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Does DIAGNOdent provide a reliable caries-removal endpoint?

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ABSTRACT

Objectives: To compare mineral density of residual dentine after excavation with different caries-removal techniques and to evaluate the diagnostic potential of laser-induced fluorescence (LIF), measured by DIAGNOdent, as a tool to determine the caries-removal endpoint.

Methods: Carious teeth were excavated by tungsten-carbide round burs (Komet), ceramic burs (CeraBurs, Komet), sono-abrasion (Cariex TC tips, Kavo), and by chemo-mechanical excavation using two enzyme-based solutions (exp. SFC-V and SFC-VIII, 3M-ESPE) or a sodium hypochlorite-based solution (Carisolv, MediTeam). The caries-excavated teeth were scanned by micro-CT (1172, Skyscan), after which the mineral density at the bottom dentine was correlated to LIF measurements at the same region. A micro-CT threshold for dentine caries was defined by comparison with surface-hardness measurements. The intensity of dentine staining was evaluated by analysing the component 'L^*' in CIE-L^*a^*b-converted images from the excavated teeth.

Results: No statistically significant difference in mineral density was found at the bottom of the cavities prepared with the different caries-excavation techniques, except for exp. SFC-V that left residual dentine with a significantly higher mineral density than when CeraBurs were used (Tukey–Kramer, $p < 0.05$). Absence of residual caries was associated with darker staining of dentine. No significant correlation was found between the distance from the deepest cavity point to the pulp-chamber roof and LIF measurements. A strong negative correlation ($R = -0.86$, $p < 0.01$) was however found between L^* values and LIF measurements, indicating that staining in residual dentine leads to higher LIF measurements.

Conclusions: LIF measured by DIAGNOdent is influenced by staining in residual dentine. Therefore, its use to determine the caries-removal endpoint is doubtful.

1. Introduction

The concept of minimal-invasive dentistry aims to maximally preserve sound tooth tissue.[1] In case of caries lesions, the highly infected and demineralised dentinal tissue should be eliminated in order to prevent lesion progression. Tooth preparation should on the other hand remain as conservative as possible in order to not unnecessarily undermine the remaining tooth structure and to preserve the tooth's mechanical resistance against intra-oral chewing.[2] However, it is not always easy to define at which point dentine excavation should be stopped. Since 'soft and wet' carious

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lesions harbour significantly more bacteria than 'hard or dry' lesions,[3] clinicians are commonly advised to remove carious dentine up to the level where it is 'firm' and no longer 'leathery'.[4]

The traditional and still most widely used method to remove caries involves steel or tungsten-carbide burs. The recently marketed CeraBurs (Komet-Brasseler, Lemgo, Germany) are all-ceramic burs intended for ultra-conservative caries removal. They are claimed to avoid over-excavation by providing a self-limiting caries-excavation property.[5] Some more recently introduced sono-abrasive tungsten carbide tips are also intended for dentine-caries removal (Cariex TC tips, Kavo, Biberach, Germany), but still need to be tested on their caries-removal efficiency. Besides mechanical caries removal, chemical agents to selectively dissolve carious dentine are currently employed as well. The sodium hypochlorite-based gel Carisolv (MediTeam, Göteborg, Sweden) appeared effective,[6] but was rather slow.[7] Initial results with an experimental pepsin-based gel (exp. SFC-V and SFC-VIII, 3M-ESPE, Seefeld, Germany) have revealed a caries-removal effectiveness that is comparable with that of Carisolv.[8]

For early caries diagnosis, laser-induced fluorescence (LIF) has proven its effectiveness. Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength induced by an energy source like a laser.[9] In the field of medical diagnostics, LIF yields information on the metabolic state of cells or presence of micro-organisms depending on the nature of the fluorescent molecule (co-enzymes or porphyrin metabolites, respectively).[10] The tissue examined is excited with a specific laser wavelength that generates a maximum fluorescent response. After a few nano/micro-seconds, the tissue will de-excite and emit light at a wavelength larger than the original excitation wavelength. This fluorescence light is then measured. The so-called 'DIAGNOdent' (Kavo, Biberach, Germany) is a laser-induced caries-detection device that is equipped with a semiconductor laser (655 nm) as excitation source. The laser light is emitted from the tip of the handpiece that also captures the fluorescence reflected from the tooth surface, by means of a photodiode (680 nm) in combination with a long-pass filter that absorbs the backscattered excitation.[11] The device quantifies the fluorescence intensity that is subsequently converted to a calibration standard, ranging from 0 to 99,[12] where sound dental tissues will exhibit the lower readings (0–12).[11]

