

Analysis of selected parameters of reparative dentin after direct pulp capping with MTA Repair HP in human teeth, using CBCT and micro-CT

Mirona Palczewska-Komsa^{1,A–F}, Adam Puszkarz^{2,B,C,F}, Bartosz Dalewski^{1,E,F}, Kinga Kaczor-Wiankowska^{3,B,C,F}, Ewa Sobolewska^{1,E,F}, Łukasz Pałka^{4,E,F}, Magdalena Gębska^{5,E,F}, Alicja Nowicka^{3,6,A,E,F}

¹ Department of Dental Prosthetics, Pomeranian Medical University in Szczecin, Poland

² Textile Institute, Faculty of Material Technologies and Textile Design, Lodz University of Technology, Poland

³ Department of Conservative Dentistry and Endodontics, Pomeranian Medical University in Szczecin, Poland

⁴ Private dental practice, Wrocław, Poland

⁵ Department of Musculoskeletal System Rehabilitation, Pomeranian Medical University in Szczecin, Poland

⁶ NovoDental Clinic, Bezrzeczce, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence

Łukasz Pałka
E-mail: dr.lpalka@gmail.com

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Conflict of interest

None declared

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Abstract

Background. The biomimetic regeneration of the pulp–dentin complex is a modern approach involving the application of a bioactive substance. Bioactive substances (e.g., cement or stem cells) have been reported to be able to control the signaling and differentiation of the pulp cells, thus limiting the inflammatory reactions and enabling the repair and regeneration of tissues. One of such materials is MTA Repair HP (MTA HP) – a type of mineral trioxide aggregate (MTA) material characterized by high plasticity, composed of hydrophilic particles of mineral oxides.

Objectives. The aim of the present study was to analyze the reaction of the pulp–dentin complex of human teeth to MTA HP using 2 radiological techniques – cone-beam computed tomography (CBCT) and micro-computed tomography (micro-CT).

Material and methods. In the study, 6 caries-free, intact maxillary and mandibular third molars or premolars from 6 patients aged 30–37 years were analyzed. The teeth were scheduled to be extracted for orthodontic or surgical reasons.

Results. No statistically significant differences in the examined parameters of the tertiary dentin were observed in the CBCT and micro-CT images ($p < 0.05$).

Conclusions. The CBCT technique, similarly to micro-CT, proved to be effective in identifying and assessing the tertiary dentin. Furthermore, CBCT has the advantage of usability in clinical settings. Knowledge on the parameters of reparative dentin identified with radiological techniques is still insufficient, and further research is recommended.

Keywords: micro-CT, CBCT, MTA HP, biomimetic material, reparative dentin

Highlights

- The biomimetic regeneration of the pulp–dentin complex is a modern approach involving the application of a bio-active substance, such as mineral trioxide aggregate (MTA).
- After applying MTA Repair HP to human teeth (maxillary and mandibular third molars or premolars), no statistically significant differences in the examined parameters of the tertiary dentin were observed in the CBCT and micro-CT images.
- The CBCT technique, similarly to micro-CT, proved to be effective in identifying and assessing the tertiary dentin.

Introduction

Vital pulp therapy (VPT) is part of the minimally invasive endodontics.^{1–3} At the molecular level, in the context of supporting wound healing and the regeneration of soft and hard tissues, the application of biomimetic materials onto the pulp–dentin complex has been widely studied, as these materials have a biostimulating effect on the regenerative processes of the pulp–dentin complex.⁴

Apart from clinical examinations, cone-beam computed tomography (CBCT) examinations are used to assess the success of VPT. However, in comparison with CBCT, micro-computed tomography (micro-CT) allows imaging with improved parameters of given structures and fewer image distortions. At the same time, in comparison with the most frequently applied histological techniques, micro-CT does not result in sample destruction, so the material can be analyzed repeatedly. The literature on the subject encompasses visual analyses and quantitative assessments of the newly formed reparative dentin, following the use of various bioactive cements, using CBCT and micro-CT. In human teeth, reparative dentin has been analyzed with the CBCT and micro-CT techniques.^{5–10} Micro-CT has also been used for visualizing reparative dentin in the teeth of rats, mice and old baboons.^{11–16}

