Clinical Update

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The Use And Predictable Placement Of Mineral Trioxide Aggregate® In One-Visit Apexification Cases

Abstract

Endodontic treatment of the pulpless tooth with an immature root apex poses a special challenge for the clinician. The main difficulty encountered is the lack of an apical stop against which to compact an interim dressing of calcium hydroxide $(Ca(OH)_2)$, or the final obturation material. In these situations the unpredictability of the result, the difficulty in creating a leak-proof temporary restoration for the duration of treatment, and the difficulty in protecting the thin root from fracture may lead to complications when using traditional (Ca(OH),-based) apexification techniques. Furthermore, given the increased mobility of today's society, lengthy treatment protocols are fraught with problems, and may not be followed through to completion. This may lead to ultimate failure of the case.

Mineral Trioxide Aggregate (MTA[®]) has recently been introduced for use in endodontics. Current literature supports its efficacy in a multitude of procedures including apexification. The focus of this paper is to propose a one-visit apexification protocol with MTA[®] as an alternative to the traditional treatment practices with $Ca(OH)_2$. One-visit apexification may shorten the treatment time between the patient's first appointment and the final restoration. The importance of this approach lies in the expedient cleaning and shaping of the root canal system, followed by its apical seal with a material that favours regeneration. Furthermore, the potential for fractures of immature teeth with thin roots is reduced, as a bonded core can be placed immediately within the root canal.

Introduction

Root canal treatment aimed at the retention of pulpless teeth comprises thorough cleaning and shaping, followed by threedimensional obturation of the root canal system (1, 2). Treatment of immature teeth poses challenges for the clinician, one of which is the lack of an apical stop, which makes controlled obturation in three dimensions demanding, if not impossible (3). In addition, the dentinal walls of an immature root may be very thin, thereby subjecting the tooth to the risk of fracture (4, 5).

Various ways of managing the pulpless tooth with a wide open apex have been suggested. These include obturation of the root canal with a customised, blunt-ended gutta-percha cone (6), filling the root canal short of the apex with gutta-percha (7), or periradicular surgery (8). Surgical intervention, however, may result in an unfavourable crown-root ratio, and thin and irregular walls at the root apex may discount surgery as a viable option (8). Furthermore, the physical and psychologic trauma of a surgical procedure on a young patient should be considered (9). "No treatment" has also been suggested as an option (10).

Apexification

Apexification is defined as a method of inducing a calcified barrier in a root with an open apex or the continued apical development of an incompletely formed root in teeth with a necrotic pulp (AAE Glossary 6th edition 1998) (11). Various materials have been suggested for use in the apexification process, but Ca(OH), has gained the widest acceptance. Kaiser first proposed the use of a paste containing Ca(OH), and camphorated monochlorophenol in 1964 at the 21st Annual Meeting of the American Association of Endodontists (12). Frank detailed a technique in 1966 that forms the basis for apexification procedures using Ca(OH), to the present day (13). Various proprietary and non-proprietary Ca(OH)₂ medicaments have been used for apexification. One product is often chosen over another on the basis of ease of handling, degree of solubility (3, 14) or radiopacity (15). To date, there is no clear evidence to indicate the superiority of any one commercially available product over Ca(OH), powder and sterile water (16).

The mechanism of apical closure using Ca(OH), is still unclear. Mitchell and Shankwalker (17) found that implantation of Ca(OH), leads to formation of calcified material in tissues not normally predisposed to calcification, thus ascribing an osseo-inductive potential to Ca(OH), Sciaky and Pisanti (18) and Pisanti and Sciaky (19) have shown that the calcium ions incorporated into the calcific barrier do not originate from the Ca(OH), but from the body's own calcium reserves. When placed in contact with vital tissue in pulp capping experiments, Ca(OH)₂ creates a necrotic layer (calcific scarring) (20). Its basic pH of 12 or more may counteract tissue acidity in areas of inflammation, impart a bactericidal effect (14) and foster the formation of calcium phosphate complexes, which in turn could act as nidi for the accumulation of calcific material (21). Other researchers, however, have questioned the need for a high pH. Studies have shown comparable degrees of apical closure with either Ca(OH), with a pH of 12.0 or tricalcium phosphate $(TriCa(PO)_{1})$ with a pH of 8.6 (22).