Recently, confocal microscopy demonstrated that detection of visible fluorescence (orange-red fluorophores), originating from by-products of bacterial metabolism in carious dentine, correlated well with the caries-removal endpoint.[13] In the same study, LIF measured at residual dentine by DIAGNOdent appeared also to correlate with fluorescence measured by confocal microscopy. Therefore, apart from the intended use of DIAGNOdent for the detection of occlusal dentine caries underneath suspicious enamel, other studies explored the use of DIAGNOdent as a diagnostic tool to check for residual caries upon caries excavation.[12,14,15] An extension of this application leads to the introduction of a laser-based caries-excavation methodology that makes use of LIF technology (feedback system) to selectively remove carious dentine.[15–17]

At the same time, however, some concerns have been raised whether LIF can correctly diagnose the end-point of caries removal. An increased LIF is apparently measured at residual dentine in the immediate proximity to the dental pulp,[15] whilst surface staining may disturb LIF readings as well.[18] There is especially growing evidence that the latter surface staining affects the accuracy of LIF to diagnose the caries-removal endpoint.[15] Therefore, the main objective of this study was to determine the mineral density of residual dentine after excavation with different caries-removal methods by means of a non-destructive technique (micro-CT), this in order to assess the applicability of DIAGNOdent to check for residual caries in teeth with different degrees of dentine staining. A secondary aim was to establish a mineral-density cut-off point for dentine caries in the micro-CT by comparison with a gold-standard technique for mechanical characterisation of carious tooth tissue (hardness measurements).

2. Materials and methods

2.1. *Selection of teeth and caries removal*

From a bulk of extracted, non-restored molars stored in 0.5% aqueous chloramine, those presenting carious lesions on the occlusal surface (presumably involving dentine) were pre-selected. Both teeth with active and inactive caries lesions were included. After cleaning off plaque, calculus and other debris with an airscaler (Soniflex 2000 equipped with a scaler

Table 1 – Caries-excavation methods and respective caries-removal endpoint used.

Caries-excavation method	Manufacturer	Caries-removal endpoint	N
Tungsten carbide round bur (n.10–23, depending on the dimensions of the lesion)	Komet-Brasseler, Lemgo, Germany	Hard cavity floor with a blunt explorer	6
CeraBurs (n.10–23, depending on the dimensions of the lesion)	Komet-Brasseler	Self-limiting cutting ability of the instrument	7
Airscaler (Soniflex 2003L) coupled to Cariex TC tips (n.71 and 72)	Kavo, Biberach, Germany	Hard cavity floor with a blunt explorer	6
SFC-V + conventional spoon excavator	3M-ESPE, Seefeld, Germany	Self-limiting caries-removal ability of the solution	8
SFC-VIII + conventional spoon excavator	3M-ESPE	Self-limiting caries-removal ability of the solution	5
SFC-VIII + polymeric instrument (star v1.3)	3M-ESPE	Self-limiting caries-removal ability of the solution	7
Carisolv + respective hand instruments (n.2–5)	MediTeam, Göteborg, Sweden	Self-limiting caries-removal ability of the solution	7

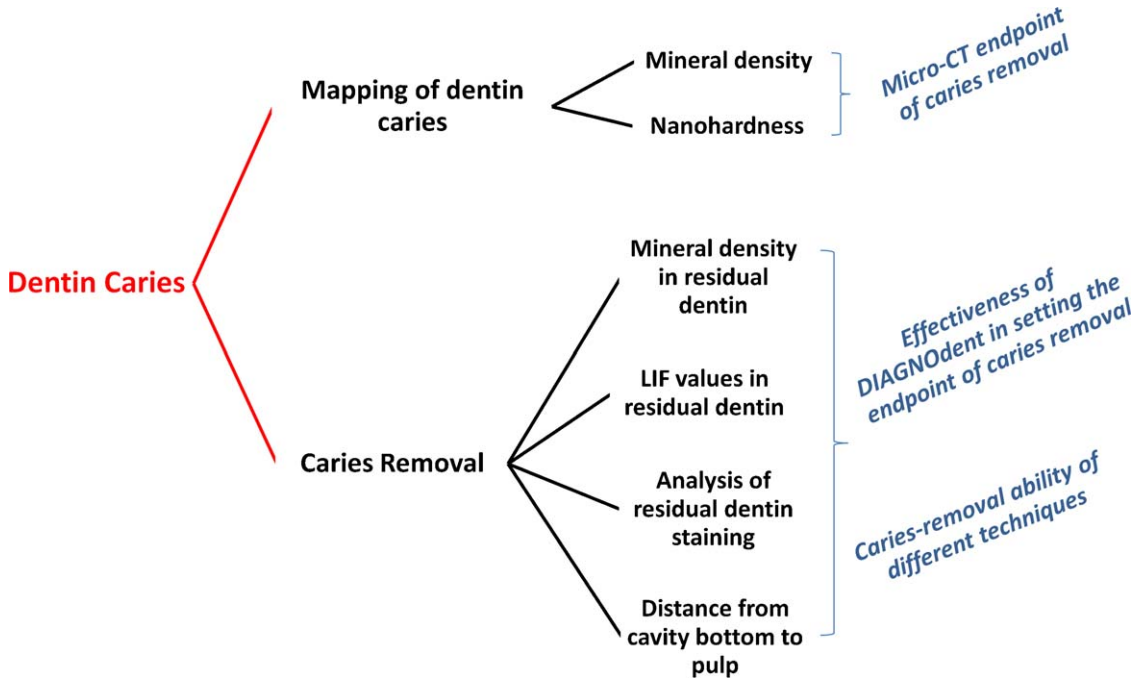

Fig. 1 – Description of each methodological step and corresponding research objective aimed for in this study.

