main advantages of the system, such as the 3D reconstruction of carious lesions and getting two-dimensional (2D) cross-sections along any axes of the 3D image, make it an ideal system to study the dynamic process of re- and demineralisation. Various researchers have shown that the concentration of minerals in sound enamel in the superficial — an intact layer of enamel white spot lesions and the deepest layer of initial caries, gradually decreases [14]. Other authors also found that examination of initial caries in the enamel using micro-CT showed no significant differences between the lesions clinically qualified as active and inactive. Only the active lesions had a more porous surface in comparison with inactive lesions [7]. Another pathology imaged in the micro-CT scan is the dentinal dead tracts extending from the exposed, damaged surface of the molar around the tooth neck to the pulp. It has been shown that the density of minerals in the dead tracts is similar to that of healthy dentine [8]. Some other micro-CT studies performed on extracted deciduous teeth evaluated the shape of carious lesions in dentin [24]. The starting point for the study were the differences between the histopathological test results confirming that carious lesions in the dentine had a characteristic shape of a cone, with a base facing the enamel-dentine junction and with the tip towards the pulp. According to micro-CT scans, the shape of the carious cavity in dentin observed in all planes had a broad foundation within the enamel-dentine border with a curved area facing the pulp. Only in the mesial-distal plane, the bottom of the cavity had a more pointed shape than that one observed in the other two planes. Consequently, probably based on X-ray scan, the cavity can be described as having a triangular shape, which results from the interpretation of 2D image. The golden standard for *in vitro* studies of caries is histological examination and transverse microradiography (TMR). In both methods, it is necessary to divide the examined tooth into scraps, which may cause changes to the results of the analysis. Micro-CT seems to be a potential solution to these problems. Comparison of TMR with micro-CT measurements of the enamel lesions under various X-ray conditions and software beam-hardening correction show that the micro-CT can be used as a non-destructive alternative to TMR for examining lesions in the enamel [12]. Another comparison of the histological examination and micro-CT scan in the assessment of occlusal surfaces of the teeth revealed that the histological measurement is more detailed for the detection and quantitative assessment of enamel demineralisation but also said that the depth of the carious cavity in the dentine could be accurately and objectively measured by micro-CT [25]. Soviero et al. [23] assessed the micro-CT in detection and evaluation of the depth of proximal deciduous teeth carious lesions comparing them with the visual assessment using the International Caries Detection and Assessment System (ICDAS), conventional bite-wing radiographs (BW) and a histological test as the golden standard [23]. Studies have shown that micro-CT vs. ICDAS and BW is the most sensitive and accurate method of assessing the demineralisation of the outer portion of the enamel (E1), and the lesions involving enamel-dentin border (D1). Micro-CT results also have shown the highest correlation with a histological study. According to the results, in order to obtain more reliable results in the micro-CT scan, a visual examination of samples of teeth should be combined with bone mineral density analysis of dental hard tissues [23]. This micro-CT scan proved a highly sensitive tool for detecting caries of the enamel and differentiation of lesions in enamel and dentin, showing that it is an important tool for detecting and evaluating the depth of proximal carious lesions *in vitro* [23]. In similar studies conducted *in vitro* [18] it was confirmed that the results of micro-CT scan had a high diagnostic value in assessing proximal caries of the enamel; it is more accurate than visual examination and radiography. However, the authors found that it cannot be an alternative for histological test and it should be verified by examining more proximal surfaces of the teeth because selection of assessed cases (the predominant percentage of the demineralised or normal surfaces) can have a significant impact on the results [18, 23]. Another study using a micro-CT method and conducting quantitative analysis of the image of initial carious lesion of human enamel formed in natural *in situ* conditions seems also very interesting. The micro-CT scan of demineralised samples of the enamel from volunteers using intraoral mandibular appliances was conducted after 21 or 29 days. The micro-CT results allowed the confirmation of the earlier results of *in vitro* studies of the four-layered structure of initial carious lesion. It was also shown that more demineralised areas have a similar layer structure as the more uniform samples; they differ in the amounts of subsurface pores [16]. However, the tested samples showed significant differences in the morphology of the enamel surface and the degree of loss of bone mineral density depending on the conditions prevailing in the mouth of the volunteer, the duration of exposure and the position of appliance [16].

