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ORIGINAL ARTICLE

Evaluation of laser fluorescence in monitoring non-cavitated caries lesion progression on smooth surfaces in vitro

J. A. Rodrigues[1] · C. S. Sarti[1] · C. M. Assunção[1] · R. A. Arthur[2] · A. Lussi[3] · M. B. Diniz[4]

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Abstract The aim of this study was to evaluate the performance of a pen-type laser fluorescence (LF) device (LFpen: DIAGNOdent pen) to detect and monitor the progression of caries-like lesions on smooth surfaces. Fifty-two bovine enamel blocks were submitted to three different demineralisation cycles for caries-like lesion induction using *Streptococcus mutans*, *Lactobacillus casei* and *Actinomyces naeslundii*. At baseline and after each cycle, the enamel blocks were analysed under Knoop surface micro-hardness (SMH) and an LFpen. One enamel block after each cycle was randomly chosen for Raman spectroscopy analysis. Cross-sectional micro-hardness (CSMH) was performed at different depths (20, 40, 60, 80 and 100 μm) in 26 enamel blocks after the second cycle and 26 enamel blocks after the third cycle. Average values of SMH (± standard deviation (SD)) were 319.3 (± 21.5), 80.5 (± 31.9), 39.8 (± 12.7), and 29.77 (± 10.34) at baseline and after the first, second and third cycles, respectively. Statistical significant difference was found among all periods ($p < 0.01$). The LFpen values were 4.3 (± 1.5), 7.5 (± 9.4), 7.1 (± 7.1) and 5.10 (± 3.58) at baseline and after the first, second, and third cycles, respectively, among all periods ($p < 0.05$). The CSMH values after the second and third cycles at 20, 40, 60, 80 and 100 μm were 182.8 (± 69.8), 226.1 (± 79.6), 247.20 (± 69.36), 262.35 (± 66.36) and 268.45 (± 65.49), and for the third cycle were 193.7 (± 73.4), 239.5 (± 81.5), 262.64 (± 82.46), 287.10 (± 78.44) and 284.79 (± 72.63) ($n = 24$ and 23), respectively. No correlation was observed between the LFpen and SMH values ($p > 0.05$). One sample of each cycle was characterised through Raman spectroscopy analysis. It can be concluded that LF was effective in detecting the first demineralisation on enamel; however, the method did not show any effect in monitoring lesion progression after three cycles of in vitro demineralisation.

Keywords Dental caries · Demineralisation · Laser fluorescence (LF) · Caries detection · Raman · Micro-hardness · DIAGNOdent pen

Introduction

In the last decades, some studies have indicated a decline in caries prevalence; however, this disease is still a serious oral health problem in many parts of the world [1–3]. The early detection of smooth surface caries lesions is important to provide proper management of caries and allows their monitoring over time [1, 4]. Conventional methods for caries detection, such as visual inspection and radiographic examination, are not capable of quantifying the mineral loss or gain occurring as a result of demineralisation and remineralisation processes, respectively [5].

Several quantitative methods have been developed and recommended to identify and qualify early caries lesions on

✉ J. A. Rodrigues
jorodrigues@ufrgs.br

[1] Department of Pediatric Dentistry, School of Dentistry of Rio Grande do Sul Federal University – UFRGS, Porto Alegre, RS, Brazil

[2] Department of Public Health, School of Dentistry of Rio Grande do Sul Federal University – UFRGS, Porto Alegre, RS, Brazil

[3] Department of Preventive, Restorative and Pediatric Dentistry, School of Dental Medicine, University of Bern, Bern, Switzerland

[4] Department of Pediatric Dentistry, School of Dentistry of Cruzeiro do Sul University – UNICSUL, São Paulo, SP, Brazil

smooth and occlusal surfaces. Devices based on fluorescence could enhance caries lesion detection. One of these methods is based on physical fluorescence, which is different for carious tissue compared with sound hard dental tissue [4, 6–9].

The DIAGNOdent pen (LFpen; DIAGNOdent 2190, KaVo, Biberach, Germany) is a laser-based instrument that emits red light at a wavelength of 655 nm from a fibre-optic bundle and captures the fluorescence emitted by oral bacterial porphyrins and other chromophores present on the demineralised dental tissue. The performance of this device in detecting or monitoring caries development on smooth surfaces has shown contradictory results in different studies [10–15].

The aim of this in vitro study was to evaluate the performance of a laser fluorescence (LF) device (LFpen) to detect and monitor the progression of caries-like lesions on smooth surfaces.

