

# Vital pulp therapy: histopathology and histobacteriology-based guidelines to treat teeth with deep caries and pulp exposure



Domenico Ricucci<sup>a</sup>, José F Siqueira Jr.<sup>b</sup>, Yuanyuan Li<sup>c</sup>, Franklin R. Tay<sup>d,\*</sup>

<sup>a</sup> Private Endodontic Practice, Cetraro, Italy

<sup>b</sup> Department of Endodontics, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil

<sup>c</sup> Department of Prosthodontics, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi 710032, PR China

<sup>d</sup> Department of Endodontics, The Dental College of Georgia, Augusta University, Augusta, GA, USA

## ARTICLE INFO

### Keywords:

caries  
direct pulp capping  
pulpotomy  
vital pulp therapy

## ABSTRACT

**Objectives:** Vital pulp therapy (VPT) encompasses distinct treatment modalities for deep caries that approximate the pulp chamber in vital teeth. Confusion exists in the literature in terms of the indication and rationale for each VPT approach. The objectives of the present study are to elucidate the indications for VPT and to present a set of histopathology and histobacteriology-based guidelines for VPT in teeth with deep caries.

**Methods:** Two hundred and sixty-four carious, unrestored and VPT-treated human teeth, which were extracted for reasons not related to the present study. The teeth were processed for histological and histobacteriological examination. Other 757 clinical cases that received different VPT procedures were followed-up to identify success rates, with the longest observational period of 30 years.

**Results:** Follow-up of the clinical cases indicated that direct pulp capping was successful in 73.2%, partial pulpotomy in 96.4% and full pulpotomy in 77.8% of the cases. Histological and histobacteriological examination showed a localised inflammatory response that commonly occurred in the subjacent pulp tissue as soon as the enamel was penetrated by caries. If the softened and infected dentine were completely excavated, without pulp exposure, and the cavity restored with an adequate restoration, pulp inflammation frequently subsided. In teeth showing pulp exposure, the extent of bacterial penetration varied and areas of infection presented severe pulp inflammation, including micro-abscesses. However, the pulp tissue apical to the infected/inflamed area was usually uninfamed and normal. Guidelines based on the present histopathological, histobacteriological and clinical findings are proposed for VPT and mainly involve direct examination of dentine and the exposed pulp tissue under deep caries for decision-making, and require strict asepsis during procedures.

**Conclusions:** Vital pulp therapy following the guidelines proposed in the present article has the potential to improve the outcome of the conservative treatment of mature teeth with deep caries and in some occasions may be an alternative to pulpectomy.

**Clinical Significance:** A guideline for VPT in the treatment of deep caries is proposed, focusing on direct observation of dentine and the exposed pulp tissue under deep caries. Stringent aseptic techniques are mandatory for VPT procedures to be successful.

## 1. Introduction

Clinicians are often faced with a dilemma in the treatment of deep caries that approximate the pulp chamber in vital teeth with closed apices: whether the dental pulp should be preserved to maintain vitality or completely removed to prevent necrosis, infection, and induction of apical periodontitis. Treatment procedures targeting at maintaining the vitality of all or part of the cariously exposed pulp have not been favoured by the majority of clinicians until recently. Concerns on the

unreliability of vital pulp therapy (VPT) procedures, which include direct pulp capping, partial pulpotomy and full pulpotomy, were mostly based on the suboptimal results derived from some follow-up studies.

Pulpectomy procedures are reported to have close to 90% success rates [1,2]. In contrast, considerably lower success rates were identified for pulp capping and pulpotomy procedures [3,4]. In a retrospective study, Barthel et al. [3] reported success rates of 37% and 13%, respectively, for 5-year and 10-year follow-ups of direct pulp capping procedures. Likewise, Bjørndal et al. [4] reported a success rate of

\* Corresponding author at: Dental College of Georgia, Augusta University, Augusta, GA, USA.

E-mail address: [ftay@augusta.edu](mailto:ftay@augusta.edu) (F.R. Tay).

<https://doi.org/10.1016/j.jdent.2019.05.022>

Received 22 March 2019; Received in revised form 28 April 2019; Accepted 18 May 2019

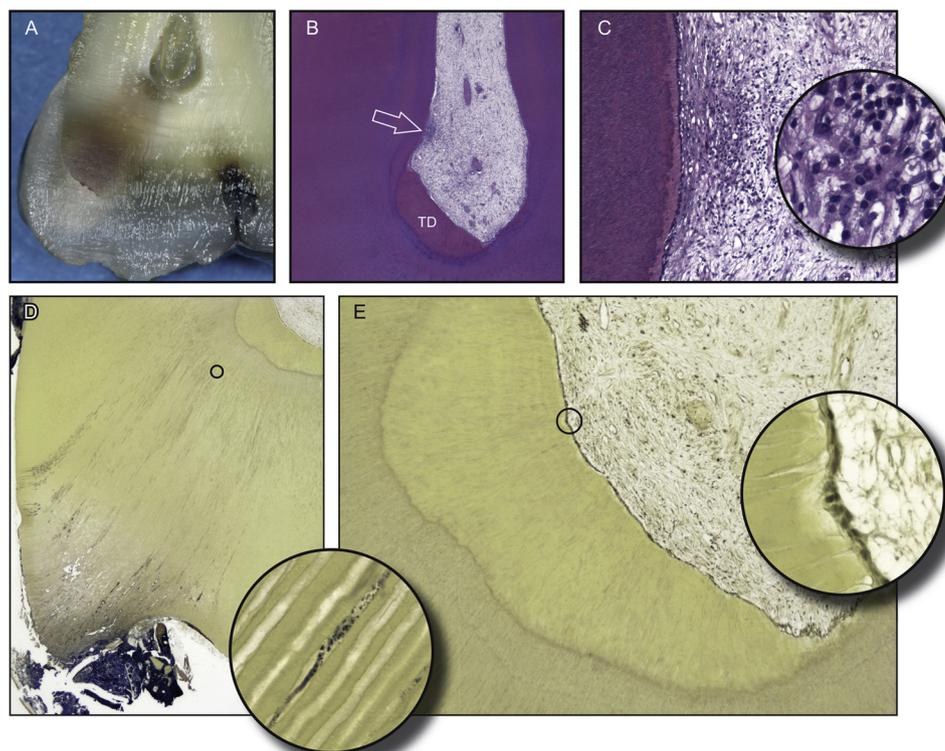
0300-5712/ Published by Elsevier Ltd.

**Table 1**  
Histobacteriologic analysis of 264 extracted teeth for location of bacteria within the dentine and pulp.

Histologic diagnosis	Number of cases	Location of bacteria		
		Circumpulpal secondary dentine	Tertiary dentine	Pulp tissue
Untreated carious teeth				
Reversible inflammation	68	68	27	-
Irreversible inflammation	92	92	92	92
Restored teeth				
Healed	28	-	-	-
Reversible inflammation	14	14	6	-
Irreversible inflammation	17	17	17	17
Teeth subjected to pulp capping				
Healed	12	-	-	-
Reversible inflammation	1	1	-	-
Irreversible inflammation	5	3	5	5
Caries-free teeth receiving experimental pulpotomy				
Healed	12	-	-	-
Reversible inflammation	2	2	2	-
Irreversible inflammation	5	5	5	5
Carious teeth receiving "partial" caries excavation				
Healed	-	-	-	-
Reversible inflammation	8	8	2	-
Irreversible inflammation	-	-	-	-

**Table 2**  
Clinical cases that received a VPT procedure prior to restoration.

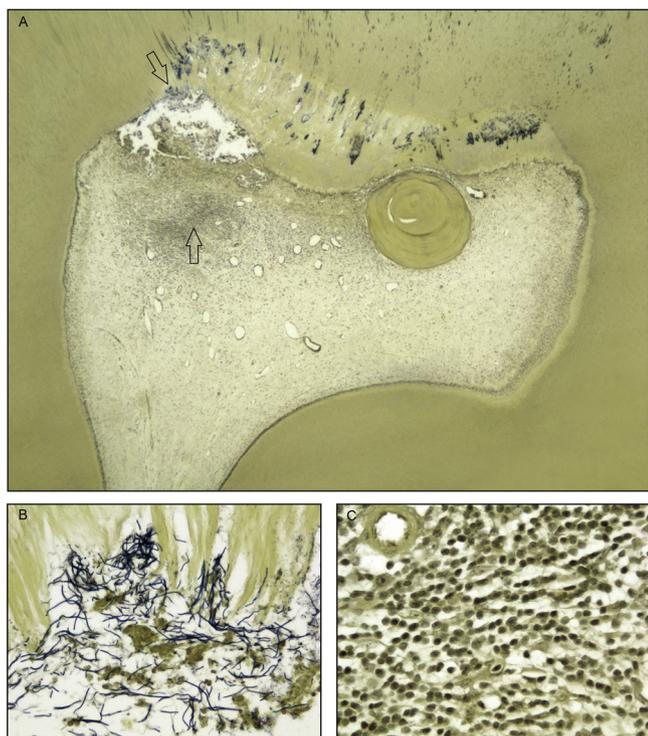
VPT procedure	Cases that were followed-up	Success		Method of evaluation		
		No.	%	Longest observation period	Clinical/ radiographic	Re-entry
Direct pulp capping	507	371	73.2	30 years	507	125
Partial pulpotomy	28	27	96.4	5 years	28	28
Full coronal pulpotomy	9	7	77.8	5 years	9	9



**Fig. 1.** Deep caries in a maxillary third molar of a 45-year-old female. The tooth was extracted for severe extrusion due to the absence of the mandibular third molar. Clinical diagnosis was reversible pulpitis. (A) Sectioning of the tooth to enable proper pulp fixation. Carious dentine was present proximal to the pulp chamber. (B) Overview of the pulp chamber. A large band of tertiary dentine (TD) can be seen subjacent the carious lesion together with scattered free calcifications (haematoxylin and eosin, original magnification  $\times 16$ ). (C) Mid-range magnification of the area of the dentine-pulp interface indicated by the arrow in (B), showing the accumulation of chronic inflammatory cells (original magnification  $\times 100$ , inset  $\times 400$ ). (D) Overview of the carious lesion showing bacteria within the dentinal tubules of the circumpulpal secondary dentine. (Taylor modified Brown & Brenn, original magnification  $\times 16$ , inset  $\times 400$ ). (E) High magnification of the tubular tertiary dentine and the subjacent pulp. Bacteria were absent in the tertiary dentine (original magnification  $\times 50$ , inset  $\times 400$ ).

