In Vitro Whitening Effect of a Hydroxyapatite-Based Oral Care Gel

Sandra Sarembe¹  Joachim Enax²  Maria Morawietz¹  Andreas Kiesow¹  Frederic Meyer²

¹Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle, Germany
²Research Department, Dr. Kurt Wolff GmbH and Co. KG, Bielefeld, Germany

Address for correspondence  Frederic Meyer, PhD, Research Department, Dr. Kurt Wolff GmbH & Co. KG, Johanneswerk Straße 34-36, Bielefeld 33611, Germany (e-mail: frederic.meyer@drwolffgroup.com).

Abstract

Objective  Oral care formulations aim to prevent oral diseases such as dental caries and gingivitis. Additionally, desire for white teeth still exists across all age groups. It is known that most whitening toothpastes are highly abrasive and can be harmful to teeth and gingiva. Therefore, a gel formulation with biomimetic hydroxyapatite (HAP; Ca₅[PO₄]₃[OH]) as active ingredient was developed. This formulation was tested with respect to its tooth whitening properties in an in vitro study.

Materials and Methods  Enamel samples were allocated to either group (a) HAP gel, (b) whitening mouth rinse with phosphates, or (c) negative control (distilled water). Test products were applied by finger (a) or were rinsed (b, c) for 1, 3, and 9 (b and c only) cycles, respectively.

Results  Color changes (ΔE) were measured spectrophotometrically. Group (a) showed a significant increase in color changes with respect to whitening after one cycle (mean ΔE = 5.4 [±2.66], p ≤ 0.006) and three cycles (mean ΔE = 11.2 [±3.11], p < 0.0001) compared to groups (b) and (c). For group (b), a significant increase in color change was measured after three (mean ΔE = 2.77 [±1.01], p = 0.02) and nine cycles (mean ΔE = 3.27 [±1.61], p = 0.006) compared to (c). Group (c) showed only minor and statistically insignificant color changes.

Conclusion  This in vitro study demonstrated a significantly higher ad hoc whitening effect of the HAP gel compared to the mouth rinse and water after short-time application.
in vivo study has shown its positive properties to release calcium in dental biofilms. This can be helpful to buffer cariogenic biofilms, and with increased calcium-levels leading to a shift from tooth demineralization to remineralization.

The aim of this in vitro study was to test the whitening effect of a newly developed gel formulation based on microcrystalline HAP. To exclude any abrasive influence by toothpaste abrasives harder than natural enamel, and more importantly by the toothbrush that is used in daily oral care, this HAP-gel formulation does not contain commonly used abrasives (e.g. hydrated silica) and was applied by finger. This fact is of importance since the whitening effect of the toothpaste is mainly determined by abrasive ingredients and the mechanical cleaning efficacy of the toothbrush. The assumed mode of action of the newly developed whitening formulation with HAP is based on its adhesion of particles on the enamel surface.

Consequently, the focus of this in vitro study was the analysis of the specific whitening effect of a HAP-containing containing gel. This would be helpful for individuals who prefer a brighter appearance of their teeth but are suffering from dentin hypersensitivity or patients suffering from gingivitis or periodontitis. Our hypothesis was that HAP would change the appearance of the natural tooth color to a brighter (whiter) color without having abrasive and/or oxidizing properties. A nonabrasive, commercially available whitening gel (whiter) color without having abrasive and/or oxidizing properties. A nonabrasive, commercially available whitening mouth rinse based on ethanol and phosphates served as positive control, and distilled water as negative control. The whitening effects of the different formulations were tested on bovine enamel samples using a pre–post design.

Materials and Methods

Treatment Products and Control Group

The whitening properties of three different groups were tested as follows:

1. Test product: HAP-based (15% w/w) oral care gel (Karex gelée; Dr. Kurt Wolff GmbH & Co. KG, Bielefeld, Germany).
   - Aqua, hydroxyapatite, glycerin, hydrogenated starch hydrolysate, calcium lactate, hydroxyethylcellulose PEG 40, hydrogenated castor oil, xylitol, calcium carbonate, hydroxyacetophenone, 1,2-hexanediol, caprylyl glycol, aroma, stevia rebaudiana leaf/stem powder, propylene glycol, sodium hydroxide, limonene, citral.
2. Positive control: whitening mouth rinse (Listerine Advance White, Johnson & Johnson GmbH, Neuss, Germany).
   - Aqua, alcohol, sorbitol, tetrasodium pyrophosphate, pentasodium triphosphate, citric acid, poloxamer 407, sodium benzoate, eucalyptol, thymol, menthol, sodium saccharin, sodium fluoride (220 ppm F), tetrasodium pyrophosphate, propylene glycol, sucralose, aroma, disodium phosphate.
3. Negative control: distilled water (pH = 6.8).

