Tooth whitening evaluation of blue covarine containing toothpastes

Danying Tao,a Richard N Smith,b Qiong Zhang,c Jianing N. Sun,c Carole J Philpotts,d Stephen R Ricketts,d Mojgan Naeeni,d Andrew Joinerd*

aDepartment of Preventive Dentistry, Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai Key Laboratory of Stomatology, Shanghai 200011, China

bSchool of Dentistry, University of Liverpool, UK.

cUnilever Oral Care, 66 Linxin Road, Changning District, Shanghai, 200335, China

dUnilever Oral Care, Quarry Road East, Bebington, Wirral CH63 3JW, UK.

Key Words: Tooth colour, bleaching, perception, aesthetics

Corresponding Author:

Dr. Andrew Joiner

Unilever Oral Care

Quarry Road East

Bebington

Wirral CH63 3JW

United Kingdom

Tel: +44 (0)151 641 3000

Fax: +44 (0)151 641 1800

Email: Andrew.Joiner@Unilever.com
Abstract

Objectives: To measure the tooth whitening effects delivered immediately after brushing with silica-based toothpastes containing blue covarine in vitro and in vivo.

Methods: Salivary pellicle coated human extracted teeth were brushed with either a slurry of a toothpaste containing blue covarine (BC), a formulation containing an increased level of blue covarine (BC+) or a negative control toothpaste containing no blue covarine. The colour of the specimens were measured in vitro using either a Minolta chromameter or a VITA Easyshade spectrophotometer, before and after brushing and changes in CIELAB values and tooth Whiteness Index (WIO) values calculated. In a double-blind cross-over clinical study, subjects brushed with either BC or BC+ toothpaste and tooth colour changes were measured with a digital image analysis system.

Results: The in vitro studies demonstrated that toothpastes containing blue covarine gave a significantly (p<0.05) greater change in b* and WIO values than the negative control toothpaste; the BC+ toothpaste gave a significantly greater increase in b* and WIO values than the BC toothpaste, and BC+ gave a significant increase in shade change versus the negative control. Clinical results showed that BC and BC+ gave a significant reduction in b* (p<0.0001) and increase in WIO (p<0.0001) from baseline indicating significant tooth whitening had occurred. The parameter changes were significantly greater when brushing with the BC+ toothpaste than with the BC toothpaste (WIO p=0.006; b* p=0.013).

Conclusions: Toothpastes containing blue covarine gave a statistically significant reduction in tooth yellowness and improvement in tooth whiteness immediately after brushing in both in vitro and clinical studies. In addition, the higher concentration blue covarine toothpaste gave statistically significant greater tooth whitening benefits than the lower concentration blue covarine toothpaste.

Clinical significance: The silica-based toothpastes containing blue covarine evaluated in the current study gave tooth whitening benefits immediately after one brush.
1. Introduction

Patients and consumers have a dissatisfaction with their current tooth colour as indicated by a number of published studies\(^1,2\) and in many regions of the world have become more interested in the cosmetic benefits derived from a healthy natural dentition. This is most readily apparent from the increased demand for orthodontic care and for products and treatments that whiten teeth.\(^3\) Approaches to improvement of tooth whiteness is currently possible via a number of routes including professional prophylaxis cleaning, placement of veneers and crowns, tooth bleaching and whitening toothpastes.\(^4\)\(^-\)\(^6\)

Traditionally, the majority of tooth whitening products work in one of two ways, either by bleaching of the teeth, or by the removal and control of extrinsic tooth stain. Tooth bleaching typically involves the direct application of hydrogen peroxide or carbamide peroxide containing gels onto the tooth surface via a range of product formats such as a mouth guard, strip or paint-on. The peroxide diffuses into the tooth structure to effect bleaching and lightening of any intrinsic colouration and thus making the teeth appear whiter.\(^7,8\) However, the effective delivery of peroxide from toothpaste is challenging in terms of formulation factors, regulatory restrictions and the relatively short exposure times during brushing.\(^9\)

Historically, one of the key functional ingredients in whitening toothpastes has been the abrasive system that helps to remove and prevent extrinsic stain formation. The abrasive cleaning system is often augmented with a range of other ingredients including surfactants, calcium chelators, enzymes and polymers, but the evidence to date suggests that the primary stain removal ingredient in toothpaste is the abrasive
However, there are international regulatory restrictions on the maximum abrasion levels permitted in a toothpaste and so there are limits to how far whitening can be safely accomplished through abrasive technologies.\textsuperscript{10,11}