31.8% for direct pulp capping and 34.5% for partial pulpotomy after 1 year. Although shortcomings were apparent in those follow-up studies, the unsatisfactory outcomes led to the opinion that VPT should be performed only in teeth with open apices [5], because the outcome of

VPT performed on cariously exposed pulps of mature teeth is unpredictable [3,6]. Another view emerging from the literature is that VPT is only successful in teeth with mechanical or traumatic pulp exposure [7,8]. The American Association of Endodontists (AAE) Glossary



**Fig. 2.** Partial necrosis in the pulp chamber of a mandibular third molar with deep caries in a 25-year-old female patient. The clinical diagnosis was reversible pulpitis. (A) Overview of the pulp chamber. A micro-abscess was present in the mesial pulp horn. The large band of tertiary dentine was penetrated by bacterial masses. A pulp stone was present in the distal portion of the pulp chamber (Taylor modified Brown & Brenn, original magnification  $\times 16$ ). (B) High magnification of the area indicated by the upper arrow in (A). Filamentous bacteria penetrated the pulp horn through dentinal tubules (original magnification  $\times 630$ ). (C) High magnification from the area of the inflammatory reaction indicated by the lower arrow in (A), with a heavy concentration of mononuclear inflammatory cells (original magnification  $\times 400$ ).

of Endodontic Terms [9] recommends the use of direct pulp capping only when pulp exposure is produced mechanically (i.e. accidentally) or traumatically.

Vital pulp therapy has been recommended as a treatment procedure in young patients only [10,11] because of the dynamic healing capacity of the dental pulp in young individuals, compared with older persons. Nevertheless, there are no studies to support this recommendation. In a systematic review on VPT in vital permanent teeth with cariously exposed pulps, the success rates of direct pulp capping were found to be highly inconsistent [12]; high success rates were reported in those studies in which only cases with the diagnosis of reversible pulpitis were included [13–16]. In their observational study of direct pulp capping, Bogen et al. [15] followed 49 teeth that were pulp capped with mineral trioxide aggregate for up to 9 years. All the cases were diagnosed with reversible pulpitis. The overall pulpal survival rate, as established by recall radiographs, subjective symptomatology and cold testing, was 98%. To date, there is an overall consensus that only teeth with the diagnosis of reversible pulp inflammation can be successfully managed with indirect or direct pulp capping procedures. The diagnosis of irreversible pulpitis in mature teeth is considered a contraindication for these procedures. These teeth are conventionally treated with nonsurgical root canal therapy [9].

Contrary to this deep-rooted professional dictum, studies published before the 2000s [17,18] and more recent studies have reported successful outcomes when pulpotomy was performed in cariously exposed pulps of vital teeth that were presented with the signs and symptoms of irreversible pulpitis and even apical periodontitis [12,19–26]. A recently published systematic review and meta-analysis indicated that

pulpotomy is a conservative treatment modality for irreversible pulpitis in permanent teeth [27]. The possibility to preserve the vitality of pulps diagnosed with irreversible pulpitis poses a challenge to the contemporary nomenclature of irreversible pulpitis. The term irreversible pulpitis implies that the pulp is irreversibly damaged, for which pulpectomy or tooth extraction is warranted. The favourable results reported in these studies contest the legitimacy of the currently well-accepted criteria for the diagnosis of pulpal health.

In contemporary endodontics, VPT of the adult permanent dentition is considered an ultra-conservative treatment modality [28]. Indirect pulp capping is a procedure in which a material is placed on a thin partition of remaining carious dentine that, if removed, there is a risk of exposing the pulp [9]. The purpose of leaving soft carious dentine is to avoid pulp exposure and to alter the microbial ecology of the caries biofilm, entombing and inactivating the remaining bacteria by virtue of the seal of the restoration, which denies further supply of fermentable sugars from diet to residual bacteria [29]. Direct pulp capping in turn involves the placement of a dental material directly on a mechanically, traumatically [9] or cariously exposed [3,15] vital pulp. Partial pulpotomy (aka Cvek pulpotomy) is the removal of a small portion of the vital coronal pulp as a means of preserving the vitality of the remaining coronal and radicular pulp tissues [9]. Miniature pulpotomy is defined as a procedure that involves gentle/limited removal of the infected dentine chips/damaged pulp tissue at the most superficial part of the pulp. According to the proponents of miniature pulpotomy, this modality produces a clean surgical wound and improves the proximity/interaction of the pulp covering agent to undifferentiated mesenchymal stem cells [30]. Complete pulpotomy (aka coronal pulpotomy or full pulpotomy) is the removal of the coronal portion of a vital pulp as a means of preserving the vitality of the remaining radicular portion [9].

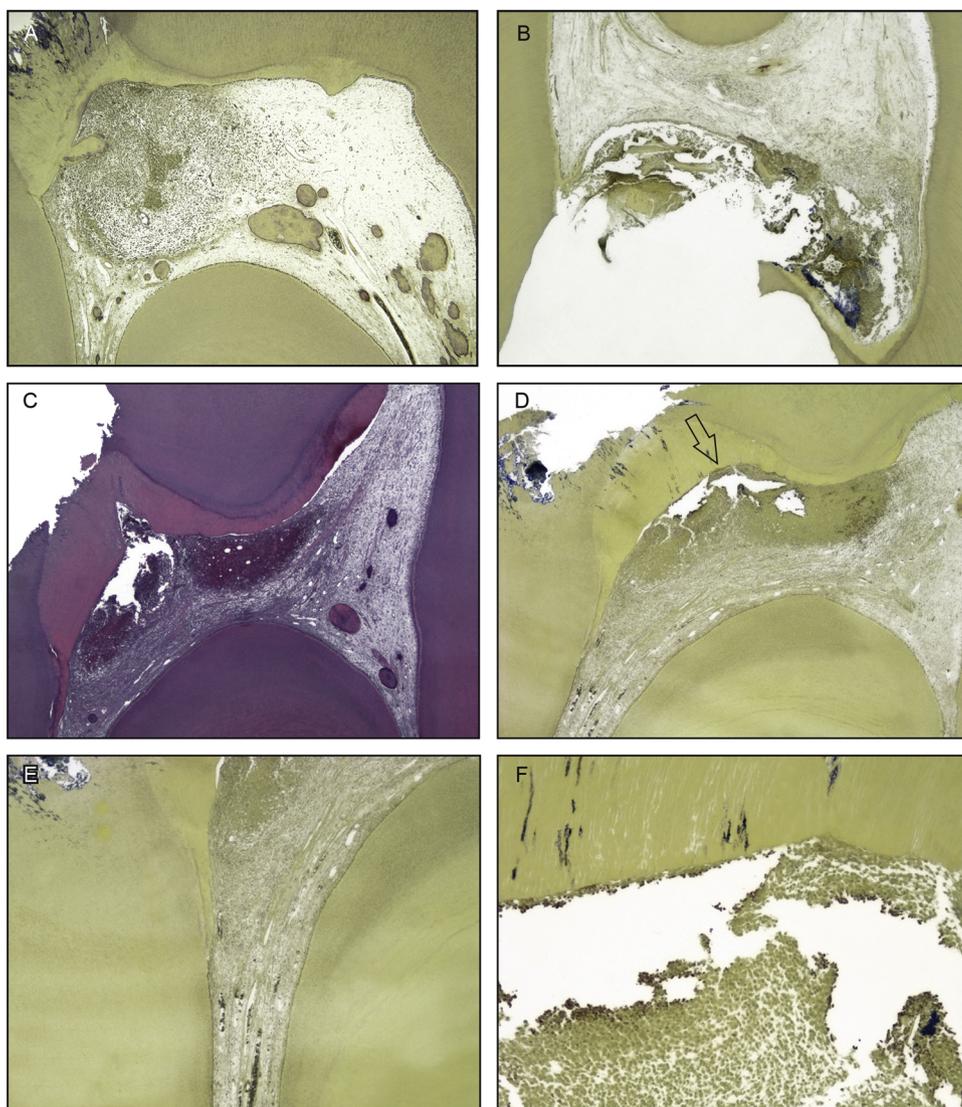
The aforementioned treatment measures are associated with different levels of pulp preservation. However, confusion exists in the literature in terms of the indication and rationale for each VPT treatment modality. In some studies, these treatment procedures were performed interchangeably [25,31], without consideration of the pathological status of the remaining pulp tissue. In other studies, the treatment modality was chosen without consideration of the clinical diagnosis, and without explaining why one procedure was preferred over the others [24,26,32–36]. Consequently, information provided by systematic reviews concerning the outcome of the various VPT procedures [12,37] is inconclusive.

The objectives of the present study were to elucidate the indications for each modality of VPT approach and to present a set of histopathology and histobacteriology-based guidelines, refined by clinical findings from numerous cases, for VPT in teeth with deep caries.

