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https://doi.org/10.1016/j.jdent.2017.08.013

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Title: Comparison of the Newer Preventive Therapies on Remineralisation of Enamel *in vitro*

Authors: M. Bataineh, M. Malinowski, M.S. Duggal, J.F. Tahmassebi

PII: S0300-5712(17)30209-9
DOI: http://dx.doi.org/10.1016/j.jdent.2017.08.013
Reference: JJOD 2827

To appear in: *Journal of Dentistry*

Accepted date: 24-8-2017

Please cite this article as: Bataineh M, Malinowski M, Duggal MS, Tahmassebi J.F. Comparison of the Newer Preventive Therapies on Remineralisation of Enamel in vitro. *Journal of Dentistry* http://dx.doi.org/10.1016/j.jdent.2017.08.013

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Title: Comparison of the Newer Preventive Therapies on Remineralisation of Enamel in vitro

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Abstract

Objectives: To investigate in vitro the effect of MI Paste, MI Paste Plus and 2800 ppm fluoride (F) toothpaste (TP) on the remineralisation of enamel subsurface lesions and to compare these to the effect of 1450 ppm and 0 ppm F toothpastes.

Methods: Enamel subsurface lesions were created in bovine enamel slabs (n=120) which were assigned randomly to five groups; (1) 0 ppm F TP, (2) 1450 ppm F TP, (3) 2800 ppm F TP, (4) 1450 ppm F TP + MI Paste (Tooth Mousse-TM,10% w/v CPP-ACP) and (5) 1450 ppm F TP + MI Paste Plus (Tooth Mousse Plus,10% w/v CPP-ACP, 900 ppm F as 0.2% w/w sodium fluoride). The enamel slabs were subjected to a pH cycling regimen for 21 days. Quantitative Light-induced Fluorescence (QLF) images were taken and analysed. Data analysis was carried out using one way ANOVA.
Results: In all groups, both $\Delta F$ (percentage fluorescence loss) and $\Delta Q$ ($\Delta F$ times the area) values improved significantly within the same group after the treatment. In addition, the mean difference in $\Delta F$ of the non-fluoride control group was significantly lower than all other groups but not for the 2800 ppm F group. Whereas the mean difference in $\Delta Q$ of the non-fluoride control of group was significantly lower when compared with all other groups ($p < 0.05$).

Conclusions: Both MI Paste and MI Paste Plus when used in conjunction with 1450 ppm F did not show a significant increase in efficacy for the remineralisation of bovine enamel subsurface lesions in the model used in this study.

Clinical Significance:

Newer preventive agents such as MI paste and MI paste plus are advocated as promoting remineralisation when used in addition to routine oral care. This in vitro study shows that they may have a limited value in promoting remineralisation over and above that of 1450 ppm F toothpaste used twice a day.

Key words: remineralisation/demineralisation, enamel, fluoride, casein phosphopeptide -amorphous calcium phosphate.
INTRODUCTION

Dental caries remains a significant public health problem in most developed countries [1, 2]. Restoration of carious teeth has significant cost implications [3]. Remineralisation of at least early lesions would provide a significant benefit to the patient in that it would avoid invasive dental treatment and also reduce costs for management of disease.

In the last decade a number of products and therapies, both for professional application and home use have been introduced into the market. These are supposed to be used in addition to normal twice a day tooth brushing with a fluoride toothpaste. In particular products based on milk proteins, such as casein phosphopeptide have been reported to enhance remineralisation of early carious lesions [4].

The anti-cariogenic properties of milk and milk products have been demonstrated in human and animal models [5,6] and the chemical effect of the phosphoprotein casein and calcium phosphate components were proposed as the main mechanism for this action [7,8]. Casein phosphopeptide (CPP) has the ability to stabilise calcium and phosphate in high concentrations at the tooth surface thereby inhibiting demineralisation and enhancing remineralisation.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been shown to have anti-cariogenic activity in laboratory, animal, human in situ and clinical experiments. The ability of CPP-ACP to enhance the remineralisation of enamel subsurface lesions has been revealed in several studies [9-13].

