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# Pathophysiology of Dental Caries

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## Abstract

Cariou lesion dynamics are dependent predominantly on the availability of fermentable sugars, other environmental conditions, bacteria, and host factors. Our current understanding of the microorganisms involved in the initiation and progression of caries is still rather incomplete. The most relevant acidogenic-aciduric bacterial species known to date are *Streptococcus mutans*, bifidobacteria, and lactobacilli. Whereas *mutans* streptococci are initiators, bifidobacteria and lactobacilli are more enhancers for progression. Boosters for microbial activity are specific environmental conditions, such as the presence of fermentable dietary sugars and the absence of oxygen. Based on these conditions, the necrotic and/or contaminated zone fulfils all criteria for disease progression and has to be removed. For those deep lesions where the pulp vitality is not affected, a selective removal of the contaminated leathery dentine should take place as this approach lowers the risk of regrowth of the few embedded microbial cells here. In repelling the microbial attack and repairing damage, the host has developed several ingenious strategies. A major resistance to carious lesion progression is mounted by the dentine-pulp tissues. The signalling molecules and growth factors released upon dentine demineralisation upregulate the odontoblast activity and act as sensor

cells. After carious stimulation, odontoblasts initiate an inflammatory reaction by producing chemokines and synthesise a protective tertiary dentine. After the destruction of these cells, the pulp still has a high capacity to synthesise this tertiary dentine thanks to the presence of adult stem cells within the pulp. Also, in addition to the systemic regulation, the pulp which is located within inextensible the confines of the dentine walls has a well-developed local regulation of its inflammation, regeneration, and vascularisation. This local regulation is due to the activity of different pulp cell types, mainly the fibroblasts, which secrete soluble molecules that regulate all these processes.

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## Microbiology of Tooth Decay

Dentistry dates as far back as 5,000 BC when people in India, Egypt, Japan, and China thought dental caries were a result of a “tooth worm.” The term “dental caries” first appeared in the literature around 1634 and is derived from the Latin word *cariēs* for decay and from ancient Irish *achrinn*, it decays. The term was originally used simply to describe holes in the teeth with little

knowledge of the aetiology and pathogenesis of the disease [1].

Concepts and beliefs about the cause of dental caries have evolved over many centuries, with the involvement of microorganisms recognised since the late 1800s. Readers interested in the concepts in caries microbiology and their development over time can find comprehensive literature on the topic [2, 3]. Despite thousands of publications, however, the central question of the relative importance of different bacteria in the disease remains unanswered.

With technical advances in our ability to identify, cultivate, and count different microorganisms, our views have evolved regarding the contribution of particular species of plaque bacteria to the caries process [4]. But it might take another 20–50 years, as a rough estimate, before deep-sequencing technologies and the gene databases have reached such a quality that the entire gene repertoire and interactions within a carious tissue can be determined. The limits so far are the length of an ambiguity-free sequence read (which is currently as short as 500 bp applying the leading Illumina sequencing technology) and, as a consequence, the need to select a short but taxon-representative region, usually a variable region, e.g., V1-V3, V3-V4, or V3-V5 of the 16S rDNA (total length 1,545 bp) or – even better – of the 23S rDNA (total length 2,905 bp). For instance, V3-V4 sequence results will leave many saccharolytic *Streptococcus* and *Actinomyces* species as “unclassified.” That means our current picture about the microorganisms involved in the initiation and progression of caries or other especially polymicrobial diseases is still rather incomplete. However, the species known to be involved so far and the correlated pathomechanisms of dental caries can be taken as a draft model and are thus discussed below.

In dental caries, we see an ecologic shift within the dental biofilm environment, driven by frequent access to fermentable dietary carbohydrates. This leads to a move from a balanced pop-

ulation of microorganisms of low cariogenicity to a consortium of high cariogenicity and to an increased production – and correlated tolerance – of organic acids promoting dental hard tissue net mineral loss. That is why we call this consortium acidogenic and acidophil (synonym aciduric). Besides the presence of fermentable dietary carbohydrates and selection of acidogenic-aciduric bacterial species, the host susceptibility, which is a rather simplified term for a multifactorial complexity, is the third major player.

