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An Evaluation of the Demineralizing Effects of Various Acidic Solutions

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Abstract: The purpose of this study was to evaluate which of the techniques and acids included in this in vitro research can induce artificial caries lesions in the most natural way. White spot lesions were created using six different demineralizing solutions in liquid form (lactic acid; orthophosphoric acid; formic acid; and an acid solution that contains calcium chloride, sodium phosphate and acetic acid) and gel form (hydrochloric acid and orthophosphoric acid). Radiographs, photographs and readings with a DIAGNODent™ pen, VITA Easyshade and a scanning electron microscope (SEM) were made in the initial situation, after 30 min, 1 h, 24 h and 96 h of demineralization. The total color change (ΔE) values in most cases presented statistically significant differences. SEM images showed different aspects of the enamel surface for each type of acid. Only in the case of exposed dentine did the DIAGNODent™ pen record significant differences. There was no noticeable radio-translucency of the teeth treated for a short period of time, but after 24 h, the absence of enamel and major demineralization of dentine were visible. Acids in the liquid state can penetrate and demineralize dental structures deeper than those that are more viscous. This study should be repeated with a protocol that includes remineralization. Using weaker acids would be another direction that could lead to more interesting findings.

Keywords: enamel demineralization; hydroxyapatite; acid solution; artificial white spot lesion

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1. Introduction

Dental caries is a multifactorial chronic disease caused by the interaction of four major factors: microorganisms, substrate, host factors and time [1]. It is caused by the action of organic acids produced during the metabolism of carbohydrates by aciduric bacteria [2]. Glycolysis (acid production) lowers the pH of the surrounding area and leads to the demineralization of dental tissue [3]. The acidic environment eventually causes a cavity on tooth surfaces [4].

When the pH in the surrounding environment decreases, the solubility of the dental enamel increases significantly. The solubility of hydroxyapatite (HAP) crystals increases 10 times with a drop of each pH unit [5,6]. When the pH drops under the value of 6, chemical dissolution of both the organic and the inorganic components takes place. This is enhanced by the water content of the tooth, which facilitates the infiltration of acid and the wash-out of minerals from the enamel [7]. During dental demineralization, calcium and phosphorus ions are drawn out from the hydroxyapatite, which has the following chemical formula: $Ca_5(PO_4)_3(OH)$ [7–10].

Dental enamel demineralization is a chemical process. The dissolution of hydroxyapatite can be described as

$$Ca_{10}(PO_4)_6(OH)_2 + 2H^+ \rightleftarrows 10Ca^{2+} + 6PO_4{}^{3-} + 2H_2O$$

$$PO_4^{3-} + H^+ \rightleftarrows HPO_4^{2-} \text{ or } H_2PO_4^- \text{ or } H_3PO_4$$

The consequences of enamel demineralization are as follows:

- An increase in interprismatic space;
- A change in the orientation of hydroxyapatite crystals and occurrence of abnormal forms;
- An expansion of the porosities of the enamel, which leads to higher penetrability of the dental tissue [11].

Optical changes (white spot lesions) occur due to the increased pore spaces between the thinned rods, which modify the refractive qualities of the enamel [12]. Simulating enamel demineralization is a complex task, and some of the steps can be accomplished using digital instruments, as in the finite elements method [13–16].

The purpose of this work is to evaluate which of the techniques and acids that are included in this study can induce artificial caries lesions in the most natural way. This is important for a better understanding of their etiology and could assist further research in finding alternative treatment options. Diagnosing and being able to treat dental caries and erosions in their early stage is essential in preventive and minimally invasive dentistry. Therefore, the null hypothesis is that all the acidic solutions (the independent variable) used in this in vitro study can create lesions for which there are no statistically significant differences ($p < 0.05$) compared to the healthy enamel, selecting as dependent variables the ΔE and the DIAGNODent™ values. Rejecting the null hypothesis could prove which acids and viscosities can mimic natural dental decay or erosion.

2. Materials and Methods

Tooth collection was approved by the Ethical Committee of U.M.F. Iuliu Hatieganu Cluj-Napoca (271/30 July 2019).

The extracted teeth were mechanically cleaned with dental curettes and professional brushing (Depural™ Neo, Pentron, Orange, CA, USA). Chemical cleaning was performed by immersing the teeth for 60 min in a Chloramine T-Sin solution from Sintofarm (20 tablets were dissolved in 1 L of distilled water).

The dental crowns were examined for enamel defects. In this study, 30 healthy molars and premolars with no caries on their vestibular and oral surfaces were included. The teeth were kept in a 0.9% saline (NaCl) solution at room temperature until they were used and in between the steps of the study.

On the vestibular and oral surface of each tooth, a 6 mm wide circle was marked with a dental burr. This helped to localize the tip of the spectrophotometer and the DIAGNODent™ pen (KaVo Kerr) every time in the same position. The teeth were covered with acid-resistant nail varnish with the circles' surface being left out.

Two silicone matrices were created for each tooth, covering half of the dental crown and root, except for the circle's surface. The base of the matrix was thin and straight. This helped to position the tooth for the X-ray so that the rays penetrated parallel to the marked circular zone of the enamel.