The role of CPP-ACP involves the localisation of ACP on the tooth surface; this in turn leads to buffering of the free calcium and phosphate ion activities, which help to maintain a state of super-saturation with respect to enamel by suppressing demineralisation and enhancing remineralisation. The presence of CPP-ACP might permit a rapid return to resting calcium concentration and allow earlier remineralisation of enamel substrate [14].

The synergistic effect of CPP-ACP and fluoride in caries prevention has been reported in a number of studies [15, 16]. This effect has been attributed to the formation of CPP-stabilised amorphous calcium fluoride phosphate (CPP-ACFP) [17], which results in an increase in the concentrations of fluoride ions together with bio-available calcium and phosphate ions and their localisation at the tooth surface by the CPP.

Most of the studies on the efficacy of CPP-ACP have investigated its effect when used alone for early lesion remineralisation [18, 19]. On the other hand, there is a lack of evidence on its
effect when added to the regular oral hygiene practice that involves brushing the teeth with fluoridated toothpaste.

Therefore, the aims of the current study were to investigate the effect of the commercially available topical crèmes containing CPP-ACP and CPP-ACFP on the remineralisation of enamel subsurface lesions in vitro when used supplementary to fluoridated toothpaste 1450 ppm F, and also, to compare their effect in vitro on the remineralisation resulting from the use of a higher concentration (2800 ppm) fluoride toothpaste alone.

**Null hypothesis:**

There is no difference in the remineralisation of enamel subsurface lesions with the following regimes: 1450 ppm F TP + MI Paste, 1450 ppm F TP + MI Paste Plus, 0 ppm F TP, 1450 ppm F TP, and 2800 ppm F TP.

**Materials and Methods**

This was an in vitro study using bovine enamel and pH cycling regime.

**Power Calculation**

Statistical advice was sought and the sample size was calculated by using data from a previous PhD thesis ‘Investigations into the effect of casein phosphopeptide-amorphous calcium phosphate on enamel demineralisation and remineralisation [20]. A total of 22 enamel slabs per group were needed. This calculation was based on the assumption that the standard deviation of the response variable was 2.0, power 90%, 0.05 significance level and a true difference between treatments would be adjusted to 3 units. This was based on the calculations by [MGH Biostatistics Centre](http://biostatistics.mgh.harvard.edu) software [21].

**Preparation of the Enamel Slabs and Sub-Surface Lesions**

All enamel slabs used in the present study were obtained from bovine incisors.

Approval for collection of bovine teeth was sought and obtained from the Food Standards Agency. The teeth were obtained from an abattoir and stored immediately in 0.1% thymol (Sigma Aldrich) solution in the fridge. Before sectioning, the teeth were cleaned using a spoon excavator and a toothbrush to remove any soft tissue remnants. To detect any defects, caries or cracks, all teeth were screened by trans-illumination and transmitted light using low-power microscopy (Leitz, Wetzlar®, Germany).
Each tooth was mounted using ‘green stick’ impression compound (Kerr, UK) on plates. The crowns were sectioned using a water cooled, diamond wire saw, cutting machine (Well@Walter EBNER, CH-2400 Le Loche). The buccal and palatal surfaces of each crown were separated, and each buccal section was cut into two slabs that were approximately 6 x 5 x 3 mm in size.

Each enamel slab was mounted on a plastic rod using “sticky wax” to hold the slab in the demineralising gel. The rod was secured to the lid of a “Sterilin” type universal tube so that when the top was screwed onto the tube, the tooth was suspended in the centre of the tube free space. Two coats of an acid resistant, coloured nail varnish (Max Factor, “Glossfinity”) were then applied on the enamel slabs, except for a small window of approximately 2 x 3 mm on the centre of each slab that was left exposed. An interval of 24 hours was left between the two applications to allow the nail varnish to dry completely.

Once the enamel slabs were prepared, they were kept in deionised water in plastic containers at room temperature to prevent dehydration.