The acidogenic-aciduric bacterial species *Streptococcus mutans* is recognised as being eminently involved in cariogenic processes, including early childhood caries, enamel carious lesions, cavitated lesions, or carious dentine. However, over time its attributed role changed from that of a true pathogen (specific plaque hypothesis [5]) to enhancer (active role) and/or indicator (passive role) of a sugar-triggered cariogenic vicious circle (extended caries ecological hypothesis [6, 7]), and the discussion still goes on [8]. As a matter of fact, *S. mutans* is detected in a few caries-free and found absent in several caries-active individuals, impairing its outstanding caries indicator potential. Furthermore, most relevant acidogenic-aciduric bacterial species are: (i) *S. mutans* relatives (called mutans streptococci or MS) with a similar virulence potential, namely *S. sobrinus*; (ii) bifidobacteria, including *Bifidobacterium dentium* and other closely related oral *Bifidobacterium* spp., but also the more distantly related species *Scardovia wiggsiae*, and (iii) lactobacilli, especially those with pellicle-adhesive potential [9].

A number of epidemiological and in vitro studies suggested that *S. sobrinus* – under circumstances yet to be determined – may be even more cariogenic than *S. mutans* [8–11]. In addition, targeted clinical studies have suggested that preschool and 15-year-old school children harbouring both *S. mutans* and *S. sobrinus* had a higher incidence of dental caries than those with *S. mutans* alone (for a review see Conrads et al. [10]).

Unlike MS, the highly aciduric bifidobacteria, especially *B. dentium*, do not colonise hard surfaces per se, since denture plaque associated with denture stomatitis harboured high levels of MS, lactobacilli, and yeasts, but not *B. dentium*. This indicates that *B. dentium* does not simply colonise intact dental hard surfaces but instead suggests that it is the lesion initiated by other species that facilitate the attachment and proliferation of *B. dentium*. In contrast to MS, the presence of this species might therefore be more a result than the cause of initial lesions. Clearly, *B. dentium* and MS are significant independent indicators [9].

A similar role (more profiteer than initiator) was recently proposed for lactobacilli, with *Lactobacillus fermentum*, *L. rhamnosus*, *L. gasseri*, *L. salivarius*, *L. plantarum*, and the *L. casei-paracasei* group as the most abundant species. According to this concept, precaries lesions become a retentive, low pH niche for lactobacilli accumulation, which take advantage of their proclivity for making and surviving in an increasingly reduced pH environment. In some cases, the lactobacilli can even outcompete and exclude the MS that created the retentive niche, which might explain why caries lesions are sometimes free of MS but not or very rarely free of lactobacilli [9].

Other less investigated but interesting caries-indicator candidates are *Atopobium* spp., *Slackia exigua* and a few others [11, 12]. The entire network of microbial organisms involved, which are not only bacteria but also saccharolytic yeasts (e.g., *Candida albicans*), *Archaea* (enhancer of fermentation processes by consuming end products such as CO<sub>2</sub> and H<sub>2</sub>), or bacteriophages (enhancer of lateral gene transfer and thus of evolution), is extremely complex and diverse.

Taken together, every cavity might have its own demineralising consortium of active organisms and genes, but the following simple principles are universal:

- 1 Presence of acidogenic-aciduric microorganisms and their ability to attach to the pellicle-coated tooth surface, either directly (pioneers

such as MS) or indirectly (beneficiaries such as bifidobacteria and lactobacilli; for a review see Conrads et al. [10]).

