Heliyon 10 (2024) e31036

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The exploration of a compound cone-beam CT contrast agent for diagnosis of human extracted cracked tooth

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ARTICLE INFO

Keywords:
Cracked tooth
Cone-beam CT
Contrast agent
Sodium iodide
Delay time

ABSTRACT

Objectives: This study aims to investigate the use of sodium iodide (NaI), dimethyl sulfoxide (DMSO), ethyl alcohol, and ethyl acetate as cone-beam CT (CBCT) contrast agents for diagnosing cracked teeth. The optimal delay time for detecting the number of crack lines beyond the dentino-enamel junction (Nd), the number of cracks extending from the occlusal surface to the pulp cavity (Np), and the depth of the crack lines was explored.

Methods: 14 human extracted cracked teeth were collected, 12 were used for enhanced scanning, and 2 were used for exploring the characteristic of crack lines. The teeth were scanned in 3 CBCT enhanced scanning (ES) modes: ES1 using meglumine diatrizoate (MD); ES2 using NaI and DMSO, ES3 using NaI, DMSO, ethyl alcohol and ethyl acetate. Three delay times (15mins, 30mins, and 60mins) were set for scanning. Nd, Np, and depth of crack lines were evaluated.

Results: There were totally 24 crack lines on 12 cracked teeth. Nd was 10 in ES1 at 60mins, 24 in ES2 at 60mins and 24 in ES3 at 15mins. Np was 1 in ES1 at 60mins, 10 in ES2 at 60mins and 21 in ES3 at 60mins, and there were significantly different among them ($p < 0.01$). The average depth presented on ES3 was significantly deeper than ES1 and ES2 ($p < 0.01$).

Conclusion: NaI, DMSO, ethyl alcohol and ethyl acetate show potential as contrast agents for enhanced CBCT scanning in diagnosis of cracked teeth and their depth *in vivo*. A delay time of 15 min is necessary to confirm the existence of crack lines, while a longer delay time is required to ascertain if these crack lines extend to the pulp cavity.

1. Introduction

Cracked teeth, a general term encompassing various types of tooth fractures, present with highly diverse and intricate clinical signs and symptoms [1]. Numerous terminologies, definitions, and classification systems have been proposed for cracked teeth [1–3]. The

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https://doi.org/10.1016/j.heliyon.2024.e31036
Received 14 October 2022; Received in revised form 27 March 2024; Accepted 9 May 2024
Available online 10 May 2024

American Association of Endodontists (AAE) classification, which is the most widely accepted, categorizes these fractures into five distinct types: craze lines, fractured cusp, cracked tooth, split tooth, and vertical root fracture [2]. The third type of cracked teeth, known as cracked tooth, is characterized by an incomplete fracture that originates in the crown and extends subgingivally, typically in a mesio-distal orientation [4]. The fracture may be confined to the coronal portion of the tooth or can propagate from the crown to the proximal root [5–7]. Epidemiological studies have revealed that cracked tooth is the third most prevalent cause of tooth loss, emphasizing its substantial clinical significance [8]. Treatment options for cracked teeth vary depending on the severity and extent of the fracture, ranging from composite resin restoration, crown placement, root canal therapy followed by cuspal coverage in cases with pulpal and periapical pathology, to even extraction in severe cases [9]. The earlier cracked tooth is diagnosed, the better prognosis it could have [3]. However, the diagnosis of cracked lines, especially the enamel microcracks is always challenging due extremely minute nature of the crack lines [10,11]. Moreover, cracked teeth may present with a diverse range of diagnostic test results, radiographic findings, and clinical manifestations [4]. Only some cracked teeth can be definitively diagnosed through direct visual inspect [4]. Therefore, multiple examinations including percussion, biting, staining, transillumination, thermal pulp tests [12–16], Cone-beam computed tomography (CBCT) [17,18] and microscopy [19] are used to verify cracked tooth clinically. In addition, several experimental techniques are being investigated for their potential in detecting dental cracks, including ultrasound [20], micro-CT [10,11, 21], optical coherence tomography [22,23] and quantitative light-induced fluorescence [24]. However, due to feasibility limitations of these techniques, none of them have been implemented in clinical practice thus far. Moreover, even if a crack is detected, assessing its extent remains challenging [25].

Our previous research revealed that the contrast agent meglumine diatrizoate (MD) can infiltrate into the fracture lines of artificially induced cracked teeth, enabling the visualization and diagnosis of these cracks on CBCT images [26]. We further developed a compound CBCT contrast agent consisting of sodium iodide and dimethyl sulfoxide (NaI and DMSO) to achieve better permeability and fluidity [25]. Compared to micro-CT, CBCT enhanced scanning utilizing NaI and DMSO as contrast agents, demonstrated superior efficiency in detecting artificial crack lines [25]. However, natural cracks *in vivo* are more complex than artificial ones, not only due to the structure of cracks but also because of the various substances present within them [7]. Therefore, in this study, based on our previous contrast agent (NaI and DMSO), we added ethyl alcohol and ethyl acetate to dissolve the organic substances inside the cracks. Human extracted cracked teeth were collected, and three enhanced scanning (ES) modes were performed on these cracked teeth. Moreover, the delay time (the interval between contrast agent application and scanning) was explored as a key factor of enhanced scanning in this study.

