Stéphane Simon, Paul Cooper, Ariane Berdal, Philip Lumley, Phillip Tomson, Anthony J Smith

Understanding pulp biology for routine clinical practice

Stéphane Simon, DCD, MSc
Associate Researcher, INSERM, UMR S 872, Centre de Recherche des Cordeliers, Paris, France
School of Dentistry, University of Birmingham, Birmingham, UK

Paul Cooper, MSc, PhD
Senior Lecturer in Oral Biology, School of Dentistry, University of Birmingham Birmingham, UK

Philip Lumley, MSc, PhD
Professor of Endodontology, School of Dentistry, University of Birmingham Birmingham, UK

Ariane Berdal, PhD
Professeur des Universités de Biologie Orale, INSERM, UMR S 872, Centre de Recherche des Cordeliers, Paris, France

Phillip Tomson
Clinical Lecturer and Specialist Registrar in Restorative Dentistry, School of Dentistry, University of Birmingham Birmingham, UK

Anthony J Smith, MSc, PhD
Professor of Oral Biology, School of Dentistry, University of Birmingham Birmingham, UK

Correspondence to:
Stéphane Simon
Laboratoire de Physiopathologie Orale Moléculaire, INSERM, UMR S 872, Escalier B, 15-21 rue de l'Ecole de Médecine, 75006 Paris, France
Tel: +33 970 40 58 87
Fax: +33 956 83 36 93
Email: srs635@bham.ac.uk

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Aim: To review the latest developments in the field of pulp biology, particularly those elements of specific interest to clinical dentists, whilst highlighting the importance of maintaining pulp vitality for conservative dentistry. Pulp biology is crucial to everyday practice in dentistry and the knowledge acquired, especially in the last five years on the pulp healing process, has highlighted simple but effective applications. However, difficulties in communication between biologists and clinicians, mostly due to the complexity of biology as a discipline, are a significant obstacle to therapeutic developments and their application on a larger scale.
Methods: A literature review was undertaken on the current understanding of the biology of the dentine-pulp complex, especially in the context of conservative dentistry.
Results: Novel biotechnological insights have recently been discovered, including the presence of stem cell-like cells within the tooth and their potential roles in reparative and regenerative processes. A greater understanding is also developing regarding the structure of the dentine-pulp complex, both macroscopically and microscopically, which may have important consequences for therapeutic choices.
Conclusions: The emergence of new adhesive systems, together with disinfecting molecules, represent a first step towards the application of new biological approaches to the treatment of pulpal disease. Improved understanding of the many pathophysiological processes of the dentine-pulp complex and the development of new materials, which are being adapted to clinical conditions, has led to significant advances for the therapeutic principles underpinning conservative dentistry.

Introduction

Significant progress in the field of carious disease management has led to much research into the mineralisation of teeth and the biological behaviour of the dentine-pulp complex. It appears that the dentine-pulp complex is able to adapt to a multitude of stimuli, invoking defence responses to maintain pulp vitality. The main role of the pulp is to secrete dentine. When tooth develop-

ment is completed, the pulp maintains the dentine through homeostatic and self-protective mechanisms. The dental pulp is able to reinitiate dentinogenesis at any time to protect itself from external injuries. However, it appears that advances in understanding the basic biology of this tissue have not yet been fully translated into better treatments in day-to-day dental practice beyond minor carious lesions. When caries has reached the dentine, it is generally assumed that it is too late to use such techniques to intervene. When the caries extends to the pulp, a pulpectomy is usually considered in order to prevent painful, infectious complications. However, no guidelines clearly define the indications for pulpectomy versus pulp vitality conservation.

Many researchers have been investigating the pulp healing process for several years, and recent advances in biotechnology have provided opportunities for the development of applications in pulp vitality maintenance, reactive dentinogenesis or revascularisation of the infected canal.

The aim of this paper is to present the relevant basic elements of pulp biology and highlight the existing and potential applications of biotechnology in the context of routine conservative dentistry.

The dentine-pulp complex

Dentine is a mineralised connective tissue that constitutes the main component of the tooth; it ensures support for the organ and confers elasticity. Seventy per cent of dentine is mineralised by hydroxyapatite crystals; it is also composed of 20% organic matrix and 10% water. The organic component is mostly composed of proteins, which play various structural, formative, signalling and homeostatic roles as well as being important in the pulp healing process. Notably, dentine and bone are very similar in their composition but show some structural differences.

Dentine provides protection to the pulp, a soft connective tissue, which ensures the 'vitality' of the tooth[1]. The 'dentine-pulp complex' is so called because of the intimate relationship of the two tissues that makes it difficult to separate their functional behaviours.

Dentine is a permeable tissue that is traversed by tubular structures called dentinal tubules, which extend from the enamel-dentine junction (EDJ) (or the cementum-dentine junction [CDJ] at the root level) to the pulp cavity (chamber or canal). These tubules contain dentinal fluid and the odontoblast processes. The extent of these odontoblast processes in the tubules remains controversial, with some authors claiming that the processes extend from the pulp chamber to the EDJ or CDJ[2-4], whereas others suggest that they are limited to the internal third of the tubules[5-8]. This structural distinction is important because the presence of cellular extensions in the tubules can influence the therapeutic approach. Tubules that have been partially occluded by cellular activity will confer different permeability properties on the tissue than those whose dimensions have been increased by carious processes and this will affect treatment planning accordingly.

The circumpulpal dentine is composed of inter-tubular dentine (between tubules) and peri- or intra-tubular dentine (secondarily deposited within the tubule, which reduces the diameter of the tubule; Fig 1). The secretion of intra-tubular dentine is continuous throughout the life of the tooth and can be accelerated in certain pathophysiological conditions (such as carious processes and tooth wear), leading to dentinal sclerosis.

The chemical composition of these two types of dentine is different, particularly in terms of their collagen contents and responses to etching procedures. These structural differences must be taken into account clinically because, for example, they can cause difficulties for bonding procedures. Protocols must differ between bonding on sclerotic dentine and filling a deep cavity in a relatively young tooth. This structural difference is also important in endodontics with the introduction of resin-based adhesive systems. Since the dentine structure in the apical region is closer to a fibrodentine structure than to a tubular one[9], questions remain regarding the sealing effectiveness of these systems in the short-, medium- and longer-term where the use of a protocol directly inspired by coronal bonding may not be optimal for endodontic use.

