THE INFLUENCE OF BASELINE HARDNESS AND CHEMICAL

COMPOSITION ON ENAMEL DEMINERALIZATION AND

SUBSEQUENT REMINERALIZATION

by

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To my loving and supporting family, thank you for being there for me every step of the way. I dedicate this project to you.

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INTRODUCTION

BACKGROUND

Tooth enamel is composed of 96 wt% inorganic material and 4 wt% organic material and water. This inorganic material is mainly composed of calcium phosphate in the form of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. It also contains many impurities including carbonate, magnesium, sodium and chloride. Carbonate in particular, along with magnesium, causes major disturbances to hydroxyapatite crystals, making them more soluble during an acid attack. Fluorine ions, on the other hand, can substitute for hydroxyl ions in hydroxyapatite to form fluorapatite, which in turn is much less soluble in acid.[1] Dental caries is a dynamic process that involves alternating demineralization and remineralization cycles. Several studies have reported that baseline physical and chemical characteristics of enamel greatly influence its behavior in demineralization and subsequent remineralization challenges.[2-4]

PURPOSE

The purposes of this study were:

1) To investigate the hardness and chemical content of sound enamel and their influence on demineralization;

2) To investigate these properties in demineralized enamel and their influence on subsequent remineralization; and

3) To investigate these properties in sound enamel and their influence on remineralization.

HYPOTHESES

Null Hypotheses

There is no correlation between surface microhardness and the susceptibility to de- and remineralization for enamel.

There is no correlation between mineral content and the susceptibility to de- and remineralization for enamel.

Alternative Hypotheses

There is a negative/positive correlation between surface microhardness and the susceptibility to de- and remineralization for enamel, respectively.

There is a negative/positive correlation between mineral content and the susceptibility to de- and remineralization for enamel, respectively.

REVIEW OF LITERATURE

BASELINE/SOUND CHARACTERISTICS
FOR DEMINERALIZATION

There have been numerous studies regarding demineralization of dental enamel. Enamel caries lesions created in vitro simulate in vivo caries in a faster, easier to control environment that allows for a better understanding of the demineralization process.[5] A wide variety of demineralization systems are available for the formation of artificial caries lesions in dental enamel. These distinct systems will lead to the creation of different lesion types, such as surface softened lesions or subsurface lesions.[6,7] For subsurface lesions, it has been found that differences in demineralizing solutions or gels with respect to degree of saturation with enamel minerals, kind of acid and viscosity can all result in differences in the physical and mechanical characteristics of the demineralized enamel, including differences in mineral distribution, chemical composition and hardness.[3,8]

Several attempts have been made to correlate the baseline characteristics of enamel with its response to demineralization. Cuy et al. showed that the hardness of dental enamel has a strong correlation with its chemical content.[9] Areas with higher concentration of hydroxyapatite constituents (P_2O_5 and CaO) were shown to have the highest nanohardness values. On the other hand, areas with higher Na_2O and MgO concentrations showed the opposite trend. Lower microhardness values with concurrently lower calcium and phosphorus contents have been demonstrated by several others.[10-12] Sabel et al. concluded that enamel specimens from primary teeth responded to demineralization by producing lesions of varying depths.[13] Deeper lesions were found to have higher amounts of carbon and nitrogen and lower amounts

of calcium and phosphorus. During lesion creation, Savory et al. found carious

enamel to have twice as much nitrogen as non-carious enamel.[14]

BASELINE/SOUND CHARACTERISTICS
FOR REMINERALIZATION

As for remineralization of enamel, it is well established that fluoride enhances

this process,[15,16] and that the greater the amount of fluoride, the less the amount of

demineralization, or the smaller the lesion depth.[17,18] Strang et al. found an increase in

remineralization rates with increases in lesion size.[4] A similar observation was found

by Lippert et al., who reported that lesions with higher R values, calculated as the

ratio of mineral loss (ΔZ) to lesion depth (L), tended to remineralize, whereas those

with lower R values further demineralized.[19] One study demonstrated that with

fluoride present, enamel specimens subjected to continuous demineralization and

remineralization cycles eventually reach an equilibrium, when remineralized lesions

are formed that are more resistant to further demineralization or remineralization.[20]

Alternatively, Feagin et al. concluded that the acid resistance of remineralized enamel

was similar to that of sound enamel.[11]

TRANSVERSE MICRORADIOGRAPHY (TMR)

TMR can be considered the "gold standard" measurement of dental hard

tissue mineral loss. Several attempts have been made to correlate the results of surface

microhardness (SMH) to those of TMR. Studies either showed a good correlation[21,22]

or a poor correlation,[5] depending on the depth and degree of demineralization of the

lesions studied. Generally, SMH gives more information about the mechanical

properties and structural integrity of surface enamel, while TMR shows the mineral

distribution of the lesion. Therefore, combining both techniques could assess changes

in both physical and chemical lesion characteristics.[23,24]

The integrated mineral loss (ΔZ) and the depth of the lesion at baseline have a profound impact on subsequent demineralization and remineralization behavior. There is an increasing tendency towards net remineralization and a decrease in further mineral loss with increasing integrated mineral loss at baseline (ΔZ_{base}).[3,25] This is likely caused by a decrease in intrinsic solubility of the lesions as a result of modification in chemical composition, i.e. loss of the more soluble material (e.g. magnesium and carbonate) in relation to the less soluble material. Lesion depth also plays a role, as deeper, more porous lesions have a higher tendency to remineralize than shallower, less porous lesions.[4,18] In the shallower lesions, the more soluble materials are more readily accessed by bacterial acids than in the deeper lesions.

APPROPRIATENESS OF SAMPLE SUBSTRATE

Many *in-vitro* studies have been carried out on bovine enamel. Bovine enamel has become a widely used substitute for human enamel, being easier to obtain in the desired quantities and providing a relatively flat surface with a more uniform thickness than human enamel. Research regarding the appropriateness of replacing bovine for human teeth has shown that subtle morphological differences do exist between the two substrates, because both tissues behave similarly, but not necessarily identically.[26,23] Bovine enamel was found to be more porous[27] and have higher carbonate[21] but lower fluoride contents.[28] However, the two behave similarly enough to provide an acceptable alternative with the advantage of reduced variability of the hard tissue substrate.[29]

GAP OF CURRENT KNOWLEDGE

During these *in-vitro* studies of demineralization and remineralization, enamel clearly responded to demineralization by producing lesions of different depths.[13]

Attempts to measure the baseline surface hardness values of enamel samples prior to any demineralization or remineralization challenges resulted in a range of hardness values.[12,17] The reason for such differences is yet to be established. Another important factor is the chemical composition of enamel. While a direct relation between chemical content and demineralization is established, no research has been done on the remineralizing potential of demineralized enamel of distinctive chemical compositions. This study aims to correlate the results of surface microhardness, chemical composition, and lesion depth in sound, demineralized and remineralized enamel.

MATERIALS AND METHODS

STUDY DESIGN

The study was a laboratory study performed on bovine enamel specimens. Incipient subsurface caries lesions were formed in the specimens at three distinct severities. After that, the specimens were remineralized using an established pH-cycling model during which they were exposed either to a diluted fluoride solution to promote remineralization, or deionized water as a negative control. Surface microhardness, chemical composition, and TMR analysis were done and compared among the sound, demineralized, remineralized and control specimens. A total of 94 specimens were included in the study, with six experimental groups (3 demineralizations x 2 treatment regimens).

SOUND ENAMEL ANALYSIS

Specimen Preparation

Extracted bovine incisor teeth were obtained from Tri State Beef Co. (OH, USA). Teeth with cracks, hypomineralized (white spot) areas, or other surface flaws were excluded. The crowns were cut into 5×5 mm specimens from the buccal surfaces only using a Buehler Isomet low speed saw (Isomet, Buehler Ltd, Lake Bluff, IL. The superficial enamel was ground to remove surface irregularities and to create a flat enamel surface using a Struers Rotopol 31/Rotoforce 4 polishing unit (Struers Inc., Cleveland, PA,) in a series of 1200-, 2400-, and 4000-grit paper. The specimens were then polished using a 1-μm diamond polishing suspension on a polishing cloth. This procedure helped to ensure the removal of approximately 200 μm to 300 μm of surface enamel (depending on the natural curvature of the enamel surface of the

specimen), which may contain relatively high concentrations of artificially introduced trace elements (e.g. F) that would otherwise compromise the comparison between the samples. The resulting specimens had a thickness range of 1.7 mm to 2.2 mm. The prepared specimens were then stored in 100-percent relative humidity at 4 °C until further use (Figure 1).

Surface Microhardness (SMH)

The specimens were mounted individually on 1-inch acrylic blocks using sticky wax. Center portions, approximately 5×3 mm, of the specimens (Section A) were used to measure the surface microhardness values. (Figure 1). A total of five baseline indentations were made using the Knoop diamond indenter (2100 HT; Wilson Instruments, Norwood, MA) with a 50-gram load along a line parallel to the external surface of the specimen approximately 100 μm apart from each other, and a dwelling time of 11 seconds (Figure 2). The Knoop hardness number (KHN) for each specimen was derived by calculating the mean of the length of the long diagonal of the five indentations. Specimens were then randomly divided into six groups based on the sound enamel KHN ensuring equal distribution of the specimens with low (<354), medium (354-375), and high KHN (>375) between the groups.

Energy Dispersive X-ray Spectroscopy (EDS)

The top portion, approximately 5 mm ×1 mm, of the specimens (Section B: Figure 1) was used for scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS, JEOL 7800F; JEOL, Peabody, MA). Section B was cut off using a Silverstone-Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories) approximately 100 μm in thickness. Any section thicker than 120 μm

(determined using drop gauge) was hand-polished using 2400-grit silicon carbide paper to the required thickness.

After that, the sections were analyzed using EDS (EDAX, Octane Super Detector) coupled with a scanning electron microscope operating at 10 kV accelerating voltage to measure the content of calcium, phosphorous, fluorine, carbonate, magnesium and nitrate in weight percent from the surface to a depth of 100 μm. The specimens were not carbon coated to minimize the risk of excess carbon being detected during analysis. A total of 11 horizontal line scans were made in each specimen, each measuring 100 μm in width, starting at the surface of the enamel up to a depth of 100 μm from the surface. The line scans were made at 10-μm increments. The SEM images were obtained at X750 magnification for comparison.

DEMINERALIZED ENAMEL ANALYSES

Demineralization

In-vitro incipient caries lesions were created in the specimens by demineralization using a method described by Lippert et al.[19] in a solution with the following composition: 0.1 M lactic acid, 4.1 mM $CaCl_2$ x 2 H_2O, 8.0 mM KH_2PO_4. 0.2 % w/v Carbopol C907 (BF Goodrich Co.), a synthetic high molecular weight polymer, was used as a surface protective agent during demineralization to create subsurface lesions. The pH of the demineralizing solution was adjusted to 5.0 using potassium hydroxide (KOH). Specimens were covered with acid resistant nail varnish except the polished enamel surface. Groups 1 (24hr/a) and 4 (24hr/b) were demineralized for 24 hours, groups 2 (48hr/a) and 5 (48hr/b) were demineralized for 48 hours, and groups 3 (96hr/a) and 6 (96hr/b) were demineralized for 96 hours. Specimens were demineralized using approximately 40 ml of said solution per

specimen at 37 °C.

The demineralization solution was not replaced during the entire

demineralization period of each specimen. After lesion creation, the specimens were

rinsed with deionized water. The prepared specimens were stored in 100-percent

relative humidity at 4 °C until further use.

Demineralized Enamel SMH

After demineralization, a second set of five indentations were made in section

A of the specimens, using the Knoop diamond indenter (2100 HT; Wilson

Instruments, Norwood, MA) with a 50-gram load along a line parallel to the external

surface of the specimen, to the left of and parallel to the sound enamel indentations,

approximately 100 μm apart from each other and approximately 200 μm from the

sound enamel indentations, and a dwelling time of 11 seconds. The Knoop hardness

number for each specimen was derived by calculating the mean of the length of the

long diagonal of the five indentations.

Demineralized Enamel Transverse Microradiography (TMR)

The bottom portion, approximately 5 mm×1 mm, of the specimens (Section C:

Figure 1) was used for TMR analysis. Section C was cut off using a Silverstone-

Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories) approximately

100 μm in thickness. Any section thicker than 120 μm (determined using drop gauge)

was hand-polished using 2400-grit silicon carbide paper to the required thickness.

The samples were mounted with an aluminum step wedge on high-resolution

glass plates type I A (Microchrome Technology Inc., San Jose, CA). Samples were

placed in the TMR-D system and x-rayed at 45 kV and 45 mA at a fixed distance for

12 seconds. The digital images were analyzed using the TMR software v.3.0.0.18

(Inspektor Research Systems BV, Amsterdam, The Netherlands). A window approximately 400 μm × 400 μm representing the entire lesion and not containing any cracks, debris, or other alterations was selected for analysis.

The following variables were recorded for each specimen: lesion depth (L) (87-percent mineral; i.e., 95-percent of the mineral content of sound enamel), integrated mineral loss (ΔZ), which is calculated as the product of lesion depth and the mineral loss over that depth, and the maximum mineral content of the surface layer (SZ_{max}).

Demineralized Enamel EDS

Following TMR analysis, section C was used for SEM and EDS analyses.

REMINERALIZED ENAMEL ANALYSES

Remineralization

All specimens were pH-cycled for 10 days using an established pH-cycling model based on that by White.[30] Specimens were covered with acid resistant nail varnish except the polished enamel surface. The demineralized enamel specimens were exposed to a daily cyclic treatment regimen consisting of one 4-hour acid challenge in the demineralization solution, four 1-minute treatments with either a sodium fluoride solution (367 ppm F simulating a 1100 ppm F dentifrice after 1:3 dilution - groups 24hr/a, 48hr/a and 96hr/a) or deionized water (negative control - groups 24hr/b, 48hr/b and 96hr/b) with storage in artificial saliva (2.20 g/l gastric mucin, 1.45 mM $CaCl_2$ x 2 H_2O, 5.42 mM KH_2PO_4, 6.50 mM NaCl, 14.94 mM KCl, pH adjusted to 7.0 using potassium hydroxide (KOH)) all other times (Table I).

The pH-cycling phase was conducted at room temperature and without stirring. After the last treatment after ten days of pH-cycling, the specimens were

placed in artificial saliva for 30 minutes before being rinsed with deionized water. The prepared specimens were stored in 100-percent relative humidity at 4 °C until further use.

Remineralized Enamel SMH

After pH-cycling, a second set of 5 indentations were made in section A of the specimens, using the Knoop diamond indenter (2100 HT; Wilson Instruments, Norwood, MA) with a 50-gram load along a line parallel to the external surface of the specimen, to the right of and parallel to the sound enamel indentations, approximately 100 μm apart from each other and approximately 200 μm from the sound enamel indentations, and a dwelling time of 11 seconds. The Knoop hardness number for each specimen was derived by calculating the mean of the length of the long diagonal of the five indentations. The extent of re-hardening, referred to as SMH recovery (%SMHr), was then calculated based on the method of Gelhard et al.[31]

$$\%SMHr = \frac{D - P}{D - B} \times 100$$

Where B is the indentation length (μm) of the sound enamel specimens at baseline, D is the indentation length (μm) after demineralization, and R is the indentation length (μm) after pH-cycling.

Remineralized Enamel TMR

The bottom portion, approximately 5 mm ×1 mm, of the specimens (Section D: Figure 1) was cut off using a Silverstone-Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories) after 10 days of pH-cycling and used for TMR.

The percent-mineral profile of each enamel specimen's demineralized and

remineralized lesion was compared with the mean sound enamel percent-mineral profile according to a method by Shen et al.[32] The difference between the areas under the densitometric profile of the demineralized lesion and the mean sound enamel, calculated by trapezoidal integration, is represented by ΔZ_d. The difference between the areas under the densitometric profile of the remineralized lesion and the mean sound enamel, calculated by trapezoidal integration, is represented by ΔZ_r. These parameters were then converted to percent-change values after remineralization, as such, percent remineralization (%R) represents the percent change in ΔZ values:

$$\%R = \frac{\Delta Z_d - \Delta Z_r}{\Delta Z_d} \times 100$$

Remineralized Enamel EDS

Following TMR analysis, section D was used for SEM and EDS analyses.

STATISTICAL ANALYSES

Pearson correlation coefficients and plots were used to evaluate the associations among surface microhardness, lesion depth, integrated mineral loss, maximum mineral content of the surface layer, and weight percentage of carbonate, nitrate, fluorine, magnesium, phosphorus and calcium at the surface at baseline, after demineralization and after remineralization.

The outcomes of surface microhardness, lesion depth, integrated mineral loss, maximum mineral content of the surface layer, and weight percentage of carbonate, nitrate, fluorine, magnesium, phosphorus and calcium at each depth were analyzed using three-way ANOVA, with factors for stage (sound, demineralized, and remineralized), demineralization time (24, 48 and 96 hours) and treatment (fluoride solution or deionized water), as well as all two-way and three-way interactions among

the factors. A repeated effect for stage was added to the model. All pair-wise comparisons from ANOVA analysis were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5 percent.

The weight percentage of carbonate, nitrate, fluorine, magnesium, phosphorus and calcium were analyzed using four-way ANOVA, with factors for depth, stage, demineralization time and treatment, as well as all two-way, three-way and four-way interactions among the factors. A repeated effect for depth with each specimen*stage was added to the model. All pair-wise comparisons from ANOVA analysis were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5 percent.

The outcomes of %R and %SMHr were analyzed using two-way ANOVA with factors for demineralization time and treatment. All pair-wise comparisons from ANOVA analysis were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5 percent.

Sample size justification: With a total sample size of 94 specimens, a one-sided 95-percent lower confidence bound for the correlation will not include zero if the correlation is at least 0.25, calculated separately by treatment regimen.

RESULTS

SURFACE MICROHARDNESS (SMH)

Table II provides the Knoop SMH values for all 6 groups at 3 stages (baseline, after demineralization and after pH cycling). The KHN was significantly different among stages ($p < 0.0001$), among demineralization times ($p = 0.0002$) and between treatments ($p < 0.0001$). The two-way interactions between stage and demineralization time ($p < 0.0001$), as well as between stage and treatment ($p < 0.0001$) were significant. The three-way interaction among stage, demineralization time and treatment was significant ($p < 0.0001$). A bar chart of the data is shown in Figure 4.

At baseline, no significant difference in the KHN was found between the groups. After demineralization, specimens that were demineralized for 24 hours had significantly higher KHN than those that were demineralized for 48 or 96 hours. Following pH-cycling, specimens that were demineralized for 24 hours had significantly higher KHN than those that were demineralized for 96 hours, irrespective of the treatment received. Within treatments, specimens that received fluoride had significantly higher KHN than the control.

The %SMHr was significantly different between treatments ($p < 0.0001$). Between demineralization times, %SMHr was significantly different between 24 and 96 hours ($p = 0.002$) and between 48 and 96 hours ($p < 0.0001$) in specimens that were treated with fluoride (test) and between 24 and 96 hours ($p = 0.030$) in specimens that were not treated with fluoride (control) as shown in Table III.

TRANSVERSE MICRORADIOGRAPHY (TMR)

Table IV provides the TMR data for all 6 groups at 2 stages (after demineralization and after pH cycling). The following variables were recorded for each specimen: lesion depth (L) (87-percent mineral; i.e., 95-percent of the mineral content of sound enamel), integrated mineral loss (ΔZ), which is calculated as the product of lesion depth and the mineral loss over that depth, and the maximum mineral density at the lesion surface zone (SZ_{max}).