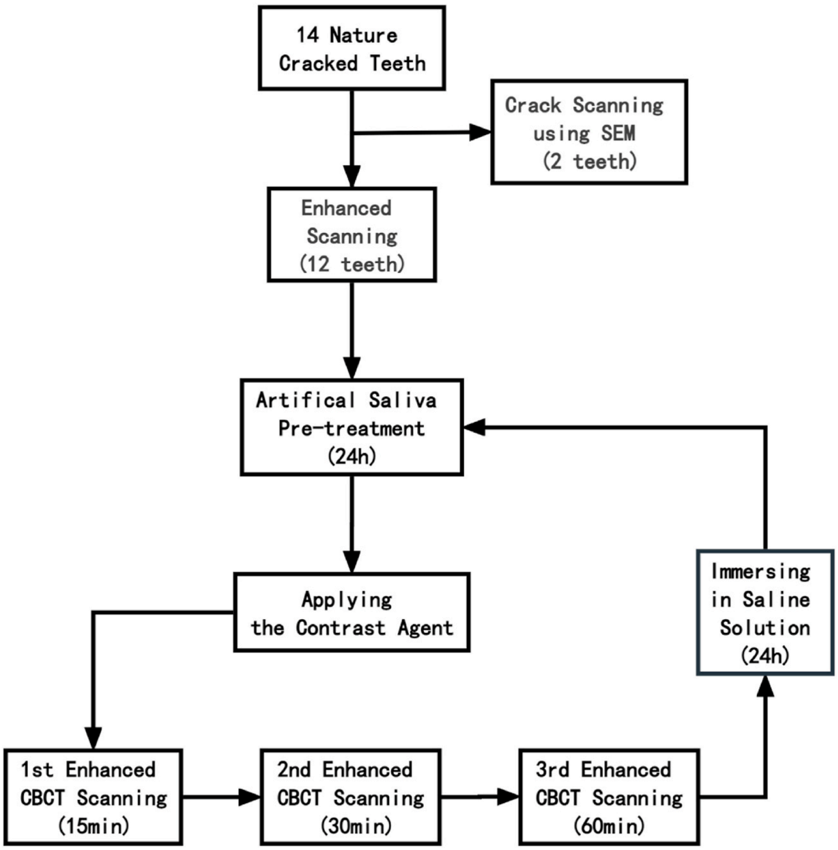

Fig. 1. The flow chart of the study protocol.

2. Methods and materials

2.1. Tooth collection

In this study, fourteen human extracted teeth (12 molars and 2 premolars) with naturally occurring cracks were collected from 14 patients. All teeth underwent routine CBCT scanning (RS) *in vivo* prior to extraction. An oral radiologist with over 10 years of experience evaluated the CBCT images, revealing observable crack lines in 5 of the 14 teeth. The decision to extract these teeth was based on a combination of factors, including clinical signs and symptoms, radiographic findings (crack lines or severe bone loss), poor prognosis, and patient wishs. Immediately following extraction, the teeth were stored in a 0.9 % isotonic NaCl solution. Of the 14 cracked teeth, 12 (10 molars and 2 premolars) were selected for enhanced scanning, while the remaining 2 (2 molars) were used to investigate the characteristics of crack lines. For the 12 teeth undergoing enhanced scanning, the number and location of crack lines on the buccal, palatal, mesial, and distal sides of the crown were recorded using transillumination. This study was conducted with the approval of our University's Ethics Committee (KY-2020NL-075), and all patients provided informed consent for the use of their extracted cracked teeth. The study protocol is outlined in Fig. 1.

2.2. Scanning electron microscope (SEM) observation

The cracked teeth were split along the cracked line using a plugger to create a wedging effect. The coronal parts were then gold-coated using a coating machine (Turbo-Pumped Sputter Coater/Carbon Coater, Q150R S, Quorum Technologies). The digitized images of the surface of the cracked coronal parts were captured with a magnification of 100× using a scanning electron microscope (SEM, S-3400N-II, Hitachi, Tokyo, Japan).

2.3. CBCT enhanced scanning

2.3.1. Contrast agent preparation

Three contrast agents were employed in this study: A: MD, B: NaI and DMSO, and C: NaI, DMSO, ethyl alcohol, and ethyl acetate. MD, a clinically used contrast agent, was obtained from Xi'an Lipont Enterprise Union Management Co., Ltd., China. For the preparation of contrast agent B, 11 g of NaI (99 %, Jiodine Chemical Co., Ltd., Qingdao, China) was dissolved in 10 mL of purified water, followed by the addition of 14 mL of DMSO (99 wt%, Aladdin Bio-Chem Technology Co. LTD., Shanghai, China). The resulting solution