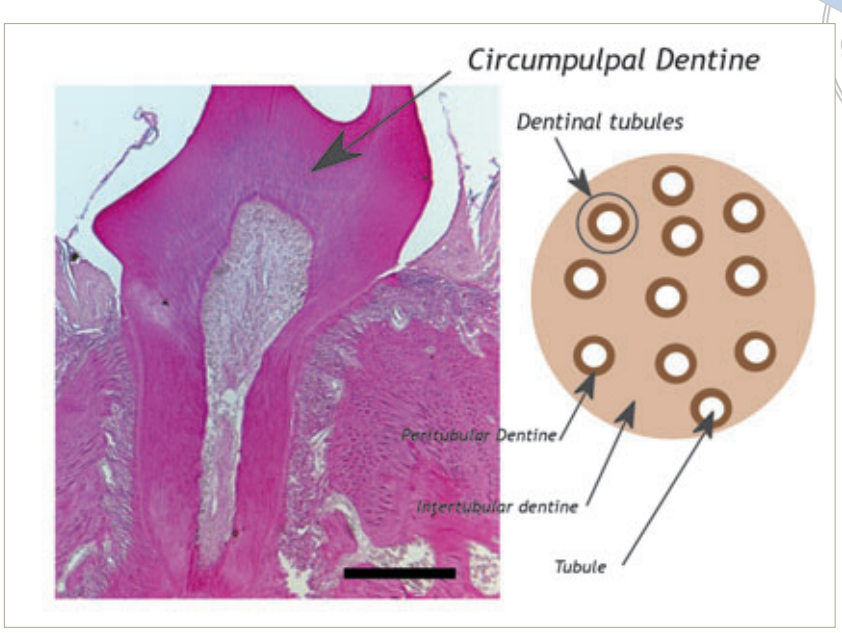

Fig 1 Schematic description of the structure of circumpulpal dentine, also called orthodentine (Van Gieson stain, bar = 500 µm).

Three types of dentine

Confusion remains regarding the terminology of the different types of dentine, i.e. primary, secondary and tertiary dentine. Many definitions have been proposed in the literature, several of which are contradictory. Although these definitions have not reached a consensus, they have been collated by Goldberg and Smith[10] who gave the following definitions:

Primary dentine

Primary dentine is the earliest dentine formed during tooth development, giving rise to the crown and root structure of the tooth. It 'patterns' the organ. The most external thin layer adjacent to the amelo-dentinal junction is formed as the odontoblasts are completing differentiation, which results in a variable tubular structure and is referred to as mantle dentine.

Secondary dentine

Secondary dentine is physiologically secreted either after the tooth has erupted into the oral cavity, or following apical closure. This dentine is generally responsible for the progressively decreasing space of the canal, which is improperly called 'calcification'. It is a physiological dentine rather than pathological in nature; its regular secretion is responsible for the asymmetrical loss of endodontic volume. This biological process explains the differences in canal volume between a young and an older tooth. The secondary dentine secretion is not uniform, but seems to occur predominantly on the roof and lateral walls of the pulp chamber, and not on its floor. The chemical composition and the histological structure of the primary and secondary types of dentine are identical. Only the rate of secretion differs: 4 µm/day for primary dentine and 0.4 µm/day for secondary dentine. The interface between primary and secondary dentine will sometimes be demarcated by a subtle calciotraumatic line. However, since both types of dentine are secreted by the same odontoblasts there will be tubular continuity and thus, there does not appear to be any clinical consequence as to whether primary or secondary dentine is present (Fig 2).

Tertiary dentine

Tertiary dentine is secreted in response to external factors, such as decay or abrasion, in order to protect the underlying pulp. In the case of moderate stress, which does not cause any destruction of odontoblasts, the secreted dentine is called 'reac-

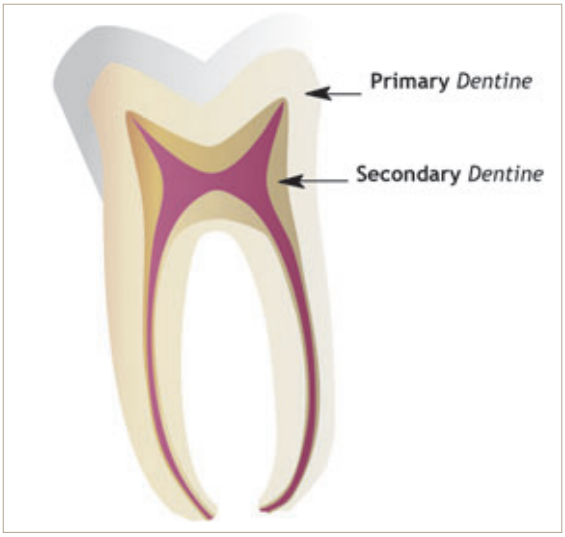

Fig 2 The histological pattern of the two types of physiological (primary and secondary) dentine.

tionary dentine'; when the stress is more intense and odontoblast survival is compromised (i.e. dentine bridge formation at sites of exposure), it is called 'reparative dentine'.

Understanding the distinction between the three types of dentine is important, and above all the distinction between secondary (physiological) and tertiary (healing) dentine must be clear, because they have generally been confused in the literature.

Dentine and bone

Many studies have shown that the compositions of these tissues are more similar than previously appreciated. Some molecules that were considered to be specific to dentine (such as dentine sialoprotein, DSP) have been found in alveolar bone. It is therefore more difficult to find specific markers to characterise these tissues. Nevertheless, the profiles of these molecules show differences between the two tissues.

On a structural level, bone and dentine have some features in common. Cells of both bone and dentine have a similar mesodermal embryonic origin, which may explain some of the similarities found in their formation and structures. In bone, secretion of the matrix is performed by osteoblasts; once embedded in the mineralised matrix, they appear to change into quiescent cells called osteocytes which communicate with each other through an extensive network of cell processes embedded in canaliculi. In the dentine-pulp complex, odontoblasts remain on the formative surface of the dentine matrix but have a cell process extending through the dentine with many lateral processes. The similarities between the morphologies of dentinal tubules and osteocyte canaliculi have recently been highlighted[11] and recent molecular data demonstrate striking similarities in the behaviour of odontoblasts and bone cells at different stages of their life cycles[12].

Pulpal cells

The pulp contains different types of cells, some of which show specialisation or differentiation for particular functions.

Odontoblasts

These are highly differentiated, post-mitotic cells, and are organised at the periphery of the pulp as a unicellular palisade. The presence of all the elements of the secretory/mineralisation machinery in the cells confirms their intense activity, notably during primary dentinogenesis. At a later stage during secondary dentinogenesis, the cells return to a quiescent state, with a reduced number of cytoplasmic organelles[13]. Cells are joined by cellular junctions such as gap junctions, thereby making a palisade of cells that provide a permeability barrier; gap junctions are also responsible for intra-cellular communication, which seems to be involved in the pulp healing process[14].

