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Tooth whitening evaluation of blue covarine containing toothpastes

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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].

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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 [6]. 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 [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 [12]. This hypothesis is supported by tooth bleaching studies [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 [15]. Using these optical principles, a silica-based toothpaste containing the pigment blue covarine was developed [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 *in vitro* and clinical studies [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 *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 min 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 (400 ml) 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) [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 s 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 s in water (50 ml) 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 [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. [20] 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 1.5 g (+/−0.1 g) of their assigned toothpaste for 90 s using their normal brushing technique, followed by a water rinse (5 ml) for 5 s. 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 Fig. 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