Sample preparation

Bovine incisors were cleaned and embedded in epoxy resin (EpoFix; Struers, Cleveland, Ohio, United States). The buccal enamel surface was grinded to 1,200 grit by SiC abrasive paper (Struers, Cleveland, Ohio, United States). All enamel samples were stored in distilled water for 24 hours before starting the experiments.

Treatment with Test Product and Control Group

To ensure a comparison of the experimental data, all enamel samples were treated for 1 minute with the test products. The whole test procedure is depicted in ►Fig. 1.

(a) HAP-gel: The HAP gel was applied by finger on the enamel surface (2 seconds, n = 8). After 1 minute, the samples were rinsed under agitation with distilled water and air dried at room temperature. The treatment procedure was repeated twice (three applications in total). Color measurements were carried out after first and third cycles.

(b) Mouth rinse and (c) control group: Enamel samples were stored in group b (mouth rinse [n = 8]) or c (distilled water [n = 4]) for 1 minute by using a laboratory shaker. Afterwards the samples were rinsed under agitation with distilled water and air dried at room temperature. The treatment procedure was repeated eight times (nine applications in total). Color measurements were carried out after first, third, and ninth cycles. Nine treatment cycles were chosen for the mouth rinse testing in order to simulate a longer treatment period which is according to the manufacturers’ instructions.

Color Measurements

Tooth color and color changes (∆E) were analyzed by a spectrophotometer (CM-3600A, Konica Minolta Sensing Europe B.V., Bremen, Germany) using a (L*a*b*) color space with coordinates: white–black (±L*), redness–greenness (±a*), and yellow–blueness (±b*). Color changes between the different measurement and the baseline measurement in each group were calculated by using the following equation:

\[ \Delta E^* = \sqrt{\Delta L^*2 + \Delta a^*2 + \Delta b^*2} \]

\[ \Delta L^* = L^*_{\text{after treatment}} - L^*_{\text{initial}} \]
\[ \Delta a^* = a^*_{\text{after treatment}} - a^*_{\text{initial}} \]
\[ \Delta b^* = b^*_{\text{after treatment}} - b^*_{\text{initial}} \]

Statistical Analysis

Statistical analyses were conducted by one-way analysis of variance (ANOVA) with post hoc Bonferroni’s test and Levene’s test for analyses of homogeneity of variance (Origin 2019b; OriginLab Corporation Company, Northampton, Massachusetts, United States). The level of significance of α was set at ±0.05.

Scanning Electron Microscopy

Surface analyses of the enamel samples at baseline and after one treatment cycle were performed by scanning electron microscopy (SEM; Quanta 3D FEG scanning electron microscope, FEI Company, Hillsboro, Oregon, United States). The samples were coated with an ultra-thin carbon film by evaporation before the SEM analyses.
Results

