

Histological Evaluation of Caries–Treated Teeth Following Clinical Visual and Tactile Inspection

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Abstract

Operators use visual and tactile criteria to evaluate the cleanliness of the prepared cavity and it is highly depending on operator's experience and judgement. The aim of this study was to determine the level of students' clinical judgement on the prepared caries-treated teeth and to compare the clinical and histological outcomes of partial and complete caries removal in extracted teeth.

Seventeen extracted teeth with occlusal or proximal caries, with no pulpal exposure were collected and divided into partially or completely caries removal preparation. Sixty-four USIM's clinical students were participated to evaluate the extracted teeth prior to gross section and hematoxylin and eosin (H&E) histological examination.

Students' clinical evaluation feedbacks revealed the mean value of the correct answer in detection of residual caries was 10.94/17 with no significant difference between Year 4 and 5. Histological findings revealed absence of any liquefaction of bacteria masses underneath the preparation which are consistent with partial or complete caries removal preparations.

This study showed there is strong correlation between clinical and histological evaluation.

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Introduction

Dental caries is defined as the destruction of localised dental hard acellular tissue by acidic by-products from the bacterial fermentation of dietary carbohydrates.¹ It is considered as the most important global oral health problems as 36% of the worldwide population having caries in their permanent teeth and primary pathological cause of tooth loss in children.¹ Dental caries can be classified according to rate of progression (acute or chronic), affected hard tissues (enamel, dentine or cementum), location (Class I to Class VI Black's Classification of Carious Lesions)⁹ and clinical visual criteria classification (International

Caries Detection and Assessment System, ICDAS).^{1,2} 'Caries prevention and management chairside guide' by FDI World Dental Federation 2017 are published with the goal of to reduce the impact of caries development by intervening caries as soon as possible before further tooth destruction, and to reverse the caries process by promoting remineralization.¹⁰ Among the listed action points are to minimize surgical intervention and to convert actively progressing caries lesions into arrested controlled ones. Professionally, dentists are encouraged to preserve tooth structure where possible and to prevent more tooth destruction.

Traditional Black's caries excavation concept proposed all remnants of caries should be were removed during cavity preparation based on the principle of 'extension for prevention' which may increase the risk of pulp exposure and post-operative symptoms.^{3,9} However, carious lesions are now treated with less invasive methods in favour of tissue conservation. Clinically, operators need to judge whether the cavity preparation is done properly

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and whether further caries removal is needed or not based on their visual and tactile perception. These judgements are really relying on knowledge and experience. Theoretically, dental students have been taught and trained on both techniques of partial and complete caries removal, therefore, they should be able to self-evaluate their own caries-treated tooth. According to Hilton and Summitt, 2000, contemporary teaching proposed dentine caries that is 'firm and leathery' should be left over the pulpal surface to avoid any further destruction and potential of pulp exposure.³ However, the interpretation of 'firm and leathery' may be differ from one supervisor to another,³ which may make it difficult for the students to understand this subjective clinical assessment. Thus practically, judgement of the students whom are lack of experience, can be difficult.

In caries removal procedure, infected or outer carious dentine which is soft, already contaminated with bacteria and would never be remineralized should be removed, whilst affected or inner carious dentine which absent or very few of bacteria and shown ability to remineralized should be left in the cavity.^{4,5} It is difficult to accurately describe these features clinically. However, through histological approach, supervisors and students can get an overview and will appreciate infected and affected dentine better.^{4,6} Since, histopathology is the clinical gold-standard technique used for diagnosis diseases and is typically used to confirm the findings of basic and translational biomedical research,^{6,7} caries-treated tooth is best undergoing histological examination as well. The aim of this study was to determine the level of students' clinical judgement on the prepared caries-treated teeth and to compare the clinical and histological outcomes of partial and complete caries removal in extracted permanent teeth. Null hypothesis was that there was no difference between clinical and histological appearance of partial and complete caries-treated tooth.