A relatively new development in tooth whitening utilises the importance of the yellow to blue tooth colour shift (i.e. reduction of $b^*$ in the CIELAB colour space) in producing an overall improvement in the perception of tooth whiteness.\textsuperscript{12} This hypothesis is supported by tooth bleaching studies\textsuperscript{13,14} that indicate that the yellow-blue ($b^*$) shift in tooth colour is an important indicator of tooth whitening and that a reduction in $b^*$ is the most important colour parameter for self-perceptual tooth whitening.\textsuperscript{15} Using these optical principles, a silica-based toothpaste containing the pigment blue covarine was developed.\textsuperscript{12,16} The toothpaste deposits blue covarine onto the tooth surface during brushing which gives a yellow to blue hue colour shift to the teeth. This reduction in yellowness makes the teeth appear whiter immediately after brushing, as confirmed in a number of \textit{in vitro} and clinical studies.\textsuperscript{12,16,17}

The silica-based toothpaste containing blue covarine has been further developed by increasing the level of blue covarine concentration in order to further enhance the optical tooth whitening benefit. The aims of the current work were to measure, after single brushing, the tooth whitening effects of two silica-based toothpastes containing different concentrations of blue covarine, using \textit{in vitro} models and in a clinical study. The null hypothesis of these studies was that an increase of concentration of blue covarine in toothpaste has no significant effect on measured change from baseline in tooth colour (CIELAB) and whiteness (WIO) after one brush.
2. Materials and Methods

2.1 In vitro study 1

Extracted human anterior teeth, obtained for research purposes according to the Human Tissue Act procedures and with informed consent, were mounted in acrylic resin blocks by embedding the roots into cold-cure acrylic resin (Simplex Rapid, Kemdent, Wiltshire, UK). Any remaining exposed dentine was sealed with two coats of nail varnish (No. 7, Colour Lock™ Nail Enamel, Boots plc, Nottingham, UK). The enamel surfaces were then cleaned with a prophylaxis paste (Clean Chemical Sweden AB, Sweden) to ensure removal of extrinsic stain. The specimens were placed in sterilised (gamma irradiated) whole human saliva for two hours to allow a pellicle to form. The baseline colour of each tooth was measured using a Minolta CR321 chromameter (Minolta Camera Co. Ltd., Japan) in the CIELAB mode. Five separate measurements of the labial surface were taken and the mean baseline colour parameters calculated.

The specimens (n=15 per group) were randomly assigned to the test products which were either a silica-based toothpaste containing blue covarine (BC), the new silica-based toothpaste containing increased levels of blue covarine (BC+) or a silica-based control toothpaste containing no blue covarine.

The specimens were brushed for 1 minute with a flat-trim toothbrush and a 1:2 slurry of toothpaste: water. The specimens were then thoroughly rinsed with water and their colour re-measured. Specimens were then placed in a beaker containing water (400ml) and the water was continuously agitated at 300 rpm using a Heidolph overhead stirrer (Heidolph Instruments GmbH, Schwabach, Germany). After two hours, the colour of the specimens was again re-measured. Changes in tooth colour were calculated as follows:
\[ \Delta L^* = L^*(t) - L^*(0) \]
\[ \Delta a^* = a^*(t) - a^*(0) \]
\[ \Delta b^* = b^*(t) - b^*(0) \]

Where \( L^*(t) \) and \( L^*(0) \) are \( L^* \) values after water rinsing for time \( t \) and pre-brushing values respectively with similar definitions for \( a^*(t) \), \( a^*(0) \), \( b^*(t) \) and \( b^*(0) \). In addition, whiteness values based on a previously described tooth whiteness index (WIO)\(^\text{18}\) were determined at each time point and mean changes in WIO calculated.

### 2.2 In vitro study 2

Human extracted incisors and premolars were prepared as above. The teeth were placed in sterilised (gamma irradiated) whole human saliva for two hours to allow a pellicle to form. The baseline CIELAB values and shade of each tooth was measured using a VITA Easyshade (VITA Zahnfabrik, Germany) in the 3D-Master mode, measuring the body colour of each tooth.