Acidified hydroxyethyl cellulose gel was prepared by adding 0.1 M sodium hydroxide (BDH Analar Grade) to 0.1 M lactic acid (Sigma Aldrich D/L GPR 87% Lactic acid) to give a pH value of 4.5 and then 6% w/v hydroxyethyl cellulose (Sigma Aldrich) was added to the solution and stirred for one hour. The mixture was left to settle for 24 hours. Each enamel slab was immersed in 15 ml of acid gel for 10 days to produce an artificial enamel subsurface lesion (Figure 1). The enamel slabs were removed from the acid gel and washed with distilled water, the nail varnish was then removed using methanol (HPLC Gradient grade, method development, Fisher Scientific) to prepare the enamel slabs for the baseline QLF measurements.

**The ΔF Range of the Artificial Lesions**

After performing the QLF baseline analysis for all enamel slabs, the range of ΔF values were found to vary between -7.66 and -31.98. The enamel slabs within the ΔF range of -14.12 to -26.65 with an average of -20.75, were selected for use in the study to enable detection of the differences in ΔF post-treatment. A sufficient number of lesions were made, in order to discard the outliers with a ΔF less than -14.00 or with ΔF more than -27.00).

**Study Materials**

- Non-fluoride toothpaste (the Boots Company PLC, Nottingham, England).
- Fluoride toothpaste 1450 ppm F (0.32% w/w sodium fluoride) (Colgate cool stripe. Colgate–Palmolive (UK) Ltd, Guildford, England).
• High fluoride toothpaste 2800 ppm F (0.619 w/w sodium fluoride) (Duraphat®. Colgate–Palmolive (UK) Ltd, Guildford, England).
• MI Paste 10% w/v CPP-ACP (GC MI Paste™, GC Corp, Tokyo, Japan).
• MI Paste Plus 10% w/v CPP-ACP, 900 ppm F (0.2% w/w sodium fluoride) (GC MI Paste Plus™, GC Corp, Tokyo, Japan).

Study and Control Groups

The enamel slabs (n=120) were randomly assigned to five groups:

- Group 1: Fluoride-free Toothpaste (0 ppm F) (negative control).
- Group 2: Fluoride toothpaste (1450 ppm F) (positive control).
- Group 3: High Fluoride toothpaste (2800 ppm F).
- Group 4: MI Paste (10% w/v CPP-ACP) + Fluoride toothpaste (1450 ppm F).
- Group 5: MI Paste Plus (10% w/v CPP-ACP + 900 ppm F) + Fluoride toothpaste (1450 ppm F).

Toothpaste slurries were prepared by mixing the toothpaste with artificial day time saliva in a volume ratio 1:4 (toothpaste: saliva) by weight, using a WhirliMixer® (Fisons) for 1 minute.

The slurries of MI Paste (10% w/v CPP-ACP) and MI Paste Plus (10% w/v CPP-ACP + 900 ppm F) were prepared by mixing 1 g of the paste with 4 ml of distilled water [22] using a WhirliMixer® (Fisons) for 1 minute.

All enamel slabs were randomly assigned to five groups using a table of random numbers. When the slabs were analysed using QLF (QLF-D Biluminator™ 2) (Inspektor Research Systems BV, Amsterdam, The Netherlands), the investigator did not know to which group each of the enamel slabs belonged to thus making the analysis completely blind.

The pH Cycling Regime

Each enamel slab was attached to a plastic rod (holder). The enamel slabs were rinsed with distilled water for 1 minute then dipped in the appropriate toothpaste slurry for 30 minutes. After that the enamel slabs were rinsed with distilled water for 1 minute and placed in daytime artificial saliva for 60 minutes. The enamel slabs were then exposed to the first demineralisation challenge by dipping in acetic acid solution (pH 4.8) for 5 minutes, then rinsed with distilled water for 1 minute and placed in daytime artificial saliva. This process was repeated until the enamel slabs were subjected to 5 demineralisation challenges, in
order to mimic five episodes of food consumption throughout the day, during which the drop
in pH to critical level lasts five minutes. After the last acid exposure the enamel slabs were
dipped in the toothpaste slurry for 30 minutes (Figures 2 and 3).