Materials and methods

Sample preparation

This study was granted ethical approval by Faculty of Dentistry Ethics Committee, Universiti Sains Islam Malaysia and conducted over April to December 2018. Twenty-one extracted permanent teeth with International

Caries Detection and Assessment System (ICDAS) Code 3, 4 and 5 were collected and divided into three groups; Group 1 (G1) that was prepared for partial caries removal (PCR), Group 2 (G2) for complete caries removal (CCR) and Group 3 (G3) as a control. The teeth were mounted with plaster and a baseline photograph and radiograph of each tooth were taken as shown in Figure 1. The caries removal methods (Table 1) following the standard protocol for partial and complete caries removal procedure adapted from Khokhar and Tewari, 2018 and Black GV, 1908 was performed using diamond and tungsten carbide burs.^{9,11} Post caries removal photograph of the prepared teeth taken as shown in Figure 2. Teeth were stored in individual plastic containers filled with normal saline and kept in incubators at 37°C at all time since initial collection period to avoid desiccation.

Partial caries removal	Complete caries removal
<ul style="list-style-type: none">Involved removal of outer layer of dentin cariesCarious tissue from the lateral walls and dentinoenamel junction (DEJ) was removed completely using low-speed metal burs and/or hand excavator.Superficial necrotic dentin was removed from the pulpal and axial wall using low-speed round bur.A layer of soft, wet carious dentin was left adjacent to pulpal wall.	<ul style="list-style-type: none">Involved complete removal of decayed tissue, irrespective of pulp exposure.All soft and demineralized dentine was removed.

Table 1. Technique of standard protocol for partial and complete caries removal procedure adapted from Khokhar and Tewari, 2018 and Black GV, 1908.

Clinical Evaluation

By using purposive sampling method, 64 subjects from 4th and 5th Year clinical dental students were participated in the clinical evaluation of the caries-treated teeth. The students were asked to examine all the seventeen caries-treated extracted teeth using visual and tactile examination. Sharp probe, mouth mirror and triple air syringe were provided for the purpose with no time limitation given to examine the teeth. The findings of the examination were recorded in the prepared evaluation sheet which adapted from Ntovas et al., 2018.⁸

Histological Examination

After clinical visual and tactile evaluation, each PCR, CCR and control teeth sample were sectioned longitudinally into two halves. After been cut, all halves were fixed in 10% formaldehyde over 24-hours in separate

containers. The samples were subjected to two different laboratory tests i.e. the histological examination (Group A) and the ground section (Group B) as in figure 3.

Group A samples were then placed in decalcifying agent. Haematoxylin and eosin (H&E) staining was performed on the sections to check the present of any purplish discoloration in dentinal tubules below the PCR and CCR area indicating the present of bacteria. Paraffin-embedded blocks were sectioned with Leica rotary microtome (Leica RM2235) at 4µm thickness and mounted on silane coated glass slides. The slides were left to dry overnight in a 35°C oven. Sections were dewaxed in a series of histolene before rehydrated in a series of different alcohol concentrations (100%, 90%, 70% and 50%). Nuclear staining was achieved Harris haematoxylin. Sections were blued by 0.5% ammonia water, followed by counterstained in eosin. Sections were dehydrated in series of alcohol solutions (90% and 100%) and cleared in histolene. Cover slipping was performed using the DPX mounting medium.

Group B samples underwent ground section slides preparation to enable histological examination. EXAKT Technovit 7230 VLC Adhesive photocuring glue was applied together with the Precision Adhesive Press to the plastic microscope slides to ensure the samples were presented evenly to the abrasive surface. Later, the samples were ground on the EXAKT Cutting/Grinding Systems machine at high speed until 4-5 mm thickness, followed by further grounding at low speed till 3-4 mm thickness was achieved. Finally, the grounded sections were cleaned in histolene for one minute.

All ground section and H&E slides were viewed under Olympus light microscope. Ground section and H&E microscopic images recorded as shown in Figure 4.

Statistical Analysis

The data were compared using independent T-test. Statistical analyses were performed using Statistical Package for the Social Science (SPSS) (Armonk, NY: IBM) software version 21.0.

Results

The conducted clinical evaluation result revealed mean values of 10.94/17 for both 4th and 5th clinical students. Separately, the

achieved mean value of correct answers in detection of residual caries among Year 4 students was 10.75, whilst Year 5 was 11.13 out of 17 questions (Figure 1). There was no significance different between Year 4 and Year 5 clinical dental students in detection of residual caries with p value 0.570 ($p > 0.005$). However, when the clinical evaluation result was analysed individually according to the 'tooth sample number', inconsistency in students' judgement to achieve correct answer regarding the prepared caries-treated teeth can be seen as presented in Table 2.

