The Anti-Gingivitis Effect of Two Commercial Toothpastes: A 21 Day, Partial-Mouth, Double Blind, Randomized Study

Giovanna Garuti1,*, Eleonora Forabosco2, Serena Benini3, Alessandra Odorici4 and Andrea Forabosco5

1University of Modena and Reggio Emilia, Surgical, Medical and Dental Department of Morphological Sciences related to Transplant, Oncology and Regenerative Medicine
2University of Modena and Reggio Emilia, Surgical, Medical and Dental Department of Morphological Sciences related to Transplant, Oncology and Regenerative Medicine
3Private practice in Modena
4University of Modena and Reggio Emilia, Surgical, Medical and Dental Department of Morphological Sciences related to Transplant, Oncology and Regenerative Medicine
5University of Modena and Reggio Emilia, Surgical, Medical and Dental Department of Morphological Sciences related to Transplant, Oncology and Regenerative Medicine

Abstract: Objective: While mechanical plaque removal represents the standard preventive practice, the high prevalence of gingival disease motivates the use of toothpaste to vehicle chemicals that aid plaque control. The objective of this study is to compare the effectiveness of two commercial toothpastes in reducing gingival inflammation and prevent dental plaque accumulation.

Materials and Methods: This study has a cross-over, randomized and double blind design with a partial mouth experimental model. The study is composed by two experimental phases of 3 weeks each, followed by a 21 day wash out interval. During the experimental phases experimental teeth were protected from toothbrushing through a toothshield filled with the tested toothpaste. At the beginning and at the end of the two experimental phases, plaque and gingival status were measured using Plaque Control Record and the Bleeding Index.

The Student t-test for paired data was used to assess whether differences between toothpastes were statistically significant (p<0.05).

Results: At day 21 the bleeding index (BOP) remains approximately constant despite the increase of Plaque Control in both groups. The toothpaste with MicroRepair/hyaluronic acid/ ZnPCA performed slightly better than the toothpaste with Triclosan/Copolymer, but the difference is not statistically significant.

Conclusions: Tested toothpastes have comparable effectiveness in the contrast of the accumulation of plaque and of gingival inflammation.

Keywords: Gingival index, Hydroxyapatite, Inflammation, Toothpastes, Triclosan.

INTRODUCTION

Dental plaque, a bacterial biofilm, is one of the major aetiologic agents involved in the initiation and progression of dental caries, gingivitis and periodontal disease [1]. Therefore, effective oral hygiene involving removal and control of dental biofilm formation plays a crucial role in prevention and successful treatment of dental disease.

The mechanical removal of supragingival plaque is an effective method of controlling plaque and gingival inflammation [2][3] [4]. According to the American Dental Association, 80% of the plaque can be removed using toothbrush and an interdental oral hygiene aid (floss or interproximal brush) [5].

However, both the absence of oral hygiene habits and the inability to perform correct toothbrushing can render mechanical plaque control insufficient. Most people cannot maintain an adequate standard of gingival health because they lack the deftness and compliance that are needed to toothbrush and floss properly. In general, common subjects are only able to remove about half of the plaque even after having brushed their teeth for 2 minutes [1, 6].

Mechanical plaque removal methods still remain the standard preventive practice that can be applied individually. However, the high prevalence of gingival disease has prompted many researchers into the
The anti-gingivitis Effect of Two Commercial Toothpastes


83

development of additional methods for the control of
dental plaque [1].

Previous work demonstrated that chemical agents
can be used as adjuncts to aid the removal of biofilms
[2, 4, 7], and since toothbrushing with a dentifrice is the
most common form of oral hygiene, toothpastes
represent the best vehicle for chemical adjuncts. The
addition of several different chemicals and antimicrobial
agents to toothpastes, aiming to inhibit the formation of
dental plaque have already been proposed in several
previous work [2, 4, 8, 1].

This study aims to compare the effectiveness of two
different commercial toothpastes in reducing gingival
inflammation and prevent dental plaque growth. One of
the two toothpastes is the Biorepair (Coswell S.p.A.,
Funo-Bologna, Italy) and contains MicroRepair /
hyaluronic acid / ZnPCA. The other toothpaste is
Colgate Total (Colgate-Palmolive S.p.A., Rome, Italy)
and contains Triclosan/copolymer.

MATERIALS AND METHODS

Study Design

This is a cross-over, randomized and double blind
study design with a partial mouth experimental model
[9]. The study is composed by two experimental
phases of 3 weeks each, followed by a 21 days wash out interval [10].

