EFFECT OF BIOTENE® ORALBALANCE MOISTURIZING LIQUID AND MOUTHKOTE® ORAL MOISTURIZER SPRAY ON HUMAN ENAMEL MEASURED BY QUANTITATIVE LIGHT-INDUCED FLUORESCENCE METHOD

by

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ABSTRACT

Objectives: The purpose of this randomized controlled experimental study was to examine how the xerostomia products Biotene® Oralbalance moisturizing liquid and MouthKote® oral moisturizer spray influence mineral content of human enamel in vitro.

Methods: 104 caries free extracted human teeth were selected and prepared, followed by baseline QLF imaging and exposure to an erosive solution of lactic acid (pH of 4.5) and/or Biotene®, MouthKote®, as well as Oral-B fluoridated rinse. Mineral loss was determined with respect to mean fluorescence loss (ΔF, %), maximum fluorescence loss (ΔQ, %), and lesion area (WS, %/mm²). Within-group and among-group comparisons were made employing independent sample t-tests, paired sample t-tests, and ANOVA for multiple comparisons with Bonferroni post hoc adjustment, or their non-parametric equivalents. For all tests, the threshold for the statistical significance was set at P < 0.05. The statistical software SPSS 17.0 was used for data analyses.

Results: The xerostomia products induced significant demineralization in extracted human teeth with prior demineralization (P=0.000) and without previous pre-demineralization (P = 0.000). There were substantial and statistically significant differences in mineral loss among all groups. The amount of demineralization (Mean ± SD) was higher in MouthKote® group 2 (-27.19 ± 6.70) and group 4 (-11.45 ± 2.94) than in Biotene group 1 (-11.15 ± 3.05) and group 3 (-7.38 ± 0.44) respectively. Oral-B fluoridated rinse aided in re-mineralization, albeit not to baseline levels.

Conclusions: Biotene® and MouthKote® induced substantial mineral loss in pre-demineralized and unaltered enamel of extracted human teeth. MouthKote® induced
greater demineralization than Biotene®. Oral-B rinse induced re-mineralization in all experimental groups except for group 2 (lactic acid/MouthKote®) where further dissolution of enamel was observed.
TABLE OF CONTENTS

ABSTRACT ....................................................................................................................... ii
TABLE OF CONTENTS ........................................................................................................ iv
LIST OF TABLES .............................................................................................................. vi
LIST OF FIGURES ............................................................................................................ viii
ACKNOWLEDGEMENTS .................................................................................................. x
1. INTRODUCTION ....................................................................................................... 1
   1.1. Saliva substitutes ............................................................................................... 2
   1.2. Quantitative light-induced fluorescence (QLF) method ....................................... 5
       1.2.1. Light interaction with hard dental tissues .................................................... 7
       1.2.2. Validation of the QLF method .................................................................. 10
       1.2.3. Applications of QLF .................................................................................. 11
       1.2.4. Limitations of QLF .................................................................................... 14
2. RATIONALE, AIM, AND HYPOTHESIS .................................................................. 16
   2.1. Rationale............................................................................................................ 16
   2.2. Aim .................................................................................................................... 16
   2.3. Hypothesis ........................................................................................................ 16
3. MATERIALS AND METHODS ................................................................................ 17
   3.1. Study design ..................................................................................................... 17
   3.2. Standardization of QLF measurements ............................................................. 19
   3.3. Specimen selection and preparation ................................................................... 20
   3.4. Test groups ....................................................................................................... 21
   3.5. Experimental protocol ....................................................................................... 21
       3.5.1. Immersion phase one .............................................................................. 21
       3.5.2. Demineralization protocol ...................................................................... 22
       3.5.3. Immersion phase two ............................................................................... 22
       3.5.4. Immersion phase three ............................................................................ 23
   3.6. Statistical analysis ............................................................................................ 24
4. RESULT ..................................................................................................................... 25
   4.1. Within-group comparisons ................................................................................ 31
   4.2. Longitudinal changes in the experimental group (EG1) during different phases of the experiment .................................................................................. 32
   4.3. Across-group comparisons ............................................................................... 36
5. DISCUSSION .............................................................................................................. 40
   5.1. Use of control groups ........................................................................................ 41
   5.2. Standardization of study conditions ................................................................... 41
   5.3. Sample selection ................................................................................................ 42
   5.4. Inclusion criteria for extracted teeth ................................................................... 42
   5.5. Pros of the internal validity ................................................................................ 43
   5.6. Cons for the internal validity ............................................................................. 44
   5.7. Pros for the external validity ............................................................................. 44
LIST OF TABLES

Table 1. Classification of xerostomia products available in Canada based on the main ingredients3 .................................................................................................................. 3
Table 2. Comparison of Biotene® moisturizing liquid and MouthKote® oral moisturizer spray3 ................................................................................................................. 4
Table 3. Studies assessing the validity and reliability of the Quantitative Light Fluorescence (QLF) device ......................................................................................... 11
Table 4. Effect of distilled water on mineral content of extracted human teeth in control group1 .................................................................................................................. 26
Table 5. Among-group comparisons - baseline comparisons among control and experimental groups .................................................................................................. 27
Table 6. Mineral changes in control group 2, experimental group 1, and experimental group 2 after immersion into lactic acid during immersion phase 1 ................................................................. 28
Table 7. Effect of xerostomia products on mineral content in control group 1, and experimental groups 1, 2, 3, and 4 during immersion phase 2 ............................................................ 29
Table 8. Effect of fluoridated Oral-B rinse on mineral content in control group 2 and experimental groups 1, 2, 3, and 4 during immersion phase 3 ................................................................ 30
Table 9. Mineral changes in the control group at immersion phases 1, 2, and 3 (∆F outcome) .................................................................................................................... 31
Table 10. Mineral changes in experimental group 1 (EG1) at different immersion phases (∆F outcome) ........................................................................................................... 32
Table 11. Mineral changes in experimental group 1 (EG1) at different immersion phases (∆Q outcome) ........................................................................................................... 32
Table 12. Mineral changes in experimental group 1 (EG1) at different immersion phases (WS outcome) ................................................................................................. 32
Table 13. Mineral changes in experimental group 2 (EG2) at different immersion phases (∆F outcome) ........................................................................................................... 33
Table 14. Mineral changes in experimental group 2 (EG2) at different immersion phases (∆Q outcome) ........................................................................................................... 33
Table 15. Mineral changes in experimental group 2 (EG2) at different immersion phases (WS outcome) ................................................................................................. 34
Table 16. Mineral changes in experimental group 3 (EG3 without demineralization) at different immersion phases (∆F outcome) ............................................................... 34
Table 17. Mineral changes in experimental group 3 (EG3 without demineralization) at different immersion phases (∆Q outcome) ............................................................... 34
Table 18. Mineral changes in experimental group 3 (EG3 without demineralization) at different immersion phases (WS outcome) ............................................................... 35
Table 19. Mineral changes in experimental group 4 (EG4 without demineralization) at different immersion phases (ΔF outcome) ................................... 35

Table 20. Mineral changes in experimental group 4 (EG4 without demineralization) at different immersion phases (ΔQ outcome) ........................ 35

Table 21. Mineral changes in experimental group 4 (EG4 without demineralization) at different immersion phases (WS outcome) ........................... 36

Table 22. Mineral changes at immersion phase 2 between CG1, non-demineralized groups EG3 and EG4 (ΔF, ΔQ, and WS outcomes) ...................... 36

Table 23. Comparisons between non-demineralized groups EG3 and EG4 at immersion phase 2 (ΔF, ΔQ, and WS outcomes) ..................................................... 37

Table 24. Mineral changes at immersion phase 2 in CG2, and demineralized groups EG1 and EG2 (ΔF, ΔQ, and WS outcomes) ........................................ 38

Table 25. Comparisons between EG1 and EG2 at immersion phase 2 (ΔF, ΔQ, and WS outcomes) .................................................................................................. 38
LIST OF FIGURES

Figure 1. Color-coding of the QLF denoting mineral loss (loss of fluorescence)\textsuperscript{23} .......... 6
Figure 2. Light interaction with hard dental tissues denoting photon reflection (a), scattering (b), transmission (c), absorption (d) and fluorescence (d)\textsuperscript{30, 31} ................ 7
Figure 3. Protein chromophore activation resulting in autofluorescence of the dental tissues ........................................................................................................... 9
Figure 4. Study profile diagram depicting each phase of the experiment ......................... 18
Figure 5. Specimens at preparation phase (polished, with 4 x 4 mm window on the buccal surface, attached to PVC square, and coded) prior to baseline QLF analysis ................................................................. 20
Figure 6. Development of erosion in control group 2 with the help of lactic acid (0.1M lactic acid adjusted to pH of 4.5) ........................................................... 22
Figure 7. Immersion phase two. Experimental group 1 was immersed in Biotene and experimental group 2 was immersed in MouthKote)................................. 23
Figure 8. Longitudinal monitoring of a specimen (1 – baseline, 2 – exposure to lactic acid, 3 – exposure to MouthKote, 4 – exposure to fluoridated mouth rinse) ...................................................................................................................... 39
Figure A1a. Comparison of $\Delta F$ change in the control group at different phases of experiment ............................................................................................................. 58
Figure A1b. Comparison of $\Delta Q$ change in the control group at different phases of experiment ............................................................................................................. 59
Figure A1c. Comparison of WS change in the control group at different phases of experiment ............................................................................................................. 60
Figure A2a. Longitudinal changes in the experimental group (EG1) at $\Delta F$ level ............ 61
Figure A2b. Longitudinal changes in the experimental group (EG1) at $\Delta Q$ level ........... 62
Figure A2c. Longitudinal changes in the experimental group 1 (EG1) at WS level ......... 63
Figure A3a. Longitudinal changes in experimental group 2 (EG2) at $\Delta F$ level ............. 64
Figure A3b. Longitudinal changes in experimental group (EG2) at $\Delta Q$ level ............. 65
Figure A3c. Longitudinal changes in experimental group 2 (EG2) at WS level ............. 66
Figure A4a. Longitudinal changes of mineral content in the experimental group 3 (EG3) at $\Delta F$ level .................................................................................................... 67
Figure A4b. Longitudinal changes of mineral content in the experimental group 3 (EG3) at $\Delta Q$ level .................................................................................................... 68
Figure A4c. Longitudinal changes of mineral content in the experimental group 3 (EG3) at WS level .................................................................................................... 69
Figure A5a. Longitudinal changes in experimental group 4 (EG4) at $\Delta F$ level ............ 70
Figure A5b. Longitudinal changes in experimental group 4 (EG4) at $\Delta Q$ level ............ 71
Figure A5c. Longitudinal changes in experimental group 4 (EG4) at WS level ............. 72
Figure B1a. Comparisons among groups (EG3 and EG4) without
demineralization at $\Delta F$ level........................................................................73

