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Effect of different storage conditions on the physical properties of bleached enamel: An *in vitro* vs. *in situ* study

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ABSTRACT

Objectives: Evaluate the effect of different storage conditions on bleached enamel using Knoop microhardness (KHN) and colour variation.

Methods: Forty-eight tooth blocks were divided into four groups ($n = 12$), based on storage media (SM): purified water (PW), artificial saliva (AS), natural saliva (NS), *in situ* (IS). Three whitening sessions were carried out using 35% hydrogen peroxide, with a week interval. Colour and KHN measurements were taken before the samples were placed in the SM (t_1), after 24 h in the SM (t_2), and after 24 h at the end of the bleaching treatment (t_3). Two extra samples from each group were analysed using a scanning electron microscope (SEM). KHN results were analysed by PROC-MIXED and Tukey–Kramer test ($\alpha = 0.05$), and colour changes were evaluated using ΔL, Δa, Δb, ΔE between the different times $\Delta 1(t_1 - t_2)$, $\Delta 2(t_2 - t_3)$ using the Kruskal–Wallis test and Dunn's test ($\alpha = 0.05$).

Results: Significant statistical difference was noted in KHN at t_3, with the lowest values found for PW. As for colour analysis in $\Delta E2$ and $\Delta b2$, IS showed values that were statistically lower when compared to AS. Likewise, there were differences between PW and AS in relation to IS when evaluating $\Delta L2$. In addition, NS showed similar values to IS.

Conclusions: The storage conditions had different effects on the physical properties of bleached enamel. NS was the only SM that showed similar behaviour to IS.

Clinical Significance: NS proved an effective SM in the protection and recovery of damage caused by bleaching and is a viable SM for *in vitro* studies.

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1. Introduction

Tooth bleaching is an efficient and conservative aesthetic treatment for discoloured teeth. The procedure uses hydrogen peroxide, which penetrates into the dental structure and releases free radicals. These free radicals oxidize pigment molecules, turning them into less complex molecules, leading to a whitened aspect of the teeth.[1–3]

Although there are no doubts with regards to the effectiveness of in-office bleaching, the safety of this technique on the tooth structure has been questioned.[4] Negative effects have been associated with dental bleaching and could be related to the pH value, oxidative effect or composition of the bleaching agents.[5] Some studies have evaluated the effects of tooth whitening and reported alterations in enamel morphology,[5–7] reduction in surface hardness,[8,9] enamel demineralization,[10] and changes in the chemical composition of the tooth[11]; however, other studies have not found changes to enamel structure.[12–18]

The inconsistency in the outcome of these studies might be due to differences in study design, type of storage condition, evaluation time, different concentrations of bleaching products, application time, or the pH of the bleaching agents.[18,19] Furthermore, the few studies that do show negative effects on dental properties generally have some limitations in the *in vitro* methodologies. These include the use of purified water and artificial saliva as storage conditions, which do not accurately reflect the clinical situation.[20] The clinical situation can be achieved using *in situ* studies, which are an intermediate stage between laboratory experiments and clinical trials by reproducing the clinical conditions and performing the analysis outside the oral cavity.[3]

The purpose of this study was to evaluate the effect of different storage conditions using *in vitro* (purified water, artificial saliva and natural saliva) *vs. in situ* methodology on bleached enamel structure, as evaluated using surface microhardness and colour analysis variation. The null hypotheses were: (1) the different storage conditions would have no effect on enamel microhardness and (2) the different storage conditions would have no influence on tooth colour change.

2. Materials and methods

This study was approved by the Institutional Ethics Committee (034/2014).

2.1. Sample preparation

Freshly extracted bovine incisors which were devoid of stain, enamel cracks or fractures were selected and stored in 0.1% thymol solution at 4 °C until required for use. Enamel-dentine blocks (4 mm × 4 mm) were obtained from the buccal surface, using a diamond cutting disc (4″ × 012 × 1/2, Buehler, IL, USA) coupled to a metallographic saw (Isomet 1000; Buehler, Lake Buff, IL, USA). Enamel and dentine thicknesses were standardized (1 mm enamel and 1.75 mm dentine). The dentine surface was flattened and the enamel surface was ground flat with sequential water-cooled silicon carbide paper discs (500-, 1000-, and 2000-SiC – Buehler, Lake Buff, IL, USA). Next, the blocks were polished using diamond paste (1 μm, 1/4 μm) and polishing cloths, and rinsed with running distilled water to remove debris between each stage and at the end of the whole process. All specimens were immersed in distilled water and ultrasonicated for 15 min to remove residual particles and the smear layer. Each specimen was marked with a diamond bur #1012 (KG Sorensen) on one side to standardize the sample position in the spectrophotometer. The dental blocks were sterilized with ethylene oxide and stored in sterilized distilled water at 4 °C until required for use. Forty-eight samples were used for the microhardness and colour analyses, and eight samples were used for the SEM analysis.

2.2. In situ aspects

Six volunteers (three male and three female), between 23 and 27 years-old, participated in the study after signing an informed consent form. These volunteers all fulfilled the inclusion criteria (absence of dental caries and/or periodontal disease, normal saliva flow) without violating the exclusion criteria (unsatisfactory restorations and prostheses in mouth, use of orthodontic appliances, use of drugs that affect salivary flow and smokers). A full-arch maxillary impression was obtained for each volunteer and a stone cast mould was fabricated. Palatal devices were made of acrylic resin containing 4 × 4 × 2.75 mm reservoirs. The palatal devices contained two reservoirs for four volunteers, and three reservoirs for two volunteers, with an added reservoir for the SEM sample. All specimens were fixed to the palatal device using sticky wax.

2.3. Saliva collection

The natural saliva used in this study was provided by the volunteers and was collected from the same individuals, at the same time of day (8:00 am), before breakfast and any oral hygiene maintenance. Salivary flow was stimulated by chewing paraffin wax (Parafilm M, American National Can, Chicago, IL) and the saliva was collected in falcon tubes retained inside a beaker filled with ice. The collected saliva was then clarified by centrifugation (JOUAN MR23i Benchtop High Speed Centrifuge Thermo Scientific MR23i, Waltham, MA, USA) at 3.800 g for 10 min at 4 °C. Next, the saliva supernatant was sterilized by filtration with a filter membrane, with a pore size of 0.22 μm using vacuum filtration systems (TPP Rapid Filtermax Vacuum Filtration Systems, Switzerland). The whole processed saliva was divided into aliquots for daily use and immediately frozen (−80 °C) until needed for use. For each daily exchange of saliva during the experiment, the aliquots of natural saliva ware thawed and mixed before use. The artificial saliva used in this study contained 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.1 mol/L Tris buffer, and pH 7.0.[21]

2.4. Peroxidase assay in natural saliva

To ensure that the salivary enzyme peroxidase remained active in natural saliva during storage, an enzyme activity test was carried out at different times, that is, after the salivary