## 2. Materials And Methods

The materials for the present study were derived from the collection of the first author, consisting of 264 human teeth that were extracted and processed for histopathological and histobacteriological examination, and 757 clinical cases that received different VPT procedures following the guidelines proposed in the present article. The longest follow-up period for the clinical cases was 30 years.

Of the 264 teeth analysed histologically, 160 were untreated carious teeth, 59 were heavily restored with amalgam or resin composite restorations, 18 had carious pulpal exposures that were managed by direct pulp capping, 19 were caries-free teeth that received experimental pulpotomy or capping with different biomaterials, and 8 were carious teeth that received “partial” or “selective” caries excavation followed by adhesive restorations. The teeth were extracted for reasons that were neither related to this study nor to failure of the adopted treatment. Reasons included prosthetic or orthodontic treatment planning, or because of the patient’s desire in not maintaining the tooth. Sectioning was performed on a mesiodistal or buccolingual plane until at least one pulp horn was exposed. The specimens were fixed in formaldehyde and



**Fig. 3.** Different extents of partial necrosis in the pulp chamber. (A) Mandibular second molar. The mesial half of the pulp was necrotic (Taylor modified Brown & Brenn, original magnification  $\times 16$ ). (B) Maxillary second premolar. The superficial portion of the pulp was necrotic, separated from the underlying vital pulp tissue by a clear demarcation line (original magnification  $\times 16$ ). (C) Mandibular first molar. Half of the pulp chamber was necrotic with an abscess (haematoxylin and eosin, original magnification  $\times 16$ ). (D) Section taken at distance from that in (C) (original magnification  $\times 16$ ). (E) Detail of the distal root canal orifice in (D). The coronal pocket of necrotic tissue faded into vital tissue (original magnification  $\times 25$ ). (F) Middle magnification of the area indicated by the arrow in (D). A micro-abscess was present. Bacteria could be found in the dentinal tubules and in the necrotic pulp (original magnification  $\times 100$ ).

demineralised in formic acid/sodium citrate solution for 4 weeks. The demineralised specimens were washed in running water for 1-2 days, dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. Serial sections were prepared and stained with haematoxylin-eosin or with the Taylor modification of the Brown and Brenn technique for histological identification of bacteria.

The clinical cases included 757 teeth with deep caries in which a pulp exposure was identified during “non-selective” caries excavation. Of these teeth, 720 were treated with direct pulp capping and 507 were followed-up for varying observation periods, ranging from a minimum of three years to a maximum of 30 years. Only cases clinically diagnosed with “reversible pulpitis”, based on clinical and radiographic findings, were subjected to direct pulp capping procedures. In none of the 507 direct pulp capping procedures was the so-called selective (partial) caries removal performed, either in a one-step or two-step manner, wherein bacteria-infected soft dentine can be left behind in a cavity preparation. The size of pulp exposure was recorded, as well as the time needed to obtain haemostasis by applying a moderate pressure on the wound with a sterile cotton pellet. In large exposures ( $> 1 \text{ mm}^2$ ), chemically pure calcium hydroxide powder was deposited on the pulp wound with a Messing gun and adapted with a gentle pressure with a sterile cotton pellet. The powder was covered with Dycal (Dentsply De Trey GmbH, Konstanz, Germany), extended to the dentine surrounding the exposure. In small exposures ( $< 1 \text{ mm}^2$ ), the pulp wound was

directly covered by Dycal. In a minority of cases ( $n = 35$ ), the wound was covered with a tricalcium silicate-based cement (Tech Bio Sealer Capping, Isasan, Rovello Porro, CO, Italy). Thirty-seven teeth were treated with pulpotomy and available for follow-up: 9 were full coronal pulpotomy and the other 28 were partial pulpotomy. Pulp wounds were capped with calcium hydroxide or tricalcium silicate cements. The follow-up ranged from 2 to 5 years. The cases were evaluated as success/failure based on clinical signs and symptoms, radiographic findings showing presence/absence of periapical disturbances, and resorption or irregular calcifications in the root canals.

Each clinical case was documented with pre-operative radiographs, as well as with pre-operative, intra-operative and post-operative digital photographs taken with a 105 mm objective (AF-S Micro Nikkor 105 mm 1:2.8 G ED VR, Nikon, Tokyo, Japan). Close-up photographs of the deepest portion of the excavated carious cavity, the exposed pulp or the pulp wound following amputation were taken with a 2x teleconverter (Nikon AF-S TC – 2E III) mounted between the camera body and the objective. All procedures were performed under magnification using either a magnifying device (EyeMag Pro 4x, Carl Zeiss Meditec Dentistry, Oberkochen, Germany) or an operating microscope (S7 / OPMI PROergo, Carl Zeiss Meditec AG, Germany). Photographs were also taken over the last few years with a video camera mounted on the operating microscope (Sony Handycam FDR-AX33, Sony Corporation, Tokyo, Japan). Patients were periodically recalled at designated time



**Fig. 4.** (A) Deep caries in a mandibular second molar of a 35-year-old male patient. The clinical diagnosis was reversible pulpitis. (B) Clinical view of the cavity after caries excavation. (C) View under microscope. The pulp can be seen through a thin layer of hard dentine, without exposure, surrounded by sound dentine.

intervals. At each follow-up, radiographs were taken and pulp vitality was determined with thermal and electric pulp tests. With the patient's informed consent, the filled restorations were re-accessed under strict asepsis in selected cases during a destined observation period. Mineralised tissues formed in the area of the previous exposure were photographed.

### 3. Results

#### 3.1. Histopathological, histobacteriological and clinical findings

Table 1 includes information on the histopathological diagnosis of the 264 teeth extracted for histological examination, and the location of bacteria within the dentine and dental pulp of each tooth after Brown and Brenn staining. Table 2 contains descriptive statistics on the success rates of clinical cases that received a VPT procedure. Direct pulp capping was successful in 371/507 (73.2%) of the cases. Partial pulpotomy was successful in 27/28 (96.4%) cases, while full pulpotomy succeeded in 7/9 (77.8%) of the cases.

Among the 160 untreated carious teeth, a localised inflammatory response could be identified in the subjacent pulp as soon as the enamel was penetrated. Bacteria were seen invading the peripheral end of dentinal tubules. With the advancement of the carious process, there were increases in the extent of inflammatory cell accumulation, as well as other changes such as tertiary dentine deposition and reduction in the number of odontoblasts in the dental pulp (Fig. 1). However, areas

of necrosis were not apparent until bacteria reached the pulp space.

The localised inflammatory response to caries approximating the pulp space was classified histologically as “reversible” if necrosis and bacterial colonisation had not occurred in the pulp. Bacteria from the caries biofilm were often present extensively in the demineralised dentine, penetrating to considerable depths, sometimes involving the tubular tertiary dentine. However, bacteria were generally absent in the subjacent pulp tissue (Fig. 1 D-E).

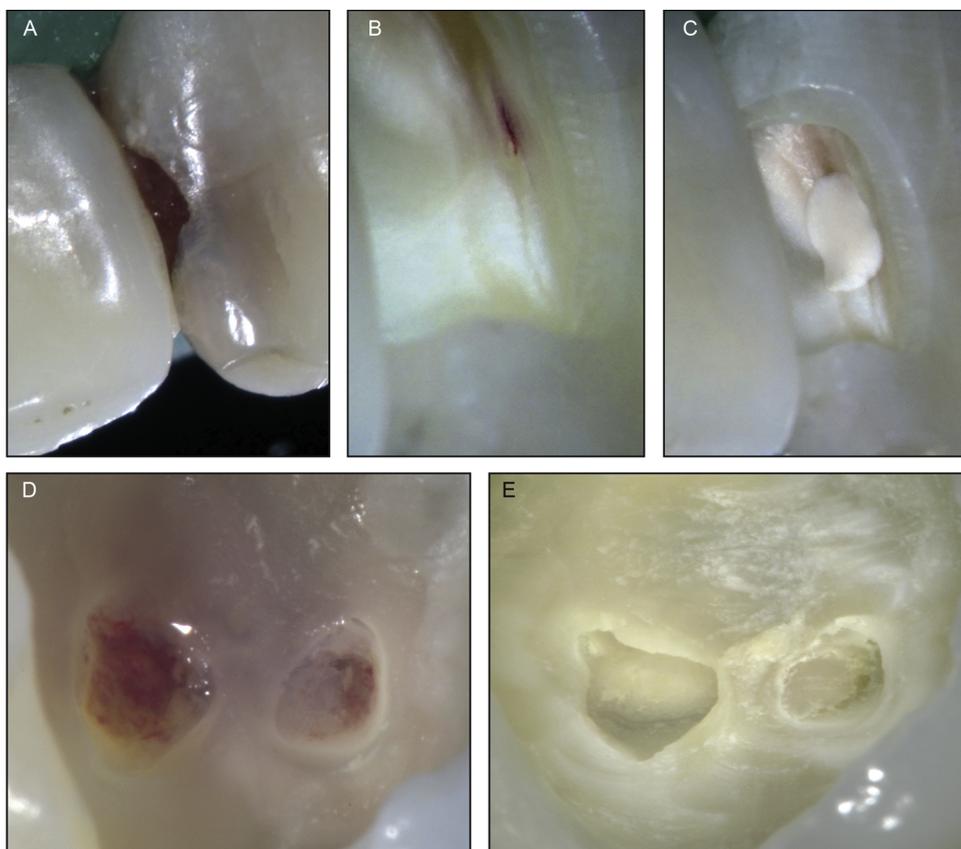
Caries progression into the dental pulp was initiated by the development of an initial area of necrosis, albeit minuscule in dimension, but consistently colonised by bacteria, in the pulp horn that was closest to the site of caries progression. From a histological standpoint, the pulp inflammation in these cases was considered “irreversible” [38,39] (Fig. 2). This condition was typically seen in histologic sections as an area of coagulation or liquefaction necrosis. The latter appeared as an apparently empty space, where the pus was washed away by histological reagents, a condition known as micro-abscess (Fig. 2A). Heavy bacterial infection was seen in the deepest part of the secondary dentine, in the residual tubules, in the tubular tertiary dentine, and along the margins of the perforation and the micro-abscess. In the absence of a frank perforation, bacteria were observed reaching the pulp through the dentinal tubules of the residual dentine (Fig. 2B). Accumulation of polymorphonuclear leucocytes (neutrophils) was present around the necrotic area. The pulp tissue surrounding the necrotic zone was infiltrated by chronic mononuclear inflammatory cells including macrophages and lymphocytes (Fig. 2C). More peripherally, however, the pulp tissue was apparently normal, uninfamed (Fig. 2A). As caries progressed deeper, micro-abscess were expanded to include increasing amounts of the pulp chamber tissue (Fig. 3). The necrotic/infected tissue was well demarcated by a halo of acute/chronic inflammatory cells, also fading into healthy uninfamed pulp tissue. Clinically, these pathologic changes could occur in the total absence of pain.