Unlike osteocytes, odontoblasts do not become incorporated in the matrix, except for their processes that are embedded in the tubule. This is why dentine must not be considered as an individual tissue but rather as the dentine-pulp complex. The odontoblast processes contain limited organelles (which are supposedly responsible for the later secretion of intra-tubular dentine), but are mostly filled by a dense network of microfilaments and other microtubules.

Increasingly, coronal odontoblasts are regarded as being different from those found in the root. The coronal odontoblasts are elongated and pyramidal, with an apical nucleus, whereas the radicular ones are more cubical, which is an indication of signifi-

cantly lower cellular activity. This fact is seldom taken into account, but would explain why therapeutic interventions that have been validated at a coronal level (pulp capping, for instance) will often fail when applied to the radicular pulp (pulpotomy).

The second layer of the pulp is a dense zone of cells (the 'Höhl's layer', separated from the odontoblasts by an acellular layer called the 'acellular Weil's layer'). During tooth development, the cellular differentiation process requires a minimum number of mitotic divisions of the odontoblast progenitor cells before they are competent to differentiate to the final cellular phenotype of the odontoblast. The last mitotic division has important spatial implications because it occurs perpendicular to the dental basement membrane resulting in two daughter cells: the one next to the basement membrane receives the inductive signal to differentiate to an odontoblast while the other does not and contributes to the Höhl layer[15]. This layer has long been considered as a potential reservoir of cells, containing incompletely differentiated cells that could be involved in the healing process for reparative dentinogenesis and dentine bridge formation if the odontoblast layer is damaged.

The capillary and nerve plexi that exist between both layers are significant; only a few nerve fibres accompany the cytoplastic extensions into the dentinal tubules and then only for a short distance. Capillaries are closely associated with the odontoblast palisade and provide these cells with the necessary nutrients for their mineralising/synthetic activities.

Pulp fibroblasts

Like all connective tissues, the major cell of pulp tissue is the fibroblast; these cells are responsible for the formation and renewal of the extracellular matrix, but at the same time they mediate its controlled remodelling. The extracellular matrix plays an important role in this connective tissue. Its viscosity changes with time (fibrosis increases with the age of the tissue) and during pathophysiological processes. Its viscoelasticity enables it to adapt to potential (and moderate) variations in pressure, inherent in the inflammatory process. Thanks to this adaptability, most episodes of pulp inflammation are clinically silent. When the intra-pulpal pressure, connected with the vasodilatation inherent in inflammation, cannot be compensated anymore, the pain increases.

Immune cells

Dendritic cells and mast cells have been identified in the pulp tissue[16], even in physiological conditions[17]. Macrophages are frequently found in healthy pulp, especially in the periphery of the tissue[18]. These phagocytic cells participate in the immune surveillance of the pulp, and enable a rapid response to invading bacteria[19,20]. Products of bacterial origin (such as toxins) diffuse via the tubules, and when in contact with pulp cells, they behave like antigens; therefore, the immune system of the pulpal parenchyma plays an important role[21]. Dendritic cells capture antigens and move these to the lymphatic nodes, where they are presented to T lymphocytes. Then, these activated T lymphocytes return to the damaged pulp. In this way, the host is immunised and will automatically respond to the future presence of these antigens. Other molecules, such as those of the transforming growth factor-beta (TGF-β) family, which have been liberated from the dentine during the mineralisation process, are able to regulate the immune system of the pulp[22].

Dendritic cells also interact with nerve fibres and blood vessels within the pulp. The neuroimmunological response of the pulp is presumably the first inflammatory reaction of the dentine-pulp complex[17].

Dental pulp stem cells

The growing interest in stem cells by the scientific community is also very apparent in dental research. The discovery of dental pulp stem cells (DPSC)[23] inside the pulp parenchyma demonstrated that the dental organ provides a 'niche' for replacement cells. Another population of stem cells has also been discovered in the pulp of deciduous teeth. These cells, or SHED (stem cells from human exfoliated deciduous teeth)[24], are particularly interesting because they are relatively easy to

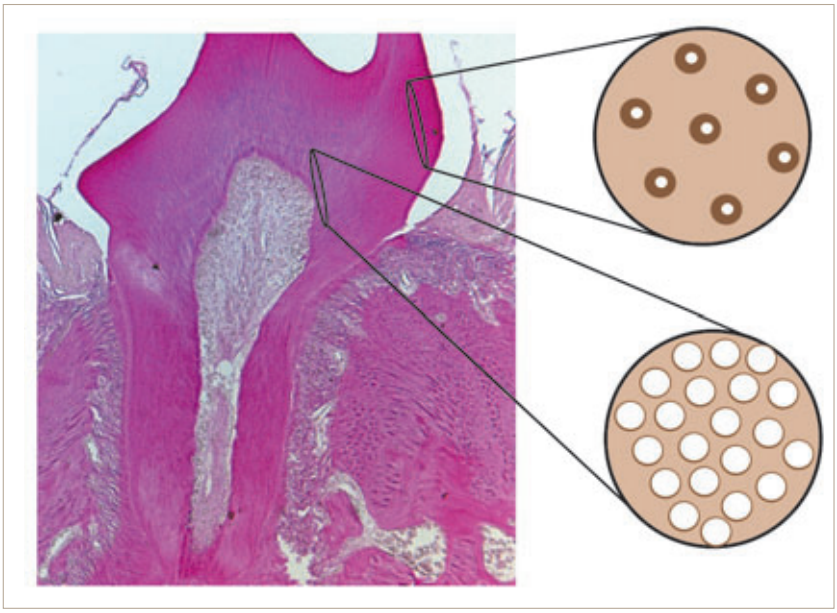

Fig 3 The density of the dentine tubules varies according to the depth of the dentine and the proximity to the endodontic cavity.

collect when the deciduous tooth is shed and replaced by the permanent successor.

The presence of stem cells in the dental pulp offers exciting possibilities for their exploitation in regenerative medicine both for dental and other diseases. They are easier to collect than bone marrow cells, which was one of the main sources of post-natal stem cells. Also, they appear to be a promising reservoir of multipotential cells, and as such offer significant potential for use in various biotechnological applications. The presence of a population of stem/progenitor cells in the dental pulp provides a local source of cells for generating new 'odontoblast-like cells' for both natural pulp wound healing or regeneration and direct pulp capping after injury to the tooth. It is still unclear whether these stem cells have a developmental derivation from the dental papilla or if they migrate to the pulp through the vasculature. An important focus of future studies will be a more precise characterisation of these stem cells and their potential to allow their most effective application in new regenerative therapies.