In the course of apexification, continued root formation is seen on occasions (23, 24). Gupta et al (25) suggest that for further root development to occur, "the area of calcific scarring must not extend to the root sheath of Hertwig or to the odontoblasts in the apical area". However, the most common appearance of the root apex following an apexification procedure is that of a dome-shaped "cap" (26). Despite radiographic and clinical evidence of formation of a hard tissue barrier, the latter is porous (10, 27) and irregular, with a layered structure, when viewed under the scanning electron microscope. A dense, acellular cementum-like tissue forms the outer layer. More centrally located, dense and irregular fibrocollagenous connective tissue with granular inclusions of foreign material and irregular fragments of calcifications are found (28). The nature of the calcified barrier thus formed has been described by various terms such as "osteo-dentine", "bone-like" (29), "cementum-like" material (30), or "osteocementum" (15, 31).

The need for placement of $Ca(OH)_2$ to encourage apexification has been questioned. Infected necrotic pulp tissue acts as an irritant to the periradicular tissues (32). The elimination of pulp tissue debris and bacteria should therefore create sufficient stimulus for completion of apical closure, which may even occur without medicament (29, 30, 33). McCormick et al (34) postulate that adequate instrumentation of the root canal, removal of necrotic tissue, microorganisms and substrate, together with a decrease in root canal space are of the utmost importance in apexification. In fact, Frank (13) does not ascribe a particular and unique effect to $Ca(OH)_2$. Instead he points to reduction of the pulpal space and ease of removal as rationale for the use of this medicament in endodontic procedures (35, 36).

There is considerable disagreement amongst members of the profession as to how often, if at all, the Ca(OH), should be changed (24). Walia et al (27) recommend replacement of the dressing if a radiograph indicates resorption of the medicament in the apical 1/3 of the canal (27). Abbott (3), however, emphasises that a radiograph is rather unreliable in both determining the degree of barrier formation or the amount of Ca(OH), remaining in the canal. He recommends replacement of the dressing in two to three month intervals, which also allows for clinical determination of barrier formation. Finucane and Kinirons (37) found that frequent changes of the dressing may speed up the formation of a calcific barrier (37). In contrast, Chosack et al (15) found no difference in the extent of the apical barrier formation between teeth receiving a single dressing, versus teeth in which the dressing was changed monthly, up to six months. In fact, a multiple-visit treatment regimen has been thought to possibly disrupt the process of apexification (26, 29).

Various authors have indicated that the success rate of apexification techniques using $Ca(OH)_2$ is high (27, 37, 38). The time required for apical closure varies among studies. Kleier and Barr (38) found that in the presence of symptoms the time to closure was extended by approximately 5 months to an average of 15.9 months. In other studies it has been shown that teeth with a narrow apex (37) and those without periradicular radiolucencies had a shorter treatment time than teeth with a wider apical diameter, or teeth with periradicular lesions (27). In teeth that had sustained displacement injuries apical closure was delayed significantly (37).

Despite the overall high success rate of $Ca(OH)_2$ in apexification, there is considerable variation in treatment outcomes (31). In addition, it is difficult to determine if and when apical closure has been achieved. Radiographic evaluation may not be reliable (3), and whilst a clinical paper point test may indicate a solid apical barrier, this may not be the case in reality (24).

Variability in treatment time makes it difficult for the clinician to advise the patient as to when the case can be completed. It is crucial that the patient, during the course of the treatment, does not get lost to recall, either by changes in his/her personal situation (job, residence) or simply by a lack of willingness to cooperate with a lengthy treatment protocol (39). Complications may occur when patients fail to adhere to their treatment schedule (3, 33). Abbott (3) draws attention to the fact that all temporary restorations have a limited lifespan. Whilst two to three month recalls are advised, patients frequently fail to return for recall assessment and treatment success may be jeopardised by breakdown of the temporary restoration may afford increased protection against leakage, as well as increased protection against fracture, a major risk factor for the immature root (40–42).

One-Visit Apexification

The concept of one-visit apexification eliminates various problems associated with traditional $Ca(OH)_2$ -based apexification techniques, such as, but not limited to:

- Recall for the purpose of completing treatment is not required, i.e. failure to successfully reappoint the patient will not have a negative effect on the treatment outcomes;
- Development of an apical seal is more predictable, both in terms of time frame and quality of the seal with a material that may display regenerative potential;
- The tooth may be restored with a bonded core or a bonded post and core at the initial appointment, thus affording increased strength and superior resistance to leakage; and
- Construction of the core is in the hands of the endodontist, who is intimately familiar with the anatomy of the root canal system.