Color Measurement
Color measurement shows a significant increase in ∆E* after one cycle (5.14 [±2.66], p ≤ 0.006) and after three cycles (11.2 [±3.11], p < 0.0001) in group a (HAP-group) compared to group b (whitening mouth rinse), and group c (water), respectively (► Fig. 2). No significant increase in ∆E was measured in group (b) after one cycle. Group (b) showed an increase in ∆E after three cycles (2.77 [±1.01], p = 0.02) and nine cycles (ΔE = 3.27 [±1.61], p = 0.006) compared to group (c). A similar trend as for ∆E* could be revealed for ∆L* and ∆a* (► Table 1). These values increased after treatment with HAP-gel (group a) and whitening mouth rinse (group b) compared to water (group c), representing an increase in brightness and reddish (minor color shift), (minor color shift). For groups (a)
Sarembe et al. study that HAP particles of a mouth rinse of 0.01 ± 0.03 O 4.51 ± 2.48. Additionally, Niwa et al. found a whitening optimum by using 15% HAP in a toothpaste formulation. This might be under in situ conditions and with an ex-vivo study design, respectively. 23,24 Our results are in good agreement with other studies that analyzed the teeth whitening effects of HAP. 3,6,25 Niwa et al., for example, analyzed the in vivo whitening effect of toothpastes with 0, 3, and 15% HAP. They found that the whitening effect could be increased by higher HAP concentrations. Interestingly, in an additional in vitro experiment it was shown that the polishing properties were not altered when higher HAP concentrations were used. 3 This clearly underlines that HAP, in contrast to abrasives with a high-relative hardness (e.g., perlite, a mineral of silicate; alumina, Al₂O₃), 26 is a suitable whitening agent which does not lead to a damage of tooth or gingiva. Moreover, HAP reduces the roughness of the teeth. 25 Dabanoglu et al. and Jin et al. showed that different calcium phosphates including HAP contribute to tooth whitening in vitro. 3,5 Besides the adhesion of HAP particles to the tooth surface as described above, remineralization effect of HAP may also contribute to tooth whitening (i.e., due to a smoother surface on which stains cannot attach). 3,11,26,27 An advantage of the use of the HAP in a gel formulation is the good adhesion to the tooth surface. It can be easily applied by using the finger after tooth brushing and also showed both remineralization effects 26 and erosion protective properties. 17,28 Bommer et al. reported that a self-assembling peptide matrix can act as an adhesive for HAP particles improving the whitening effect of HAP alone. 6 A similar effect could be observed in HAP group of our study. The matrix of the HAP-gel may further increase the HAP-adhesion to the tooth surface compared to HAP-particles alone (which already show a good adhesion to tooth surfaces). 5,10,25 Future studies should be carried out to analyze the whitening effect of the HAP-gel also under in vivo conditions. To date, only a few in vivo studies on the whitening effects of HAP have been published. 6,8,25 In situ studies show an efficient reduction of bacterial colonization to enamel surfaces by using HAP-based mouth rinses. 18,23,36 This might be also an important factor for tooth whitening since stains are often incorporated into dental plaque. 1

In contrast to the HAP-gel, the tested mouth rinse showed only minor whitening effects in our in vitro setting after one,

| ΔL* | After cycle 1 | After cycle 3 | After cycle 9 |
|-----|--------------|--------------|--------------|
| HAP-gel | 4.51 ± 2.48 | 10.66 ± 2.97 | Not performed |
| Mouth rinse | 0.93 ± 0.81 | 2.31 ± 1.02 | 3.08 ± 1.43 |
| Water | 0.15 ± 0.33 | 0.32 ± 0.43 | 0.07 ± 0.22 |

| Δa* | HAP-gel | 0.59 ± 0.17 | 1.10 ± 0.19 | Not performed |
|-----|--------|-------------|-------------|--------------|
| Mouth rinse | 0.08 ± 0.13 | 0.15 ± 0.16 | 0.29 ± 0.35 |
| Water | 0.15 ± 0.33 | 0.06 ± 0.06 | 0.01 ± 0.03 |

| Δb* | HAP-gel | -1.77 ± 1.60 | -2.87 ± 1.39 | Not performed |
|-----|--------|--------------|-------------|--------------|
| Mouth rinse | -0.62 ± 1.00 | -0.88 ± 1.19 | -0.07 ± 1.12 |
| Water | 0.16 ± 0.35 | -0.29 ± 0.35 | -0.23 ± 0.33 |

Abbreviation: HAP, hydroxyapatite.
three, and nine cycles. This may be explained by the different mode of action compared to HAP. The SEM images showed that HAP adheres to the tooth surface; however, only a thin layer of unknown deposits was detectable in the mouth rinse group (Figs. 3B and 3C). Thus, the mouth rinse may support (e.g. by its ingredients ethanol and phosphates, as well as by an acidic pH) the stain removal action of toothbrush and toothpaste, but does not lead to the formation a white (protective) layer on the tooth surface. Other test approaches are necessary to understand completely the whitening mechanisms of such mouth rinses. Further testing should also involve model refinement, i.e. it would be of interest to include experimental steps in the protocol in order to examine the stability of the whitening effect against chemical (mimicking acid challenges of daily food intake) and/or mechanical (mimicking tongue and mucosa movement or saliva flow or tooth brushing) stress.