Photographs of the teeth were taken in the initial situation, and their color was recorded. The baseline color was measured for all specimens and recorded using a spectrophotometer (VITA Easyshade® Advance 4.0, Vita Zahnfabrik, Bad Säckingen, Germany) according to the CIE (Commission Internationale de l'Eclairage) L*, a*, b* system. The values of L* represent lightness (white-black); a* and b* are the chromaticity coordinates (green-red and blue-yellow) [17]. Positive a* values indicate the red color range and negative values represent the green color range. Positive b* values show the yellow color range, while negative values indicate the blue color range. The teeth were measured three times (with recalibration after each tooth), and the mean values for L*, a* and b* were recorded.

The VITA Easyshade® system measures the remission spectrum with a defined light, which passes perpendicular to the tooth (the measurement range is 400–700 nm). The color measurements were standardized by using the same black background, one operator and

the same lighting conditions. The measurements were repeated after each demineralization cycle, and the total color change (ΔE) was calculated using the following formula [18,19]:

$$\Delta E_{ab} = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

The teeth were examined with a DIAGNODent™ pen (Kavo Kerr, Brea, CA, USA) which uses laser fluorescence to detect caries within the tooth structure. When the incident laser light is dispersed on the enamel, a carious tooth structure will exhibit fluorescence directly related to the depth of the caries, resulting in higher reading values. Clean and healthy tooth structure reveals little or no fluorescence and will give low scale readings.

The readings were performed using a probe with a flat tip, this being recommended for smooth surfaces. Measurement time was standardized to ten seconds [20].

The instrument was calibrated against a ceramic standard before each measurement session, and maximum readings were recorded. The values obtained can be classified to indicate the diagnosis [21,22]. This classification is presented in Table 1.

Table 1. The DIAGNODent™ pen readings classification.

DIAGNODent™ Reading Values	Tooth Tissues Status
1–13	Healthy enamel
14–20	Enamel caries
21–29	Deep enamel caries
>30	Dentine caries

The surface morphology was examined with a scanning electron microscope (SEM Inspect™ S, FEI, Hillsboro, OR, USA)—a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with the atoms of the enamel, producing various signals that present information about the surface topography and composition of the tooth [23]. The SEM parameters used for the measurements were 1000× magnification, a 50 mm scale and 4.5 spot. The last step was to make bitewing radiographs of the initial situation. The tooth was placed in its silicone matrix and positioned over the image receptor. Using the parallel technique, the X-ray beam from the tube-head was oriented perpendicular to the tooth surface.

Six types of demineralizing solutions were prepared:

1. Hydrochloric acid gel 15% (Icon Etch, DMG); pH 1.
2. Orthophosphoric acid gel 35% (Vococid, Voco); pH 0.8.
3. Lactic acid 80%; pH 2.
4. Liquid 37% orthophosphoric acid; pH 1.5.
5. Formic acid 85%; pH 2.5.
6. An acid solution that contains 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid; pH 4.5.

In each acidic solution $n = 5$ teeth were immersed, each tooth having 2 demineralizing sites (one on the vestibular and one on the oral surface). Therefore, $n = 10$ treated sections were analyzed for each acid.

The teeth were taken out of the silicone matrix and put in the demineralizing solutions for 30 min. Each tooth was washed under running tap water and scanned with the DIAGNODent™ pen, the Vita Easyshade® and SEM. Pictures and X-rays of the affected area were taken.

The teeth were reinserted into the demineralizing solutions. After 1 h, they were taken out and washed, and all of the readings were performed again.

Until the next day, the teeth were kept in saline water for rehydration. They were then reinserted in the demineralizing solutions for 24 h. All recordings were repeated for each tooth.

The last demineralizing cycle involved keeping the teeth in the prepared solutions for 96 h. The solutions were renewed every day. All determinations (DIAGNODent™ pen, Vita Easyshade®, SEM, photograph and radiograph) were repeated for each tooth.

The data were collected into an Excel database. The results were statistically analyzed to compare the variables of the demineralization periods in relation to the type of acidic solution. Therefore, the ANOVA one-way test was used to determine the statistical differences between the groups. For the post hoc comparisons, the Tukey test was applied, the significance level being set to $a = 0.05$. The statistical analysis was performed using the graphics from Origin2019b Graphing & Analysis (OriginLab), the results being considered significant for $p \leq 0.05$.

The present research was conducted as a pilot study, as extracted teeth without any caries lesions—even teeth without early-stage caries lesions—are difficult to collect, and increasingly so. The problem has been exacerbated by the COVID-19 pandemic, which has resulted in many patients having to postpone non-urgent dental care, including orthodontic treatment, which might have required the extraction of healthy teeth due to lack of space.

In the future, we plan to repeat the demineralizing protocol on more teeth, using weaker concentrations of acids and including remineralizing cycles.

3. Results and Discussion

3.1. Photography

Dental photography is of multilevel importance. It is simple, fast and extremely useful in documenting the techniques, stages and results of this study [24].

Figure 1 represents the aspect of a demineralizing site in the initial state; during the action of hydrochloric acid; and after the acidic treatment of the tooth for 30 min, 1 h, 24 h and 3 days.