Materials and methods

Ethical considerations

This study was conducted according to the requirements of the Ethics Committee on Animal Use of the Rio Grande do Sul Federal University—UFRGS (protocol 20576), Porto Alegre, Brazil.

Sample preparation

In this study, bovine teeth were selected because they are convenient to obtain in significant quantities, are in good condition and have more uniform composition than human teeth. Furthermore, bovine teeth have similar chemical composition to human teeth [16]. One hundred teeth were initially collected.

Bovine incisors (*Bos taurus*) without cracks and structural defects were used in this study. The teeth were cleaned using an ultrasonic scaler (Sonics & Schuster Laxis) to remove calculus and debris, after which they were brushed under running water for 15 s. The crowns were separated from the roots using a precision cutting machine with a diamond disc under cooling using a metallographic cutter (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA). The flattest portions of the buccal surfaces were cut in enamel blocks of 4 × 4 mm.

Sixty enamel blocks were embedded in epoxy resin. Then, the samples were grinded and polished (grinding papers 220, 400, 600 and 1200, polishing cloth with 1 and 1/4 μm diamond abrasive, under constant cooling, APL4, Arotec Industry & Commerce S/A, Cotia, SP, Brazil). A 1 × 4 mm portion was covered with nail varnish to protect this area from the development of artificial caries, providing a sound reference area for further measurements.

The initial surface micro-hardness (SMH; five indentations in the central area, 100 μm away from each other, 50 g for 5 s, HMV-2T, Shimadzu, Kyoto, Japan) measurements were performed to select samples with similar SMH values (14, 18). After these measurements, a total of 52 enamel blocks were selected to start the experimental procedures (Fig. 1).

Artificial caries induction

To induce caries-like lesions, strains of *Streptococcus mutans*, *Lactobacillus casei* and *Actinomyces naeslundii* were used in a tryptic soy broth (TSB) medium with 0.5% sucrose (adapted from Arthur et al., 2015) [17]. All blocks remained initially for 48 h in this bacterial inoculum at 37 °C. After this period, the blocks were rinsed in tap water and stored in relative humidity for SMH, LFpen and Raman microscopy analysis. After the first demineralisation cycle and measurements, all blocks were submitted to hydrogen peroxide plasma sterilisation without damaging the samples to avoid cross infection, and a second demineralisation cycle was performed. At the end of the second cycle, the blocks were randomly divided, and 26 blocks were submitted to the third demineralisation cycle. At this moment, the other 26 blocks were submitted to cross-sectional micro-hardness (CSMH) measurements.

Fluorescence measurements

The LF measurements were carried out using a DIAGNOdent pen (LFpen; DIAGNOdent 2190, KaVo, Biberach, Germany). The LFpen was calibrated against a ceramic reference (standard calibration) according to the manufacturer's specifications before each sample measurement. The probe tip, which was designed for smooth surfaces, was placed perpendicularly on the test site and moved until the maximum value (peak) was reached; this value was recorded three times, and the mean was calculated. The measurements were performed by a single, previously trained examiner (intra-class correlation (ICC) mean values 0.95–1.0). These procedures were performed at baseline and after each demineralisation cycle.

Surface and cross-sectional micro-hardness measurements

The SMH assessment was performed at baseline and after each demineralisation cycle (n = 26 after third cycle). Six indentations were made at a central area, 50 μm from each other (50 g for 5 s, HMV-2T, Shimadzu, Kyoto, Japan). The examiner performed a blind assessment.

The samples were longitudinally sectioned (26 samples after two cycles and 26 after three cycles) through the centre of the exposed enamel (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA), including the sound area, for CSMH. The

