IS A pH- CYCLING MODEL A SUITABLE SIMULATOR OF ACTUAL PHYSICOCHEMICAL PROCESSES IN INITIAL CARIOUS LESIONS?

Hasić Branković Lajla*[1], Tahmiščija Irmina[1], Korać Samra[1], Konjhodžić Alma[1], Džanković Aida[1], Hadžiabdić Naida[2]

[1] Department of Dental Pathology and Endodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina
[2] Department of Oral Surgery, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

*Corresponding author

Assistant Professor
Lajla Hasić Branković, M.Sc, Ph.D,
Dental Pathology and
Endodontics specialist,
University of Sarajevo,
Faculty of Dentistry with Clinics,
Department of Dental Pathology
and Endodontics,
Bolnička 4a,
71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 (33)214 249
e-mail: lajlabrankovic@gmail.com

ABSTRACT

Artificial caries-like models were developed to understand processes in dental caries. Numerous different models were introduced during the past few decades. One of the first models was a pH-cycling model. It remains a basic model for simulation of various processes in enamel and dentin during carious lesion development.

The paper aims to present leverages and disadvantages, features and acquiring procedures for the basic pH-cycling model.

Material and methods: 60 enamel slabs made from the first and the second human premolars extracted from orthodontic reasons, were divided into three experimental groups (native enamel (NE) control group, white spot (WS)-experimental group, and demineralized enamel (DE) - negative control group). All three groups were positioned on the same experimental model. The native enamel (NE) group didn't receive any de-or remineralization treatment. WS group was submitted to a pH cycling regime to create artificial caries lesion, and demineralized enamel was created by constant demineralization during 24 hours being then submerged into distilled deionized water, without remineralization treatment.

Cross-sectional microhardness (CSMH) measurements were done through the outer peripheral layer of enamel. Measurement results are expressed as a Knoop Hardness Number (KHN) and subsequently converted into the volume of the mineral percentage.

Results: CSMH showed the formation of artificial caries like lesion with an average depth of 50-60 micrometres. Significant differences ($p \leq 0,05$) appeared in the first 40 micrometres in the surface enamel layer. Differences in enamel cross-sectional microhardness were lost in deeper portions of the enamel.

Conclusion: pH cycling models are suitable for the investigation of physical and chemical processes in enamel during early caries lesion progression. This model enables monitoring changes in depth, construction of lesions mineral profile as an additional experimental procedure in the same sample.

Keywords: artificial caries, pH-cycling model, enamel demineralization, enamel remineralization, enamel CSMH.

Introduction

pH-cycling models as simulators of actual physic-chemical processes in dental caries

In-vitro models, developed a few decades ago, have proven to be very useful models in examining the dynamics of caries formation and the appearance of carious lesions. These models realistically reflect clinical *in-vivo* conditions and provide insight into changes in the biological substrate of enamel. [1] A well-designed experimental model allows us to easily adjust the level of caries risk by changing the conditions of enamel exposure. Furthermore, enamel samples can be additionally analyzed microscopically, functionally, subjected to another type of tests or became a biological substrate for detail examination in microbiological models (for example substrate for biofilm growth), etc. [2, 3] *In-vitro* models are still the most widely used form of testing performed for this purpose. [4, 5]

Clinical studies are very expensive and pertinently time-consuming. It often takes 1-3 years to evaluate for example the preventive effect of an experimental agent. Hence, other models of shorter-term clinical studies have been developed. They are equally expensive but significantly faster to perform. [6]

Additional possibilities of testing enamel samples underwent in some experimental procedure in clinical studies are technically difficult, if not even unmanageable. [3] Clinical studies remain the gold standard for assessing the efficacy of individual agents, but due to their shortcomings, they have largely been replaced by well-controlled *in-vitro* models. [5, 6]

A well-controlled *in-vitro* model involves an experimental system precisely defining the dose-dependent sensitivity of the administered agent, as well as potential differences in efficacy and/or rate of action. [6] The models allow the isolation and emphasis of certain aspects of the carious process, such as the microbiological, biochemical or physicochemical aspects of the carious process. [3, 7] Leading among these are pH -cycling models.