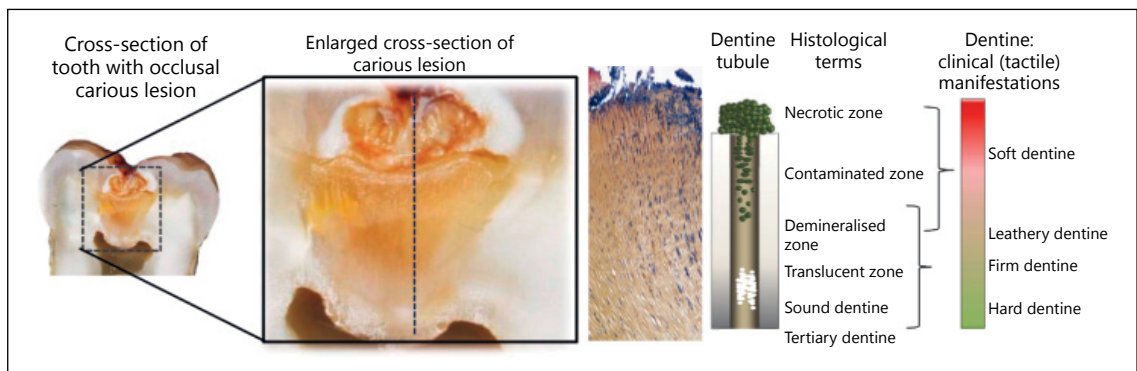
- 2 Environmental conditions favouring the multiplication and metabolism of such species: access to low-molecular sugars, especially sucrose, and low redox potential at the same time. High sugar and low oxygen leads to rapid fermentation and acid production.

With these simple principles, it is possible to identify (constitute) what a carious tissue actually is and how much tissue must or should be removed or excavated to stop further decay.

### **Histology of a Carious Tissue – The Microbiological Perspective**

The degree of success in eliminating bacteria during cavity preparation and prior to the insertion of a restoration may increase the longevity of the restoration and therefore the success of the restorative procedure. The complete eradication of bacteria in a caries-affected tooth during cavity preparation is considered a difficult clinical task and – from the perspective of a microbiologist – almost impossible, and also not required anymore, as is discussed in the chapter by Bjørndal [this vol., pp. 68–81]. Attempts to excavate completely extensive carious tissue may affect the vitality of the pulp and weaken the tooth structure. In principal, disinfection of the cavity preparation after caries excavation can aid in the elimination of bacterial remnants, reducing the risk for recurrent caries and failure of the restoration. However, the side effects of chemical disinfectants (e.g., chlorhexidine or benzalkonium chloride) on the restorative treatment, including reduced dentine bond strength, have been a major concern for both dental clinicians and researchers [13], and therefore alternatives still have to be found and their efficacy proven.

As shown in Figure 1, the carious tissue consists of 4 different zones, but only 3 clinically noticeable



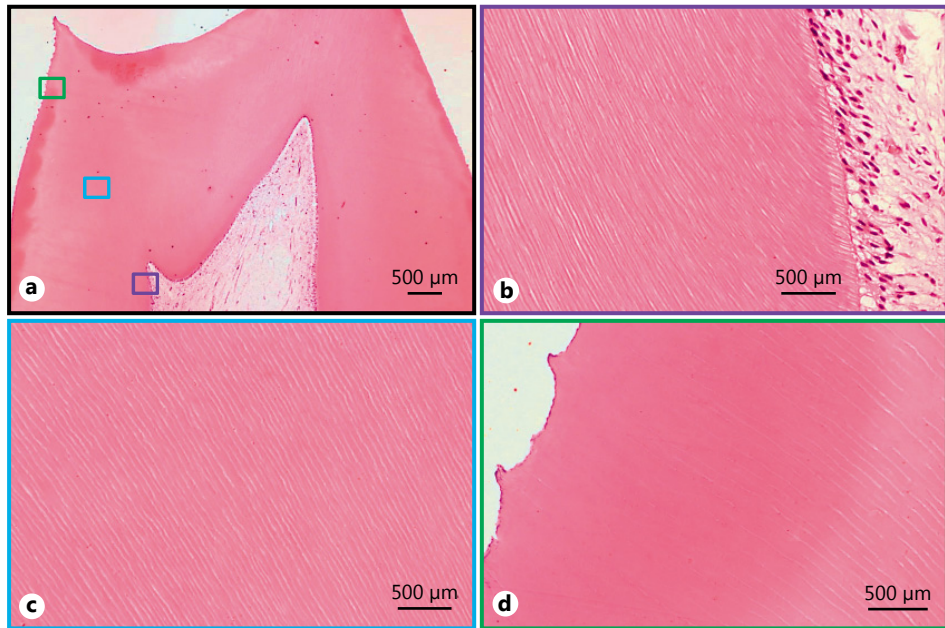
**Fig. 1.** Histology of carious tissue. Note the correlations between cross-section, ultrastructure zones, and clinical (tactile) manifestations. modified from Innes et al. [14] and Ogawa et al. [15].