Another application of micro-CT is the assessment of different techniques of cavity preparation [26–28]. Currently in the restorative dentistry, the principle of economical preparation of the carious cavity tissue is important. The main goal of clinicians is to remove irreversibly damaged dentine, and leave the tissue, which can be remineralised at the bottom of the cavity, but the observation, even in the high-resolution light microscopy, shows that the boundary between these two layers is not well marked. Evaluation of complete removal of carious dentin is therefore subjective and based on the examination of hardness of tissue being prepared and can lead to excessive removal of healthy dentin from the cavity rated from 8.5% to 44.3% by volume [27]. With using micro-CT it was possible to assess a conventional technique for removing caries using the excavator and chemo-mechanical one using Carislov gel and to find that healthy dentin was removed in the conventional and chemo-mechanical technique respectively at 4% and 2.1% [1]. Also Neves et al. [20] found that chemo-mechanical preparation of cavities was the most selective method for removing dental caries while preserving the healthy tissue. By using micro-CT, they compared the effectiveness and minimal invasiveness potential of nine techniques for dental caries removal, including an Erbium-YAG laser, drills: tungsten, CeraBur and tungsten with Caries Detector, chemo-mechanical and ultrasonic removal of caries and three experimental methods [20]. Thanks to micro-CT, the effectiveness of caries removal was possible to study using two parameters, measured after removal of caries: the (mean) relative volume of residual caries and the mean mineral density of the dentine at the bottom of the cavity. According to the micro-CT results, it has been shown that the Erbium-YAG laser cannot be considered as selective technique for removing decay due to variable efficacy of results and minimally invasive potential. The use of rotary and oscillatory techniques for removal of caries showed a better efficiency of removal of dental caries, except for CeraBur and ultrasounds, which showed a clear tendency to leave residues of caries at the bottom and walls of the cavity [20].

BIOMATERIALS ASSESSMENT IN MICRO-CT

X-ray microtomography is also used, inter alia, in biomedical engineering. The possibilities offered in the analysis of complex structures of biomedical materials turn out to be indispensable. An example of the use of microtomography in imaging biomaterials used in medicine/dentistry is the research on the quality of tooth filling. In this case, there is no other opportunity to assess the filling without destroying the samples besides tomographic analysis. Classical X-ray image does not reflect all the details and does not allow accurate representation of the degree of penetration of the material in the tooth structure or the impact of biomaterial on the surrounding tissue [6, 8, 9, 17]. Modern research methods, including undoubtedly the X-ray microtomography, make a precise analysis of biomaterials used in dentistry possible, for example of their surface area, surface structure, internal structure, porosity and the nature of the connection between them and the living tissue. Analysis of the properties of several materials allows predicting the final result of their actions and choosing the most appropriate biomaterial in the given clinical situation, even bioactive one (e.g. Biodentine) (Fig. 3) [4, 5].

Micro-CT is also a very useful device for the development of a standard method of measuring the marginal seal with 50% silver nitrate solution as a marker [6, 9]. The proportion of penetration depth of silver nitrate visible in microtomography at the contact surface in the area of the neck of the tooth ranged from 10.2% to 92.6% depending on the method of preparation of the cavity (conventional or ART) and the material used (composite or glass-ionomer) [9]. This method made the evaluation of the deepest penetration of the marker possible, which in turn provides an accurate measurement of the level of connection of the sealant or filler material and the tooth tissues [9, 22]. By using

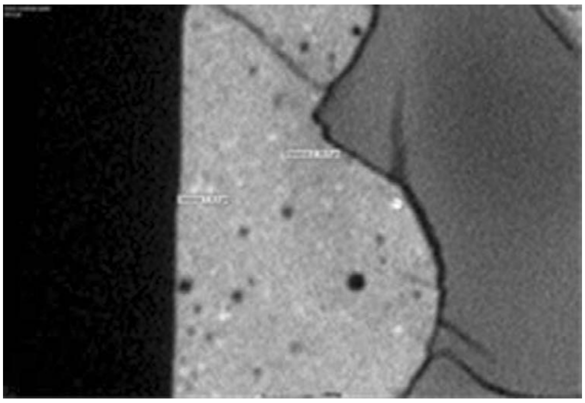

Figure 3. Bioactive material Biodentine in contact with dentin 1: 0.023 (X-ray microtomography v | tome | x m General Electric-Measurement & Control Solutions, Germany).

micro-CT, the new high-viscosity glass-ionomers were assessed to have a significantly lower marginal leakage than composite sealants [6]. An *in vitro* research by Lee et al. [17] shows that micro-CT is an effective method to assess the effect of remineralisation of caries at contact surface adjacent to the glass ionomer restorations. The study evaluated a change in the density of minerals of carious lesions before applying samples of materials and after 90, 180 and 270 days. Average values of changes in mineral density were compared between the groups in order to assess the effects of remineralisation. The study showed in micro-CT that remineralisation of proximal carious lesions was greater in the vicinity of conventional and resin-modified glass ionomers and compomers in comparison with composites. In addition, Davis et al. [8] showed the glass-ionomer activity on partially demineralised dentin in micro-CT. The most significant change observed in micro-CT after 1 week is an increase in radio-opacity within the demineralised dentin confirming the release of strontium ions from the cavity-filling material. This is also confirmed by an increase in the LAC of demineralised dentin, which also corresponds with a decrease in radio-opacity in glass-ionomer immediately adjacent to the cavity [8].

CONCLUSIONS

X-ray micro-CT is a non-destructive method of testing materials, which allows obtaining with high precision of flat or spatial scan of material or element in question. This method allows 3D analysis of the material structure properties, including its comprehensive qualitative and quantitative description as well as, to a limited extent, analysis of chemical composition. A great advantage of this method is the ability to study solid samples for imaging internal architecture and performance of 3D models that describe the geometry. In summary, the capabilities of the X-ray microtomography offering the analysis of mineralised tissues — complex structures of bone, teeth and biomedical materials, turn out to be indispensable since it opens new opportunities for cognitive and implementation research.

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