A literature review shows that in the last few decades, several *in-vitro* models have been developed on which numerous experiments have been performed. Knowledge about the nature and dynamics of the carious process, the content of fluoride and other elements in it largely derive from these *in- vitro* experimental models. [4]

Various pH-cycling models have been developed for different purposes. Some are designed to evaluate demineralization, others to study remineralization and some to evaluate the effect of fluoride in these processes. [5, 8, 9] There are numerous examples of successful implementation of pH-cycling models containing enamel and dentin blocks, not just in restorative dentistry, but in dentistry in general. [10, 11, 12] They all had tremendous success in cariology. [4]

In-vitro experimental protocols, however, also show limitations.

Therefore, these models should be evaluated very carefully, especially when assessing the preventive potential of individual drugs, depending on administered doses. [13, 14] For instance, the preventive fluoride effect is observable in such models. A well-designed pH-cycling model, preconcert to simulate conditions in a specific moment of the carious process, can determine predominant component of this process is: demineralization or remineralization. Additionally, the impact of fluoride on the degree of enamel hardness, as well as the reduction of the enamel lesion depth can be evaluated. The precise chemical structure of fluoride "enriched" enamel can be also determined. [15, 16, 17, 18]

Various studies were performed to develop and adapt pH-cycling models to be more faithful representative of *in-vivo* conditions. One of the variable parameters is the substrate in pH-cycling models. Bovine enamel showed numerous advantages over humans. It is low-cost, easy to purchase and it´s chemical structure is very similar to human enamel. [13, 14, 19] Therefore, the method described in this paper is applicable for bovine enamel, likewise.

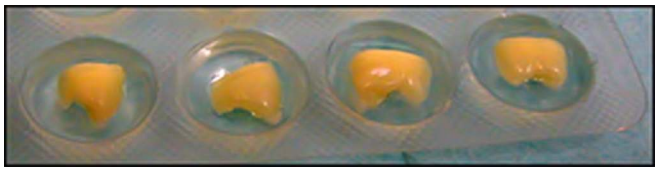
The aim of the paper is an accurate description of pH cycling protocol, as well as lesions microhardness evaluation.

Material and methods

The research was conducted based on the approval of the Ethics Committee of the Faculty of Dentistry, decision No: 09-545-3 / 11.

The research was managed on enamel slabs originating from 30 permanent premolar teeth, extracted from orthodontic reasons. The inclusive criteria were: without obvious initial carious lesions, without white spots, no enamel cracks (infractions) on the crown of the tooth. The root and crown were separated with a diamond burr, and the crown was cut in half in vestibule-oral direction. Thus, two samples of enamel were obtained from each tooth.

Enamel samples were placed in blocks of transparent self-adhesive acrylate, remaining 9 mm^2 (3 X 3 mm) free. Acrylate and samples were placed in standardized PVC blisters intended for the packaging of Paracetamol à 500 mg tablets. (**Picture 1**)



Picture 1.
Enamel blocks embedded in self-adhesive acrylate poured into PVC blisters.

The surface of the enamel blocks was polished with abrasive discs under water cooling to remove a surface layer about 30 μm thick. The aforementioned layer is naturally richer in fluorides and contains "anamnesis "of different treatments from the period of intraoral time.

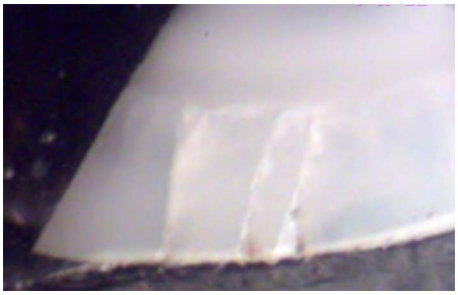
Samples were divided into 3 groups, each containing 20 samples.