layers. The outer layer, clinically the soft dentine, consists of the necrotic zone with the microbial biofilm attached, and the contaminated zone. The soft dentine is characterised by a gradient of microorganisms with cell-numbers between  $10^1$  and  $10^8$  per mg (measured from the inside to outside, pulpal to coronal), including aciduric, facultative anaerobic bacteria. Comparing the conditions here with the principles mentioned above, this necrotic and/or contaminated zone fulfils all criteria for disease (demineralisation) progression as it is anaerobic (low redox potential demanding a fast substrate turnover for sufficient energy resourcing) and, at least temporarily, fed by high concentrations of fermentable dietary carbohydrates. This layer has to be removed.

The next layer is the demineralised zone, which correlates clinically with leathery dentine. This zone is characterised by few microorganisms per milligram, very little nutrients (since already consumed by the bacteria and yeasts in the outer layer), and a strictly anaerobic atmosphere. While the latter condition favours demineralisation by acid production, the sheer low number of fermenting bacteria and the very low nutritional source prohibits substantial multiplication and metabolism. It is the consensus that for deep lesions, extending beyond the inner (pulpal) third or quarter of dentine radiographically, selective

removal (incomplete excavation to protect the pulp) should be limited to soft dentine, excluding the removal of contaminated leathery dentine [14]. From the microbiological point of view, this approach is tolerable as electron transport within, and acid production by, the few cells is also very low in this zone. However, bacteria have several strategies to overcome harsh conditions and – after preparation, disinfection if applicable, infiltration if applicable, and restoring – might still be alive although in a dormant state [16, 17]. This means the lesion and the bacteria are arrested, but only temporarily. If there is gap formation at the tooth-restoration interface, possibly further supported by the microleakage of fluids and salivary proteins to the gap, this leads to inevitable microbial colonisation from saliva, but also to the possible regrowth of dormant cells and, ultimately, secondary caries formation. Therefore, for less deep lesions, selective removal should take place down to firm dentine, which not only has clinical advantages (more depth for a solid restoration), but also lowers the risk of regrowth of surviving microbial cells.

Finally, pulpally the translucent zone of firm softer dentine is characterised by demineralisation since acids, but not the bacterial cells, penetrate to this depth. Here, the plate-form apatite crystals apparently dissolve and recrystallise into



**Fig. 2.** **a** Histology of sound dentine in a premolar. **b** Dentine tubules are numerous and wide open on the pulp side. **c** They are less numerous and appear more narrow at mid-distance between the pulp and the dentine-enamel junction. **d** Dentine tubules are very narrow and many of them appear completely obliterated upon approaching the dentine-enamel junction.

a rhomboid form, defined as whitlockite  $[\text{Ca}_9(\text{MgFe})(\text{PO}_4)_6\text{PO}_3\text{OH}]$ . This crystalline form seems to be softer and less resistant to cutting and acids [15]. This layer might not be absolutely sterile, but metabolism of aciduric microorganisms is almost impossible and thus negligible. For repelling and combatting the microbial attack and repairing damages, the host has developed several ingenious strategies.

### Pulp Response to Dental Caries

Host pulp response to dental caries is a key element in understanding the carious process and its consequences. In this regard, enamel acts as a physical mineralised barrier preventing bacterial infiltration into the dentine and pulp. Also, the underlying dentine histology, composition, and function provide significant information on how

bacteria invasion is hindered by the dentine itself and how this dentine provides signalling molecules to induce dentine regeneration during the carious process. In the case of a deep carious lesion reaching the odontoblasts, the pulp tissue itself has also elaborated efficient strategies to hinder or even arrest the carious lesion progression and the bacterial infiltration into the pulp.