The discovery of stem cells in the dental pulp has been a significant advance for dentistry. Although many questions remain unanswered, the identification of 'post-natal stem cells' in the tooth provides a new platform for the development of exciting biological approaches for vital pulp therapy.

Dentinal tubules – a means of communication

The tubular structure

The tubular density of dentine is high (30,000 mm^2 on average), and these measure approximately 1 to 3 μm in diameter on average in humans; their distribution is unequal throughout the dentine and their density increases near the pulp cavity, reflecting the crowding of odontoblasts as they move pulpally (Fig 3).

They course in a sinusoidal manner in the crown rather than a rectilinear way, due to a change in the radius of curvature from the outer to the inner aspect of the dentine and have numerous lateral extensions, which provide a potential means of communication between them. Thus, much of the area of the dentine matrix is in direct communication with the odontoblasts on its formative surface through their processes. This has significant clinical implications in terms of the communication between the inner and outer regions of the tooth and how disease or surgical intervention can have an impact on this.

The tubular density differs significantly at outer and inner areas of the dentine. It is estimated that the surface occupied by these tubules represents 1% of the dentinal surface in the periphery of the

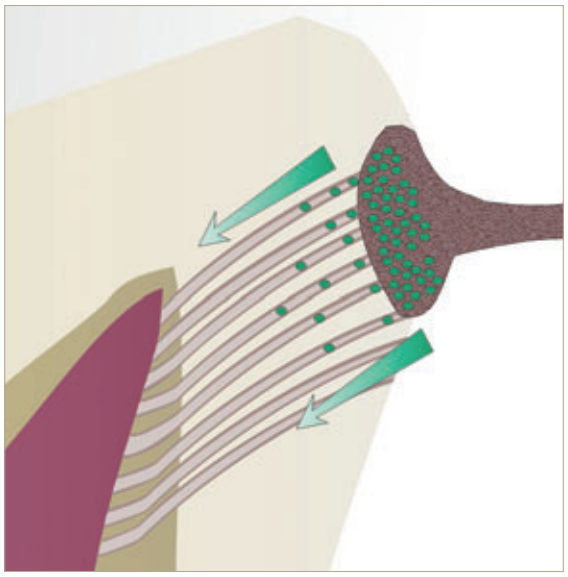

Fig 4 The principle of the diffusion phenomenon in the tubules.

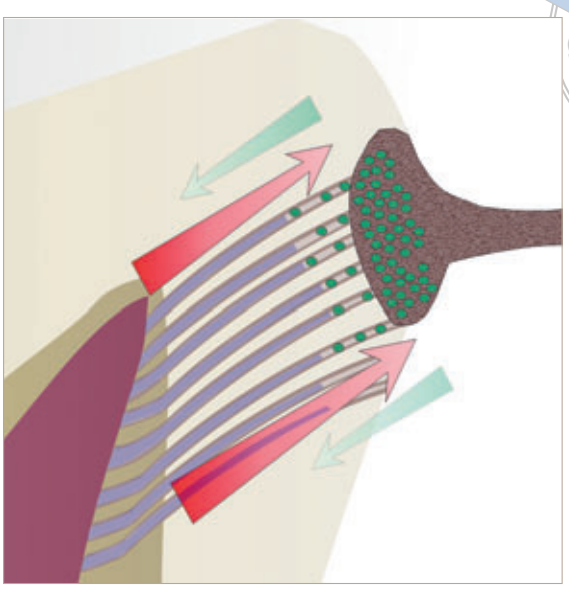

Fig 5 The difference in the pressure between the intra-pulpal cavity and the outside area is protective of the pulp parenchyma.

dentine (under the enamel) and 22% near the pulp. This variable distribution underlines the importance of location when drilling a cavity or making a coronal preparation, due to the opening of a diffusion pathway into the pulp tissue and potentially allowing the ingress of bacteria, toxins and other adverse agents.

The entire length of the tubules will contain dentinal fluid, which probably represents a cellular exudate from the pulp parenchyma together with any local odontoblast secretions. Two different phenomena are encountered from the moment when open tubules are exposed.

- Diffusion (Fig 4): When two biological environments (i.e. the pulp and the external environment of the oral cavity) are separated by a filter (i.e. the tubular dentine), a concentration gradient will be seen for agents in those two environments. Diffusion occurs from the most concentrated to the least concentrated milieu in order to equilibrate the concentrations. In the case of teeth, the presence of bacteria at high concentration in the saliva will lead to their passive diffusion to the sterile pulp parenchyma. Nevertheless, the relatively large diameter of bacteria relative to the diameters of tubules is an obstacle to their movement through the tubules, although toxins and other molecules can still readily pass through.
- The phenomenon of intra-pulpal pressure, which is greater than that external to the tooth: physiologically, the pressure in the pulp relative to that outside the tooth is such that there tends to be an outward flow of fluid if open tubules are exposed, and thus limits the risk of contamination from inward diffusion (Fig 5). Fluid mechanics represents a complex science, especially in the context of the irregular architecture within a dentinal tubule, but this outward pressure in the tubule ensures that the tubular dentine matrix does not act as a simple permeable sieve.

The greater pressure intra-pulpally is directly relevant to clinical procedures in restorative dentistry. In the bonding process, it is recommended to 'dry but not desiccate' the dentinal surface. It is worth noting that a few seconds after drying, the dentinal surface is wet again. At this stage, it is not recommended to further dry the surface otherwise desiccation of the dentine may occur, causing unnecessary damage to the pulp, which would inevitably lead to post-surgical pain. New generations of adhesives take this into account and tolerate the presence of moisture in the dentine to ensure optimal adhesion.