Group I, "Native Enamel" (NE) was placed in the solution for remineralization, natural saliva equivalent. Samples were kept in this solution until the moment of cross-section micro hardness measure. This "positive control" group represents enamel under conditions in which it is normally found in the oral cavity. Such enamel is surrounded by saliva with a high potential for remineralization.

Twenty samples from group II, "White Spot" (WS) underwent pH-cycling regimen containing demineralization in a demineralizing solution for three hours (3h), and the rest of the time the enamel samples were submerged in the remineralizing solution, the equivalent of artificial saliva. The daily demineralization regime was established on the basis of estimated average of about person's 5 meals consumption daily. Assuming there is normal salivary flow, the pH drop after each meal lasts on average about half an hour. [5, 7]

The daily regimen was repeated cyclically for 8 days. Samples were submerged every time in a fresh solution. The amount of demineralization solution is precisely specified to prevent solution saturation with ions originating from the enamel. The experimental conditions were adjusted so that each enamel block had 20 ml of demineralization solution. After each period of demineralization, blocks were washed in a sufficient amount of distilled deionized water and then returned to the same remineralizing solution. Samples were stored at room temperature during treatment and experimental procedures. Daily regimen can be set differently, depending on what lesion depth is expected and enough for specific experiment. Total demineralization time can be shorter or longer (2-14 days). Eight day cycles were average time for lesion formation. [20, 21]

The third group "Demineralized Enamel"(DE) received demineralization protocol containing constant exposure to the demineralization solution for 24 hours, followed by immersion in distilled deionized water. The enamels from this group of samples were not "proposed" the possibility of remineralization, hence they were placed in distilled deionized water, instead of in a demineralizing solution. This group was "negative control". Total time of demineralization was same in DE and WS group (24 hours). The idea was to examine the impact of demineralization/remineralization cycle shift on the physical properties of enamel. This group ensured evidences regarding "simple" dissolution of the enamel under the acid attack. Differences between WS and DE group should provide evidences that the enamel is able to



Picture 2.
Enamel infractions that occurred after sample cutting. All these samples were excluded from further research. The image was taken using a digital camera on a stereo microscope, magnification 30X.

restore, reabsorb part of the lost minerals if it is surrounded by a medium supersaturated with minerals.

The demineralizing solution composition: 2.2 mM $CaCl_2$; 2.2 mM NaH_2PO_4, 0.05 M lactic acid in 50 mM acetate buffer with adjusted pH to 4.3 with 50% NaOH. 20,21

Remineralization solution Composition (artificial saliva): 3,9 mM Na_3PO_4; 4.29 mM $NaCl_{2;}$ 17,98 mM KCl; 1,1 mM $CaCl_2$; 0,08 mM $MgCl_2$; 0,5 mM H_2SO_4; 3,27 mM $NaHCO_3$, in destilled deionized water with pH set on 7,2. 21 The amount of artificial saliva is also specified, 10 ml of solution per each enamel block.

Sample preparation as well as microhardness measurements were performed at the Dental Materials Department on ACTA (Academic Center for Dentistry in Amsterdam; NL).

To measure micro hardness through the depth of the lesion, samples were cut perpendicular to the surface of the enamel using Isomet ™ 1000 precision saw Buehler equipped with a Mitutoyo micrometer. After polishing, 1,5mm thick samples were glued with instant cyano-acrylate adhesive to the microscope slides.

At this point, it should be noted that the cutting process was the weakest link in the preparation procedure. Certain numbers of samples were lost, either they fell out of the acrylic mold or the enamel exhibit infractions that appeared only in cross-section.

It cannot be determined with certainty whether enamel infractions occurred during sample cutting or existed before but were not visible. Due to previous, during the experiment, it was decided to examine all samples under a stereomicroscope equipped with a digital camera. (**Picture 2**) After reviewing all samples, it was decided to include only samples without visible infractions.