tip #5, Kavo, Biberach, Germany), the teeth were embedded for ease of manipulation by their roots in gypsum with their occlusal surface left free.

Digital radiographs were obtained (MiniRay, Soredex, Tuusula, Finland) with the aid of a CCD detector (Vista Ray CCD System, Dürr Dental AG, Bietigheim-Bissingen, Germany), in order to exclude teeth without distinct dentine caries. The remaining teeth ($n = 56$) were then divided in groups according to the 7 different caries-excavation methods to be employed (Table 1). Before excavation, the overlying enamel was removed with a cylinder round diamond bur (ref. 838.314.010, Komet-Brasseler, Lemgo, Germany) coupled to a high-speed water-cooled air turbine. Five teeth were further excluded as they exhibited only a small caries into dentine, whilst another five teeth were excluded after caries-excavation due to pulp exposure, resulting in a total of 46 evaluated specimens. Fig. 1 shows a detailed description of each study phase and the corresponding research objective aimed for in this study.

2.2. *DIAGNOdent measurements*

After caries excavation following the respective caries-removal endpoints described in Table 1, the deepest point of the excavated cavity was identified with a periodontal probe and the maximum LIF reading was taken with a DIAGNOdent

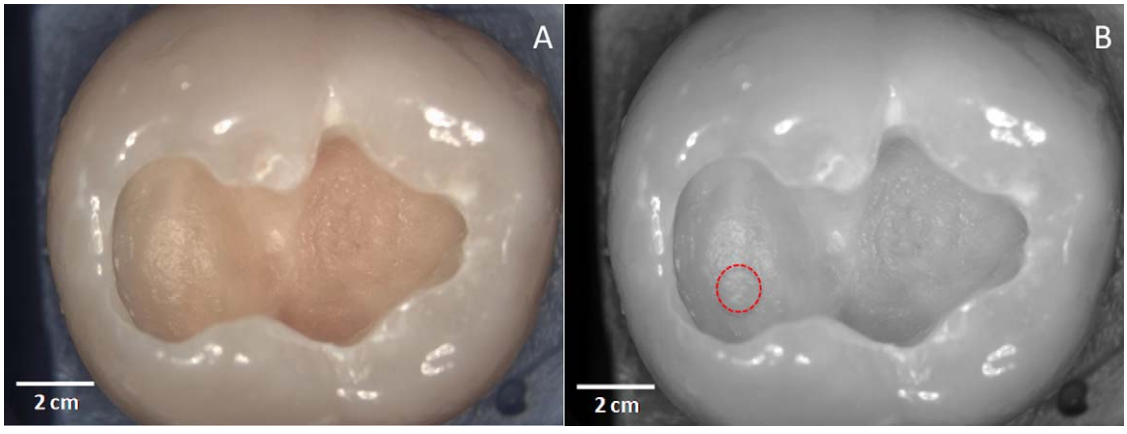

Fig. 2 – (A) RGB colour stereomicroscopic image of a tooth after caries removal and colour-adjustment following the colour-calibration scheme. (B) L^* channel image after converting image (A) to $L^*a^*b^*$ colour space. The dotted circle indicates the region of interest at the deepest part of the cavity corresponding to the region where the DIAGNOdent measurement was performed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

pen 2190 coupled to a sapphire fissure probe after calibration with a standard stone (C86), as indicated by the manufacturer (Kavo). Three consecutive measurements were performed, and when differences were recorded, the average value was taken as the final reading. Immediately after this DIAGNOdent measurement, the deepest point of the excavated cavity was marked on a stereomicroscopic photo taken from the tooth, for further determination of the staining intensity of the residual dentine surface.

2.3. Colour image acquisition and calibration

Colour stereomicroscopic images of the occlusal surface of the excavated teeth were taken in a stereomicroscope (Wild M5A, Wild-Heerbrugg, Heerburg, Switzerland) illuminated by a 150 W light source (FOT 150, Fiber Optic, Spreitenbach, Switzerland) coupled to a digital camera (Moticam 2300, Motic, Xiamen, China).