Dentinal permeability is an unavoidable factor to take into account in therapeutic procedures in den-

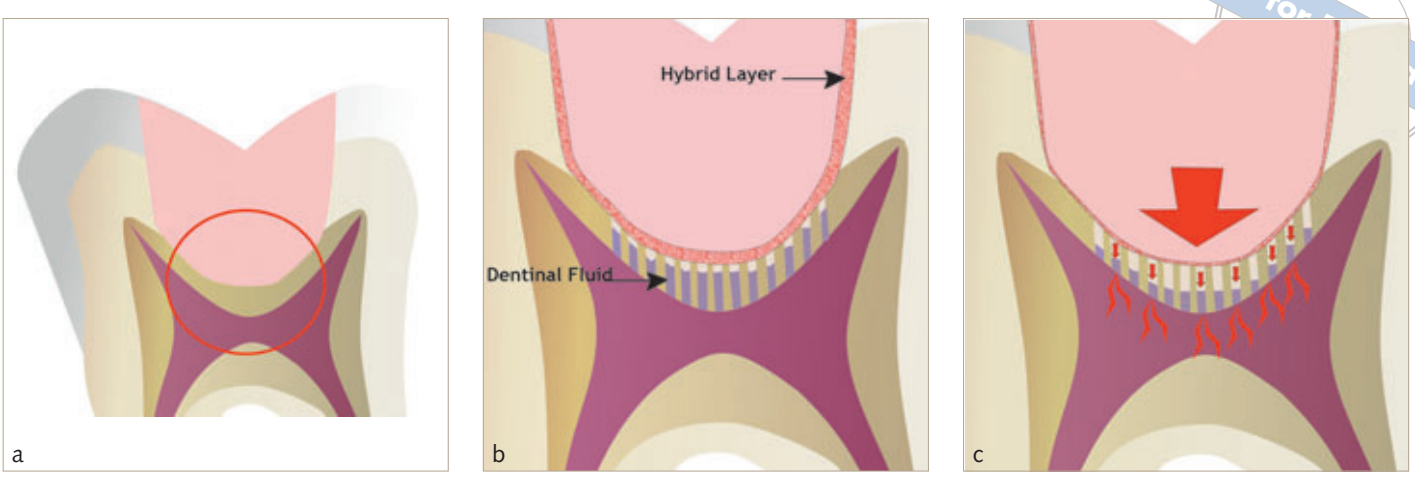

Fig 6 The principle of Brännström's hydrodynamic theory: (a) the application of forces on the filling can lead to a displacement of the dentinal fluid into the tubuli, and create an over-pressure in the pulp, which can cause post-surgical discomfort (b and c).

tistry. Surgical intervention with teeth will inevitably cause some damage to the pulp; the responding defence processes are numerous and complex and their interplay are not fully understood. Nevertheless, they are the basis of the healing process, and post-surgical pain is sometimes encountered after even conservative treatment. It is necessary to recognise the defence processes in order to optimally manage the post-treatment discomforts.

The pulpal hydrodynamic theory[25] probably provides the best understanding of pulpal pain and helps to explain how one tooth can become very sensitive to cold after a dentinal exposure, whereas other teeth in the same mouth remain painless, or why for experimental purposes, application of a few drops of sugar solution can provoke violent pain whereas patients can tolerate much more aggressive stimuli on other teeth? On the basis of histological and physiological observations, Brännström demonstrated that dentinal sensitivity is closely associated with very fast and brief movements of dentinal fluid inside the tubules, which may trigger responses within the vascular and neural structures at the pulpal level.

Since Brännström's seminal publication, new theories have been proposed to extend the understanding of dentinal sensitivity, such as the possibility of partial innervation of the tubules. Recent findings suggest the possible existence of sensory properties for the odontoblast and its role in sensory transmission of stimuli to the tooth is a topic of much study[26,27].

Although the precise mechanisms of pain transmission in teeth remain to be fully elucidated, present knowledge already allows understanding of some of the clinical responses encountered in day-to-day practice. It is quite common to find patients complaining of pain after they had received a bonded inlay using an indirect technique. Sensitivity is mainly encountered while chewing and can be explained by the hydrodynamic theory. When chewing, some bondings have a 'shock absorber' effect owing to their viscoelastic properties. During each micro-movement, fluid movements in the tubules and in the hybrid layer are triggered and may be responsible for the extra external pressure being exerted on the pulp (Fig 6). Thus, acute pain can be the result of routine restorative treatment.

Movement of the dentinal fluid may explain the peculiar pain described by patients presenting with a root crack or fracture. This pain is considered as peculiar as it is not a reaction to pressure, but it is felt during relaxation (opening). In the case of a cleft, the pressure within the tooth during occlusion causes micro-movements and tends to separate the two edges of the cleft; therefore, the pulpal interstitial fluid tends to invade the space created. When pressure is released, the two dentinal edges will close up, thus creating increased intra-pulpal pressure resulting in very sharp and transient pain. Therefore, the appropriate treatment for such pathology is to retain the edge gap and provide a tooth covering (for example, with a crown). In this case, endodontic treatment has little merit.

Consequences of new technological developments

Knowledge of the dentine-pulp complex provides an important foundation for a new era of dentistry with a biological focus. For example, the concept of cavity disinfection is of primary importance to prevent bacterial penetration and diffusion of toxins beneath restorations and this is being exploited in the development of new restorative products. Bonding systems have now been developed, which aim to complement the adhesive properties with antibacterial molecules to control residual and recurrent caries. Clearfil™ Protect Bond (Kuraray, Frankfurt, Germany), in which 12-methacryloyloxy-dodecylpyridinium bromide (MDPB) has been added to the bonding agent, is a relevant example of this new generation of products. New research is underway to consider the addition of other molecules, which would play a role in stimulating pulpal healing.

Towards a biological approach

The concept of vital pulp therapy is one that focuses on using biological principles to maintain pulp vitality. This requires an understanding of the many pathophysiological processes of the dentine-pulp complex and the development of new materials for clinical use, which exploit the pulpal healing processes and better mimic the physiological tissues being restored. However, this evolution of new therapeutic principles within conservative dentistry dictates that techniques should be developed in which new materials are used in the context of minimising damage to the dental tissues during restorative preparation.

The gradual replacement of amalgams by composites, which are considered to have better aesthetic properties in coronal restorations has been justified in part by the toxicity of the former both to the patient and environment, although resins are far from being inert themselves. However, adhesive restorations have a biological advantage in minimising the amount of dental tissue needing to be removed during preparation. Apart from the biomechanical advantages of maximal retention of dental tissue, injury to the tissues is minimised and diffusion pathways to the pulp are reduced with the decreased number of open tubules. The improvement in the long-term prognosis offered by these approaches is evident[28]. Therefore, minimalist dentistry, also called 'non-invasive' dentistry or Minimal Intervention Therapy, has significant potential[8,29].