A consistent observation in the 59 teeth that had extensive amalgam or composite restorations is that pulp inflammation would eventually subside if the softened and infected dentine was completely excavated and the cavity restored with an adequate (fluid- and bacteria-tight) restoration. The only histological changes that remained permanently in the pulp were reduction in the number of odontoblasts, varying amounts of tertiary dentine, and segregated calcifications within the dental pulp.

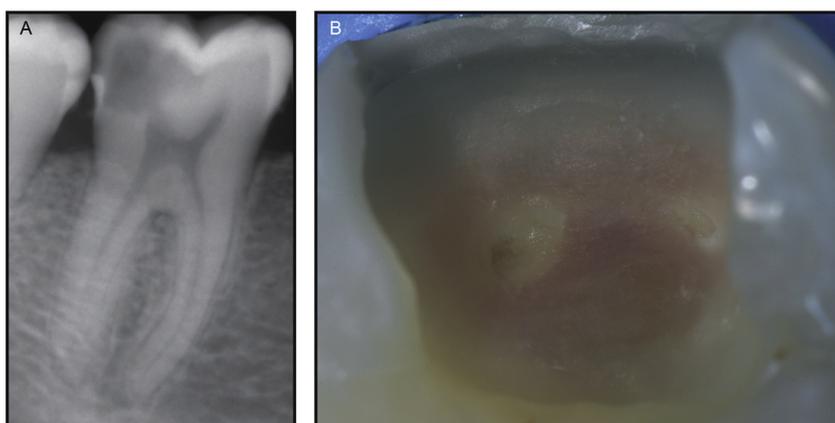
Of the 18 teeth processed histologically at varying times after receiving direct pulp capping, 12 had been clinically assessed as successful. They showed uninfamed pulp tissue and a mineralised barrier filling the previous exposure. This tissue was atubular and irregular, with tunnels containing necrotic tissue, and lined on the pulpal side by fibroblasts. In five cases scattered dentine spicules were observed, embedded in the newly formed tissue. No bacteria were observed in this mineralised tissue and on the cavity floor. One tooth, clinically assessed as success and extracted 15 month after pulp capping for orthodontic reasons, showed a moderate accumulation of chronic inflammatory cells in the pulp subjacent the mineralised bridge, and small numbers of bacteria on the portions of the cavity floor adjacent to the exposure. The remaining teeth, clinically assessed as failures, exhibited partial necrosis and bacterial colonization in the exposed pulp horns. Bacteria were also observed in the lacunae of the newly formed mineralised tissue, in the tubules of the dentine surrounding the exposure, and on the cavity floor (Table 1).

Twelve of the 19 intact teeth receiving experimental pulpotomy were considered histologically healed, with uninfamed pulps and irregular mineralised tissue repairing the exposure. Bacteria leaking through the margins of coronal restorations were found to be the cause of reversible pulpitis in two cases and of irreversible pulpitis with partial necrosis in five cases (Table 1).

The eight carious teeth treated with “partial” excavation to firm dentine and restored with adhesive techniques, all showed severe infection of the secondary dentine, with bacteria invading the tertiary



**Fig. 5.** (A) Interproximal caries lesion in a maxillary central incisor. Palatal view. The clinical diagnosis is reversible pulpitis. (B) Following thorough caries excavation, a tiny exposure of the pulp horn was identified. The surrounding dentine was hard and clean. (C) Direct pulp capping was performed. A calcium hydroxide lining was placed over the exposed pulp and the surrounding dentine. The cavity was subsequently restored with resin composite. (D) Large pulp exposure of the mesial horns of a mandibular first molar with deep caries. The clinical diagnosis was reversible pulpitis. Direct pulp capping with Biodentine, a tricalcium silicate-based restorative material, was performed. (E) Re-entrance into the cavity after 3 months revealed mineralised tissue barriers “repairing” both exposures. The tooth responded normally to pulp tests.



**Fig. 6.** 24-year-old male patient seeking treatment for severe spontaneous pain originating in the right mandible. The clinical diagnosis was irreversible pulpitis. (A) Periapical radiograph shows that the caries lesion is close to the distal pulp horn. (B) After meticulous caries excavation, two exposures appeared in the area of the distal pulp horn, containing yellowish avascular tissue that was indicative of partial necrosis. The surrounding pulp tissue that was visualised through a thin layer of remaining dentine had a reddish hue, which was indicative of hyperaemia. The tooth was treated with partial pulpotomy.

dentine in two cases (Table 1). Inflammatory changes of varying degree were observed in all cases. It is noteworthy that all cases were asymptomatic at the moment of extraction, and responded normally to pulp sensibility tests.

### 3.2. Recommendations and guidelines

In cases diagnosed clinically with “reversible pulpitis”, according to agreed criteria [38,39], two scenarios were encountered at the end of caries excavation. In the first scenario, there was no pulp exposure and hard sound dentine, albeit discoloured, could be identified in the deepest part of the cavity. The discoloured dentine could not be removed with sharp hand excavators from the base of those cavities. In those cases, cavities were restored by placing a calcium hydroxide-based liner to cover the thin layer of remaining dentine where the underlying pulp was showing through (Fig. 4).

In the second scenario, the pulp was exposed to a variable extent

(Fig. 5), ranging from a tiny spot over a single pulp horn (Fig. 5B), to large areas involving one or more pulp horns (Fig. 5D). Based on these findings, careful observation with an operating microscope was undertaken to analyse the status of the involved tissues and guide the subsequent decision-making process. A direct pulp capping procedure was indicated when the four following conditions were met: 1) the dentine surrounding the exposure was sound; 2) red, homogeneous and blood filled tissue was observed on the surface of the pulp wound, with no yellowish liquefied areas or dark non-bleeding zones; 3) no dentine chips, displaced during excavation, were present on the wound; and 4) haemostasis could be achieved within 2-3 minutes after rinsing with a mild disinfectant such as chlorhexidine or 1% sodium hypochlorite, followed by the application of a sterile cotton pellet on the surgical wound. The pulp wound was then covered with a biocompatible and possibly bioactive restorative material, extended to the dentine surrounding the exposure, and the cavity restored appropriately. Chemically pure calcium hydroxide, calcium hydroxide-containing bases,



**Fig. 7.** Contraindications for direct pulp capping. (A) Pulp tissue is dark and avascular. (B) Superficial pulp tissue at the exposure site is deconstructed and avascular. Surrounding dentine is soft. (C) Close-up of the major exposure in (B). (D) Exposed pulp tissue is avascular. (E) Exposed pulp is pale and deconstructed, surrounded by reddish pulp tissue. (F) A dentine chip displaced into avascular exposed pulp tissue. (G) Several dentine fragments displaced into a barely vascular pulp. The surrounding dentine is soft. Partial pulpotomy was performed. (H) Appearance of the case in (G) after removal of the soft dentine and partial pulpotomy. The surgical wound appeared clean after haemostasis.

mineral trioxide aggregate (MTA) or tricalcium-silicate cements were used as pulp-capping materials. In the followed-up clinical cases with favourable outcome, the capped exposure site was repaired with a mineralised tissue filling in the previous exposure within 3-6 months. Such a feature was evident when a cavity was re-accessed upon the subject's approval (Fig. 5E; Table 2).

Redness of the exposed pulp tissue that was evident through a thin layer of remaining dentine is indicative of hyperaemia, an indirect sign of inflammation (Figs. 6–7). It was not uncommon to observe hyperaemia surrounding necrotic areas that were deprived of circulation (Figs. 6B; 7 E). A dark colour of the cavity floor and of the exposed pulp horns, in the absence of bleeding, is also indicative of necrosis (Fig. 7A). The pulp tissue in the exposed pulp horns might contain alternating avascular and vascular tissues (Figs. 7 B-C). The exposed pulp horns sometimes exhibited a yellowish, pale hue, which is indicative of the absence of blood circulation and necrosis (Figs. 6B, 7D-E). In some instances, pus was evident in the exposed pulp horn. Dentine spicules could be observed through the exposure, displaced into the vascular or avascular pulp tissues during excavation (Fig. 7 F-G). In all these circumstances, the diagnosis of partial pulpal necrosis was made and considered as contraindication for direct pulp capping.