Before image acquisition, a colour-calibration scheme was applied. First, the camera was calibrated by applying a white-balance correction as described by the camera manufacturer. Secondly, a greyscale patch (n.21, neutral 6.5) from a standard colour-calibration card (GretagMacbeth Mini Color Checker, X-Rite, Regensdorf, Switzerland) was imaged and the RGB histogram was adjusted to the standard value of the grey patch ($R = 160$, $G = 160$, $B = 160$) in the acquisition mode of the camera software (Motic Images Plus 2.0, Motic). Next, the excavated teeth were photographed with a calibrated colour scale in the RGB-colour space.

Since this first colour calibration was qualitative, the images were additionally corrected within the open-source software interface of ImageJ.[19] From an ROI selected in the standard grey-patch image, an RGB histogram was produced and the mean values of the red, green and blue channels were taken and multiplied by the respective integer to reach the standard value of 160. Next, each tooth image was converted to an RGB stack containing three 8-bit greyscale images corresponding to the red, green and blue channels, which were multiplied by the correction integer obtained from the grey-patch image. The stack was then converted back to RGB colour (Fig. 2A).

2.4. Quantitative analysis of the staining intensity of residual dentine

Quantitative evaluation of the staining intensity of residual dentine in the excavated cavities was performed after converting the RGB images to 'Commission Internationale de l'Éclairage' $L^*a^*b^*$ colour space (CIE $L^*a^*b^*$) and analysing the component "L" in the converted images. In the CIE L^*a^*b colour space, the three coordinates (L, a, b) represent the lightness of the colour ($L = 0$ yields black and $L = 100$ indicates white), its position between red/magenta and green (a^*, negative values indicate green, whilst positive values indicate magenta) and its position between yellow and blue (b^*, negative values indicate blue and positive values indicate yellow).[20]

Conversion of the RGB images to CIE $L^*a^*b^*$ colour space was performed by means of the ImageJ-based plugin "Color Space Converter".[21] For each tooth, a stack containing three grey-scale images corresponding to each colour channel (L^*, a^*, or b^*), was obtained. The image corresponding to the "L" channel was selected and a 150-pixel diameter ROI (±1 mm diameter) was placed over the previously marked area that corresponds to the deepest area in the excavated cavity. The corresponding "L" value from the ROI corresponding to the area evaluated by DIAGNOdent, was then obtained (Fig. 2B).

2.5. Mineral density in residual dentine

The excavated teeth were also submitted to a micro-CT scanning procedure and the projection files were further processed as described elsewhere.[22] Briefly, scanning was performed in a micro-CT device (Skyscan 1172, Skyscan, Kontich, Belgium) at 100 kV, 100 μA and 14.66 μm pixel size. Calibration of grey values into mineral density of hydroxyapatite (HAp) was undertaken by scanning HAp phantoms with different mineral densities and obtaining a calibration curve for the used micro-CT parameters.[22] After reconstruction of the projection images (Nrecon 1.51, Skyscan), the cross-section slices were processed to reduce the presence of noise and to alleviate the partial volume effect. Mineral density was calculated in g/cm^3 of HAp over a 70-μm thick region around the deepest cavity area, as depicted in Fig. 3A.

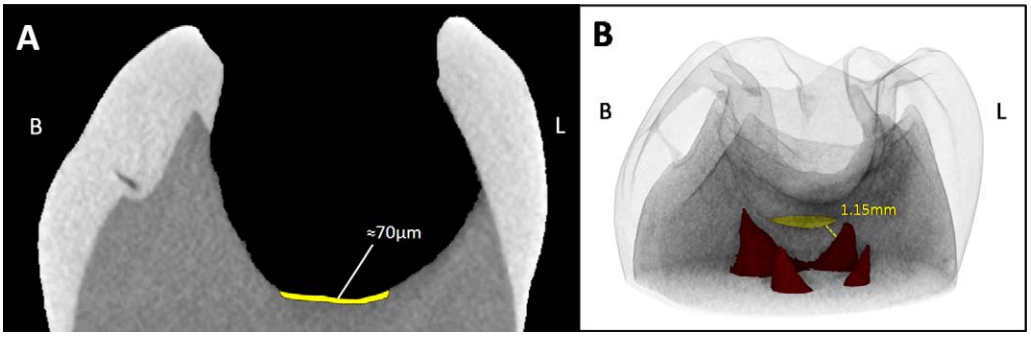

Fig. 3 – (A) Relative position and thickness of the volume of interest used for mineral-density measurement at residual dentine. (B) Volumetric representation of one tooth after caries removal. In red, the pulp chamber is depicted, whilst in yellow the area corresponding to the deepest part of the cavity used for mineral-density measurements, is marked. The minimum distance from the deepest part of the cavity to the pulp-chamber roof was measured. B, buccal; L, lingual. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2.6. *Distance from the bottom of the excavated cavity to the pulp-chamber roof*

The minimal distance between the deepest point in the excavated cavity and the highest point of the pulp-chamber roof was measured in mm after 3D-volumetric rendering of the micro-CT slices (VGStudio Max 2.0, Volume Graphics, Heidelberg, Germany), as shown in Fig. 3B.