Figure B1b. Comparisons among groups (EG3 and EG4) without
demineralization at $\Delta Q$ level........................................................................74

Figure B1c. Comparisons among groups (EG3 and EG4) without
demineralization at WS level...............................................................................75

Figure B2a. Comparisons among groups (EG1 and EG2) with demineralization
at $\Delta F$ level ........................................................................................................76

Figure B2b. Comparisons among groups (EG1 and EG2) with demineralization
at $\Delta Q$ level........................................................................................................77

Figure B2c. Comparisons among groups (EG1 and EG2) with demineralization
at WS level...............................................................................................................78
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1. **INTRODUCTION**

Xerostomia is an abnormal dryness of the mouth resulting from decreased saliva secretion and/or complete loss of salivary flow\(^1\). Individuals with dry mouth are at a greater risk of developing caries and mucosal lesions, as well as having problems with mastication, swallowing, taste perception, speaking, and halitosis. Numerous conditions are implicated in the cause of dry mouth, including salivary gland destruction as a result of head and neck irradiation treatment, uncontrolled diabetes mellitus, Sjögren’s syndrome, renal failure, HIV-associated salivary gland disease, and xerostomia-inducing medications\(^1\). If left unattended, dry mouth can induce rapid deterioration in oral health.

The treatment of xerostomia is mostly palliative and is done with the use of oral moisturizers and lubricants\(^2\). There are several non-prescription products designed to palliate dry mouth, and these vary greatly in composition and active ingredients, delivery form, effectiveness, availability, and price. The purpose of saliva substitutes and stimulants is to maintain a healthy oral environment and improve “lubricity” of oral tissues by direct lubrication (in individuals with complete loss of saliva), stimulation of residual salivary flow (partial loss of saliva), or a combination of both. Unfortunately, many of these products have little clinical evidence to support their use in xerostomic individuals\(^3\). Previous studies of xerostomia products evaluated the subjective effects of the dry mouth remedies, assessing their effectiveness in xerostomic individuals by using questionnaire surveys. Although questionnaire surveys have many advantages, such as application in both small and large populations, cost-effectiveness and time efficiency, they also have some major disadvantages including problems with reliability, validity, and misinterpretation of questions\(^4\). It is difficult for patients to self-quantify xerostomia, as it is a subjective sensation, and may be perceived differently from one patient to
another. Based on the review of studies\textsuperscript{3} on the palliation of xerostomia conducted between 1960 and 2006, several inferences can be made:

- Controversial results of the re- and demineralizing effects of xerostomia products available in Canada were reported previously\textsuperscript{3}.
- The majority of over the counter dry mouth products lack adequate information on their packaging, such as pH, side effects, and recommendations for use in dentate and edentulous patients.
- Inappropriate labeling of xerostomia remedies could lead to poor choices in selection of an appropriate product, and potentially cause irreversible damage if used indiscriminately\textsuperscript{3}.
- There is a need for more rigorously designed clinical studies and new diagnostic tools enabling exploration into new avenues to manage xerostomia.

1.1. Saliva substitutes

Xerostomia remedies are designed to lubricate, stimulate salivary flow, or both depending on the etiology and severity of condition. Xerostomia products can be classified into four groups based on the active ingredients: biopolymer based including plant mucilage, xantham gum and/or animal mucin; salivary enzyme based; acid based; and petroleum based\textsuperscript{3}. 
Table 1. Classification of xerostomia products available in Canada based on the main ingredients

<table>
<thead>
<tr>
<th>Biopolymer based (carboxymethylcellulose, hydroxyethylcellulose) abbr.: CMC, HEC</th>
<th>Salivary enzyme based (lactoperoxidase, lyzozyme, glucose oxidase)</th>
<th>Acid based (malic, citric, ascorbic acids)</th>
<th>Petroleum based gum (petroleum derivative)</th>
</tr>
</thead>
</table>
| → **Plant mucilage:**  
- Moi-stir (CMC)  
- MouthKote (Yerba santa)  
- Biotene Oralbalance products (HEC)  
- Aloe vera gel (Aloe barbadensis)  
- Salinum (Linum usitatissimum)*  
- Glandosane (CMC)*  
- Oralube (CMC)*  
- Salivart (CMC)* | Biotene Oralbalance products:  
- Moisturizing gel  
- Moisturizing liquid  
- Antibacterial toothpaste  
- Chewing gum  
- Mouthwash | Salivix*  
Salivin*  
Thayers  
Saliva Sure | Trident chewing gum  
Extra chewing gum  
Biotene chewing gum |
| → **Animal mucin:**  
- Saliva Orthana (bovine mucin)*  
- Saliva Medac (porcine mucin)* | | | |
| → **Xantham gum:**  
- Xialine (Xanthomonas campestris byproduct) * | | | |

* denotes products currently unavailable in Canada, June (2010)

Two of the most readily available xerostomia products in Canada are Biotene® (Laclede International) and MouthKote® (Parnell Pharmaceuticals). A number of studies demonstrated their effectiveness in alleviating dry mouth symptoms, producing long-lasting effects, and showing good acceptance by the patients. These studies have not reported adverse effects from the use of MouthKote® and Biotene®. However, several in vitro studies reported potential demineralizing effect from the use of dry mouth products.

Biotene® Oralbalance moisturizing liquid is a clear, odorless, mild-tasting viscous preparation, and is based on hydroxyethylcellulose (HEC) which is a hygroscopic, odorless, water-soluble powder derived from the plant mucilage. The product also
includes salivary enzymes that are patented. Biotene® Oralbalance moisturizing liquid is available in 1.5 oz (45 ml) squeezable bottles\textsuperscript{18}.

Table 2. Comparison of Biotene® moisturizing liquid and MouthKote® oral moisturizer spray\textsuperscript{3}

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Manufacturer’s recommendations</th>
<th>Reported side effects</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotene® Oralbalance moisturizing liquid; 1.5 oz (45 ml)</td>
<td>Use whenever necessary to relieve dryness. When using the Biotene Moisturizing liquid, squirt directly into the mouth as often as moisture is needed. It is safe to swallow excess liquid to assist in throat lubrication. Repeat several times daily as needed.</td>
<td>n/a</td>
<td>For dentate/edentulous individuals Complete or partial loss of saliva During the day and at night</td>
</tr>
</tbody>
</table>

| MouthKote® oral moisturizer spray bottle (60 ml, 240 ml); or packets – 5ml, 50 packets/box | Spray into mouth to relieve dry mouth discomfort as needed; may be swallowed or expectorated; shake well before using. Swirl thoroughly in the mouth the content of the packet for 10 seconds, gargle and spit out. Repeat as necessary. | n/a | Edentulous individuals Complete or partial loss of saliva Daytime |

MouthKote® oral moisturizer spray is a yellow, lemon scented, and flavored liquid, and is based on yerba santa (a vegetable cacti) plant extract, as well as ascorbic and citric acids for stimulation of the salivary flow. It is available in 60 ml and 240 ml spray bottles, and individual 5 ml packets (50 packets/box)\textsuperscript{18}. A comparison is provided in Table 2. Biotene® and MouthKote® are most readily available xerostomia products in Canada, and were reported to be effective in alleviating dry mouth symptoms, produce a long-lasting effect, and showed acceptance by the individuals with dry mouth\textsuperscript{3}.

Demineralization of enamel in vitro caused by xerostomia products has been also previously reported\textsuperscript{14-17, 19-22}.

A need exists for more rigorously designed controlled clinical studies, and new diagnostic techniques to enable exploration into new avenues of xerostomia.
management. With the emergence of QLF technology, which enables quantitative longitudinal monitoring of mineral changes in the hard dental tissues \textit{in vitro}, \textit{in situ}, and \textit{in vivo}, it would be beneficial to determine what effects Biotene® Oralbalance dry mouth moisturizer and MouthKote® oral moisturizer may have on the mineral content of human dentition. Such information will be useful to dental practitioners and individuals with dry mouth.

There is no definitive cure for dry mouth, and the management of the condition remains palliative through the use of dry mouth products. Such products are designed to ameliorate the dry mouth condition and promote oral health. Some scientific evidence suggests that the use of such products can cause irreversible damage to human and bovine dentition \textit{in vitro}. At the same time, there is little evidence to support the beneficial effect of dry mouth products on oral homeostasis in individuals with xerostomia. With the help of QLF technology, the erosive potential of MouthKote® and Biotene® two xerostomia products on human enamel in vitro may be investigated, with the prospect of further \textit{in vivo} study in the future.

1.2. Quantitative light-induced fluorescence (QLF) method

In order to estimate the effect of dry mouth products on hard dental tissues, it was important to utilize a precise quantitative longitudinal measurement of mineral changes in human enamel.

Quantitative light-induced fluorescence is a novel technology for early caries detection, and has been tested for quantitative longitudinal monitoring of mineral changes in the hard dental tissues \textit{in vitro}, \textit{in situ}, and \textit{in vivo}, and has been recommended as an adjunct dental diagnostic method in clinical examination\textsuperscript{32-39}. QLF uses the natural fluorescence of hard dental tissues to discriminate between compromised and sound
structures and to quantify the demineralization of the lesion. The QLF method is based on the principle that compromised dental tissue will fluoresce less due to impaired density, thereby producing a darker fluorescent image than that of sound mineral tissue$^{23}$. The parameters measured with this method include mean fluorescence loss ($\%$), maximum fluorescence loss in the lesion ($\%$), and extent of the lesion area ($\%/mm^2$)$^{23}$. The QLF equipment is comprised of a 35W arc lamp with a violet-blue band-pass filter, a handpiece with a charge coupled device (CCD) based digital camera, a PC with display monitor, framegrabber, and Inspektor™ Pro software$^{23}$. When the violet-blue light illuminates the tooth surface, a digital fluorescent image is captured by the camera, and then transferred to the computer and displayed on the monitor in real-time$^{23}$. After manually outlining the contour of the lesion on the display, the QLF software calculates the mean and the maximum grey level value of each pixel within the lesion. By subtracting the grey level value within the lesion from grey level values in the surrounding tissue, the difference in fluorescence is obtained. Loss in fluorescence is graphically presented on the computer display according to a color-code: blue, purple, red and yellow identifying minimal, low, moderate, and higher fluorescence loss respectively as shown in Figure 1. The size of the lesion is calculated based on the number of pixels within the lesion.