For the 0 ppm F, 1450 ppm F and 2800 ppm F TP groups, enamel slabs were then placed in
the night time artificial saliva. While for the 1450 ppm F + TM and 1450 ppm F + MI plus
groups, and following the toothpaste dipping in 1450 ppm F toothpaste slurry, the enamel
slabs were rinsed gently with distilled water and then dipped in the MI Paste or MI Paste
Plus slurries for 30 minutes and finally were placed in the night time artificial saliva without
rinsing. The enamel slabs were kept in an incubator at 37˚C at all times except during
dipping in the slurries or the demineralisation solution. MI Paste and MI Paste Plus were
used in slurry form with artificial saliva in order to mimic the dilution by saliva in the mouth.
The composition of the day and night time artificial saliva has been published [22]

Quantitative Light-Induced Fluorescence (QLF) Measurements

For each enamel slab, QLF measurements were obtained after the creation of the enamel
subsurface lesions and at the end of the 21 days experiment period using the QLF machine
(QLF-D Biluminator™ 2), under controlled conditions. All the slabs were dried for 15
seconds with compressed air prior to imaging, and were examined in a dark room using the
following settings: shutter speed of 1/30 s, aperture value of 6.7, and ISO speed of 1600. All
digital images were stored automatically on a personal computer with image-capturing
software (C3 version 1.16; Inspektor Research Systems, Amsterdam, The Netherlands). All
fluorescence images were examined with analysing software (QA2 version 1.16; Inspektor
Research Systems). The analyses were performed by a single trained examiner.

To ensure that images of the enamel slabs were always captured in the same camera
positions and from the same angles, the camera was attached to a stand in the same
position for all the images. The QLF camera was fixed at a position that provided optimum
illumination of the enamel block surface. The camera specimen distance was standardised
using the jig thereby controlling specimen stability light intensity and magnification.

A patch was drawn around the white spot lesion site by the study examiner with its borders
on sound enamel. Inside this patch, the fluorescence levels of sound tissue were
reconstructed by using the fluorescence radiance of the surrounding sound enamel. The
percentage difference between the reconstructed and the original fluorescence levels was
calculated. The same area of interest was used for the baseline and endpoint white spot
lesion image identification.
For each enamel lesion the following three measurements were obtained: \( \Delta F \): Percentage fluorescence loss with respect to the fluorescence of sound tooth tissue; related to lesion depth (%), Area: The surface area of the lesion expressed in pixels\(^2\) (px\(^2\)), \( \Delta Q \): The \( \Delta F \) times the Area. Percentage fluorescence loss with respect to the fluorescence of sound tissue times the area; related to lesion volume (% px\(^2\)).

**Intra-Examiner Reproducibility**

The study investigator randomly retested 15% of the enamel slabs with the QLF at the end of the experiment. Intra-examiner reproducibility was tested using the Bland-Altman plot as well as using Intra-class Correlation Coefficient (ICC). The intra-examiner reproducibility was tested using the Bland-Altman plot. The mean of the differences or the bias was 0.04% which is very close to 0 indicating that there was a good level of agreement. The Intra-class Correlation Coefficient was found to be (0.99) which also represents a very good reproducibility.

**Statistical Analysis**

The data were analysed using SPSS statistical software package for windows version 20.0. Descriptive statistics were used to calculate the mean, median, range, and standard deviation. The normality of the data distribution was assessed using Shapiro-Wilk test and Kolmogorov-Smirnov test.

Paired sampled t-tests were performed to compare the changes in remineralisation between baseline and post-treatment measurements within the same group. Bonferroni correction was applied to account for multiple testing.

One way ANOVA was used to compare between the five groups when the data were normally distributed, and Kruskal-Wallis test was used when the data were not normally distributed. Furthermore, Mann-Whitney U test was used to assess if there was any significant difference between each of the groups. The test calculated the 95% confidence interval and the significance level was set at a level of \( p < 0.05 \).