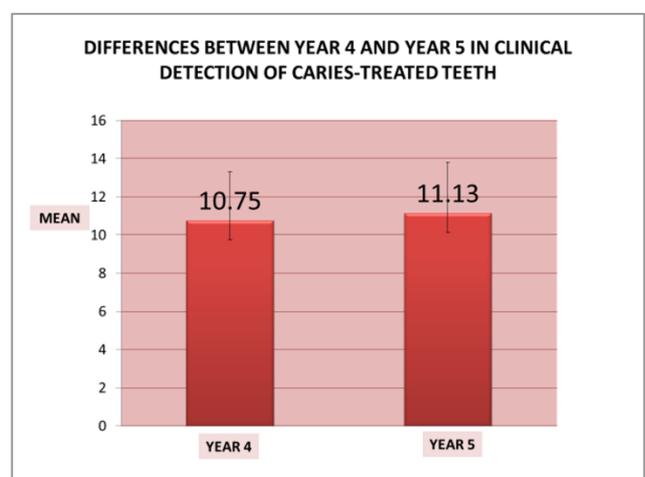


Figure 1. Result of Year 4 and Year 5 clinical dental students' clinical detection of residual caries through visual and tactile sensation.

Clinically, fresh carious teeth were easily detected through visual brown discoloration and soft mushy tactile consistency. Histological H&E stained slides of all four controlled sample of fresh carious teeth, present of purplish colours in the dentinal tubules indicating conspicuous presence of bacteria (Figure 4). The complete caries-removed teeth were all visually 'very clean cavity preparation' which histologically proven by the absence of the purplish colours in the dentinal tubules in all examined samples indicating the absenteeism of bacteria (Figure 5). Hence, there is no infected dentine left in the completely caries removal teeth. Interestingly, clinically visually brown discoloration but firm, leathery tactile sensation also histologically showed absence of purplish colours in the dentinal tubules in all examined partially caries-removed teeth samples (Figure 6). Hence, no need further caries removal is needed since infected dentine is already absent.

Tooth no	Tooth Preparation	Type of tooth	Is the tooth still need caries removal	
			Yes	No
1	Complete	Incisor	12	52
2	Complete	Canine	13	51
3	Partial	Molar	50	14
4	Complete	Canine	30	34
5	Complete	Premolar	20	44
6	Partial	Molar	22	44
7	Partial	Incisor	32	32
8	Complete	Premolar	10	54
9	Partial	Molar	36	28
10	Partial	Molar	42	22
11	Complete	Canine	26	38
12	Partial	Molar	40	24
13	Partial	Molar	44	20
14	Complete	Molar	5	59
15	Complete	Premolar	10	54
16	Complete	Canine	2	62
17	Complete	Premolar	13	51

Table 2. Result of students' clinical evaluation of caries-treated teeth.

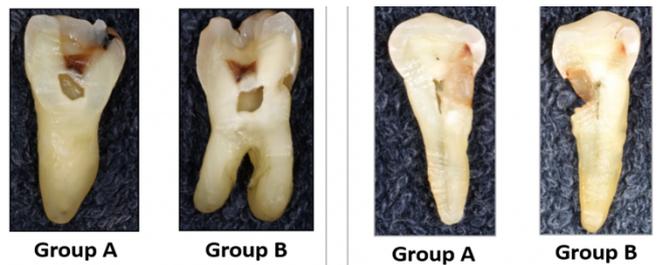


Figure 3. Teeth were cut into halves for two separate laboratory examination; Group A for H&E and Group B for ground section.