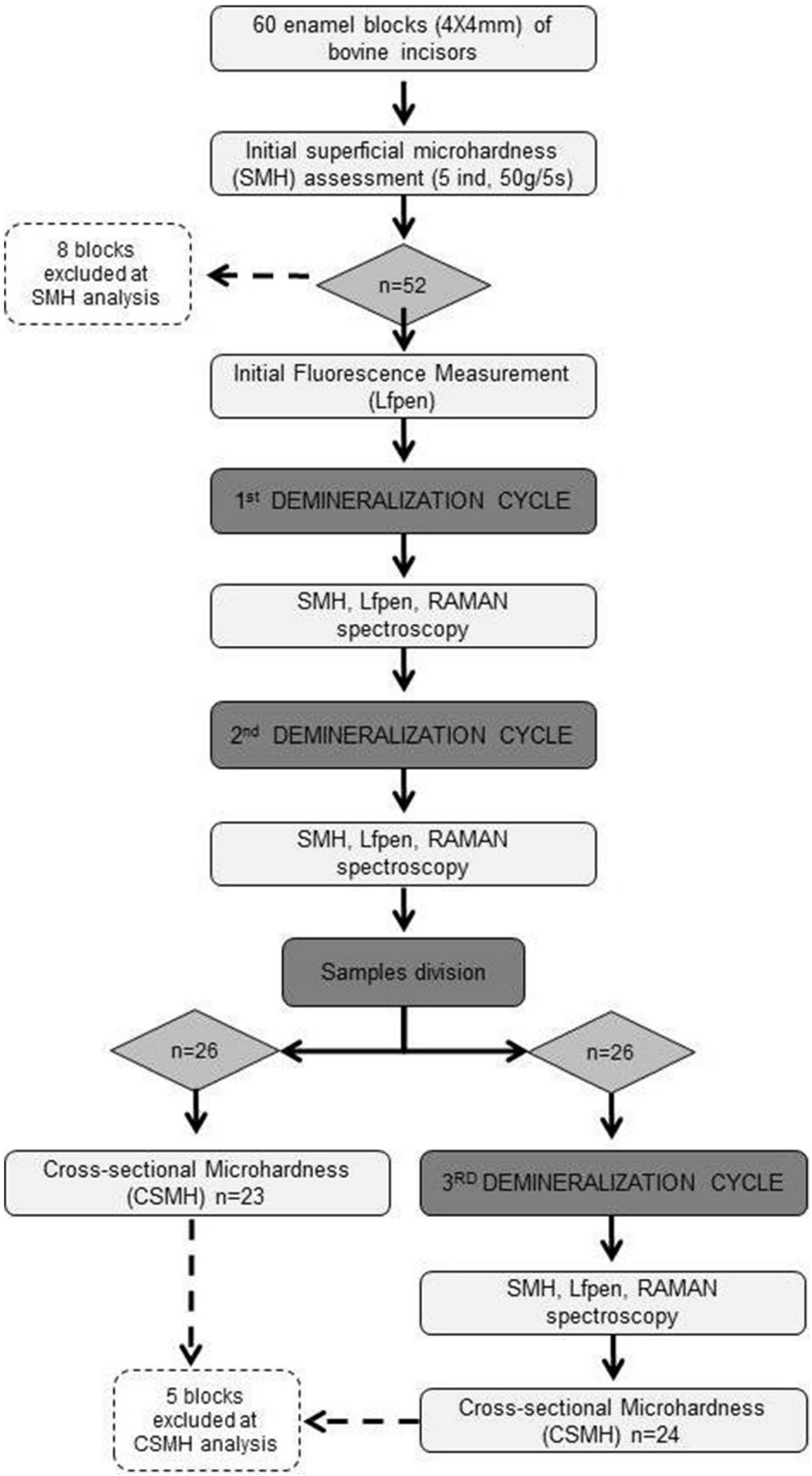


Fig. 1 Study design

samples were polished again (polishing cloth with 1 and 1/4 μm diamond abrasive, under constant cooling). The indentations were made at 20, 40, 60, 80 and 100 μm of the surface, on both sound and demineralised areas (three columns, 50 μm distance). The mean value of each distance was calculated. The SMH change (%SMH), integrated hardness (KHN × μm) and integrated loss of hardness (ΔKHN) were calculated for each sample [5, 18].

Table 1 The LFpen SMH and percentage of change in SMH (%SMH) mean values (analysis of variance (ANOVA) repeated measures (RM) followed by the Holm-Sidak test, data-log transformed)

	Baseline (n = 52)	First cycle	Second cycle	Third cycle (n = 26)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
FLpen	4.31 (± 1.53)^{A}	7.58 (± 9.70)^{B}	7.16 (± 7.16)^{B}	5.10 (± 3.58)^{AC}
SMH	320.35 (± 20.75)^{A}	80.49 (± 31.92)^{B}	39.82 (± 12.77)^{C}	29.77 (± 10.34)^{D}
%SMH Change	–	74.95 (± 9.39)^{A}	87.61 (± 3.66)^{B}	88.52 (± 9.35)^{B}

Different letters on the same line represent significant difference ($p \leq 0.005$)

Raman spectroscopy analysis

To characterise the demineralisation area, one sample after each cycle was randomly chosen for Raman spectroscopy analysis (Senterra Raman microscope, Bruker Optics, Bileria, EUA). The phosphate and calcium peaks were selected, at sound and demineralised areas. The examiner performed a blind analysis.