**RESULTS**

Three main parameters for QLF were statistically analysed, these were: \( \Delta F \), \( \Delta Q \), and Area.

One way ANOVA test was performed to assess if there was any statistically significant difference in \( \Delta F \), \( \Delta Q \) and Area values at the baseline between the lesions assigned to the five groups. No statistically significant difference was found.
The results showed a statistically significant improvement in the ΔF and ΔQ values post-treatment compared with that at baseline in all groups, paired sampled T-test (p < 0.001). While improvement in the lesion area was seen in all groups (p<0.001) except the control group (p=0.481).

The means and standard deviations for the differences in the ΔF, ΔQ, and Area (baseline – post-treatment) for the study groups are presented in Table 1. Figures 4-6 represent the box-and-whisker plots for the difference in ΔF, ΔQ, and Area of all the study groups.

Tables 2 and 3 show the multiple comparisons of the differences in ΔF and ΔQ at baseline and post-treatment between all test and control groups using One-Way Anova with Pairwise comparisons with Bonferroni correction test. The mean difference in ΔF of the 0 ppm F control group was significantly lower compared to the 1450 ppm F, 1450 ppm F + TM and 1450 ppm F + MIPlus groups, (p< 0.05). However, no significant difference was observed between the 0 ppm F and 2800 ppm F groups. The mean difference in ΔQ of the 0 ppm F was statistically significant lower compared to all groups (p < 0.05).

The results showed the mean difference in the lesion area was statistically significant between the groups (p < 0.001) and further statistical test revealed that the mean difference in the lesion area of the 0 ppm F group was statistically significantly lower compared to all groups (p < 0.001).

**DISCUSSION**

The null hypothesis was rejected as significant differences were found in the enamel remineralisation for ΔF, ΔQ and area between the test groups and the negative control group.

The potential of the remineralisation of enamel lesions has been demonstrated by Dirks [23], who noticed spontaneous disappearance of the white spot lesions in young individuals as a result of the remineralising ability of saliva. In the present study the effects of both CPP-ACP (MI Paste) and CPP-ACFP (MI Paste Plus) on the remineralisation of enamel subsurface lesions were assessed. Both materials were used as one application after the use of fluoridated toothpaste (1450 ppm F).

The results showed that in all QLF parameters (ΔF, ΔQ and lesion area) the 1450 ppm + TM and 1450 ppm + MI plus groups produced remineralisation of the enamel subsurface lesions which was statistically significant compared with the baseline as well as in comparison to the remineralisation produced in the negative control group (0 ppm F). This remineralisation was
also found to be greater in comparison with that following the use of the 1450 ppm F and 2800 ppm F groups but this difference failed to reach significance.

In the current model both CPP-ACP and ACP-ACFP were used directly after the use of the 1450 ppm F toothpaste. The use of F toothpaste may have caused a blockage of the surface pores of the lesions by the formation of fluorapatite in the surface layer which in turn could prevent the diffusion of the calcium and phosphate ions from the CPP-ACP into the body of the lesions to promote remineralisation.

The positive effect of CPP-ACP (MI Paste) and CPP-ACFP (MI Paste Plus) in the remineralisation of enamel lesions has been shown in previous in vitro studies [24-26]. However, most of these have used surface softened enamel rather than the sub-surface lesions, which was used in the current study. Such lesions are likely to be easier to remineralise due to the lack of the surface zone present in the lesions. The porous nature of the lesions in these studies compared to the less porous surface layer could account for the lack of synergistic effect of fluoride and CPP-ACP as the CPP-ACP may not be as able to penetrate into the lesion. Also, several of these studies have used the these remineralising crèmes on their own, and this does not reflect the actual way they are usually used in for home oral care where they are always used in addition to routine use of fluoride toothpaste.