Additionally, oral-care formulations based on HAP show not only whitening properties, but was also tested...
to be effective in preventing cavities, improvement of gingival health, and reduction of dentin hypersensitivity.\textsuperscript{11} Biomimetic ingredients based on HAP used in oral care show a high compatibility, since the mineral phase of the teeth and bones mainly consists of calcium and phosphate.\textsuperscript{12,13}

**Conclusion**

To conclude, the *in vitro* model tested in this study can be used as basic-approach for further testing of nonabrasive whitening agents.

In this study, the nonabrasive HAP-gel showed the highest values on tooth-whitening when compared to a mouth rinse with whitening-agents. Therefore, HAP particles may be suited to be a gentle, fast, and biomimetic approach for cosmetic tooth whitening.

**Authors’ Contributions**

S.S., J.E., M.M. A.K., and F.M. prepared the study protocol; S.S. performed conduction and analysis of the experiments; S.S., J.E., M.M. A.K., and F.M. took part in preparation of the publication manuscript.

**Funding**

This study was funded by Dr. Kurt Wolff GmbH & Co. KG, Bielefeld, Germany.

**Conflict of Interest**

None declared.

**Acknowledgments**

The authors would like to thank Carolin Ufer for the support in the sample preparation and Jasmin Zuehlke for performing the scanning electron microscopy analysis (both Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle, Germany).