LESION DEPTH (L)

The lesion depth was significantly different between stages (p = 0.0030), between demineralization times (p < 0.0001) and between treatments (p = 0.0066). As shown in Figure 5, lesions were significantly deeper after demineralization than after pH-cycling in all groups, irrespective of the treatment received, except group 24hr/b, in which the specimens were demineralized for 24 hours and received distilled water (control), where there was no significant difference in lesion depth after demineralization and after pH-cycling. Lesions were significantly deeper in specimens that were demineralized for 96 hours than those that were demineralized for 24 or 48 hours. Within treatments, specimens that received fluoride were significantly shallower in depth than the control.

INTEGRATED MINERAL LOSS (ΔZ)

The integrated mineral loss was significantly different between stages (p < 0.0001), between demineralization times (p < 0.0001) and between treatments (p < 0.0001). The two-way interactions between stage and demineralization time (p = 0.0309), as well as between stage and treatment (p = 0.0111) were significant. As shown in Figure 6, mineral loss was significantly greater after demineralization than

after pH-cycling in all groups, irrespective of the treatment received, except group 24hr/b, in which the specimens were demineralized for 24 hours and received distilled water (control), where there was no significant difference in mineral loss after demineralization and after pH-cycling. Mineral loss was significantly greater in specimens that were demineralized for 96 hours than those that were demineralized for 24 or 48 hours. Within treatments, specimens that received fluoride had significantly less mineral loss than the control. Mean mineral distribution graphs are shown in Figure 7 and Figure 8.

MAXIMUM MINERAL DENSITY OF THE SURFACE (SZ_{MAX})

The maximum mineral density at the lesion surface zone was significantly different between stages ($p < 0.0001$), between demineralization times ($p = 0.2011$) and between treatments ($p < 0.0001$). The two-way interaction between stage and treatment was significant ($p < 0.0001$). Following demineralization, no significant differences were found in the mineral density of the surface zone between the groups at all three demineralization times. However, following pH-cycling, there was significantly higher surface zone mineralization in specimens that were demineralized for 48 hours and 96 hours than those that were demineralized for 24 hours. Specimens had significantly higher surface zone mineralization after pH-cycling than after demineralization in all groups, irrespective of the treatment received. Within treatments, specimens that received fluoride had significantly higher mineral density of the surface zone than the control. A bar chart is shown in Figure 9.

% REMINERALIZATION (%R)

The %R was significantly different between treatments (p < 0.0001) only after demineralization for 24 hours. Between demineralization times, %R was significantly different between 24 and 48 hours (p = 0.004) and between 24 and 96 hours (p = 0.011) in specimens that were not treated with fluoride (control) as shown in Table V.

ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDS)

The surface weight% of phosphorus was not significantly different among stages. However, the surface weight% was significantly lower after pH-cycling for calcium (p = 0.0006), magnesium (p = 0.0115) and nitrate (p = 0.0115), and significantly higher after pH-cycling for fluorine (p = 0.0248) and carbonate (p < 0.0001). The data for the weight% of all six elements at the surface in the six groups at three stages (baseline, after demineralization and after pH-cycling) are shown in Table VI and Table VII and Figure 10.

Calcium, phosphorus, magnesium, carbonate and nitrate at the surface were not significantly affected by demineralization time or treatment. Surface fluorine was significantly affected by treatment, as specimens that received fluoride had higher surface fluorine weight% than the control (p < 0.0001), irrespective of demineralization time as shown in Figure 11. The two-way interaction between stage and treatment was also significant for fluorine (p = 0.0009).

The depth (p < 0.0001) and the two-way interaction between depth and treatment (p < 0.0001) were significant for fluorine. Specimens that received fluoride treatment during pH-cycling had significantly higher weight% of fluorine at the surface than at 10 μm (p = 0.0146) and at 10 μm than at 20 μm (p < 0.0001). The depth profile for fluorine is shown in Figure 12.

The depth profiles for calcium, phosphorus, carbonate, nitrate and magnesium are shown in Figure 13 through Figure 17. The weight% of both calcium and phosphorus was significantly lower at the surface than at 10 μm and at 10 μm than at 20 μm (p < 0.0001), irrespective of demineralization time, stage, or treatment. On the other hand, the weight% of both carbonate and nitrate was significantly higher at the surface than at 10 μm and at 10 μm than at 20 μm (p < 0.0001), irrespective of demineralization time, stage, or treatment. The weight% of magnesium remained unchanged along the entire depth of the specimens examined (p < 0.0001).

CORRELATIONS

Considering the TMR data, as shown in Figure 18, a strong positive correlation can be seen between the lesion depth and integrated mineral loss both after demineralization (p < 0.0001, r = 0.91) and after pH-cycling (p < 0.0001, r = 0.91). The maximum mineral density of the surface zone also shows a moderate negative correlation with both the lesion depth (p < 0.0001, r = -0.42) and integrated mineral loss (p < 0.0001, r = -0.55) after pH-cycling as seen in Figure 19.

There was a statistically significant but weak positive correlation between baseline Knoop hardness and Knoop hardness after demineralization (p = 0.002, r = 0.31), and between Knoop hardness after demineralization and after pH-cycling (p = 0.0006, r = 0.35), however, there was no statistically significant correlation between baseline Knoop hardness and Knoop hardness after pH-cycling (p = 0.4929, r = 0.07) as displayed in Figure 20.

After demineralization, there was a statistically significant but weak negative correlation between the hardness and lesion depth (p = 0.0172, r = -0.25) and between the hardness and integrated mineral loss (p = 0.0017, r = -0.32) of the specimens. A similar relation was found between the hardness after demineralization and the lesion

depth (p = 0.0338, r = -0.22) and integrated mineral loss (p = 0.0055, r = -0.28) after pH-cycling. The plots are displayed in Figure 21 and Figure 22.

After pH-cycling, there was a statistically significant moderate correlation between the hardness and lesion depth (p = 0.0005, r = -0.35), integrated mineral loss (p < 0.0001, r = -0.49), and the maximum mineral density of the surface zone (p < 0.0001, r = 0.58) as shown in Figure 23.

The hardness of the specimen after pH-cycling was found to be greater when the weight% of fluorine was greater at baseline (p = 0.0265, r = 0.23), after demineralization (p = 0.0216, r = 0.24), or after pH-cycling (p = 0.002, r = 0.32), although these correlations are considered weak as demonstrated in Figure 24. Furthermore, as seen in Figure 25, a weak correlation was found between the weight% of fluorine and the maximum mineral density of the surface zone after demineralization (p = 0.019, r = 0.24), while a stronger correlation was found between the weight% of fluorine and the maximum mineral density of the surface zone after pH-cycling (p < 0.0001, r = 0.43).

SEM IMAGES

Figure 26 shows the SEM images taken at X750 magnification. The SEM images show that at baseline, the enamel structure is smooth and the enamel rods are visible. After demineralization, the enamel became rough and the rods are no longer visible. After pH-cycling, the lesion depth decreased for all groups, but more in the groups that received fluoride than the control.
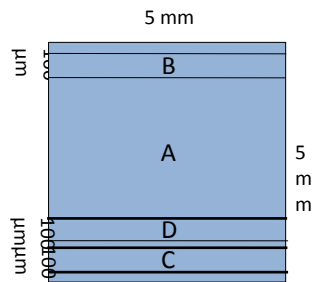
FIGURES AND TABLES

FIGURE 1.   Sections of the specimen. Section A was used for microhardness measurement. Section B was used for chemical analysis of the sound specimen. Section C was used for TMR and chemical analysis of the demineralized specimen. Section D was used for TMR and chemical analysis of the remineralized specimen.

Demineralized Enamel Sound Enamel Remineralized Enamel

2.3 mm

1.5 mm

B

A

D

C

100 μm

200 μm

(a)

(b)
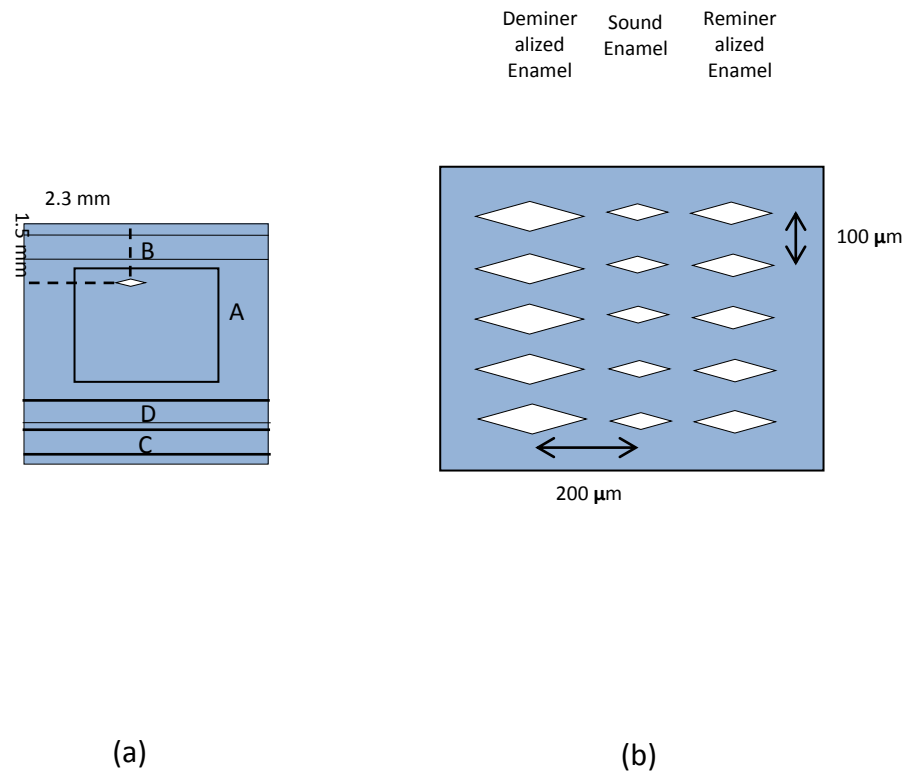
FIGURE 2.   Location of the microhardness indentations (a) location of the
              first sound enamel indentation (b) location of the hardness
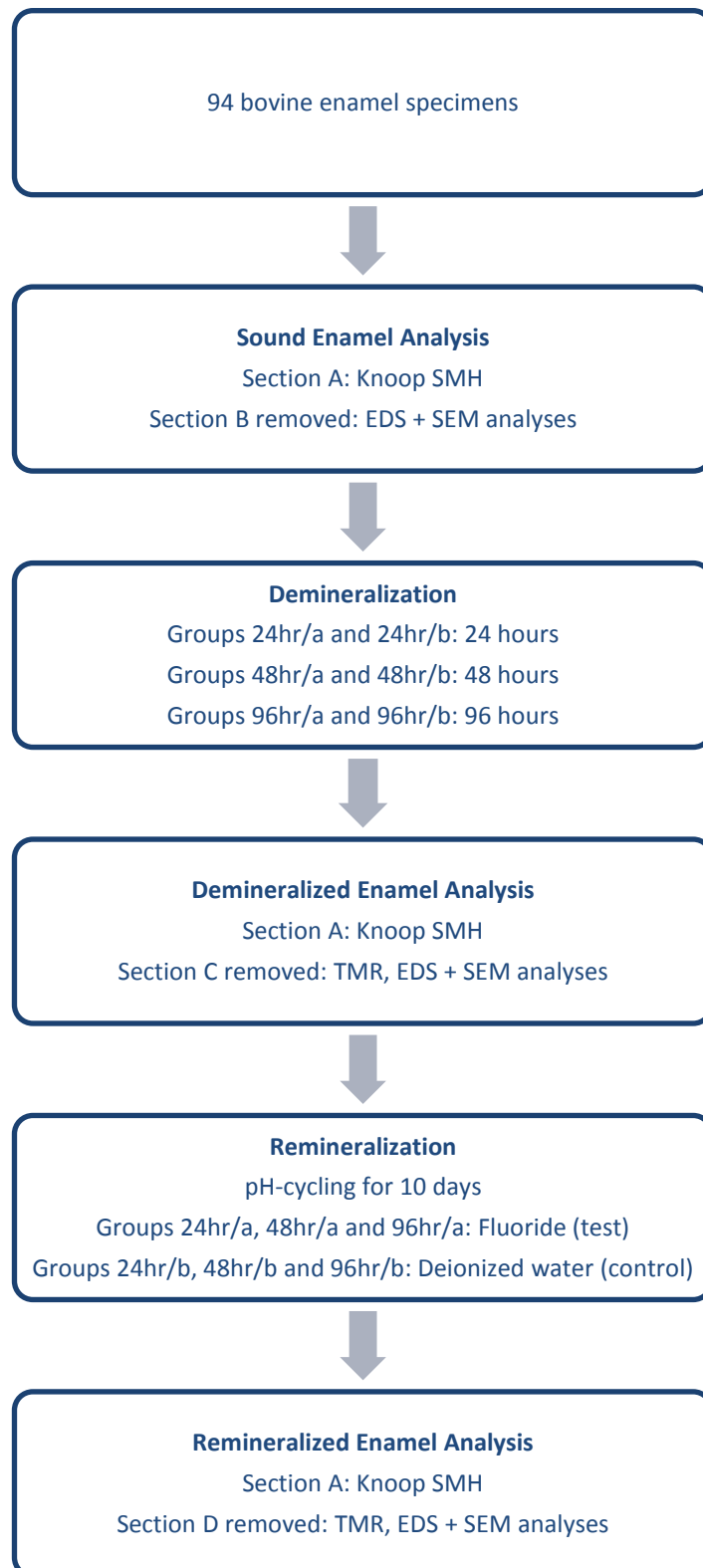   indentations in section A.

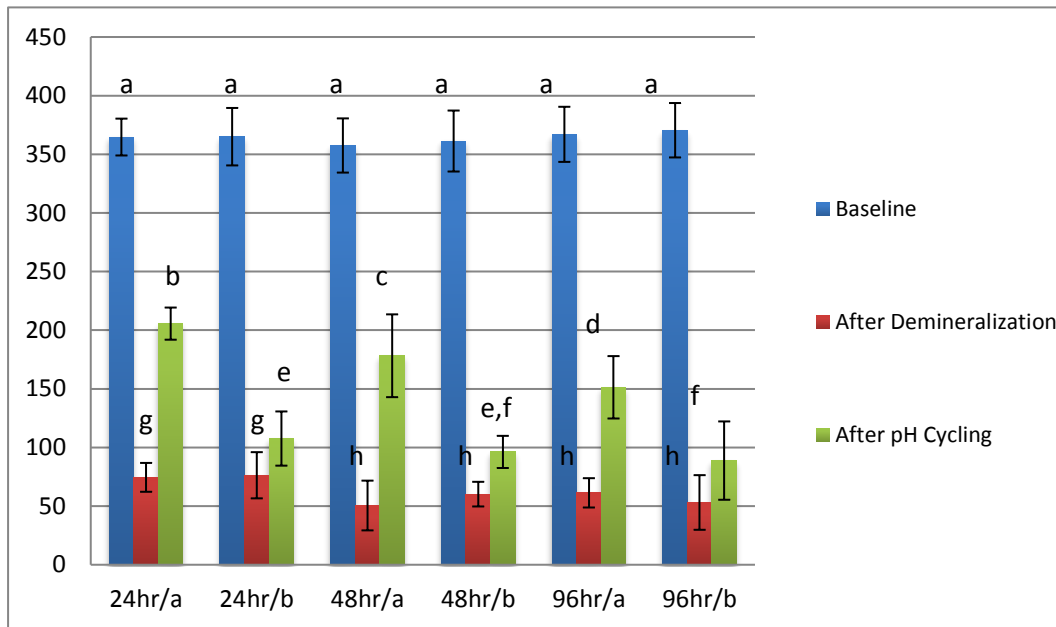FIGURE 3.　　　　　　　Flowchart of the experimental design.

FIGURE 4.   Knoop surface microhardness for all 6 groups at different stages
(Lowercase letters indicate statistically significant differences
between groups).
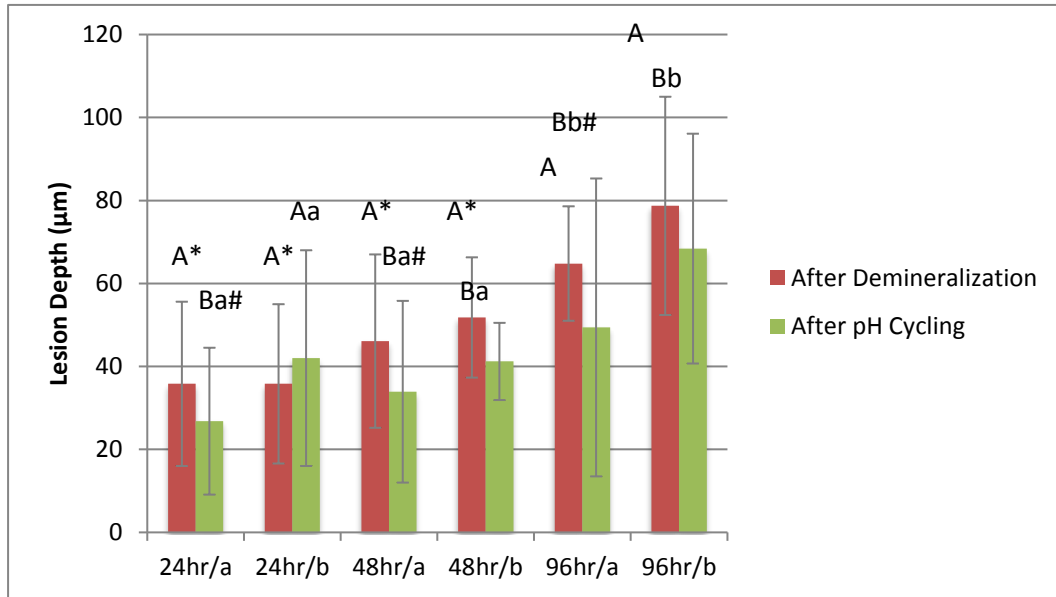
FIGURE 5.   Lesion depth for all 6 groups at different stages.
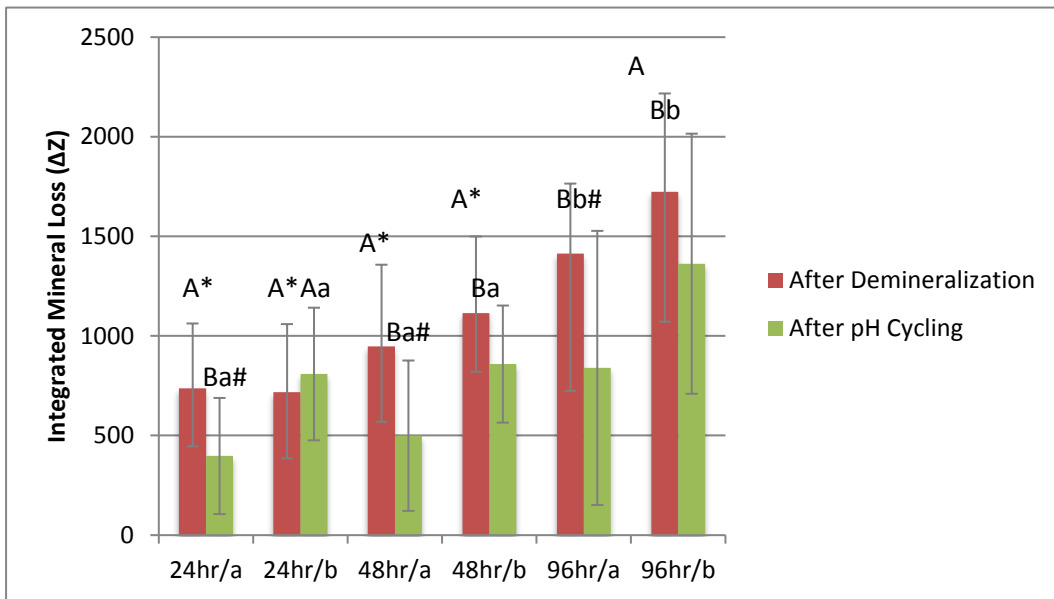
FIGURE 6.   Integrated mineral loss for all 6 groups at different stages.

