

# A macroscopic and histological analysis of radiographically well-defined deep and extremely deep carious lesions: carious lesion characteristics as indicators of the level of bacterial penetration and pulp response

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## Abstract

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**Aim** To investigate the relationship between radiographically and macroscopically well-defined carious lesions and the dentine–pulp complex with regard to: (i) level of bacterial penetration; (ii) inflammatory status including the presence of hyperplastic pulp stroma; and (iii) formation of hard and/or ectopic connective tissue.

**Methodology** The material comprised 68 untreated cavitated permanent teeth divided into well-defined radiographic categories based on the lesion penetration depth: (i) deep lesions ( $\geq 3/4$  of the dentine thickness with a radio-dense zone separating the lesion from the pulp) and (ii) extremely deep lesions (the carious lesion penetrated the entire thickness of the dentine, without a radio-dense zone). After extraction, the teeth were processed for histology. The material was scored with regard to coronal breakdown, macroscopic variables describing caries activity and histological variables describing the dentine–pulp complex (bacteria, inflammatory infiltrate, partial pulp necrosis, hyperplastic changes and hard tissue/ectopic presence of connective

tissue). Interrater agreement was assessed using Cohen's kappa. Associations between variables were assessed using Pearson's chi-squared or Fisher's exact test. The effect size was reported by odds ratio (OR) and associated 95% confidence interval (CI). Level of significance was set to 5%.

**Results** There were significant associations between a closed environment (1–2 surfaces involved) and the presence of biofilm, retrograde demineralization and light-coloured demineralized dentine. Whereas radiographically defined deep lesions tended to have bacteria only in the primary dentine ( $P < 0.001$ , OR = 20.55, 95% CI [4.44, 107.89]), extremely deep carious lesions tended to have bacteria in contact with the pulpal tissue ( $P = 0.007$ , OR = 6.84, 95% CI [2.00, 62.83]), presence of an inflammatory infiltrate (Fisher's exact;  $P < 0.001$ ) and partial pulp necrosis. Hyperplastic pulps were seen only in extremely deep lesions.

**Conclusions** Unlike deep lesions, extremely deep carious lesions were often associated with severe pulp inflammation and infection. A radiographic threshold between deep and extremely deep lesions is suggested as indicator of the bacterial penetration level and the severity of the pulpal response prior to intervention.

**Keywords:** caries, dental pulp, inflammation, lesion activity, polyps.

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## Introduction

Although increasing evidence indicates the success of less invasive carious tissue removal procedures in deep carious lesions associated with asymptomatic, vital pulps (Ricketts *et al.* 2013, Schwendicke *et al.* 2013, 2016a), priority is usually given to more invasive treatment methods (Schwendicke & Göstemeyer 2016b). The disagreement between proponents of the different strategies is due mainly to contrasting appreciations of the ontology of caries as a disease. Groups that advocate less invasive procedures interpret the carious lesion as a manageable ecosystem, in which an effective change in the cariogenic biofilm arrests further progress of the lesion (Marsh 1994, Bjørndal *et al.* 1998) and subsequently induces an inflammatory pulp response compatible with healing (Cooper *et al.* 2014). In contrast, groups favouring invasive and nonselective carious tissue removal understand caries as an irreversible and infectious disease that requires complete elimination of the infectious agent, followed by removal and repair of the damaged tissue (Barrieshi-Nusair & Qudeimat 2006, Ricucci *et al.* 2019).

Whatever the school of opinion, the success of a pulp-preserving treatment strategy rests on a precise evaluation of the state of the pulpal tissue. As several studies have shown, such evaluations cannot be made with great precision (Seltzer *et al.* 1963, Mejare *et al.* 2012), and evaluation is effectively a prediction procedure. To limit errors in diagnostic ‘prediction’ and to limit the clinical consequences of potential over- or undertreatment, awareness might thus be raised of the following: (i) the reason the pulp is inflamed (e.g. due to traumatic exposure of the pulp, a deep carious lesion or iatrogenic damage); (ii) the radiographic penetration depth of the carious lesion; and (iii) the activity status of the carious lesion.

Although all three factors are known to affect the inflammatory response of the pulp (Reeves & Stanley 1966, Bjørndal & Thylstrup 1998; Bjørndal & Darvann 1999), they are not often included in the diagnostic process or choice of treatment strategy (Bjørndal *et al.* 2014). As recently suggested (Bjørndal *et al.* 2019, ESE 2019), this may explain divergences between the outcomes of vital pulp treatments.

It is therefore hypothesized that, if such variables were included in its assessment, more information about the pulpal status before treatment might be generated. This might help practitioners select the most appropriate management strategy of an extensively progressed carious lesion.

This study aimed to investigate whether there is a link between distinct radiographic levels of lesion penetration depth and the resulting pulpal response. For this purpose, untreated permanent molar teeth with extensive carious destruction were divided into two distinct and well-defined radiographic categories of lesion penetration depth (i.e. deep carious lesions vs. extremely deep carious lesions). Based on this grouping, the teeth were used to investigate the relationship between the radiographic penetration level and variables relevant to the carious lesion (i.e. radiographic penetration depth, number of tooth surfaces involved in the carious lesions and carious lesion activity), as well as variables related to the dentine-pulp complex (bacterial penetration level, inflammatory status of the pulp and the pulpal formation of hard and/or ectopic connective tissue).

