Advances since the paper by Zander and Glass (1949) on the pursuit of healing methods for pulpal exposures: Historical perspectives

Gunnar Bergenholtz, DDS, Odont Dr (PhD),[a] Göteborg, Sweden
SAHLGRENSKA ACADEMY, GÖTEBORG UNIVERSITY

Clinical observations and experimental studies in humans and laboratory animals have demonstrated that healing and repair of pulpal exposures by caries, trauma, or iatrogenic causes are possible with a variety of wound treatment methods. Yet clinical trials have shown that predictable long-term pulp tissue preservation may be an elusive goal and has led to doubts about pulp capping and pulpotomy as valid clinical procedures. Nevertheless substantial knowledge has accumulated over the years on the mechanisms and the treatment factors that are important to promote/support continued vital pulp functions. This article highlights some key contributions to our current knowledge base, which have come to light during the more than 50 years since a pioneering experimental study by Zander and Glass was published in the Triple O journal. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:S102-8)**

In Volume 2 of the 1949 Triple O, a most remarkable and trend-setting paper appeared.[1] The authors were Helmuth A Zander (later to become a legendary periodontist) and RL Glass from Tufts College Dental School in Boston. Zander and Glass had applied liquefied phenol of 90% strength directly onto exposed pulps of human teeth prior to capping the wounds with either a thick creamy paste of calcium hydroxide in water (CH) or zinc-oxide eugenol cement (ZOE). The idea of the study was to follow, by histology, the healing pattern and thereby observe the extent to which it would result in a seal of the exposure sites with new dentin. They used a new approach at the time to conceive of the dynamics of the wound healing events by extracting the experimental teeth after various time periods following the initiation of the test procedures. This study design, also employed by the authors in another paper the same year,[2] came to stand as a norm for experimental pulpal research. Also noteworthy is that cluster effects were balanced by employing 2 teeth in each patient by which the healing responses to both capping measures could be compared in one and the same individual.

But what would the rationale be for adding such a tissue-destructive agent as phenol to the pulpal wounds? Already calcium hydroxide was known to be severely caustic and thus potentially harmful. Yet surprisingly, prior clinical[3] and histologic observations[4] had indicated that CH promoted pulpal healing and hard tissue repair, something that most other materials previously

tested had been unable to. The background to the study was as follows.

In the JDR report[2] they had observed rapid healing of pulpal wounds cut in healthy young bicuspids capped with CH, whereas ZOE had maintained inflammatory lesions throughout the experimental period. Both dentin repair and the development of a new continuous layer of odontoblasts were unsuccessful with ZOE instead of capping with CH. Another conspicuous finding was that CH caused a limited tissue necrosis, whereas the remainder of the pulp retained normal tissue structures. The zone of necrosis was distinct already 24 hrs after capping and appeared to persist. Adjacent to the tissue necrosis they further noted that "a well-defined zone of new dentin, with an adherent layer of odontoblasts . . . both continuous with the walls of the pulpal chamber" had emerged already at 4 weeks after the exposure. In the Triple O paper they hypothesized that the tissue necrosis was crucial to the healing response. A previous study had reported good clinical follow-up results following "phenolizing" pulps prior to capping decid-uous teeth,[5] and another paper had advocated phenol for disinfection of the wound site following caries excavation.[6] Zander and Glass reasoned that it could well be that the calcium hydroxide paste per se was not the critical factor but more so the tissue necrosis it induced. That prompted them to include phenol to cauterize the exposed pulps to be capped by either CH or ZOE and thereby explore, for the first time, a potential mechanism inherent in the healing process of the pulp to a direct exposure.

The findings revealed that a 2-minute application of phenol, although causing an initial but superficial tissue necrosis, did not change the pattern of tissue responses for either capping mode. Although pulps remained vital, ZOE-capped pulps continued to display inflammation

and absence of hard tissue repair regardless of being "phenolized." Similarly, phenol pretreatment of CH-capped wounds had no influence in either direction, ie, it neither enhanced nor impaired the healing response. Zander and Glass interpreted their data to show that an alkaline environment and possibly access to calcium ions is beneficial to pulpal wound healing.