One-visit apexification, in its traditional sense, has been described in the literature as the non-surgical compaction of a biocompatible material into the apical end of the root canal, thus creating an apical stop and enabling immediate obturation of the root canal (16). Hence, one-visit apexification may, strictly speaking, not fall within the definition of apexification, as there is no implication of inducing either a calcified barrier, or continued apical root development. Dimashkieh (43) used resorbable oxidised cellulose as a matrix, against which amalgam was compacted. Other researchers, however, endeavoured to use the apical stop in order to encourage the deposition of calcified tissues, using a resorbable calcium phosphate ceramic material (44) or dentine chips (45), with some degree of success. A one-visit apexification technique using $Ca(OH)_2$ was proposed by Schumacher and Rutledge (46).

Apexification Techniques Using MTA®

Mineral Trioxide Aggregate (MTA*) is marketed in the USA by Dentsply Tulsa Dental Corporation (Tulsa, OK, USA). On the Material Safety Data Sheet (MSDS) (http://www.tulsadental.com/ PDFs/MTA-MSDS-W 01-02C.pdf) the major ingredients of "white MTA*" are listed as:

- Dicalcium Silicate
- Tricalcium Silicate
- Tricalcium Aluminate
- Bismuth Oxide
- Calcium Sulphate Dihydrate (gypsum)
- Other trace elements (impurities).

The manufacturer lists the pH as of MTA" mixed with water as between 12 and 13. MTA" was approved for use in humans by the FDA in 1998 (47). Most research on MTA" has been conducted with grey MTA". In 2002 a light-coloured type of MTA" was introduced by the manufacturer ("white MTA""), in order to address concerns of discolouration of teeth treated with the conventional MTA" (8, 48, 49). Whilst the published literature regarding the use of white MTA" is scant at this point, unpublished data (50, 51) indicates an equally favourable tissue response to both materials. The main difference between the two materials is a reduction in iron content in white MTA", as compared to grey MTA".

Various properties of MTA* suggest its usefulness in cases where one-visit apexification is desired. MTA*, when used as a rootend filling, has shown better marginal adaptation and less leakage than either Super-EBA, Intermediate Restorative Material (IRM*) (Dentsply Caulk, Milford DE, USA) or amalgam (52, 53). Its sealing ability is unaffected by the presence of blood (54). The method of placement, however, has an influence on leakage, with intracanal delivery contributing to greater leakage than surgical placement as a root-end filling (4). This may be due to the inability of the operator to visualise delivery of the material and the need to apply only light compaction forces in order to prevent gross apical extrusion. Neither pre-medication with a Ca(OH), containing paste, as recommended by the manufacturer of MTA* (http://www.tulsa dental.com/PDFs/MTA DFU W-01-02C.pdf), nor thickness of the MTA" plug have an impact on leakage (4). MTA" exhibits good antibacterial properties (55), and it is suggested that it may have osseo-inductive properties (5, 56), thus fulfilling the requirements of the definition of "apexification" as defined in the AAE glossary (11).

Whilst the literature is replete with articles describing apexification techniques using $Ca(OH)_2$, there is a paucity of literature dealing with MTA^{*} as a stimulus to root apexification. Furthermore, long-term prospective randomised clinical trials comparing the use of the two materials (Ca(OH)₂ and MTA^{*}) in apexification procedures have not been carried out to-date.

In an animal model involving immature teeth with induced periradicular lesions Shabahang et al (5) found that treatment for three months with $Ca(OH)_2$ or MTA^{*} resulted in comparable amounts of hard tissue formation. The MTA^{*} group, however, showed significantly greater consistency and predictability, with a tendency towards less inflammatory infiltration. The successful apexification of a primary mandibular second molar with no permanent successor was described by O'Sullivan & Hartwell (57). Giuliani et al (58) describe three case reports, all of which had presented with a buccal sinus tract and a radiographically discernable periradicular rarefaction. In all cases, the root canal system was debrided and dressed with $Ca(OH)_2$ during the initial appointment. In a second appointment an apical plug of MTA^{*} was placed, and a moist cotton pellet was sealed into the root canal. The remainder of the root canal was filled using gutta-percha during a third appointment. In each of these cases clinical function at one-year follow-up was adequate, the size of the periradicular lesion was reduced, and the sinus tract had disappeared.