## Materials and methods

The study comprised 68 permanent molars with extensive carious lesions. The teeth had been extracted in a group of young individuals (aged 12–18 years) by Danish dentists from the Greater Copenhagen area as part of a dental mission concerning improved oral health effects on Mactan Island, the Philippines. This work was undertaken in accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008), and approved by Department of Education Lapu-Lapu, Mactan Island, the Philippines. The biologic human material was fully anonymized, and the inclusion of the extracted teeth in this study was reviewed and approved by the Danish National Committee on Health Research Ethics under protocol number H-1-2012-FSP.

After extraction, the teeth were immediately immersed in 4% neutral buffered formalin (VWR, Søborg, Denmark) for a minimum of 24 h. All teeth were rinsed in phosphate-buffered saline (pH 7.2) (The Substrate and Sterile laboratory University of Copenhagen, Copenhagen, Denmark). A radiograph was acquired in a buccal-lingual direction using a Planmeca ProSensor® HD, Planmeca ProX Intraoral X-ray Unit and the Planmeca Romexis Software (Pludent, Helsinki, Finland). An exposure time of 0.6 s using 8 mA and 6 kV was used. All surfaces of each tooth were then macroscopically photographed using an InfinityX camera (Teledyne Lumenera, Ottawa, Ontario, Canada) under a SteREO Discovery.V8 stereomicroscope (Carl Zeiss MicroImaging

GmbH, Oberkochen, Germany). Using a MIKRO-TRENN saw (Hofer, Aathal-Seegräben, Switzerland), each tooth was sectioned in half. The saw was adjusted to cut through the central part of the occlusal cavitated lesion in a mesio-distal direction.

Each of the resulting sections was examined in transmitted light and, using the stereomicroscope, was photographed imbibed in phosphate-buffered saline (pH 7.2). Each specimen was demineralized in 10% EDTA (pH 6.9) (VWR) until complete demineralization as evaluated radiographically. Specimens were cleared in methyl salicylate (VWR) and double-embedded in 1% celloidin (Ampliqon, Odense, Denmark) and paraffin (VWR; Culling 2013). The specimens were sectioned (4 µm) serially at central carious lesion sites until exhaustion of the specimen. Due to the differences between the teeth with regard to tooth type and overall section plane, the total number of sections from each specimen varied greatly. Sectioning was carried out on a Leica sliding microtome (Leica Biosystems, Copenhagen, Denmark). Each specimen was assessed according to macroscopic parameters related to the carious lesion, including a histological examination of the dentine–pulp complex.

#### Macroscopic and radiographic evaluation

Surface breakdown caused by caries was described as the number of tooth surfaces involved in the carious lesion. The results were dichotomized and, following the terminology used for bacterial ecosystems (Edwardsson 1987), used to describe the degree of openness of the lesion environment, a closed lesion environment being indicated by 1–2 surfaces involved in the carious lesion (Fig. 1a–b) and an open lesion environment by 3–5 such surfaces (Fig. 1c–e). The penetration level of the carious lesion was assessed by means of radiographic examination, and each lesion was graded into two distinct categories according to recently published guidelines (ESE 2019) as either: (i) deep (i.e. the carious extending  $\geq 3/4$  of the dentine thickness, with a visible–radiopaque zone separating the carious lesion from the pulp, Fig. 1f) or (ii) extremely deep (i.e. the carious lesion extending the entire thickness of the dentine, Fig. 1g).

The activity status of the lesion environment was estimated on the basis of the following macroscopic variables: visible biofilm accumulation either present (Fig. 1h) or absent (Fig. 1i); colour of demineralized dentine either light (Fig. 1h) or dark (Fig. 1i); and retrograde demineralization either present or absent

(Fig. 1h). These variables are well-known markers for assessing lesion activity status (Bjørndal & Darvann 1999). In order not to destroy the interface between the carious lesion and the pulp, and not to compromise the subsequent histologic examination, the texture of the demineralized dentine was omitted from the panel of variables. Scoring was done by two of the authors (SD and LB). In cases of disagreement, the results were discussed until consensus was reached.

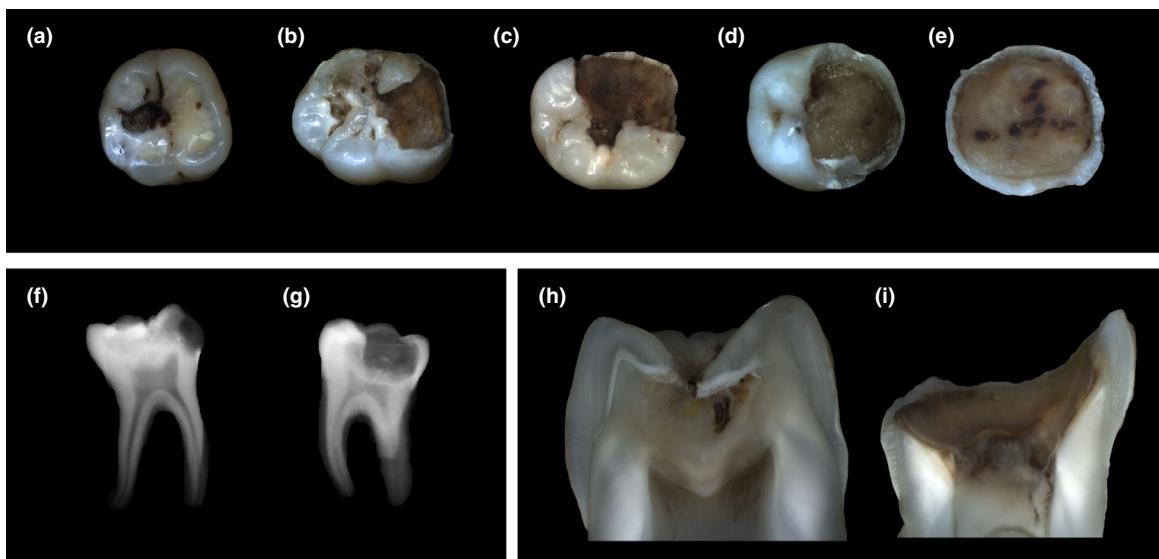
#### Histologic evaluation

Haematoxylin and eosin staining was used to examine tissue architecture, and the modified Brown and Brenn staining method (Taylor 1966) was used to establish the presence or absence of bacteria. Stained and mounted tissue samples were visualized using an Axio Scan.Z1 slide scanner (Zeiss, Jena, Germany). The specimens were examined (yes/no) for the following variables:

1. *Bacterial penetration level*: bacteria present only in primary dentine (Fig. 2a); bacteria reaching tertiary dentine (Fig. 2b); or bacteria reaching the pulp (Fig. 2c).
2. *Status of the pulp*: presence of a hyperplastic pulp stroma (pulp polyp; Fig. 2d); presence of an inflammatory infiltrate (Fig. 2e). In the event of an inflammatory infiltrate, it was assessed whether the infiltrate was confined solely to the coronal pulp or whether it also involved the radicular pulp. In case of necrotic changes, it was noted if there was partial necrosis (Fig. 2e) or full necrosis (Fig. 2f).
3. *Formation of hard and/or connective tissue*: presence of tertiary dentine (Fig. 2g); presence of a pulp stone (Fig. 2h); and/or ectopic connective tissue resembling neither pulp stone nor tubular dentine matrix (Fig. 2i). The histologic variables were scored by two of the authors (SD and LB). Disagreements were discussed until consensus was reached.

#### Data analysis and statistics

Conventional descriptive statistics were used for the general classification of the material. Associations between dichotomous variables were assessed using Pearson's chi-squared or Fisher's exact test where necessary (degrees of freedom = 1). The effect size was reported by computing the odds ratio (OR) with



**Figure 1** Macroscopic variables: (a–e) degree of openness defined as the involvement in the carious lesion of between 1 and 5 tooth surfaces. Well-defined radiographic penetration depth: (f) a deep carious lesion has reached the pulpal  $\frac{1}{4}$  of the dentine with a zone of radiopaque dentine between the lesion and the pulp. (g) An extremely deep carious lesion penetrates the entire thickness of the dentine. Variables related to carious activity: (h) a closed lesion environment with retrograde demineralization seen as a white line of demineralization undermining the enamel. The underlying demineralized dentine is light/yellow, and clear signs of biofilm accumulation are noted. (i) an open lesion environment with darkening of the demineralized dentine, only plaque accumulation in the central part of the specimen, where the carious lesion has exposed the pulp chamber. Note that the hyperplastic changes (\*) are macroscopically visible.

95% confidence intervals (CI). The level of significance was set to 5%. Interrater agreement was assessed by calculating Cohen's kappa ( $\kappa$ ). Statistical calculations were done using SPSS statistics package, edition 26 (IBM Corp., Armonk, NY, USA).

## Results

The interrater evaluation showed perfect agreement for most scores ( $\kappa = 1.00$ ). The interrater agreement between the following variables had relatively lower  $\kappa$ -values: retrograde demineralization ( $\kappa = 0.77$ ;  $P < 0.001$ ); macroscopic presence of biofilm ( $\kappa = 0.81$ ;  $P < 0.001$ ); colour of demineralized dentine ( $\kappa = 0.53$ ;  $P = 0.038$ ); and ectopic connective tissue ( $\kappa = 0.84$ ;  $P < 0.001$ ).

### Macroscopic characteristics of the lesion environment

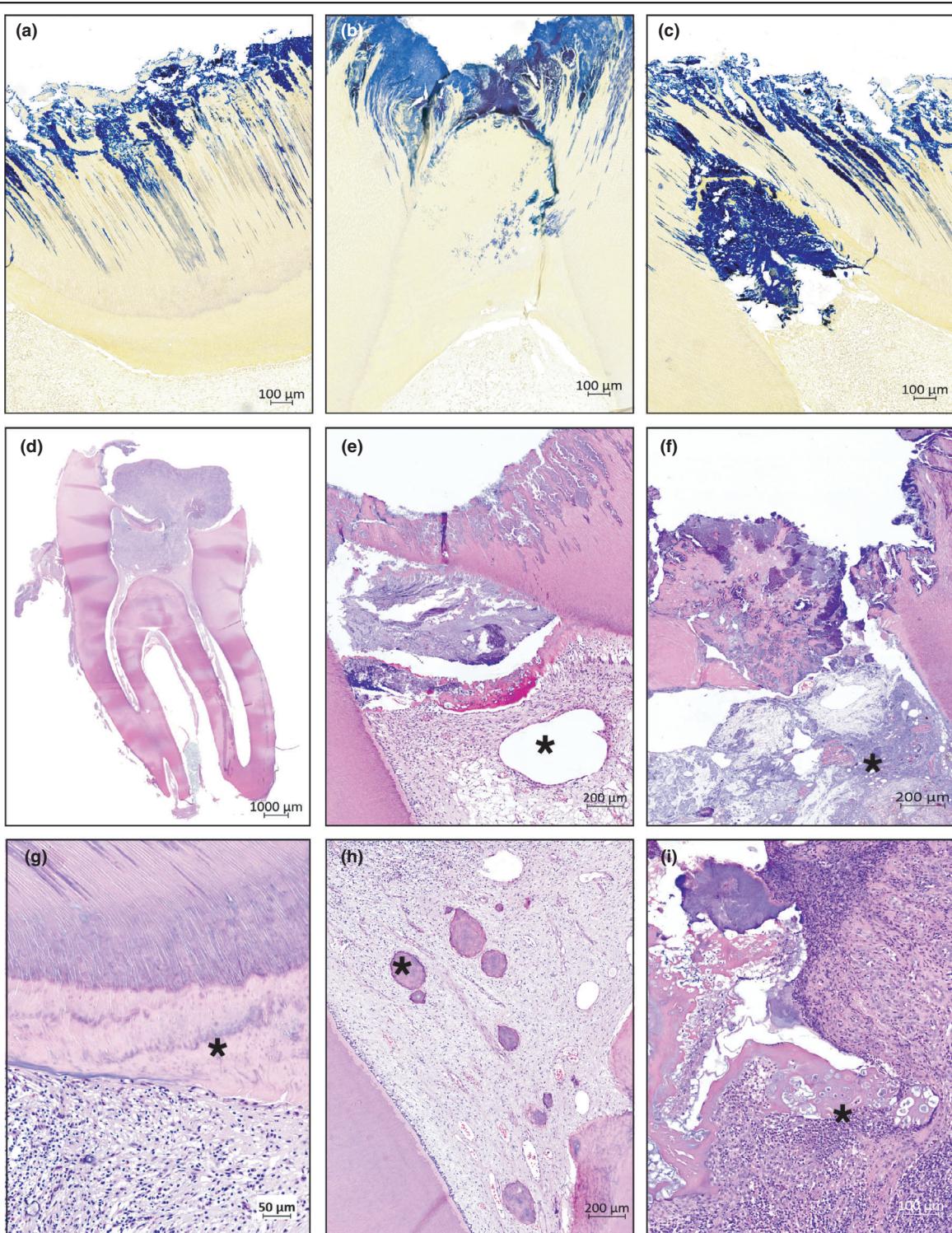
Over three-quarters of the carious lesions (84%) were extremely deep (57/68), and 53% had an open lesion environment (36/68). Their degree of openness was