![Figure 1. Color-coding of the QLF denoting mineral loss (loss of fluorescence)$^{23}$](image)

Low mineral loss $\rightarrow$ High mineral loss
1.2.1. Light interaction with hard dental tissues

In order to understand how QLF works, it is important to understand how light interacts with hard dental tissues. Dental enamel is the hardest and most mineralized human tissue. Its mechanical, physical and chemical properties are a function of its mineral composition and structure. Calcium hydroxyapatite crystals are the basic constituents of enamel, and are arranged in densely packed prismatic structures, perpendicular to the surface, providing the tooth with considerable mechanical properties. A number of resources are available that deal specifically with tooth enamel formation, and the reader is referred to these for a comprehensive discussion of the subject. The small amounts of organic matter embedded in the interprismatic spaces, such as structural proteins, lipids, and carbohydrates, can play an important role in tissue plasticity and autofluorescence. Light can interact with hard dental tissues in a variety of ways. It can be reflected, scattered, transmitted or absorbed. The interaction of light energy with the target tissue is mainly determined by the specific wavelength of the light, and the optical properties of the target tissue.

Figure 2. Light interaction with hard dental tissues denoting photon reflection (a), scattering (b), transmission (c), absorption (d) and fluorescence (d).

Figure 2 illustrates light interaction with hard dental tissues as follows:

a) Photon reflection by the material;
b) photon scattering within the medium;

c) photon transmission through the material;

d) photon absorption and conversion into heat, and;

e) photon absorption and fluorescence.

These types of interactions can happen alone or in combination\textsuperscript{25,26}. Fluorescence of hard dental tissues occurs due to light interaction with the tissue, resulting in electromagnetic radiation. During photon absorption, electrons are moved from a lower to higher state, followed by the return to the initial state resulting in energy emission in the form of light, or fluorescence. The precise nature of dental fluorescence remains unclear\textsuperscript{27}. The majority of the fluorescence can be attributed to the organic components and protein chromophores within the tooth structure, as well as the hydroxyhyapatite structure\textsuperscript{27}. The protein chromophore is a part of a visibly colored protein molecule responsible for light absorption over a wide range of wavelengths, thus giving rise to color\textsuperscript{27,28}. This ability of the protein chromophores to produce color should not be confused with the color-coding of the QLF denoting mineral loss. It had been suggested that differences in fluorescence of hard dental tissues are due to organic and inorganic complexes\textsuperscript{27-29}. Where the tissue is sound, there may be a higher probability that emitted photons will hit a chromophore due to a longer path length of travel, resulting in less absorption of the light and thus producing a pronounced fluorescence\textsuperscript{27-29}. In contrast, the demineralized hard dental tissue results in a shorter path length, and consequently a loss of autofluorescence is observed. A possible explanation for this phenomenon was proposed by Angmar-Mansson and ten Bosch (2001)\textsuperscript{30}; with the suggestion of several contributing factors:

1) shorter path length in compromised tissue and hence less light absorption and weaker fluorescence;
2) scattering of the light within the lesion preventing the excitation light from reaching the underlying fluorescent dentine, thus preventing fluorescent light from the dentine from reaching the surface;

3) fluorescence is disturbed by a change in the molecular environment of the protein chromophores, and;

4) protein chromophores are destroyed by the caries process\textsuperscript{30,31}.

Quantitative light-induced fluorescence is a clinically tested, nondestructive, longitudinal tool for use \textit{in vitro}, \textit{in situ}, and \textit{in vivo} which will be employed in this study for the purpose of detection on mineral changes in extracted human teeth immersed xerostomia products.
1.2.2. Validation of the QLF method

The quantitative light-induced fluorescence method was developed and introduced into dentistry over two decades ago. It has been validated and applied *in vitro*, *in situ*, and *in vivo*\(^3\). Table 1 presents a summary of studies validating the use of the QLF method. QLF technology has been effectively tested on enamel, dentin and root surfaces for detection of smooth surface lesions. It was also validated against transverse microradiography (TMR) with regard to the quantification of enamel erosion *in vitro*\(^4\) and longitudinal microradiography for incipient caries lesions\(^3\). A multi-site laboratory study by ten Cate et al. 2000\(^4\) evaluated the ability of QLF to detect the lesion area in exfoliated deciduous teeth. Teeth were sectioned and examined by transverse microradiography, histology and polarized light microscopy. It was concluded that under certain conditions, QLF analysis demonstrated a sensitivity of 79% and a specificity of 75% for bucco-lingual specimens. A study of *in vivo* detection of smooth surface caries lesions by Al-Khateeb *et al.*\(^3\) showed an inter-observer reproducibility of 0.95-0.99. Another *in vivo* study on the reliability and reproducibility of the QLF method demonstrated that QLF is a good tool with respect to identification of the variables of lesion area, and average and maximum changes in lesion fluorescence. Analysis of inter-examiner reliability (agreement) demonstrated an intra-class correlation coefficient of 0.95 - 0.98, and for the analytical stage the inter-examiner agreement showed 0.93 - 0.99\(^3\). QLF was also concluded to be a sensitive method, suitable for longitudinal quantification of incipient caries lesions on smooth surfaces in children, as well as orthodontic patients (Tranæus S, 2001)\(^4\), and also appropriate for *in vivo* monitoring of mineral changes in incipient caries lesions\(^4\).
Evidence suggests that the correlation between QLF and absolute mineral loss (as measured by radiography) may be as high as $r = 0.92^{44}$.

In general, QLF presented as a sensitive, specific, reproducible method with good inter-examiner reliability in the quantification of enamel lesions.

**Table 3. Studies assessing the validity and reliability of the Quantitative Light Fluorescence (QLF) device**

<table>
<thead>
<tr>
<th>Study assessments</th>
<th>Authors</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth surface caries (in vivo)</td>
<td>Al-Khateeb S. et al., 1998 (37)</td>
<td>Inter-observer reproducibility (0.95 – 0.99)</td>
</tr>
<tr>
<td></td>
<td>Shi X-Q et al., 2001 (45)</td>
<td>Sensitivity 0.76, specificity 0.92</td>
</tr>
<tr>
<td>Buccal surfaces vs. transverse microradiography (TMR), histology and polarized light microscopy</td>
<td>Ten cate JM. et al., 2000 (41)</td>
<td>Under clinical conditions QLF showed sensitivity 79% and specificity 75% for buccal-lingual specimens</td>
</tr>
<tr>
<td>Repeatability and reproducibility</td>
<td>Tranæus S. et al., 2001 (38)</td>
<td>Excellent in vivo repeatability and reproducibility</td>
</tr>
<tr>
<td>Evaluation of caries prevention</td>
<td>Tranæus S. et al., 2001 (39)</td>
<td>Sensitive method useful for clinical trials</td>
</tr>
<tr>
<td>Monitoring of mineral changes (in vivo)</td>
<td>Ferreira Zandon A. et al., 2000 (43)</td>
<td>Appropriate for in vivo monitoring of mineral changes in incipient carious lesions in orthodontic patients</td>
</tr>
<tr>
<td>Root caries</td>
<td>Pretty I. et al., 2003 (46)</td>
<td>Correlation with gold standard 0.89</td>
</tr>
<tr>
<td>Secondary caries</td>
<td>Benedict SL et al., 1996 (47)</td>
<td>Sensitivity 0.95, specificity 0.85</td>
</tr>
<tr>
<td>Enamel erosion</td>
<td>Elton V et al., 2009 (48)</td>
<td>Appropriate for measuring subsurface mineral loss, non-destructive</td>
</tr>
<tr>
<td>QLF vs. profilometry* and TMR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Profilometry: The use of a laser or white-light, computer-generated projections to perform surface measurements of three dimensional objects*.

1.2.3. Applications of QLF

The QLF method provides a fluorescent image of a smooth surface demineralized lesion, quantifying the lesion with respect to relative mineral loss and size of the lesion.

This method is commonly used for quantitative longitudinal monitoring of mineral changes in the visually accessible hard tooth structures (smooth surface lesions) *in vitro*,
in situ, and in vivo. Numerous studies have investigated the use of QLF in the following areas:

- Assessing erosive potential of commercially available mouth rinses on enamel,
- Monitoring and management of incipient and hidden dental caries,
- Quantitative assessment of stain,
- Detection of demineralization around orthodontic brackets,
- Detection of demineralization adjacent to the existent restorations,
- Quantification of dental plaque,
- Discrimination between dental composite resin and tooth structure,
- Diagnosis of smooth and occlusal caries.

A study by Adeyemi A et al. compared QLF method with digital imaging in relation to the detection and quantification of the development and removal of stains on teeth in vitro. For both staining and stain removal there was a statistically significant (p < 0.01) reverse correlation between QLF values and digital imaging values. QLF demonstrated a high correlation with digital imaging as a technique for detecting and monitoring tooth stains and tooth whitening in vitro.

A randomized controlled longitudinal study by Trancœus S et al. evaluated the effects of fluoride varnish and professional tooth cleaning on remineralization of white spot lesions (n = 21; n = 62 white spot lesions) using the QLF method. Group I underwent cleaning and fluoride treatment, and demonstrated a significant decrease in lesion area and increased fluorescence radiance over a period of six months, suggesting that the repeated application of fluoride increases remineralization on white spot lesions. Group II was subjected only to cleaning and did not show any changes in white spot lesions over the period of six months. This study demonstrated the beneficial effect of fluoride on white spot lesion remineralization, and also demonstrated QLF as being a sensitive and suitable method for longitudinal monitoring of incipient caries lesions on smooth
tooth surfaces. Although the study states a sample size of \( n = 21 \) (human subjects), the statistical data analysis was carried out on \( n = 62 \) (white spot lesions).