The specimens (n=32 per group) were manually brushed for 10 seconds with a flat-trim toothbrush and a 1:2 slurry (toothpaste: water) of the test toothpaste. The toothpastes used were either the silica-based toothpaste containing increased levels of blue covarine (BC+) or a control silica-based toothpaste containing no blue covarine. Specimens were rinsed for 5 seconds in water (50ml) before re-measuring the tooth colour and mean changes in colour parameters calculated. The tooth shades obtained by the Vita Easyshade were converted to numerical values obtained from a published visual whiteness ranking study of VITA 3D Master shades\(^\text{19}\) and change in tooth shades were calculated.
2.3 Clinical evaluation

Eighty subjects were recruited to participate in this controlled double blind, two products, cross-over design study. The objective of the study was to measure the change in tooth colour and tooth whiteness immediately after brushing with either BC or BC+ toothpaste using a non-contact camera-based digital imaging system (DIS). Smith et al.\textsuperscript{50} have reported a full description of the DIS and its validation previously.

The protocol for the study was reviewed and approved by an Independent Ethics Committee of the Shanghai Clinical Research Centre. Male and female subjects in good general health aged 18-65 were accepted onto the study. Subjects had to have two suitable natural upper central incisors (no caps, crowns, veneers, cracks, gum recession or restorations on the distal, mesial, buccal or incisal edges). The teeth had to be free of extrinsic stain (or if present could be removed by the study dentist) and free of intrinsic stain (e.g. tetracycline, fluorosis).

At the start of each test session, subjects were asked about their compliance with the requirements of the protocol and whether there had been any change in their health or medication. A pre-brushing image of the two upper central incisor teeth of each subject was taken using the DIS. Subjects then brushed with 1.5g (+/- 0.1g) of their assigned toothpaste for 90 seconds using their normal brushing technique, followed by a water rinse (5 ml) for 5 seconds. Immediately after brushing and rinsing, a post-brushing image was taken. The study toothpastes were tested in a randomised order. Subjects had to refrain from eating or drinking for at least one hour before a test session. Subjects used their own toothbrush and toothpaste at home for the duration of the study and were not allowed to use any other forms of oral hygiene. An overview of the study flow is shown in Figure 1.
Tooth colour and whiteness parameters (CIELAB and WIO) were determined. The outcome variable per subject was defined as the average of the colour parameter values measured on the two upper central incisor teeth. Paired t-tests were used to test for significant differences between the pre-images and immediately after (post) images for each product. Between product differences were assessed for statistical significance using repeated measurement analysis in order to take into account for the interdependence of variables measured for each subject. The level of significance was set at p<0.05.

3. Results

3.1 In vitro study 1
The mean changes in CIELAB values immediately after brushing plus water rinse and after 2 hours immersed in water are shown in Table 1. This shows that both toothpastes containing blue covarine gave an immediate reduction in b* and an increase in WIO, indicating a yellow to blue shift in the colour of the teeth and an increase in tooth whiteness respectively, whereas the control toothpaste did not. Statistical analyses were applied to changes from baseline (ANOVA, Tukey-Kramer using JMP, version 8, SAS Institute Inc.) and showed that there was a significant difference for all product comparisons (p<0.05) for Δb* and ΔWIO at both time points. There were no statistical differences between products for ΔL* at both time points. For Δa*, BC+ gave a statistically greater reduction than both BC and control only immediately after brushing and water rinse whereas all other product comparisons at both time points were not significant.

3.2 In vitro study 2
The change in CIELAB colour parameters, WIO and tooth shade following treatment with the blue covarine containing toothpaste and control toothpaste are shown in Table 2. Statistical analyses were performed on changes from baseline and showed
that the toothpaste containing blue covarine gave a greater reduction in a* and b*, an increase in tooth whiteness (WIO) and a greater improvement in tooth shade compared to the control toothpaste. The product difference for these parameters were of statistical significance (p<0.05, Student’s t-test).

3.3 Clinical Evaluation
Eighty subjects were recruited with seventy-nine completing the study. The only one subject withdrawal was due to personal reasons. The mean pre, post and difference in CIELAB and WIO parameters are shown in Table 3. It can be seen that both blue covarine containing toothpastes significantly increased tooth whiteness from baseline as shown by an increase in WIO (WIO change for BC and BC+ was 2.70 and 4.18 respectively) and by a reduction in b* (b* reduction for BC and BC+ was 1.04 and 1.39 respectively). The increase in WIO was greater after brushing with the BC+ toothpaste than after brushing with the BC toothpaste and the product difference was of statistical significance (p=0.006).