significantly associated with the following variables: biofilm, retrograde demineralization and colour of demineralized dentine. Closed lesion environments tended to be covered by a biofilm ( $P = 0.007$ , OR = 11.92, 95% CI [1.43, 99.40]). The colour of the demineralized dentine in a closed lesion environment tended to be light ( $P < 0.001$ , OR = 12.08, 95% CI [3.12, 47.00]); and retrograde demineralization tended to be associated with a closed lesion environment ( $P < 0.001$ , OR = 9.00, 95% CI [3.00, 27.03]).

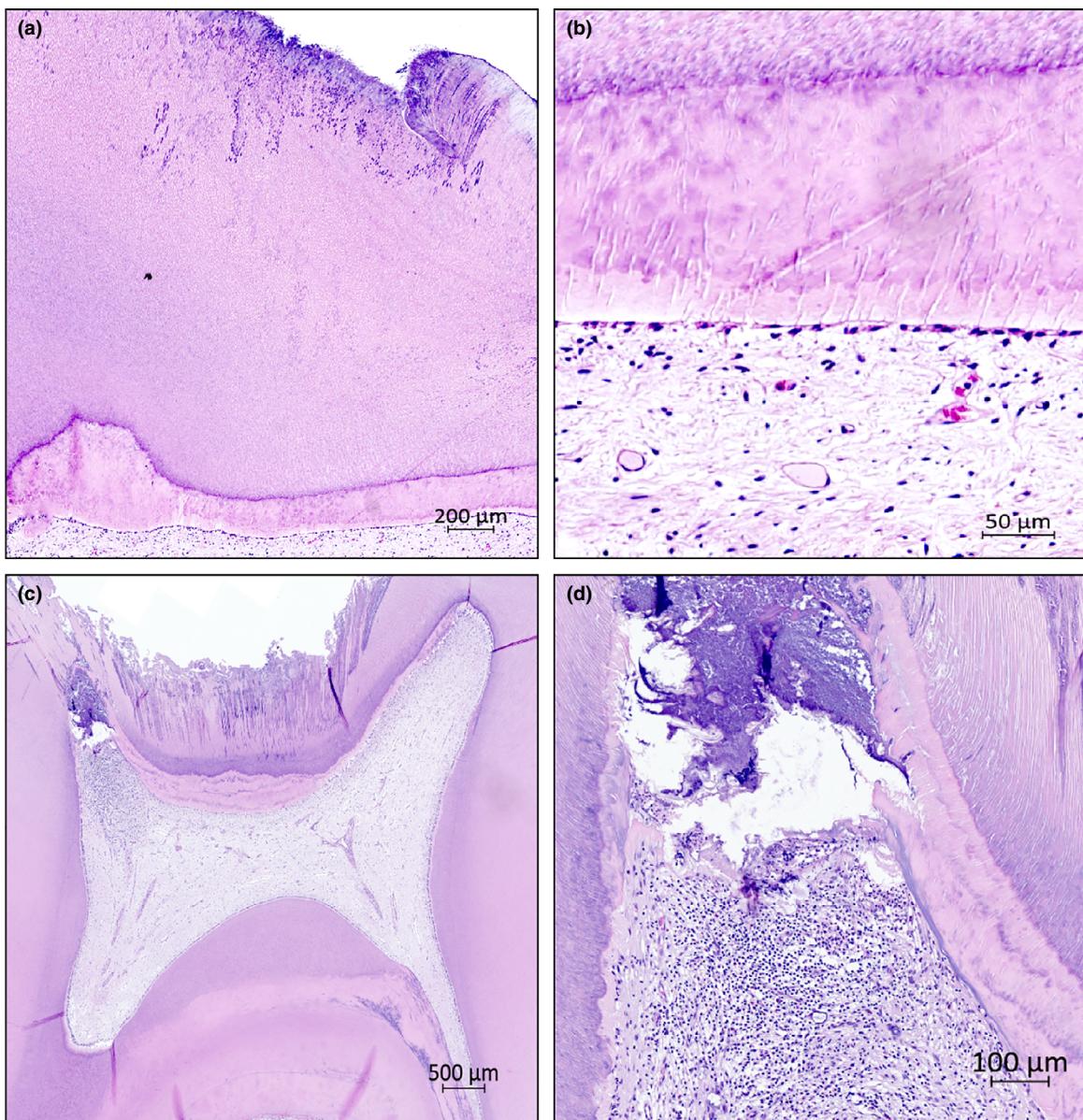
### Histological characteristics versus radiographic depth and lesion environment