a.
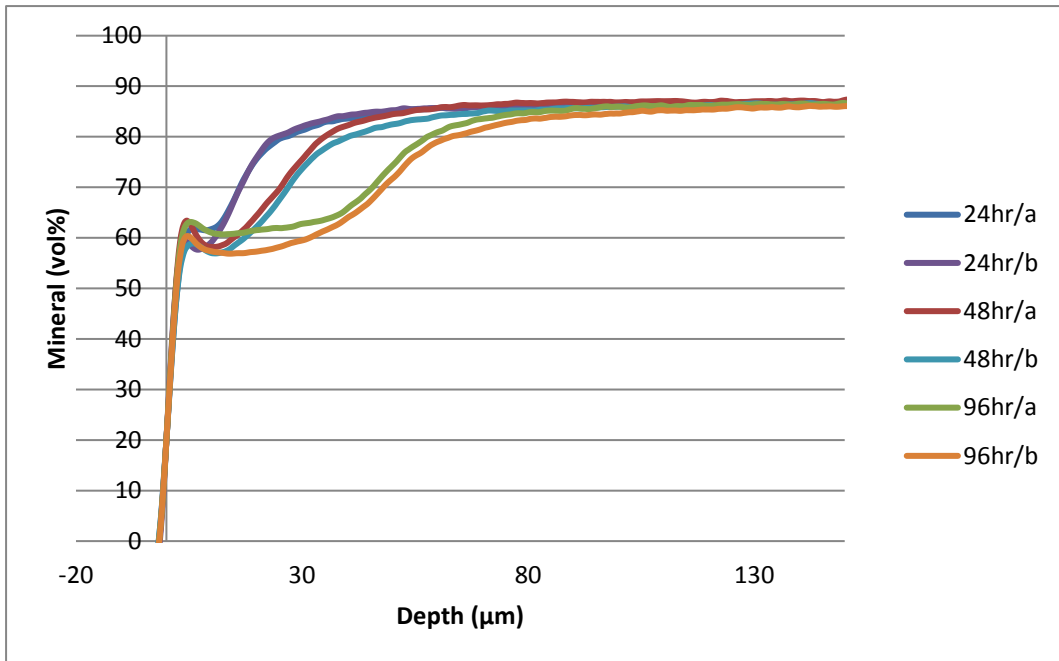
b.
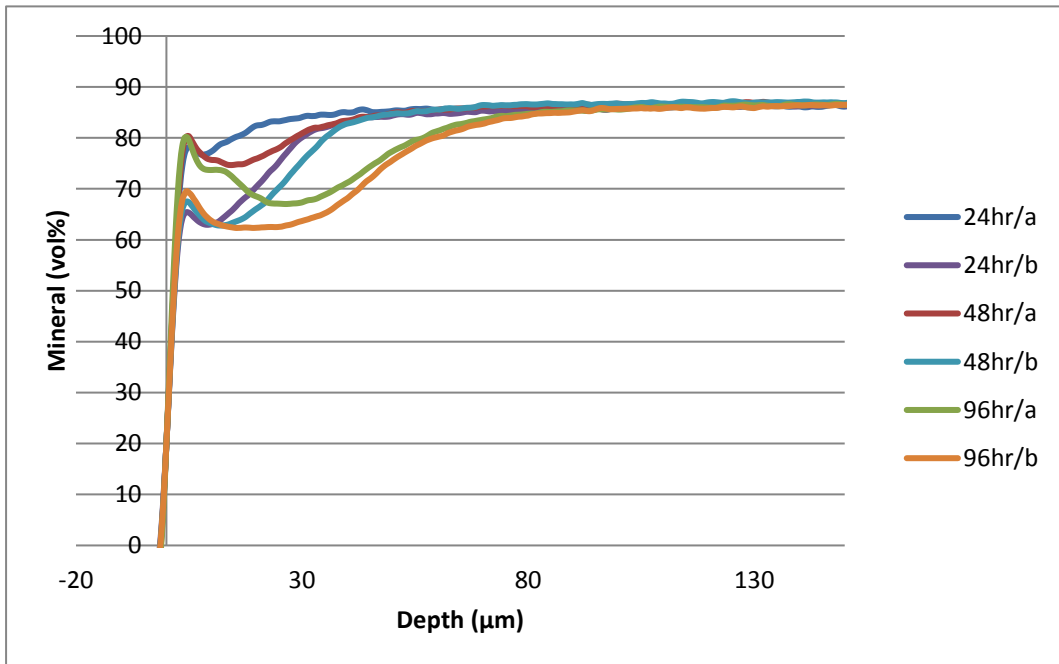
FIGURE 7.   Mean mineral distribution for all 6 groups by stage a. after demineralization and b. after pH-cycling.
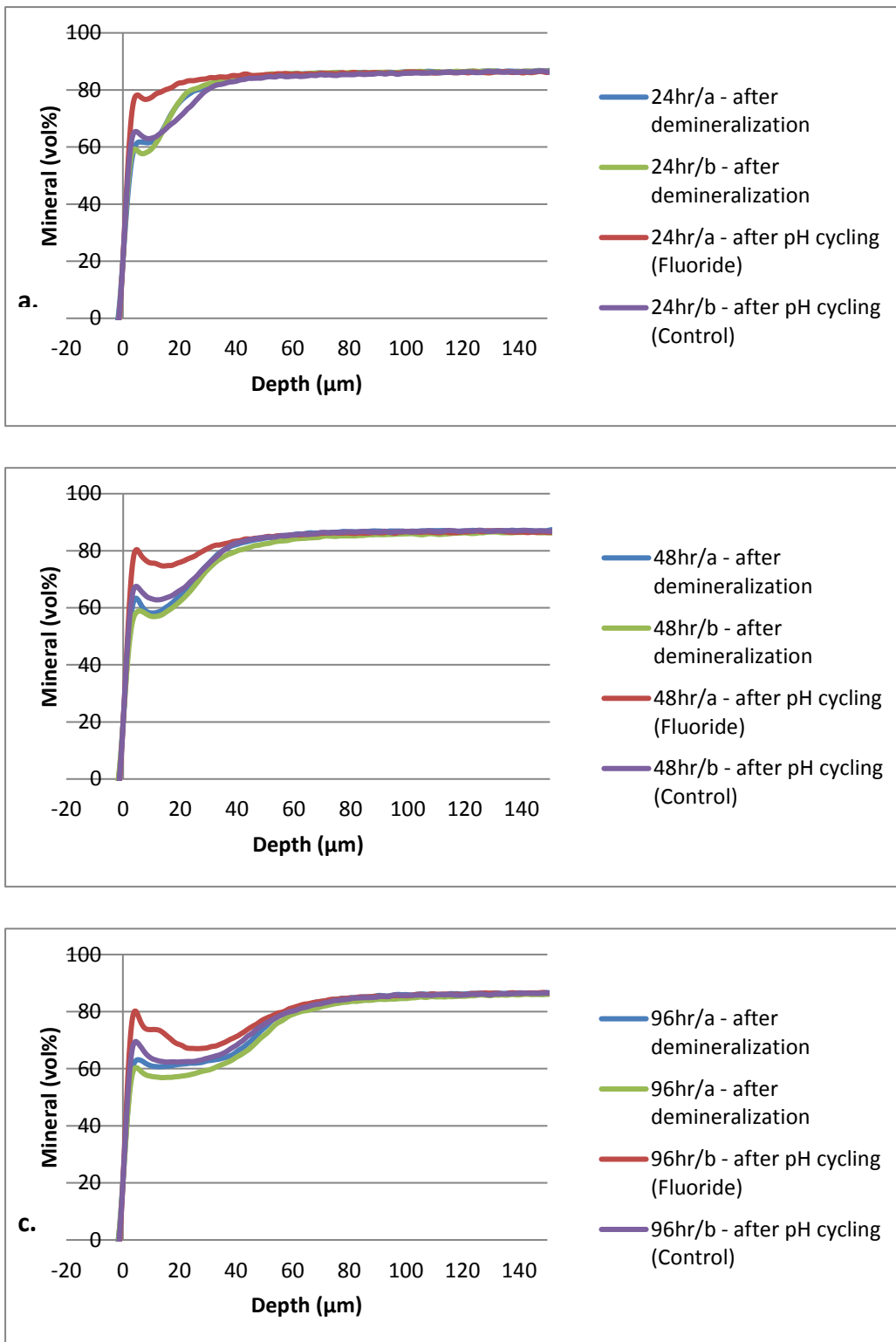
FIGURE 8.   Mean mineral distribution for all 6 groups by demineralization time a. groups 24hr/a and 24hr/b, b. groups 48hr/a and 48hr/b, and c. groups 96hr/a and 96hr/b.
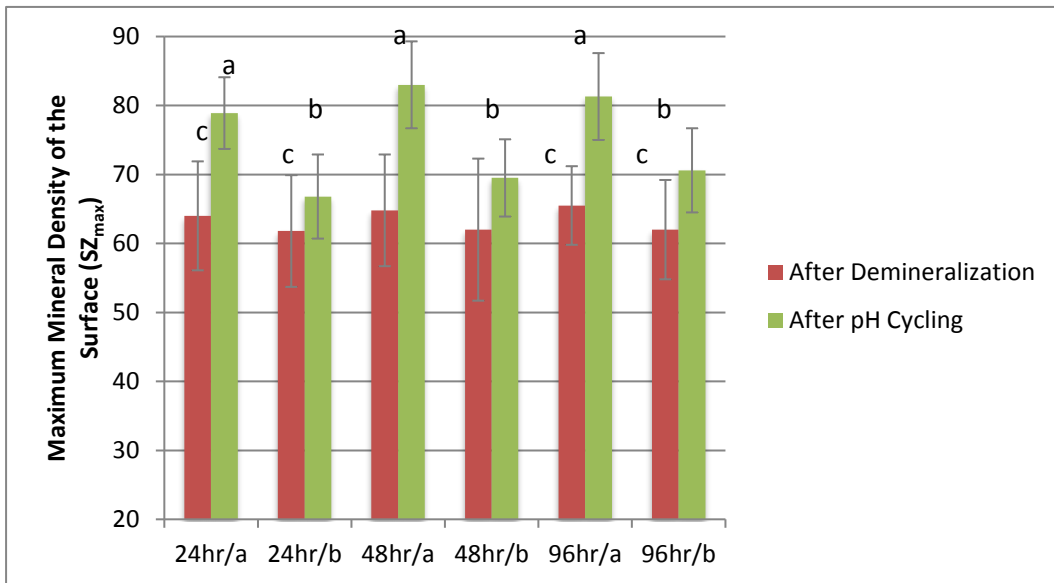
FIGURE 9.   Maximum mineral density of the surface zone for all 6 groups at different stages.
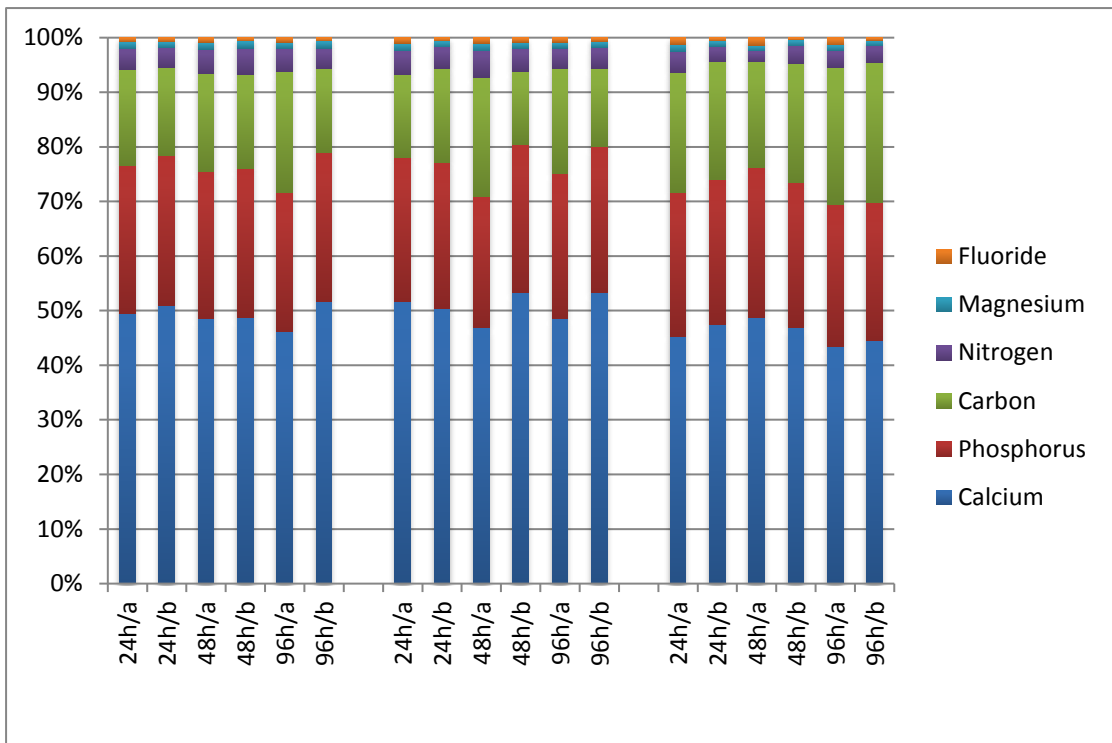
FIGURE 10.   Weight% of chemical elements at the surface in 6 groups at different stages.
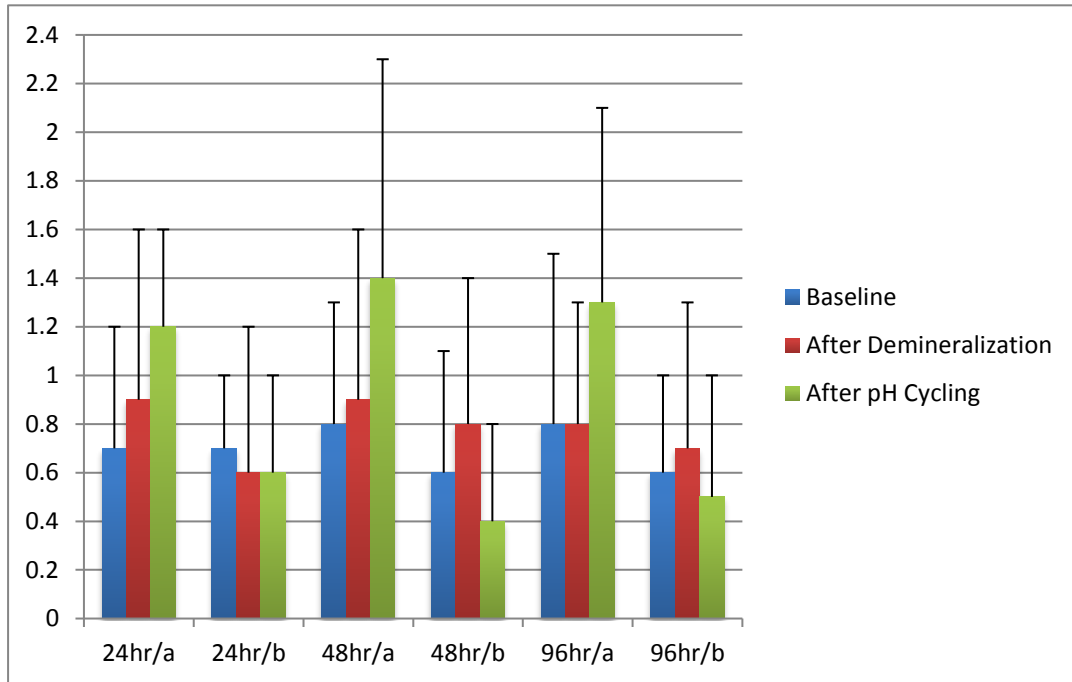
FIGURE 11.   Weight% of fluorine at the surface in 6 groups at different
stages (* indicate statistically significant differences between
treatments).
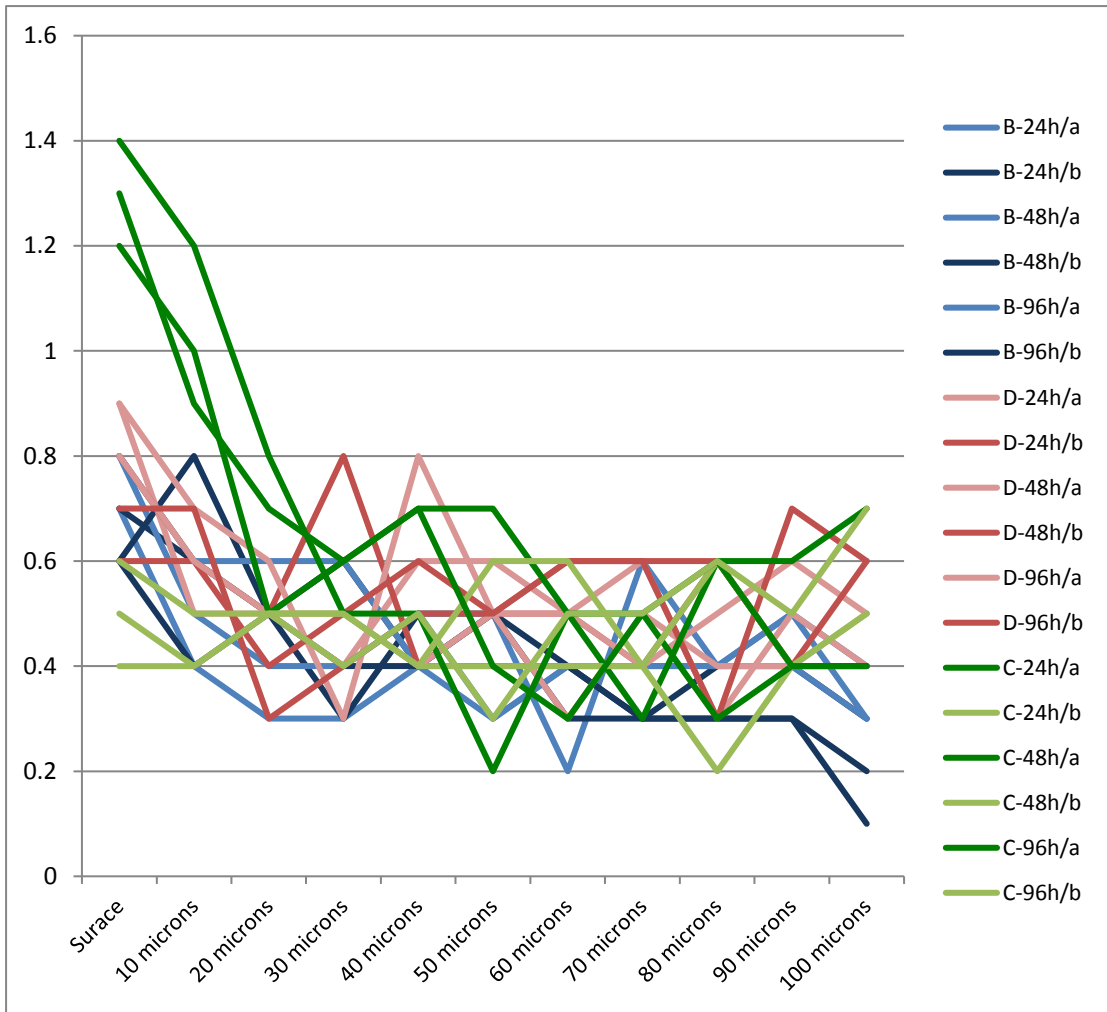
FIGURE 12.   Weight% of fluorine from the surface to 100-μm depth in 6
              groups at different stages B- at baseline, D- after
              demineralization, and C- after pH-cycling.
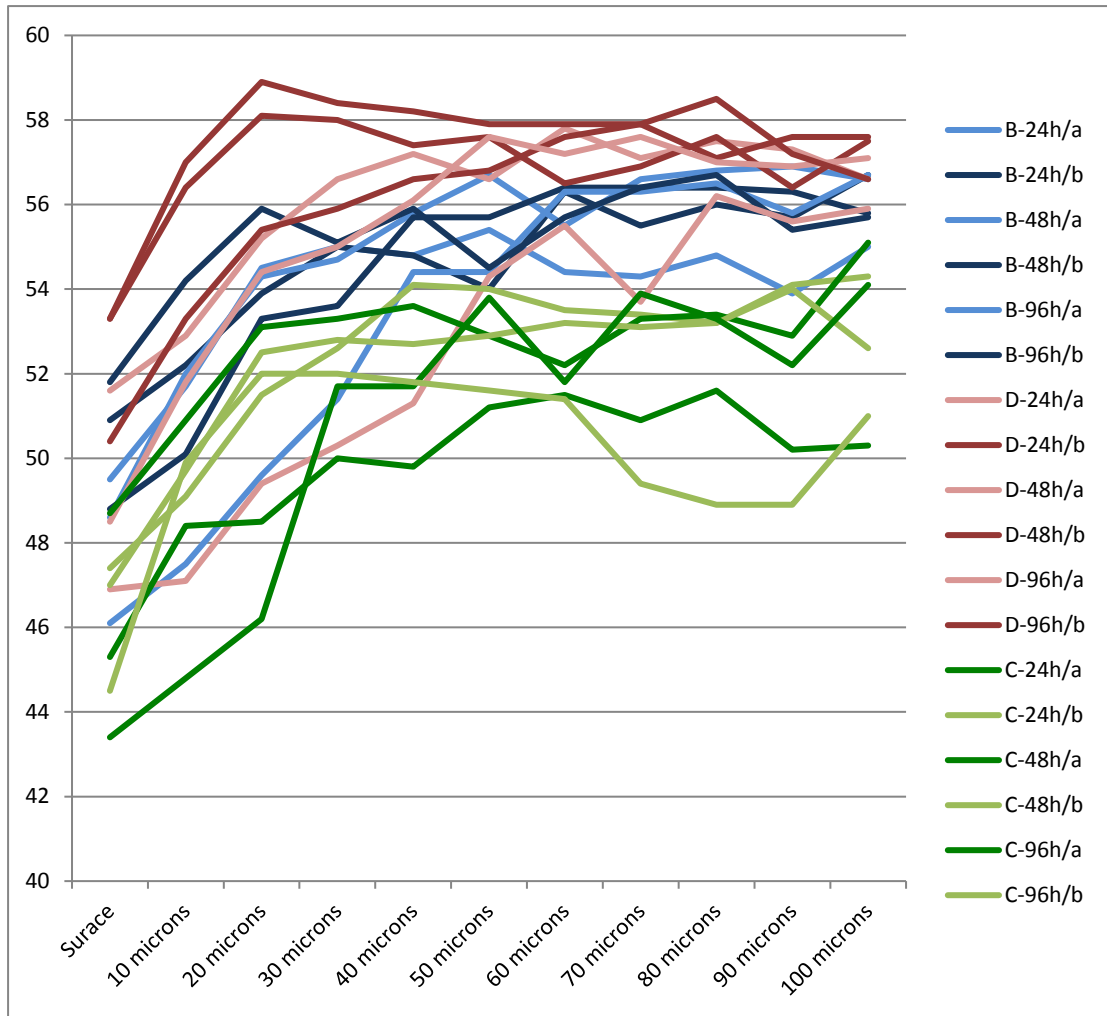
FIGURE 13.   Weight% of calcium from the surface to 100-μm depth in 6 groups
at different stages B- at baseline, D- after demineralization, and C-
after pH-cycling.
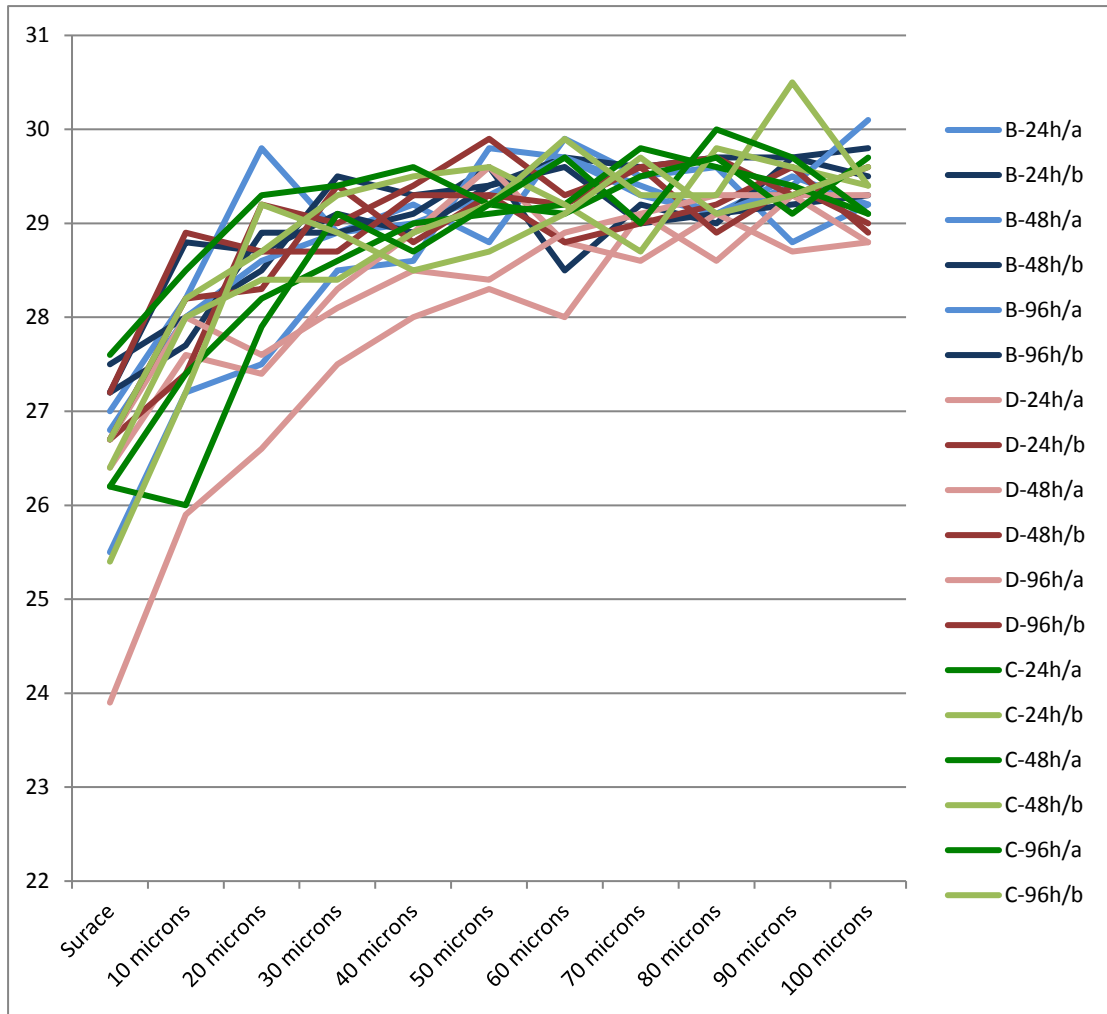
FIGURE 14.   Weight% percent of phosphorus from the surface to 100-μm depth in 6 groups at different stages B- at baseline, D-after demineralization, and C- after pH-cycling.