4. Discussion
The findings of the present study demonstrated that blue covarine containing toothpaste formulations gave a measureable and statistically significant increase in tooth whiteness (WIO) immediately after one brushing in vitro and in vivo. In addition, the higher concentration blue covarine toothpaste gave a statistically significantly greater increase from baseline in a*, b* and WIO values than the lower concentration blue covarine toothpaste. Thus, the null hypothesis is rejected for these parameters. However, the higher concentration blue covarine toothpaste did not gave a statistically significantly greater increase from baseline in L* values than the lower
concentration blue covarine toothpaste and hence the null hypothesis is accepted for this parameter.

The evaluation of tooth whitening products using *in vitro* methods is important for the testing of hypotheses and the identification of efficacious formulations. Although there are numerous *in vitro* models described in the literature for evaluating tooth whitening, extracted human teeth are the most relevant substrate when evaluating changes in intrinsic tooth colour following product treatment and these have been used successfully to evaluate the efficacy of a range of tooth whitening products. The results of the current studies clearly demonstrate that the *in vitro* models used are able to discriminate between a negative control silica toothpaste and the test toothpastes containing blue covarine in terms of measuring changes in the intrinsic colour of extracted teeth following brushing, thus confirming the validity of the model and approach.

The first *in vitro* study has shown that the blue covarine containing toothpastes gave a significant reduction in tooth yellowness (b*) and increase in tooth whiteness (WIO) compared to the negative control toothpaste, and that the higher blue covarine concentration toothpaste gave greater changes compared to the lower blue covarine concentration toothpaste. This confirms previous *in vitro* evidence where a dose response for blue covarine concentration versus b* reduction and WIO increase has been reported. The colour changes (b* and WIO) after 2 hours water rinsing showed a continued significant product difference and indicates significant substantivity of the blue covarine to the tooth surface under the current experimental conditions.
There are many methods currently used to assess tooth colour, ranging from subjective visual comparisons with coloured porcelain or acrylic shade guides to objective instrumental measurements using spectrophotometers, colorimeters and image analysis techniques.\textsuperscript{23-25} In order to measure objectively tooth shade values, a VITA Easyshade spectrophotometer was used in the second \textit{in vitro} study. These types of devices have been developed to reduce or overcome imperfections and inconsistencies of traditional tooth shade matching via visual comparison with a shade guide.\textsuperscript{24} A number of studies have shown that the VITA Easyshade has excellent repeatability for tooth shade and colour measurement.\textsuperscript{26-30} For example, in a study measuring the ability of the VITA Easyshade to measure commercial shade guides, the reliability and accuracy of the device was found to be 96.4\% and 92.6\% respectively.\textsuperscript{31} In addition, it has been successfully used to monitor tooth colour changes following various whitening treatments (32-34). Thus, the VITA Easyshade is appropriate for evaluating tooth colour and assessing tooth colour changes following product treatments. This is confirmed in the current study where tooth whitening effects were successfully measured using the VITA Easyshade and demonstrating that the blue covarine containing toothpaste gave a significantly greater increase in WIO and tooth shade than the control toothpaste.

The clinical data (Table 3) shows similar whitening behaviour as the \textit{in vitro} data. Paired comparisons showed that both the blue covarine containing toothpastes gave a statistically significant reduction in tooth yellowness ($b^*$) and a statistically significant improvement in tooth whiteness (WIO). In addition the statistical analysis showed that the reduction in $b^*$ value and the increase in WIO was greater for the BC+ group than for the BC group, demonstrating that increasing the blue covarine concentration increases the overall tooth whitening benefit.
Tooth whitening products are marketed in many different formats. However, toothpaste is perhaps the most accessible product format for many patients and consumers. The incorporation of blue covarine into a silica-based toothpaste formulation has been shown in the current studies to be an effective vehicle for delivering improved tooth whiteness and shade immediately after brushing.

5. Conclusions

Toothpastes containing blue covarine gave a statistically significant reduction in tooth yellowness and improvement in tooth whiteness immediately after brushing in both in vitro and clinical studies. In addition, the higher concentration blue covarine toothpaste gave statistically significant greater tooth whitening benefits than the lower concentration blue covarine toothpaste.