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS 21, Chicago, USA). The ICC for intra-examiner reproducibility analysis and analysis of variance (ANOVA) repeated measures (RM) was followed by the Holm-Sidak test, which was used to evaluate the correlation among the LFpen, SMH and percentage of change in SMH mean values (%SMH). Integrated hardness (KHN × μm) and integrated loss of hardness (ΔKHN) were also determined. The significance level was set at 0.05.

Results

Intra-examiner reproducibility for the LFpen showed ICC mean values of 0.95 (baseline), 0.99 (after the first cycle), 0.95 (after the second cycle) and 1.0 (after the third cycle), indicating an excellent agreement between the examiner measurements in each period.

For the LFpen, SMH and (%SMH) mean values are shown in Table 1. The CSMH values at different depths (20, 40, 60, 80 and 100 μm) are presented in Table 2.

The integrated hardness (KHN × μm) of sound and demineralised enamel was calculated at a depth of 100 μm using the trapezoidal rule [18]. The integrated loss of hardness (ΔKHN) was calculated by subtracting the integrated hardness of demineralised enamel from the integrated hardness of sound enamel [5] (Table 3).

Figure 2 shows scatter plots using the LFpen values according to the SMH at each assessment period and the comparison of the three periods. Figure 3 shows the decrease of the intensity of the phosphate peak from the interface/boundary between sound enamel (Z = 0) and demineralised enamel (Z = 20).

Discussion

Few studies have been conducted focusing on the performance of the LF method for the detection and monitoring of caries lesions on smooth surfaces, and many questions regarding its effectiveness have been pointed out. To our knowledge, no study has combined a bacterial model to induce caries lesions for LF measurements and Raman spectroscopy as an assessment tool for caries lesion progression.

The detection of demineralisation on smooth surfaces and its progression was evaluated in this study. The LFpen emits a diode laser at 655 nm wavelength, which is absorbed by the dental tissues. Part of this light is re-emitted as fluorescence. The fluorescence increases with the progression of caries lesion. According to Lussi and Hellwig (2006) [19], high reproducibility values indicate that the method may be able to monitor the carious process. This statement seems to be true when caries lesions are evaluated in later stages. In this study, in the

Table 2 CSMH values in different depths (analysis of variance (ANOVA) repeated measures (RM) followed by Holm-Sidak test, data-log transformed)

Depth	Sound (n = 24)	Second cycle (n = 23)	Third cycle (n = 24)
	Mean KNH (SD)	Mean KNH (SD)	Mean KNH (SD)
20 μm	236.97 (± 58.16)^{A}*	182.8 (± 69.8)^{B}	193.7 (± 73.4)^{B}
40 μm	260.8 (± 89.8)	226.1 (± 79.6)	239.5 (± 81.5)
60 μm	273.75 (± 84.23)	247.20 (± 69.36)	262.64 (± 82.46)
80 μm	288.93 (± 80.68)	262.35 (± 66.36)	287.10 (± 78.44)
100 μm	292.37 (± 85.93)	268.45 (± 65.49)	284.79 (± 72.63)

Different superscrit letters show significant difference between sound and demineralised CSMH values only at 20 μm depth

Table 3 Integrated hardness (KHN × μm) of sound and demineralised enamel

Cycle	Sound	Demineralised enamel	ΔKHN
	Mean (SD)	Mean (SD)	Mean (SD)
Second cycle	16,440.4 (± 4003.2)	17,527.5 (± 4764.2)	1367.6 (± 2716.3)
Third cycle	15,072.8 (± 3887.0)	16,131.8 (± 4416.5)	1395.8 (± 2831.5)

evaluation of non-cavitated lesions, the ICC values were excellent (0.95–0.99) in terms of intra-examiner agreement. This shows that the method is reproducible, however not necessarily valid. These values indicate the use of the LFpen for caries lesion monitoring over time. Similar results were also found in previous studies [7, 19].