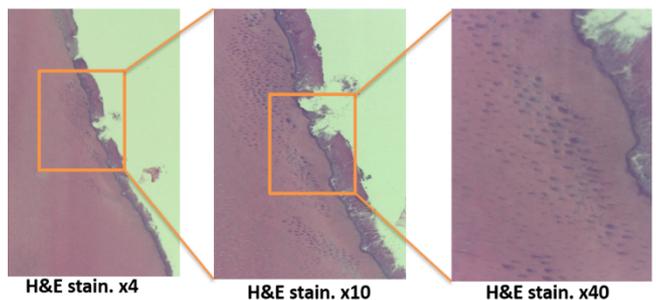
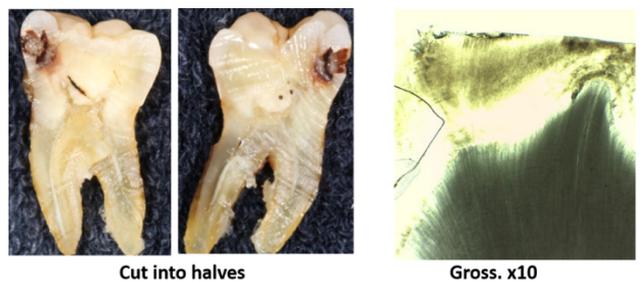


Figure 4 Clinical and histological images of the control fresh caries teeth.

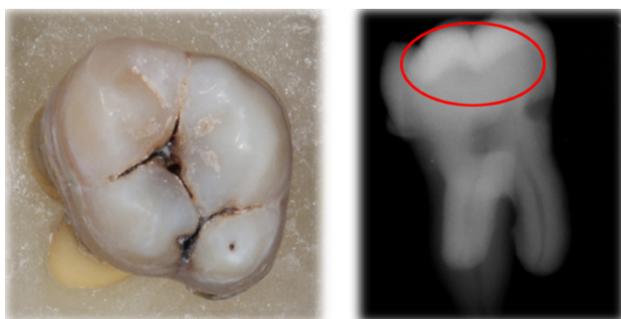


Figure 1. Preoperative photograph and radiograph.

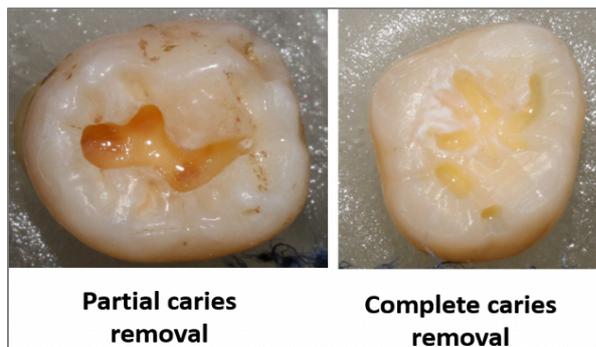


Figure 2. Example of teeth sample undergone partial and complete caries removal procedure.

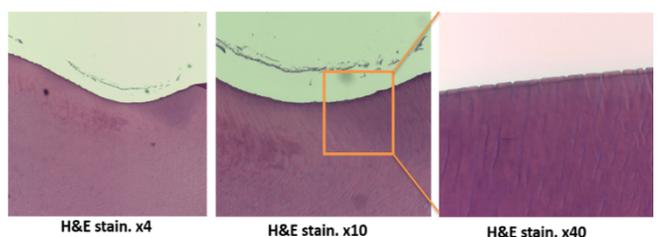


Figure 5. Clinical and histological images of complete caries-removed teeth.

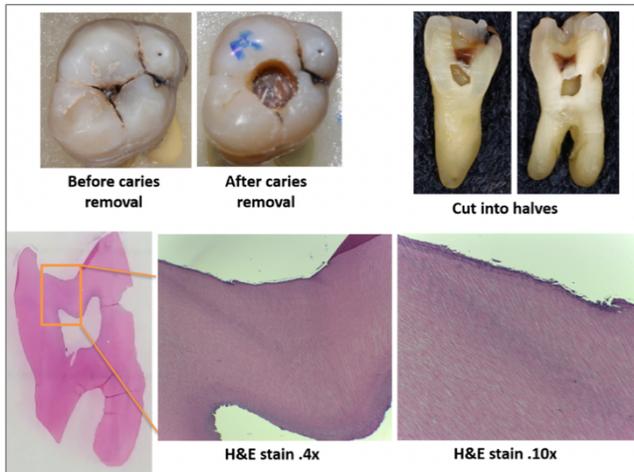


Figure 6. Clinical and histological images of partial caries-removed teeth.