2.7. *Determination of the carious dentine threshold using micro-CT*

For determination of a dentine-caries threshold using micro-CT, mineral-density values of dentine obtained by micro-CT were correlated to hardness measurements, which can be considered a ''gold-standard'' for dentine-caries determination.[23] One tooth presenting dentine caries was scanned by micro-CT using the parameters described previously, prior to being prepared for dentine-hardness measurements.

The tooth was embedded by the crown in epoxy resin (Epofix Kit, Struers, Ballerup, Denmark) and sectioned in a mesio-distal direction at the centre of the occlusal caries lesion with the aid of a diamond cut-off wheel (231CA, Struers, Ballerup, Sweden) mounted in an Accutom-50 machine (Struers, Ballerup, Sweden). Metallographic polishing was accomplished with alumina slurries (10, 6, and 3 μm) and polishing cloths. Next, the cut tooth surface was mapped with indentations performed with a Berkovich diamond indenter in a Nano/Micro-Hardness Tester CSM at approximately every 200 μm (CSM instruments, Peseux, Switzerland) (Fig. 4A and B). The indenter approach speed was set to 300 mN/min with a maximum load of 150 mN. As the most central and hence ''soft'' parts of the carious lesions could not be appropriately mapped, measurements were done at peripheral regions of the carious lesion (Fig. 4B, asterisk). The whole mapping of indentations was transferred to the corresponding micro-CT slice of the tooth by means of masks consisting of 5-pixel diameter ROIs (Fig. 4C). The mean mineral density in each ROI in the micro-CT slice was calculated and correlated to the hardness measurements.

As the original micro-CT projections were generated into cross-section slices of the tooth, a slice-to-volume registration method was manually implemented, where the single stereomicroscopic ''slice'' was iteratively compared to best match the contour of the Y-re-sliced micro-CT data slices.

This transformation from mineral-density micro-CT values to hardness measurements was necessary to define an objective endpoint for caries removal. Based on this value, the excavated teeth were classified according to the mineral density at the bottom of the excavated cavity. If the mineral density was lower than the caries-removal endpoint, the tooth was considered to have residual dentine caries. If the mineral density was higher, the tooth was considered caries-free.

2.8. *Statistical analysis*

Pearson's correlation coefficients (R) were calculated between the L^* values, mineral density and LIF measurements. Student's t-test was used to assess differences between L^* values amongst teeth presenting residual caries or not.

Fig. 4 – (A) Stereomicroscopic image of the tooth used for hardness measurements and complete mapping of indentations. (B) Reflected light microscopy image of the region of interest depicted by dotted lines in (A). Indentation in carious dentine is marked by the asterisk and in sound dentine by the arrow. (C) Micro-CT slice corresponding to the measured slice in (A) after transfer of the selected ROIs.

Table 2 – Mineral-density values, DIAGNOdent measurements and distance from the deepest point in the cavity to the pulp-chamber roof according to the excavation methods.

Caries-excavation method	Mean mineral density (g/cm^3) ± SD	Mean LIF measurements ± SD	Mean distance to pulp-chamber roof (mm) ± SD
Conventional bur	1.18 ± 0.17	38.33 ± 34.93	1.75 ± 0.85
Cerabur	0.97 ± 0.19*	24.43 ± 24.89	1.47 ± 0.51
Cariex	1.19 ± 0.21	34.67 ± 39.78	1.12 ± 0.49
SFC-V	1.29 ± 0.24*	41.25 ± 37.07	1.47 ± 0.48
SFC-VIII	1.01 ± 0.15	47 ± 36.14	1.54 ± 0.62
SFC-VIII + instrument	1.07 ± 0.12	48.14 ± 37.94	1.96 ± 0.56
Carisolv	1.13 ± 0.10	29.71 ± 20.63	1.60 ± 0.59

* Statistically significant difference (Tukey–Kramer test, $p < 0.05$).

ANOVA with a Tukey–Kramer procedure was used to check for differences between the excavation groups. Significance was measured to the level of 5%.

3. Results

Mineral-density values at the bottom of the cavity, mean LIF values and the mean distance from the bottom of the cavity to the pulp-chamber roof are shown in Table 2 for the different excavation methods. No statistical difference was found between these variables for the different excavation groups, except for the mineral density measured in the exp. SFC-V group, which was statistically significantly higher than the values obtained in the CeraBur group (ANOVA, Tukey–Kramer procedure, $p < 0.05$). Although for the mean LIF values some excavation groups resulted in almost 100% difference, the high standard deviations probably accounted for the lack of statistical significance.