The largest loss of samples occurred in the third group (DE). The surface enamel layer of these samples was remarkably softened and simply lost during cutting and polishing. (**Pictures 3,4 and 5**).

CSMH (Cross-section microhardness) measurements were performed on Microhardness tester HM-124 Hardness testing machine Mitutoyo, Akashi, Japan; equipped with Knoop diamond and microscope camera MIO magnification lens 25, 100 and 200X. 22

A load of 25 g for 5 seconds was used to measure the microhardness; hence it was proven that higher loads than e.g. 50g can cause cracks in the enamel. 4

Microhardness testing was performed on every 10 μm, up to a depth of 80 μm, and ups to a depth of 200 μm every 20 μm. Measurements were performed under 100X magnification. Enamel microhardness was measured and registered in Knoop Hardness Number (KHN).

Pictures 3, 4, and 5.
Surface layer porosities in group DE. Magnification 15, 30,80 X.

Results and statistical analysis

Descriptive statistics are given in **tables 1 and 2.**

ANOVA statistical analysis of results was used for testing differences between the groups. Post-hoc LSD tests were complete to show the significance of founded differences. The mean significant difference was set at the 0,05 level and signed with an asterisk symbol. Performed tests showed statistically significant differences only in the first 50μm depth. Those results and significant differences are specified in **Table 3.**

The pH-cycling process produced a lesion with an average depth of 50-60 μm, as a global review of the results and statistical analysis demonstrate.

The most pronounced differences were in the surface layers and successively decreased in depth toward the healthy, deeper layer of the enamel (**Chart 1 and 2**).

Table 3 and Chart 3 shows the value of the volume percentage of minerals in the test groups. The mineral volume percentage was calculated based on the measured microhardness values, and a detailed procedure is explained in the "Discussion" section.

Descriptive statistics	Mean ±SD						
Depth of CSMH Measurement	10μm	20μm	30μm	40μm	50μm	60μm	70μm
NE (I group)	284,11 ± 58,40	291,22 ± 70,69	321,11 ± 58,45	320,00 ± 76,706	313,72 ± 91,842	290,06 ± 80,767	299,72 ± 92,927
WS (II group)	90,33 ± 69,44	212,27 ± 80,56	257,20 ± 89,41	279,80 ± 87,184	285,60 ± 49,231	300,27 ± 56,358	289,13 ± 65,953
DEMIN (III group)	24,46 ± 26,74	76,62 ± 56,032	106,85 ± 65,39	163,15 ± 95,994	188,23 ± 73,741	277,46 ± 73,839	296,46 ± 93,375

Table 1. Descriptive statistics for CSMH measurements in the first 70 μm.
Legend: NE-native enamel, WS-white spot lesion, DEMIN- enamel demineralized in extreme conditions, without remineralization possibility.

Descriptive statistics	Mean ±SD						
Depth of CSMH Measurement	80μm	100μm	120μm	140μm	160μm	180μm	200μm
NE (I group)	338,06 ± 144,216	320,72 ± 109,384	338,17 ± 84,244	303,44 ± 109,217	301,39 ± 104,127	332,28 ± 160,811	285,06 ± 138,858
WS (II group)	331,53 ± 62,277	349,87 ± 78,363	363,33 ± 94,827	342,67 ± 69,849	330,33 ± 55,045	366,20 ± 118,788	369,27 ± 155,924
DEMIN (III group)	270,92 ± 116,514	293,77 ± 97,616	316,15 ± 74,653	311,69 ± 83,485	317,38 ± 112,853	314,77 ± 53,176	331,31 ± 60,883

Table 2. Descriptive statistics for CSMH measurements in-depth 80-200 μm.
Legend: NE-native enamel, WS-white spot lesion, DEMIN- enamel demineralized in extreme conditions, without remineralization possibility.