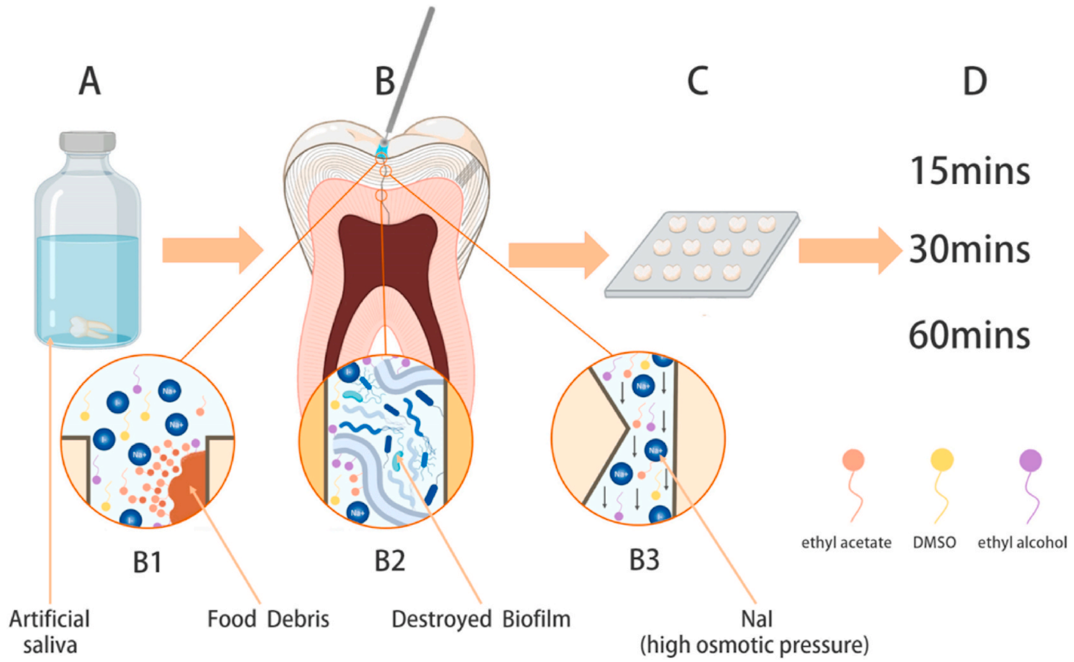

Fig. 2. A: the cracked tooth is immersed in artificial saliva for 24 h; B: a disposable fiber-tip sterile applicator is used for painting the contrast agent on the surface of the crack; B1: the ethyl acetate dissolve in many organic solvents in the cracks and it increases the solvent power of the contrast agent for debris; B2: ethyl alcohol destroys the hydrophobic layer of biofilm. B3: ethyl acetate and ethyl alcohol, as surfactants, reduce the surface tension between liquids and the surface; help the contrast agent go deeper. DMSO increases hydrophilia of the contrast agent and helps the contrast agent dissolve into the artificial saliva; and the high osmotic pressure of NaI makes the contrast agent reaches deeply; C: the teeth were left undisturbed in a foam plate for contrast agent infiltrating; D: CBCT enhanced scanning was performed after 15mins, 30mins and 60mins respectively.

contained 30 wt% NaI and 42 wt% DMSO. Contrast agent C was prepared by first dissolving 25 g of NaI in 13 mL of purified water. Subsequently, 23 mL of DMSO was added to the NaI aqueous solution, followed by the sequential addition of 10 g of ethyl alcohol (99 wt%, Aladdin Bio-Chem Technology Co. LTD., Shanghai, China) and 10 g of ethyl acetate (99 wt%, Aladdin Bio-Chem Technology Co. LTD., Shanghai, China). The final composition of contrast agent C was 30 wt% NaI, 12 % ethyl alcohol, 12 % ethyl acetate, and 30 wt% DMSO in an aqueous solution.

2.3.2. *Teeth pre-treatment with artificial saliva*

Artificial saliva was prepared by dissolving six components in 500 mL of purified water at 40 °C: 0.025 g of magnesium chloride, 0.42 g of sodium chloride, 0.31 g of potassium chloride, 0.375 g of sorbic acid, 0.17 g of dipotassium hydrogen phosphate, and 2 g of hypromellose (all obtained from Xilong Scientific Co., Ltd., Guangdong, China) [27]. The viscosity of the resulting solution, measured using a viscosimeter (IKA Werke GmbH & CO. KG, Germany), was 9.25 mm^2/s. Subsequently, all cracked teeth were immersed in the prepared artificial saliva for a period of 24 h [Fig. 2 (A)] [25].

2.3.3. *CBCT enhanced scanning*

In this study, three enhanced scanning (ES) modes were employed: ES1, ES2, and ES3, utilizing MD, NaI and DMSO, and NaI, DMSO, ethyl alcohol, and ethyl acetate as contrast agents, respectively. All scanning was performed using a NewTom VGi scanner (QR SRL, Verona, Italy) with the following parameters: 110 kV, 3 mA, 10 × 10 cm field of view, 0.15 mm voxel size, and 5.4 s exposure time.

For each ES mode, the surface of the cracked tooth was briefly air-dried using compressed air. Subsequently, the contrast agent was applied to the surface of the crack lines using a disposable fiber-tip sterile applicator (TPC, Dongguan, China). The treated teeth were then placed in a foam plate (phantom on CBCT) and left undisturbed. After painting, the cracked teeth were scanned with CBCT at three delay times: 15mins, 30mins and 60mins. To eliminate the influence of any residual contrast agent within the cracks, samples were immersed in a saline solution (0.9 % isotonic NaCl) for 24 h following each ES scan to clear the agent from the cracks. A routine CBCT scan was subsequently conducted to confirm the absence of any remaining contrast agent in the crack lines. All CBCT images were scanned at rest to ensure optimal quality and minimize artifacts, such as motion or beam hardening artifacts [Fig. 2 (B-D)].