Clinical techniques for the application of MTA^{*} in one-visit apexification cases have been addressed by a number of authors (48, 58, 59) and a detailed discussion on one-visit apexification procedures was published by Witherspoon and Ham (8). Handling characteristics of MTA^{*}, however, together with the anatomic characteristics of a wide open apex, make the predictable application of the MTA^{*} technique sensitive and difficult to achieve.

A Method For The Predictable Placement of MTA® In One-Visit Apexification Procedures

MTA" requires moisture for its setting reaction. The manufacturer recommends sealing a moistened cotton pellet into the root canal for a minimum of four hours (http://www.tulsadental.com/PDFs/MTA DFU W-01-02C.pdf), a protocol supported by multiple studies (4, 58, 60). This implies that obturation of the remainder of the root canal with placement of a core may have to be delayed for a minimum of four hours, which in turn may require reappointment of the patient. In apexification cases a wide open apex allows direct access of tissue fluids to the apical extent of the MTA" plug. Together with the brief application of moisture from the coronal end, followed by the placement of a coronal core using a bonded composite (with or without a post) can be accomplished without delay.

An *in vitro* model has been devised to illustrate the technique for the predictable placement of MTA" in a wide-open apex situation. This *in vitro* simulation can be reproduced easily to permit practising the technique prior to applying it in an *in vivo* situation.

Preparation of the model (Figs I and 2):

 Obtain an extracted immature tooth. Prepare the access cavity, and clear the tooth of any pulpal remnants and debris. Close the apices by applying soft wax, and thinly apply some Vaseline" to the external root surface.



Figure 1: Teeth set in test tubes.



Figure 2: Coronal access -- note the presence of gelatine (periradicular tissues) in the apical flare.

AUSTRALIAN ENDODONTIC JOURNAL VOLUME 29 No. 1 APRIL 2003

- 2) Mix some cooking gelatine (available in supermarkets) and pour it into a test tube, leaving the top 1 cm of the tube empty. Refrigerate until gelatine is just beginning to set – check every few minutes as time may vary!
- 3) Place the apical extent of the tooth into the test tube, so that approximately 5 mm of the root is covered with gelatine. Then refrigerate until the gelatine is firm.
- 4) With gentle rocking movements, remove the tooth from the gelatine, **remove the wax from the tooth**, and replace the tooth in its indentation in the gelatine. Exerting light pressure will cause some gelatine to move upwards and occupy some of the space of the apical flare. Gelatine occupying the "periradicular area" of the extracted tooth will simulate the consistency of the periradicular tissues in an *in vivo* case.
- 5) Prepare a thin mix of dental stone and, using a spatula, flow it on top of the gelatine in the test tube to fix the tooth in its position.
- 6) Wait for the stone to set, then follow the MTA^{*} placement technique as outlined below.

Step I: Determination of working length (W/L)



Figure 6: Healt softening a guttabercha point.

> Figure 7: Custom-moulding a softened gutta-percha point.



smaller than the one binding 1--2 mm short of the established W/L will often be appropriate (Fig. 5).

In teeth with markedly irregular or ovoid shaped canals, a plugger can be custom-moulded by repeated insertion of the reverse end of a large size gutta-percha point that has been carefully heatsoftened or softened in chloroform (Figs. 6 and 7).

Step 3: Set the instruments to the required length

The technique presented is based on both **tactile sensation**, and on strict adherence to **measurements**. The thickness of the



Figure 8: Plugger flush with the outlet.



Figure 3: Working length determination.

In a tooth with an immature root the obturation should reach the level of maximum constriction, and not protrude grossly beyond the apical flare (61–63). It is important to remember that root development in the labiolingual plane tends to lag behind root development in the mesiodistal plane (9). The working length should be determined radiographically, as an electronic apex locator may be unreliable in a wide open apex situation (64) (Fig. 3). Rinse the root canal system gently with water (*in vitro* – see "*in vivo* application" below) and dry with paper points.

Step 2: Selection of the appropriate plugger

Stainless steel pluggers are preferred over nickel-titanium instruments for this purpose, as they are inflexible and allow for better tactile sensation. A plugger must not be too large so as to bind against the fragile dentinal walls (Fig. 4), and not too small, so as to pierce the MTA*. Experience indicates that a plugger one size





Figure 4: Plugger too large.