The additive effect of the CPP-ACP and fluoride was reported to result from the interaction between the CPP-ACP with fluoride ions to produce ACFP [16, 17] which in turn promote the localisation of the fluoride ion at the tooth surface. The presence of fluoride ions along with the calcium and phosphate ions in plaque increases the level of fluorapatite saturation; consequently promoting the remineralisation of enamel with fluorapatite in response to the acid challenge. Unexpectedly, in the present study, the CPP-ACP group produced more remineralisation than the CPP-ACFP group. However, this difference was not found to be statistically significant. The reason for such a finding may have been caused by the use of a higher fluoride concentration in the CPP-ACFP group which might have resulted in the formation of the surface hyper-mineralised layer that inhibited the diffusion of calcium and phosphate into the body of the lesion. Bearing in mind that the use of the CPP-ACFP as well as the CPP-ACP crèmes were performed after the application of the 1450 ppm F twice daily which in turn could have been responsible for the formation of the surface mineral rich zone.

Another explanation for this finding is the formation of calcium fluoride precipitate (crystals) when the fluoride ions are present along with calcium ions from CPP-ACP resulting in less fluoride and calcium ions available to diffuse down the body of the lesion to promote remineralisation. This is particularly significant when using an in vitro model that is lacking in natural salivary proteins especially the calcium stabilising proline-rich protein and statherin.
In vivo, CPP-ACP will likely exert an enhanced effect, due to the ability of CPP to be incorporated into the salivary pellicle and at the surface of bacteria in the plaque biofilm [9,10, 14].

In the current study, a 21 day period of pH cycling was implemented in order to allow sufficient time to produce changes in the pre-demineralised enamel slabs. This demineralisation challenge represented the acid in the cariogenic challenge. The pH cycling protocol used for the current study was developed at the University of Leeds and it has been used in a previous caries study [27] at the Leeds School of Dentistry, Department of Paediatric Dentistry.

Bovine enamel with artificial subsurface caries-like lesions was used in the present study. The mineral distribution in carious lesions of bovine teeth is reportedly similar to that found in human teeth, and the structural changes are comparable in human and bovine teeth [28]. Bovine teeth are easier to obtain, have a more uniform composition when compared to human teeth, and have been generally demonstrated to perform similarly to human teeth [29]. Previous studies have also shown that the method used in the present study to produce subsurface caries-like lesions produces uniform subsurface lesions, when examined using Transverse Microradiography [22].

In the present study the results showed that all toothpastes led to remineralisation of the enamel subsurface lesions, with the largest effect seen with the 1450 ppm F toothpaste followed by that produced by the 2800 ppm F toothpaste. It was noted that the higher fluoride concentration of 2800 ppm toothpaste did not follow the dose response curve. However, when ΔQ was used as the outcome measure, a significant difference was observed between 0 ppm F and 2800 ppm F, but this was not evident for ΔF. A possible explanation for this anomaly is that ΔQ reflects the total demineralisation which also takes into account the area of the lesion and the depth. ΔF is an average measure of fluorescence loss which is a reflection of the average demineralisation in the measured lesion. With 2800 ppm F, although the area of the lesion seemed to have been reduced leading to an improved ΔQ, the average mineral loss and depth of the lesion across the remaining lesion did not significantly improve with 2800 ppm F compared to the 0 ppm F control.

This was an interesting result, given that it has been shown in a randomised, double-blind clinical trial [30] that 2800 ppm F dentifrice reduced caries experience by 20.4%, which was an 85% greater reduction than that produced by the 1700 ppm F, and statistically significantly comparable to the 1100 ppm F. This clinical study had clearly shown a dose response. The F dose response was not observed for remineralisation of subsurface caries-like lesions in the current study; a possible explanation could be that a high F concentration
in the toothpaste slurry produced a surface mineral rich layer which in turn compromised the remineralisation of the body of the lesion. Some evidence to support this hypothesis can be found in the literature. The remineralisation of white spots lesion by fluoride was investigated in previous studies [31], which showed that the topical applications of high concentration fluoride solutions resulted in a higher initial, but lower subsequent, rates of mineral deposition. The deposition of fluorapatite in the surface layer can result in the blockage of the surface pores and subsequently restrict diffusion into the lesion. The resulting surface would probably be much more caries-resistant compared to the original enamel, but it still retained a demineralised appearance [32].