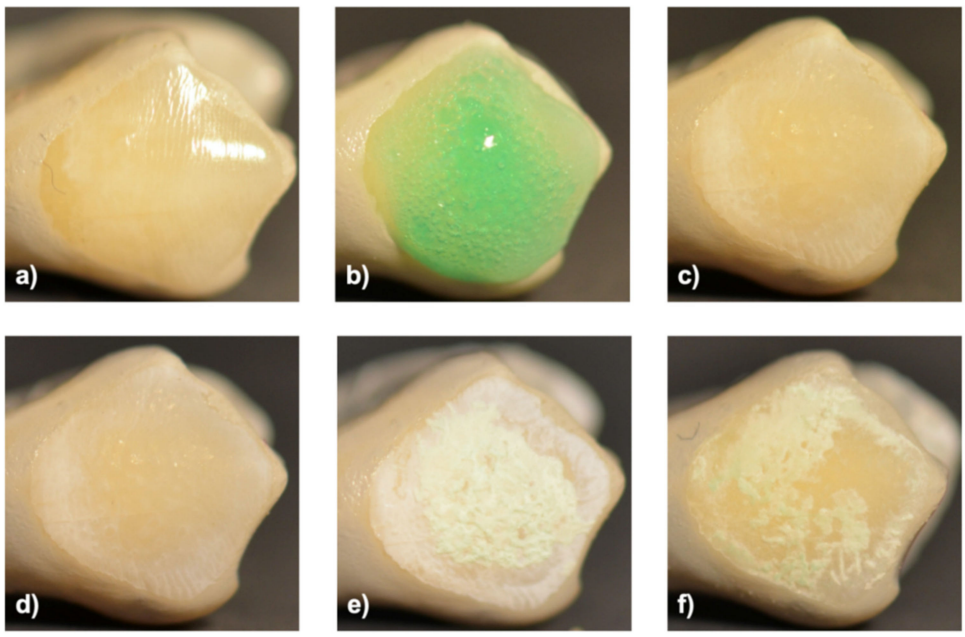

Figure 1. (**a**) The initial aspect of a tooth. (**b**) Hydrochloric acid in action. (**c**) After 30 min of demineralization. (**d**) After 1 h of demineralization. (**e**) After 24 h of demineralization. (**f**) After 96 h of demineralization.

The appearance of the teeth after 30 min of demineralization, as shown in Figure 2, is as pure white patches, as if the teeth were covered with a thin layer of chalk. The acid solution that contained $CaCl_2$, NaH_2PO_4 and acetic acid did not affect the enamel as the other acids did; the surfaces of these teeth were smooth and relatively glossy.

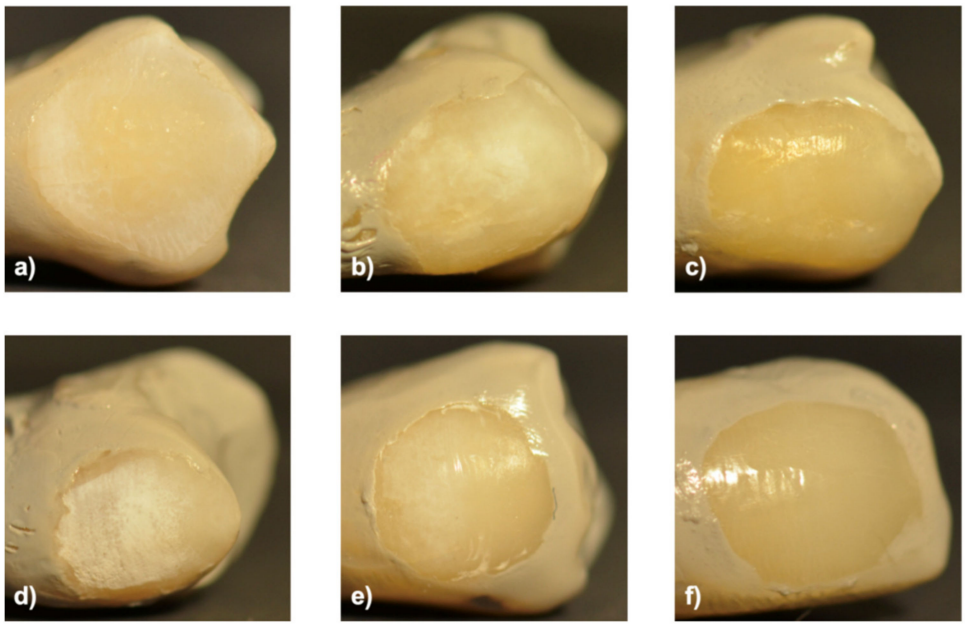

Figure 2. The enamel after being placed for 30 min in: (**a**) hydrochloric acid; (**b**) orthophosphoric acid; (**c**) lactic acid; (**d**) orthophosphoric acid solution; (**e**) formic acid; (**f**) $CaCl_2$, NaH_2PO_4 and acetic acid solution.

After 1 h of acidic treatment, the aspect of the enamel became more matte and chalk-like, except for the teeth treated with the acetic acid solution (shown in Figure 3).