A controlled experimental study by Heinrich-Weltzien et al.\(^{63}\) evaluated QLF’s ability to detect early enamel lesions \textit{in vitro}, which was validated with microradiographic and chemical analyses. Within the study sample, \((n = 25 \text{ bovine specimens and } n = 30 \text{ human specimens})\) calcium loss during demineralization was measured to calculate the severity of demineralization, followed by QLF measurement, and then the results compared with TMR. A strong correlation was found between fluorescence changes and mineral loss \((r = 0.79 - \text{laser system and } r = 0.84 - \text{portable lamp system})\). QLF was demonstrated to be a reproducible and sensitive method in the determination of the severity of incipient smooth surface caries lesions\(^{63}\).

QLF is able to effectively monitor changes in erosion and mineral changes in carious lesions, quantify stain and plaque, discriminate between tooth structures and restorations, and is a good “patient-operator” educational tool\(^{49,62}\). There have been recent developments in the application of QLF in the detection of occlusal caries and fissure lesions\(^{61,64}\).

QLF is capable of measuring small changes in tooth mineral content and produces quantitative measurements. It is particularly useful in longitudinal assessments of new and existing caries lesions located on smooth tooth surfaces. QLF offers a unique opportunity for the dental practitioner to educate, motivate patients, and promote oral health. QLF also has a potential application in dental education where students are able to detect and monitor longitudinal changes in the patients they see in the clinic\(^{49}\).
1.2.4. Limitations of QLF

It has been suggested that the QLF measurements may be influenced by the presence of dental plaque, calculus, stain, ambient light conditions, the degree of hydration of the tooth surface, the location of the lesion, the experience of the QLF operator, and QLF camera angulation.

The presence of dental plaque, calculus and stain may influence the QLF recording of the demineralized lesion. Plaque, stain and calculus may contain fluorescing particles, leading to false positive results. It is imperative to have a clean tooth surface prior to QLF application. Ambient light will influence the quality of the QLF image. The use of an ambient light meter is strongly recommended, and QLF must be used in a fixed ambient lighting environment of non-fluorescent source, with an optimal light level of 0 - 88 lux, which is the equivalent of a semi-dark environment. This amount of light is sufficient for clinical note taking and observance of practitioner and subject. Bright light may cause reflection scattering of light at the enamel surface. Hydration of the lesion may influence the reliability of QLF measurements. A study by Pretty et al. (2004) examined how lesion hydration might affect QLF measurements. This controlled in vitro study examined the effect of hydration (distilled water and saliva) on dental lesions (n=10) in order to determine an optimal drying method for QLF readings. Teeth were demineralized and then immersed into solutions, followed by compressed air-drying or drying with a cotton roll application. Air-drying for 15 seconds produced reliable results with distilled water and saliva-hydrated lesions. Cotton wool roll (CWR) application produced reliable results in 89 seconds with distilled water and 110 seconds with saliva. More time was needed to dry saliva-hydrated lesions as compared to distilled water. As lesion severity increased, time to reach reliable measurement significantly increased under control drying and CWR. It was suggested that when reporting QLF data the
method for dehydrating the lesions should be described, including both the technique and application time, so that in future the data can be accurately compared between studies\textsuperscript{67,68}.

Another major limitation of QLF is the location of the demineralized lesion. If QLF use is restricted to smooth surfaces, then this introduces a substantial limitation in the application of the QLF method. The experience of the QLF practitioner and angulation of the camera are also important factors to consider\textsuperscript{69,70}. Significant differences in QLF reading (i.e. fluorescence loss and lesion size) were observed in deviations larger than 20 degrees from the perpendicular were permitted\textsuperscript{41}. Differences in inter-examination reliability between experienced and novice examiners were also previously reported\textsuperscript{41}. Therefore, training is essential for novices of the QLF technique. All aforementioned factors are important to consider while designing or evaluating QLF study.
2. **RATIONALE, AIM, AND HYPOTHESIS**

2.1. **Rationale**

Although the xerostomia products Biotene® Oralbalance Moisturizing liquid and MouthKote® Oral Moisturizer spray are commonly available in Canada, their effects on human dentition were not extensively studied. Scientific evidence to support their use is limited, and the manufacturer’s recommendations for use that are included with the products are not comprehensive. Such an investigation will provide important information on the erosive potential of Biotene® and MouthKote®, help in providing recommendations for use, and improve the knowledge of dental practitioners in the palliation of xerostomia. Future similar studies will also benefit from sample size calculations based on the present study.

2.2. **Aim**

The aim of the present study is to investigate how the xerostomia products Biotene® Oralbalance moisturizing liquid and MouthKote® oral moisturizer spray influence mineral content of human enamel using Quantitative Light-induced Fluorescence (QLF) method.

2.3. **Hypothesis**

Hypothesis: Biotene® Oralbalance moisturizing liquid and MouthKote® oral moisturizer spray can potentially cause demineralization of hard tooth structures.
3. **MATERIALS AND METHODS**

3.1. Study design

A randomized, controlled and partially blinded experimental design has been chosen for the present inquiry. This *in vitro* study investigated the effects of xerostomia products on human enamel. A total of one hundred and four human teeth were used in this study. The QLF method was employed to measure mineral changes in enamel. The study profile diagram (Figure 4) provides a graphical representation of the study process.
Figure 4. Study profile diagram depicting each phase of the experiment

NOTES:
- Teeth and solution contained in beakers and maintained at 35°C within shaker/incubator. Solutions changed every day; pH and temperature of solutions measured daily.
- All teeth rinsed with DH2O for 5 minutes and open-air dried for 30 minutes after immersion in solution.
- QLF measurement under standardized conditions (use of ambient light meter and magnetic-base holder). QLF images captured and stored on disc.
- CG = Control Group
- EG1 - EG4 = Experimental Group 1 - 4
The teeth were randomly divided into five groups (1 control and 4 experimental groups), which were subjected to four experimental phases: specimen preparation phase (SP), immersion phase 1 (IM Phase 1), immersion phase 2 (IM Phase 2), and immersion phase 3 (IM Phase 3). For the SP phase, all teeth were specially prepared for the study, followed by the IM phase 1 where the Experimental Group 1 (EG 1) and the Experimental Group 2 (EG 2) were immersed into the Lactic acid solution and the Control Group (CG) was immersed into the DH$_2$O. For IM Phase 2, CG was placed into the Lactic acid solution, and the previously demineralized EG1 and EG2 were immersed into the Biotene® and MouthKote® respectively. Non-demineralized EG3 and EG4 were also immersed into the Biotene® and MouthKote® respectively. For IM Phase 3 all groups (1 control and 4 experimental) were placed into the Oral-B mouth rinse. QLF images were taken before and after each phase using standardized conditions.

3.2. Standardization of QLF measurements

All QLF measurements were taken under standardized conditions, which included the use of the ambient light meter for control of ambient light conditions, and a magnetic base holder for setting of the QLF handpiece. Measurement of the ambient light was taken before each QLF analysis in order to verify that an appropriate ambient light level of 80 lux was maintained. The magnetic base holder was permanently attached to the QLF trolley during this experiment to insure standardized positioning and angulation of the QLF handpiece. All QLF images were taken and analyzed by a single partially blinded (to control group) examiner (MG). All images were saved onto a disk and analyzed using the QLF Inspektor® Pro software for mineral loss parameters measured by mean fluorescence loss (%), maximum fluorescence loss (%), and the extent of the area lesion (%/mm$^2$).
3.3. Specimen selection and preparation

A total of one hundred and four (104) cavity-free, permanent molars and premolars were collected from several dental clinics in the greater area of Vancouver, BC, Canada. Teeth were extracted for orthodontic purposes in individuals between the ages of 16-25. A verbal informed consent was obtained from the individuals who agreed to donate their extracted teeth for the study. Selection of specimens was carried out according to stringent criteria: no visible cavitations or white spot lesions, no presence of calculus, and no presence of any visible enamel defects.

![Figure 5. Specimens at preparation phase (polished, with 4 x 4 mm window on the buccal surface, attached to PVC square, and coded) prior to baseline QLF analysis.](image)

The selected teeth were randomly divided into five groups (one control and four experimental) and prepared by gentle polishing with a rubber cup followed by coating in an acid-resistant nail varnish (OPI-Base Coat), leaving a window on the buccal surface, approximately 4 x 4 mm square. Each individual tooth was mounted on a square piece of PVC thermoplastic (SIMONA® VERSADUR® RIGID PVC 250, Type II,
Polyvinylchloride, Industrial Plastics and Paint, Langley, BC) with the help of sticky dental wax. The PVC thermoplastic material was selected due to its thermal, corrosion and chemical resistant properties.

3.4. Test groups

Teeth were divided into five groups: control group (CG) \( n = 26 \), experimental group one (EG1) with a sample size \( n=26 \), experimental group two (EG2) \( n = 26 \), experimental group three (EG3) \( n = 13 \), and experimental group four (EG4) \( n = 13 \). Two control types were employed in the present study. Firstly, without exposure to lactic acid, the control group CG1 served as a control for the experimental groups without erosion. Secondly, after being exposed to lactic acid, the control CG2 served as a control for the experimental groups with erosion. Consequently, the control group prior to immersion into lactic acid is noted as control group CG1 and after the immersion into lactic acid as control group CG2.

3.5. Experimental protocol

3.5.1. Immersion phase one

During the Immersion Phase I the control group CG1 (\( n = 26 \)) was exposed to distilled water, and experimental groups EG1 (\( n = 26 \)) and EG2 (\( n = 26 \)) were exposed to a standardized demineralizing solution of lactic acid (0.1 M lactic acid adjusted to a pH of 4.5). Demineralization with Lactic acid solution was carried out according to the method described by Pretty I et al. and Mishra P et al.\(^{41,71}\). Teeth and solutions were contained in beakers and maintained at 35 C° within a shaker/incubator for 48 hours. Solutions were changed daily, and pH and temperature of the solutions were monitored daily. After 48 hours of exposure, the teeth were rinsed in distilled water for 5 minutes and open-air dried for 30 minutes. Subsequently, the QLF
images were taken under the aforementioned standardized conditions, and the QLF images were stored on a disc.