2.4. *Crack lines evaluation on CBCT images*

CBCT images were reconstructed and analyzed using the inbuilt software NNT 9.0 (QR SRL, Verona, Italy). The number of crack lines extending beyond the dentino-enamel junction (Nd) and the number of cracks propagating from the occlusal surface to the pulp

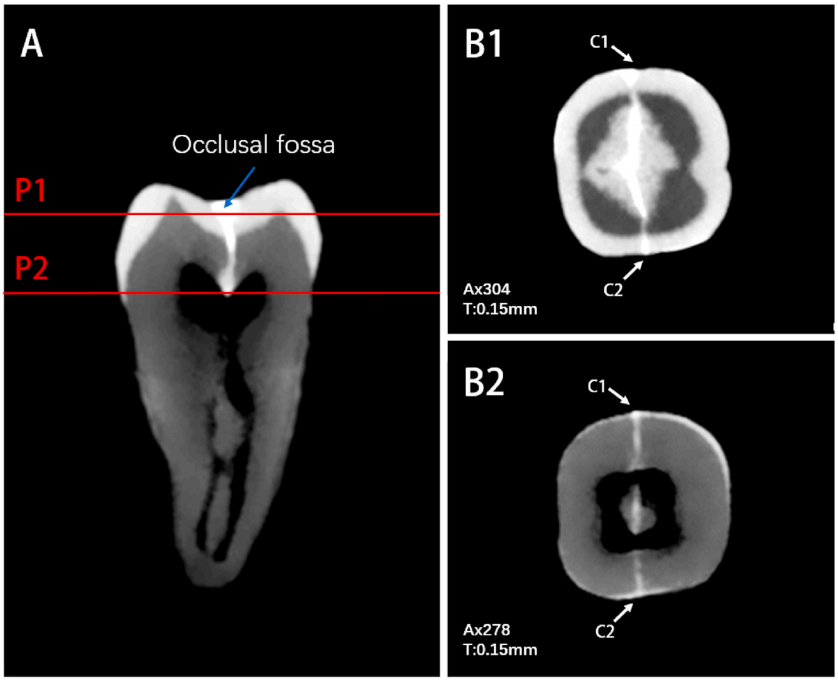

Fig. 3. A is the coronal image of a cracked tooth in ES3 mode, P1, P2 represent the beginning and end position of the crack. B1, B2 are axial images corresponding to P1 and P2, respectively. Ax304, Ax278 are the number of the axial images. The voxel size of the CBCT image is 0.15 mm. Depth of crack equals the number of slices times the voxel size (Depth=(304-278 × 0.15 mm = 3.90 mm). For this tooth, two cracked lines were counted in mesial and distal side respectively (C1, C2).

cavity (Np) were evaluated. A crack was counted for Nd and Np if a hyperdense line was present on at least two consecutive axial images. The number of cracked lines was assessed separately on the buccal, palatal, mesial, and distal sides. To determine the depth of a crack line, regions of interest were reconstructed along the tooth's long axis. Crack depth was calculated by multiplying the number of slices displaying the crack line by the voxel size of the CBCT (Depth = Number of slices × 0.15 mm) [Fig. 3 (A1, B1, B2)].

Two observers independently evaluated the CBCT images for Nd and Np. In case of disagreement, a senior radiologist was consulted. After four months, the same two observers reassessed Nd and Np to analyze intra-examiner agreement for all three delay times. An experienced radiologist evaluated the depth of crack lines on all CBCT images. Before evaluation, calibration, including unified training on diagnostic standards, was performed. The radiologist reassessed the depth of crack lines after four months, and the intraclass correlation coefficient (ICC) was analyzed for all three delay times.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was employed to compare the depth of cracks between CBCT RS, ES1, ES2, and ES3 at the three delay times. McNemar's test was used to analyze the differences in Nd and Np between CBCT RS and ES3, ES1 and ES3, and ES2 and ES3. All statistical analyses were performed using SPSS 23.0 software (IBM SPSS Statistics Base Integrated Edition 23, Armonk, NY, USA).

3. Result

3.1. SEM observation

There were irregular microcracks within main cracks were found in SEM [Fig. 4 (A, B)], indicates that natural crack lines are not straight and singular but rather irregular with numerous branching microcracks.

3.2. Nd and Np detected on three ES modes at three delay time

There were totally 24 crack lines on 12 extracted cracked teeth (mesial, distal, buccal and palatal side was recorded respectively), the number and position of the cracks of the 12 teeth using transillumination were shown in Table 1. Nd and Np on three ES modes at three delay times were shown in Table 2 and Table 3. Significant differences were found for Np between ES3 and RS, ES3 and ES1, ES3

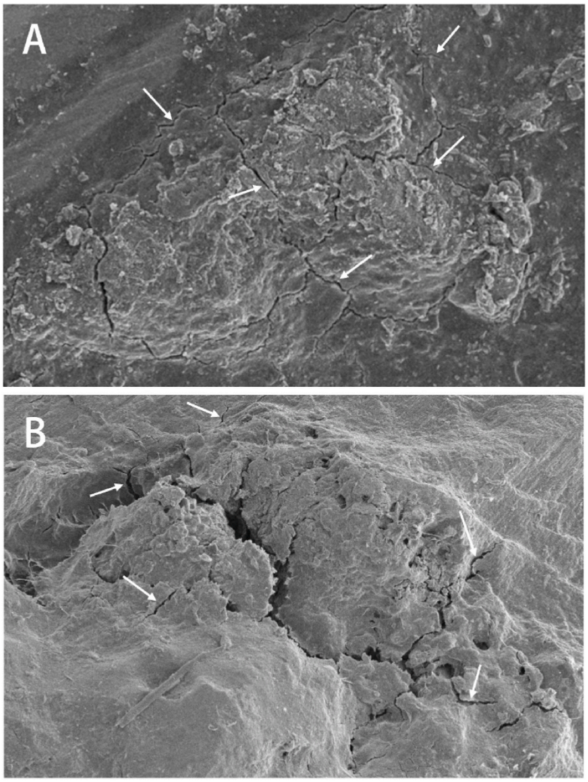

Fig. 4. A and B are SEM images (× 100 magnificence, × 200 magnificence) of the cracked teeth, respectively. The microcracks within the main crack are marked.