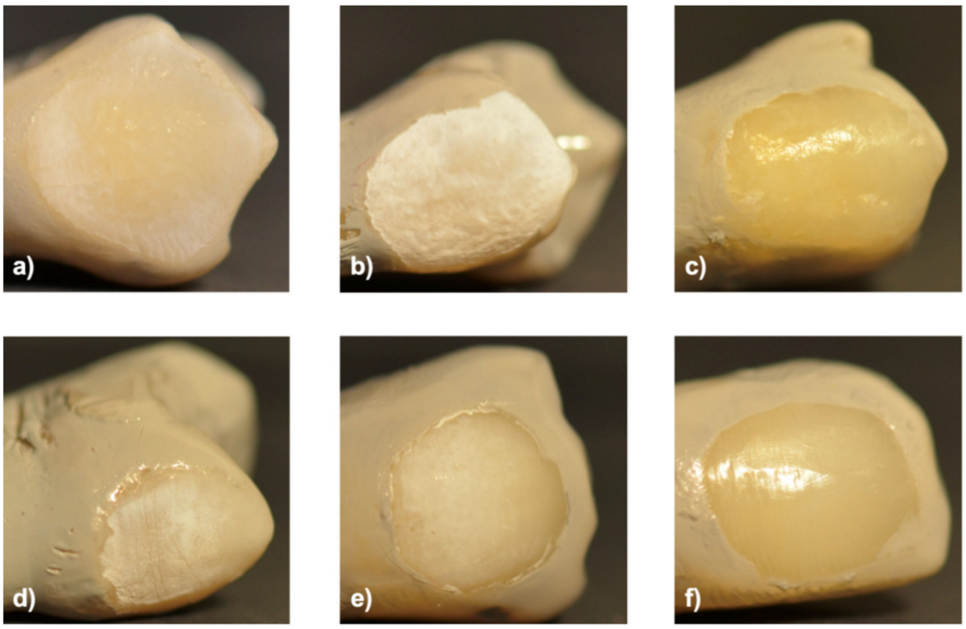

Figure 3. The enamel after being placed for 1 h in: (**a**) hydrochloric acid; (**b**) orthophosphoric acid; (**c**) lactic acid; (**d**) orthophosphoric acid solution; (**e**) formic acid; (**f**) $CaCl_2$, NaH_2PO_4 and acetic acid solution.

Figure 4 presents an important change in the teeth after the 24 h etching period. The enamel started to erode and the treated area became concave. The teeth immersed in the $CaCl_2$, NaH_2PO_4 and acetic acid solution remained complete and white with no loss of substance.

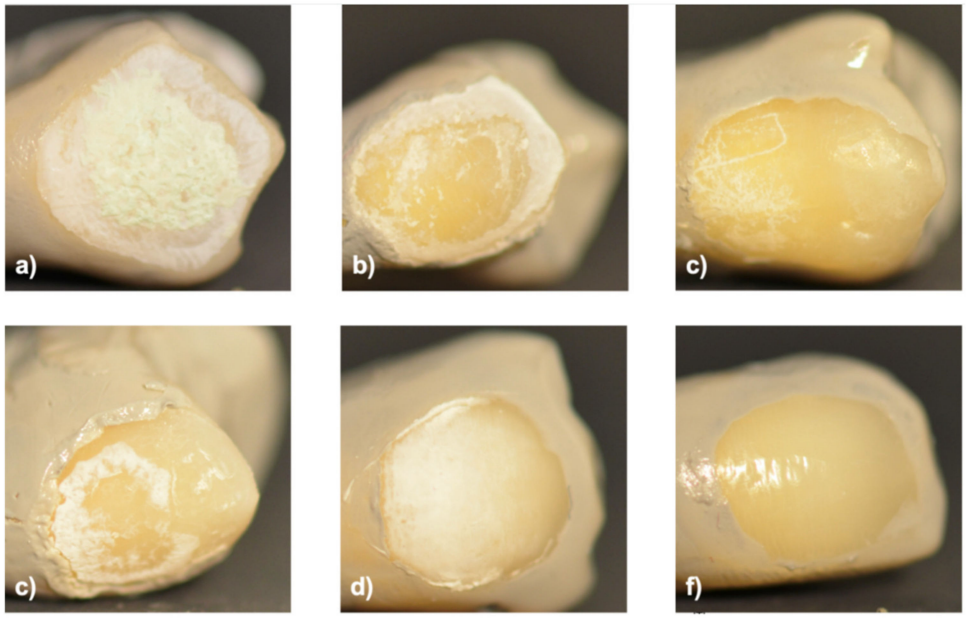

Figure 4. The enamel after being placed for 24 h in: (**a**) hydrochloric acid; (**b**) orthophosphoric acid; (**c**) lactic acid; (**d**) orthophosphoric acid solution; (**e**) formic acid; (**f**) $CaCl_2$, NaH_2PO_4 and acetic acid solution.

Figure 5 shows the demineralizing site after 96 h of acidic treatment. The teeth in the 37% orthophosphoric acid solution and some of those immersed in lactic acid lost their entire enamel layer, and the dentine was completely visible. The other dental crowns had patches of exposed dentine, except for those treated with the acetic acid solution, which exhibited only superficial alterations.