### Dentine Histology at the Dentine Enamel Junction Is Different from that of Deep Dentine

Dentine has a unique histological tubular appearance. The tubules close to the dental pulp are numerous and wide open. Their diameter decreases gradually as they get away from the pulp and become very narrow or completely obliterated upon approaching the dentine-enamel junction (Fig. 2).

This suggests that the superficial dentine hinders bacterial infiltration when they reach the dentine surface. On the other hand, when bacteria reach the open dentine tubules they can invade the dental pulp more rapidly through the tubules. This may suggest significant consequences to the underlying pulp only if we consider the bacterial invasion consequences. However, the dental pulp established efficient protective mechanisms against this invasion.

### **Dentine Matrix Contains Sequestered Signalling Molecules**

While the major dentine inorganic component is hydroxyapatite, its organic matrix is mainly composed of collagen I and non-collagenous proteins such as dentine sialoprotein [18] and dentine matrix protein-1 [19]. These are involved in the initiation and the regulation of dentine mineralisation. In addition, different signalling molecules have been reported to be secreted by the odontoblasts and sequestered in the dentine matrix, mainly in an inactive form. Among others, these include transforming growth factor- $\beta_1$  (TGF- $\beta_1$ ), basic fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF), and platelet derived growth factor (PDGF) [20, 21]. During the carious dissolution of the dentine matrix, these molecules can be released and reach the underlying odontoblasts leading to the upregulation of their synthetic activity.

In addition to the responsiveness to these growth factors, recent data demonstrated that odontoblasts act as sensor cells as they express transient potential channel receptors. These receptors allow the odontoblast to be responsive to the external stimulations, such as noxious heat, noxious cold, as well as chemical and mechanical stimulations [22]. Thus, upon stimulation, odontoblasts synthesise a new tertiary dentine at the pulp periphery facing the stimulation site. This focally secreted dentine can also be deposited

within the dentine tubules to decrease their permeability to cariogenic bacteria and their toxins, leading to the protection of the underlying pulp.

Thus, odontoblasts represent the first defence mechanism in case of carious lesion development. Indeed, these cells also express receptors called Toll-like receptors (TLRs) 2 and 4 [23, 24], which recognise specific structures on Gram-positive and Gram-negative bacteria, respectively. These TLRs belong to a big family of pattern recognition receptors that are activated after contact with common molecules on the pathogen surface. In moderate carious injuries, TLRs 2 are highly expressed in the underlying odontoblasts [25]. Upon activation, these TLRs induce the secretion of antimicrobial molecules such as  $\beta$ -defensins and nitric oxide by the odontoblasts which have an antibacterial effect against *S. mutans*, thus limiting cariogenic bacteria progression towards the pulp [26]. Also, upon activation of their receptors, odontoblasts secrete proinflammatory chemokines which lead to dendritic cell recruitment in order to eliminate the pathogenic agents [27].

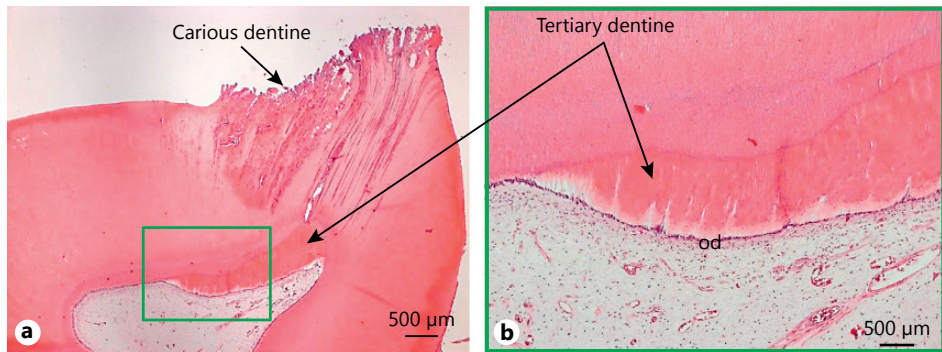
Overall, in the case of moderate dentine carious lesions, the odontoblasts act as a barrier exerting antimicrobial effects and initiating the secretion of a tertiary dentine to protect the underlying pulp (Fig. 3).