Conclusions

Both MI Paste and MI Paste Plus when used in conjunction with 1450 ppm F did not show a significant increase in efficacy for the remineralisation of bovine enamel subsurface lesions in the model used in this study.

Acknowledgements

We would like to thank Dr Simon Strafford and Professor Jack Toumba for their help and advice with this study.

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addition of citric acid and casein phosphopeptide-amorphous calcium phosphate to a sugar-

enamel subsurface lesions in situ by the use of three commercially available sugar free


**Figures**

Figure 1. QLF image taken with the blue light showing the demineralised lesion with an intact surface in the centre of the enamel slab as well as a patch drawn around the lesion with the border in sound enamel. (1: sound enamel, 2: demineralised enamel, 3: patch drawn around the lesion to include sound enamel for reference).
Figure 2: Flow chart of the pH cycling protocol for the 0 ppm F, 1450 ppm F and 2800 ppm F toothpastes groups.
Figure 3: Flow chart of the pH cycling protocol for the 1450 ppm F + MI and 1450 ppm F + MI Plus groups.
Figure 4: Boxplot for the difference in ΔF at baseline and post-treatment for all study groups. Error bars represent SD; the line in the Box-and-whisker plot is the median value of the data. The median values were close in the 1450 ppm F, 1450 ppm F + TM and 1450 ppm F + MI Plus groups.
Figure 5: Boxplot for the difference in ΔQ at baseline and post-treatment for all study groups. The medians are close in the 1450 ppm F, 2800 ppm F, 1450 ppm F + TM and 1450 ppm F + MI Plus groups.

Figure 6: Boxplot for the difference in the lesion area at baseline and post-treatment for all study groups. It shows skewness of the data in the 0 ppm F group. The medians are close in the 1450 ppm F, 1450 ppm F + TM and 1450 ppm F + MI Plus groups.
Table 1: Mean difference in ΔF, ΔQ and Area for all study groups between baseline and post treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean Difference in ΔF at baseline and post-treatment± SD</th>
<th>Mean Difference in ΔQ at baseline and post-treatment± SD</th>
<th>Mean Difference in area at baseline and post-treatment± SD</th>
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</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>24</td>
<td>3.647 ± 1.89</td>
<td>15124.3 ± 25091.00</td>
<td>165 ± 1129.03</td>
</tr>
<tr>
<td>1450 ppm</td>
<td>24</td>
<td>5.887 ± 2.03</td>
<td>45447.8 ± 17787.43</td>
<td>-1082 ± 746.53</td>
</tr>
<tr>
<td>2800 ppm</td>
<td>24</td>
<td>4.869 ± 2.66</td>
<td>37226.3 ± 22747.10</td>
<td>-971 ± 1018.20</td>
</tr>
<tr>
<td>1450 ppm + MI</td>
<td>24</td>
<td>6.219 ± 2.57</td>
<td>53651.2 ± 25712.28</td>
<td>-1607 ± 1292.69</td>
</tr>
<tr>
<td>1450 ppm + MI Plus</td>
<td>24</td>
<td>6.107 ± 2.53</td>
<td>45335.4 ± 22596.46</td>
<td>-1134 ± 630.32</td>
</tr>
</tbody>
</table>

No.: number, SD: standard deviation
Table 2: Multiple comparisons of the difference in ΔF at baseline and post-treatment between test and control groups.

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>1450 ppm</td>
<td>-2.24004*</td>
<td>.68065</td>
<td>.013</td>
<td>-.4.1882 - .2919</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2800 ppm</td>
<td>-1.22179</td>
<td>.68065</td>
<td>.753</td>
<td>-.3.1700 .7264</td>
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Table 3: Multiple comparisons of the difference in $\Delta Q$ at baseline and post-treatment between test groups and control.

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*: The mean difference is significant at the 0.05 level.
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*. The mean difference is significant at the 0.05 level.