3.5.2. Demineralization protocol

Specimens from the groups EG1 and EG2 were immersed into beakers containing a demineralizing solution of lactic acid (0.1 M lactic acid adjusted to pH of 4.5) freshly made by Macdonald’s Compounding Pharmacy (Vancouver) and maintained at 35°C within a shaker/incubator for forty-eight hours. Solutions were changed daily, and pH and temperature of the solutions were also monitored daily.

![Image of beakers containing lactic acid solution](image_url)

Figure 6. Development of erosion in control group 2 with the help of lactic acid (0.1M lactic acid adjusted to pH of 4.5)

3.5.3. Immersion phase two

During immersion phase 2 the control group (n=26) was demineralized in Lactic acid, experimental groups previously immersed in lactic acid EG1 (n = 26) and EG3 (n = 13) were immersed into Biotene®, and the experimental groups not immersed in lactic acid EG2 (n = 26) and EG4 (n = 13) were immersed in MouthKote® for 48 hours following the study protocol. QLF images were taken and stored on disk.
3.5.4. Immersion phase three

During immersion phase three, all five groups (one control and four experimental groups) were immersed into an Oral-B fluoridated mouth rinse for 48 hours following the study protocol. Subsequently, QLF images were taken and stored on disk. Teeth and solutions were contained in beakers and maintained at 35°C within the shaker/incubator for forty-eight hours per study phase. Solutions were changed daily, and pH and temperature of the solutions were monitored daily. All teeth were rinsed with DH₂O for five minutes and open air-dried for thirty minutes prior to taking the QLF images. QLF measurements were taken under standardized conditions. QLF images were captured, stored and subsequently blindly analyzed by a single examiner (MG).

Figure 7. Immersion phase two. Experimental group 1 was immersed in Biotene and experimental group 2 was immersed in MouthKote®.
3.6. Statistical analysis

Within-group comparisons were made by analyzing the before and after measurements of each specimen, and among group comparisons were made by comparing results among groups acquired at the different stages of experiment. For all data analyses, the SPSS version 17.0 statistical software was used. For comparisons, Independent Sample t-test, paired sample t-test, and ANOVA for multiple comparisons with Bonferroni post hoc adjustment were used. Two groups of comparisons were done. For the within-group comparison a paired sample t-test was used; and for the across-group comparison one-way ANOVA with Bonferroni post hoc adjustments was employed. For all tests, the threshold for the statistical significance was set at $P < 0.05$. 
4. RESULTS

One hundred and four specimens were analyzed by quantitative light induced fluorescence with respect to mean fluorescence loss over the lesion ($\Delta F$, %), maximum fluorescence loss in the lesion ($\Delta Q$, %), and the extent of the area of the lesion (WS, %/mm$^2$).

Three levels of comparisons were performed. Firstly, distilled water (DH$_2$O) was tested with respect to its effect on the extracted teeth, in order to evaluate DH$_2$O appropriateness as a rinsing medium. Secondly, within-group comparisons were employed in order to compare each group at the different phases of the experiment. Thirdly, among-group comparisons were made to compare the findings across groups. The results are presented in Tables 4 – 25 and box plots in figures A1a, A1b, A1c through to figures B2a, B2b, B2c.

Table 4 presents the effects of the immersion of teeth in DH$_2$O. This testing was performed to evaluate if immersion into distilled water will produce a mineral loss in extracted human teeth. This testing was performed on the control group (CG1).
Table 4. Effect of distilled water on mineral content of extracted human teeth in control group 1

<table>
<thead>
<tr>
<th>QLF measurements</th>
<th>Control group CG1 (N=26)</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>△F</td>
<td>Baseline</td>
<td>-6.79 ± 1.08</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>-7.06 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>△Q</td>
<td>Baseline</td>
<td>-5.10 ± 12.20</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>-7.47 ± 17.87</td>
<td></td>
</tr>
<tr>
<td>WS</td>
<td>Baseline</td>
<td>0.06 ± 1.23</td>
<td>0.163</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>0.80 ± 1.64</td>
<td></td>
</tr>
</tbody>
</table>

* Means ± SD were compared by paired sample t-test
△F denotes mean fluorescence loss (%)
△Q denotes fluorescence loss in the lesion (%)
WS denotes extent of the lesion area (%/mm²)

There were no statistically significant differences between the means and SD of different measures of mineral content, when the baseline values were compared with the values obtained after immersion of teeth into DH₂O. This testing demonstrated that DH₂O did not produce significant demineralization of extracted human teeth, and therefore, it was considered safe for use for the subsequent rinsing procedures as outlined in the study protocol. Baseline values among all groups are compared in Table 5. These comparisons demonstrated that there were no statistically significant differences between CG1 (control group) and three experimental groups in any of the measured parameters at the baseline level. This may indicate that the random allocation into groups was successful.
Table 5. Among-group comparisons - baseline comparisons among control and experimental groups

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Study Groups</th>
<th>N</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ΔF</strong></td>
<td>CG1 Control Group 1</td>
<td>26</td>
<td>-6.79 ± 1.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-6.62 ± 0.92</td>
<td>0.121</td>
</tr>
<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>-6.90 ± 1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>-6.18 ± 0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>-6.39 ± 0.54</td>
<td></td>
</tr>
<tr>
<td><strong>ΔQ</strong></td>
<td>CG1 Control Group 1</td>
<td>26</td>
<td>-5.10 ± 12.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-3.16 ± 6.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>-7.78 ± 17.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>-1.00 ± 2.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>-0.96 ± 1.10</td>
<td></td>
</tr>
<tr>
<td><strong>WS</strong></td>
<td>CG1 Control Group 1</td>
<td>26</td>
<td>0.59 ± 1.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>0.42 ± 0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>0.91 ± 1.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>0.23 ± 0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>0.54 ± 0.91</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD were compared by one-way ANOVA with post-hoc Bonferroni adjustments.  
ΔF denotes mean fluorescence loss (%)  
ΔQ denotes fluorescence loss in the lesion (%)  
WS denotes extent of the lesion area (%/mm²)

Table 6 compares the mean values of three parameters related to mineral loss after immersion into Lactic acid (the immersion phase I) among one control (CG2) and two experimental groups (EG1 and EG2). This testing demonstrated that similar amounts of mineral loss occurred in the groups that were immersed into Lactic acid (CG2 and EG1 and EG2) (P > 0.005).
Table 6. Mineral changes in control group 2, experimental group 1, and experimental group 2 after immersion into lactic acid during immersion phase 1

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Study Groups</th>
<th>N</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG2 Control Group 2</td>
<td>26</td>
<td>-11.10 ± 2.79</td>
<td></td>
</tr>
<tr>
<td>ΔF</td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-11.14 ± 2.95</td>
<td>0.611</td>
</tr>
<tr>
<td></td>
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<td>26</td>
<td>-11.80 ± 3.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>ΔQ</td>
<td>CG2 Control Group 2</td>
<td>26</td>
<td>-122.63 ± 78.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-106.95 ± 86.93</td>
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<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>-119.23 ± 93.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>WS</td>
<td>CG2 Control Group 2</td>
<td>26</td>
<td>9.44 ± 5.30</td>
<td>0.851</td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>8.59 ± 5.42</td>
<td></td>
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<td></td>
<td>EG2 Experimental Group 2</td>
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<td>9.21 ± 5.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*Mean ± SD were compared with one-way ANOVA with post-hoc Bonferroni adjustment. After immersion into lactic acid, control group one (CG1) became control group two (CG2).

ΔF denotes mean fluorescence loss (%)
ΔQ denotes fluorescence loss in the lesion (%)
WS denotes extent of the lesion area (%/mm²)

Table 7 presents the levels of mineral loss in all four experimental groups after the immersion into the xerostomia products. There were substantial and statistically significant differences in mineral loss among all four groups. The groups that were previously immersed into Lactic acid (EG1 and EG2) showed higher levels of mineral loss in comparison to the groups EG3 and EG4 that were not previously immersed into Lactic acid.
Table 7. Effect of xerostomia products on mineral content in control group 1, and experimental groups 1, 2, 3, and 4 during immersion phase 2

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Study group</th>
<th>N</th>
<th>Mean ± SD*</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF</td>
<td>CG 1 Control Group 1</td>
<td>26</td>
<td>-6.79 ± 1.08</td>
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</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-11.15 ± 3.05</td>
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<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>-7.38 ± 0.44</td>
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</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>-11.45 ± 2.94</td>
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<tr>
<td>ΔQ</td>
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<td>-5 ± 12.20</td>
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<td>EG4 Experimental Group 4</td>
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</tr>
<tr>
<td>WS</td>
<td>CG 1 Control Group 1</td>
<td>26</td>
<td>0.59 ± 1.23</td>
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<td></td>
<td>EG1 Experimental Group 1</td>
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<td>7.48 ± 5.00</td>
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<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>0.48 ± 0.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>7.22 ± 4.35</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD were analyzed with one-way ANOVA with post-hoc Bonferroni adjustment. ΔF denotes mean fluorescence loss (%), ΔQ denotes fluorescence loss in the lesion (%), WS denotes extent of the lesion area (%/mm²).

Table 8 presents changes in mineral levels in all study groups after immersion into a fluoridated rinse. This testing showed that the control group CG2 had the lowest level of mineral loss and the experimental groups with erosion (ECG1 and EG2) had higher levels of mineral loss than the experimental groups without erosion (EG3 and EG4). All differences were highly statistically significant (P = 0.000).
Table 8. Effect of fluoridated Oral-B rinse on mineral content in control group 2 and experimental groups 1, 2, 3, and 4 during immersion phase 3

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Study group</th>
<th>N</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG1 Control Group 2</td>
<td>26</td>
<td>-9.05 ± 1.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-8.62 ± 1.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>-28.89 ± 1.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>-6.63 ± 0.51</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>-9.99 ± 1.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CG1 Control Group 2</td>
<td>26</td>
<td>-25.46 ± 25.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>-23.48 ± 33.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>-510.81 ± 45.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>-2.70 ± 5.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>-66.40 ± 45.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CG1 Control Group 2</td>
<td>26</td>
<td>2.49 ± 2.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG1 Experimental Group 1</td>
<td>26</td>
<td>2.24 ± 2.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG2 Experimental Group 2</td>
<td>26</td>
<td>15.70 ± 3.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG3 Experimental Group 3</td>
<td>13</td>
<td>0.365 ± 0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG4 Experimental Group 4</td>
<td>13</td>
<td>6.09 ± 3.55</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD were analyzed with one-way ANOVA with post-hoc Bonferroni adjustment.