However, in the case of severe and rapidly progressive carious lesions, tertiary dentine focal synthesis may not be enough and bacteria may destroy the newly synthesised tertiary dentine, reach the underlying pulp, and induce an inflammatory reaction (Fig. 4).

### **The Dental Pulp Defence Strategies**

When the odontoblastic barrier is destroyed by the carious lesion and either bacteria or their toxins reach the underlying pulp, a tertiary dentine secretion can still be observed. This dentine, which is secreted after the odontoblast destruction, is synthesised by another cell type originat-





**Fig. 3. a** Histology of a moderate carious lesion in a molar. It is characterised by a disorganised dentine with a loss of the typical tubular structure at the carious site. **b** Tertiary dentine is secreted locally by the underlying odontoblasts (od). At this stage, bacterial infiltration is arrested at a distance from the pulp, which has a normal aspect.

ing for the differentiation of adult pulp stem cells. This reparative dentine usually contains fewer tubules than the physiological one. This might decrease the bacterial infiltration or their toxins to the underlying pulp. While little was known about the cells secreting this dentine, the discovery of adult stem cells within the dental pulp provided a significant step forward. Indeed, all dental pulps in permanent and primary teeth and at all ages comprise, at least, a population of adult stem cells [28]. This was first demonstrated in a culture system of cells isolated from the pulps of third molars, where pulp cells were able to produce a mineral matrix with molecular and mineral characteristics of dentine [29]. Additionally, when isolated with specific mesenchymal stem cell markers such as STRO-1 and transplanted after mixing with hydroxyapatite/tricalcium phosphate ceramic powder subcutaneously in mice, they generated a dentine/pulp-like tissue [30]. There is converging evidence that one of the niches of these stem cells is located in the perivascular area. After pulp injury, these cells are activated and migrate to the injury site to synthesise the tertiary dentine [31]. It has been reported that TGF- $\beta_1$ , which can be released after the dissolution of dentine, is involved in the recruitment of these cells and their differentia-

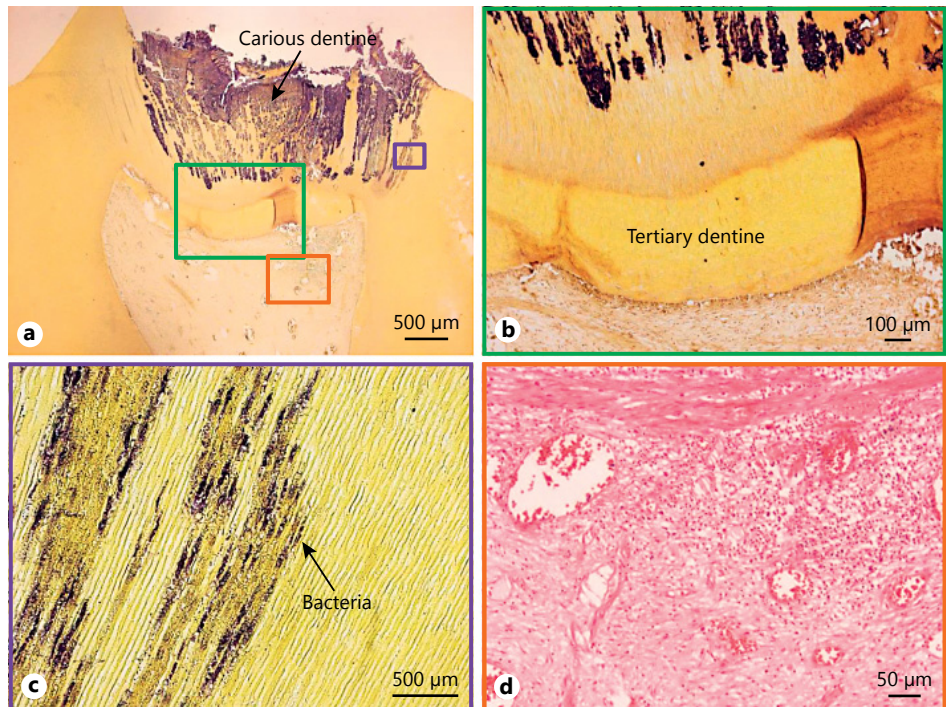
tion into odontoblast-like cells secreting the tertiary dentine [32].