Various biomimetic preparations are used in VPT, all aimed at supporting natural defensive and regenerative mechanisms, and the eventual healing of pathological changes.¹⁷ One of such materials is MTA Repair HP (MTA HP). Mineral trioxide aggregate (MTA) has a wide range of applications in endodontics, i.e., for direct pulp capping (DPC), closing root canal perforations, as well as the perforations which occur within the furcation regions due to iatrogenic reasons, treating carious lesions or resorption, and retrograde root canal filling within periapical surgery.^{18–21}

The aim of the present study was to analyze the reaction of the pulp–dentin complex of human teeth to the direct application of MTA HP, using 2 radiological techniques – CBCT and micro-CT. The study used both techniques to assess the structural parameters of the reactionary dentin (the dentin bridge) formed underneath the MTA HP material in human teeth. The null hypothesis states that the CBCT and micro-CT techniques provide comparable imaging of reparative dentin structures in human teeth.

Material and methods

Material

In the study, 6 caries-free, intact maxillary and mandibular third molars or premolars from 6 patients aged 30–37 years were analyzed. The teeth were scheduled to be extracted for orthodontic or surgical reasons. The study was conducted in full accordance with ethical principles, including the World Medical Association (WMA) Declaration of Helsinki (2008). The patients received a thorough explanation of the experimental rationale, clinical procedures and possible complications. The experiments were undertaken with the understanding and written consent of each patient, and according to the abovementioned principles. All experimental protocols were independently reviewed and approved by the Local Ethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval No.: KB/2020/47/NK).

Operating procedure

Periapical radiographs were taken for all the analyzed teeth (MINRAY®, Soredex, Tuusula, Finland). Tooth sensitivity was assessed with thermal testing (Kältespray; M+W Dental, Bidingen, Germany) and electric sensitivity testing (Vitality Scanner Electric Pulp Tester; SybronEndo, Orange, USA). All clinical procedures were performed by the same clinician, who used an operating microscope with ×4 magnification (OPMI Pico; Carl Zeiss, Jena, Germany). Occlusal class I on the occlusal surfaces was prepared with a sterile round diamond bur at a high speed, with standard abundant water cooling. When approaching the pulp chamber, a new sterile round diamond bur (ISO size 012) was used to gently produce an exposure of at least 1 mm². Bleeding was controlled by irrigation with a saline solution (0.9% NaCl) and a sterile cotton pellet. The exposed pulp was capped with a 2–3-millimeter-thick layer of MTA HP (MTA Repair HP; Angelus USA Inc., Vernon, USA). MTA-HP was covered with a thin layer of glass ionomer (Riva Light Cure HV; SDI, Chicago, USA). Finally, the cavity was filled using Single Bond™ Universal with the self-etch technique and Filtek™ Ultimate (3M ESPE, Seefeld, Germany).

Clinical examination

The evaluation of the pulp status was conducted based on patient-reported symptoms with a verbal pain intensity scale, clinical examinations, and thermal and electrical tests. Immediately after cavity restoration, the patients were asked to register their level of discomfort. Clinical assessment was performed 6 weeks after the procedure.

Radiological examination

Six weeks after DPC with MTA-HP, before extraction, periapical radiographs were taken (DIGORA™ Optime UV; Soredex) using the right angle technique with positioners. The identical exposure parameters were applied in all cases. Subsequently, the teeth were extracted as atraumatically as possible by a qualified oral surgeon under local anesthesia with mepivacaine without a vasoconstrictor (Scandonest®, Septodont, Saint-Maur-des-Fossés, France). The CBCT and micro-CT examinations were performed after tooth extraction. Each tooth was scanned separately, using the following exposure parameters: source voltage [kV]: 60–70 automatic control (CBCT), 90 (micro-CT); source amperage [μA]: 100–750 automatic control (CBCT), 111 (micro-CT); resolution [pixels]: 341 × 341 (CBCT), 4,032 × 2,688 (micro-CT); pixel size [μm]: 500 (CBCT), 4.5 (micro-CT); exposure time [ms]: 5,500 (CBCT), 1,306 (micro-CT); and filter: Cu 0.2 (CBCT), Al 0.5 + Cu 0.038 (micro-CT). In the CBCT analysis of a given tooth, reparative dentin was identified in original axial areas and in multidimensional reconstructions with the Veraview IC5 HD tomograph (J. Morita, Ina-machi, Japan). The three-dimensional (3D) image analysis procedures were performed using the open-source software ITK-SNAP, v. 3.8 (<http://www.itksnap.org/pmwiki/pmwiki.php>) and 3D Slicer (SlicerCMF extension of 3D Slicer, v. 4.11.0; <https://www.slicer.org>). The original de-identified DICOM files were converted to nii.gz files using ITK-SNAP.²² In the micro-CT analysis of a given tooth, the identification of reparative dentin was performed using SKYSCAN 1272 (Bruker Corporation, Kontich, Belgium), and the 3D reconstructions of the teeth were obtained using the NRecon 1.7.4.2 and CTvox 3.3.0 r1403 software by Bruker. The geometrical parameters of the tested reparative dentin were calculated using the CTAn 1.17.7.2+ software by Bruker.