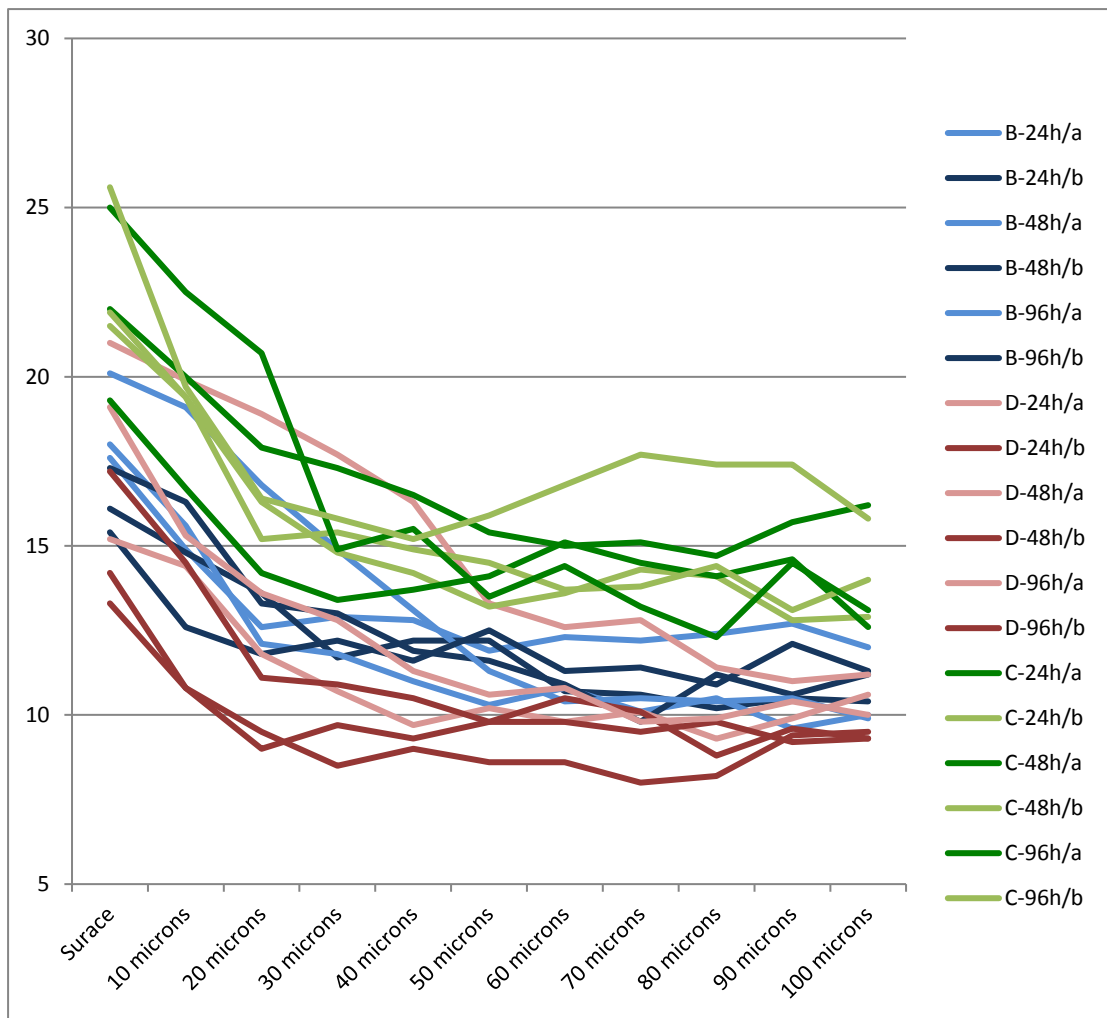
FIGURE 15. Weight% of carbonate from the surface to 100-μm depth in 6 groups at different stages B- at baseline, D- after demineralization, and C- after pH-cycling.
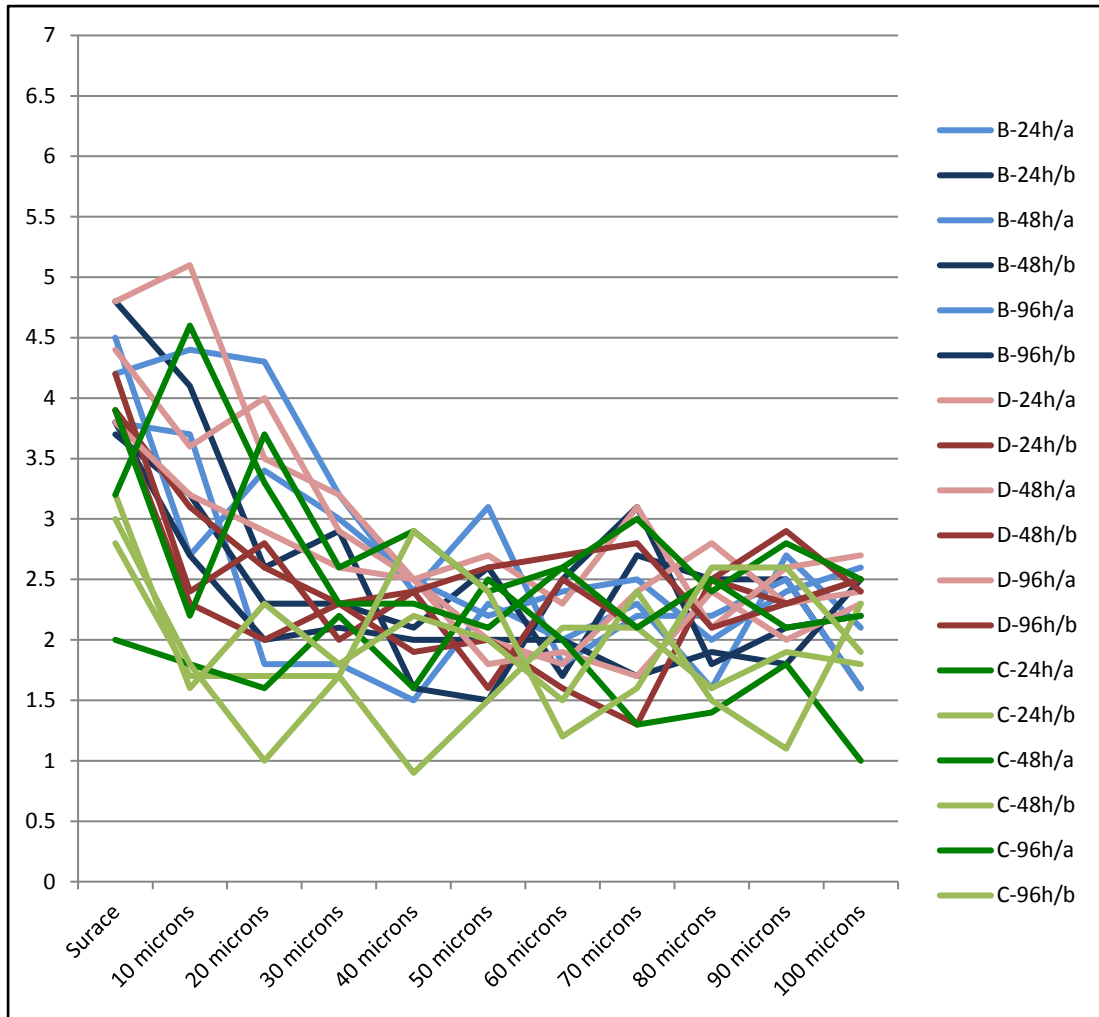
FIGURE 16.   Weight% of nitrate from the surface to 100-µm depth in 6
              groups at different stages B- at baseline, D- after
              demineralization, and C- after pH-cycling.
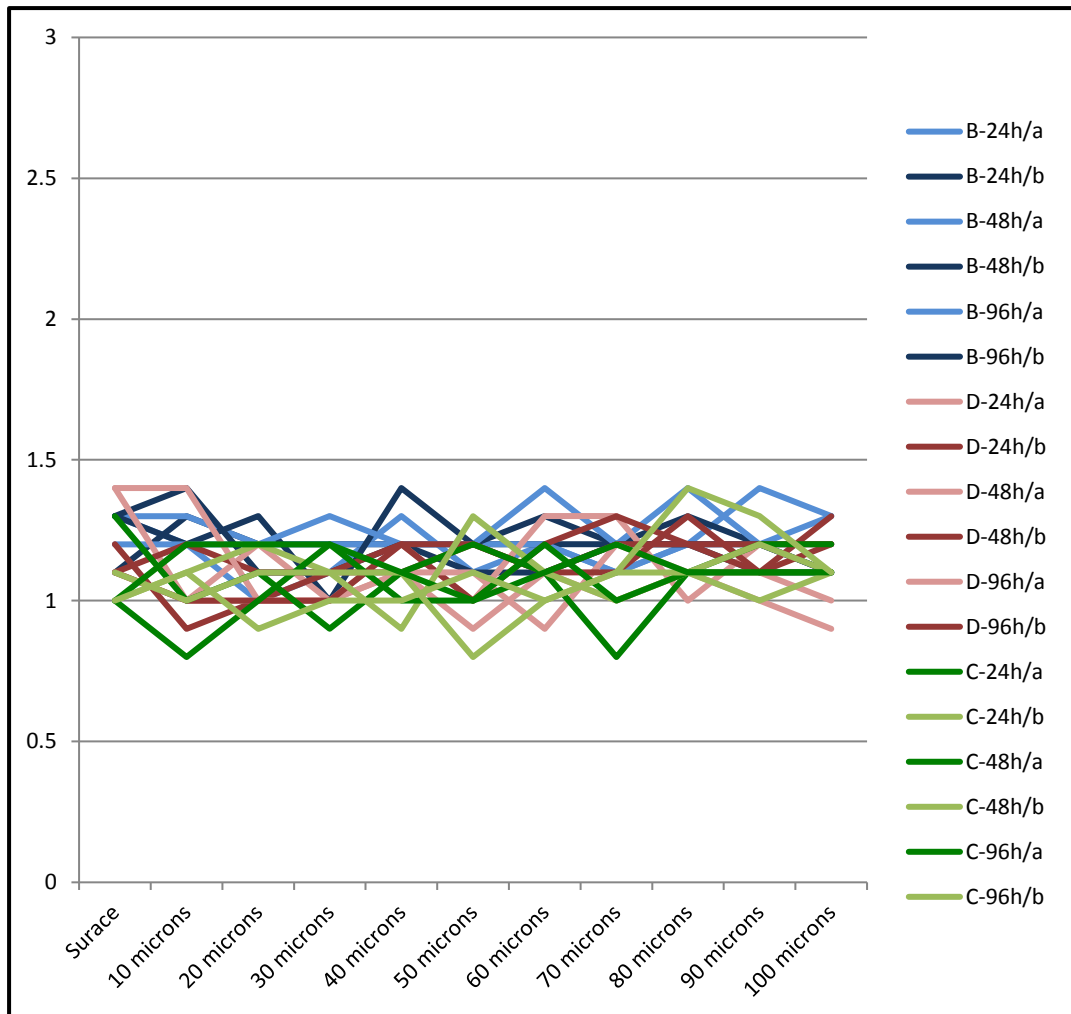
FIGURE 17.   Weight% of magnesium from the surface to 100-µm depth in 6 groups at different stages B- at baseline, D- after demineralization, and C- after pH-cycling.
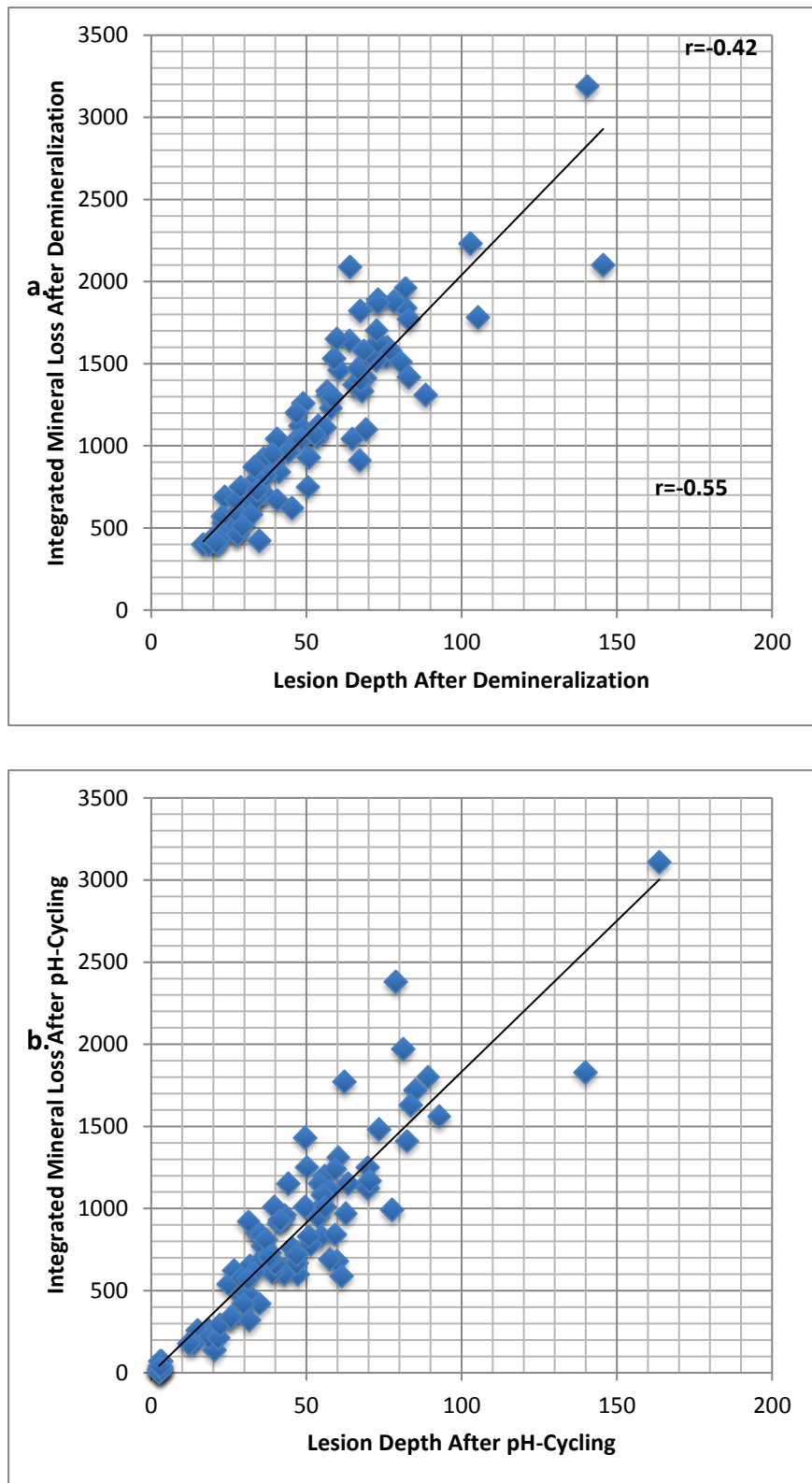
FIGURE 18.   Pearson correlation coefficients between lesion depth and integrated mineral loss a. after demineralization, and b. after pH-cycling.
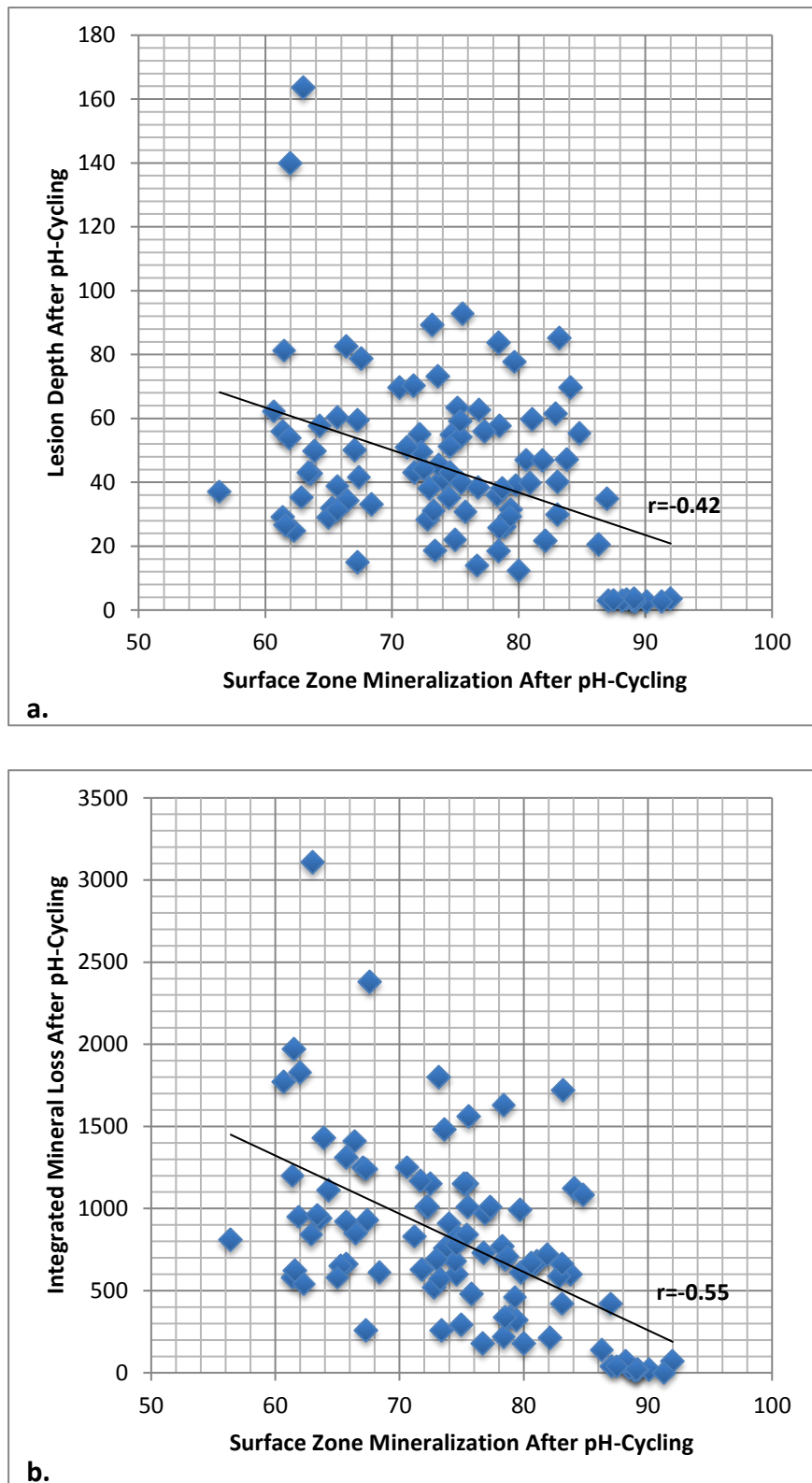
FIGURE 19. Pearson correlation coefficients between maximum mineral density of the surface zone and a. lesion depth, and b. integrated mineral loss after pH-cycling.
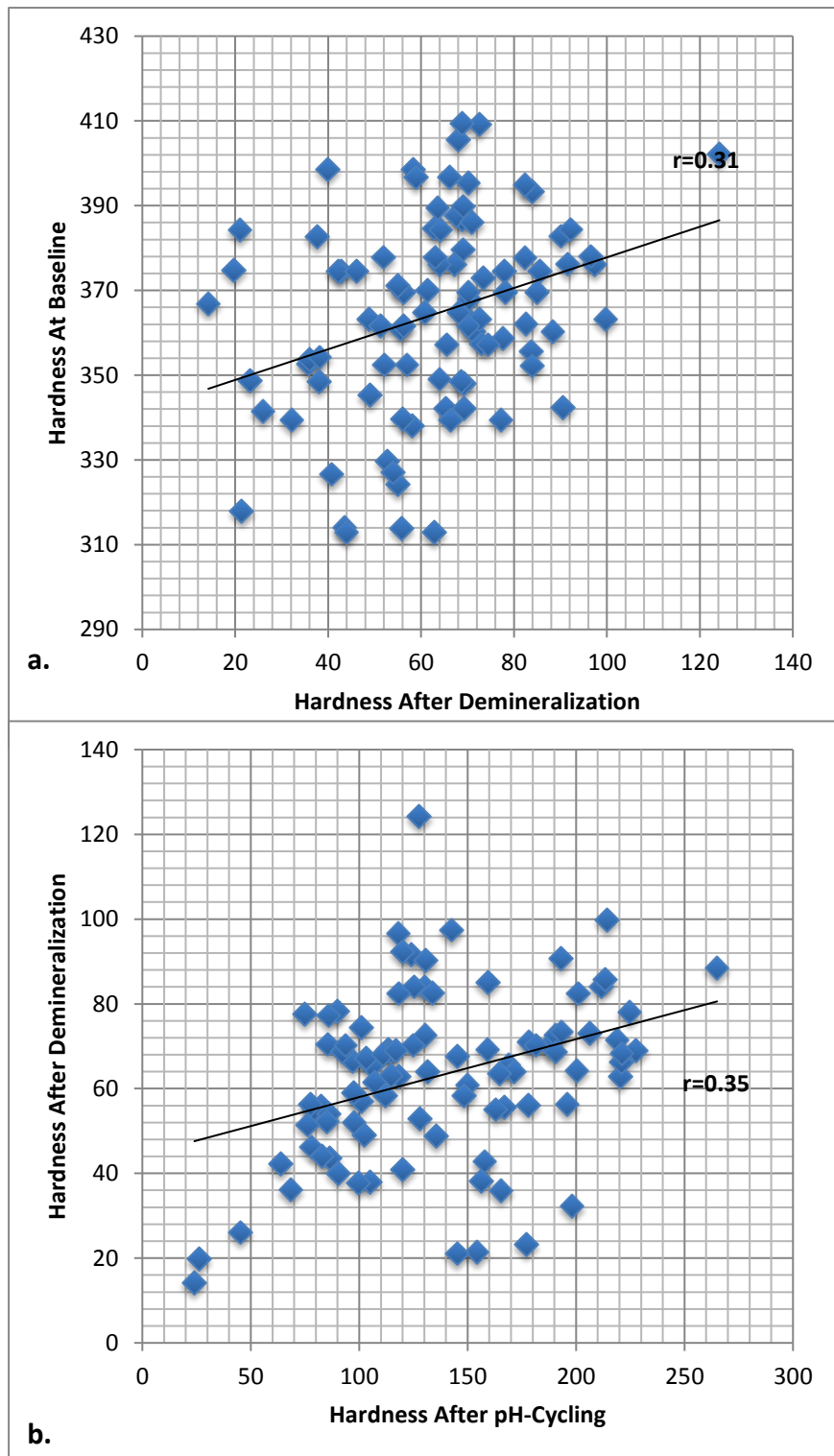
FIGURE 20(a).   Pearson correlation coefficients between Knoop hardness a. at
                baseline and after demineralization, and b. after
                demineralization and after pH-cycling.
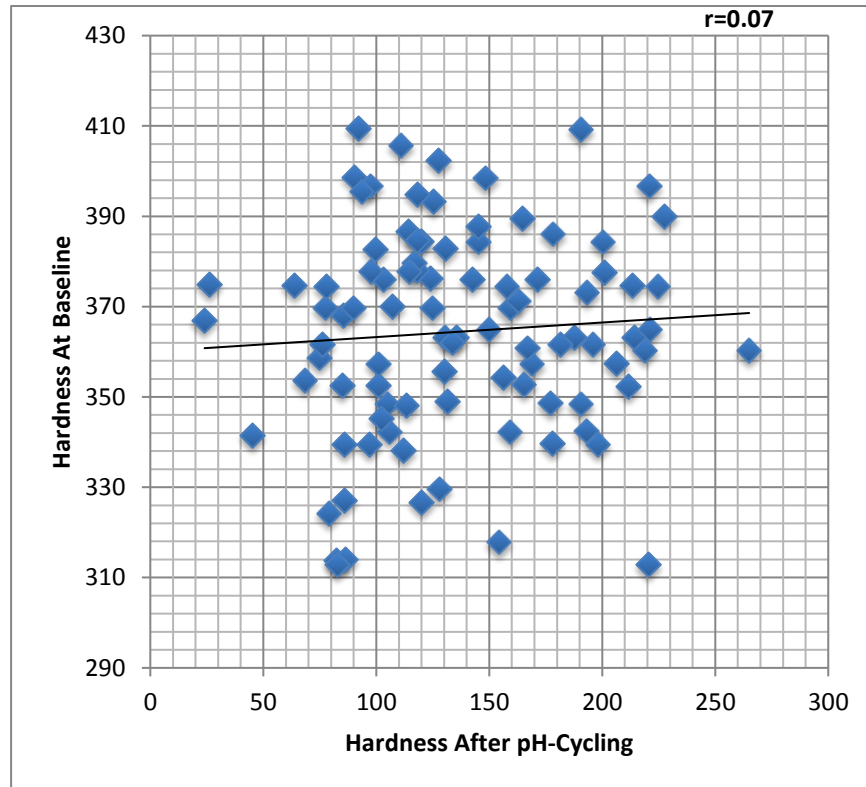
FIGURE 20(b).   Pearson correlation coefficients between Knoop hardness at baseline and after pH-cycling.