Pulpal responses to injury

Progress in tissue regeneration has enabled researchers to understand better how the odontoblasts and, more generally, the pulp react after an injury. The tertiary dentine secreted in the absence of pulpal exposure is commonly reactionary in nature and helps to both restore the structural integrity of the tooth and increase the distance between the injurious agent and the pulp. After the initial secretion and development of the primary dentine, the secretory odontoblast seems to fall into a semi-quiescent, semi-inactive state during which it continues its secretory activity but at a much slower rate. This process of secondary dentinogenesis is responsible for the gradual reduction in size of the pulp chamber, although the molecular control of the odontoblasts responsible for the decrease in activity remains to be elucidated.

During injury to the tooth (caries, trauma or wear), a cascade of pulpal responses will be initiated. The exquisite regenerative or healing potential of the pulp though means that transient histological changes do not necessarily lead to clinically significant manifestations[30]. Depending on the nature of the injury (whether it is brief or prolonged, or low or high intensity), the scope of the pulpal responses will likely differ. Injury of weak or moderate intensity will often be resolved by a brief inflammatory response followed by reactionary dentinogenesis. With injury of a greater intensity, odontoblast death may well ensue (e.g. deep caries or severe trauma), and as long as inflammation does not become uncontrolled, differentiation of a new generation of odontoblast-like cells may occur leading to dentine bridge formation at sites of exposure: this process is called reparative dentinogenesis (Fig 7).

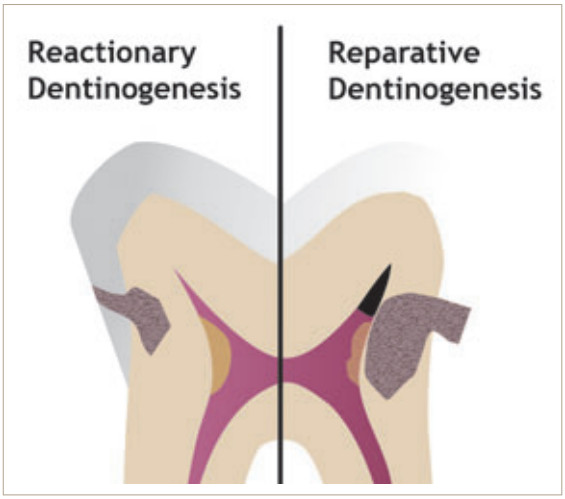

Fig 7 The two kinds of tertiary dentinogenesis.

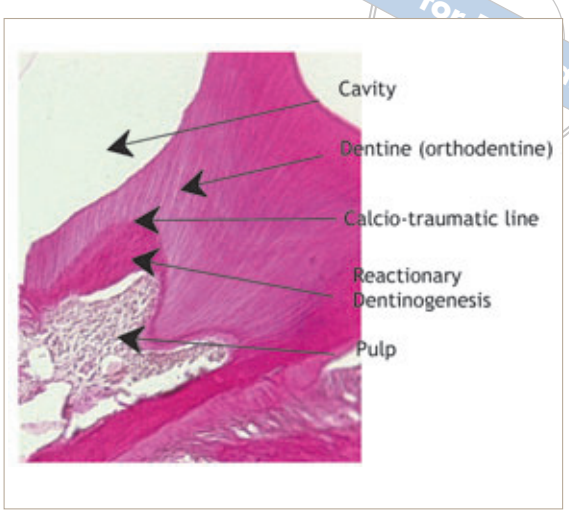

Fig 8 Frontal histological section of a mouse molar which has been treated with a coronary filling. The staining of reactionary dentinogenesis is more pronounced than the other dentine.

Reactionary dentinogenesis

After injury, odontoblasts abandon their quiescent state and begin to secrete again at a faster pace depositing reactionary dentine. Histologically, the calciotraumatic line signals the beginning of this new activity in the dentine matrix (Fig 8). Importantly, because the same cells are responsible for secretion of the reactionary dentine, tubular continuity is seen together with consequent maintenance of dentine permeability. This clearly has consequences in regard to the choice of restorative material, which should not allow leaching of any cytotoxic components.

Although still poorly understood, it can be postulated that the molecular control processes that are responsible for switching off the odontoblast during the change from primary to secondary dentinogenesis may be reversed to stimulate the odontoblasts again. Decryption of the phenomenon of cell reactivation is necessary, and will allow development of new therapeutics that control cell activity.

It is also important to consider the nature of the signalling process between the injurious agent and the odontoblasts. Bacteria and their toxins are key candidates in the direct stimulation of odontoblasts during caries[31]. Also, lipopolysaccharides and other bacterial toxins initiate intra-pulpal inflammatory processes, but other signalling processes may also be critical in the overall balance of pulpal cell responses leading to healing and tissue regeneration[32,33].

Dentine is a mineralised connective tissue rich in collagen, but it also contains trace amounts of very potent bio-active molecules including cytokines and growth factors, which are sequestered within the matrix during the mineralisation process and become essentially fossilised therein. During the decay process, demineralisation of the tissue is accompanied by the release of these molecules, which were initially fossilised[34]. In this pool of substances, many growth factors can be found, especially those of the TGF-β family[35,36]. These growth factors have a variety of cell signalling properties and act at very low concentrations. Once liberated, these factors traverse the tubules to the pulp and induce various cellular responses, including activation of the odontoblasts[37]. Once stimulated, these formerly quiescent cells enter an active state, and secrete tertiary reactionary dentine (Fig 9).

From this concept, it is possible to imagine opportunities for therapeutic stimulation, inducing a targeted release of these proteins. For example, cleaning the cavity with EDTA solution, which is well known for its ability to dissolve the mineral phase, would be a potential way to liberate growth factors and to induce the stimulation of odontoblasts[38-40]. Etching with orthophosphoric acid, used for conditioning the dentine in bonding