In both the CBCT and micro-CT analyses, the region of the exposed pulp was identified as the drilled area of the pulp chamber roof. The largest diameter of the exposed pulp was calculated (in millimeters).

Identification of reparative dentin

In both the CBCT and micro-CT examinations, the area of the tertiary dentin was determined entirely manually. This was due to the low contrast between the tertiary and

secondary dentin. Two-dimensional (2D) sections were obtained in the transverse plane to determine the first and the last layer in the crown-apical direction so that reparative dentin could be identified. In comparison with the primary dentin, it was assumed that the calcified region underneath the pulp-capping material is the region of the induced tertiary dentin. The tertiary dentin was marked green, using special software (SKYSCAN 1272) with a tool for coloring the regions of interest (ROI).

The visual assessment of reparative dentin was conducted through the micro-CT analysis in 3 planes normalized with the color change scale. Following the reconstruction of the images, grey areas were displaced by color, taking advantage of the fact that the areas of correct pulp, dentin and enamel are X-ray-permeable to different extent. It was adopted that in a correctly formed tooth, the green and blue colors represent fillings, and purple represents pulp. Therefore, reparative dentin was defined as orange, and different color saturation of the tissue was considered as differences in calcification as compared to physiological dentin. The 3D reconstructions of the tested teeth were obtained using the NRecon 1.7.4.2 and CTvox 3.3.0 r1403 software.

Quantitative assessment of reparative dentin

Thickness of reparative dentin

The reparative dentin thickness was determined using a linear measurement tool and measuring the distance from the base of the restoration to pulp in the sagittal, coronal or cross-sectional images generated from the CBCT or micro-CT scans after the orientation of the orthogonal planes with the use of the software vertical and horizontal reference lines.²³ The serial profile of reparative dentin formed in the direction from the crown (the first virtual layer) to the root (the last virtual cross-section) was determined using tooth axial layers in the CBCT analysis size 0.125 mm, whereas in the micro-CT analysis, the size was 0.0045 mm; it allowed the calculation of the estimated thickness of the tertiary dentin (in millimeters).

Volume of reparative dentin

A solid figure was isolated from the obtained images of all planes, corresponding to the formed tertiary dentin; its volume (in cubic millimeters) was mathematically calculated in the CBCT analysis using ITK-SNAP, v. 3.8., and in the micro-CT analysis, the CTAn 1.17.7.2+ software by Bruker.

Statistical analysis

The mean (*M*), standard deviation (*SD*), median (*Me*), minimum (*min*), and maximum (*max*) values, and the coefficient of variation (*CV*) were calculated for the thickness and volume of the tertiary dentin, and the diameter of the exposed pulp. The distribution of values deviated

from normal distribution in the Shapiro–Wilk test. Therefore, the non-parametric Mann–Whitney test was used to compare the variables.

Results

Clinical examination

All the analyzed teeth were characterized by a normal sensitivity response to a cold stimulus (pain lasting up to 10 s). Sensitivity to electric current was within the normal values in the range of 23–47 units. None of the patients reported pain after treatment.

Radiological examination

Based on the X-ray images, it was found that in all teeth under study there was a lack of pathological lesions, such as the presence of mineralization in the pulp chamber, or internal or external resorption, which would suggest the failure of DPC.