#### Bacterial penetration level

Radiographic lesion depth was significantly associated with the penetration level of the bacteria. Deep lesions tended to have bacteria only in the primary dentine ( $P = 0.000$ , OR = 20.55, 95% CI [4.44, 107.89], Fig. 3a–c), whereas extremely deep carious lesions tended to be associated with bacteria in the pulpal stroma ( $P = 0.007$ , OR = 6.84, 95% CI [2.00, 62.83], Fig. 3d–f). Radiographic lesion depth was not



**Figure 2** Bacterial penetration level: (a) bacteria in primary dentine, (b) bacteria in tertiary dentine and (c) bacteria reaching the pulp. Modified Brown and Brenn staining. Pulpal status: (d) hyperplastic pulp stroma (polyp), (e) partial necrosis (\*) and associated inflammatory infiltrate, and (f) total pulp necrosis (\*). Formation of hard and/or connective tissue: (g) tertiary dentine (\*) and primary dentine with biofilm. (h) Pulp stones (\*) in an uninflamed part of the coronal pulp. (i) Aggregates of ectopic connective tissue with forming cells (\*) situated in lacunae. (d-i) Haematoxylin and eosin stain.



**Figure 3** (a) Histological features of a well-defined deep lesion. (b) Detail shows that the bacteria have not reached the pulp. The tertiary dentine is partly atubular, and the pulp has no inflammatory infiltrate. (c) The bacteria have reached tertiary dentine in the extremely deep carious lesion. (d) Detail showing the region to be partly necrotic and with a subjacent inflammatory infiltrate. Haematoxylin and eosin stain.

significantly associated with the bacteria present in tertiary dentine.

#### *Status of the pulp*

Due to complete necrosis (13% of the specimens) and procedural errors, histologic scoring of the pulp was limited to 46 of the 68 teeth (68%). The

extremely deep lesions were significantly associated with the presence of an inflammatory infiltrate (Fisher's exact;  $P < 0.001$ ). The inflammatory infiltrate affected both the coronal and the radicular pulps in 62% of the carious lesions scored as extremely deep. Generally, the radicular inflammatory changes were seen to affect the most coronal part of the radicular

pulp. Nearly one-third of the material with vital tissue (30%) was hyperplastic. Such changes were seen only in extremely deep lesions. In some of these teeth, the initial stages of polyp formation barely reached the upper margins of the pulp cavity. The polyp stroma was infiltrated by inflammatory cells and had no epithelial cover (Fig. 4a). Other teeth were at more developed stages, in which the polyp was protruding from the pulp chamber. The lateral dentine was covered with bacteria but there was no bacterial penetration (Fig. 4b–c). Few of the pulp polyps were covered by a mature, stratified squamous epithelium (Fig. 4d). The largest polyps were macroscopically visible (Fig. 4e), and the lateral dentine had signs of dark-coloured demineralized dentine (Fig. 4f).

Radiographic lesion depth was significantly associated with partial necrosis of the pulp (Fisher's exact;  $P < 0.001$ ), which was seen only in extremely deep carious lesions. An open lesion environment was significantly associated with the presence of an inflammatory infiltrate ( $P = 0.017$ , OR = 8.08, 95% CI [1.52, 42.83]) and with hyperplastic changes  $P < 0.001$ , OR = 28.60, 95% CI [3.28, 249.73]). Regardless of the hyperplastic changes, the tertiary dentine appeared as a mixture of tubular and atubular areas with a clear demarcation between primary and tertiary dentine, and with a layered appearance (Fig. 5a).