Although Hermann[3] often is recognized as the inventor of the treatment method, the research presented by Zander and Glass[1,2] laid a most important ground for CH as a vehicle for capping clinical exposures of the pulp. No doubt it spurred a tremendous interest to exploring not only why CH works but also the extent to which it would function in the clinical setting. An abundance of experimental studies and clinical trials followed and generated strong support for CH. However, not a few clinical failures were reported. This led to doubts about pulp capping and pulpotomy with CH as valid clinical procedures.[7] The phenomenon of pulpal healing and hard tissue repair following application of a highly caustic agent such as CH was nevertheless intriguing to many experimentalists. As a result, considerable knowledge has been gained over the years on the factors that may impact healing/repair of pulpal wounds. It is the purpose of this article to highlight some of the key contributions to current knowledge, generated over the more than 50 years since the paper by Zander and Glass[1] was published.

UNDERSTANDING THE BASIS FOR THE FORMATION OF DENTIN BARRIERS
Role of calcium ions

The ambiguous mechanism by which calcium hydroxide promotes dentin repair of pulpal wounds attracted the interest of 2 dental scientists at the Hebrew University of Hadassah School of Dentistry in Jerusalem, Sara Pisanti and Ino Sciacy. Three factors of importance for the initiation of the process had been proposed at the time: (1) the wound dressing per se by providing calcium to the build up of the new dentin barrier, (2) the neutralizing effect of CH on the acidity produced during the wound healing process, and (3) the tissue coagulating effect, as examined by Zander and Glass.[1]

In 2 papers[8,9] they took on the issue of the origin of calcium in the repair process. In a first experiment[8] they mixed CH powder with radioactively labeled calcium in calcium chloride and applied the paste onto mechanically exposed pulps in dogs. The tissue responses were observed over time by both conventional histology and autoradiography of ground sections cut longitudinally through the exposure sites. In all instances radioactivity could be traced to the capping material and none of the healing sites displayed evidence of incorporation of

^{45}Ca in the newly formed dentin bridge. In a second report[9] they copied the experimental set up of the first paper, except that the dogs were given ^{45}Ca intravenously instead of labeling the capping material. Now, radioactivity was observed in the bridge. Pisanti and Sciacy[9] concluded that other mechanisms than calcium ions, deriving from the capping material, must be instrumental in promoting the combined pulpal healing and dentin repair.

Role of the induced tissue necrosis

The notion, initiated by Zander and Glass,[1] that a superficial pulp tissue necrosis was crucial and served as a stimulus for the initiation of the hard tissue repair process remained firm for years. Ulla Schröder, in her 1973 thesis,[10] not only confirmed the findings of Zander and Glass[1,2] and others,[11-13] but also detailed the initial events of its formation.

She carried out experiments in lower healthy premolars of young individuals to be extracted for orthodontic reasons. The study protocol included a gentle technique for cutting into the pulpal tissue developed by Granath and Hagman,[14] later advocated by Cvek for partial pulpotomies in clinical cases.[15,16] By operating an end-cutting diamond in a high-speed engine under rigorous irrigation with sterile saline, a minimally lacerated wound surface was established at the level of the cementoenamel junction. Following a strict aseptic protocol, CH was subsequently placed and the tissue responses were recorded over time mainly by conventional histologic methods and light microscopy. Also transmission electron microscopy[17] and scanning electron microscopy[18] were used to study the repair process. Very-short-term observations revealed that the tissue necrosis assumed a multilayered appearance. Within 3 hours a clear demarcation of the coagulated tissue was discernible against the underlying normal pulp. During the subsequent days inflammatory cell infiltrates accumulated to a slight or moderate degree and later disappeared. Mineral deposits were seen 1 month after exposure as spherical globules developing in and near the coagulated tissue. The rising hard tissue had an irregular appearance and was fibrous and atubular in nature, similar to mantle dentin. This early formation of hard tissue in ectopic pulpal sites was later termed fibrodentin.[19] Nearby a predentin-like tissue had also emerged, adjacent to which elongated odontoblast-like cells, seemingly involved in matrix production, were lined up. This part of the mineralization process displayed tubules, although not in as a regular pattern as that of normal dentin.

Although circumstantial, the findings were interpreted to support the view that CH triggers a key stimulus for the wound healing process by virtue of the

induced tissue coagulation. The release of hydroxyl ions into the tissue from the capping material was considered the critical factor. The significant role ascribed the tissue necrosis, however, became challenged when experimental pulp capping with hard-setting CH compounds resulted in matrix and hard tissue depositions directly at the capping material−pulp tissue interface without an intervening zone of necrosis.[20-22] These reports, based on light-microscopic observations, were later confirmed by ultrastructural analysis.[23] The reason for the absent tissue necrosis is explained by the lower pH of these compounds relative to CH water slurry. Thus, less tissue damage would ensue. However, the findings did not refute the belief that CH generated a stimulus that was vital for the hard tissue repair process to be initiated.