Identification and visual assessment of reparative dentin

In all teeth under analysis, the presence of reparative dentin was identified. The visual assessment of reparative dentin in the CBCT and micro-CT analyses was performed in 2D and 3D projections. The data concerning each sample is presented in Fig. 1, comparing the X-ray, CBCT and micro-CT images of tooth 18 after covering pulp with the MTA HP material.

Quantitative assessment of reparative dentin

Table 1 shows the descriptive statistics of the selected geometrical parameters of reparative dentin and the pulp exposure in CBCT and micro-CT. Concerning the geometric parameters of reparative dentin, it was found that the mean thickness and volume of reparative dentin were 0.39 ± 0.24 mm and 0.42 ± 0.29 mm³ in CBCT, and 0.31 ± 0.18 mm and 0.30 ± 0.22 mm³ in micro-CT, respectively. There were no statistically significant differences in the

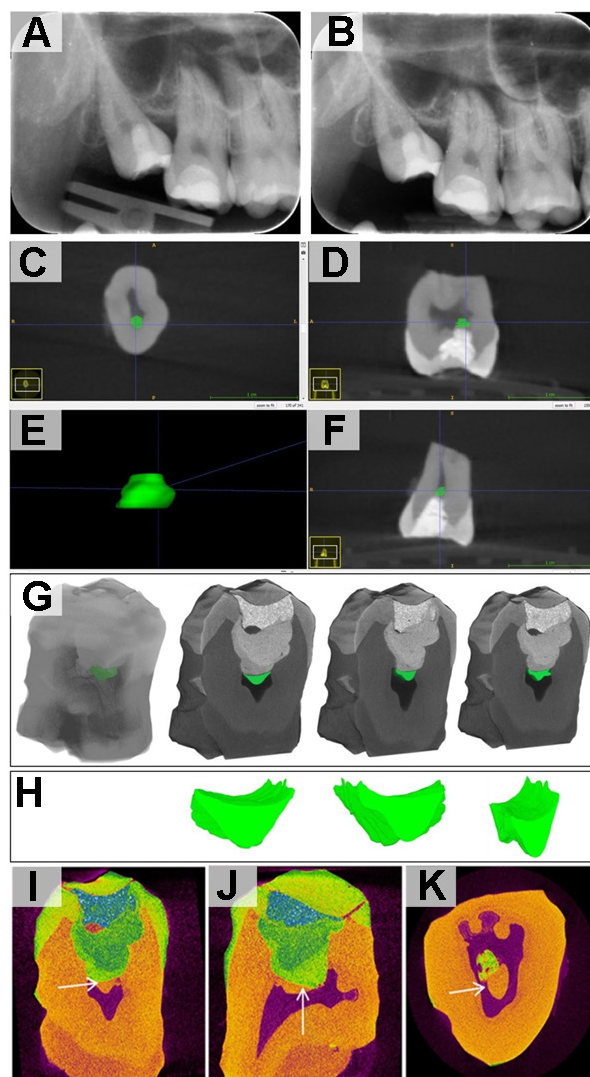


Fig. 1. Comparison of the X-ray, cone-beam computed tomography (CBCT) and micro-computed tomography (micro-CT) images of tooth 18 after covering pulp with the MTA (mineral trioxide aggregate) Repair HP (MTA HP) material

A – X-ray: tooth 18 equipped with the MTA HP material immediately after the direct pulp capping (DPC) procedure; B – X-ray: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure; C–F – CBCT: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure (green color – reparative dentin); G – micro-CT: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure (green color – reparative dentin); H – micro-CT: tooth 18 – the isolated solid figure of reparative dentin; I–K – micro-CT: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure (white arrows – reparative dentin, orange color – dentin, green and blue colors – filling, purple color – pulp).