#### *Hard tissue formation and/or ectopic connective tissue*

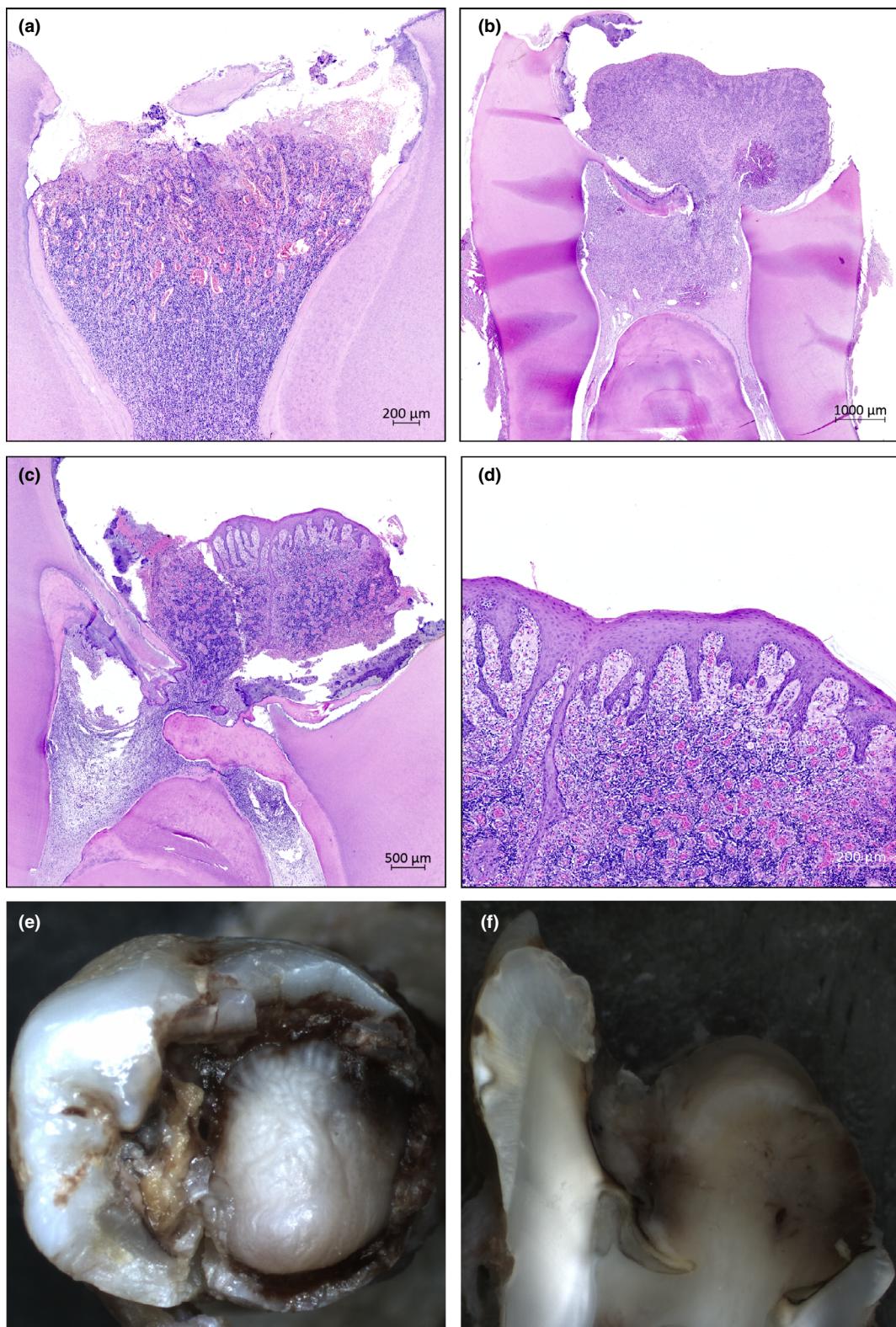
More extremely deep lesions than deep lesions presented with signs of ectopic connective tissue (Fisher's exact test;  $P = 0.009$ ). The ectopic tissue occurred in three different patterns: (i) aligned towards the necrotic parts of the pulp in extremely deep lesions (Fig. 5b); (ii) attached to the walls of the pulp chamber, but with a clearly discernible difference between primary dentine and the newly formed tissue (Fig. 5c); or (iii) embedded within the inflamed pulpal tissue (Fig. 5d). The level of radiographic penetration was significantly associated with the formation of tertiary dentine, with extremely deep lesions tending to have more tertiary dentine (Fisher's exact test;  $P = 0.044$ ). The level of radiographic penetration was not significantly associated with the occurrence of pulp stones. The openness of the lesion environment was not significantly associated with the presence of ectopic connective tissue, tertiary dentine formation or the presence of pulp stones.