The common hardness threshold for ''infected'' or irreversibly denatured dentine found in the literature ranges around 300 GPa.[23] After finding the corresponding point of this threshold in the correlation obtained in the present study (Fig. 5), a mineral-density value of 1.11 g/cm^3 was found as the cut-off point for the end-point of caries removal. After using this cut-off value, evaluation of staining intensity at residual dentine showed that for teeth where no residual caries was left ($n = 22$) statistically significant (two-tailed t-test, $p < 0.01$) lower L^* values (41.19 ± 17.63) were found than in teeth where residual caries was left (58.5 ± 14.18; $n = 24$). This indicates that absence of residual caries was frequently associated with more intense staining.

Regardless of the excavation method, no significant correlation was found between the distance from the deepest cavity point to the pulp-chamber roof and LIF measurements (Fig. 6A). On the other hand, a strong negative correlation ($R = -0.86$, $p < 0.01$) was found between the L^* values and LIF measurements (Fig. 6B), indicating that staining in residual dentine accounts for statistically significantly higher LIF measurements. In the same way, a significantly negative correlation ($R = -0.622$, $p < 0.01$) was found between the L^* values and mineral-density values (Fig. 6C), pointing out that stained dentine is generally more mineralised. As a result, a significantly positive correlation was also found between the mineral-density values and the LIF measurements ($R = 0.57$, $p < 0.01$), as shown in Fig. 6D.

4. Discussion

The potential of micro-CT in providing quantitative data regarding mineral density of hard tissues has previously been demonstrated,[24] but in order to achieve this, reliable instrument calibration is required.[25] Previous studies using similar equipment have shown that the medium camera resolution (17.34 μm voxel size) was sufficient to assess mineral changes

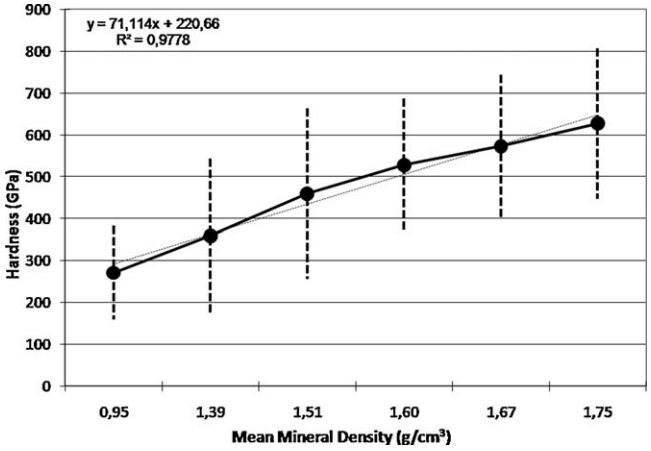

Fig. 5 – Correlation between hardness (GPa) obtained by nano-indentation and mineral density measured by micro-CT.