Table 1. Comparison of the geometrical parameters of reparative dentin and the pulp exposure in CBCT and micro-CT

Parameter	Technique	N	M ±SD	Me	min	max	CV
Reparative dentin thickness [mm]	CBCT	6	0.39 ±0.24	0.32	0.16	0.78	61.32
	micro-CT	6	0.31 ±0.18	0.29	0.14	0.62	57.11
Reparative dentin volume [mm ³]	CBCT	6	0.42 ±0.29	0.42	0.10	0.87	70.11
	micro-CT	6	0.30 ±0.22	0.33	0.05	0.56	73.54
Pulp exposure diameter [mm]	CBCT	6	1.47 ±0.53	1.56	0.75	2.25	35.76
	micro-CT	6	1.48 ±0.56	1.27	0.89	2.36	38.16

M – mean; SD – standard deviation; Me – median; min – minimum; max – maximum; CV – coefficient of variation.

thickness and volume of the tertiary dentin, and in the diameter of the exposed pulp in CBCT and micro-CT ($p < 0.05$). A moderate correlation ($r = 0.67$), at the confidence level of 0.95, was marked between the pulp exposure values obtained with both techniques (CBCT vs. micro-CT). For CBCT, there was a strong positive correlation between the minimum and maximum thickness values ($r = 0.91$), and for micro-CT, the correlation between the values was very weak, expressed with $r = 0.48$. The correlation between the reparative dentin volume values in CBCT and micro-CT was very weak ($r = 0.43$).

Discussion

The formation of compact, hard reparative dentin without bacterial invasion is the crucial key to the success of VPT.^{13,24} The average diameter of the exposed pulp in the examined teeth was 1.47 mm in the CBCT examination and 1.48 mm in the micro-CT examination. Statistical analysis showed no difference in the size of the exposed pulp with regard to the technique used. The obtained values are consistent with those mentioned by other authors.^{5,8}

Reparative dentin is a highly heterogeneous structure. Isolating a whole solid is a complex and time-consuming endeavor. However, the parameters of reparative dentin have been calculated and discussed in various studies.^{10–20,22,25} In all the analyzed teeth, the presence of the newly formed reparative dentin was identified through both CBCT and micro-CT; however, the tissue showed differences in terms of shape and size. Reparative dentin was formed in an irregular and unpredictable manner, though its form was closely related to the shape and size of the exposed pulp and the MTA HP material covering the wound. Therefore, it can be cautiously concluded that the actions of the operator conducting DPC affect the shape of the newly formed reparative dentin. With greater insertion of the material to the pulp wound, the reparative dentin bridge is more irregular and larger. More homogeneous reparative dentin, without defects such as a tunnel defect, provides better protection for the exposed pulp.

The visual analysis of the tertiary dentin with the use of CBCT relies on identifying the presence of islands of calcified tissue, as well as detecting complete (continuous) tertiary dentin.⁵ Nowicka et al. used the CBCT technique to show the presence of 25 tertiary dentin bridges out of the 37 histologically identified bridges after the DPC of human teeth with various materials.⁸ In another similar study on humans, 8 weeks after the application of various materials, the highest number of complete tertiary dentin bridges was recorded in the premolar group, following pulp capping with MTA and EndoSequence Root Repair Material (ERRM).⁹

The visual analysis of the parameters of reparative dentin is frequently carried out in micro-CT.^{10,12,13,15,16}

The analyses of reparative dentin morphology were most frequently conducted on animal teeth in the procedure of indirect or direct pulp capping with various MTA cements.^{13,16,23–25} In a micro-CT based study on 10 molar rat teeth, after 4-week observations, the areas of the newly formed reparative dentin and the pulp cavity were measured in 5 randomly selected transverse sections, followed by the calculation of the relative ratio of the reparative dentin area to the pulp cavity area.¹⁶ Following reconstruction, in order to show mineral density, grey images were displaced by color images. In visual assessment, greater development of the tertiary dentin was identified in the teeth capped with the MTA material.¹⁶ In our study, similarly to the study by Kim et al.,¹⁶ the color change scale was adopted using the NRecon 1.7.4.2 and CTvox 3.3.0 r1403 software.

Apart from the visual assessment of reparative dentin, the quantitative assessment of its physical parameters, such as thickness and volume, is important. In comparison with visual assessment, quantitative assessment is more precise and objective.