Subject

Subject has been selected among students of
Hygiene Dental School at University of Modena and
Reggio Emilia.

All subjects of this study have been recruited after
having read and signed a written informed consent,
and after having filled a medical history questionnaire.

All procedures of the study were detailed according
to the 2008 Helsinki Declaration 24 and to applicable
Italian Law. Contrary to public and private health centers (DM 18/3/1998 published in the Official
Gazette, GU n. 122 of 28-05-1998), Italian law does not
require ethical committee approval for clinical work
performed in private dental offices and therefore no
ethical committee resolution is released. The study
have been conducted in a private dental offices in
Modena (Italy). The sample size for t-test at 0.05 level,
power of 80/85% was 15 patients for each group.

Inclusion Criteria

All subject have at least 18 years old. All volunteers
had a good oral hygiene and had at least 20 natural
teeth and 4 experimental posterior teeth in the lower
left quadrant. None of the participants showed any form
of periodontal disease. All participants were in good
general health; they were not affected by diabetes, HIV
or AIDS and/or malignancy.

Exclusion Criteria

Subjects with medical disorders, acute infections,
undergoing antimicrobial therapy during the trial or in
the month before, periodontal disease (probing depth >
4mm), use of mouthwash and oral antimicrobial gel
e.g. chlorhexidine) during the trial, smokers and
pregnant or lactating women were excluded.

Randomization and Blinding

Volunteers were then randomized into two groups:
eighteen subject were allocated in the first group and
seventeen in the second group. Subjects were
allocated to the two groups according to an allocation
plan generated with the online tool “randomization
plan”, available from www.randomization.com. The
allocation follows the block randomization approach,
with variable block sizes between two and six.

In this study three operators were involved. The first
operator knew the composition of the toothpastes and
the assigned group. He selected the volunteers, gave
them the assigned toothpaste and the at home
instructions (that were equal for both groups).

The second operator allocated patients into two
groups and performed the statistical analyses. He was
blinded about which toothpaste was assigned to test
group A and which to test group B.

The third operator (executor) measured the clinical
parameters at day 0 and at day 21 and performed
professional oral hygiene; he was blinded with respect
to the patient’s allocation for all the duration of the
study.

Patients were blinded with respect to assigned the
toothpaste: toothpaste samples were packed in
identical and anonymous tubes.

The study is composed by two experimental phases
of 3 weeks each, followed by a 21 days wash out interval. Each group tested both toothpastes.
Pre-Experimental Phase

Volunteers were selected and underwent a complete periodontal screening to check the absence of periodontal pocket (probing depth > 4 mm) and the presence of four posterior natural teeth on the left inferior quadrant.

Each participant underwent a professional oral hygiene to eliminate dental plaque 21 days before starting the experimental phase.

A dental impression was taken to prepare the toothshield (Figure 1).

![Termoforming machine used to produce toothshields.](image)

To favor plaque growth, the teeth considered in this study (molars and premolars in the 4th quadrant) were protected from the mechanical action of toothbrushing by means of a toothshield. The toothshields were made of a thermoplastic mouthguard material and cover the four teeth considered in the study and their gingival margin, thus preventing the mechanical removal of dental plaque (Figure 2). Toothshields allowed for a space of 1 mm around the tooth surface. This space was filled with the tested toothpaste, thus making it possible to measure its antibacterial and anti-inflammatory effects.

![Example of a toothshield used for our study.](image)

Clinical Parameters

At the day 0 and at the day 21 for each experimental periods, plaque and gingival status were measured using two indices:

- Plaque Control Record: PCR (O’Leary Index) [12, 13]
- Bleeding Index: BI [14-16].

Plaque Control Record measures the amount of plaque on tooth surface [13].

Bleeding on probing (BOP) is an indicator of tissue inflammatory response to bacterial pathogens [17, 18]. The operator used WHO ball-end probe on all patients.

Toothpaste Tested

In this study we tested two commercial dentifrices: Biorepair (containing MicroRepair / hyaluronic acid / ZnPCA) and Colgate Total (containing Triclosan and Copolymer).

To prevent any influence over patients and operators, toothpaste samples were packed in identical and anonymous tubes. Tubes were marked with the letter “P” or the letter “C”. Letter “P” is associated to the toothpaste containing MicroRepair / hyaluronic acid / ZnPCA, while letter “C” is associated to the toothpaste containing Triclosan /Copolymer.
During the experimental phases, all subjects brushed with the toothpaste that was assigned to them. During the 21 days of washout all subjects used the same dentifrice containing fluoride.