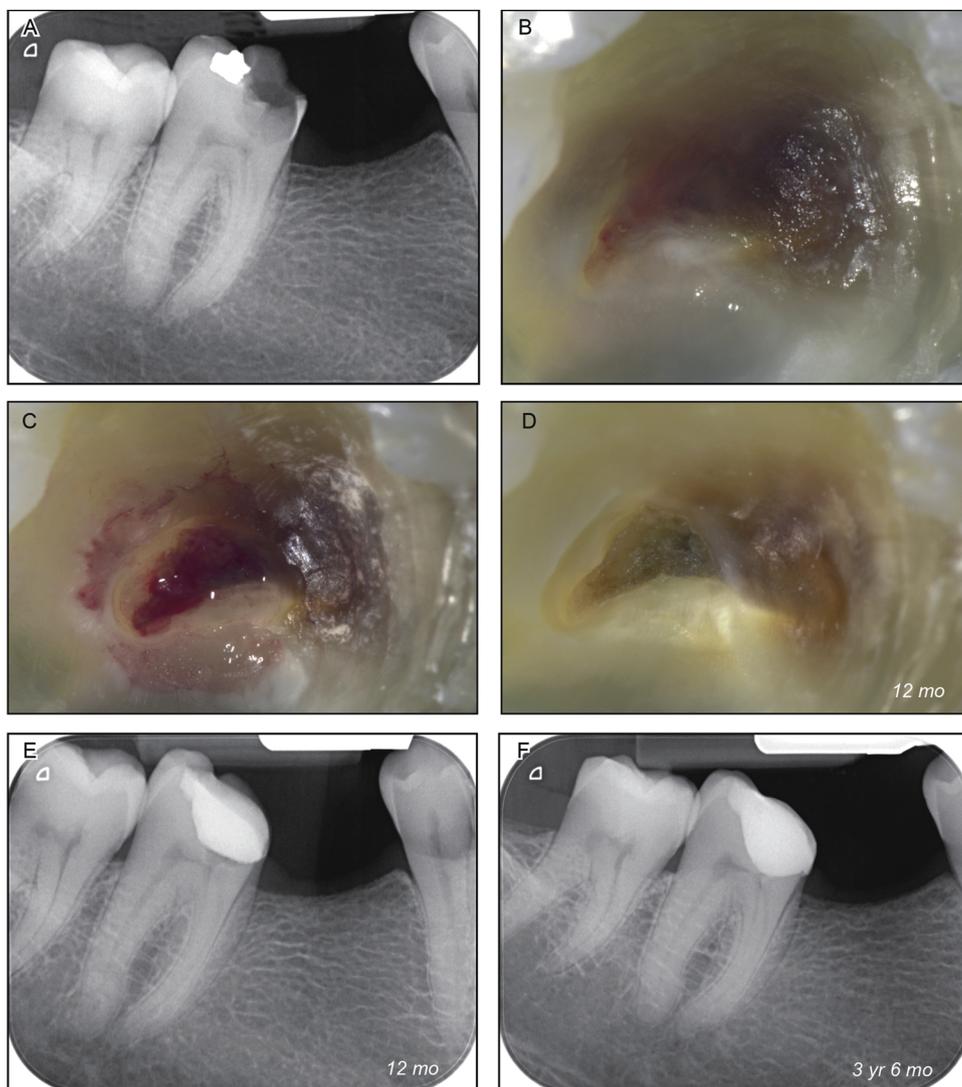
In those cases, part of the affected pulp tissue were removed until the wound lied on apparently sound tissue. Pulpotomy was performed as alternative to pulpectomy and root canal treatment (Fig. 8). The choice of partial or full pulpotomy was based on the location in the pulp chamber in which healthy bleeding pulp tissue could be identified and that haemostasis could be easily attained. Pulpotomy was performed using a round or conical high-speed diamond bur under ambient water spray. The perforation was enlarged, the adjacent soft dentine was

removed and a superficial layer of the pulp was gently shaved off. The resulting wound was rinsed with an antiseptic solution and a sterile cotton pellet was applied for haemostasis. The surgical site was then carefully examined under high magnification. If healthy pulpal tissue was not encountered or when haemostasis could not be achieved within a reasonable time, a successive layer of pulp tissue was removed and the surgical wound placed more apically. The procedure was repeated until the optimal features of a “healthy” wound were observed. In molar teeth, a full chamber pulpotomy sometimes had to be performed to satisfy the aforementioned conditions, depending on the extent of degeneration of the pulp chamber. Pulpectomy or root canal treatment was indicated in the event that those optimal conditions were not encountered in the surgical wounds of both multi-rooted or single-rooted teeth.

Root canal treatment was sometimes required even in cases that the preliminary clinical diagnosis was “reversible pulpitis”. In the selection of VPT vs root canal treatment, it was the clinical intraoperative observation under an operating microscope that ultimately dictated the operative procedures that were undertaken, and not the preoperative clinical diagnosis. At the same time, it was the direct clinical observation of the surgical site that determined the invasiveness of the pulpotomy. Large portions of the pulp chamber roof and of the pulp tissue might have to be removed. Nevertheless, the extent of the exposure should not constitute a contraindication for the selected pulpotomy procedure (Fig. 9).

#### 4. Discussion

From a histological perspective, the clinical management of pulpitis



**Fig. 8.** A 62-year old male patient seeking treatment for severe spontaneous pain caused by his mandibular second molar. (A) Radiograph showing deep recurrent caries proximal to the mesial pulp horns. (B) Appearance of the cavity after excavation. (C) Pulp wound after pulpotomy and haemostasis. The pulp was capped with calcium hydroxide. (D) The cavity was re-entered after 12 months. The tooth responded normally to vitality tests. Mineralised tissue was present over the area of previous pulpotomy. (E) Radiograph taken at the 12-months follow-up, prior to re-opening. (F) The newly formed hard tissue was covered with a base and the cavity restored with resin composite. A radiograph taken after 3 year and 6 months showed normal periapical conditions. The tooth responded positively to pulp vitality tests.

should be based on prognosis of treatment. That is, the pulp may be preserved in its entirety if it is reversibly inflamed, i.e., with no areas of necrosis, signs of micro-abscesses and foci of infection (Figs. 1,4,5). On the contrary, if the pulp is irreversibly inflamed (Figs. 2–3), it has to be partially or totally resected for improved treatment outcome [38,39] (Figs. 6–9). It is important to highlight that determination of the extent of pulpal degeneration based on clinical signs and symptoms has never been demonstrated [40]. Accordingly, attempts to establish a precise treatment plan based on the clinical signs and symptoms [41] remain questionable. Therefore, the authors propose that the final decision for selecting the most appropriate treatment to be performed in each case be taken after examining the deepest part of dentine and the exposed pulp tissue after non-selective total caries removal. This is based on the common observation that the extent of the degeneration of the exposed pulp varies from case to case and the most apical area of tissue is usually uninfamed and amenable to preservation. Therefore, based on these histopathological and histobacteriological findings, one may assume that the terms “reversible” and “irreversible” pulpitis actually refer to the conditions of part and not the entire pulp tissue. If the clinician can infer the conditions of the pulp tissue by direct examination, more predictable treatment may be provided.

The rationale behind the partial pulpotomy protocol is the selective removal of the diseased pulp segments where necrosis and bacterial colonisation have taken place, as well as the removal of the soft, potentially infected dentine around the exposure and dentine spicules

with tubules that may harbour bacteria. The ultimate goal is to obtain a clean surgical wound in non-infected pulp connective tissue that is surrounded by mineralised non-infected dentine. If these objectives are achieved, the new environment is expected to be conducive to healing. That is, a reparative process is triggered with the production of mineralised tissues filling the area of the previous exposure (Figs. 5 D-E; 8 C-D; 9 D, F, H). Inflammation is part of the repair process, and it sets the stage for healing to occur. It has to be stressed that the favourable conditions can only be maintained if a fluid and bacteria-tight seal is provided by the overlying restoration or crown. This will prevent bacterial leakage and re-colonization of the surgical wound.

The clinical appearance of the exposed pulp has not been used as a parameter for indication of VPT. Several cases are capped only because the pulp is exposed and sometimes bleeding, without considering the clinical aspects that are taken into consideration in this article. For instance, Fig. 10 shows an example of pulp capping failure. That pulp should not have been directly capped, according to the criteria proposed herein, because the aspect of the exposed tissue should have advised against this procedure. Instead, partial pulpotomy had more chances to result in success in cases like that. At that time, decision was made for pulp capping, the treatment failed, the patient did not accept root canal treatment and the tooth was extracted. Histobacteriological analysis demonstrated bacterial infection in the pulp horn and in the dentine surrounding the exposure (Fig. 10 E-F).

The guidelines proposed in this article were the result of a learning



**Fig. 9.** A 15-year old boy referred for root canal treatment of a mandibular first molar with the diagnosis of irreversible pulpitis. (A) Radiograph showing extensive occlusal caries reaching the mesial pulp horns. (B) Occlusal aspect of the carious lesion after rubber dam isolation. (C) All pulp horns were exposed after caries excavation, with dentine chips displaced into the vascular pulp. Partial pulpotomy was performed. (D) Occlusal view of the cavity after partial pulpotomy and haemostasis. (E) The wound was capped with chemically pure calcium hydroxide powder and the cavity restored temporarily. (F) Re-accessing the cavity after 3 months revealed the formation of a hard tissue barrier that filled the pulpotomised site. (G) Radiograph taken at the 3 months follow-up, prior to re-opening. (H) Close-up of the newly formed calcified barrier.