With regard to CBCT imaging, the thickness of reparative dentin has been analyzed in human teeth. In a study by Nowicka et al., the thickness of the tertiary dentin in the CBCT images was 0.23 mm in the MTA group.⁸ In the present study, the mean thickness of the dentin bridge in the 6 analyzed samples was 0.39 mm. The difference could be due to the size of the evaluated samples and the measurement technique.

Kim et al. analyzed the thickness of reparative dentin with micro-CT in 30 premolar old baboon teeth, in which the exposed pulp was capped with calcium hydroxide ($\text{Ca}(\text{OH})_2$), ProRoot® MTA White or white Portland cement (PC).¹⁶ Following the use of ProRoot MTA White, the reparative hard tissue achieved a thickness of 0.43 ± 0.05 mm.¹⁶ AlShwaimi et al. analyzed the exposed pulp of 18 human premolar teeth, which were capped with betamethasone/gentamicin (BG) cream and MTA.¹³ It was found that the thickest reparative dentin was formed in the teeth capped with MTA. The mean thickness of the reparative hard tissue was 0.078 mm.¹³ The present study shows that the identified range of the thickness values 6 weeks after DPC with MTA HP was 0.14–0.78 mm. There was no statistically significant difference in the thickness of the tertiary dentin between the CBCT and micro-CT techniques. The mean thickness calculated based on the thickness value ranges of the individual 6 teeth under analysis was 0.39 mm, which is in line with the results obtained in the abovementioned studies. However, it should be noted that the calculation of the mean thickness of reparative dentin presented herein should be treated as a guidance value, since other factors must be taken into account as well, e.g., the number of samples (6–30), the type of teeth under analysis, study time (between 8 weeks and 4 months), species (humans, old baboons), and the material used for DPC.

Generally, determining the volume of reparative dentin is not possible using conventional radiovisiography, and is difficult using the CBCT and micro-CT techniques. A study using CBCT in the analysis of human teeth, 6 weeks after extraction demonstrated that the tomography resolution negatively affected the detection of the minimum measurable distance, and therefore histological images were necessary to measure very small bridges.⁸ It was found that in the Ca(OH)₂ group, the volume of the formed reparative dentin was moderate, whereas in the MTA (0.45 mm³) and Biodentine groups (0.47 mm³), it was moderate to high, with no significant differences between the latter two groups.⁸ In the present study, when employing the CBCT analysis, the mean volume of dentin bridges amounted to 0.42 mm³, which is slightly lower than the results obtained by Nowicka et al.⁸ The difference may result from different materials used in the studies.

In our study, the volume of reparative dentin in human teeth was measured with the micro-CT technique for the first time. To achieve the highest precision in the measurement of the geometrical parameters of the dentin bridge, the ROI enclosing the analyzed reparative dentin was determined manually. This principle was adopted in the present study with respect to both the CBCT and micro-CT techniques, and there was no statistically significant difference in the volume of the tertiary dentin between these techniques. CBCT, similarly to micro-CT, was effective in identifying and assessing tertiary dentin, so the null hypothesis was accepted. However, the limitation of the present study is the small number of samples, which was due to the difficulty in obtaining the study material.

Conclusions

The CBCT technique, similarly to micro-CT, proved to be effective in identifying and assessing the tertiary dentin, and CBCT has the advantage of being applicable in clinical settings.

In the future, the scope of studies on the issue discussed here is to be expanded, as knowledge on the parameters of reparative dentin identified with radiological techniques is still insufficient.

Ethics approval and consent to participate

The study was approved by the Local Ethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval No.: KB/2020/47/NK). All patients provided written informed consent to participate in the study.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.









Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Mirona Palczewska-Komsa  <https://orcid.org/0000-0003-3875-488X>
 Adam Puszkarz  <https://orcid.org/0000-0002-1765-9093>
 Bartosz Dalewski  <https://orcid.org/0000-0002-0652-664X>
 Kinga Kaczor-Wiankowska  <https://orcid.org/0000-0003-2201-8167>
 Ewa Sobolewska  <https://orcid.org/0000-0001-7932-9631>
 Łukasz Pałka  <https://orcid.org/0000-0002-9365-7882>
 Magdalena Gębska  <https://orcid.org/0000-0002-1477-8302>
 Alicja Nowicka  <https://orcid.org/0000-0002-0455-4209>

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