The microbiological model used in this study was more suitable than the pH-cycling models for the evaluation of caries-like lesions. Chemical experimental models, such as pH cycling and immersion using demineralising agents, do not simulate the demineralisation process that occurs in the oral cavity, owing to the absence of microorganisms. Therefore, they reflect only the physical-chemical aspects of enamel dissolution. An in vitro model that uses bacterial biofilm would be more representative than the chemical experimental models since dental caries is a multifactorial disease. Moreover, the LFpen detects changes in organic tooth content, such as fluorophores and other chromophores produced by cariogenic bacteria. Thus, the induction model of caries-like lesion used in this study seems to be closer to clinical reality [15, 20, 21].

In addition to LF, this study also used micro-hardness and Raman spectroscopy to assess the caries lesions. The microhardness technique has been used to assess tooth mineral loss [7]. Pioneer studies have suggested that this technique is able to distinguish among distinct caries lesion depths by the assessment of changes in physical properties of the tooth surface by means of load applied perpendicularly to a subsurface

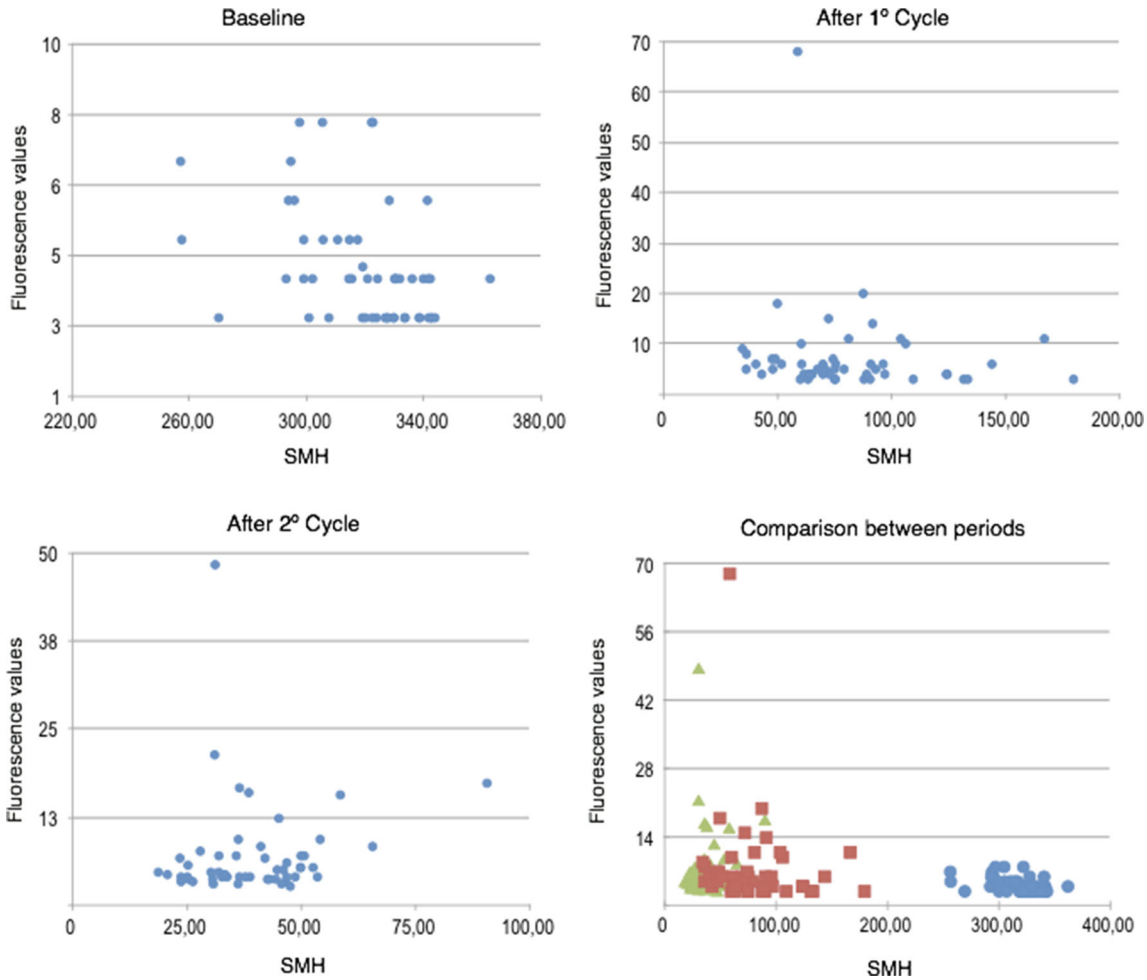
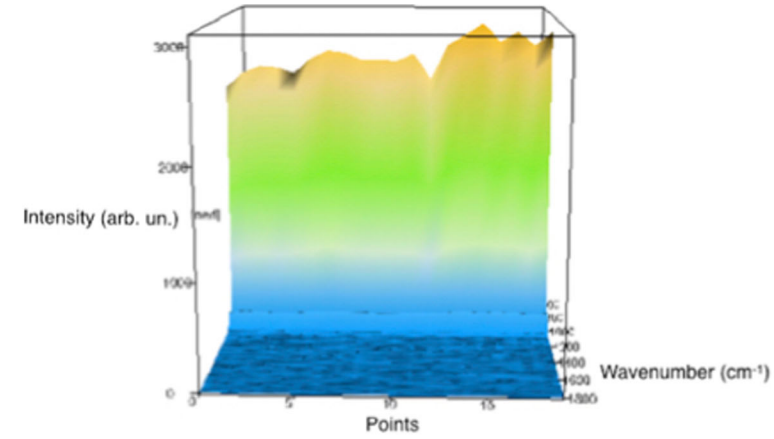
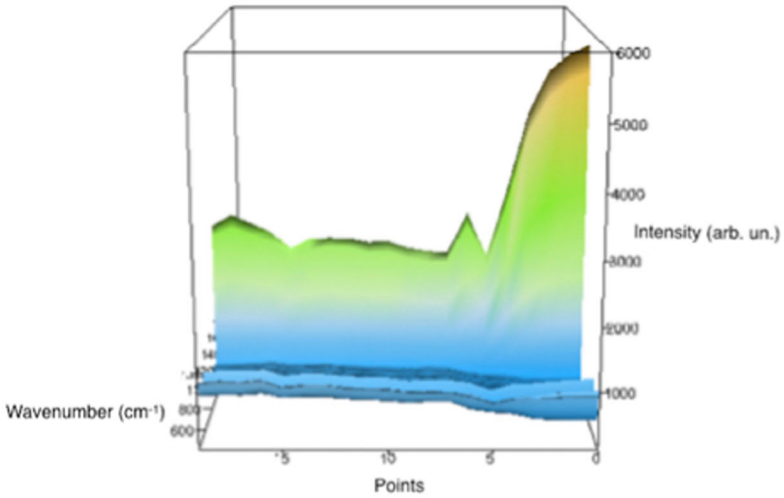