Impact of the surgical trauma

A factor that was not generally controlled for in the early studies was the impact of the surgical trauma. It would not have been unreasonable to assume that the injury per se, caused by the mechanical exposure, and the associated release of inflammatory mediators would generate sufficient stimuli to promote the dentin repair process. After all, the classic studies in germ-free rats by Kakehashi et al[24,25] had demonstrated that without wound treatment, dentin repair occurred in germ-free animals but was absent in conventional rats, thus demonstrating the crucial negative influence of wound infection. In their first paper,[24] the wounds became covered by debris, hair, and food particles and this matter could have generated a stimulating effect. Nonetheless, in their second paper[25] they attained the same kind of repair response to a carrier substance loaded with or without prednisolone.

The assumption that the wound injury per se causes a sufficient drive for dentinal repair in humans and nonhuman primates, however, was proven invalid by experiments in which pulpal wounds were covered with thin polytetrafluoroethylene (Teflon) disks.[14,26,27] Heys et al,[27] at the University of Michigan in Ann Arbor, examined the tissue responses from the time of the immediate mechanical exposure of healthy pulps in the monkey and onward over 8 weeks. While the pattern of healing events appeared identical to that of capping with a hard-set CH compound, the final differentiation of repairing odontoblasts failed to occur against the Teflon material. By 8 weeks after exposure, inflammatory responses and formation of blood clots were resolved. But only pulpal fibroblasts had migrated to the wound site and were seen aligned parallel to and against the Teflon surface. Most importantly, no deposition of hard tissue had emerged, which would normally be the case at that point in time against a CH-containing compound. A similar observation had also been observed against

amalgam after 3 weeks of capping.[28] Pulp tissue in that study was completely restored and was without inflammatory infiltrates, suggesting that neither the wound injury nor the amalgam used had been able to initiate reparative dentinogenesis.

The fact that some sort of stimulus, in addition to the mere wound injury, is required for starting up the dentin repair process became obvious from several key observations. For example, Cvek et al[26] applied CH onto fresh pulpal exposures in the monkey for only 10 minutes and then, following its removal, covered the exposure sites with Teflon and sealed the access cavity with a fortified ZOE. By that measure only, hard tissue repair of the same extent and character developed as that of continual placement of CH over 12 weeks. The authors, together with several other investigators, also demonstrated that dentin repair of pulpal exposures by no means is unique to substances based on release of calcium or hydroxyl ions or both. In fact, repair responses similar to that of CH were reported for a plethora of other materials including, for example, polycarboxylate cement,[29] cyanoacrylate,[30,26] bioactive ceramics,[31] silicate cement,[28] zinc phosphate cement,[28] resin-composites,[28,32,33] and recently a compound based on Portland cement termed mineral trioxide aggregate (MTA).[34] Crucial to these results was the extent to which wound infection had been prevented from interfering with the healing process and that the materials per se did not maintain a pulpal inflammatory lesion, which was the obvious case for ZOE. In the experimental studies referred to, various efforts were undertaken to control leakage of bacterial elements along the surface restoration.

To conclude, numerous experimental studies carried out over more than 50 years have demonstrated that dentinal repair of pulpal wounds is possible with a variety of dental restorative materials. An important proviso has been that pulpal healing occurs in an environment free from wound infection and that the materials, after the initial exposure to the pulp, become relatively innocuous. However, the histologic observations of hard tissue repair led to much speculation as to the mechanisms involved in the wound healing process and especially regarding the elements that may promote the recruitment and differentiation of repairing odontoblasts. The alleged role of tissue necrosis is just 1 example. It must be recognized that these interpretations were done in a time when molecular biology was a relatively underdeveloped field. Obviously the molecular events involved in regulating the differentiation of specialized cells responsible for dentinal repair are now in the process of becoming unraveled (see further below). Yet, the interest CH generated, because of its beneficial effects, must be acknowledged as an important

impetus for the continued efforts undertaken to develop improved treatment methods for capping exposed dental pulps.