**References**

1. Epple M, Meyer F, Enax J. A Critical Review of Modern Concepts for Teeth Whitening. Dent J (Basel) 2019;7(3):E79
2. Joiner A. Whitening toothpastes: a review of the literature. J Dent 2010;38(suppl 2):e17–e24
3. Vieira GHA, Nogueira MB, Gaio EJ, Rosing CK, Santiago SL, Rego RO. Effect of whitening toothpastes on dentin abrasion: an in vitro study. Oral Health Prev Dent 2016;14(6):547–553
4. Omar F, Ab-Ghani Z, Rahman NA, Halim MS. Nonprescription bleaching versus home bleaching with professional prescriptions: which one is safer? A comprehensive review of color changes and their side effects on human enamel. Eur J Dent 2019;13(4):589–598
5. Dabanoglu A, Wood C, Garcia-Godoy F, Kunzelmann KH. Whitening effect and morphological evaluation of hydroxyapatite materials. Am J Dent 2009;22(1):23–29
6. Bommer C, Flesia HP, Xu X, Kunzelmann KH. Hydroxyapatite and Self-assembling peptide matrix for non-oxidizing tooth whitening. J Clin Dent 2018;29(2):57–63
7. Jin J, Xu X, Lai G, Kunzelmann KH. Efficacy of tooth whitening with different calcium phosphate-based formulations. Eur Oral Sci 2013;121(4):382–388
8. Kim BL, Jeong SH, Jang SO, Kim KN, Kwon HK, Park YD. Tooth whitening effect of toothpastes containing nano-hydroxyapatite. Key Eng Mater 2006;309–311:541–544
9. Niwa M, Sato T, Li W, Aoki H, Aoki H, Daisaku T. Polishing and whitening properties of toothpaste containing hydroxyapatite. J Mater Sci Mater Med 2001;12(3):277–281
10. Fabritius-Vilpoux K, Enax J, Herbig M, Raabe D, Fabritius H-O. Quantitative affinity parameters of synthetic hydroxyapatite and enamel surfaces in vitro. Biosens Biomim Nan 2019;8:141–153
11. Enax J, Fabritius HO, Fabritius-Vilpoux K, Amaechi BT, Meyer F. Modes of action and clinical efficacy of particulate hydroxyapatite in preventive oral health care – state of the art. Open Dent J 2019;13:274–281
12. Hu M-L, Zheng G, Zhang Y-D, Yan X, Li X-C, Lin H. Effect of desensitizing toothpastes on dentine hypersensitivity: a systematic review and meta-analysis. J Dent 2018;75:12–21
13. Hiller K-A, Buchalla W, Grillmeier I, Neubauer C, Schmalz G. In vitro effects of hydroxyapatite containing toothpastes on dentin permeability after multiple applications and ageing. Sci Rep 2018;8(1):4888
14. Harks I, Jockel-Schneider Y, Schlagenhauf U, et al. Impact of the daily use of a microcrystal hydroxyapatite dentifrice on de novo plaque formation and clinical/microbiological parameters of periodontal health. A randomized trial. PLoS One 2016;11(7):e0160142
15. Hagenfeld D, Prior K, Harks I, et al. No differences in microbiome changes between anti-adhesive and antibacterial ingredients in toothpastes during periodontal therapy. J Periodontal Res 2019;54(4):435–443
16. Schlagenhauf U, Kunzelmann K-H, Hannig C, et al. Impact of a non-fluoridated microcrystalline hydroxyapatite dentifrice on enamel caries progression in highly caries-susceptible orthodontic patients: A randomized, controlled 6-month trial. J Investig Clin Dent 2019;10(2):e12399
17. Cieplik F, Rupp CM, Hirsch S, et al. Ca2+ release and buffering effects of synthetic hydroxyapatite following bacterial acid challenge. BMC Oral Health 2020;20(1):85
18. Meyer F, Enax J. Hydroxyapatite in oral biofilm management. Eur J Dent 2019;13(2):287–290
19. Sudradjat H, Meyer F, Loza K, Epple M, Enax J. In vivo effects of a hydroxyapatite-based oral care gel on the calcium and phosphorus levels of dental plaque. Eur J Dent 2020;14(2):206–211
20. Enax J, Epple M. Die Charakterisierung von Putzkörperrn in Zahnpasten. Dtsch Zahnarztfl Z 2018;73:116–124
21. León K, Mery D, Pedreschi F, León J. Color measurement in L’a*b’ units from RGB digital images. Food Res Int 2006;39:1084–1091
22. Ceci M, Viola M, Rattalino D, Beltrami R, Colombo M, Poggio C. Discoloration of different esthetic restorative materials: a spectrophotometric evaluation. Eur J Dent 2017;11(2):149–156
23. Keschke A, Holder C, Basche S, Tahan N, Hannig C, Hannig M. Efficacy of a mouthrinse based on hydroxyapatite to reduce initial bacterial colonisation in situ. Arch Oral Biol 2017;80:18–26
24. Lelli M, Putignano A, Marchetti M, et al. Remineralization and repair of enamel surface by biomimetic Zn-carbonate hydroxyapatite containing toothpaste: a comparative in vivo study. Front Physiol 2014;5:333
25. Reis PQ, da Silva EM, Calazans FS, et al. Effect of a dentifrice containing nanohydroxyapatite on the roughness, color, lightness, and brightness of dental enamel subjected to a demineralization challenge. Gen Dent 2018;66(4):66–70
26. Amaechi BT, AbdulAzeez PA, Alshareef DO, et al. Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. BDJ Open 2019;5:18
27. Tschoppe P, Zandim DL, Martus P, Kiellbassa AM. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. J Dent 2011;39(6):430–437
28 Colombo M, Beltrami R, Rattalino D, Mirando M, Chiesa M, Poggio C. Protective effects of a zinc-hydroxyapatite toothpaste on enamel erosion: SEM study. Ann Stomatol (Roma) 2017;7(3):38–45

29 Steinert S, Zwanzig K, Doenges H, Kuchenbecker J, Meyer F, Enax J. Daily application of a toothpaste with biomimetic hydroxyapatite and its subjective impact on dentin hypersensitivity, tooth smoothness, tooth whitening, gum bleeding, and feeling of freshness. Biomimetics (Basel) 2020;5(2):E17

30 Hannig C, Basche S, Burghardt T, Al-Ahmad A, Hannig M. Influence of a mouthwash containing hydroxyapatite microclusters on bacterial adherence in situ. Clin Oral Investig 2013;17(3):805–814

31 van Loveren, C, ed. Toothpastes. Amsterdam: Karger Publications; 2013. Doi: 10.1159/isbn.978-3-318-02207-0

32 Epple M. Review of potential health risks associated with nanoscopic calcium phosphate. Acta Biomater 2018;77:1–14

33 Meyer F, Amaechi BT, Fabritius HO, Enax J. Overview of calcium phosphates used in biomimetic oral care. Open Dent J 2018;12:406–423