Discussion

Clinically, operators' judgement whether or not a cavity preparation is properly done, and whether or not further caries removal is needed, is based on their visual and tactile perception. The reliability of using dental explorer to detect residual caries as conducted in our study is depending on the operator's experience and familiarity. Tactile perception with fingertip is a subjective method as sensor nerve function are employed. These judgements are really knowledge- and experience-dependant.⁸ Lack of knowledge and experience among undergraduate dental students may be the main reason behind the inconsistency in students' answer during the clinical evaluation as reflected in Table 2. However, the more exposure of Year 5 students to clinical hours does help increase their knowledge and experience compared to Year 4 students, therefore reflected in the slightly higher mean value of correct answer (Table 1). We believed photographic reproduction of photographs and histological slides of this study, and using them for teaching purposes in our dental school will help to improve the students' understanding regarding cariology and histology, hence also will help improving their clinical judgement.⁶

Histological examination has been established as the gold standard of diagnosis.⁸ Only through histologic examination, the sound, infected and affected zone of carious tissue lesion of can be distinguished with reproducible

in vitro studies results. In our study, interestingly all caries-treated teeth sample showed no bacteria invasion within the dentinal tubules under the partial and complete caries-removed area. Therefore, we can safely assume that when technique of standard partial caries removal is executed correctly, it is safe to leave the partial caries-treated tooth as shown in all partially caries-treated teeth in our study. Thus, the risk of pulp exposure can be avoided. This finding is consistent with Kidd et al., 2004 review that showed there is no clear evidence that it is harmful to leave infected dentine before sealing the cavity, even if it is soft and wet since re-enter caries studies showed no distinct differences seen between conventional complete vs incomplete caries removal, and between post 1-year vs post 10-years incomplete caries removal.³

We also suggested that the knowledge that it is safe to leave the partial caries-removed tooth as it is provided the technique was correctly executed as the required standard even by the less experience dental undergraduates might answer Ricketts et al. 2013 suggestion indirectly.^{12,20} Ricketts et al. found that 219 per 1000 teeth treated (0.22%) have been pulp or nerve exposed when the complete caries removal technique was used, whilst only 50 out of 1000 treated teeth (0.05%) pulp or nerve exposure were found when the partial caries removal technique was used.^{13,20} However, those fillings were mostly placed by specialist dentists. Thus, Ricketts et al. suggested for future studies to be carried out by non-specialist dentists to check whether the results would be similar.¹² Correct execution of partial caries removal technique promises less pulpal exposure and no interference in pulpal vitality, thus preserves tooth defensive mechanisms, tooth sensitivity and proprioception, and ensure no postoperative complications.¹¹

Unfortunately, we faced difficulty with our ground sections. Many of our halved ground section samples lifted off from the slides during the grinding process, causing the final microscopic examination were impossible. Used of EXAKT Technovit 7230 VLC Adhesive glue to the plastic microscope slides had not work as we hope for. The teeth were not stable enough, hence parts of the already halved samples dislodged during the ground section preparation. The best protocol to perform ground section is by

to process and embed the tooth in a resin block. Thus, the tooth will be stable, and the ground section will give promising microscopic result.^{6,14} Furthermore, although we kept all extracted permanent teeth samples in normal saline since collection stage to avoid dry out, to achieve the best result, the extracted teeth for ground sections should be preserved in 10% formalin until used.¹⁵

Conclusion

Having the ability to identify potential risk factors help dental care providers to play an active role in dental screening, preventive services and dental treatment including restoration. This study showed there is strong correlation between clinical and histological evaluation. A proper caries-treated tooth with all the infected dentine removed and only affected dentine left in situ showed bacterial absenteeism in the dentinal tubules underneath the cavity preparation. Thus, it is proven that good theoretical knowledge will lead to good clinical judgement, hence the restoration will be last longer.

However, Taylor's Modified Brown and Brenn staining technique should be able to show the presence of bacteria in dentinal tubules far better than hematoxylin and eosin (H&E) staining. Furthermore, cariology and histology research will improve better if only a way to examine and monitor the ongoing activity in underlying pulp of the treated-carious lesion in vivo can be found.

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Declaration Of Interest

The authors report no conflict of interest

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