and ES2 respectively ($p < 0.01$). The invisible crack lines on RS images were observable on ES3 images [Fig. 5 (A-D)].

3.3. Depth of crack lines

The average crack depth detected on CBCT ES1, ES2, and ES3 images at the three delay times were shown in Table 4. Significant differences were found between ES3 and RS, ES3 and ES1, ES3 and ES2 ($p < 0.01$) respectively. The ES3 has higher diagnostic efficiency than ES1 and ES2 at all the three delay times [Fig. 6 (a1-a3, b1-b3, c1-c3)].

3.4. Inter and intra-examiner agreement

The inter- and intra-examiner reproducibility (kappa value) for Nd and Np were shown in Table 5. For ES3, the inter- and intra-examiner agreement for Nd was almost perfect. The inter-examiner agreement for Np in ES3 was substantial, while the intra-examiner agreement for Np was substantial for observer 1 and almost perfect for observer 2. Table 4 also displays the intraclass correlation coefficient for crack line depth, which was excellent in ES3.

4. Discussion

Cracked tooth consistently pose a diagnostic challenge in dental clinic [1]. In recent years, CBCT has gained widespread use in dental radiology due to its ability to provide accurate three-dimensional images with high spatial resolution [27]. However, routine use of CBCT for diagnosing cracked teeth is not recommended, as most crack lines are too narrow to be detected [27]. Our previous study suggested that CBCT enhanced-scanning with contrast agents (MD, NaI, and DMSO) could potentially improve the accuracy of crack line detection [25,26]. Nevertheless, the cracked teeth in our previous study were artificial created, and the natural crack lines are more complicated than artificial ones [25,26]. The infiltration of contrast agents into natural crack lines could be significantly more challenging than infiltration into artificial ones. Therefore, in this study, human extracted cracked teeth were used, and ethyl alcohol and ethyl acetate were added to dissolve substances within the crack lines. We compared the diagnostic accuracy of this novel compound contrast agent (NaI, DMSO, ethyl alcohol, and ethyl acetate) with our previously used contrast agents (MD, NaI, and DMSO), and the appropriate delay time was explored in this study.

To improve the infiltration of contrast agents into natural crack lines, it is crucial to understand the characteristics of these cracks. Previous studies have indicated that 1) tensile microcracks in coronal dentin may not propagate along the tubules but are often deflected to adjacent tubules [28]. 2) there are bacteria biofilm and food debris in crack lines [29,30]. Similarly, our SEM observations showed the presence of microcracks within the main cracks. Thus, natural crack lines are not straight and singular but rather irregular with numerous branching microcracks. Moreover, these cracks contain various substances [29,30]that, despite appearing as air-like density on CBCT images, can obstruct the pathway of contrast agents [7,25]. In this study, there were only 45 % crack lines that were detected with MD as contrast agent at 60mins, however, in our previous study, 65 % artificial crack lines were detected with MD as contrast agent at 10 min [26]. Therefore, the natural cracks are more difficult to be infiltrated by contrast agents.

In this study, the ES3 demonstrated higher diagnostic efficiency compared to ES1 and ES2 at all three delay times. The crack lines detected on ES3 images were found to be deeper than those on ES1 and ES2 images ($p < 0.01$). Ethyl alcohol, known as a disinfector, effectively inhibits biofilm formation by bacteria [31] and can destroy the hydrophobic layer of biofilm [32]. Ethyl acetate could easily dissolve in many organic solvents and enhances the solvent power of the contrast agent for debris [33]. Additionally, both ethyl acetate and ethyl alcohol act as surfactants, reducing the surface tension between liquids and surfaces, thereby facilitating better fluid penetration. By lowering the surface tension, surfactants make molecules more 'slippery' and less likely to stick together [34]. Therefore, our contrast agent can infiltrate deeper into natural cracks. The invisible crack lines on CBCT routine *in vivo* scanning were observable on ES3 images, proving it is potential for future *in vivo* usage.

Determining whether the cracks have reached or are in close proximity to the pulp cavity is crucial for treatment planning. In these situations, root canal treatment may be needed due to pulpitis, pulp necrosis and subsequent apical periodontitis caused by bacteria

Table 1
The number and position of cracks on the 12 cracked teeth using transillumination.

No.	Teeth position[a]	Cracks position	Number of cracks
1#	47	Mesial to distal	2
2#	27	Mesial to distal	2
3#	16	Mesial to distal	2
4#	47	Mesio-buccal to distal	2
5#	36	Mesial to distal	2
6#	15	Mesial to distal	2
7#	25	Mesial to distal	2
8#	26	Buccal to palatal	2
9#	36	Mesio-buccal to disto-lingual	2
10#	46	Mesio-buccal to distal	2
11#	17	Mesial to distal	2
12#	46	Buccal to disto-lingual	2

[a] The numbering of teeth position using FDI tooth numbering system.