UNRESOLVED ISSUES

Failure to preserve vital pulp functions in CH-capped teeth on a long- term basis has been an important observation in some clinical follow-up studies.[12,35-36] Although early pulpal deaths, occurring within months or the first year subsequent to treatment, may be related to the effects of a primary infection, emergence of late failures is likely to be caused by re-infection of the pulp. Pathways for penetration of infectious elements are often present in the so-called dentin bridge. While appearing solid macroscopically or by radiography, large nonmineralized areas may permeate it.[12,37-39,26] Should infectious threats emerge such channels are likely to serve as avenues for dissemination of bacteria and bacterial breakdown-products to the pulp.

In an extensive follow-up of clinical cases, assembled in a general practice, of which histological examinations were carried out of 81 teeth, Hilding Nyborg[12] observed that the barrier to CH assumed many different configurations. It sometimes had a blister-like appearance. In other cases it also extended deep into the pulp and interfered greatly with the tissue volume. He speculated that these patterns were a result of tissue changes induced prior to capping. As many of the cases were prompted by a caries pulp exposure, it is reasonable to assume that the new hard tissue was triggered not only by the capping material *per se* but also by the process leading to healing of the inflammatory lesion. Hence following attempts to attain healing and repair of an infected and inflamed pulp, tissue changes are to be expected that may impair its long-term function (see also Bergenholtz and Spångberg[7]).

Clearly experimental studies employing healthy teeth are unable to control for the influence of wound infection and the associated inflammatory processes. Although animal models have been developed to mimic these effects,[28,40-45] modeling of caries-induced pulpitis for wound healing studies is difficult because of the variations in infectious load and tissue reactions occurring in clinical cases.[46] Therefore, much knowledge has yet to be gained on how inflammation in the pulp is regulated and how it can be monitored to optimize the outcome of various wound treatment approaches.[7]

Porosities and defects in the bridge have also been described for capping of healthy, previously noninjured pulps in humans and nonhuman primates.[37-39,26] The fact that such defects are weak spots and give impaired protection to secondary infections from either breakdown of the surface restoration or along its margins was demonstrated by Cox et al.[43] In a monkey model, pulpal wounds were treated with a hard-set CH compound either immediately or after prior exposure to the oral environment for 24 hours and one week to induce a pulpal infection. Regardless, tissue responses were similar. Many pulps became properly healed whereas others either displayed severe inflammation or complete tissue necrosis in spite of the fact that the wound site had been closed with hard tissue. The adverse tissue responses were observed after a follow-up period of 1-2 years and correlated well with presence of stainable bacteria in the capping material as well as in the defects of the bridge. Already Zander[4] had reported on "complete pulp degeneration" in a clinical case, where dentin repair had covered the pulpal exposure. However, he did not recognize the potential of it being caused by bacterial leakage.

Collectively, these observations have indicated a need to understand how inflammation in the pulp shall be managed to avoid adverse tissue responses following conservative treatment of pulpal exposures. They also show that repair of pulpal wounds with hard tissue not necessarily will result in a homogenous protective barrier of a function similar to primary dentin. Therefore, treatment methods should be sought that ideally result in dentin regeneration rather than hard tissue repair of unpredictable quality.

TRENDS IN CURRENT RESEARCH

Recent approaches to pulpal wound treatment have essentially followed 2 lines. One has continued the traditional path and has sought to find improved synthetic materials that provide better seals than CH. Another line has taken a biologic approach and explores the molecular and cellular basis for pulp tissue regeneration with the hope to identify a biologically based strategy for treatment of clinical exposures.[47,48]

Improving the wound barrier by nonbiologic materials

Primarily, 2 nonbiologic materials have recently been proposed for clinical usage: (1) adhesive restoratives based on resin composites and (2) MTA. It goes without saying that if a synthetic material provides a long-lasting seal of the wound site, there would be no need for additional hard tissue coverage as long as the underlying pulp maintains a healthy state. Interestingly, the fact that resin composites were found to stimulate dentin repair of exposed noninflamed pulps in laboratory animals[31-33,49] gave rise to their popularity as pulp capping agents. Onoe,[32] for example, provided evidence that the barrier became more solid than that formed in response to CH and therefore felt that resin composites had an edge. However, experimental observations in healthy human teeth have not been equally encouraging. While pulps

maintained evidence of vital functions, reports were given that capping with resin composites often does not result in hard tissue repair.[50-52] That in itself would not be of concern as long as the wound site integrity is maintained. But findings that healing was also delayed and resulted in lingering inflammatory infiltrates and signs of foreign body responses[53,50,51] have led to cautioning the clinical use of resin composites for pulp capping purposes.[52,54] The risk for deficient bonding to the dentin substrate or loss of adhesion over time,[55] resulting in leakage potentials, is an added disadvantage of this type of dental material.[46,52] Furthermore, properly designed clinical trials with adequate long-term follow-ups of pulp capping with resin composites are lacking.