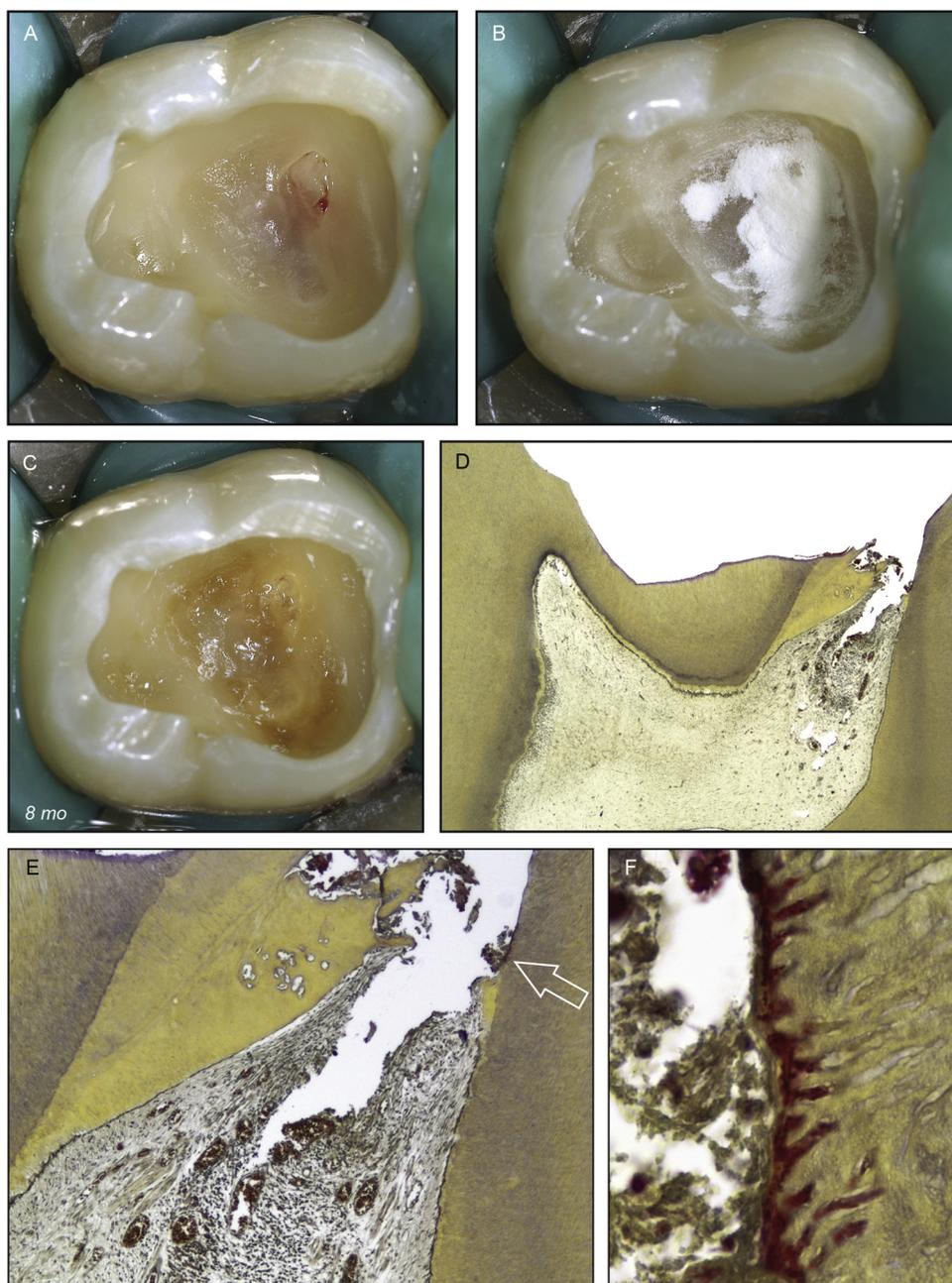
and refinement process over the years based on histological, histobacteriological and clinical observations. A flow chart encompassing the clinical procedures to be adopted for VPT is presented (Fig. 11). They mostly rely on direct observation of dentine and the exposed pulp tissue under deep caries. Inspection of these tissues has been substantially improved with the increasing use of magnification by dental professionals. Although loupes under abundant illumination may be used, the operating microscope is expected to provide a more refined and detailed inspection of the affected tissues. The rationale of this histopathology and histobacteriology-based treatment protocol is to estimate the localisation of the advancing front of infection, both in the dentine and in the subjacent pulp. No pulp healing is expected to occur in the presence of infection, and the goal is elimination of the infected tissues, as well as protection of the uninfected pulp wound with biocompatible and potentially bioactive dental materials.

Removal of infected tissues should be performed progressively. In the deepest part of the cavity, no burs should be used for excavation, only sharp hand excavators. If pulp exposure occurs, one may assume that it is caused by the carious process itself and not “accidental”. Direct pulp capping should only be performed if the surrounding dentine is sound and the exposed pulp does not exhibit suggestive signs of infection. If these conditions are not met, progressively more invasive procedures are indicated, until the clinical appearance indicates absence of infection in the dentine and pulpal wound. This procedure will allow the clinician to distinguish cases where the pulpotomy procedure may be indicated from those in which tissue degradation has advanced to the root canal orifices or beyond. For advanced infection cases,

pulpectomy represents the only treatment option.

A poor correlation between clinical and histologic data has long been reported [40,42,43]. Consequently, the profession has over the years accepted the notion that it is not possible to classify accurately the pulp condition of painful teeth, or to differentiate clearly between pulps that can be saved without definitive root canal treatment. Those previous studies had limitations in the histologic procedures used at that time, including the absence of serial sectioning and bacterial staining. In addition, the degree and depth of infection in the pulp tissue cannot be ascertained only with haematoxylin and eosin staining alone. Using defined criteria for clinical and histologic classification of pulp conditions and strict laboratory methodologies, a recent study found a good correlation between clinical and histologic diagnosis of pulp conditions [39]. The clinical diagnosis of normal pulp/reversible pulpitis matched the histologic diagnosis in almost 97% of the teeth, while matching of the clinical and histologic diagnoses of irreversible pulpitis occurred in 84% of the cases [39]. In that study, the clinical diagnosis of reversibility/irreversibility of pulp inflammation was correlated exclusively to the presence/absence of bacterial penetration; no correlation was demonstrated between clinical symptoms and the extent of pulp degeneration [39].

The literature is contentious with respect to the level in which VPT should end. Removal of inflamed tissue to the level of healthy uninfamed pulp is generally considered critical for healing [12,31,44–46]. Visual observation of the exposed pulp under magnification has not been recommended until recently for evaluating the extent of inflammation within the dental pulp. Matsuo et al. [13] suggested that



**Fig. 10.** A 32-year old man sought treatment for severe pain to chewing and cold stimuli in the right mandible. A radiograph showed that the mandibular first molar had a deep occlusal caries lesion, proximal to the mesial pulp horns. (A) After anaesthesia, rubber dam isolation, and excavation, exposure of the mesio-lingual pulp horn was visible. (B) Attention was not paid to the partly avascular exposed pulp tissue, and pulp capping with chemically pure calcium hydroxide powder was accomplished. The powder was covered with Dycal and the cavity restored with IRM. (C) The patient was asked to return after 4 months, but he returned only after 7 months because of severe spontaneous pain. The cavity was re-accessed and a probe could penetrate into the previous defect, which did not appear to have been repaired. The patient did not accept any treatment aimed at the conservation of the tooth and requested extraction. (D) A section cut through the previous exposure shows that a portion of the mesial pulp tissue chamber is unstructured, while the remainder exhibits normal appearance (Taylor modified Brown & Brenn, original magnification  $\times 16$ ). (E) Detail of the mesial pulp horn in (D) (original magnification  $\times 100$ ). (F) High power view of the area indicated by the arrow in (E) showing bacteria colonising tubules of the dentine surrounding the exposure (original magnification  $\times 630$ ).

profuse pulp bleeding that is difficult to arrest is indicative of advanced pulpal inflammation. If the bleeding cannot be stopped within a reasonable time, the treatment procedure should be modified, shifting from partial pulpotomy to full pulpotomy, or from full pulpotomy to pulpectomy.

The deep-rooted concept of leaving soft infected dentine in the deepest part of the cavity for avoiding pulpal exposure [47] is to be challenged from a biological perspective. The concept has many proponents, and the fear of pulpal exposure is the recurring theme [29,48–50]. However, there are not microbiological and histopathological data to support this approach. The “selective” caries removal approach, which recommends removal to soft or firm dentine, is based on the assumption that pulp exposure is a negative prognostic factor in teeth with deep carious lesions [4,51–53]. It is beyond argument that a carious process is involved if an exposure is encountered during hand excavation. However, leaving soft carious dentine over the pulp is analogous to leaving bacteria or allowing bacteria to colonise the vicinity of a surgical wound [54], which may sustain inflammation and

lead to necrosis. Although caries is expected to be arrested if the bacterial access to fermentable sugars in diet is denied, the microbiota in the deepest zones of affected dentine is dominated by asaccharolytic anaerobic bacteria that may derive nutrients in the form of proteins and glycoproteins from the demineralised collagen of dentine and from the pulp tissue fluids that infiltrate the dentinal tubules [55–59]. There is a potential for residual bacteria to maintain pulp injury and inflammation. In addition, partial caries excavation precludes the possibility of clinically evaluating the true extent of the carious process, the appearance of the exposed pulp tissue, the presence of necrosis, and the amount of pulp bleeding.