ΔF denotes mean fluorescence loss (%)

ΔQ denotes fluorescence loss in the lesion (%)

WS denotes extent of the lesion area (%/mm²)

The xerostomia products Biotene® Oralbalance dry mouth moisturizer and MouthKote® oral moisturizer produced significant demineralization in extracted human teeth with prior demineralization (P=0.000) and without previous pre-demineralization in Lactic acid (P = 0.000). The amount of demineralization (Mean ± SD) was higher in MouthKote® groups EG2 (-27.19 ± 6.70) and EG4 (-11.45 ± 2.94) than in Biotene groups EG1 (-11.15 ± 3.05) and EG3 (-7.38 ± 0.44), respectively. More demineralization was observed in previously pre-demineralized teeth as compared to sound teeth. Analysis of variance (ANOVA) demonstrated significant demineralization after the lactic acid immersion period (P = 0.000), as well as significant demineralization in the experimental groups
after immersion into the xerostomia products ($P = 0.000$). Paired t-tests demonstrated significant differences between “test” and “control” lesions.

### 4.1. Within-group comparisons

Findings from within-group comparisons are presented in Tables 9 – 21 below and figures A1a, A1b, A1c through to A5a, A5b, A4c (box plots) in Appendix A.

Table 9. Mineral changes in the control group at immersion phases 1, 2, and 3 ($\Delta F$ outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-6.79 ± 1.08</td>
<td>0.073</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>-7.06 ± 1.40</td>
<td>0.000</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>-11.10 ± 2.79</td>
<td>0.010</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-9.05 ± 1.78</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment.
$\Delta F$ denotes mean fluorescence loss (%)

Table 9 and Figures A1a, A1b, A1c (see appendix A) illustrate mineral changes in the control group at different immersion phases. There were no statistically significant differences between the mean values at the baseline and after immersion into distilled water.

There was a statistically significant mineral loss after immersion into lactic acid, and a slight mineral gain upon subsequent immersion into the Oral-B mouth rinse.

Immersion into lactic acid produced a substantial variation in mineral loss. Similar patterns were observed with respect to all of the three outcome measurements.

There was a statistically significant gain in mineral content after immersion into fluoridated mouth rinse, but this mineral gain did not reach the baseline level. Similar patterns were observed in $\Delta F$ and $\Delta Q$. With regard to WS, a remineralization tendency was observed, albeit not statistically significant.
4.2. *Longitudinal changes in the experimental group (EG1) during different phases of the experiment.*

Table 10. Mineral changes in experimental group 1 (EG1) at different immersion phases (\(\Delta F\) outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-6.62 ± 0.92</td>
<td>0.000</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>-11.14 ± 2.95</td>
<td>0.963</td>
</tr>
<tr>
<td>Biotene</td>
<td>-11.15 ± 3.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-8.62 ± 1.77</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. \(\Delta F\) denotes mean fluorescence loss (%)

Table 11. Mineral changes in experimental group 1 (EG1) at different immersion phases (\(\Delta Q\) outcome)

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-3.16 ± 6.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>-106.95 ± 86.93</td>
<td>0.156</td>
</tr>
<tr>
<td>Biotene</td>
<td>-94.92 ± 85.77</td>
<td>0.000</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-23.48 ± 33.39</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. \(\Delta Q\) denotes fluorescence loss in the lesion (%)

Table 12. Mineral changes in experimental group 1 (EG1) at different immersion phases (WS outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.42 ± 0.78</td>
<td>0.000</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>8.59 ± 5.42</td>
<td>0.000</td>
</tr>
<tr>
<td>Biotene</td>
<td>-7.48 ± 5.00</td>
<td>0.072</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-2.24 ± 2.66</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. WS denotes extent of the lesion area (%/mm²)

A substantial variation in mineral loss was noted between the baseline and lactic acid immersion phases. There was a slight mineral gain tendency, albeit not a statistically
significant change between the lactic acid and Biotene® immersion phases. A statistically
significant improvement in mineral content was noted between immersion into Biotene®
and subsequent immersion into Oral-B fluoridated rinse.

Table 13. Mineral changes in experimental group 2 (EG2) at different immersion phases (ΔF outcome)

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-6.90 ± 1.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>-11.80 ± 3.41</td>
<td>0.000</td>
</tr>
<tr>
<td>MouthKote</td>
<td>-27.19 ± 6.70</td>
<td>0.338</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-28.89 ± 1.75</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. ΔF denotes mean fluorescence loss (%)

A statistically significant demineralization occurred from the baseline to the lactic acid immersion phases. Further demineralization took place when the specimens were immersed in MouthKote® after lactic acid. No statistically significant mineral gain was observed between the MouthKote® and Oral-B immersion phases.

Table 14. Mineral changes in experimental group 2 (EG2) at different immersion phases (ΔQ outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-7.78 ± 17.02</td>
<td>0.000</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>-119.23 ± 93.19</td>
<td>0.000</td>
</tr>
<tr>
<td>MouthKote</td>
<td>-540.93 ± 282.54</td>
<td>0.552</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-510.81 ± 45.76</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. ΔQ denotes fluorescence loss in the lesion (%)

At the ΔQ level, a statistically significant demineralization was observed from baseline to lactic acid immersion. Further demineralization took place when teeth were taken from lactic acid and immersed into the MouthKote®. Immersion into Oral-B rinse did not induce statistically significant mineral gain.
Table 15. Mineral changes in experimental group 2 (EG2) at different immersion phases (WS outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.91 ± 1.79</td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>9.21 ± 5.85</td>
<td>0.000</td>
</tr>
<tr>
<td>MouthKote</td>
<td>-18.75 ± 6.97</td>
<td>0.188</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-15.7069 ± 3.55</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. WS denotes extent of the lesion area (%/mm²)

A mineral loss was induced from the baseline to the immersion into lactic acid. Further substantial mineral loss occurred after immersion into MouthKote® took place. Upon immersion into Oral-B rinse, a slight but not statistically significant mineral gain was noted.

Table 16. Mineral changes in experimental group 3 (EG3 without demineralization) at different immersion phases (ΔF outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-6.18 ± 0.26</td>
<td>0.000</td>
</tr>
<tr>
<td>Biotene</td>
<td>-7.38 ± 0.44</td>
<td>0.000</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-6.63 ± 0.51</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment. ΔF denotes mean fluorescence loss (%)

A clear gain in mineral content was observed, albeit not to the baseline levels.

Table 17. Mineral changes in experimental group 3 (EG3 without demineralization) at different immersion phases (ΔQ outcome)

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-1.00 ± 2.90</td>
<td>0.000</td>
</tr>
<tr>
<td>Biotene</td>
<td>-3.49 ± 6.44</td>
<td>0.057</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-2.70 ± 5.77</td>
<td></td>
</tr>
</tbody>
</table>

ΔQ denotes fluorescence loss in the lesion (%)

A marginal gain in minerals was noted, albeit not statistically significant.
Table 18. Mineral changes in experimental group 3 (EG3 without demineralization) at different immersion phases (WS outcome)

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.23 ± 0.57</td>
<td>0.000</td>
</tr>
<tr>
<td>Biotene</td>
<td>-0.48 ± 0.88</td>
<td>0.053</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-0.37 ± 0.77</td>
<td></td>
</tr>
</tbody>
</table>

WS denotes extent of the lesion area (%/mm²)

A marginal gain in minerals was noted, albeit not statistically significant.

Table 19. Mineral changes in experimental group 4 (EG4 without demineralization) at different immersion phases (ΔF outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-6.39 ± 0.54</td>
<td>0.000</td>
</tr>
<tr>
<td>MouthKote</td>
<td>-11.45 ± 2.94</td>
<td>0.002</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-9.99 ± 1.75</td>
<td></td>
</tr>
</tbody>
</table>

ΔF denotes mean fluorescence loss (%)

A statistically significant loss in mineral content was observed.

Table 20. Mineral changes in experimental group 4 (EG4 without demineralization) at different immersion phases (ΔQ outcome)

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-0.96 ± 1.10</td>
<td>0.000</td>
</tr>
<tr>
<td>MouthKote</td>
<td>-88.90 ± 53.03</td>
<td>0.000</td>
</tr>
<tr>
<td>Oral-B</td>
<td>-66.40 ± 45.76</td>
<td></td>
</tr>
</tbody>
</table>

ΔQ denotes fluorescence loss in the lesion (%)

A statistically significant loss in minerals was noted.
Table 21. Mineral changes in experimental group 4 (EG4 without demineralization) at different immersion phases (WS outcome)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.54 ± 0.91</td>
<td>0.000</td>
</tr>
<tr>
<td>MouthKote</td>
<td>-7.22 ± 4.35</td>
<td>0.018</td>
</tr>
<tr>
<td>Oral-B</td>
<td>6.09 ± 3.55</td>
<td></td>
</tr>
</tbody>
</table>

WS denotes extent of the lesion area (%/mm²)

A statistically significant loss in mineral content was noted from the baseline upon MouthKote® immersion, whereas statistically significant increase in mineral content was observed from the MouthKote® into Oral-B immersion.

4.3. Across-group comparisons

Tables 22-23 and Figures B1a, B1b and B1c (see Appendix B) depict changes in control group and experimental groups EG3 and EG4 (ΔF, ΔQ, and WS) at baseline and after immersion into xerostomia products.