.

a.

r=-0.25
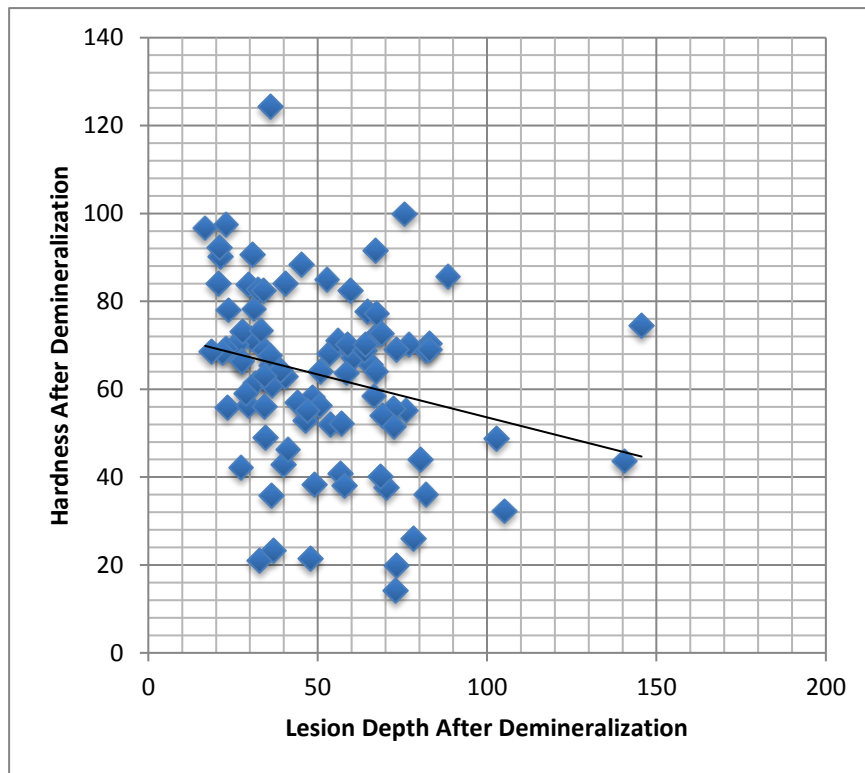
b.

r=-0.32
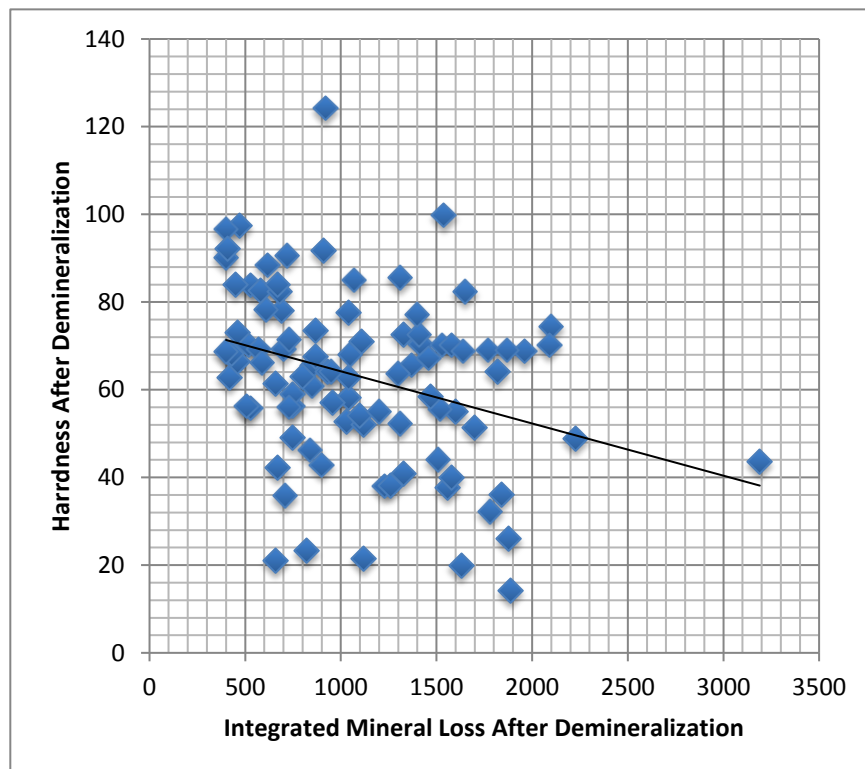
FIGURE 21. Pearson correlation coefficients between Knoop hardness a. at baseline and after demineralization, and b. after demineralization and after pH-cycling.