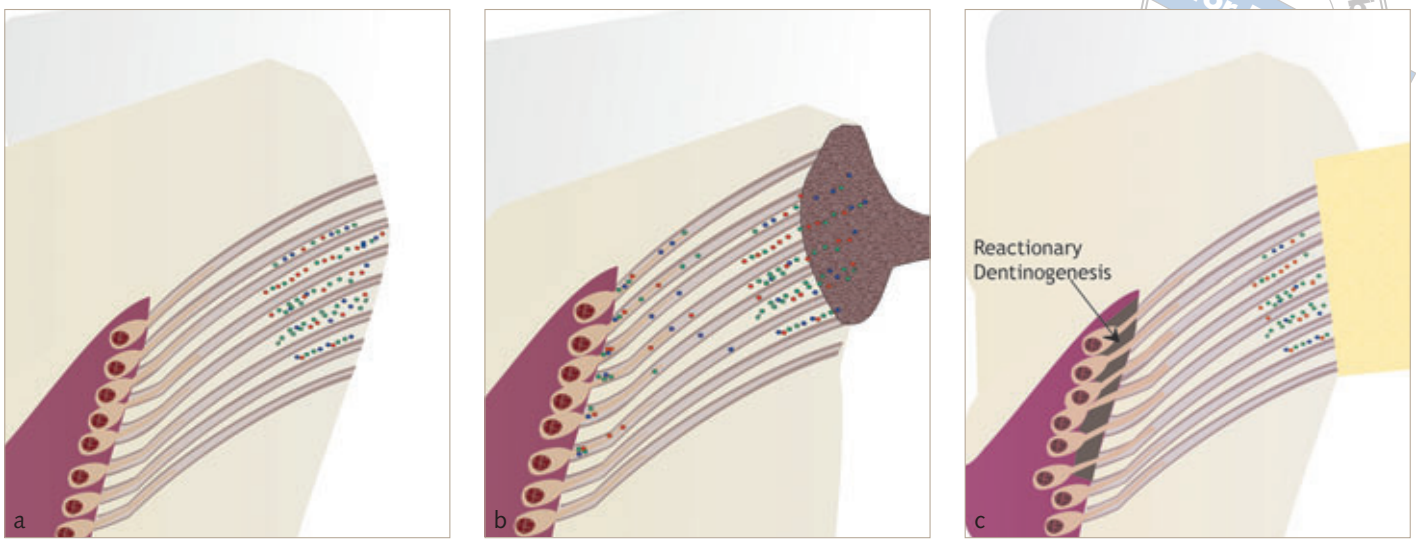

Fig 9 Numerous matrix proteins are fossilised in the collagen matrix of the dentine during the mineralisation process: (a) these factors are released by the dissolution of the mineral matrix (whether pathological or therapeutic); (b) they join the odontoblast layer via the tubules; and (c) nowadays, they are considered to be a potential signalling pathway in the dentine pulp healing process.

procedures, also promotes demineralisation of the dentine and liberation of biological factors. Other products that are presently in the dentist's therapeutic arsenal might come back into favour with new indications. For a long time, calcium hydroxide had been used as a protective lining, especially beneath amalgam fillings, but has more recently fallen out of favour. Nevertheless, this material certainly has the ability to release components from the dentine, including growth factors[41]. Unlike the chelating agents which only have brief contact with the dentine, calcium hydroxide remains in place beneath restorations and favours a gentle and continued dissolution, thus releasing growth factors; its action is prolonged and potentially controllable depending on the form of the product.

More recently, the action of mineral trioxide aggregate (ProRoot™ MTA, Dentsply Maillefer, Ballaigues, Switzerland) in releasing growth factors has been demonstrated[42], although the amounts released differ from those liberated by calcium hydroxide. These differences are interesting because they might explain the differences in the behaviour of both materials. If the processes underlying this action were better understood, then the use of such linings under coronal restorations might readily find favour again, soon thereby providing a new focus for research in bioactive materials.

Reparative dentinogenesis

Odontoblasts are the only cells that secrete dentine. If they suffer injury, the formation of a dentine bridge at sites of pulpal exposure is still possible, providing that new odontoblast-like cells are 'available' in the area. Conventionally, wound healing involves cell migration from the edge of the injury and with cell division, the superficial cells move to the centre of the injury regenerating and repairing the tissue and allowing its reorganisation.

Odontoblasts are differentiated post-mitotic cells, and are unable to divide to produce new secretory cells. When these cells are lost, another form of replacement occurs involving stem or progenitor cells resident in the pulp[43,44] (Fig 10). After pulp exposure, and after placement of an appropriate material, a dentine bridge is formed in a few weeks (Fig 11) by 'new odontoblast-like cells'. The origin of these odontoblast-like cells in the dentine pulp healing process is not clearly established. Several authors believe that these processes are likely to be the same as those involved in tooth development[45]; however, the origin of these cells is still unclear and an origin from outside the tooth via the vasculature cannot be excluded.

Various clinical procedures for repair of a pulp exposure have been proposed and calcium hydroxide has long been considered as the mate-