Table 22. Mineral changes at immersion phase 2 between CG1, non-demineralized groups EG3 and EG4 (ΔF, ΔQ, and WS outcomes)

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Immersion Phase 2 (without demineralization)</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF</td>
<td>Control (CG1)</td>
<td>Biotene (EG3)</td>
<td>-7.38 ± 0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-7.06 ± 1.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MouthKote (EG4)</td>
<td>-11.45 ± 2.94</td>
</tr>
<tr>
<td>ΔQ</td>
<td>Control (CG1)</td>
<td>Biotene (EG3)</td>
<td>-3.49 ± 6.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-7.47 ± 17.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MouthKote (EG4)</td>
<td>-88.90 ± 53.03</td>
</tr>
<tr>
<td>WS</td>
<td>Control (CG1)</td>
<td>Biotene (EG3)</td>
<td>0.48 ± 0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.80 ± 1.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MouthKote (EG4)</td>
<td>7.22 ± 4.35</td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment.
ΔF - denotes mean fluorescence loss (%)
ΔQ - denotes fluorescence loss in the lesion (%)
WS - denotes extent of the lesion area (%/mm²)
More loss in mineral content (ΔF, ΔQ, and WS) was noted in experimental group 4 upon immersion into MouthKote®, as compared to control group (CG1) and experimental group 3.

Table 23. Comparisons between non-demineralized groups EG3 and EG4 at immersion phase 2 (ΔF, ΔQ, and WS outcomes)

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Groups</th>
<th>Immersion Phase 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biotene (EG3)</td>
<td>-7.38 ± 0.44</td>
<td>0.00</td>
</tr>
<tr>
<td>ΔF</td>
<td>MouthKote (EG4)</td>
<td>-11.45 ± 2.94</td>
<td></td>
</tr>
<tr>
<td>ΔQ</td>
<td>Biotene (EG3)</td>
<td>-3.49 ± 6.44</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>MouthKote (EG4)</td>
<td>-88.90 ± 53.03</td>
<td></td>
</tr>
<tr>
<td>WS</td>
<td>Biotene (EG3)</td>
<td>0.48 ± 0.88</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>MouthKote (EG4)</td>
<td>7.22 ± 4.35</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment.
ΔF - denotes mean fluorescence loss (%)
ΔQ - denotes fluorescence loss in the lesion (%)
WS - denotes extent of the lesion area (%/mm²)

During immersion phase 2, experimental group 4 (MouthKote®) demonstrated more mineral loss as compared to experimental group 3 (Biotene®).

Tables 24-25 and Figures B2a, B2b and B2c (see Appendix B) illustrate changes of mineral content (ΔF, ΔQ, and WS) in the control group and experimental groups (EG1, EG2) after lactic acid and/or xerostomia products exposure.
Table 24. Mineral changes at immersion phase 2 in CG2, and demineralized groups EG1 and EG2 (ΔF, ΔQ, and WS outcomes)

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Immersion Phase 2 (without demineralization)</th>
<th>Mean ± SD*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF</td>
<td>Control (CG2)</td>
<td>Biotene (EG1) -11.15 ± 3.05</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-11.10 ± 2.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MouthKote (EG2) -27.19 ± 6.70</td>
<td>0.00</td>
</tr>
<tr>
<td>ΔQ</td>
<td>Control (CG2)</td>
<td>Biotene (EG1) -94.92 ± 85.77</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-122.63 ± 78.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MouthKote (EG2) -540.93 ± 282.54</td>
<td>0.00</td>
</tr>
<tr>
<td>WS</td>
<td>Control (CG2)</td>
<td>Biotene (EG1) 7.48 ± 5.00</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.44 ± 5.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MouthKote (EG2) 18.75 ± 6.97</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment.
ΔF - denotes mean fluorescence loss (%)
ΔQ - denotes fluorescence loss in the lesion (%)
WS - denotes extent of the lesion area (%/mm²)

Table 25. Comparisons between EG1 and EG2 at immersion phase 2 (ΔF, ΔQ, and WS outcomes)

<table>
<thead>
<tr>
<th>QLF Measurements</th>
<th>Groups</th>
<th>Immersion 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF</td>
<td>Biotene (EG1)</td>
<td>-7.38 ± 0.44</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>MouthKote (EG2)</td>
<td>-11.45 ± 2.94</td>
<td></td>
</tr>
<tr>
<td>ΔQ</td>
<td>Biotene (EG1)</td>
<td>-3.49 ± 6.44</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>MouthKote (EG2)</td>
<td>-88.90 ± 53.03</td>
<td></td>
</tr>
<tr>
<td>WS</td>
<td>Biotene (EG1)</td>
<td>8.59 ± 5.42</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>MouthKote (EG2)</td>
<td>7.22 ± 4.35</td>
<td></td>
</tr>
</tbody>
</table>

* Means compared by one-way ANOVA with post-hoc Bonferroni adjustment.
ΔF - denotes mean fluorescence loss (%)
ΔQ - denotes fluorescence loss in the lesion (%)
WS - denotes extent of the lesion area (%/mm²)

Mineral loss in the control group 2 (erosion) and immersion into Biotene® was similar, however the mineral loss observed in the control group 2 and immersion into MouthKote® was substantially larger than the aforementioned groups. Moreover, there was a substantial variation in mineral loss within the MouthKote® group as compared to other groups in this study. The groups exposed to lactic acid and subsequently immersed into various xerostomia products are compared in the figures B2a, B2b, B2c and Tables 24, 25.
Figure 8. Longitudinal monitoring of a specimen (1 – baseline, 2 – exposure to lactic acid, 3 – exposure to MouthKote, 4 – exposure to fluoridated mouth rinse)
5. **DISCUSSION**

The present study examined the effects of xerostomia products Biotene® Oralbalance moisturizing liquid and MouthKote® oral moisturizer on mineral changes in human enamel. Biotene® and MouthKote® induced significant mineral loss in both predemineralized and unaltered specimens. More demineralization was produced in MouthKote® experimental groups with and without demineralization as compared to the Biotene® groups with and without demineralization.

The fluoride-containing mouth rinse aided in re-mineralization in all experimental and control groups, albeit not to the baseline levels. The exception was the experimental group, which was immersed in lactic acid and MouthKote®, and where further enamel mineral loss occurred.

Although xerostomia is not a life-threatening condition, it can significantly affect the well-being of an individual. This is particularly true for the institutionalized elderly, those suffering from head and neck irradiation treatment side effects, and haemodialysis patients. Some of the serious consequences of xerostomia include rampant tooth decay, tooth loss, and candida albicans infection, and these may ultimately lead to difficulties with chewing, swallowing, and possibly malnutrition. There are a variety of products available to manage xerostomia symptoms including sialogogues, over-the-counter saliva substitutes and stimulants. Despite the wide availability of OTC xerostomia products, their use in not always supported by clinical evidence. Nor are the results of studies typically published in professional peer-reviewed journals. First and foremost, the management of xerostomia should (1) not result in harm to the existing dentition, (2) palliate the dry mouth condition, and (3) prevent recurrent decay and associated oral-mucosal lesions. It is not an uncommon practice to use either antibacterial and / or fluoride-containing dentifrices in the institutionalized elderly for the
purpose of amelioration of oral health. Treatment protocols such as daily rinsing with a 0.12% chlorhexidine (CHX) solution for controlling caries and regular application of fluoride have been tested, although clinical trials have shown this to be ineffective in preserving sound tooth structures in the elderly. Fluoride, on the other hand, has been shown to be very effective against rampant decay if properly applied on a regular basis. Fluoride rinse was able to reduce the incidence of caries among elderly residents of long-term care facilities. Many xerostomia products available in Canada fall under the category of “over-the-counter” (OTC) products. Being an over-the-counter product, clinically testing prior to the marketing of the product is not always required as the control and scrutiny of such products are not the same as for prescription drugs. There is no uniformity in the way the OTC xerostomia products are labeled. The recommendations for use are vague and misleading.

In view of these issues, xerostomia products available in Canada should clearly indicate the pH on the packaging, as well as outline the possible side effects and recommendations for use in edentulous and dentate patients.

5.1. Use of control groups

In order to resemble a clinical situation in the study, the control without demineralization (sound teeth) and the control with demineralization by lactic acid served as two different types of controls. Firstly, the control group prior to any exposure served as a control group for the experimental groups without erosion, whilst the same control group after being exposed to lactic acid served as a control group for the experimental groups with erosion.

5.2. Standardization of study conditions

All relevant variables between the experimental and control groups were standardized, such as the dry mouth products used in this study, adherence to the experimental
protocol, eliminating bias in the sample selection, baseline randomization, and partial blindness of the investigator.

5.3. Sample selection

A sample size calculation was not possible in the present study as there were no similar previous studies performed, and therefore no relevant findings from previous studies were available to be used as a basis for sample size calculations. However, this study employed a relatively high sample size as compared to previous studies examining extracted human teeth with the help of QLF method. Numerous QLF studies employed smaller sample sizes for the purpose of detection of in vitro demineralization in primary teeth (n = 12)\textsuperscript{72}, the effect of hydration on QLF readings (n = 10)\textsuperscript{73}, formation of artificial enamel lesions (n = 30)\textsuperscript{35}, in vivo monitoring of incipient caries lesions (n = 21)\textsuperscript{38}, and in vivo comparison of QLF and visual inspection for detection of initial carious lesions in high caries risk adolescents (n = 34)\textsuperscript{73}. The sample size selected for this study may have been much larger than necessary. However, calculation of sample size for future similar studies will be possible based on the sample size of the current study.

5.4. Inclusion criteria for extracted teeth

The extracted human teeth (n = 104) of similar age ranging from 17 to 25 years were used. A stringent selection protocol was employed (no visible cavitations or white spot lesions, no visible enamel defects, and no presence of calculus). The strict inclusion protocol was used to reduce the variation in the observations that could arise because of the systematic differences in the sample\textsuperscript{74}. However, this standardization also leads to a limitation as the present study findings may be generalized to “younger teeth” only as opposed to “elderly teeth” of individuals with dry mouth.
5.5. Pros of the internal validity

Pros of the internal validity are as follows:

(1) A relatively large sample size was used as compared to other in vitro QLF/human teeth studies, which could contribute to higher validity, but it is not always a prerequisite in achieving so. For example, Pretty et al.\textsuperscript{72} examined the ability of QLF to detect and longitudinally monitor changes in demineralized enamel in primary teeth \textit{in vitro} (n=12) in a randomized, controlled, experimental \textit{in vitro} study with combined \textit{in vivo} study.