Differences between groups I, II, and III	Mean Difference	Sig
NE (10) ↔ WS (10)	193,778(*)	,000
NE (10) ↔ DEMIN (10)	259,650(*)	,000
WS (10) ↔ DEMIN (10)	65,872(*)	,016
NE (20) ↔ WS (20)	78,956(*)	,003
NE (20) ↔ DEMIN (20)	214,607(*)	,000
WS (20) ↔ DEMIN (20)	135,651(*)	,000
NE (30) ↔ WS (30)	63,911	,024
NE (30) ↔ DEMIN (30)	214,265(*)	,000
WS (30) ↔DEMIN (30)	150,354(*)	,000
NE (40) ↔ WS (40)	40,200	,186
NE (40) ↔ DEMIN (40)	156,846(*)	,000
WS (40) ↔DEMIN (40)	116,646	,001
NE (50) ↔ WS (50)	28,122	,322
NE (50) ↔ DEMIN (50)	125,491(*)	,000
WS (50) ↔DEMIN (50)	97,369	,002
NE (60) ↔ WS (60)	10,211	,704
NE (60) ↔ DEMIN (60)	12,594	,653
WS (60) ↔ DEMIN (60)	22,805	,435

Table 3.
Description of statistically significant differences between the three groups given by a layer of depth.

Discussion

The natural remineralization potential of the initial carious lesion is extremely high in a solution supersaturated with necessary minerals, such as saliva. In our research, a remineralization solution was virtually identical to saliva's mineral composition.

A measurable difference in microhardness between native enamel and the untreated carious lesion is already lost at depth of 40 µm, while differences in chemical composition as well as in microstructure certainly exist.

The initial carious lesion designed in the pH-cycling model in this study probably does not have the same healing mechanism as the white spot formed *in-vivo* conditions.

The untreated initial carious lesion produced in our study apparently

Depth in µm	Volume %Mineral		
	I group NE	II group WS	III group DE
10 µm	83,77892	52,1681	32,56653
20 µm	84,68023	73,9488	48,93913
30 µm	88,35403	80,26106	55,74836
40 µm	88,22074	83,22706	66,22398
50 µm	87,46221	83,96873	70,29468
60 µm	84,53394	85,81169	82,92566
70 µm	85,74342	84,41644	85,33746
80 µm	90,36155	89,59425	82,07648
100 µm	88,30723	91,73069	85,0008
120 µm	90,37442	93,26323	87,75661
140 µm	86,20398	90,8988	87,2154
160 µm	85,95053	89,45243	87,9052
180 µm	89,68276	93,58632	87,58956
200 µm	83,9	93,93052	89,56827

Table 4.
Values of the relative enamel mineral content

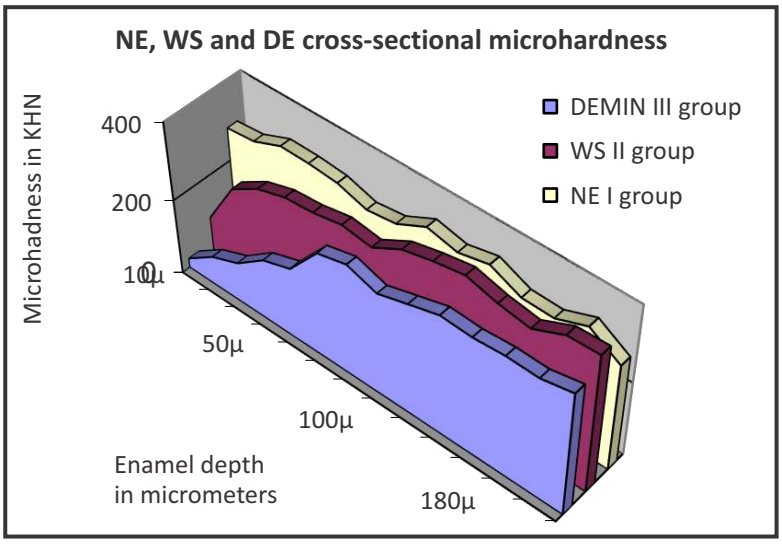


Chart 1. Microhardness for three groups given in KHN, in outer 200 micrometres of enamel.