Conflict of interest statement

Jianing Sun, Qiong Zhang, Carole Philpotts, Stephen Ricketts, Mojgan Naeeni and Andrew Joiner are employees of Unilever.
REFERENCES


Table 1. *In vitro* study 1: Mean changes from baseline (s.e.) in CIELAB and WIO values immediately following toothpaste brushing plus water rinsing and 2 hours immersion in water

<table>
<thead>
<tr>
<th></th>
<th>After brush and rinse</th>
<th>After 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BC</td>
</tr>
<tr>
<td>ΔL*</td>
<td>0.65(0.17)a</td>
<td>0.63(0.16)a</td>
</tr>
<tr>
<td>Δa*</td>
<td>-0.01(0.04)a</td>
<td>-0.01(0.02)a</td>
</tr>
<tr>
<td>Δb*</td>
<td>0.23(0.10)a</td>
<td>-0.21(0.03)b</td>
</tr>
<tr>
<td>ΔWIO</td>
<td>-0.89(0.31)a</td>
<td>0.89(0.21)a</td>
</tr>
</tbody>
</table>

Change from baseline values with different letters within each time point and within each colour parameter are statistically different (p<0.05, ANOVA, Tukey-Kramer).

Table 2. *In vitro* study 2: Mean changes from baseline (s.e.) in CIELAB values, WIO values and tooth shade immediately following toothpaste brushing plus water rinsing

<table>
<thead>
<tr>
<th></th>
<th>ΔL*</th>
<th>Δa*</th>
<th>Δb*</th>
<th>ΔWIO</th>
<th>Δ Tooth Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Covarine Toothpaste (BC+)</td>
<td>0.05 (0.19)a</td>
<td>-0.28 (0.04)a</td>
<td>-0.72 (0.20)a</td>
<td>3.10 (0.79)a</td>
<td>1.81 (0.30)a</td>
</tr>
<tr>
<td>Control Toothpaste</td>
<td>0.26 (0.17)a</td>
<td>0.13 (0.02)b</td>
<td>-0.07 (0.09)b</td>
<td>-0.02 (0.30)b</td>
<td>0.11 (0.11)b</td>
</tr>
</tbody>
</table>

Change from baseline values with different letters within each colour parameter are statistically different (p<0.05, Student’s t test)
Table 3. Clinical study results: Mean (s.e.) CIELAB and WIO whiteness index values at baseline, post brushing and mean difference

<table>
<thead>
<tr>
<th>Product</th>
<th>Baseline</th>
<th>Post Brushing</th>
<th>Mean Difference Post-Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>BC</td>
<td>78.14 (0.38)</td>
<td>77.82 (0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BC+</td>
<td>78.11 (0.38)</td>
<td>77.76 (0.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product comparison</td>
<td></td>
<td>ns(^b)</td>
<td></td>
</tr>
<tr>
<td>a*</td>
<td>BC</td>
<td>7.51 (0.17)</td>
<td>6.93 (0.17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BC+</td>
<td>7.55 (0.17)</td>
<td>6.54 (0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product comparison</td>
<td></td>
<td>p=0.0005(^b)</td>
<td></td>
</tr>
<tr>
<td>b*</td>
<td>BC</td>
<td>30.44 (0.35)</td>
<td>29.40 (0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BC+</td>
<td>30.44 (0.34)</td>
<td>29.05 (0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product comparison</td>
<td></td>
<td>p= 0.013(^b)</td>
<td></td>
</tr>
<tr>
<td>WIO</td>
<td>BC</td>
<td>-35.85 (1.85)</td>
<td>-33.16 (1.78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BC+</td>
<td>-35.97 (1.77)</td>
<td>-31.78 (1.74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product comparison</td>
<td></td>
<td>p=0.006(^b)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Statistical analysis of change from baseline using paired t-test. \(^b\) Product comparison using repeated measurement analysis.
Visit 1: Screening/Enrolment
Informed consent.
Medical history, oral examination, decision on eligibility.
Suitable subjects will have stain removal from upper central incisors if required.
No eating / drinking for at least 1 hour prior to test session.

Visit 2 & 3: Test Sessions
At least two days between each test session
Health and compliance questions
Pre-brushing digital image capture of subject's teeth
Supervised use of test product
(Randomised, brushing with 1.5g (+/- 0.1g) of toothpaste for 90sec, water rinse
Immediate post-brushing digital image capture of subjects teeth
Image analysis and data entry
Statistical analysis