**Experimental Phase**

Each experimental phase took 21 days. Volunteers were randomly assigned to two groups and they received a new toothbrush, a test dentifrice and a toothshield.

They were instructed to brush and floss their teeth twice a day with the assigned dentifrice except for the four teeth in the lower left quadrant, that had to be covered by the shield. Before seating the toothshield, they had to fill it with the tested dentifrice.

At day 0 and at day 21 of each experimental phases researchers measured PCR and BOP of all the patients. The researcher who recorded BOP and PCR did not know the experimental group to which patients belonged.

At the end of each experimental phases, volunteers underwent a professional oral hygiene.

After the first experimental phase, all subject underwent a wash-out period of 21 days. At the end of the wash-out period, the two test groups switched dentifrice. Hence, test group A was assigned to the dentifrice P in the first experimental phase and to the dentifrice C in the second experimental phase. On the other hand, test group B was assigned to dentifrice C in the first experimental phase and to dentifrice P in the second experimental phase.

**Statistical Analysis**

The results are presented as mean and standard deviation of PCR and BI indexes.

The standard two sample Student t-test for paired data was used to assess whether differences among different treatments were statistically significant (significance level $p<0.05$).

**RESULTS**

**Subject**

Thirty-five subjects participated to the double-blind study. Volunteers comprised 29 female and 6 male subjects (83% female). Volunteers age ranges from 19 to 34 years. The average age is 23.9 with a standard deviation of 4.8 years. All thirty-five subjects completed both the experimental phases without any drop out. All the enrolled subjects received the intended treatment, and all their data have been considered in the statistical analyses without any exclusion.

As the study has a cross over design, results are computed over the two groups with the same toothpaste. So we analyzed data about thirty-five subjects for both toothpaste C and toothpaste P.

**Plaque Control Record and Bleeding on Probing at Day 0**

The mean value and standard deviation for BOP and PCR at day 0 for the tested toothpastes are collected in Table 1. There are no statistically significant differences at day 0 between the two groups for both PCR ($p=0.97$) and BOP ($p=0.28$).

**Plaque Control Record and Bleeding on Probing at Day 21**

The mean value and standard deviation for BOP and PCR at day 21 for tested toothpaste are collected in Table 2. During the 21 days of experimental phases plaque grew on unbrushed tooth surface. As expected, the plaque index (PCR) increased.

To evaluate if the difference in PCR value between day 21 and day 0 is statistically significant we apply the t-test for paired data. The analyses performed on the data showed that this was statistically significant both

---

**Table 1**: Mean Value and Standard Deviations of PCR and BOP at day 0 for Toothpastes MicroRepair / Hyaluronic Acid / ZnPCA (P) and Triclosan/Copolymer (C)

<table>
<thead>
<tr>
<th>Toothpaste</th>
<th>Plaque Control Record</th>
<th>Bleeding on Probing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>MicroRepair / hyaluronic acid / ZnPCA (P)</td>
<td>0.179</td>
<td>0.116</td>
</tr>
<tr>
<td>Triclosan/Copolymer (C)</td>
<td>0.179</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Comparison Between Toothpastes

To compare the effectiveness of the tested toothpastes we analyze the differences between average values of PCR and BOP before and after experimental phases.

As regards the parameter of plaque index (PCR), difference of the means (day 21 – day 0) is 0.100 for toothpaste Triclosan/Copolymer (C) and 0.089 for MicroRepair / hyaluronic acid / ZnPCA (P). The increase in PCR for the toothpaste “P” is slightly lower with respect to that of toothpaste “C”.

The null hypothesis was tested and p value is 0.363 (no statistical significance).

For the Bleeding on Probing (BOP) index, difference of the means (day 21 – day 0) is 0.004 for toothpaste Triclosan/Copolymer (C) and -0.020 for MicroRepair / hyaluronic acid / ZnPCA (P). We applied the student t-test and verified that the difference between the average values of BOP is not statistically significant (p = 0.106).

DISCUSSION

Volunteers recruited in this study were Dental Hygiene student at University. They adopted the best practices for toothbrushing and have a strong compliance with respect to the requirements of this study. Compliance is also increased using a toothshield covering only four posterior teeth. Similar toothshields have already been used in other studies [10, 9, 19].

Toothpastes effectiveness was evaluated through two indices: Plaque Control Record (PCR) and Bleeding on Probing (BOP). Plaque Control Record measures the amount of plaque on tooth surface, while Bleeding on Probing evaluates the presence of gingival inflammation.