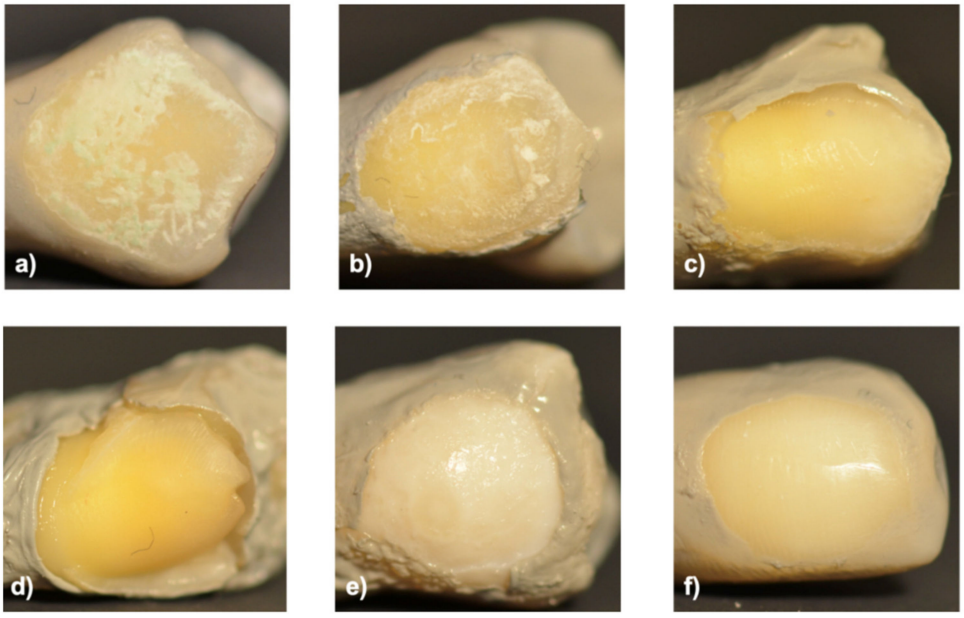

Figure 5. The enamel after being placed for 96 h in: (**a**) hydrochloric acid; (**b**) orthophosphoric acid; (**c**) lactic acid; (**d**) orthophosphoric acid solution; (**e**) formic acid; (**f**) $CaCl_2$, NaH_2PO_4 and acetic acid solution.

There was a significant difference, visible to the naked eye, between the opacity of wet and dry demineralized enamel. The white spot lesion started to dry out approximatively

1 min after the tooth was removed from the saline solution. Interestingly, the aspect of the wet and dry dentine is relatively alike.

3.2. Scanning Electron Microscopy (SEM)

The SEM 1000× magnified view of the teeth shows the chemically eroded dental enamel. Figure 6a–e show the eroded prismatic enamel and the interprismatic enamel left out, protruding. This aspect of the demineralized enamel compares with the aspects found in other studies. In these images, relatively regular etching patterns are visible [25–27].

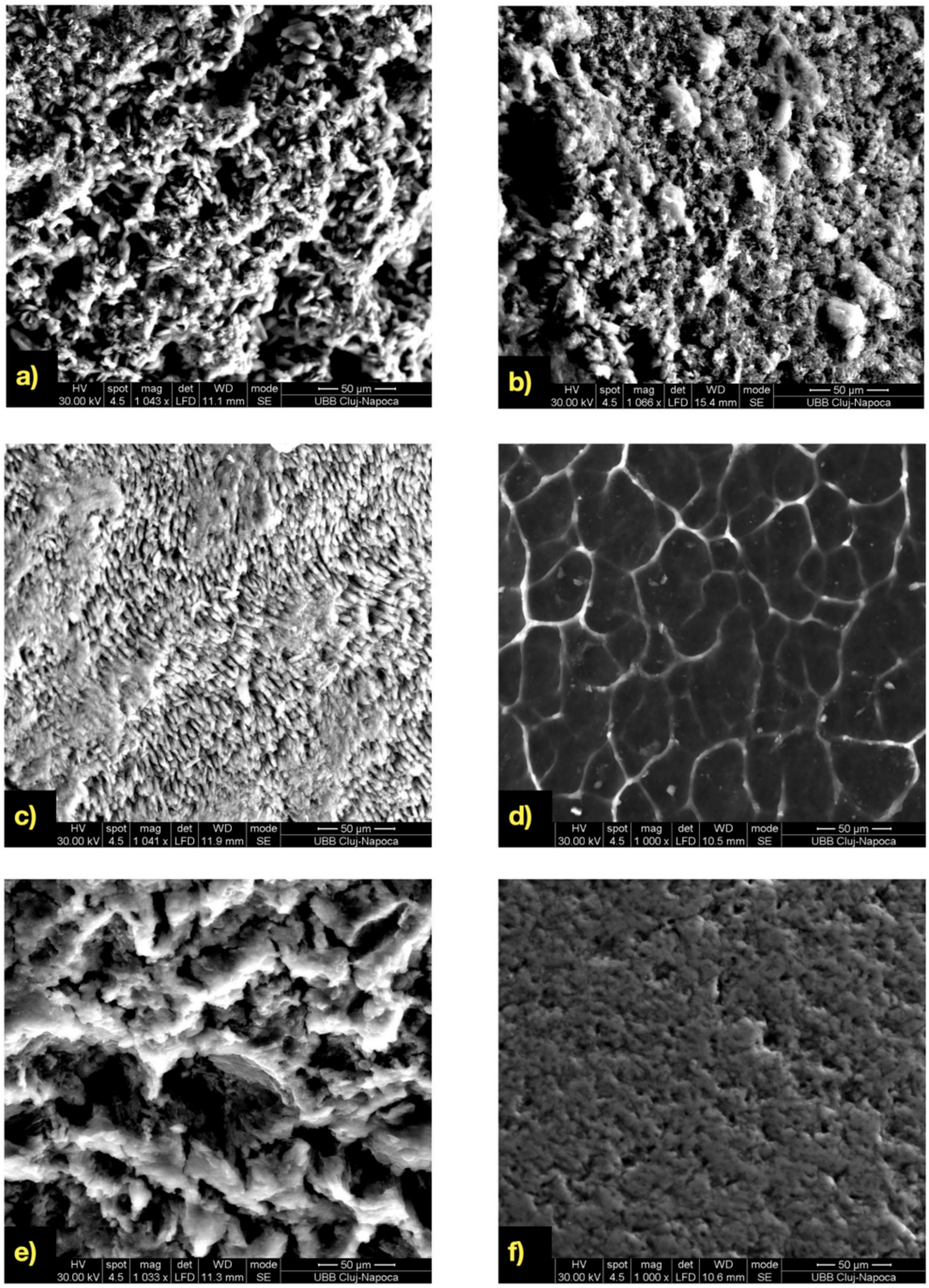

Figure 6. The SEM aspect of the teeth demineralized for 96 h in: (**a**) hydrochloric acid; (**b**) orthophosphoric acid; (**c**) lactic acid; (**d**) orthophosphoric acid solution; (**e**) formic acid; (**f**) $CaCl_2$, NaH_2PO_4 and acetic acid solution.