Similar to other parts of the body [60,61], inflammation is part of the wound healing process in the dental pulp [62]. Inflammation caused by bacterial infection cannot resolve if bacteria are not eliminated [63]. Macrophages are one of the first lines of the body's innate defence against bacteria; their mode of activation will determine the success or failure of the host's response to pathogen aggression. These mononuclear cells exist as the pro-inflammatory (M1) phenotype and

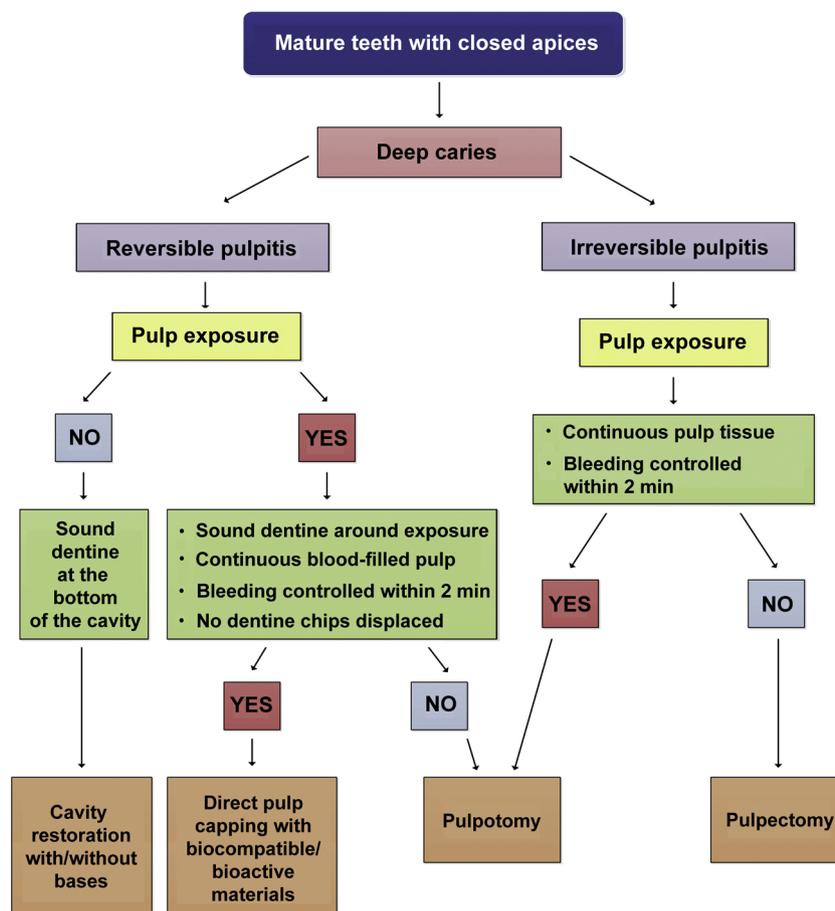


Fig. 11. Flow chart depicting the guidelines for vital pulp therapy as an alternative approach in the treatment of deep caries.

the anti-inflammatory (M2) phenotype. During the early stage of infection, macrophages are predominantly polarised toward the M1 phenotype. The M1 macrophages possess bactericidal activity, induce an inflammatory response and release pro-inflammatory cytokines and chemokines that contribute to killing of the invading bacteria and resolution of inflammation. Such responses, however, must be controlled to prevent excessive tissue damage. Hence, M2-polarised macrophages are activated to produce anti-inflammatory mediators that regulate the M1 macrophages, suppress inflammation and promote tissue repair, remodelling and vasculogenesis [64]. Failure in eliminating bacteria from the infected part of the dental pulp is likely to result in sustaining of the M1 macrophage phenotype or reverting of the M2 phenotype to the M1 phenotype, both of which intensify the pro-inflammatory responses and prevent wound healing [65]. Although the concept of leaving infected dentine in carious teeth is supported by randomised clinical trials, one of which was conducted with patient follow-up for 10 years [66], this conservative method of caries management does not appear to be biologically robust to justify its adoption. Moreover, no study has consistently examined the pulp tissue reaction to this procedure and determined if histological success ensues.

Dentine barrier formation only occurs when pulp inflammation and infection are minimised to enable reinstatement of tissue homeostasis and pulpal health [67]. That said, VPT procedures must be based on stringent aseptic techniques. Prophylaxis, rubber dam isolation, field disinfection and the use of sterile instruments after caries removal are mandatory measures for these procedures to be successful.

#### Declaration of interests

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

The authors report no conflict of interest associated with the present work.

#### References

- [1] Y.L. Ng, V. Mann, S. Rahbaran, J. Lewsey, K. Gulabivala, Outcome of primary root canal treatment: systematic review of the literature - part 1. Effects of study characteristics on probability of success, *Int. Endod. J.* 40 (2007) 921–939.
- [2] D. Ricucci, J. Russo, M. Rutberg, J.A. Burleson, L.S. Spångberg, A prospective cohort study of endodontic treatments of 1,369 root canals: results after 5 years, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 112 (2011) 825–842.
- [3] C.R. Barthel, B. Rosenkranz, A. Leuenberg, J.F. Roulet, Pulp capping of carious exposures: treatment outcome after 5 and 10 years: a retrospective study, *J. Endod.* 26 (2000) 525–528.
- [4] L. Bjørndal, C. Reit, G. Bruun, M. Markvart, M. Kjaeldgaard, P. Näsman, et al., Treatment of deep caries lesions in adults: randomized clinical trials comparing stepwise vs. direct complete excavation, and direct pulp capping vs. partial pulpotomy, *Eur. J. Oral Sci.* 118 (2010) 290–297.
- [5] American Academy of Pediatric Dentistry (AAPD), Guideline on pulp therapy for primary and immature permanent teeth, *Clin. Prac. Guid.* 37 (2014) 244–252.
- [6] M. Cvek, A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture, *J. Endod.* 4 (1978) 232–237.
- [7] E.J. Swift Jr, M. Trope, A.V. Ritter, Vital pulp therapy for the mature tooth – can it work? *Endod. Topics* 5 (2003) 49–56.
- [8] M. Trope, Regenerative potential of dental pulp, *Pediatr. Dent.* 30 (2008) 206–210.
- [9] American Association of Endodontists (AAE), Glossary of Endodontic Terms, ninth ed., (2015) (accessed 3 January 2019), <https://www.aae.org/specialty/clinical-resources/glossary-endodontic-terms/>.
- [10] P. Hørsted, B. Sandergaard, A. Thylstrup, K. El Attar, O. Fejerskov, A retrospective study of direct pulp capping with calcium hydroxide compounds, *Endod. Dent. Traumatol.* 1 (1985) 29–34.