a.
r=-0.22
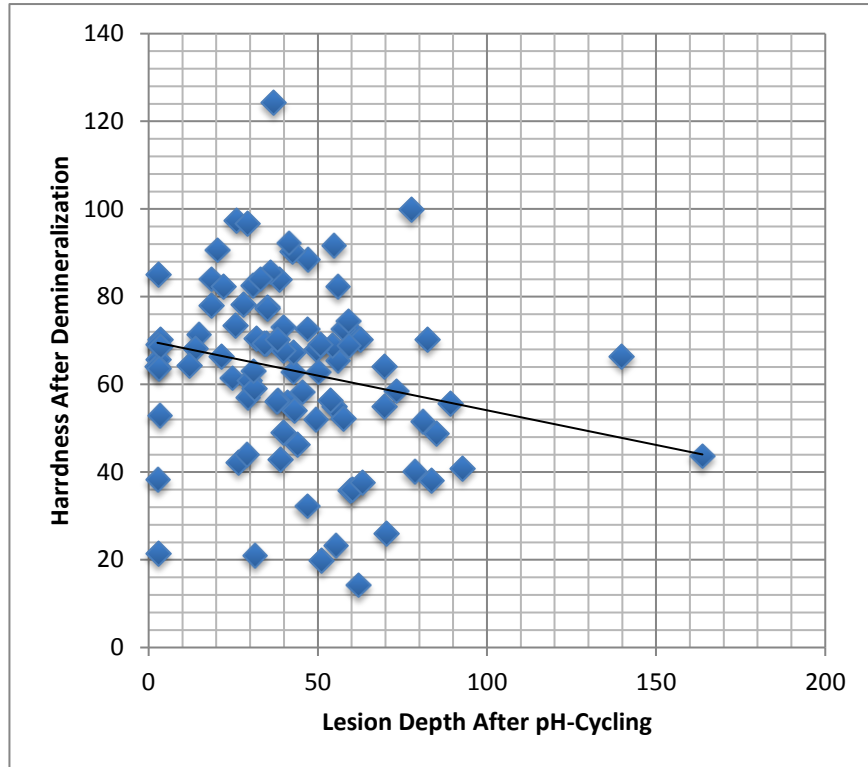
b.
r=-0.28
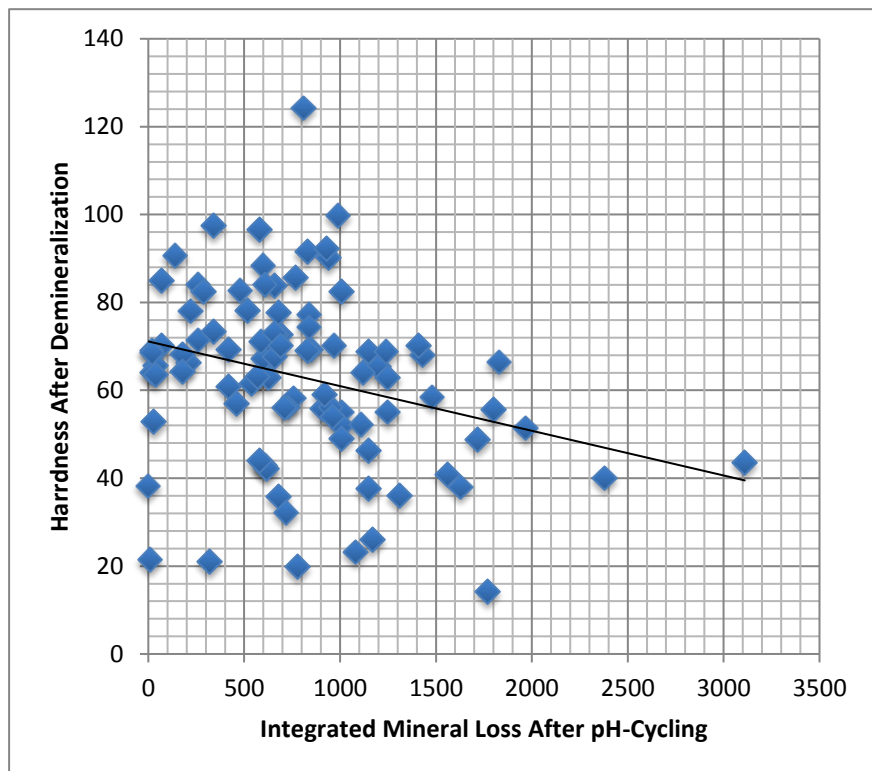
FIGURE 22.   Pearson correlation coefficients between Knoop hardness after demineralization and a. lesion depth after pH-cycling, and b. integrated mineral loss after pH-cycling.

49

a.

r=-0.35
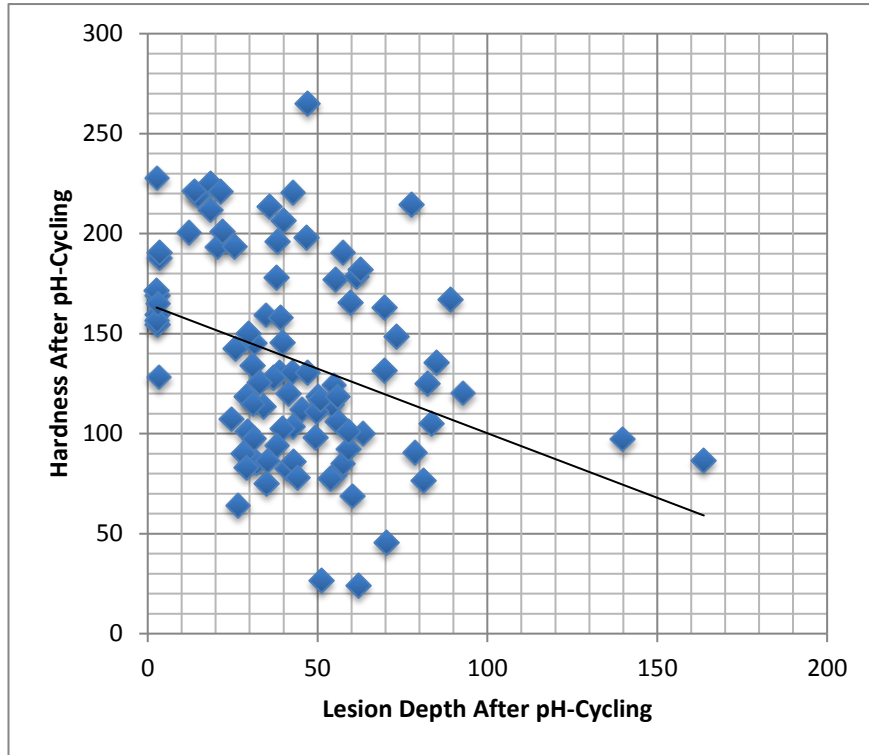
b.

r=-0.49
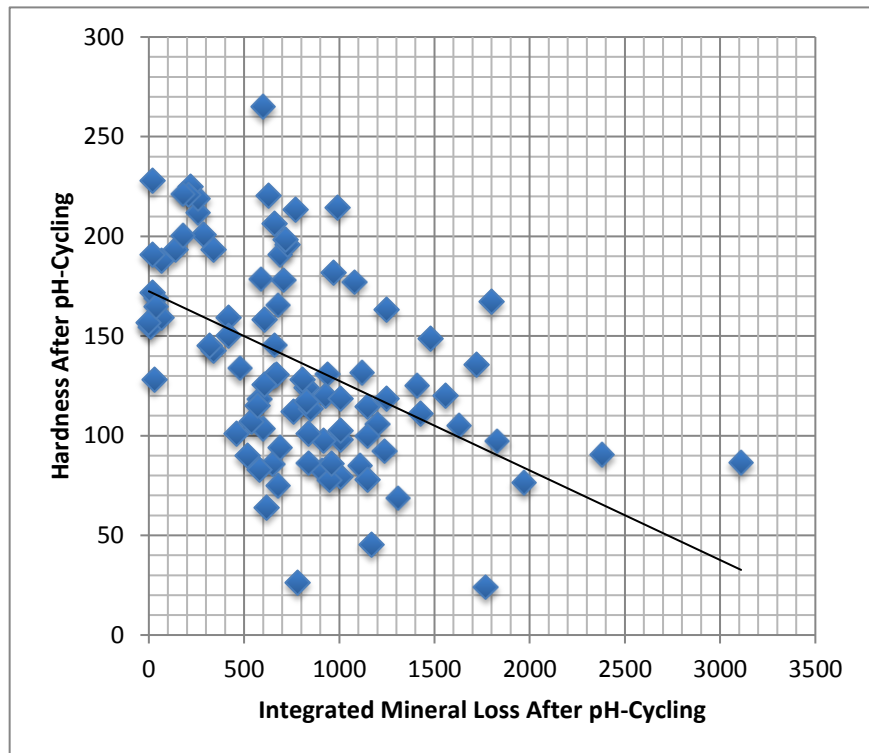
FIGURE 23(a)   Pearson correlation coefficients between Knoop hardness
after pH-cycling and a. lesion depth and b. integrated
mineral loss after pH-cycling.
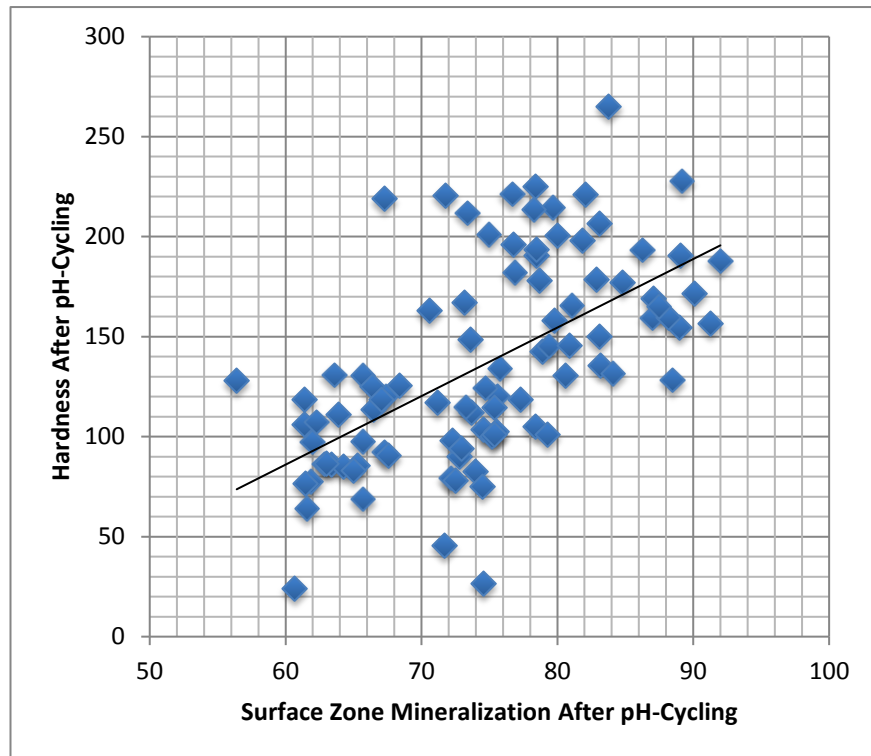
FIGURE 23(b).   Pearson correlation coefficients between Knoop
hardness after pH-cycling and maximum mineral
density of the surface zone after pH-cycling.
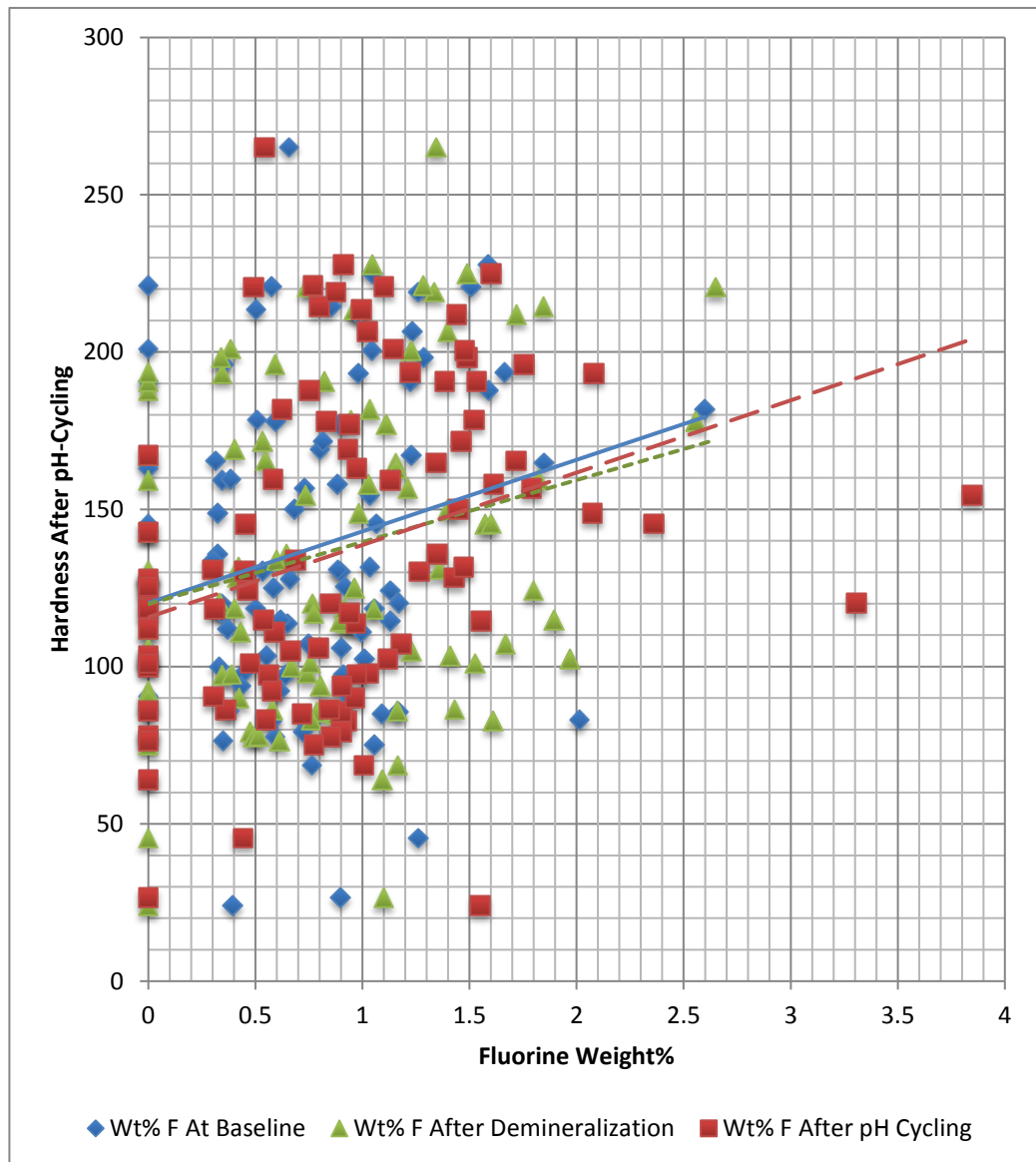
FIGURE 24.   Pearson correlation coefficients between Knoop hardness
after pH-cycling and the weight% of fluorine at baseline,
after demineralization, and after pH-cycling.

a.

r=0.24
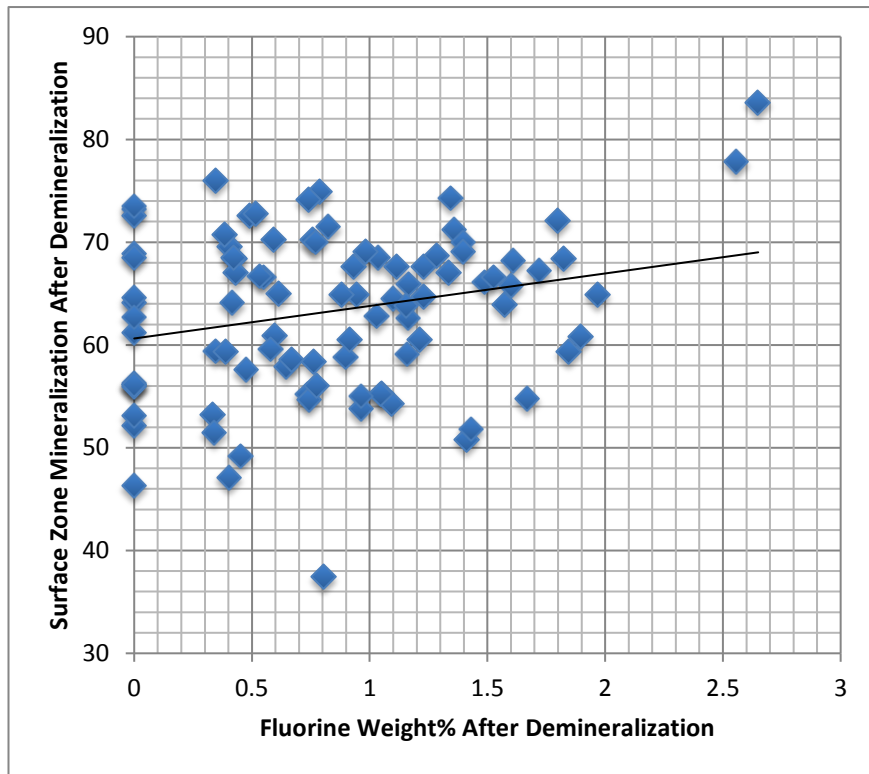
b.