Table 2
The number of crack lines beyond dentino-enamel junction (Nd) on RS, ES1, ES2 and ES3 images.

	15mins	30mins	60mins
Nd(RS)	10(41.7 %)		
Nd(ES1)	4 (16.7 %)	8 (33.3 %)	10 (41.7 %)
Nd (ES2)	14 (58.3 %)	20 (83.3 %)	24 (100 %)
Nd(ES3)	24 (100 %)	24 (100 %)	24 (100 %)
p (ES3 vs. RS)[a]	0.000	0.000	0.000
p (ES3 vs. ES1)[a]	0.000	0.000	0.000
p (ES3 vs. ES2)[a]	0.002	0.125	/

RS, routine CBCT *in vivo*; ES1, CBCT scanning with MD as contrast agent; ES2, CBCT scanning with NaI and DMSO as contrast agent; ES3, CBCT scanning with NaI, DMSO, ethyl alcohol and ethyl acetate as contrast agent.
[a] McNemar's test.

Table 3
The number of cracks presented from the occlusal surface to the pulp cavity (Np) on RS, ES1, ES2 and ES3 images.

	15mins	30mins	60mins
Np (RS)	6 (25 %)		
Np (ES1)	0 (0 %)	1 (4.2 %)	1 (4.2 %)
Np (ES2)	2 (8.3 %)	6 (25 %)	10 (4 %)
Np (ES3)	14 (58.3 %)	18 (75 %)	21 (87.5 %)
p (ES3 vs. RS)[a]	0.000	0.000	0.000
p (ES3 vs. ES1)[a]	0.000	0.000	0.000
p (ES3 vs. ES2)[a]	0.000	0.000	0.001

RS, routine CBCT *in vivo*; ES1, CBCT scanning with MD as contrast agent; ES2, CBCT scanning with NaI and DMSO as contrast agent; ES3, CBCT scanning with NaI, DMSO, ethyl alcohol and ethyl acetate as contrast agent.
[a] McNemar's test.

invading the pulp [35]. Given the challenges in infiltrating natural cracks, exploring of delay times becomes necessary. Therefore, three delay times were set, and the results showed that the longer delay time, the deeper the contrast agent infiltrated. On ES3 mode, only 14/24 (58.3 %) crack lines were observable from occlusal surface to pulp cavity at 15 min, while 18/24 (75 %) were observable at 30mins, and 21/24 (87.5 %) were observable at 60mins. However, due to the front of cracks being much narrower than the cracks at the beginning, the infiltration speed decreased as the contrast agent infiltrated deeper. In this study, the average increased depth from 30min to 60min (0.13 mm) is only half of from 15mins to 30mins (0.26 mm). Therefore, we suggest using different delay time for different diagnosis purpose. To verify the existence of cracked lines, a 15-min delay time is needed, while a longer delay time is required to detect whether the crack lines reach the pulp cavity.

Regarding the toxicity of the four components of our new compound contrast agent, no adverse reactions have been reported when they were used in small doses [36–39]. NaI, the first-generation X-ray contrast agent [37] has been extensively used in medicine for treating respiratory and thyroid diseases. DMSO, a highly efficient solvent for water-insoluble compounds, is widely employed as a vehicle for drug therapy [39,40]. Ethyl acetate, a flavoring agent, is commonly used in food manufacturing, while ethyl alcohol is frequently utilized for clinical disinfection [41].

There are still limitations in this study. Firstly, the extracted teeth were stored in saline solution. Due to the difficulty of collection of cracked teeth, some teeth were immersed in saline solution for more than one year. The structures and substances inside the cracks may be not entirely consistent with those of fresh ones. Secondly, the sample of cracked tooth is small; the contrast agent needs to be verified in more cracked teeth. Thirdly, the teeth were left dry on the foam plate after painting with the contrast agent. An oral environment imitating experiment is needed in the future. Fourthly, the crack lines in this study may be wider than many early cracked teeth in clinic. Fifthly, the cracked teeth in this study were not performed CBCT scanning without contrast agent *in vitro*. Therefore, the diagnostic efficiency of this contrast agent still needs to be verified in more cracked teeth.

5. Conclusion

NaI, DMSO, ethyl alcohol and ethyl acetate maybe a potential contrast agent used in CBCT enhanced scanning of cracked tooth. A delay time of 15 min is necessary to confirm the existence of crack lines, while a longer delay time is required to ascertain if these crack lines extend to the pulp cavity.

Data availability statement

Data will be made available on request.