- [11] J. Ward, Vital pulp therapy in cariously exposed permanent teeth and its limitations, *Aust. Endod. J.* 28 (2002) 29–37.
- [12] P. Aguilar, P. Linsuwanont, Vital pulp therapy in vital permanent teeth with cariously exposed pulp: a systematic review, *J. Endod.* 37 (2011) 581–587.
- [13] T. Matsuo, T. Nakanishi, H. Shimizu, S. Ebisu, A clinical study of direct pulp capping applied to carious-exposed pulps, *J. Endod.* 22 (1996) 551–556.
- [14] N. Farsi, N. Alamoudi, K. Balto, A. Al Mushayt, Clinical assessment of mineral trioxide aggregate (MTA) as direct pulp capping in young permanent teeth, *J. Clin. Pediatr. Dent.* 31 (2006) 72–76.
- [15] G. Bogen, J.S. Kim, L.K. Bakland, Direct pulp capping with mineral trioxide aggregate: an observational study, *J. Am. Dent. Assoc.* 139 (2008) 305–315.
- [16] J. Mente, B. Geletneky, M. Ohle, M.J. Koch, P.G. Friedrich Ding, D. Wolff, et al., Mineral trioxide aggregate or calcium hydroxide direct pulp capping: an analysis of the clinical treatment outcome, *J. Endod.* 36 (2010) 806–813.
- [17] U. Zilberman, E. Mass, H. Sarnat, Partial pulpotomy in carious permanent molars, *Am. J. Dent.* 2 (1989) 147–150.
- [18] I. Mejäre, M. Cvek, Partial pulpotomy in young permanent teeth with deep carious lesions, *Endod. Dent. Traumatol.* 9 (1993) 238–242.
- [19] E. Mass, U. Zilberman, Long-term radiologic pulp evaluation after partial pulpotomy in young permanent molars, *Quintessence Int.* 42 (2011) 547–554.
- [20] S. Asgary, M.J. Eghbal, J. Ghoddsi, S. Yazdani, One-year results of vital pulp therapy in permanent molars with irreversible pulpitis: an ongoing multicenter, randomized, non-inferiority clinical trial, *Clin. Oral Investig.* 17 (2013) 431–439.
- [21] S. Asgary, M.J. Eghbal, J. Ghoddsi, Two-year results of vital pulp therapy in permanent molars with irreversible pulpitis: an ongoing multicenter randomized clinical trial, *Clin. Oral Investig.* 18 (2014) 635–641.
- [22] Z. Li, L. Cao, M. Fan, Q. Xu, Direct pulp capping with calcium hydroxide or mineral trioxide aggregate: a meta-analysis, *J. Endod.* 41 (2015) 1412–1417.
- [23] P. Linsuwanont, K. Wimonstithikul, U. Pothimoke, B. Santiwong, Treatment outcomes of mineral trioxide aggregate pulpotomy in vital permanent teeth with carious pulp exposure: the retrospective study, *J. Endod.* 43 (2017) 225–230.
- [24] N.A. Taha, M.A. Khazali, Partial pulpotomy in mature permanent teeth with clinical signs indicative of irreversible pulpitis: a randomized clinical trial, *J. Endod.* 43 (2017) 1417–1421.
- [25] S. Asgary, R. Hassanizadeh, H. Torabzadeh, M.J. Eghbal, Treatment outcomes of 4 vital pulp therapies in mature molars, *J. Endod.* 44 (2018) 529–535.
- [26] N. Uesrichai, A. Nirunsittirat, P. Chuveera, T. Srisuwan, T. Sastraruji, P. Chompu-Inwai, Partial pulpotomy with two bioactive cements in permanent teeth of 6- to 18-year-old patients with signs and symptoms indicative of irreversible pulpitis: a noninferiority randomized controlled trial, *Int. Endod. J.* (2019), <https://doi.org/10.1111/iej.13071> [Epub ahead of print].
- [27] Y. Li, D. Bing, C. Dahl, B. Bergeron, P. Shipman, L. Niu, et al., Pulpotomy for carious pulp exposures in permanent teeth: a systematic review and meta-analysis, *J. Dent.* (2019) [in press].
- [28] S. Asgary, M. Fazlyab, S. Sabbagh, M.J. Eghbal, Outcomes of different vital pulp therapy techniques on symptomatic permanent teeth: a case series, *Iran. Endod. J.* 9 (2014) 295–300.
- [29] E.A. Kidd, Clinical threshold for carious tissue removal, *Dent. Clin. North Am.* 54 (2010) 541–549.
- [30] S. Asgary, M. Ahmadyar, Can miniature pulpotomy procedure improve treatment outcomes of direct pulp capping? *Med. Hypotheses* 78 (2012) 283–285.
- [31] S. Asgary, M.J. Eghbal, M. Fazlyab, A.A. Baghban, J. Ghoddsi, Five-year results of vital pulp therapy in permanent molars with irreversible pulpitis: a non-inferiority multicenter randomized clinical trial, *Clin. Oral Investig.* 19 (2015) 335–341.
- [32] M.K. Caliskan, Pulpotomy of carious vital teeth with periapical involvement, *Int. Endod. J.* 28 (1995) 172–176.
- [33] I.V. Nosrat, C.A. Nosrat, Reparative hard tissue formation following calcium hydroxide application after partial pulpotomy in cariously exposed pulps of permanent teeth, *Int. Endod. J.* 31 (1998) 221–226.
- [34] P. Chailertvanitkul, J. Paphangkorakit, N. Sooksantisakoonchai, N. Pumas, W. Pairojamornyoat, N. Leela-Apiradee, et al., Randomized control trial comparing calcium hydroxide and mineral trioxide aggregate for partial pulpotomies in cariously exposed pulps of permanent molars, *Int. Endod. J.* 47 (2014) 835–842.
- [35] N.A. Taha, M.B. Ahmad, A. Ghanim, Assessment of mineral trioxide aggregate pulpotomy in mature permanent teeth with carious exposures, *Int. Endod. J.* 50 (2017) 117–125.
- [36] M. Galani, S. Tewari, P. Sangwan, S. Mittal, V. Kumar, J. Duhan, Comparative evaluation of postoperative pain and success rate after pulpotomy and root canal treatment in cariously exposed mature permanent molars: a randomized controlled trial, *J. Endod.* 43 (2017) 1953–1962.
- [37] H. Alqaderi, C.T. Lee, S. Borzangy, T.C. Pagonis, Coronal pulpotomy for cariously exposed permanent posterior teeth with closed apices: a systematic review and meta-analysis, *J. Dent.* 44 (2016) 1–7.
- [38] D. Ricucci, J.F. Siqueira Jr, *Endodontology, An Integrated Biological and Clinical View*, Quintessence Publishing, London, 2013.
- [39] D. Ricucci, S. Loghin, J.F. Siqueira Jr, Correlation between clinical and histologic pulp diagnoses, *J. Endod.* 40 (2014) 1932–1939.
- [40] S. Seltzer, I.B. Bender, M. Ziontz, The dynamics of pulp inflammation: correlations between diagnostic data and actual histologic findings in the pulp, *Oral Surg. Oral Med. Oral Pathol.* 16 (1963) 846–871.
- [41] W.J. Wolters, H.F. Duncan, P.L. Tomson, I.E. Karim, G. McKenna, M. Dorri, et al., Minimally invasive endodontics: a new diagnostic system for assessing pulpitis and subsequent treatment needs, *Int. Endod. J.* 50 (2017) 825–829.
- [42] L.J. Baume, Diagnosis of diseases of the pulp, *Oral Surg. Oral Med. Oral Pathol.* 29 (1970) 102–116.
- [43] P.M. Dummer, R. Hicks, D. Huws, Clinical signs and symptoms in pulp disease, *Int. Endod. J.* 13 (1980) 27–35.
- [44] L. Tronstad, I.A. Mjör, Capping of the inflamed pulp, *Oral Surg. Oral Med. Oral Pathol.* 34 (1972) 477–485.
- [45] M. Trope, R. McDougal, L. Levin, K.N. May Jr, E.J. Swift Jr, Capping the inflamed pulp under different clinical conditions, *J. Esthet. Restor. Dent.* 14 (2002) 349–357.
- [46] J. Ghoddsi, M. Forghani, I. Parisay, New approaches in vital pulp therapy in permanent teeth, *Iran. Endod. J.* 9 (2014) 15–22.
- [47] J. Tomes, *A System of Dental Surgery*, John Churchill, London, UK, 1859.
- [48] E.A. Kidd, How 'clean' must a cavity be before restoration? *Caries Res.* 38 (2004) 305–313.
- [49] M. Maltz, E.F. Oliveira, V. Fontanella, G. Carminatti, Deep caries lesions after incomplete dentine caries removal: 40-month follow-up study, *Caries Res.* 41 (2007) 493–496.
- [50] L. van der Sluis, E. Kidd, R. Gruythuysen, L. Peters, Preventive endodontics – an argument for avoiding root canal treatment, *Endod. Pract. Today* 7 (2013) 259–274.
- [51] F. Schwendicke, J.E. Frencken, L. Bjørndal, M. Maltz, D.J. Manton, D. Ricketts, et al., Managing carious lesions: consensus recommendations on carious tissue removal, *Adv. Dent. Res.* 28 (2016) 58–67.
- [52] L. Bjørndal, H. Fransson, G. Bruun, M. Markvart, M. Kjældgaard, P. Näsman, et al., Randomized clinical trials on deep carious lesions: 5-year follow-up, *J. Dent. Res.* 96 (2017) 747–753.
- [53] H.F. Duncan, K.M. Galler, P.L. Tomson, S. Simon, I. El-Karim, R. Kundzina, G. Krastl, T. Dammaschke, H. Fransson, M. Markvart, M. Zehnder, L. Bjørndal, European Society of Endodontology position statement: management of deep caries and the exposed pulp, *Int. Endod. J.* (2019), <https://doi.org/10.1111/iej.13080>.
- [54] J. Turtiainen, T. Hakala, T. Hakkarainen, J. Karhukorpi, The impact of surgical wound bacterial colonization on the incidence of surgical site infection after lower limb vascular surgery: a prospective observational study, *Eur. J. Vasc. Endovasc. Surg.* 47 (2014) 411–417.
- [55] I.N. Rôças, F.R. Alves, C.T. Rachid, K.C. Lima, I.V. Assunção, P.N. Gomes, J.F. Siqueira Jr, Microbiome of deep dentinal caries lesions in teeth with symptomatic irreversible pulpitis, *PLoS One* 11 (2016) e0154653.
- [56] I.N. Rôças, K.C. Lima, I.V. Assunção, P.N. Gomes, I.V. Bracks, J.F. Siqueira Jr, Advanced caries microbiota in teeth with irreversible pulpitis, *J. Endod.* 41 (2015) 1450–1455.
- [57] K.C. Lima, L.T. Coelho, I.V. Pinheiro, I.N. Rôças, J.F. Siqueira Jr, Microbiota of dentinal caries as assessed by reverse-capture checkerboard analysis, *Caries Res* 45 (2011) 21–30.
- [58] J.S. Paddick, S.R. Brailsford, E.A. Kidd, D. Beighton, Phenotypic and genotypic selection of microbiota surviving under dental restorations, *Appl Environ Microbiol* 71 (2005) 2467–2472.
- [59] S. Edwardsson, Bacteriological studies on deep areas of carious dentine, *Odontol Revy (Suppl)* 32 (1974) 1–143.
- [60] T.J. Koh, L.A. DiPietro, Inflammation and wound healing: the role of the macrophage, *Expert Rev. Mol. Med.* 13 (2011) e23.
- [61] H. Sorg, D.J. Tilkorn, S. Hager, J. Hauser, U. Mirastschijski, Skin wound healing: an update on the current knowledge and concepts, *Eur. Surg. Res.* 58 (2017) 81–94.
- [62] A. Turabelidze, L.A. DiPietro, Inflammation and wound healing, *Endod. Topics* 24 (2011) 26–38.
- [63] S. Kakehashi, H.R. Stanley, R.J. Fitzgerald, The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats, *Oral Surg. Oral Med. Oral Pathol.* 20 (1965) 340–349.
- [64] C. Atri, F.Z. Guerfali, D. Laouini, Role of human macrophage polarization in inflammation during infectious diseases, *Int. J. Mol. Sci.* 19 (2018) E1801.
- [65] A. Shapouri-Moghaddam, S. Mohammadian, H. Vazini, M. Taghadosi, S.A. Esmaeili, F. Mardani, et al., Macrophage plasticity, polarization, and function in health and disease, *J. Cell. Physiol.* 233 (2018) 6425–6440.
- [66] V. Thompson, R.G. Craig, F.A. Curro, W.S. Green, J.A. Ship, Treatment of deep carious lesions by complete excavation or partial removal: a critical review, *J. Am. Dent. Assoc.* 139 (2008) 705–712.
- [67] J.C. Farges, B. Alliot-Licht, E. Renard, M. Ducret, A. Gaudin, A.J. Smith, et al., Dental pulp defence and repair mechanisms in dental caries, *Mediators Inflamm.* 2015 (2015) 230251.