The extent of the enamel lesion depends on the chemical composition of the tooth, the type and concentration of the acidic solution and the duration of the etching, as well as the prismatic or aprismatic structure of the enamel [26,28]. This is the reason why in Figure 6c, the aspect of the demineralized site is different.

In severe dental erosion, where dentine is exposed, the rounded openings of the tubules are visible, as is the missing collagen matrix. This is shown in Figure 6d, which displays a tooth that was treated with orthophosphoric acid solution for 96 h.

On the other hand, the aspect of the lesions is different for the teeth eroded with the acetic acid solution; the demineralization is much lighter and affects only the superficial part of the enamel, as seen in Figure 6f.

3.3. *VITA Easyshade*®

The study groups recorded variables where the ΔE values were taken at four time intervals (ΔE1, ΔE2, ΔE3 and ΔE4) in six different demineralizing solutions (hydrochloric gel; orthophosphoric acid gel; lactic acid; orthophosphoric acid solution; formic acid; and $CaCl_2$, NaH_2PO_4 and acetic acid solution). N = 3 measurements were made for each assessment.

In the chart below, the ΔE values were calculated between two etching cycles. Thereby, ΔE1, 2, 3 and 4 represent the color differences between the different measurements (see Table 2).

Table 2. ΔE values and their signification.

ΔE Values Calculated between Two Etching Cycles	Signification
ΔE1	Initial situation–30 min of demineralization
ΔE2	30 min–1 h acid cycle
ΔE3	1 h–24 h of etching
ΔE4	24–96 h of acidic treatment

Statistical analysis was performed in order to compare the ΔE values obtained for the same demineralizing intervals and in different acidic solutions. For the ΔE1, the ANOVA one-way test showed a statistically significant difference ($p = 0.0354$) between the formic acid–orthophosphoric acid gel and $CaCl_2$, NaH_2PO_4 and acetic acid solution–orthophosphoric acid gel. In ΔE2, a statistically significant difference was found ($p = 0.25443$) but without differences in the Tukey test. Regarding ΔE3, the ANOVA test showed no statistically significant difference ($p = 0.25443$) between any of the demineralizing solutions. In ΔE4, a statistically significant difference was found ($p = 2.12339 \times 10^{-9}$), except for the comparison between the lactic acid–hydrochloric gel; formic acid–orthophosphoric acid solution; and $CaCl_2$, NaH_2PO_4 and acetic acid solution–hydrochloric gel.

Vita Easyshade® readings, shown in Figure 7, show a medium color change after etching the tooth for 30 min and a small difference after 1 h. It is obvious that after 24 h, the ΔE3 value is higher. The most outstanding results were obtained for the teeth treated for 96 h with the 37% orthophosphoric acidic solution. This remarkable deviation is explained by the spectrophotometric difference between the enamel and dentine aspects.

In the following figure, ΔE values represent the difference between the initial situation and that after each etching period. Thus, ΔE1 is calculated between the initial state and after a 30 min etching, ΔE2 between the initial state and 1 h, ΔE3 between the initial state and 24 h and ΔE4 between the initial state and 96 h of acidic treatment.

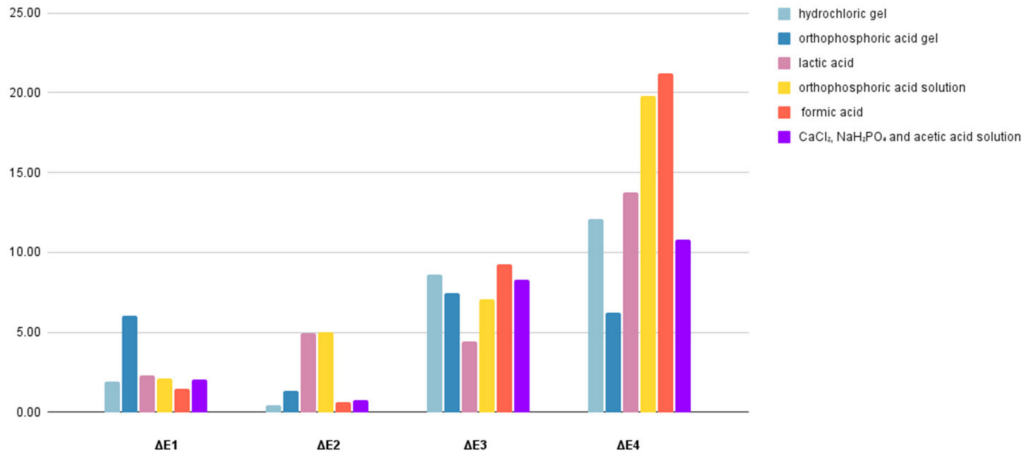

Figure 7. ΔE values for the acidic solutions used.