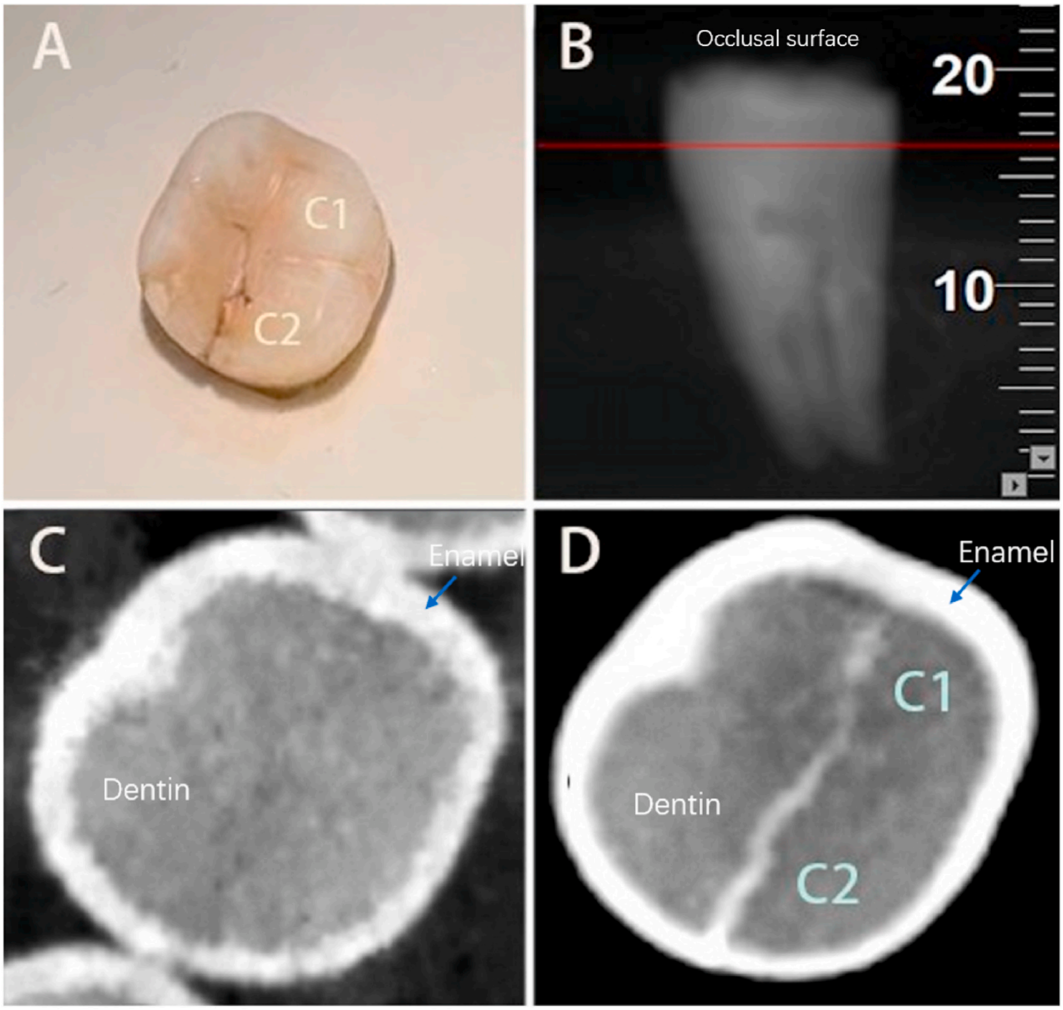

Fig. 5. A shows a cracked line on the occlusal surface. There are two cracks marked C1 and C2. C2 is clear whereas C1 is subtle. B shows the position of the axial images in the tooth (red lines); C is the CBCT image of the cracked tooth scanned *in vivo* (RS) and no obvious cracked line is seen; D is the extracted tooth scanned in ES3 mode, the crack lines were demonstrated well. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Crack depth on RS, ES1, ES2 and ES3 images.

	15mins	30mins	60mins
Depth *(RS)*	1.43 ± 1.79 mm		
Depth *(ES1)*	0.67 ± 1.00 mm	0.91 ± 1.25 mm	1.05 ± 1.35 mm
Depth *(ES2)*	2.11 ± 1.34 mm	2.52 ± 1.34 mm	2.82 ± 1.33 mm
Depth *(ES3)*	3.32 ± 1.09 mm	3.58 ± 0.84 mm	3.71 ± 0.67 mm
p (ES3 vs RS)[a]	0.000	0.000	0.000
p (ES3 vs ES1)[a]	0.000	0.000	0.000
p (ES3 vs ES2)[a]	0.000	0.003	0.008

RS, routine CBCT *in vivo*; ES1, CBCT scanning with MD as contrast agent; ES2, CBCT scanning with NaI and DMSO as contrast agent; ES3, CBCT scanning with NaI, DMSO, ethyl alcohol and ethyl acetate as contrast agent.

[a] One-way analysis of variance (ANOVA).

Ethical approval and consent to participants

This study was approved by the Ethics Committee of the Nanjing Stomatological. Hospital, Medical School of Nanjing University (KY-2020NL-075). All methods were carried out in accordance with relevant guidelines and regulations or declaration of Helsinki. The