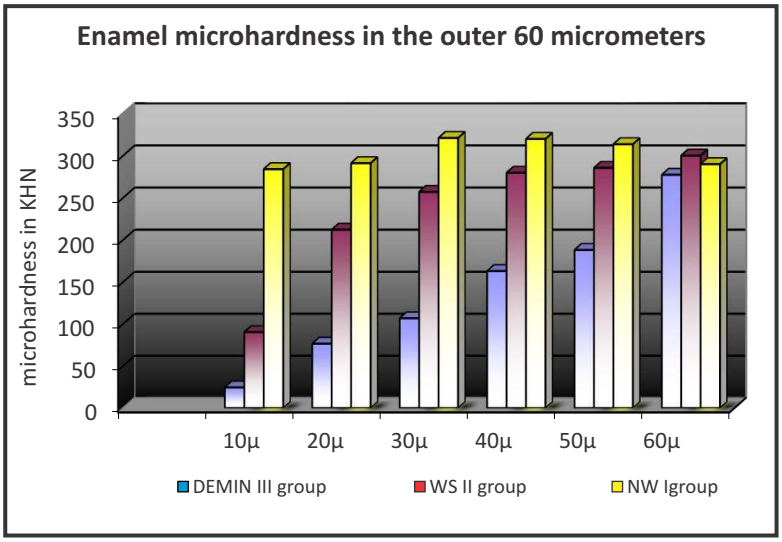


Chart 2. Enamel microhardness in outer 60 micrometres.

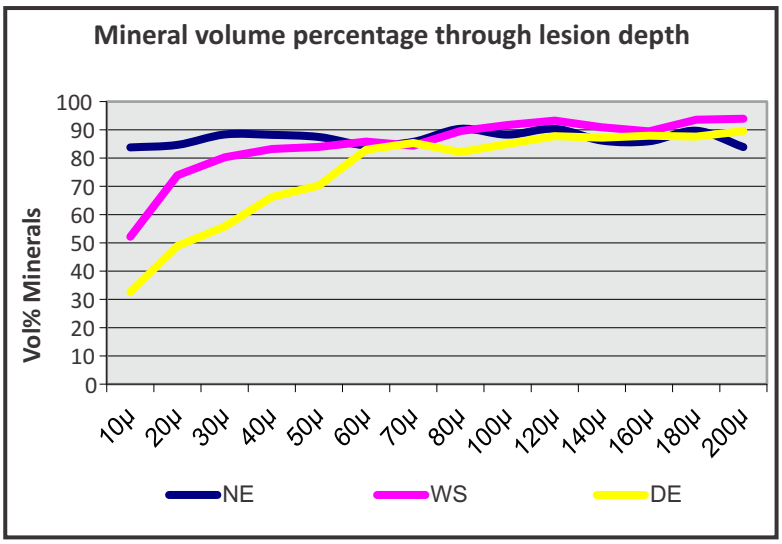


Chart 3: Changes of mineral volume content through lesions depth.

remineralizes by the same pattern as erosion does *in -vivo*. [23, 24]

It is well-known that the white spot lesion is *in - vivo* healing by forming a well-mineralized superficial zone on its surface below which is the body of the lesion with larger pores. The thickness of the well-mineralized surface zone is different according to different authors and amounts to 10-50 μm, while the body of the lesion can extend up to 350 μm into the depth of the enamel. [23, 24, 25]

To be precise, this morphological feature of the initial carious lesion was not examined by measuring the microhardness but by different microscopy methods, undoubtedly more precise than the microhardness examination.

Microhardness is only one of the physical property parameters. Other properties can be indirectly established on this basis including the mineral composition of enamel.

Measuring the microhardness in the cross-section could not prove the "body of a lesion" existence since the WS (group II) showed to be softest on the surface, but going deeper, the hardness approached the values found in native enamel.