The only demineralizing solution that presented a statistically significant difference with $p = 0.02042$ for the ANOVA test and no difference for the Tukey test was the orthophosphoric acid gel. The lactic acid and orthophosphoric acid solution showed statistically significant differences for all the time intervals, except for the first two: between the initial state and 30 min, and between the initial state and 60 min. For the hydrochloric gel; formic acid; and $CaCl_2$, NaH_2PO_4 and acetic acid solution, the ANOVA one-way test presented statistically significant differences ($p = 7.21066 \times 10^{-7}$, $p = 5.59125 \times 10^{-9}$ and $p = 1.03103 \times 10^{-7}$) for all the time intervals, except for the first one (between the initial situation and after 30 min).

Figure 8 represents the visual changes over time for each acidic solution used in the study. ΔE values represent the difference between the initial state and after each etching period. There is a relatively small difference between ΔE1 and ΔE2 and a remarkable increase in ΔE4 for each demineralizing solution. Exceptionally large values were obtained after 24 h for the orthophosphoric acid solution, probably because at this point, dentine was exposed.

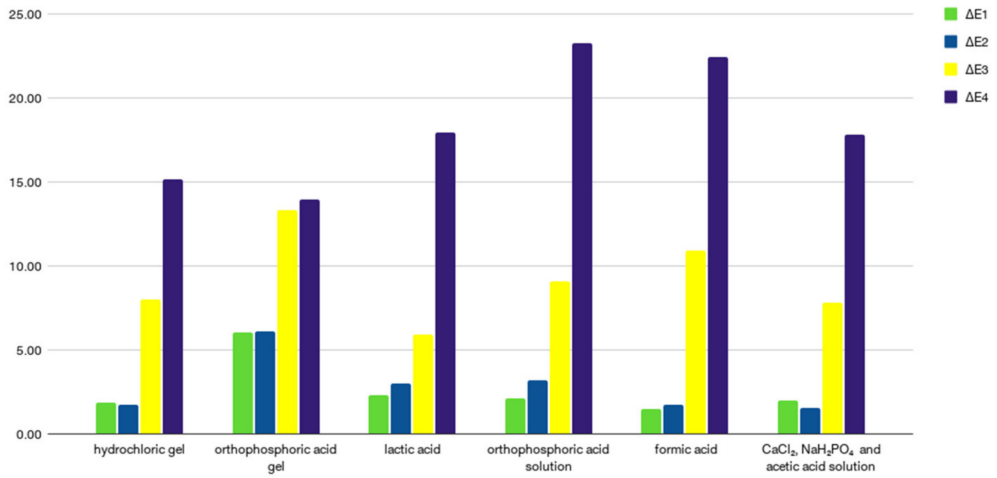

Figure 8. The changes in time for each acidic solution.

According to reviews, ΔE values greater than 3.3 are perceptible by ordinary individuals. In this study, significant differences in ΔE values were found mostly after the longer demineralizing periods [29–31].

3.4. DIAGNODent™ Pen

An ANOVA one-way test was made for the values obtained by the readings with a DIAGNODent™ pen. The only demineralizing solution that showed no statistically significant difference was the orthophosphoric acid gel ($p = 0.07092$). For the hydrochloric gel, a statistically significant difference was found ($p = 0.03822$), but without differences between the values for the ad hoc Tukey test. Lactic acid; formic acid; and the $CaCl_2$, NaH_2PO_4 and acetic acid solution presented a statistically significant difference ($p = 0.01393$, $p = 0.02189$ and $p = 0.00357$). The orthophosphoric acid solution shows the biggest difference ($p = 2.76479 \times 10^{-8}$) with a statistically significant difference for the Tukey test as well.

The DIAGNODent™ mean values are shown in Table 3, and their graphical representation can be seen in Figure 9. The readings were either between 1–5 or over 30. Increased numbers were obtained when dentine was exposed, but for all other situations very low values were recorded, and there were just a few results in between. This could be explained by the fact that the acid solutions eroded the demineralized enamel completely, so no caries lesion-like defects were present. Alternatively, it could have been that the DIAGNODent™ pen did not provide a satisfactory correlation with histopathological investigations on smooth surfaces, as it was found to do so in a few previous studies [20,22].

Table 3. The mean results from the DIAGNODent™ pen before and after the application of acid solutions on the enamel surface.

	Initial	30 Min	1 Hour	24 Hours	96 Hours
Hydrochloric gel	2.46	3.90	3.68	19.70	27.31
Orthophosphoric acid gel	3.44	3.51	5.71	23.30	29.59
Lactic acid	2.50	2.77	2.69	5.94	18.86
Orthophosphoric acid solution	4.50	5.12	5.03	28.33	32.20
Formic acid	2.31	2.09	3.48	10.21	23.04
$CaCl_2$, NaH_2PO_4 and acetic acid solution	2.07	2.13	3.41	3.19	4.96