## Discussion

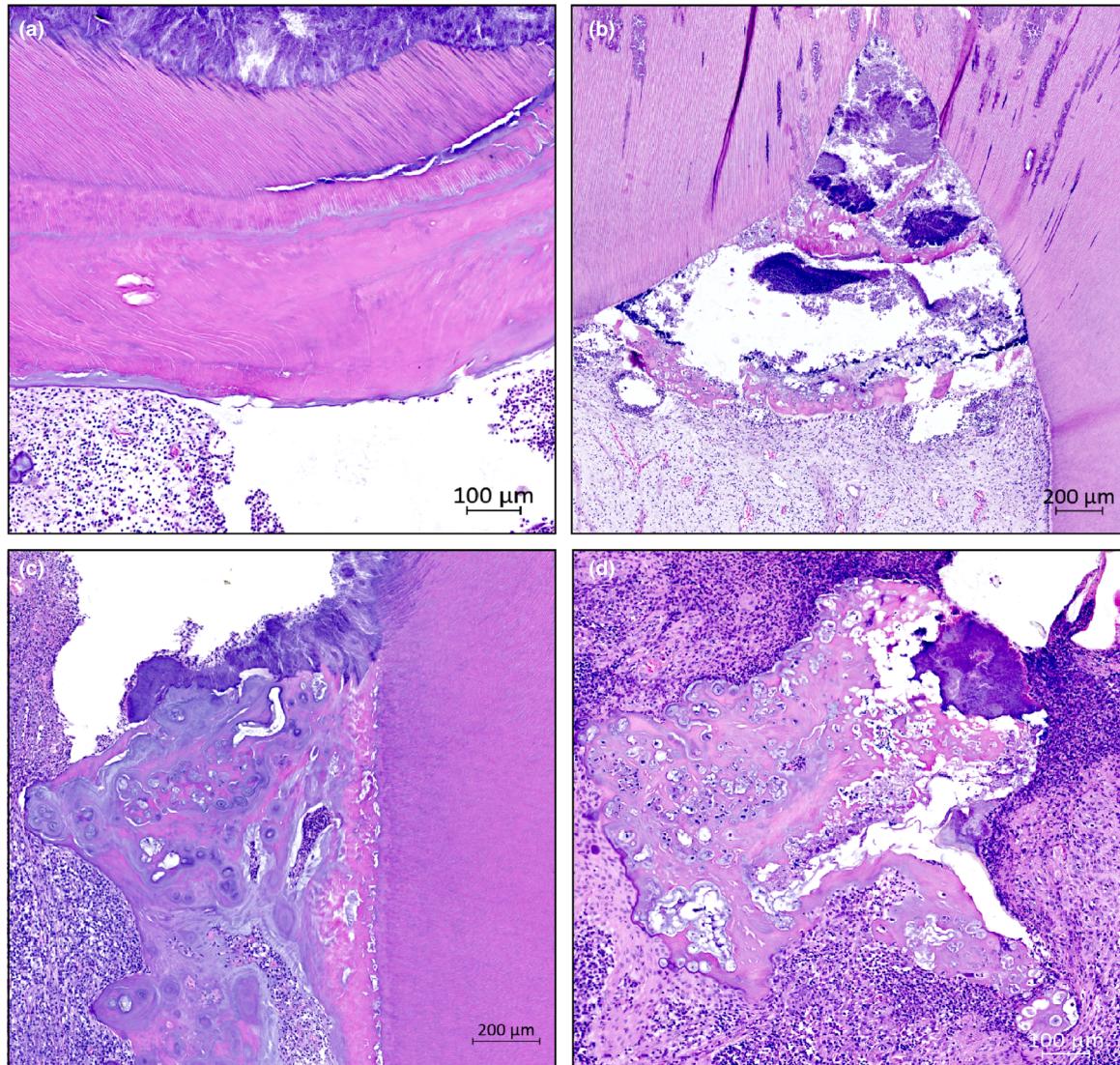
In this study, a clinically based radiographic grouping of deep and extremely deep carious lesions (Bjørndal *et al.* 2019, ESE 2019) was used as a basis for describing the macroscopic and histological characteristics of the dentine–pulp complex in cases of extensive carious destruction. The link between such well-defined radiographic groupings and the reactions of the dentine–pulp complex has not been studied before and may support and improve the treatment decision process for vital pulp therapy. These results showed that, unlike open lesion environments, closed lesion environments (Fig. 1a,b) were significantly associated with the presence of biofilm. The colour of demineralized dentine in closed lesion environments tended to be light, accompanying retrograde demineralization.

Although this study was based on extracted teeth, it is suggested that, as more carious tooth surfaces are broken down, the untreated lesion environment becomes more open. This reduces the accumulation of biofilm within the lesion and leads to darkening of the carious dentine. Darkening of the latter has previously been linked to arrested carious lesions, whose colour is caused by nonenzymatic glycation (Matsuda *et al.* 2016), which is also known as the Maillard reaction. In principle, the concept of converting an ecosystem from an active closed environment into an open arrested one reflects the philosophy underlying less invasive carious removal procedures (Massler 1967, Edwardsson 1974). Similarly, the two-stage removal of carious tissue, also known as a stepwise excavation procedure, is intended to convert the active cariogenic environment into an inactive lesion environment, in which the carious dentine left behind will darken and the lesion not progress further. The inactive lesion environment is further characterized by a lower bacterial load in the cavity in the second stage of the excavation process than in the first (Bjørndal *et al.* 1997).

After division of the material on the basis of the depth of radiographic penetration into deep and extremely deep lesions, it was evident that in deep carious lesions, the bacteria were more usually present only in the primary dentine (Fig. 3a–c). In contrast, extremely deep lesions were more usually associated with bacteria that had reached the pulp (Fig. 3d–f), and also with an inflammatory infiltrate ( $P = 0.000$ ) and subsequent partial necrosis ( $P = 0.009$ ). These findings support recent clinical guidelines stating that the



**Figure 4** (a) Initial stages of polyps have highly vascular and inflamed tissue that barely protrudes from the pulp chamber, (b) to large polyps (c) occasionally covered in a mature, stratified, squamous epithelium (detailed in d). (b, c) Biofilm is noted on the lateral borders of dentine and not on the surface of the polyps. (e) Stereo-macroscopic view of a large polyp protruding through the crown. (f) The macroscopic specimen shows the polyp penetrating the roof of the pulp chamber, with lateral remnants of thin dark carious dentine. Haematoxylin and eosin.



**Figure 5** Hard tissue and/or ectopic connective tissue: (a) tertiary dentine with tubular and atubular areas formed in layers, of which the first zone underneath primary dentine is mainly tubular in appearance. The surface of carious dentine is covered in biofilm. There are three clear patterns of ectopic connective tissue formation: (b) towards bacteria, creating a barrier against the infection front; (c) attached to the walls of the pulp chamber, with a clear distinction between the connective tissue aggregate and the primary dentine; and (d) embedded within the inflamed pulpal stroma. Haematoxylin and eosin.

category of teeth with deep carious lesions should be managed by selective and stepwise excavation procedures, as bacteria in these cases are not expected to have reached the pulp (ESE 2019).