r=0.43
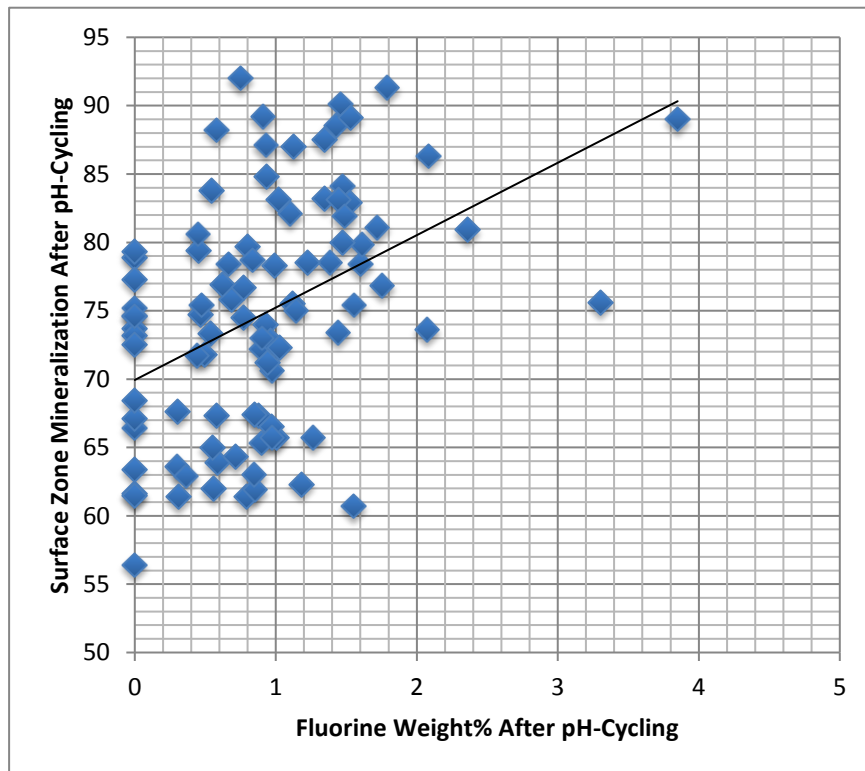
FIGURE 25.   Pearson correlation coefficients between the weight% of fluorine and the maximum mineral density of the surface zone a. after demineralization and b. after pH-cycling.
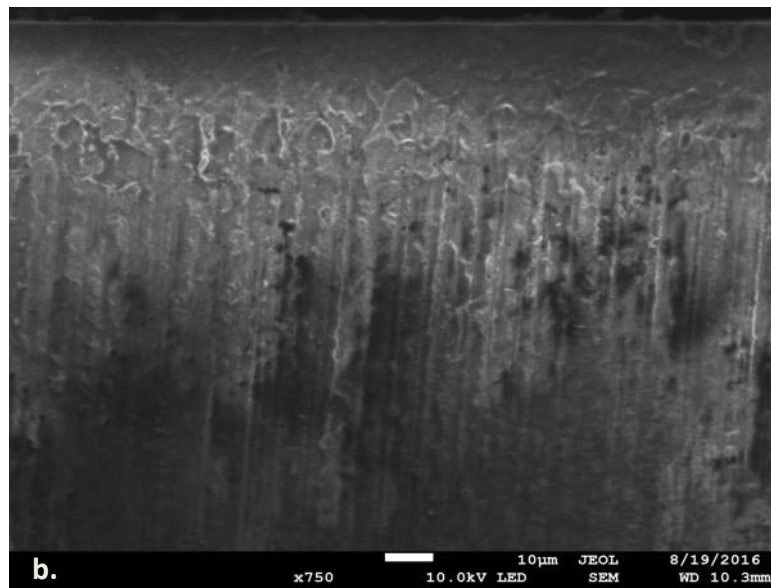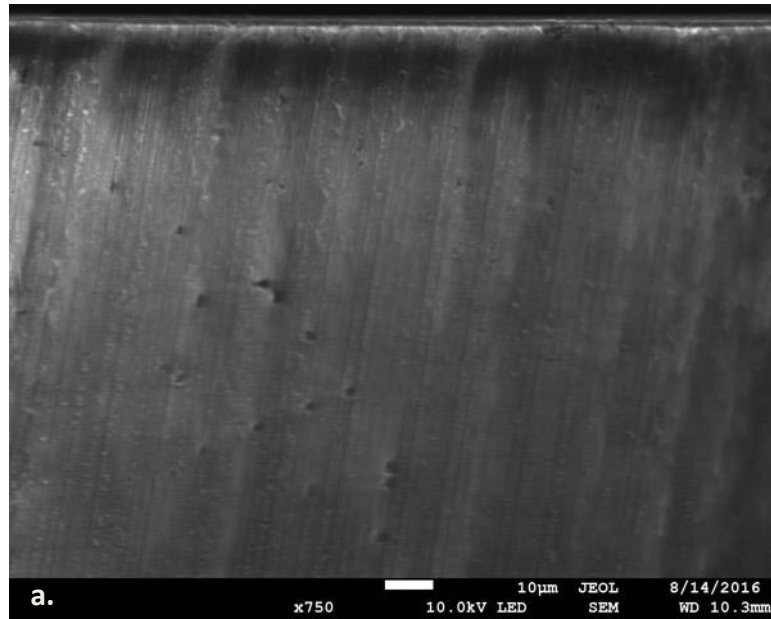
FIGURE 26(a)   SEM images taken at X750 magnification a. at baseline, and b. after 24 hr demineralization.
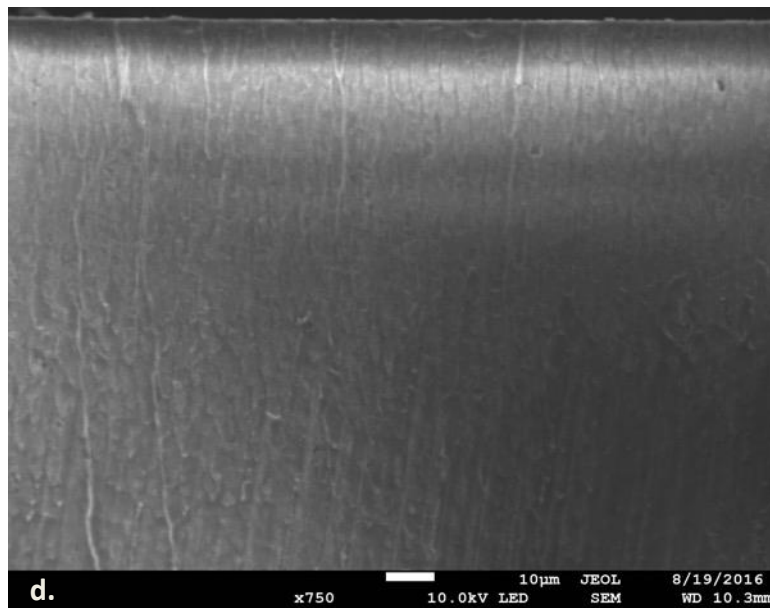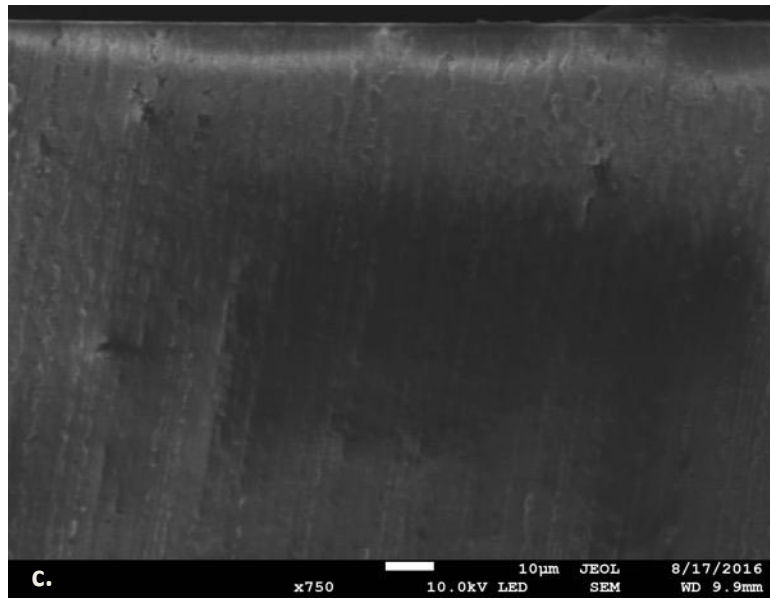
FIGURE 26(b). SEM images taken at X750 magnification c. after 48 hr
demineralization, and d. after 96 hr demineralization.
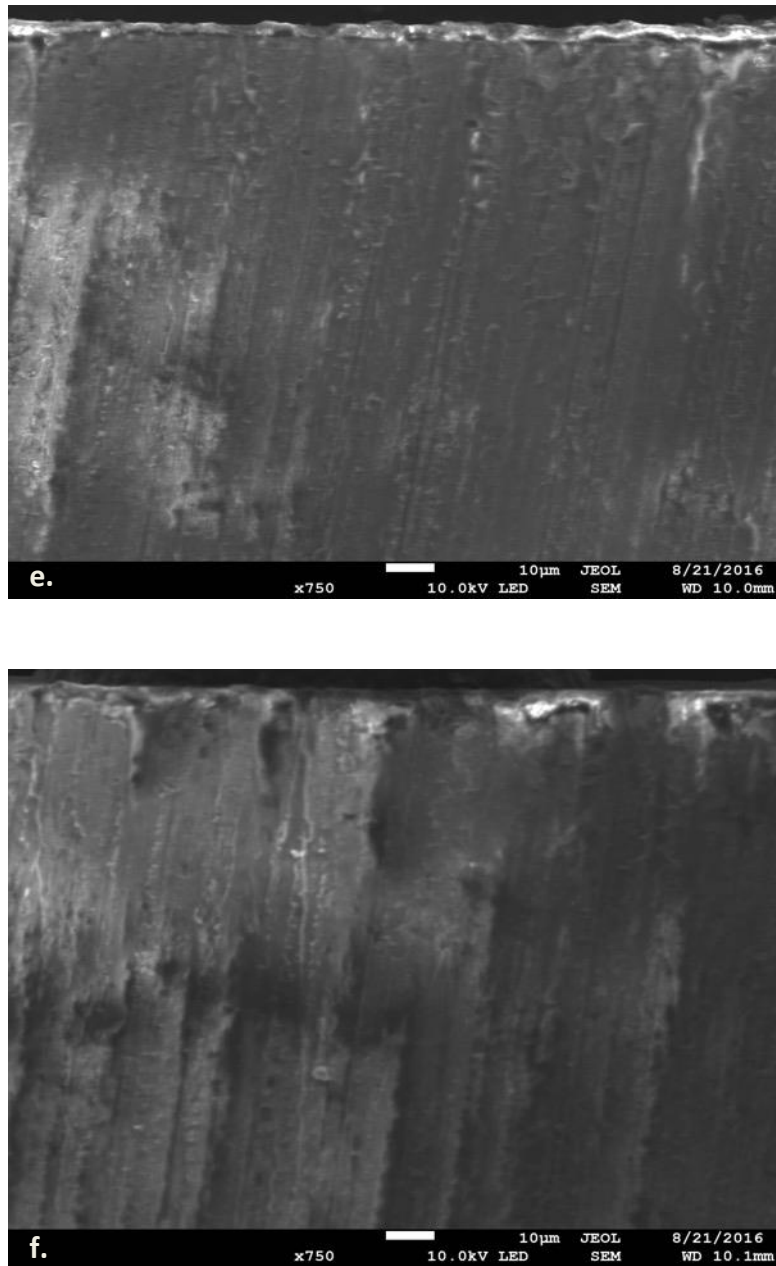
FIGURE 26(c). SEM images taken at X750 magnification e. after 24 hr demineralization and fluoride treatment, and f. after 48 hr demineralization and fluoride treatment.
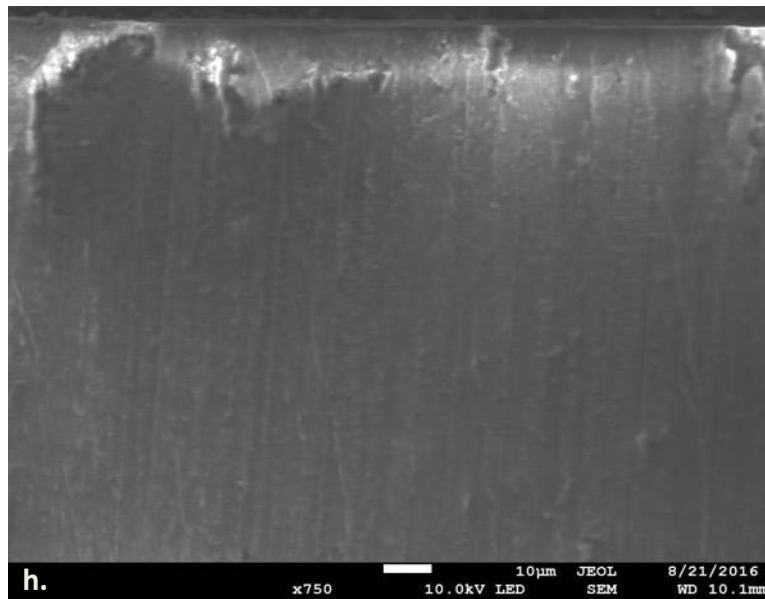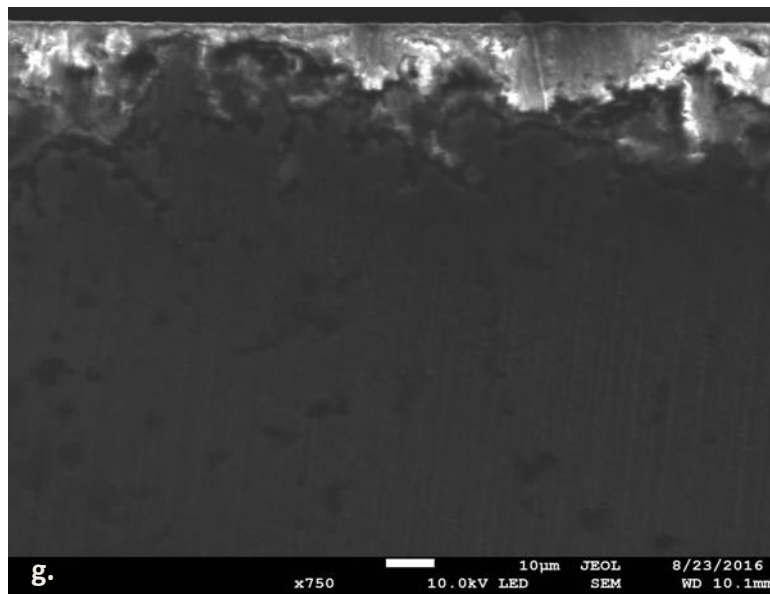
FIGURE 26(d). SEM images taken at X750 magnification g. after 96 hr
demineralization and fluoride treatment, and h. after 24 hour
demineralization and control treatment.
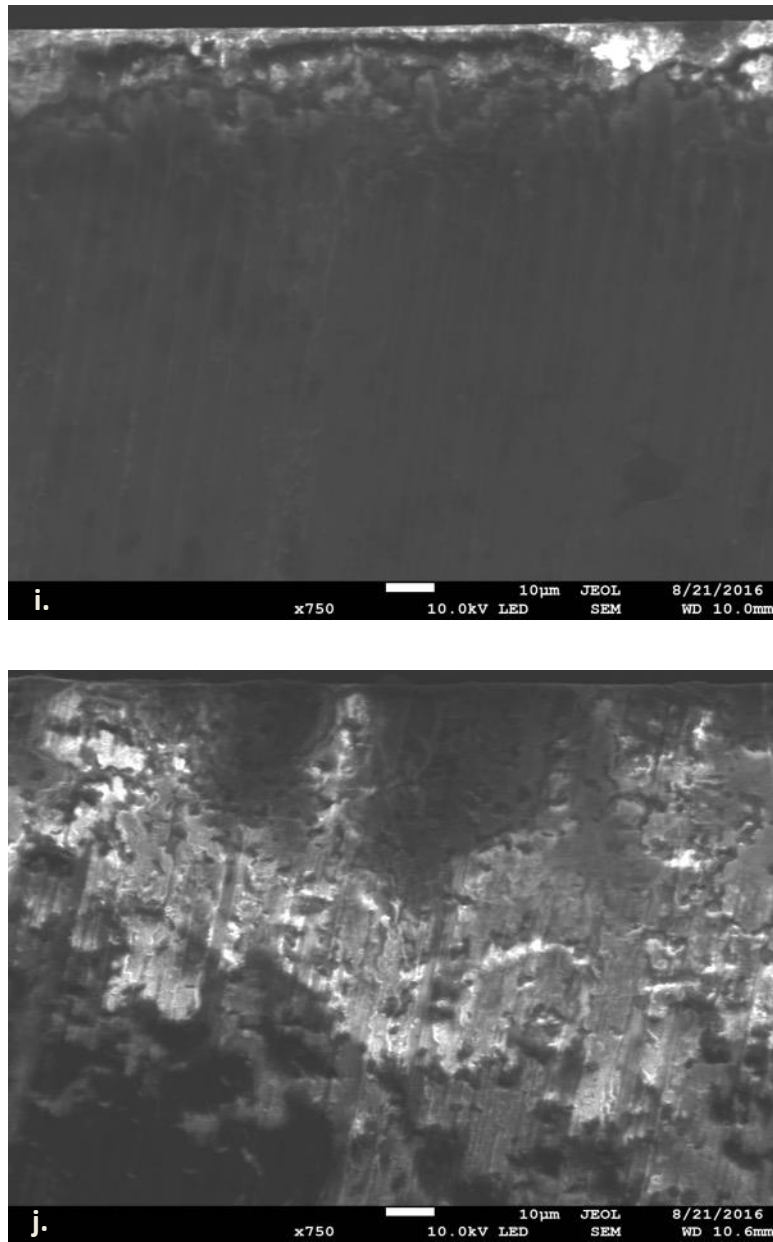
FIGURE 26(e). SEM images taken at X750 magnification i. after 48 hr demineralization and control treatment, and j. after 96 hr demineralization and control treatment.

TABLE I

Daily pH-cycling treatment regimen

Duration	Specimen treatment
1 min	Treatment
60 min	Artificial saliva
1 min	Treatment
60 min	Artificial saliva
240 min	Acid challenge
60 min	Artificial saliva
1 min	Treatment
60 min	Artificial saliva
1 min	Treatment
Overnight	Artificial saliva

TABLE II

Mean Knoop surface microhardness for all 6 groups
at different stages (SD in parentheses)

Groups	n	Baseline	After demineralization	After pH-cycling
24hr/a	15	364.7 (15.7) A	74.5 (12.3) C*	205.6 (13.7) Ba#
24hr/b	18	365 (24.5) A	76.3 (19.7) C*	107.6 (23.1) Bc
48hr/a	15	357.5 (23.1) A	50.5 (21.2) C	178.2 (35.3) Bab#
48hr/b	15	361.3 (26) A	60.2 (10.5) C	96.2 (13.7) Bd
96hr/a	15	367 (23.5) A	61.3 (12.5) C	151.3 (26.6) Bb#
96hr/b	16	370.5(23.2) A	53.1(23.3) C	88.8(33.4) Be

Uppercase letters indicate statistically significant differences between stages.
* indicate statistically significant differences between demineralization times after demineralization.
Lowercase letters indicate statistically significant differences between demineralization times after pH-cycling.
indicate statistically significant differences between treatments.

TABLE III

Least square means and standard error of the least square means for %SMHr

Baseline lesion severity	Treatment	n	Indentation length IL (μm)			%SMHr
			Baseline	After demineralization	After pH-cycling	
24hr	Fluoride	15	44.2	98.65	58.91	72.6±2.6% Aa
	Deionized water	18	44.22	98.89	82.83	28.6±2.38% Cb
48hr	Fluoride	15	44.68	128.05	63.94	75±2.6% Aa
	Deionized water	15	44.45	109.87	86.62	34.6±2.6% CDb
96hr	Fluoride	15	44.1	109.52	69.38	61.1±2.6% Ba
	Deionized water	16	43.88	127.16	97.64	36.3±2.52% Db
Uppercase letters indicate statistically significant differences between lesion severities within each treatment. Lowercase letters indicate statistically significant differences between treatments within each lesion severity.						

TABLE IV

Mean TMR variables for all 6 groups at different stages (SD in parentheses)

Groups	n	Stage	ΔZ (vol%min x μm)	L (μm)	SZ_{max} (vol%min)
24hr/a	15	After demineralization	737 (325) A*	36 (20) A*	64 (8) A*
		After pH-cycling	397 (292) Ba#	27 (18) Ba#	79 (5) Ba#
24hr/b	18	After demineralization	718 (341) A*	36 (19) A*	62 (8) A*
		After pH-cycling	809 (333) Aa	42 (26) Aa	67 (6) Ba
48hr/a	15	After demineralization	947 (411) A*	46 (21) A*	65 (8) A*
		After pH-cycling	499 (378) Ba#	34 (22) Ba#	83 (6) Bb#
48hr/b	15	After demineralization	1114 (386) A*	52 (15) A*	62 (10) A*
		After pH-cycling	859 (294) Ba	41 (9) Ba	70 (6) Bb
96hr/a	15	After demineralization	1413 (352) A	65 (14) A	66 (6) A*
		After pH-cycling	839 (688) Bb#	49 (36) Bb#	81 (6) Bb#
96hr/b	16	After demineralization	1724 (493) A	79 (26) A	62 (7) A*
		After pH-cycling	1363 (653) Bb	68 (28) Bb	71 (6) Bb

Uppercase letters indicate statistically significant differences between stages.
* indicate statistically significant differences between demineralization times after demineralization.
Lowercase letters indicate statistically significant differences between demineralization times after pH cycling.
indicate statistically significant differences between treatments.