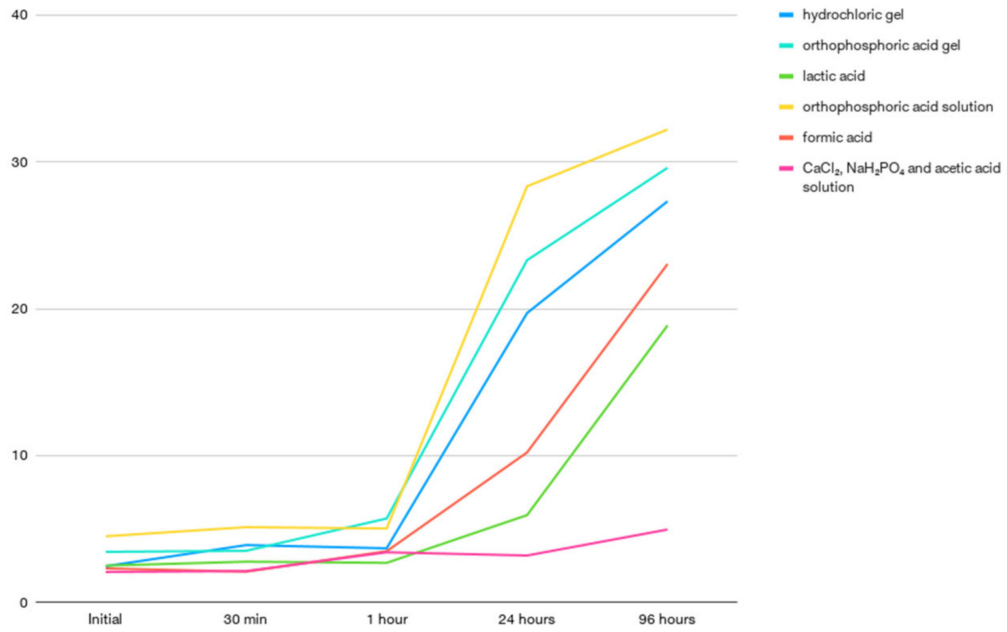

Figure 9. The mean values measured with the DIAGNODent™ pen.

3.5. X-ray

Analyzing the X-rays, no noticeable radio-translucency was found on the surface of the dental enamel of the teeth treated for 30 min and 1 h with different acid solutions. Radiologically, one can observe a major demineralization of the dentine and the complete

absence of dental enamel on the teeth immersed for 24 and 96 h (mostly on those treated with liquid 37% orthophosphoric acid). This is shown in Figure 10b.

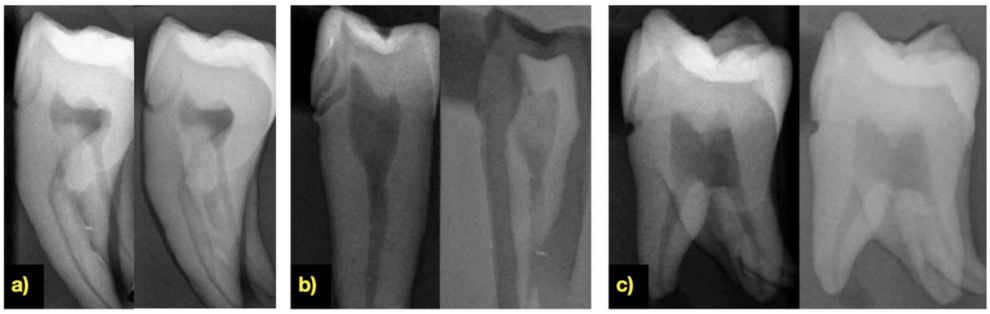

Figure 10. The radiologic aspect of the teeth before and after demineralization for 24 h with (**a**) hydrochloric acid gel; (**b**) orthophosphoric acid solution; (**c**) orthophosphoric gel.

Nowadays, the quantity and frequency of acidic food and beverage consumption have both increased [21]. As a result, examining the etiology, prevalence, prevention and treatment of dental erosion has become greatly important. There are many factors that can cause enamel erosion: diet, oral hygiene routine, drugs and professional environment. To prevent its progression, the causative factors should be identified as soon as possible so that protective measures can be implemented [23].

Studies show that beverages with low pH cause dental erosion of different degrees [32–34]. Case reports and other studies suggest that dietary acid erosion is playing a significant role in tooth wear [35,36]. How demineralizing a solution is depends on the type of acid it contains, its pH value, its acid concentration and its temperature [37–43]. The results of this in vitro study show that acids in liquid state are more harmful to the dental hard tissues than gels; therefore, beverages and acid reflux represent a greater concern. In our research, different demineralizing periods were studied, and it has been shown that prolonged exposure (exposure for more than 24 h) to strong acidic solutions causes total dissolution of the dental enamel. However, an acid solution that contains calcium chloride, sodium phosphate and acetic acid with a pH of 4.5 did not demineralize the teeth, even after 96 h of exposure.

This in vitro research was limited due to the small number of extracted teeth that were treated, a shortcoming that will be overcome with a new, larger study that will use a protocol that includes remineralizing cycles and weaker acids.

4. Conclusions

The present study aimed to compare six different acidic solutions with respect to enamel demineralization. These acidic solutions were in liquid form (lactic acid, orthophosphoric acid; formic acid; and an acid solution that contains calcium chloride, sodium phosphate and acetic acid) and gel form (hydrochloric acid and orthophosphoric acid). The results of this in vitro research showed that acids in liquid state can penetrate the dental structures and demineralize them to a deeper extent than the gels. Orthophosphoric acid solution was the most efficient in dissolving the enamel, while the acetic acid solution affected only the superficial part of the enamel.

The SEM aspects of etched teeth presented different types of enamel and dentine demineralization depending on the acidic solution used.

Radio translucency was not observed, as the acids eroded the surface of the enamel completely. The present study could contribute to an initial understanding of enamel demineralization and the effect of acid erosion on HAP crystals. However, more research should be performed to further investigate the effects of different concentrations of acids on dental structures.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of U.M.F. IULIU HATIEGANU CLUJ-NAPOCA (271/30 July 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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