Figure 5: Correct size plugger.

AUSTRALIAN ENDODONTIC JOURNAL VOLUME 29 No. 1 APRIL 2003





Figure 9: Plugger flush with the outlet — in this position, the handle is withdrawn by approx. 1 mm.

Figure 10: Handle withdrawn by an additional 3 mm.

apical barrier of MTA* is not of consequence in terms of leakage (4), and with the use of a bonded composite material as backfill there is no requirement for the MTA* to resist substantial displacement forces as would be the case in a gutta-percha backfill. As such, the placement of a 2–3 mm apical plug may be sufficient. In the example presented, the total working length is 13 mm. An increment of MTA* of 3 mm in length will be deposited and compacted, anticipating some lateral movement, resulting in an apical plug of approximately 2 mm in thickness. Hence, the plugger is set at 13 mm - 2 mm = 11 mm.



Figure 11: MTA plug with a predictable length of 3 mm.

Clinically the Dovgan* carrier (Quality Aspirators, Duncanville, Texas, USA) is quite useful. Upon close inspection it is apparent that in its end position the plunger protudes by approximately 1 mm beyond the outlet of the carrier. It is of utmost importance to start with the plunger *flush* with the outlet, which means in this case, the handle will need to be withdrawn by approximately 1 mm (see Figs, 8 and 9). A further withdrawal of the handle by 3 mm (i.e. a total of 4 mm) will result in an MTA* plug of a predictable length (3 mm) (Figs. 10 and 11).

Next, mix the MTA" to a dry consistency and load the pre-set Dovgan" carrier.

Step 4: Placement of the MTA*

Deposit the MTA^{*} plug into the canal (Fig. 12). Using the pre-set plugger, tease the material gently into position. In this example (working length 13 mm, MTA^{*} plug thickness of 3 mm, and plugger set at 11 mm) a slight resistance will be felt as soon as the plugger



Figure 12: MTA plug placed into the root canal.



Figure 13: MTA plugs dabbed into position.



Figure 14: Coronal view of apical MTA plug.

reaches a distance of approx. 0.5 to 1 mm from the pre-set length. At this point, it is not advisable to compact the material further – rather, use the plugger circumferentially to clean the walls down to the level of the MTA". A large calibre paper point is then dipped in sterile water and used to moisten the coronal aspect of the MTA", whilst gently dabbing it into position with light compaction pressure (Fig. 13 and 14). Note that MTA" can be irrigated from the canal quite easily prior to setting.

Step 5: Placement of an intermediate material

The placement of an intermediate material is an important step in this technique. Having added moisture to the MTA" in the previous step, the intermediate material will seal in the moisture and prevent dehydration of the MTA" during the following steps. It will allow the use of bonding procedures in the construction of the core, without risk of damage to the as-yet unset MTA". The main





Figure 15: Application of the glass ionomer cement.

Figure 16: MTA plugs and glass ionomer intermediate material.



Figure 17: Coronal view of glass ionomer cement.

seal against coronal leakage will be afforded by the bonded core, not the intermediate material. Self-curing glass ionomer cement is used for this purpose, such as FUJI IX " (Espe Dental AG, 82229 Seefeld, Germany). The cement is mixed in accordance with the manufacturer's instructions, and applied with the Centrix " syringe system (Shelton, CT, USA). A stopper on the metal tip of the syringe should be set just short of the length set on the plugger. Apply the glass ionomer cement under visual control, using magnification, when possible (Figs. 15–17).

Step 6: Placement of the core build-up

The use of a dual- or auto-curing bonding system, such as Allbond 2[°] (Bisco, Schaumburg, IL, USA) and a composite based auto-curing core system, such as TiCore[°] (EDS, South Hacken-



80

Figure 18: Pre-operative radiograph.

Figure 19: Pre-operative - apical view.





Figure 21: Post-operative – apical view.

Figure 20: Post-operative radiograph.

sack, NJ, USA) or CorePaste⁺ (Den-Mat, Santa Maria, CA, USA) (40) is recommended. The use of the Centrix⁺ syringe system may be helpful for the application of the core material.

The end result is depicted in Figures 18 and 19, which show the pre-treatment condition and Figures 20 and 21, which are post-treatment.

In vivo application

When applying this technique in a clinical setting, general principles of endodontic treatment should be adhered to, such as the administration of an appropriate local anaesthetic and rubber dam isolation.