The dominance CH has enjoyed, thus far, as the most preferred agent for clinical treatment of pulpal exposures may be overtaken by MTA. This material, based on Portland cement as the major ingredient, was first conceived for root-end fillings in conjunction with apical surgeries.[56] After being mixed with water it generates, in its unset stage, a rather high pH, which similarly to CH causes cell coagulation.[57] Recent electron probe microanalyses have indicated that lime, silica, and bismuth oxide are predominating compounds in both an original and a modified composition.[58] Its ability to set in a moist environment without undergoing dimensional changes, together with beneficial tissue compatibility in a set stage, has given this material several clinical applications including capping of exposed pulps.[34,59]

The key point events in the responses of the pulp to MTA have been found to be almost identical to those of CH. Tziafas et al[60] observed in a dog model, 3 weeks after capping, depositions of dentin-like tissue adjacent to "a firm osteodentinal zone" to which elongated polarized cells were lined up. Longer observations in dogs have shown consistent pulpal healings with hard tissue barrier formations of substantial thickness.[61] Thus, its composition and the pulp tissue responses it evokes give no reason to believe that the mode of action is different to the one for CH. However, a distinct advantage of MTA, in comparison with CH, is that it sets hard. Therefore, this material is likely to better resist dissolution in tissue fluids and may thus serve as a more dependable barrier than CH in case the subjacent dentin bridge becomes defective. However, experimental studies in humans and clinical trials have yet to show the real clinical potential of this material.

Exploration of the potentials of bioactive molecules

Growing optimism is emerging about the prospect of using biologic macromolecules for treatment of pulpal exposures.[47,48,62,63] These expectations stem from the extraordinary progress reached in recent years on the understanding of the molecular nature of the signals that regulate the differentiation of odontoblasts during tooth morphogenesis.[47,63] Thus far, applications of that knowledge to reparative dentinogenesis have resulted in numerous experimental studies exploring the dentinogenic potential of a variety of biologic molecules (growth factors, enamel and dentin matrix proteins, extracellular matrix molecules) that are active during different phases of tooth development[64-69] —see the excellent review by Michel Goldberg and Anthony Smith.[47] Dentin repair of a varying morphology and extent have generally been reported to occur following application of these molecules onto direct exposures of the pulp in animal teeth. However, in most instances experiments have utilized teeth with healthy pulps and when an inflammatory pulp model was employed, reparative dentinogenesis failed to occur.[70] It is obvious that much fundamental work has yet to be conducted before this new technology will find clinical applications. Yet the prospective is promising. It will be even more exciting when we understand how the effects of these powerful agents can be tempered for control of the healing process so that a homogeneous hard tissue results that will not assume more than a limited portion of the pulpal chamber. An even more daring goal would be to regain the tooth substance that was lost prior to the pulpal exposure.[62] Of course research will also have to address safety aspects, delivery systems, and finally clinical feasibility.[48,71]

CLINICAL INFERENCES

Since the 1949 paper by Zander and Glass[1] pulp biology research has generated a tremendous wealth of knowledge on the behavior and function of the dental pulp under normal and diseased conditions. Given that knowledge and the potentials that stem cell research and gene therapy also may offer, the chances to develop reliable clinical methods for treatment of pulpal exposures have probably never been brighter. Researchers and clinicians together with molecular biologists have now a golden opportunity to address and finally resolve a treatment problem in clinical dentistry that has yet defied a robust solution.