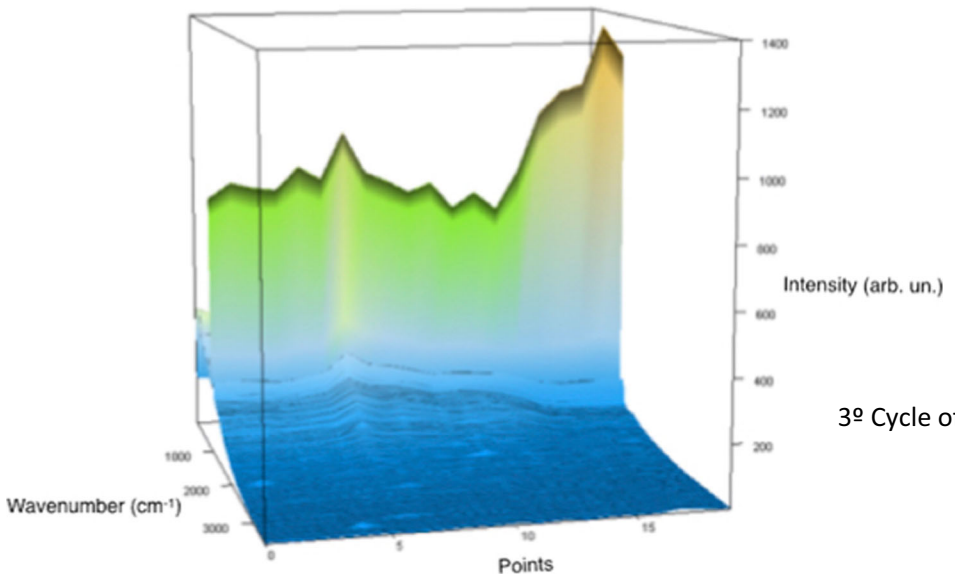
Fig. 2 Scatter plots of fluorescence values and SMH at baseline, after the first and second cycles and comparing the three periods



1º Cycle of demineralization



2º Cycle of demineralization



3º Cycle of demineralization

Fig. 3 Spectrum generated by Raman spectroscopy after each cycle of demineralisation

lesion [22, 23]. Furthermore, integrated hardness can be determined in response to distinct lesion depths [23]. On the other hand, Raman spectroscopy allows the quantification of mineral content of specimens in terms of calcium and phosphate, which are closely related to the pattern of tooth demineralisation [21, 24]. Therefore, the use of these three techniques allowed a more in-depth characterisation of the caries-like lesions produced under the tested conditions.