TABLE V

Least square means and standard error of the least square means for %R

Baseline lesion severity	Treatment	n	ΔZ_d (vol%min x μm)	ΔZ_r (vol%min x μm)	%R
24hr	Fluoride	15	737	397	44.3±13.6% Aa
	Deionized water	18	718	809	-33.1±12.4% Bb
48hr	Fluoride	15	947	499	37.6±13.6% Aa
	Deionized water	15	1114	859	14.9±13.6% Aa
96hr	Fluoride	15	1413	839	40.4±13.6% Aa
	Deionized water	16	1724	1363	20.7±13.2% Aa

Uppercase letters indicate statistically significant differences between lesion severities within each treatment.
Lowercase letters indicate statistically significant differences between treatments within each lesion severity.

TABLE VI

Mean weight percent of calcium, phosphorus and carbonate at
the surface in 6 groups at different stages (SD in parentheses)

Groups	n	Stage	Calcium	Phosphorus	Carbonate
24hr/a	15	Baseline	49.5 (8.7) A	27 (2.6) A	17.6 (8.2) A
		After demineralization	51.6 (7.6) A	26.4 (2.7) A	15.2 (7.1) A
		After pH-cycling	45.3 (5.1) B	26.2 (2.8) A	22 (6) B
24hr/b	18	Baseline	50.9 (5.6) A	27.5 (2.3) A	16.1 (5.5) A
		After demineralization	50.4 (8.4) A	26.7 (2.2) A	17.2 (7.9) A
		After pH-cycling	47.4 (7.6) B	26.7 (3.3) A	21.5 (8.6) B
48hr/a	15	Baseline	48.6 (6.2) A	26.8 (2.8) A	18 (7.9) A
		After demineralization	46.9 (13.5) A	23.9 (4.4) A	21.9 (14) A
		After pH-cycling	48.7 (5.1) B	27.6 (2.8) A	19.3 (5.8) B
48hr/b	15	Baseline	48.8 (7.4) A	27.2 (4) A	17.3 (8.6) A
		After demineralization	53.3 (4.2) A	27.2 (2) A	13.3 (4.1) A
		After pH-cycling	47 (7.4) B	26.4 (2.8) A	21.9 (8.6) B
96hr/a	15	Baseline	46.1 (11.7) A	25.5 (3.9) A	22.1 (14.6) A
		After demineralization	48.5 (6.6) A	26.7 (1.9) A	19.1 (6.7) A
		After pH-cycling	43.4 (8.1) B	26.2 (3) A	25 (8.1) B
96hr/b	16	Baseline	51.8 (4.9) A	27.2 (2.3) A	15.4 (5.8) A
		After demineralization	53.3 (4.1) A	26.7 (1.2) A	14.2 (4.3) A
		After pH-cycling	44.5 (9.8) B	25.4 (3.5) A	25.6 (10.5) B

Uppercase letters indicate statistically significant differences between stages.

TABLE VII

Mean weight percent of nitrate, magnesium and fluorine at the
surface in 6 groups at different stages (SD in parentheses)

Groups	n	Stage	Nitrate	Magnesium	Fluorine
24hr/a	15	Baseline	3.8 (2.9) A	1.3 (0.5) A	0.7 (0.5) A
		After demineralization	4.4 (2.9) A	1.4 (0.6) A	1 (0.7) A
		After pH-cycling	3.9 (3.3) B	1.3 (0.5) B	1.2 (0.4) B
24hr/b	18	Baseline	3.7 (1.9) A	1.19 (0.5) A	0.7 (0.3) A
		After demineralization	3.9 (1.9) A	1.1 (0.5) A	0.6 (0.6) A
		After pH-cycling	2.8 (2.1) B	1 (0.4) B	0.6 (0.4) A
48hr/a	15	Baseline	4.5 (1.8) A	1.3 (0.4) A	0.8 (0.5) A
		After demineralization	4.8 (3.7) A	1.4 (0.7) A	1 (0.7) A
		After pH-cycling	2 (1.9) B	1 (0.3) B	1.4 (0.9) B
48hr/b	15	Baseline	4.8 (3.4) A	1.3 (0.2) A	0.6 (0.5) A
		After demineralization	4.2 (2.4) A	1.2 (0.6) A	0.8 (0.6) A
		After pH-cycling	3.2 (2.3) B	1.1 (0.4) B	0.4 (0.4) A
96hr/a	15	Baseline	4.2 (2.1) A	1.2 (0.3) A	0.8 (0.7) A
		After demineralization	3.8 (2.5) A	1.1 (0.5) A	0.8 (0.5) A
		After pH-cycling	3.2 (3.2) B	1 (0.7) B	1.3 (0.8) B
96hr/b	16	Baseline	3.8 (1.9) A	1.3 (0.4) A	0.6 (0.4) A
		After demineralization	3.9 (2.2) A	1.1 (0.4) A	0.7 (0.6) A
		After pH-cycling	3 (3.2) B	1 (0.4) B	0.5 (0.5) A

Uppercase letters indicate statistically significant differences between stages.

DISCUSSION

This *in-vitro* study evaluated the sound (baseline), demineralization, and remineralization characteristics of bovine enamel at three demineralization times and treated with either fluoride or deionized water as a negative control. The null hypothesis was partially rejected, since a correlation was found between the microhardness and fluorine content and between the susceptibility to de- and remineralization, but no correlation was found for any other minerals examined.

SURFACE MICROHARDNESS RESULTS

Microhardness tests provide information on the physical property of surface enamel in response to de- and remineralization protocols. Microhardness testing has been proven to be a valid method to measure alterations in dental hard tissue.[33,34] In this study, enamel microhardness decreased as demineralization time increased, although there was no significant difference in KHN between 48 hours and 96 hours of demineralization. Considering %SMHr, lesions that were demineralized longer showed less %SMHr irrespective of treatment received. This behavior is in accordance with other studies.[12,21,23]

Microhardness values increased following pH-cycling in both fluoride and control groups, with fluoride groups showing a greater increase. %SMHr results also demonstrated that fluoride was able to cause significantly greater enamel re-hardening than the control. The role of fluoride in reducing enamel demineralization and enhancing remineralization has been previously established.[11,17] However, even in the absence of fluoride, salivary pellicle has been shown to have a protective effect against demineralization of enamel.[35-37] Pearson correlation coefficients also show a significant positive, although weak, correlation between the weight% of fluorine at

the surface and the surface microhardness following pH-cycling. The presence of

fluoride has been shown to increase the rate of enamel rehardening *in vitro*.[11,38]

TRANSVERSE MICRORADIOGRAPHY RESULTS

The lesions showed a tendency for greater integrated mineral loss (ΔZ) and

lesion depth (L) with increased demineralization time. This strong relation has been

shown in several studies.[23,39,40] Following pH-cycling, specimens that received

fluoride had lower lesion depths and integrated mineral loss and greater %

remineralization than the control, which further emphasizes the role of fluoride in

enhancing remineralization.[17,24] In fact, % remineralization indicated that all groups

demonstrated remineralization following pH-cycling, irrespective of demineralization

time or treatment, and lesion depth and integrated mineral loss decreased following

pH-cycling, irrespective of treatment, except for specimens that were demineralized

for 24 hours and did not receive fluoride. This group conversely demonstrated no net

remineralization and instead further demineralized according to %R results, and

lesion depth and integrated mineral loss increased (non-significantly). The increase,

or rather, lack of significant decrease in L and ΔZ could be explained by the behavior

of smaller lesions during dissolution. Smaller lesions are thought to have greater

solubility than larger ones, or those that are demineralized for longer, and thus have a

greater tendency to demineralize further.[19,41] As specimens are placed in

demineralization solutions, the more soluble material in the lesion (i.e. magnesium

and carbon) is removed more readily than the less soluble material. Therefore, as

demineralization continues, a greater proportion of less soluble material remains,

thereby reducing the bulk solubility of the specimen.[3] Conversely, larger lesions have

a greater ability to remineralize. Possible reasons include their greater porosity

allowing more diffusion of remineralizing solutions, greater enamel area per unit

volume of remineralizing solution, or the fact that smaller lesions reach SZ_{max} faster than larger lesions, thereby allowing larger lesions greater time to remineralize.[18] This is confirmed in the present study by the %R, which shows that larger, more demineralized lesions exhibited more remineralization after pH-cycling.

Regarding the maximum mineral density of the surface zone (SZ_{max}), the ability of fluoride to form a highly mineralized surface layer in initially demineralized enamel and dentin specimens has been previously demonstrated.[41,42] The surface layer exhibits greater mineralization in the presence of fluoride than in its absence.[43] Nonetheless, this surface layer was present even in the absence of fluoride.[43] Salivary pellicle has been shown to have a protective effect on the surface of enamel,[36] and can prevent demineralization of the surface layer even in the absence of fluoride.[44] On the other hand, in the presence of fluoride, further dissolution is prevented as a result of re-precipitation of the dissolved minerals in the form of a fluoride-rich surface layer. This protective effect can be confirmed by the present findings.

The weight% of fluorine showed a significant positive correlation with the mineralization of the surface zone, which became stronger following pH-cycling in this current study. Additionally, a negative moderate correlation was found between the mineralization of the surface layer and both the lesion depth and integrated mineral loss of the specimens. This indicates that the presence of fluoride facilitated the incorporation of minerals into the lesion thereby decreasing susceptibility to further demineralization. This effect of fluoride in reducing enamel demineralization in a dose-dependent manner was previously proven by Argenta et al.[17] The existence of this relatively intact surface layer also functions to distinguish the subsurface caries lesions created in this study from the chemical etching of enamel.[40]

ENERGY DISPERSIVE X-RAY SPECTROSCOPY RESULTS

The results of the energy dispersive x-ray spectroscopy (EDS) show that the chemical composition of the surface enamel did not change significantly for any of the minerals examined following demineralization. Davidson et al. demonstrated that when bovine enamel was demineralized for up to 8 days at a pH of 5, the weight percent of calcium at the surface only changed a few percent in weight compared to that of the sound specimens.[45] Microradiographic analysis in this study confirmed mineral loss following demineralization and mineral gain following pH-cycling. This suggests that the minerals may have been lost and gained at a fixed ratio. This can be seen in the Ca:P ratio at the three stages; which remained 1.8 irrespective of demineralization time or treatment. Several studies have shown that the Ca:P ratio was stable at various mineralization stages, which indicates the stoichiometric dissolution and redeposition of minerals in bovine enamel.[11,46] Armstrong et al. similarly established that the composition of enamel did not differ between sound and caries teeth.[47] Sabel et al. found significantly lower amounts of calcium and phosphorus parallel to greater amounts of carbon and nitrogen in lesions compared with sound enamel.[13] However, this study was performed on primary human enamel, which is of greater porosity and has a higher tendency for dissolution.[48,49] Furthermore, the demineralization protocol used in this study was done using methylcellulose gel for 30 days.

Regarding the depth profiles of the chemical elements, the greater weight% of carbonate and nitrate detected at the surface has been seen in several studies. Glauche et al. found high concentrations of carbon and nitrogen at the surface of the specimens, which decreased steeply.[50] This was explained by the presence of a biofilm layer rich in organic material on the surface of the specimens. Kuboki et al.

studied the chemical composition of enamel in specimens either exposed to saliva or not. The results showed significantly higher concentrations of carbon and nitrogen at the surface of specimens that were covered in a layer of pellicle following exposure to saliva.[51] This study was able to demonstrate the selective adsorption of salivary proteins into enamel. The amino and carboxyl groups that make up gastric mucin can easily penetrate the micro-porosities on the enamel surface, which will lead to an increase in the concentration of carbonate and nitrate detected in the outer layers of enamel.

Considering the results of fluorine, the outcomes of this study demonstrate that a mineralized surface zone could be observed in both fluoridated and non-fluoridated specimens; however, EDS results show that significantly higher fluorine levels were detected in the fluoridated groups. Ten Cate et al. has also demonstrated that microradiograms of fluoridated and non-fluoridated samples were able to show a distinct surface zone.[20] Nonetheless, fluoride seems to be the predominant factor influencing remineralization.[43,52] The depth profile also exhibits that the greatest amount of fluorine is deposited in the surface layer and gradually decreases up to a depth of approximately 20 um. This is in agreement with Petersson, who showed that fluoride uptake in enamel by topical fluoride application is limited to the first 10 um[53] and decreases significantly in deeper layers up to about 40 um.[54]

CORRELATION RESULTS

The strong positive correlation between lesion depth and integrated mineral loss observed in this study is in accordance with several other studies.[19,23] Regarding the correlations between Knoop hardness values at various stages, it can be seen that specimens with higher surface microhardness at baseline and following demineralization also had higher surface microhardness after demineralization and

pH-cycling, respectively, although these correlations were weak. If surface microhardness (SMH) is considered a measurement of the presence, mineralization, or thickness of the surface layer, as well as a measurement of the subsurface demineralization, then these results may indicate that when a mineralized surface layer was present, the specimens maintained their structural integrity throughout de- and remineralization challenges. However, due to the weak correlation and relatively large amount of scattering, this data should not be over-interpreted. Comparably, Lippert et al. did not find significant correlations between the indentation length of sound specimens and the change in indentation length after demineralization of the specimens for up to 48 hours, using either Knoop or Vickers indenters.[23]

In this study, the surface hardness correlated weakly to moderately with the mineral loss and lesion depth determined by transverse microradiography. Previous studies have either shown similar[5,24] or conflicting[21,23,55] results. One possible explanation for the difference in results could be the protocol used for demineralization. Lippert et al. showed weaker correlations for carbopol lesions compared to the other demineralization protocols.[24] Additionally, deeper lesions with greater subsurface mineral loss, such as those produced with carbopol in comparison to MeC or HeC lesions, show weaker relations between hardness and TMR data.[5,24] Furthermore, Arends et al. demonstrated that the linearity between indentation length and lesion depth is strongly load dependent; and as such is much weaker for 50-gram than 500-gram loads.[55] Interestingly, a significant correlation between hardness and surface zone mineralization could only be found after pH-cycling, which stresses the role of fluoride in creating a highly mineralized surface layer which has rehardened as a result of remineralization.[56]

In the future, focus should be on studying the physical and chemical structure of natural white spot lesions. The similarity between human and bovine enamel does not eliminate the fact that bovine enamel is more porous and has higher carbon content that human enamel. Furthermore, lesions produced by different systems and with distinctive mineral distributions may influence the de- and remineralization characteristics.

SUMMARY AND CONCLUSIONS

This *in vitro* model aimed at evaluating the physical and chemical characteristics of sound, demineralized and remineralized enamel by creating subsurface caries lesions in bovine specimens and subjecting them to a well-established pH-cycling regimen. The specimens were studied using Knoop surface microhardness (SMH), energy dispersive x-ray spectroscopy (EDS) and transvers microradiography (TMR), and the results were analyzed and compared based on demineralization time (24, 48 or 96 hours) and treatment (fluoride or deionized water).

It can be concluded that increased demineralization time led to a decrease in SMH values and an increase in lesion depth and integrated mineral loss. Overall, SMH values were able to show that harder specimens at the sound stage (baseline) and after demineralization remained hard after demineralization and pH-cycling, respectively, although this correlation was weak. Furthermore, there was no correlation between SMH values at baseline and after pH-cycling. Additionally, results from SMH show that harder lesions were less susceptible to demineralization, as they showed less lesion depth and integrated mineral loss measured by transverse microradiography. Additionally, harder lesions showed greater surface zone mineralization.

The minerals examined in this study demonstrated a stoichiometric dissolution and redeposition behavior, which can be seen by the stable Ca:P ratio at various mineralization stages. Regarding fluorine, its increase correlated well with the

increase in both SMH and surface zone mineralization. The increase in surface zone mineralization, in turn, made lesions less susceptible to demineralization, as they showed less lesion depth and integrated mineral loss measured by transverse microradiography. The deposition of fluorine was limited to the outer 20 um of enamel, with the greatest amount being at the surface.

Following pH-cycling, fluoride and, to a lesser extent, non-fluoride groups were able to remineralize. In the non-fluoride or control groups, shallower lesions had a greater tendency to further demineralize, while deeper lesions remineralized. The artificial saliva used in this study played a role in remineralization of the enamel in the deeper lesions, i.e. those that were demineralized longer, as evident by the increase in SMH, decrease in lesion depth, gain in mineral volume%, and the formation of a mineralized surface zone.

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ABSTRACT

THE INFLUENCE OF BASELINE HARDNESS AND CHEMICAL

COMPOSITION ON ENAMEL DEMINERALIZATION AND

SUBSEQUENT REMINERALIZATION

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BACKGROUND

Several studies have reported that harder enamel with higher contents of calcium (Ca), phosphorus (P) and fluorine (F) coupled with lower contents of carbonate (C), magnesium (Mg) and nitrate (N) was found to be more resistant to demineralization. Additionally, the hardness of dental enamel was found to have a strong correlation with its chemical content. However, yet to be established is the relation between the physical and chemical structure of enamel and its response to de- and remineralizing conditions.

OBJECTIVES

The aims of this laboratory study were: 1) To investigate the hardness and chemical content of sound enamel and their influence on demineralization; 2) To investigate these properties in demineralized enamel and their influence on remineralization; and 3) To investigate these properties in sound enamel and their influence on remineralization.

MATERIALS AND METHODS

Incipient subsurface caries lesions were created in 94 bovine enamel specimens using Carbopol C907 using three demineralization times. The specimens were then pH-cycled and treated using either 367 ppm F sodium fluoride or a placebo. Knoop surface microhardness (SMH), Energy dispersive X-ray spectroscopy (EDS) and Transverse microradiography (TMR) were performed on the specimens at all stages and compared between them. TMR variables included integrated mineral loss (ΔZ), Lesion depth (L) and maximum mineral density of the surface zone (SZ_{max}). Data were analyzed using three- and four-way ANOVA and Pearson correlation coefficients were calculated.

RESULTS

SMH, ΔZ, L and SZ_{max} were significantly different among stages, demineralization times and treatment. The weight% of F at the surface was significantly affected by treatment, irrespective of demineralization time. A statistically significant moderate correlation was found between SZ_{max} and ΔZ and SZ_{max} and L after pH cycling. SMH also correlated weakly to moderately with TMR data.

CONCLUSIONS

SMH and SZ_{max} decreased while ΔZ and L increased with increased demineralization time. Both fluoride and non-fluoride specimens were able to remineralize, which emphasizes the role of saliva in mineralization. The Ca:P ratio remained stable at various stages, indicating the stoichiometric dissolution and redeposition of minerals. The greatest deposition of F was at the surface and its increase led to an increase in SMH and SZ_{max}. SMH values showed that harder specimens at baseline and after demineralization remained hard after demineralization and pH-cycling, respectively, although this correlation was weak. Additionally, harder lesions showed less L and ΔZ and greater SZ_{max}.

RELEVANCE

This *in-vitro* study will help better understand the caries process and the impact of physical and chemical characteristics of enamel on de- and remineralization challenges.

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