At a depth of 100 to 160 μm the WS group shows even slightly higher hardness, but not statistically significant compared to native enamel. This can be an indication increased density zone of enamel formation, a zone generally formed below the body of the lesion.

Queiroz and associates produced an initial carious lesion about 80 μm deep, working on bovine enamel. The total demineralization time was extended to 64 hours, and the pH of the demineralizing solution was about 5. [14]

Featherstone produced a lesion with an average depth of 75 μm in a 14-day cycle with a daily demineralization period of 6 hours. [26]

Puig-Silla produced a lesion with a depth of 45 to 60μm, working also on bovine enamel, with a pH of the demineralizing solution set at 4, 4. The total duration of the demineralization cycle was 48 hours. [27]

In a 14-day cycle with a pH set on 4.3, Shirahatti produced a lesion average depth 115 to 150 μm, depending on the agent with which the enamel was pretreated before pH cycling. [28]

Referred to the above studies, the depth of the lesion was evaluated by microscope (polarized, confocal) or by microradiographs, not based on the measurement of microhardness as it was the case in our study.

Based on the above, it is reasonable to expect that our research generated similar depth lesions since the experimental conditions were similar. Hence, cross-sectional microhardness measurement as a method is not sufficiently sensitive to detect fine differences in the microstructure.

Therefore, parameters are introduced to directly connect enamel microhardness with its mineral composition, as an indicator of the microstructure. The ratio of the mentioned quantities can be expressed by the formulation established by Featherston and associates in 1983. 26:

$$\text{Volume \%Mineral} = 4,3 \times \sqrt{\text{KHN}} + 11,3$$

Therefore, calculation based on the previous formula, the cross-sectional microhardness values can be converted to the percentage mineral content of the enamel. The values of the relative enamel mineral content are given in the **table 4** and **chart 3.**

Conclusions

1. The pH cycling method proved to be valid *in - vitro* method for testing changes related to the initial carious lesion.
2. The CSMH method allows the creation of the mineral profile of the lesion and monitoring of changes in depth.
3. The natural potential for remineralization of the initial carious lesion is large, statistically significant difference in microhardness between native enamel and untreated initial carious lesion in our study is lost at a depth of 40μm.

Declaration of interest:

The authors declare no conflict of interest.