In addition to exploring means by which pulpal tissue can be regenerated and stimulated to predictably heal pulpal exposures, research must also address, as alluded to above, how pulpal infections and associated inflammatory involvements should be managed. After all, the large majority of clinical exposures are related to caries and the treatment often entails infected wounds. Although caries into the pulp may not necessarily be synonymous with an irreversibly inflamed condition, there are no means, as yet, to determine the extent inflammation has

progressed.[7] Thus, capping of pulps that have become exposed in conjunction with a caries excavation procedure will involve a great deal of uncertainly as to the survival potentials of the tissue. Removing caries may per se also worsen the condition by inadvertent transfer of infected debris into the soft tissue. Except for causing an exacerbation of the inflammatory lesion, displaced infected debris may begin a lingering tissue irritation that finally may result in a pulpal breakdown. Together with leakage of bacterial elements from the oral environment along the restoration margins, this mechanism may explain failures of pulp capping not seldomly encountered by clinicians as well as seen after many years of clinical follow-up.[12,35,36]

The risk for pulp capping failure (clinical pulpitis and subsequent pulp necrosis), in either the short or long term, suggests that an indiscriminate use of the capping instrument for the management of pulpal wounds, and especially those by caries, should not be applied. Not even today are there reasons to deviate from the long-held view that pulp capping, or rather partial pulpotomy, as advocated by Miomir Cvek,[15,16] should be restricted to (1) clinically healthy pulps in fairly young individuals, where (2) the case is easily managed and (3) the inflammatory involvement can be deemed minimal as suggested by a bleeding that is easy to stop.[73-76] These procedures should furthermore not be carried out unless (4) the patient can be enrolled in a long-term recall program.[72]

REFERENCES

1. Zander HA, Glass RL. The healing of phenolized pulp exposure. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1949;2: 803-10.
2. Glass RL, Zander HA. Pulp healing. J Dent Res 1949;28:97-107.
3. Hermann BW. Dentinobliteration der Wurzelkanäle nach Behandlung mit Calzium. Zahnärztl Rundschau 1930;39:888-98.
4. Zander HA. Reaction of the pulp to calcium hydroxide. J Dent Res 1939;18:373-9.
5. Rosenstein SN. Pulp capping in deciduous teeth. J Am Dent Assoc 1942;19:1632-43.
6. Easlick RA. Management of pulp exposures in the mixed dentition. J Am Dent Assoc 1943;30:179-87.
7. Bergenholtz G, Spångberg L. Controversies in endodontics. Crit Rev Oral Biol Med 2004;15:99-114.
8. Sciaky I, Pisanti S. Localization of calcium placed over amputated pulps in dog's teeth. J Dent Res 1960;39:1128-32.
9. Pisanti S, Sciaky I. Origin of calcium in the repair wall after pulp exposure in the dog. J Dent Res 1964;43:641-4.
10. Schröder U. Reaction of human dental pulp to experimental pulpotomy and capping with calcium hydroxide. Odontol Rev 1973;24(Suppl 25):5-22.
11. Nyborg H. Healing process in the pulp on capping. Acta Odontol Scand 1955;13(Suppl 16):1-130.
12. Nyborg H. Capping of the pulp. The processes involved and their outcome. A report of the follow-ups of a clinical series. Odontol Tidskr 1958;66:296-364.
13. Langer M, Ulmansky M, Sela J. Behaviour of human dental pulp to Calxyl with and without zinc oxide and eugenol. Arch Oral Biol 1970;15:189-94.
14. Granath L-E, Hagman G. Experimental pulpotomy in human bicuspids with reference to cuttting technique. Acta Odontol Scand 1971;29:155-61.
15. Cvek M. A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. J Endod 1978;4:232-7.
16. Mejàre I, Cvek M. Partial pulpotomy in young permanent teeth with deep carious lesions. Endod Dent Traumatol 1993;9: 238-42.
17. Schröder U, Sundström B. Transmission electron microscopy of tissue changes following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. Odontol Rev 1974;25:57-68.
18. Schröder U, Granath L-E. Scanning electron microscopy of hard tissue barrier following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. Odontol Rev 1972; 23:211-304.
19. Tziafas D. Reparative dentinogenesis. A monograph on the dentinogenic potential of dental pulp. Thessaloniki: University Studio Press; 1997. p. 15.
20. Stanley HR, Lundy T. Dycal therapy for pulp exposures. Oral Surg 1972;34:818-27.
21. Tronstad L. Reaction of the exposed pulp to Dycal treatment. Oral Surg 1974;38:945-53.
22. Fitzgerald M. Cellular mechaniscs of dentinal bridge repair using 3H-thymidine. J Dent Res 1979;58:2198-206.
23. Mjör IA, Dahl E, Cox CF. Healing of pulp exposures: an ultrastructural study. J Oral Pathol Med 1991;20:496-501.
24. Kakehashi S, Stanley HR, Fitzgerald R. Effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1965;20:340-9.
25. Kakehashi S, Stanley HR, Fitzgerald R. The exposed germ-fee pulp: effects of topical corticosteriod medication and restoration. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1969;27: 60-7.
26. Cvek M, Granath L, Cleaton-Jones P, Austin J. Hard tissue barrier formation in pulpotomized monkey teeth capped with cyanoacrylate or calcium hydroxide for 10 and 60 minutes. J Dent Res 1987;66:1166-74.
27. Heys DR, Fitzgerald M, Heys RJ, Chiego DJ. Healing of primate dental pulps capped with Teflon. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1990;69:227-37.
28. Cox CF, Keall CL, Keall HJ, Ostro E, Bergenholtz G. Biocompatibility of surface-sealed dental materials against exposed pulps. J Prosthet Dent 1987;57:1-8.
29. McWalter GM, El-Kafrawy AH, Mitchell DF. Pulp capping in monkeys with a calcium-hydroxide compound, an antibiotic, and a polycarboxylate cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1973;36:90-100.
30. Berkman MD, Cucolo FA, Levin MP, Brunelle LJ. Pulpal repsonse to isobutyl cyanoacrylate in human teeth. J Am Dent Assoc 1971;83:140-5.
31. Oguntebi B, Clark A, Wilson J. Pulp capping with Bioglass and autologous demineralized dentin in miniature swine. J Dent Res 1993;72:484-9.
32. Onoe N. Study on adhesive bonding systems as a direct pulp capping agent. Japan J Conserv Dent 1994;37:429-66.
33. Kitasako Y, Inokoshi S, Tagami J. Effects of direct resin pulp capping techniques on short-term response of mechanically exposed pulps. J Dent 1999;27:257-63.
34. PittFord TR, Torabinejad M, Abedi HR, Backland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp capping material. J Am Dent Assoc 1996;127:491-4.
35. Hörsted P, Söndergaard B, Thylstrup A, El Attar K, Fejerskov O. A retrospective study of direct pulp capping with calcium hydroxide compounds. Endod Dent Traumatol 1985;1:29-35.
36. Barthel CR, Rosenkranz B, Leuenberg A, Roulet JF. Pulp capping of carious exposures: treatment outcome after 5 and 10 years: a retrospective study. J Endod 2000;26:525-8.