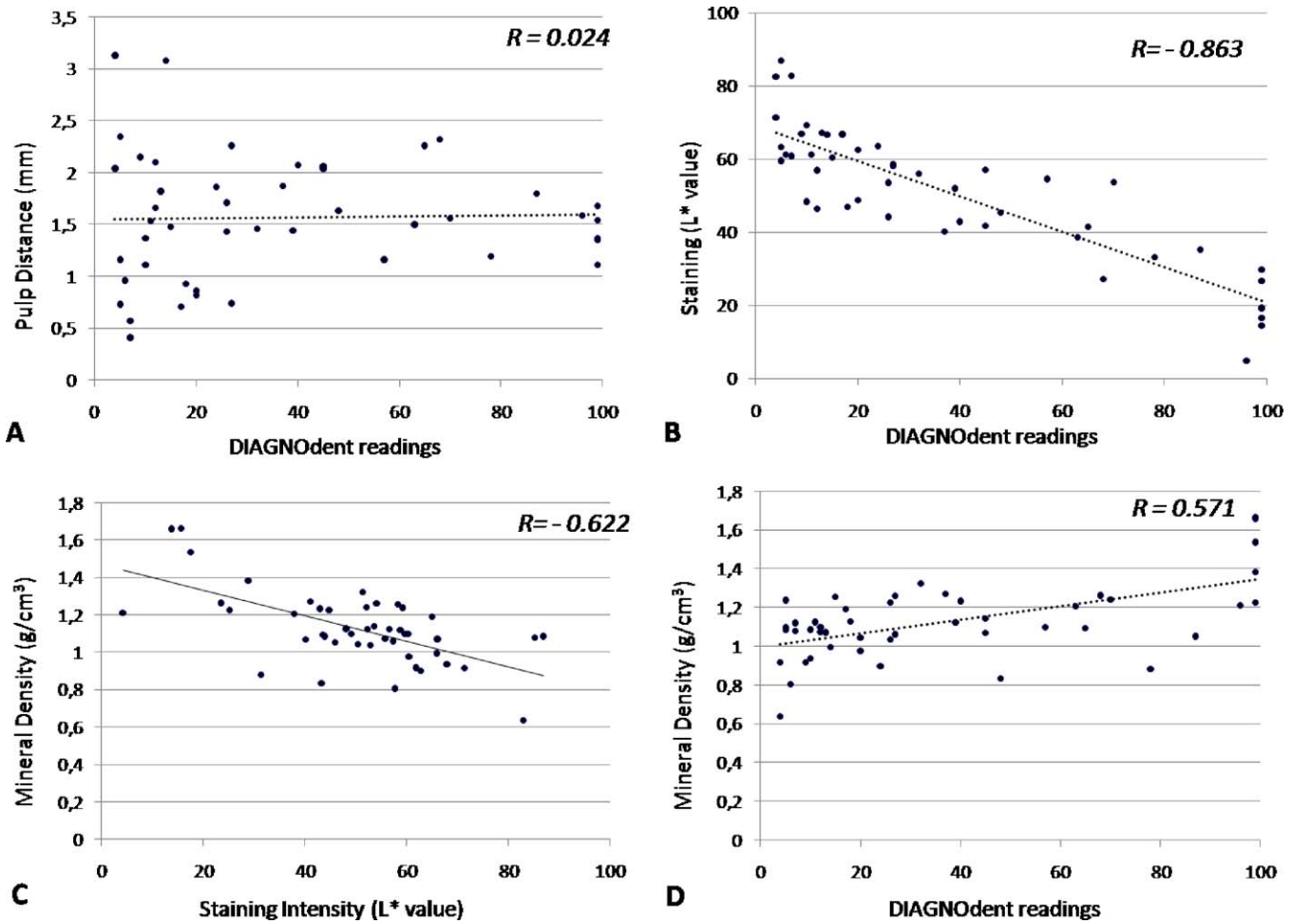

Fig. 6 – (A) Correlation between LIF measured by DIAGNOdent at the deepest part of the cavity and distance to the pulp chamber. (B) Correlation between LIF measured by DIAGNOdent and the staining intensity of residual dentine (L^* value). (C) Correlation between the staining intensity of residual dentine (L^* value) and the mineral density at the deepest part of residual dentine. (D) Correlation between LIF measured by DIAGNOdent and the mineral density at the deepest part of residual dentine.

in dentine.[26] In this study, a resolution of 14.66 μm was found appropriate to scan a full volume of the tooth in a suitable time period (≈30 min), whilst providing reliable mineral-density data.

The dentine-caries threshold used was based on hardness measurements described in the literature,[23] which in our data corresponded to 1.11 g/cm^3 of HAp. This threshold falls within the range of mineral-density values measured by micro-CT in previously published papers. Willmot et al.[27] for instance found a mineral concentration of 1.42 g/cm^3 HAp for sound deciduous dentine (1.77 for the upper limit and 1.27 for the lower limit), whilst Clementino-Luedemann and Kunzelmann[24] found values between 1.36 and 1.45 g/cm^3 for sound permanent dentine.

Regarding the efficacy of caries removal, the present study has shown that one of the experimental versions of the pepsin-based caries-removing gel (exp. SFC-V) presented more aggressive caries-removing properties, as appeared from statistically higher mineral-density values measured after excavation, as compared to those of CeraBurs (Table 2). On the other hand, exp. SFV-VIII presented similar caries-removal efficiency as Carisolv. In a previous study,[8] micro-CT demonstrated that exp. SFC-V and Carisolv presented similar caries-removal properties when caries was removed by a prototype plastic instrument. The latter may have been responsible for the less aggressive caries removal. According to the manufacturer (3M ESPE), differences between SFC-V and SFC-VIII reside on the thickening agent, which in SFC-V was not stable enough after a short storage period. It resulted in a thinner solution, which penetrated more into the demineralised dentine and therefore lead to an increased dentine dissolution and removal.

Regarding the CeraBurs, the lower mineral-density values obtained could have resulted from the caries-removal endpoint that relied only on the self-limiting cutting properties of the bur. These burs are made of ceramic materials, which are expected to present increased resistance to dulling during excavation, as compared to the previous self-limiting burs made of polymeric materials (SmartBurs, SSWhite, Lakewood, USA). We believe that the force used by the operator can also to some extent influence the cutting ability of this instrument, indicating that thus an operator-learning curve may be involved. One previous study compared the caries-removal efficacy by these burs with that of tungsten-carbide burs.[5] They concluded that although Ceraburs left somewhat more residual carious tissue, the difference was not statistically significant with that obtained by the tungsten-carbide burs.