Additionally, recent investigations on dental pulp, which has a terminal circulation, revealed that, in addition to the systemic regulation, it has a local regulation of its vascularisation, inflammation, and regeneration. This allows the dental pulp to resist bacterial invasion by different mechanisms, as explained below.

### Dental Pulp Local Regulation

It is well established that carious injury leads to pulp hypoxia. Different pulp cell types, such as fibroblasts, endothelial, and stem cells, have been reported to upregulate the synthesis of hypoxia-inducible factor, which increases the synthesis of angiogenic growth factors such as VEGF, FGF-2, and PDGF. This leads to vasodilation and the increased formation of blood vessels at the carious injury site. Overall, this increases nutrient, blood, and oxygen supply to the injured tissue. This also allows inflammatory cell recruitment to carry out phagocytosis of pathogens. After complete pulp healing, there is a downregulation of angiogenic factor secretion and a return to normoxia with normal pulp vascularisation [33].





**Fig. 4.** **a** Histology of a severe carious lesion in a molar. It is characterised by a disorganised dentine at the carious site. **b** Tertiary dentine is produced in the form of a bridge by the underlying odontoblasts. At this stage, bacteria infiltrated the dentine tubules (**c**) and the pulp, which appears inflamed and infiltrated by numerous inflammatory cells (**d**).

Carious injury also leads to a pulp inflammatory reaction initiated by complement activation. Complement is the name given to about 40 proteins synthesised mainly by the liver and released in the plasma. During the inflammatory process, the complement is activated, leading to the synthesis of biologically active complement fragments. These play a major role in eliminating pathogenic agents. Pulp fibroblasts have been recently reported as the only non-immune cell capable of synthesising all complement proteins [34]. After complement activation, biologically active fragments are released. Recent investigation of these fragments revealed their involvement in the pulp anti-inflammatory and regeneration processes. Indeed, pulp complement can be activated by lipoteichoic acids of Gram-posi-

tive bacteria, such as *S. mutans* and *S. sanguinis*. Upon activation, several biologically active molecules are released. Among these, C5a has been shown to be involved in the recruitment of pulp stem cells [35] and in the guidance of nerve growth to the stimulation site [36]. Another fragment, C3a, is involved in the proliferation of both pulp fibroblasts and stem cells and in guiding fibroblast migration to the stimulation site [37]. This clearly illustrates the involvement of complement in the pulp regeneration process facing bacterial infiltration during carious disease. Indeed, reparative dentine is efficient in arresting the carious injury progression (Fig. 3). This may be partially explained by the fact that pulp complement activation also leads to the synthesis of a complex molecular structure called membrane

attack complex. This complex structure can be produced by the fibroblasts and has been shown not only to be fixed on *S. mutans* and *S. sanguinis*, but also to kill these cariogenic bacteria [33]. When this complex polymerises on bacteria walls, it creates numerous holes leading to the entry of electrolytes and water, which results in bacteria destruction. Thus, the fibroblasts dampen down, and may even arrest the bacterial invasion to the

pulp and provide the adequate signals not only to kill cariogenic bacteria, but also to initiate the regeneration process by recruiting the stem cells and nerve regeneration.

Overall, a carious lesion should be regarded as a dynamic process. Its progression does not only depend on the bacterial infiltration and the local environment, but also on the host pulp response.

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