(2) QLF was found to be an effective monitoring method of the changes in the mineral loss over time. Al-Khateeb S \textit{et al.}\textsuperscript{35} controlled experimental study evaluated QLF’s ability to detect early enamel lesions \textit{in vitro}, and was able to demonstrate a strong correlation between fluorescence changes and mineral loss.

(3) Other pros for the internal validity in the present study are as follows:

- random allocation;
- blinded assessments;
- quantitative, accurate measurement of the outcome variables;
- two conditions mimicking clinical situations (sound and demineralized teeth), where two types of controls were employed;
- stringent study protocol was used to reduce variation, and potential bias in the measurements;
- light source control by a light meter in order to assure consistent ambient light levels in order to obtain valid QLF readings;
- stringent protocol for drying teeth (30 minutes open air dried) to assure consistency in dryness of the teeth prior to QLF imaging. This is an essential requirement as it has been previously shown that the wetness on the tooth surface influences QLF readings\textsuperscript{67};
to ensure that storing teeth in DH$_2$O as well as rinsing in it during the experiment has no additional effect on the teeth, a test of the effects of DH$_2$O was performed at the primary stage of the experiment.

Given these considerations, the internal validity of the present study should be considered sufficient. The present study findings may be useful for calculating the necessary sample size in future in vitro studies.

5.6. Cons for the internal validity

(1) Although the camera angulation was fixed, some minimal discrepancies could still occur which might, to a certain extent, influence the QLF readings.

(2) There is a possibility that the xerostomia remedy products used in the present study came from different batches. This might result in minor variation in pH, content and consistency among the products used. Given that the expiry dates were always observed, and pH of the solutions was measured at each phase of the experiment, the potential variation among products would only have had a slight effect, if any at all, and consequently this is judged to have no bearing on the findings of the present study.

5.7. Pros for the external validity

(1) The fact that DH$_2$O did not induce enamel dissolution confirms the findings of other studies$^{17,19}$ that it was adequate to use distilled water for the storage of extracted teeth as well as during the rinsing procedures in this experiment.

(2) This present study demonstrated that QLF was able to effectively trace changes in teeth immersed into xerostomia products at different phases of the experiment. Overall, lactic acid and xerostomia products induced substantial demineralization in extracted teeth.

(3) Immersion into fluoride-containing mouth rinse resulted only in partial remineralization in all of the groups except the group previously immersed into lactic acid.
and MouthKote®. After the immersion into fluoridated mouth rinse, further enamel dissolution was observed, and this may be attributed to the tooth damage threshold having been exceeded, causing irreversible damage. In clinical experience, some hard tissue lesions present as white spot lesions or cavitations. Perhaps erosion in the lactic acid followed by immersion in erosive MouthKote® product produced irreversible damage to the teeth. Although substantial re-mineralization upon immersion into fluoridated rinse was observed in the rest of the groups, it never reached the baseline levels.

5.8. Cons for the external validity

(1) The experimental design of the present study does not represent real life conditions of real mouth and dentition, such as the presence of saliva, its buffering capacity, lubricity, and chemical composition. Controlled conditions present in an in vitro experiment differ significantly from those in vivo, and the results may not translate well to real life settings. As experimental conditions were not identical to the oral environment, it is necessary to test these products in future in vivo studies. These limitations are to be expected from a laboratory experiment.

(2) Although human teeth were studied, the teeth used were extracted for orthodontic purposes and included molars and premolars of young individuals between ages of 17-25. Besides natural variation of specimens, the “young” teeth are most likely to be different from the “older” teeth found in xerostomic individuals. With age, human enamel wears progressively (which is manifested as wear facets), becomes less permeable, and discolors due to a deepening layer of dentin and/or addition of organic material to the enamel from the environment. Using relatively “young” teeth is a limitation as it limits the possibility of generalizing the findings of this study to “older” teeth (limitations to external validity).
(3) As part of the study design, creation of artificial erosion lesions was necessary, which was accomplished with a solution of lactic acid (0.1 M lactic acid adjusted to pH of 4.5). A standard protocol for creating lesions was strictly followed. Because of these limitations, the study findings should not be generalized to the population of older individuals with dry mouth. A well-designed clinical double-blinded randomized trial may validate or invalidate the findings of the present *in vitro* study.
6. **CONCLUSIONS**

Biotene® and MouthKote® xerostomia products produced substantial mineral loss in pre-demineralized as well as in unaltered enamel of extracted human teeth. MouthKote®, as compared to Biotene®, produced greater demineralization.

The fluoride-containing mouth rinse aided in re-mineralization of the lesions, albeit not to the baseline levels, in all experimental groups except for the lactic acid/MouthKote® group where further dissolution of enamel was observed.

The study hypothesis was partially accepted because only MouthKote® induced demineralization in both demineralized and sound enamel, whereas Biotene® only induced demineralization in previously demineralized enamel but not in sound enamel.

Given the clinical limitations of this study protocol, until further evidence is acquired, the use of MouthKote® xerostomia product could safely be recommended only for edentulous individuals with dry mouth.

6.1. **Suggestions for future research**

Considering the limitations of the present study but given that potential harmful effects of the xerostomia products are possible, the findings of the present study need to be validated in controlled *in vivo* clinical study. There is also a need for more rigorously designed clinical studies enabling exploration into new avenues of xerostomia management.

Disclaimer: The author has no interest in any dental manufacturer.


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APPENDIX A - WITHIN-GROUP COMPARISON

The box plots are used as a visual illustration in this study, which can give a quick overview of the patterns of longitudinal changes within each group and understand what is happening during each phase of the experiment.

Interpreting box plots: Numbers represent actual cases (teeth) which can be traced; an asterisk symbol (*) denotes that the case is further from the main distribution, while a circle symbol (O) denotes that the case is closer to the distribution.

Findings from within-group comparisons are presented in Tables 9 to 21 and figures A1a, A1b, A1c to A5a, A5b, and A5c.
Figures A1a, A1b, and A1c illustrate the patterns of longitudinal changes in the control group for the $\Delta F$, $\Delta Q$, and WS measurements from the experiment. Refer to table 9 for comparison of means and SD.

![Figure A1a. Comparison of $\Delta F$ change in the control group at different phases of experiment](image)

$\Delta F$ - indicates mean fluorescence loss (%)
Figure A1b. Comparison of $\Delta Q$ change in the control group at different phases of experiment

$\Delta Q$ - indicates fluorescence loss in the lesion (%)
Figure A1c. Comparison of WS change in the control group at different phases of experiment

WS - indicates extent of the lesion area (%/mm²)
Figures A2a, A2b, A2c illustrate longitudinal changes in the experimental group (EG1) during different phases of the experiment. Tables 10, 11, and 12 present mineral changes in experimental groups at different immersion phases, in particular from lactic acid into the xerostomia products.

![Figure A2a. Longitudinal changes in the experimental group (EG1) at ΔF level]

ΔF - indicates fluorescence loss in the lesion (%)
Figure A2b. Longitudinal changes in the experimental group (EG1) at ΔQ level

ΔQ - denotes fluorescence loss in the lesion (%)
No gain, very slight changes were observed for some specimens.
Statistically significant gain in minerals was noted.
Figure A2c. Longitudinal changes in the experimental group 1 (EG1) at WS level

WS - denotes extent of the lesion area (%/mm²)
Figures A3a, A3b, A3c illustrate longitudinal changes (ΔF, ΔQ, and WS) in experimental group (EG2) during experiment.

Figure A3a. Longitudinal changes in experimental group 2 (EG2) at ΔF level

ΔF - indicates mean fluorescence loss (%)
Figure A3b. Longitudinal changes in experimental group (EG2) at $\Delta Q$ level

$\Delta Q$ - indicates fluorescence loss in the lesion (%)
Figure A3c. Longitudinal changes in experimental group (EG2) at WS level

WS - indicates extent of the lesion area (%/mm²)
Figures A4a, A4b, A4c illustrate longitudinal changes of mineral content (ΔF, ΔQ, and WS) in the experimental group 3 (EG3) at different stages during experiment.

Figure A4a. Longitudinal changes of mineral content in the experimental group 3 (EG3) at ΔF level

ΔF - indicates mean fluorescence loss (%)
Figure A4b. Longitudinal changes of mineral content in the experimental group 3 (EG3) at ΔQ level

ΔQ - indicates fluorescence loss in the lesion (%)
Figure A4c. Longitudinal changes of mineral content in the experimental group 3 (EG3) at WS level.

WS - indicates extent of the lesion area (%/mm²)
Figures A5a, A5b, A5c illustrate longitudinal changes ($\Delta F$, $\Delta Q$, and WS) in experimental group 4 (EG4) during experiment.

Figure A5a. Longitudinal changes in experimental group 4 (EG4) at $\Delta F$ level

$\Delta F$ - indicates mean fluorescence loss (%)
Figure A5b. Longitudinal changes in experimental group 4 (EG4) at ΔQ level

ΔQ - indicates fluorescence loss in the lesion (%)
Figure A5c. Longitudinal changes in experimental group 4 (EG4) at WS level

WS - indicates extent of the lesion area (\%/mm²)
APPENDIX B - ACROSS-GROUP COMPARISONS

Figures B1a, B1b and B1c depict changes in control group and experimental groups EG3 and EG4 (ΔF, ΔQ, and WS) at baseline and after immersion into xerostomia products.

ΔF - indicates mean fluorescence loss (%)
Figure B1b. Comparisons among groups (EG3 and EG4) without demineralization at \(\Delta Q\) level

\(\Delta Q\) - indicates fluorescence loss in the lesion (%)
Figure B1c. Comparisons among groups (EG3 and EG4) without demineralization at WS level

WS - indicates extent of the lesion area (%/mm²)
Figures B2a, B2b and B2c illustrate changes of mineral content (ΔF, ΔQ, and WS) in the control group and experimental groups (EG1, EG2) after Lactic acid and/or xerostomia products exposure.

Figure B2a. Comparisons among groups (EG1 and EG2) with demineralization at ΔF level

ΔF - denotes mean fluorescence loss (%)
Figure B2b. Comparisons among groups (EG1 and EG2) with demineralization at $\Delta Q$ level

$\Delta Q$ - denotes fluorescence loss in the lesion (%)
Figure B2c. Comparisons among groups (EG1 and EG2) with demineralization at WS level

WS - denotes extent of the lesion area (%/mm²)