Although the SMH was statistically different between the baseline and the three cycles, the CSMH only showed a difference on the first layer (20 μm), when comparing the second and third cycles. The superficial SMH change (%SMH) presented a significant difference only between the sound enamel (baseline) and initial caries lesion (three demineralisation cycles). Considering the LFpen values, it can be observed that the cycles for lesion induction were effective in demineralising enamel blocks and a significant increase in fluorescence values was found only after the first cycle. This shows that the method was effective to detect and quantify the initial demineralisation of the enamel blocks.

However, when the blocks were submitted to the second and third cycles, simulating the process of lesion progression, the LFpen was not effective for monitoring lesions in a stage previous to cavitation. These findings support previous results obtained by Moriyama et al. (2014) [7]. A positive aspect that can be understood from this result is that the method is able to discriminate the sound enamel from the enamel initially demineralised. In an in vivo study, Gokalp and Baseren (2005) also found that the LF showed good ability to detect healthy tooth surfaces [25]. This fact emphasises the idea that the LFpen device might to be able to detect the first changes in the enamel since its fluorescence shows more differences between the sound tissue and a progressed lesion. Moreover, micro-hardness and Raman analysis were more sensitive to such mineral changes up to the structural level and therefore were better to characterise lesion progression over time.

In this study, Raman spectroscopy was used to identify changes in the inorganic tooth components. Raman spectroscopy shows the microbiochemical structure of the tissues. It is a non-destructive vibrational technique, which allows simultaneous characterisation of organic and inorganic matter and could show the pattern of demineralisation through the calcium of phosphate peaks (about 960 cm^{-1}) [25]. According to the increase of demineralisation of the enamel surface during the three experimental cycles, the obtained spectrum peak of phosphate (around 960 cm^{-1}) also decreased, indicating that their phosphate content (mineral content) is lower than that of the sound enamel. This is in agreement with a recent study that used this methodology to observe the initial demineralisation of enamel surfaces [26] and corroborates with Diniz et al. (2009), who also observed that the LF device was not effective in monitoring in vitro demineralisation and remineralisation of smooth enamel surfaces [24].

Conclusion

Based on the results of the present study, which combined an investigation of LF with micro-hardness and Raman spectroscopy, it can be concluded that the LF pen was effective in detecting the first demineralisation in the enamel; however, the method did not show any effect in monitoring enamel lesion progression after three cycles of demineralisation. These findings indicate that the LFpen does not accurately measure small changes in mineral content.

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Compliance with ethical standards This study was approved by the Research and Ethics Committee on Animal Use of the Federal University of Rio Grande do Sul (process no. 20576) since bovine teeth were used. Therefore, no informed consent was necessary.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Pretty IA, Maupome G (2004) A closer look at diagnosis in clinical dental practice: part 5. Emerging technologies for caries detection and diagnosis. J can Dent Assoc 70:540–540a–540i
2. Gimenez T, Bispo BA, Souza DP et al (2016) Does the decline in caries prevalence of Latin American and Caribbean children continue in the new century? Evidence from systematic review with meta-analysis. PLoS One 11:e0164903. doi:10.1371/journal.pone.0164903
3. Tagliaferro EPS, Meneghim MC, Ambrosano GMB et al (2008) Distribution and prevalence of dental caries in Bauru, Brazil, 1976–2006. Int Dent J 58:75–80
4. Bader JD, Shugars DA (2006) The evidence supporting alternative management strategies for early occlusal caries and suspected occlusal dentinal caries. J Evid Based Dent Pract 6:91–100. doi:10.1016/j.jebdp.2005.12.004
5. Spiguel MH, Tovo MF, Kramer PF et al (2009) Evaluation of laser fluorescence in the monitoring of the initial stage of the de-/remineralization process: an in vitro and in situ study. Caries Res 43:302–307. doi:10.1159/000218094
6. Lussi A, Francescut P (2003) Performance of conventional and new methods for the detection of occlusal caries in deciduous teeth. Caries Res 37:2–7
7. Moriyama CM, Rodrigues JA, Lussi A, Diniz MB (2014) Effectiveness of fluorescence-based methods to detect in situ demineralization and remineralization on smooth surfaces. Caries Res 48:507–514. doi:10.1159/000363074
8. Rodrigues JA, Hug I, Diniz MB, Lussi A (2008) Performance of fluorescence methods, radiographic examination and ICDAS II on occlusal surfaces in vitro. Caries Res 42:297–304. doi:10.1159/000148162