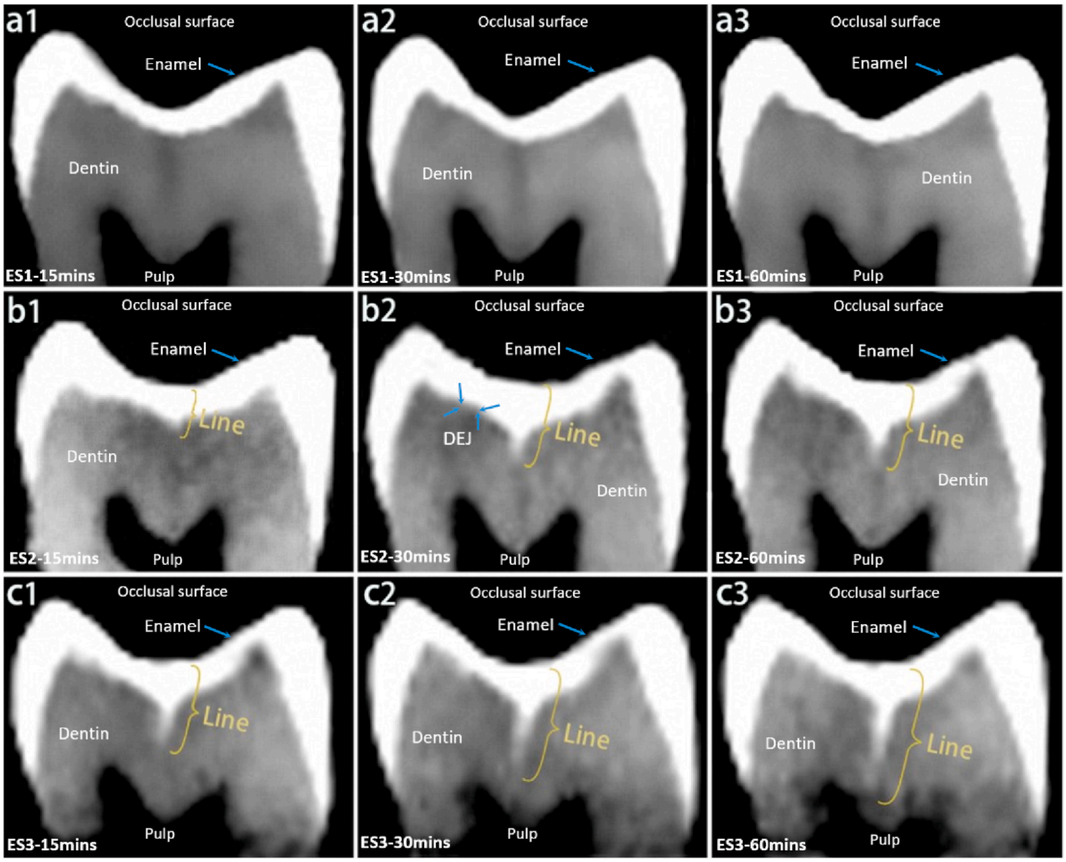

Fig. 6. a1, a2 and a3 are CBCT images with MD as contrast agent at 15mins, 30mins and 60mins respectively. No contrast agent infiltrated into crack line. b1, b2, and b3 are CBCT images acquired at 15, 30, and 60 min, respectively, utilizing NaI and DMSO as the contrast agent. The contrast agent reaches the dentin. c1, c2 and c3 are CBCT images obtained at 15, 30, and 60 min, respectively, employing a combination of NaI, DMSO, ethyl alcohol, and ethyl acetate as the contrast agent. This combination of contrast agents exhibited deeper penetration compared to MD, NaI, and DMSO alone, reaching near the pulp cavity at 60 min. The yellow line is the crack line presented on CBCT images. Anatomical tooth structures (pulp, dentin, dento-enamel junction (DEJ), enamel) are marked. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Repeatability analysis of Nd, Np and depth of cracks in CBCT scanning.

	Nd (ES1)	Nd (ES2)	Nd (ES3)	Np (ES1)	Np (ES2)	Np (ES3)	Dc (ES1)	Dc (ES2)	Dc (ES3)
Inter-examiner agreement	1	1	1	1	0.745	0.734	/	/	/
Intra-examiner agreement (observer 1)	1	1	1	1	0.875	0.792	/	/	/
Intra-examiner agreement (observer 2)	1	1	1	1	0.898	0.830	/	/	/
ICC (radiologist)	/	/	/	/	/	/	0.991	0.977	0.942

Nd: The number of crack lines beyond dentino-enamel junction; Np: The number of cracks presented from the occlusal surface to the pulp cavity; Dc: The depth of crack lines; ES1: CBCT scanning with MD as contrast agent; ES2: CBCT scanning with NaI and DMSO as contrast agent; ES3: CBCT scanning with NaI, DMSO, ethyl alcohol and ethyl acetate as contrast agents.

data are anonymous, and the requirement for written informed consent was therefore waived by the Ethics Committee of the Nanjing Stomatological Hospital, Medical School of Nanjing University (KY-2020NL-075).

Funding

This work was supported by the National Natural Science Foundation of China (Nos. 82201135), General project of Jiangsu Provincial Health Commission (No. M2021077), “2015” Cultivation Program for Reserve Talents for Academic Leaders of Nanjing Stomatological School, Medical School of Nanjing University (No. 0223A204).

CRediT authorship contribution statement

Ziyang Hu: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Formal analysis. **Yanni Hu:** Writing – review & editing, Resources, Investigation. **Shi Xu:** Validation, Resources, Investigation. **Jia Zhuang:** Investigation, Data curation. **Dantong Cao:** Software, Resources, Investigation, Formal analysis, Data curation. **Antian Gao:** Resources, Investigation, Data curation. **Xin Xie:** Investigation, Data curation. **Zitong Lin:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Lin, Dr. Hu, Dr. Cao, Dr. Gao, and Dr. Xie has patent A compound Cone-beam CT contrast agent and its configuration method licensed to Application number: ZL 2021 1 0957963.0. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Not applicable.

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