Following access cavity preparation, thorough debridement and



Figure 23: Selection of the plugger.



Figure 22: Pre-operative, accessed alio loco.

shaping of the canal is recommended, using sodium hypochlorite (NaOCI) as the irrigant (65, 66), followed by removal of the smear layer using 17% EDTA and NaOCI (67). Disinfection of the root canal may be enhanced by the use of a 2% chlorhexidine solution (68–70) (Premier Dental Products, Plymouth Meeting, PA, USA), which is placed into the root canal system for 1–2 minutes and then removed with an NaOCI rinse. The canal is gently dried with sterile



Figure 24: Placement of MTA.

paper points, and the apexification procedures are performed as previously described.

The one-visit approach is useful in cases where access to the patient may be limited to one appointment. An example would be the case of a very young patient or of a patient who indicates his or her unwillingness to be reappointed for completion of the procedure. Furthermore, when vital pulp extirpation from an immature



Figure 25: Intermediate GIC and bonded composite ore in situ.



Figure 26: One year review.

tooth is inevitable, the above protocol may be considered as encouraging apexogenesis. The treatment can easily be completed in one visit, provided bleeding can be controlled with gentle pressure of a paper point.

In other cases, where patient cooperation is not a problem, it may be desirable to carry out cleaning and shaping procedures

during a separate visit prior to the apexification appointment. In this case, debridement and shaping of the canal are performed using NaOCI (65, 66), followed by removal of the smear layer (67). It is recommended that an interim dressing of $Ca(OH)_2$ and 2% chlorhexidine solution be placed into the root canal (71). The access cavity is then closed with a double seal of Cavit" (Espe Dental AG, 82229 Seefeld, Germany) and IRM". The patient is reappointed one week later and the dressing is removed using files and copious irrigation with NaOCI. After removal of the smear layer, the canal is gently dried with sterile paper points, and the apexification procedures are performed as previously described.

One-visit apexification using MTA" is not limited to young teeth with immature roots. It may also be used in the treatment of mature teeth that have undergone apical resorption. The difference between these two different case scenarios is that the roots in the resorptive cases may have a reduced risk of fracture when compared to those of immature teeth. However, the advantages of the one-visit apexification concept (such as predictability, increased resistance to leakage after early placement of a permanent core, and the ability to complete treatment in one visit, without the need to reappoint the patient) apply equally to both.

An example of an *in vivo* case with a one-year follow-up is illustrated in Figures 22–26. (Case courtesy of Dr Joy Field, Dallas, Texas. Note: access cavity prepared *alio loco* during emergency visit.)

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From The Journals

Fracture-Toughening Mechanisms Responsible For Differences In Work To Fracture Of Hydrated And Dehydrated Dentine

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This study investigates the nature of deformation and differences in the mechanisms of fracture and properties of dentine where there has been a loss of moisture. It has been suggested that the fluid filled dentinal tubules could function to hydraulically transfer and dissipate the occlusal forces applied to teeth. From the perspective of theoretical mechanics, the structural stability of dentine is a function of mineralisation and moisture content. Although differences in biomechanical properties of hydrated and dehydrated dentine have been reported, the influence that moisture plays in the biomechanical behaviour is still not well understood or fully investigated. Controlled *in vitro* fracture toughness testing was conducted on bovine teeth to determine the influences (p<0.01) were observed between the fracture toughness of hydrated (554 \pm 27.7 J/m²) and dehydrated (113 \pm 17.8 J/m²) dentine. Observations of the crack tip region during crack extension revealed that extensive "ligament" formation occurred behind the crack tip. These ligaments provide considerable stability to the crack by significantly increasing the work of fracture, thereby acting as a fracture toughening mechanism. Micro-cracking, reported as a fracture toughening mechanism in bone, is also clearly seen. A zone of inelastic deformation may occur as hydrated specimens revealed upon crack extension, a region about the tip that appeared to suck water into the structure and to exude water behind the crack tip. In dehydrated dentine, no inelastic zone was observed. Microcracking is present though the cracks are smaller, straighter and with less opening than hydrated dentine. Only limited ligament formation just behind the crack tip was observed. These differences resulted in a significantly lower work of fracture with unstable brittle fracture characteristics. These findings may be relevant for bone, a similar mineralised hydrated tissue.