37. Ulmansky M, Sela J, Sela M. Scanning electron microscopy of calcium hydroxide induced bridges. J Oral Pathol 1972;1:244-8.

38. Hörsted P, El Attar K, Langeland K. Capping of monkey pulps with Dycal and a Ca-eugenol cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1981;52:531-53.

39. Goldberg F, Massone EJ, Spielberg C. Evaluation of the dentinal bridge after pulpotomy and calcium hydroxide dressing. J Endod 1984;10:318-20.

40. Tronstad L, Mjör IA. Capping of the inflamed pulp. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1972;34:477-85.

41. Cotton WR. Bacterial contamination as a factor in healing of pulpal exposures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1974;38:441-9.

42. McWalter GM, El-Kafrawy AH. Mitchell. Long-term study of pulp capping in monkeys with three agents. J Am Dent Assoc 1976;93:105-10.

43. Cox CF, Bergenholtz G, Heys DR, Syed SA, Fitzgerald M, Heys RJ. Pulp capping of the dental pulp mechanically exposed to the oral microflora—a 1-2 year observation of wound healing in the monkey. J Oral Pathol 1985;14:156-68.

44. Cox CF. Bergenholtz. Healing sequence in capped inflamed dental pulps of Rhesus monkeys (*Macaca mulatta*). Int Endod J 1986;19:113-20.

45. Heide S, Kerekes K. Delayed partial pulpotomy in permanent incisors of monkeys. Int Endod J 1986;19:78-89.

46. Bergenholtz G. Evidence for bacterial causation of adverse pulpal responses in resin-based dental restorations. Crit Rev Oral Biol Med 2000;11:467-80.

47. Goldberg M, Smith AJ. Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. Crit Rev Oral Biol Med 2004;15:13-27.

48. Tziafas D. The future role of a molecular approach to pulp-dentinal regeneration. Caries Res 2004;38:314-20.

49. Akimoto N, Momoi Y, Kohno A, Suzuki S, Otsuki M, Suzuki S, Cox CF. Biocompatibility of Clearfil Liner Bond 2 and Clearfil AP-X system on nonexposed and exposed primate teeth. Quintessence Int 1998;29:177-88.