The methodology used to measure the staining intensity of residual dentine after caries removal was partially based on methods used in previous literature.[28,29] In these studies, staining of residual dentine was evaluated after application of a red caries-disclosing solution (Caries Detector, Kuraray Europe, Frankfurt, Germany). Although a clear association between the L^* values and the staining typically present in inactive caries was seen, the most direct correlation was found between the a^* values and the staining induced by the caries-disclosing dye. As in the present study no external staining was used, the L^* values indicated the degree of staining based on a black to white scale.

It has been shown that the fluorescence of bacterial metabolites under red light excitation is responsible for the fluorescence of carious teeth.[30] This fact forms the basis for the use of DIAGNOdent to diagnose occlusal ''hidden'' caries lesions. However, bacterial metabolites may not be the only fluorophores that increase the DIAGNOdent readings, as the baseline fluorescence of whiter teeth is expected to be lower compared with that of darker teeth.[11] Indeed, the presence of stain and brown-spot lesions results in an additive fluorescence signal and thus definitely affects the DIAGNOdent performance as a diagnostic tool.[18]

The rationale behind the use of DIAGNOdent to detect residual carious dentine is also based on fluorescence induced by bacterial metabolites present in carious dentine.[13] As the red fluorescent indicator is exhibited by the metabolites, but not by the cariogenic bacteria themselves,[31] its detection merely means that at some point during caries progression bacteria had been metabolic active. It does not reflect the current state of bacterial metabolism in the region, which in the case of heavily stained, typical inactive caries is normally very low.[32]

The typical discolouration found in some carious lesions can be attributed to the so-called ''Maillard reaction'', which occurs between carbohydrates and a protein amino group and forms brownish polymers causing a typical dentine-caries discolouration.[33] Since products of the Maillard reaction are able to fluoresce,[34] they may thus dramatically increase the DIAGNOdent readings in stained lesions. Considering that the colour of carious dentine is not correlated to the levels of bacterial activity in residual dentine,[35] the use of this technology as a caries-removal endpoint can consequently be impaired.

The general agreement in previous studies that investigated the potential to use DIAGNOdent to set the caries-removal endpoint, was that a cut-off point for ''sound'' or ''affected'' dentine tissue could be set between 11 and 20.[13,14,16] These studies, however, did not report on the activity of the lesions (active or arrested), nor on the status of residual dentine staining. In one study, where only molars with typical active caries lesions were used, the lowest DIAGNOdent value, at which bacteria were detected, was 15.6 ± 1.2 (mean $\pm$ SD), whilst the largest value without bacterial detection was 40.8 ± 2.0 (mean $\pm$ SD).[36] This corroborates to set at the safe side a probable interval between 11 and 20 as an indication that all infected tissue is removed from active lesions, where much staining is not expected.

Mineral-density determination can be considered as an indirect measurement of the hardness of dentine. Whereas previously a significant negative correlation has been found between dentine hardness and level of bacterial infection, such correlation was not found for tissue colour/staining and bacterial infection.[35] As hardness in carious dentine is not directly correlated to staining, it was not surprising that mineral-density values in this study did not positively correlate with staining in residual caries (Fig. 6C). On the contrary, the fact that darker lesions were more mineralised, indicates that a more conservative removal of primary occlusal caries should include retaining stained and hard tissue at the bottom of the cavity.

LIF measurements of root caries also disclosed a direct correlation to tissue staining.[37,38] Although the ability to correctly identify the presence of caries by DIAGNOdent was very good, in those studies the distinction between ''sound'' and ''carious'' dentine was made histologically by means of polarised light microscopy. The latter identifies any microscopic change in ''sound'' tissue, but does not register the actual lesion activity. Interestingly, the higher LIF measurements were associated with heavily stained, but shallow cavities. LIF measurements were in both studies only weakly correlated to histological caries assessment.

According to some authors, another possible limitation to the use of laser-induced fluorescence to diagnose residual caries is that DIAGNOdent values near the dental pulp are increased when measured *in vivo*.[15] In accordance with the present *in vitro* study, others found no significant increase in DIAGNOdent readings within the proximity to the pulp.[38] These high values measured *in vivo* should perhaps be attributed to other factors related to living pulpal tissue. However, fluorescence-aided caries-removal with an Er:YAG laser (Key Laser III, Kavo, Biberach, Germany) showed that the observation of increased fluorescence closer to the dental pulp was probably not clinically significant.[15,39]

5. Conclusion

Regarding the caries-excavation methods, the experimental pepsin-based chemical caries-removal agent 'exp. SFC-V' exhibited the most aggressive caries-removing properties, whilst the CeraBurs left residual dentine with a lower mineral density than the actually measured threshold for dentine caries. The significant increase in LIF measured by DIAGNOdent with increased staining of residual caries may impair the use of laser-induced fluorescence to set the endpoint of caries removal during cavity preparation, especially in inactive or stained lesions. The results of this study indicate that DIAGNOdent should not be used beyond the manufacturers' recommendation to diagnose occlusal caries in suspicious fissures.

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