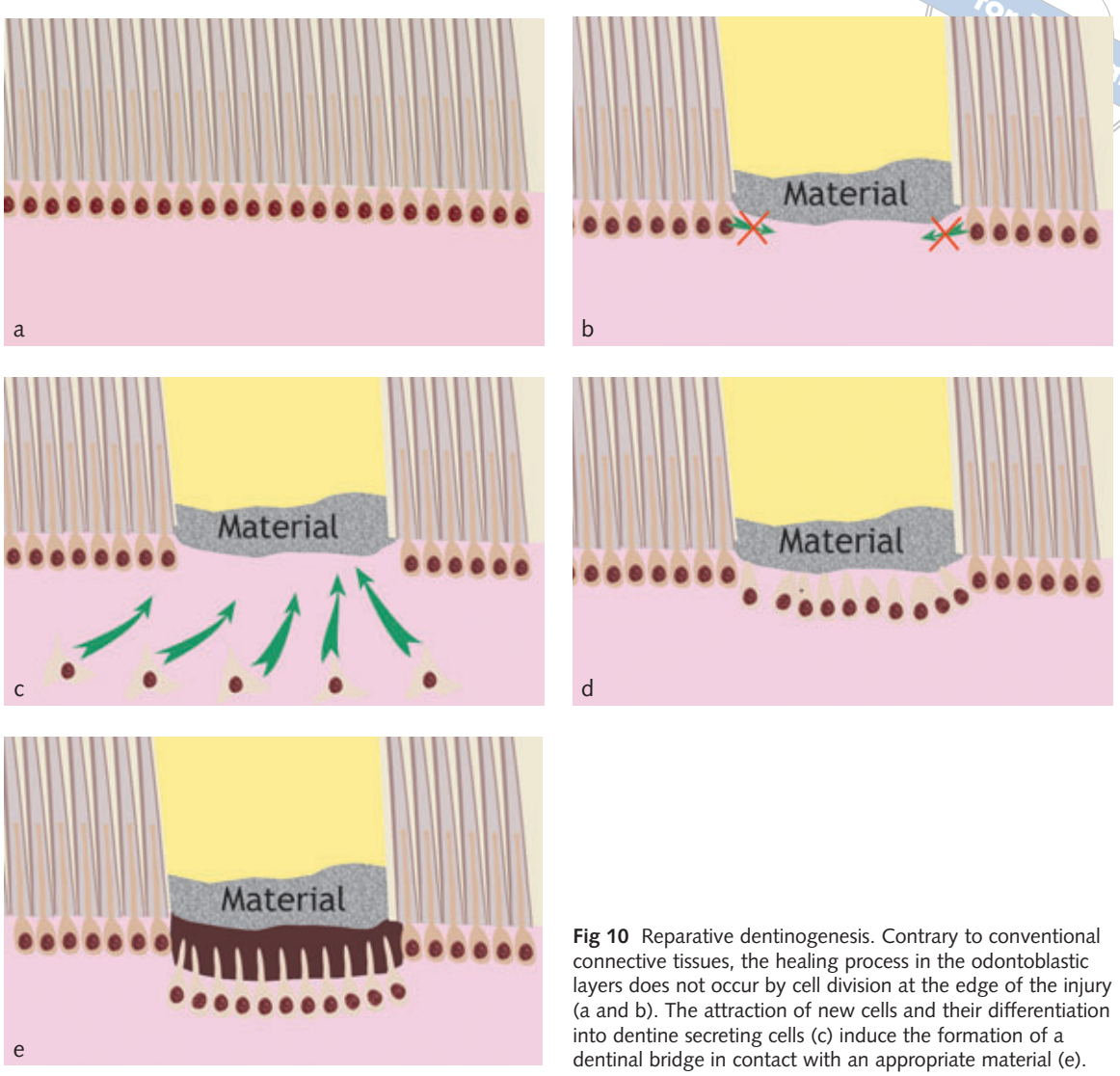

Fig 10 Reparative dentinogenesis. Contrary to conventional connective tissues, the healing process in the odontoblastic layers does not occur by cell division at the edge of the injury (a and b). The attraction of new cells and their differentiation into dentine secreting cells (c) induce the formation of a dentinal bridge in contact with an appropriate material (e).

rial of choice. However, the poor quality of the dentinal bridge and its lack of a hermetic seal are potential sources of failure with this material. There is still a lack of trust in pulp capping due to the inability to predict prognosis and practitioners will frequently remove the pulp of the tooth rather than try to keep it alive. Several *in vivo* studies have shown that MTA induces the creation of a dentine bridge of good quality, with an effective hermetic seal, which can merge with the dentinal walls at the edge of the defect[46]. The advantage of the quality of the dentine bridge obtained with MTA versus calcium hydroxide as a pulp capping agent has been recently demonstrated in a randomised clinical trial[47]. Although several authors have proposed capping the pulp with the filling material itself, there is no histological evidence that this can induce dentine bridge formation. Even if, clinically, the results seem to be satisfactory, this approach cannot be considered to be reliable in the long term. This example perhaps illustrates what distinguishes the clinician from the scientist. Whereas clinicians focus on the sealing properties of materials and the prevention of any bacterial or fluid leakage, scientists are more interested in the bio-activity of these materials and their ability to induce a wound healing response. Both are complementary, and it is essential that these two approaches are brought together. For the future, existing materials will provide an important stepping stone towards the development of more specific biomolecules as dentistry develops in a biotechnological direction and may encourage greater practitioner uptake of such approaches.

The clinical consequences

Translation of biological knowledge into the clinical setting can simply involve the adoption of a more biological frame of mind during treatment planning, but it is not always that easy. For instance, the impact of tooth structure on biological events can be fairly readily appreciated, such as in the thickness of the residual dentine separating a cavity from the pulp which can be an important factor in its protection. In deeper cavities, where the thickness of the cavity floor is less than 0.5 mm, the number and the height of the 'opened' tubules is such that the communication with the pulp is possibly comparable to that of an actual cavity exposure[48]. In contrast, clinically, it can be very difficult, if not impossible, to know the inflammatory status of the pulp, and the cytological state of the odontoblasts since pulpal histology does not necessarily correlate well with clinical presentation. This makes it very difficult to effectively plan treatment in such situations and there is an urgent need for more reliable means of diagnosing of the activity of carious lesions and the level of pulpal inflammation. While thermal or electrical tests provide information on the innervation of the pulp, there is a need for tests that evaluate the vasculature and, in particular, the effects thereon of any inflammation that may be present. Unfortunately, the hard tissue shell enclosing the pulp constrains the use of techniques such as Laser Doppler scanning or scintigraphy, which provide valuable information in other medical specialities.

Conclusions

The application of biotechnology to the field of restorative dentistry has immense potential. For many years, there has been a gap between basic research and dental practice, but the recent arrival of new adhesive systems containing antibacterial molecules provide a first step towards biological treatment of the pulp. It is important that research in dentistry goes beyond the study of alloys, sizes of drills, disinfecting power of solutions, or mechanical resistance and embraces the opportunities offered by biotechnology, which will change the perception and practice of dentistry.

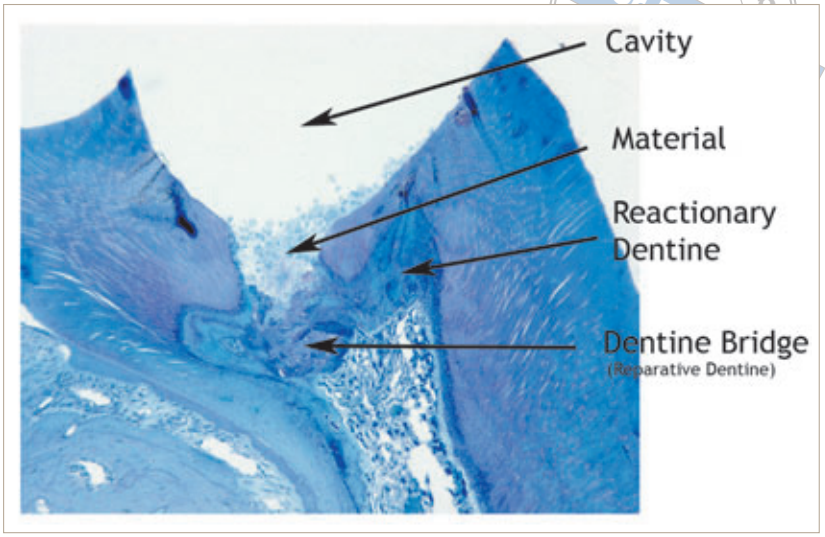

Fig 11 Dentine bridge 5 weeks after pulp capping with MTA of a mouse first molar (frontal semi-thin section, x50 magnification, methylene blue-azur II).

Above all, the training of dentists will have to adapt to these new approaches to dental treatment. Progress has been made in the last ten years in aesthetic management and restoration of functionality. Very soon, the modern dentist will have to be aware that biological approaches, rather than the purely mechanical functions, will add a new dimension to restorative dentistry.

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