50. Hebling J, Giro EM, Costa CA. Biocompatibility of an adhesive system applied to exposed human dental pulp. J Endod 1991;25:676-82.

51. Pereira JC, Segala AD, Costa CA. Human pulpal response to direct pulp capping with an adhesive system. Am J Dent 2000;13:139-47.

52. Hörsted-Bindslev P, Vilkinis V, Sidlauskas A. Direct capping of human pulps with a dentin bonding system or with calcium hydroxide cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:591-600.

53. Gwinnett AJ, Tay FR. Early and inetrmediate time response of the dental pulp to an acid etch technique in vivo. Am J Dent 1998;(Spec iss)S35-44.

54. deSouza Costa CA, Hebling J, Hanks CT. Current status of pulp capping with dentin adhesive systems: a review. Dent Mat 2000;16:188-97.

55. DeMunck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 2005;84:118-32.

56. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19:591-5.

57. Saidon J, Jianing H, Zhu Qiang, Safavi K, Spångberg LSW. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:483-9.

58. Asgary S, Parirokh M, Eghbal MJ, Brink F. Chemical differences between white and gray mineral trioxide aggregate. J Endod 2005;31:101-3.

59. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999;25:197-205.

60. Tziafas D, Pantelidou O, Alvanou A, Belibasakis G, Papadimitriou S. The dentinogenic effect of mineral trioxide aggregate (MTA) in short-term capping experiments. Int Endod J 2002;35:245-54.

61. Menezes R, Bramante CM, Letra A, Gomes Carvalho VG, Garcia RB. Histologic evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white Portland cements as wound dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:376-9.

62. Rutherford B, Fitzgerald M. A new biological approach to vital pulp therapy. Crit Rev Oral Biol Med 1995;6:218-29.

63. Murray P, Windsor J, Smyth T, Hafez A, Cox CF. Analysis of pulpal reactions to restorative procedures, materials, pulp capping, and future therapies 2002;13:509-20.

64. Nakashima M. The induction of reparative dentine in the amputated dental pulp of the dog by bone morphogenetic protein. Arch Oral Biol 1990;35:493-7.

65. Rutherford RB, Wahle J, Tucker M, Rueger D, Charette M. Induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. Arch Oral Biol 1993;38:571-6.

66. Goldberg M, Six N, Decup F, Buch D, Soheili Majd E, Lasfargues JJ, et al. Application of bioactive molecules in pulp-capping situations. Adv Dent Res 2001;15:91-5.

67. Nakamura Y, Hammarström L, Matsumoto K, Lyngstadaas SP. The induction of reparative dentine by enamel proteins. Int Endod J 2002;35:407-7.

68. Olsson H, Davies JR, Holst KE, Schröder U, Petersson K. Dental pulp capping: effect of Emdogain gel on experimentally exposed human pulps. Int Endod J 2005;38:186-94.

69. Lovschall H, Fejerskov O, Flyvbjerg A. Pulp-capping with recombinant human insulin-like growth factor I (rhIGF-I) in rat molars. Adv Dent Res 2001;15:108-12.

70. Rutherford RB, Gu K. Treatment of inflamed ferret dental pulps with recombinant bone morphogenetic protein-7. Eur J Oral Sci 2000;108:202-6.

71. Bergenholtz G. Factors in pulpal repair after oral exposure. Adv Dent Res 2001;15:84.

72. Hörsted-Bindslev P, Bergenholtz G. Vital pulp therapies. In: Bergenholtz, G, Hörsted-Bindslev P, Reit C, editors. Textbook of Endodontology. Munksgaard: Blackwell; 2003. p. 66-91.

73. Baume LJ, Holz J. Long-term clinical assessment of direct capping. Int Dent J 1981;31:251-60.

74. Christensen GJ. Pulp capping 1998. J Am Dent Assoc 1998;129:1297-9.

75. Stockton LW. Vital pulp capping: a worthwhile procedure. J Can Dent Assoc 1999;65:328-31.

76. Ward J. Vital pulp therapy in cariously exposed permanent teeth and its limitations. Aust Endod J 2002;28:29-37.

Reprint requests:

Gunnar Bergenholtz
Faculty of Odontology
Box 450, S-405 30
Göteborg, Sweden
gunnar.bergenholtz@odontologi.gu.se