It was shown by laboratory studies using test cavities in sound dentine that leaving microorganisms behind, whether alive or dead, may create a reservoir of endotoxins that cause a chronic inflammatory reaction in the pulp (Cooper *et al.* 2010). Such bacterial metabolites have been shown experimentally to be able to diffuse through dentinal tubules in test cavities (Love 2002, Love & Jenkinson 2002). This explains why, even in the absence of bacteria in direct contact with the pulpal tissue, a chronic inflammatory reaction can be maintained in the pulp – a process caused by the release of inflammatory mediators from the odontoblasts and macrophages present in the pulp after stimulation by the bacterial endotoxins (Schweikl *et al.* 2017). In the group of natural teeth scored as having deep carious lesions as opposed to the group scored with extremely deep lesions examined in this study, a significantly less frequent appearance of an inflammatory infiltrate was found. Based on the findings of the current study, it is not possible to investigate the fate of well-defined deep lesions where an inflammatory response is maintained (e.g. if carious dentine is left behind during a stepwise excavation procedure), but clinical data have proven that avoiding pulp exposure in the treatment of teeth with deep carious lesions (as opposed to extremely deep lesions) leads to long-term success, particularly when the stepwise excavation procedure is used (Bjørndal *et al.* 2017).

Some decades ago, histological observations showed that, when bacteria are present in tertiary dentine, the pulp is often irreversibly inflamed (Reeves & Stanley 1966). However, the study in question did not include information on radiographic penetration depth. But although a remaining dentine thickness of at least 0.5 mm towards the pulp has been proposed as a prerequisite for avoiding severe pulpal injury (Murray *et al.* 2003), this is equally difficult to handle in a clinical setting. Whilst it would have been preferable if the present material had been larger, as reflected by the wide CIs and distributed more evenly between deep and extremely deep carious lesions, the findings of this study support the rationale of radiographically classifying an extensive carious lesion as either deep or extremely deep prior to intervention. The clinical consequence of this classification is that whereas stepwise or

selective carious removal is indicated in deep lesions, a more invasive treatment approach (e.g. pulpotomy or root canal treatment) should be taken in cases of extremely deep carious lesions. In this material, the majority of teeth with extremely deep carious lesions had an inflammatory infiltrate that also included the radicular pulpal tissue. However, the fact that 38% of the teeth scored with extremely deep carious lesions that had an inflammatory infiltrate had unaffected radicular pulp tissue may indicate that pulpotomy is an option even in extremely deep stages of caries progression.

Until an extremely deep carious lesion reaches a completely unroofed stage, tertiary dentine is formed subjacent to the lesion. This was previously found to consist of tertiary dentine that was mainly atubular and tubular only in some parts (Ricucci *et al.* 2014). Earlier observations on human carious teeth in pre-cavitated stages of lesion formation showed that primary odontoblast cells can be involved in early tertiary dentine formation (Bjørndal *et al.* 1998, Ricucci *et al.* 2014). Although a general qualitative description of the tertiary dentine was not included in this material, a layered appearance of the tertiary dentine in extremely deep lesions was noted, whereas the area closest to the primary dentine had a more tubular structure resembling the early pattern of tertiary dentine (reactionary dentine; Fig. 5a). This layered appearance of the tertiary dentine may reflect the inflammatory changes occurring in the pulp as the lesion environment changes over time (Kuwabara & Massler 1966) (Fig. 1a–e). As discussed by others (Cooper *et al.* 2014), it is hypothesized that a conversion of the lesion environment from active to arrested is accompanied by a low-grade or desirable inflammatory response compatible with healing.

It is currently uncertain to what degree the ectopic connective tissue that is also found within the pulp can be viewed as a variation of dentine or simply as a dystrophic intrapulpal mineralization caused by another developmental pathway.

In this study, a high prevalence of pulp polyps was noted, which were found only in extremely deep carious lesions in which the pulp was completely exposed to the oral environment. In this stage, where most of the central coronal carious dentine was absent, the lesion appeared to have been largely arrested, which might be an important prerequisite for polyp formation, as opposed to a closed lesion environment, where biofilm is maintained, sustaining pulp

inflammation and eventually leading to necrosis and periapical disease.

Whilst it is not known whether a polyp is a stable phenotype, the polyps in the present study varied considerably in size (Fig. 4). The fact that some of them were epithelialized may indicate that a pulp polyp has a distinct developmental pattern. The epithelium, which in some cases covered the polyps, has been proposed to originate from the oral mucosa (Southam & Hodson 1974). The complex interplay between destruction and repair that occurs during pulpal inflammation is highlighted by the successful 'transplantation' of one type of tissue (epithelium) onto another (the inflamed pulpal tissue).

Whilst pulp polyps were once associated with the formation of ectopic connective tissue (Boulger 1931), the present material did not provide evidence of a significant association between a pulp polyp and the presence of ectopic connective tissue. The presence of an ectopic connective tissue was noted only in several extremely deep carious lesions without polyps. It is hypothesized that this may represent signs of late repair. Further studies are needed to elucidate this.

This study demonstrated that both the bacterial penetration level and the inflammatory status of the pulp were significantly linked to distinct radiographic categories of extensive carious penetration level. Including such information in the clinical evaluation of teeth with extensive carious breakdown prior to treatment is suggested, as this might lead to a more predictable outcome of both noninvasive and invasive vital pulp treatment strategies.

## Conclusions

Dividing teeth into two distinct radiographic groups, that is teeth with deep carious lesions versus teeth with extremely deep carious lesions, is suggested as an indicator of the bacterial penetration level and the related pulp response. In cases of teeth with extensive carious breakdown, it may be possible to optimize the choice of treatment methods by including such information in a preoperative case assessment. Unlike in extremely deep lesions, the risk of bacteria in the pulp of well-defined deep lesions is low. In extremely deep lesions, two different pulpal reaction patterns were noted: either an inflamed pulp in which bacteria and necrosis were present, or the presence of polyps within an open lesion environment, accompanied by signs of inactivation of the carious lesion.

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## Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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