References

1. ten Cate JM. Models and role models. Caries Res. 2015;49 Suppl 1:3-10.
2. Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? Braz Oral Res. 2009;23 Suppl 1:23-30.
3. Skucha-Nowak M, Gibas M, Tanasiewicz M, Twardawa H, Szklarski T. Natural and Controlled Demineralization for Study Purposes in Minimally Invasive Dentistry. Adv Clin Exp Med. 2015;24(5):891-8.
4. Argenta RM, Tabchoury CP, Cury JA. A modified pH-cycling model to evaluate the fluoride effect on enamel demineralization. Pesqui Odontol Bras. 2003;17(3):241-6.
5. Featherstone JD. The continuum of dental caries--evidence for a dynamic disease process. J Dent Res. 2004;83 Spec No C: C39-42.
6. Casals E, Boukpessi T, McQueen CM, Eversole SL, Faller RV. Anticaries potential of commercial dentifrices as determined by fluoridation and remineralization efficiency J Contemp Dent Pract. 2007 1;8(7):1-10
7. A. Featherstone JD.Dental caries: a dynamic disease process. Aust Dent J. 2008 53(3):286-91.
8. Moi GP, Tenuta LM, Cury JA. Anticaries potential of a fluoride mouth rinse evaluated in vitro by validated protocols. Braz Dent J. 2008;19(2):91-6.
9. Corry A, Millett DT, Creanor SL, Foye RH, Gilmour WH. Effect of fluoride exposure on the cariostatic potential of orthodontic bonding agents: an in vitro evaluation Orthod. 2003;30(4):323-9; discussion 298-9.
10. (De Campos PH, Sanabe ME, Rodrigues JA, Duarte DA, Santos MT, Guaré RO, Duque C, Lussi A, Diniz MB. Different bacterial models for in vitro induction of non-cavitated enamel caries-like lesions: Microhardness and polarized light microscopy analyses. Microsc Res Tech. 2015;78(6):444-51.
11. Mohammadipour HS, Maghrebi ZF, Ramezanian N, Ahrari F, Daluyi RA. The effects of sodium hexametaphosphate combined with other remineralizing agents on the staining and microhardness of early enamel caries: An in vitro modified pH-cycling model. Dent Res J (Isfahan). 2019 ;12;16(6):398-406.
12. (Al-Angari SS, Lippert F, Platt JA, Eckert GJ, González-Cabezas C, Li Y, Hara AT. Dental bleaching efficacy and impact on demineralization susceptibility of simulated stained-remineralized caries lesions. J Dent. 2019; 81:59-63.
13. Gängler P, Kremniczky T, Arnold WH. In vitro effect of fluoride oral hygiene tablets on artificial caries lesion formation and remineralization in human enamel.BMC Oral Health. 2009 2;9:25.
14. Queiroz CS, Hara AT, Paes Leme AF, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Braz Dent J. 2008;19(1):21-7.
15. (Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. Dent Mater J. 2013;32(1):138-43.
16. Almeida LF, Marín LM, Martínez-Mier EA, Cury JA. Fluoride Dentifrice Overcomes the Lower Resistance of Fluorotic Enamel to Demineralization. Caries Res. 2019;53(5):567-575.
17. Velo MM, Tabchoury CP, Romão DA, Cury JA. Evaluation of low fluoride toothpaste using primary enamel and a validated pH-cycling model. Int J Paediatr Dent. 2016 ;26(6):439-447.
18. Rodrigues E, Delbem AC, Pedrini D, Cavassan L. Enamel remineralization by fluoride-releasing materials: proposal of a pH-cycling model. Braz Dent J. 2010;21(5):446-51.
19. Ayoub HM, Gregory RL, Tang Q, Lippert F. Comparison of human and bovine enamel in a

microbial caries model at different biofilm maturation. J Dent. 2020; 96:103328.

20. Exterkate RA, Ten Cate JM. Effects of a new titanium fluoride derivative on enamel de- and remineralization.Eur J Oral Sci. 2007;115(2): 143-7.
21. S, Varghese NO, Varghese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phosphopeptide on enamel lesions: An in vitro comparative evaluation. J Conserv Dent 2010; 13:42-6.
22. Zero DT, Zhang JZ, Harper DS, Wu M, Kelly S, Waskow J, Hoffman M.The remineralizing effect of an essential oil fluoride mouthrinse in an intraoral caries test. J Am Dent Assoc. 2004;135(2):231-7.
23. Mount GJ, Hume WR. Preservation and reconstruction of tooth structure. Mosby International Ltd; 1998.
24. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc. 2000;131(7):887-99.
25. Wefel JS, Harless JD. The effect of several topical fluoride agents on artificial lesion formation. J Dent Res 1982; 61(10):1169-1171.
26. Featherstone JD, Shariati M, Brugler S, Fu J, White DJ. Effect of an anti-calculus dentifrice on lesion progression under pH cycling conditions in vitro.Caries Res 1988; 22:337-341.
27. Puig-Silla M, Montiel-Company JM, Almerich-Silla JM. Comparison of the remineralizing effect of a sodium fluoride mouthrinse versus a sodium monofluorophosphate and calcium mouthrinse: an in vitro study.Med Oral Patol Oral Cir Bucal. 2009 1;14(5): E257-62.
28. Shirahatti RV, Ankola VA, Nagesh L, Hallikerimath S. The effect of three different pastes on enamel caries formation and lesion depth progression – An in vitro study. J Oral Health Comm Dent 2007; 1 (1): 1-6.