A correct toothbrushing technique is sufficient to prevent gingival inflammation and maintain an appropriate oral hygiene. In these conditions, at least 6 months of trial and very large groups of patients are needed to observe gingivitis and evaluate the effectiveness of a toothpaste (or mouthwash) [9, 3, 20, 21].

In particular, a large number of patients would be required to avoid the inherent bias caused by the different toothbrushing habits and techniques used by different patients.

To avoid this bias this study leverages the methodologies that are already proposed in the literature [10, 9, 1, 22, 23, 20]. To favor plaque growth, the teeth examined in this study (molars and premolars in the 4th quadrant) are protected from the mechanical action of toothbrushing thanks to a toothshield.

It is demonstrated that in 2-4 weeks dental plaque can grow, mature and induce gingivitis. For this reason, according to literature, our study is composed by two experimental phases each of 21 days with 21 day of wash out interval between them [10, 7, 19, 24].

This study follows a cross-over design to avoid differences between experimental groups [10, 24, 21].

The difference between PCR values at day 21 and day 0 is positive and statistically significant for both the toothpastes tested. This outcome is expected because

<table>
<thead>
<tr>
<th>Toothpaste</th>
<th>Plaque Control Record</th>
<th>Bleeding on Probing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>MicroRepair / hyaluronic acid / ZnPCA (P)</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>Triclosan/Copolymer (C)</td>
<td>0.29</td>
<td>0.14</td>
</tr>
</tbody>
</table>
dental plaque accumulates on unbrushed surface. These data do not agree with a study that shows a slight reduction in plaque accumulation [2]. However other studies are in accordance with ours and recorded an increasing in dental plaque on unbrushed surface [9, 10, 5, 22].

However, the increase of the plaque index value for toothpaste MicroRepair / hyaluronic acid / ZnPCA (P) is smaller than the increase of PCR related to the toothpaste Triclosan/Copolymer (C). This result suggests a slightly higher efficacy of the toothpaste MicroRepair / hyaluronic acid / ZnPCA (P) in fighting dental plaque growth, but statistical analyses show that the difference between the two tested toothpastes is not significant.

The difference between BOP values at day 21 and day 0 is positive for toothpaste Triclosan/Copolymer (C) and negative for toothpaste MicroRepair / hyaluronic acid / ZnPCA (P). BOP is an index of gingival inflammation [18, 17] and the toothshield was filled with tested toothpaste, thus making it possible to measure its antibacterial and anti-inflammatory effects. This method has already been proposed in other studies [2, 10, 19, 9].

When using toothpaste Triclosan/Copolymer (C), gingival inflammation increased and this is consistent with plaque growth [5] and in agreement with other studies [10, 21].

When using toothpaste MicroRepair / hyaluronic acid / ZnPCA (P), gingival inflammation decreased even if plaque grows. This result is unexpected and suggests that toothpaste is effective in countering gingival inflammation and bleeding.

Data show that MicroRepair / hyaluronic acid / ZnPCA have an anti-inflammatory effect even in the total absence of mechanical dental plaque control.

However, the t-test for paired data does not confirm the statistical significance of the difference in BOP between the tested toothpastes.

CONCLUSION

The study found that, due to the absence of brushing, dental plaque growth measured by PCR increased both in the experimental group treated with toothpaste Triclosan/Copolymer (C) and in the group treated with toothpaste MicroRepair / hyaluronic acid / ZnPCA (P). However, the group treated with toothpaste MicroRepair / hyaluronic acid / ZnPCA (P) presented a lower increase of the PCR index, due to its effectiveness against plaque accumulation and bacterial aggressions.

The bleeding index (BOP) remains approximately constant despite the increase of the accumulation of plaque. This highlights the effectiveness of both toothpastes in fighting gingival inflammation. In particular, the bleeding index improves slightly after the use of the dentifrice containing MicroRepair / hyaluronic acid / ZnPCA (P), thus showing the effectiveness of toothpaste P in fighting gum inflammation and gingival bleeding, main causes of periodontitis.

Although the toothpaste MicroRepair / hyaluronic acid / ZnPCA (P) performed slightly better than the toothpaste Triclosan/Copolymer (C), it was not possible to demonstrate the statistical significance of the higher effectiveness of the dentifrice with MicroRepair / hyaluronic acid / ZnPCA compared to toothpaste with Triclosan /Copolymer. It is still possible to conclude that MicroRepair / hyaluronic acid / ZnPCA is a toothpaste whose properties and use can help fighting and preventing gum’s bleeding and inflammation, also in case of minor gingivitis.

REFERENCES

Garuti et al.