9. Braun A, Krause F, Jepsen S (2005) The influence of the calibration mode of a laser fluorescence device on caries detection. Caries Res 39:144–149. doi:10.1159/000083161
10. Aljehani A, Yang L, Shi X-Q (2007) In vitro quantification of smooth surface caries with DIAGNOdent and the DIAGNOdent pen. Acta Odontol Scand 65:60–63. doi:10.1080/00016350601058051
11. Mendes FM, Hissadomi M, Imparato JCP (2004) Effects of drying time and the presence of plaque on the in vitro performance of laser fluorescence in occlusal caries of primary teeth. Caries Res 38:104–108. doi:10.1159/000075933
12. Mendes FM, Siqueira WL, Mazzitelli JF et al (2005) Performance of DIAGNOdent for detection and quantification of smooth-surface caries in primary teeth. J Dent 33:79–84. doi:10.1016/j.jdent.2004.10.010
13. Paes Leme AF, Tabchoury CPM, Zero DT, Cury JA (2003) Effect of fluoridated dentifrice and acidulated phosphate fluoride application on early artificial carious lesions. Am J Dent 16:91–95
14. Pinelli C, Campos Serra M, de Castro Monteiro Loffredo L (2002) Validity and reproducibility of a laser fluorescence system for detecting the activity of white-spot lesions on free smooth surfaces in vivo. Caries Res 36:19–24
15. Shi XQ, Tranaeus S, Angmar-Mansson B (2001) Validation of DIAGNOdent for quantification of smooth-surface caries: an in vitro study. Acta Odontol Scand 59:74–78
16. Yassen GH, Platt JA, Hara AT (2011) Bovine teeth as substitute for human teeth in dental research: a review of literature. J Oral Sci 53:273–282
17. Arthur RA, Martins VB, de Oliveira CL et al (2015) Effect of over-the-counter fluoridated products regimens on root caries inhibition. Arch Oral Biol 60:1588–1594. doi:10.1016/j.archoralbio.2015.07.018
18. Cury JA, Rebelo MA, Del Bel Cury AA et al (2000) Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res 34:491–497
19. Lussi A, Hellwig E (2006) Performance of a new laser fluorescence device for the detection of occlusal caries in vitro. J Dent 34:467–471. doi:10.1016/j.jdent.2005.11.002
20. Lussi A, Imwinkelried S, Pitts N et al (1999) Performance and reproducibility of a laser fluorescence system for detection of occlusal caries in vitro. Caries Res 33:261–266
21. De Campos PH, Sanabe ME, Rodrigues JA et al (2015) Different bacterial models for in vitro induction of non-cavitated enamel caries-like lesions: microhardness and polarized light miscroscopy analyses. Microsc Res Tech 78:444–451. doi:10.1002/jemt.22493
22. Purdell-Lewis DJ, Groeneveld A, Arends J (1976) Hardness tests on sound enamel and artificially demineralized white spot lesions. Caries Res 10:201–215
23. Arends J, Schuthof J, Jongebloed WG (1979) Microhardness indentations on artificial white spot lesions. Caries Res 13:290–297
24. Diniz MB, Paes Leme AF, Cardoso K d S et al (2009) The efficacy of laser fluorescence to detect in vitro demineralization and remineralization of smooth enamel surfaces. Photomed Laser Surg 27:57–61. doi:10.1089/pho.2007.2230
25. Gokalp S, Baseren M (2005) Use of laser fluorescence in monitoring the durability and cariostatic effects of fluoride and chlorhexidine varnishes on occlusal caries: a clinical study. Quintessence Int 36:183–189
26. Mohanty B, Dadlani D, Mahoney D, Mann AB (2013) Characterizing and identifying incipient carious lesions in dental enamel using micro-Raman spectroscopy. Caries Res 47:27–33. doi:10.1159/000342432