Fygg

Feed Your Good Guys

ENHANCED with nanoXIM

NANO-HYDROXYAPATITE by FLUIDINOVA

Fygg™ - Feed Your Good Guys™

15.5% nano XIM at 57.34% concentration (8.6% nano-HAp)

Technical Data Sheet

Fygg™ - Feed Your Good Guys™

15.5% nano XIM at 57.34% concentration (8.6% nano-HAp) varnish

is a nano-hydroxyapatite-containing varnish with added prebiotics and L-arginine that can be used to help aid in remineralization, desensitization, and whitening, as well as for supporting and nourishing the oral microbiome. Fygg varnish can be easily applied to enamel and dentin, without the undesirable stickiness and aftertaste of a typical fluoride varnish and is nearly unnoticeable after application. Saliva and the addition of micro-hydroxyapatite help to activate the formula, and it will adhere to dry or teeth that are slightly moist, spreading through our saliva and oral cavity after application. The patent-pending formula contains no unnecessary chemicals or ingredients like hexanes or propylene glycol-derivatives, making it safe to swallow and apply in all ages.

COMPOSITION

Fygg™ - Feed Your Good Guys™ contains 15.5% nanoXIM.CarePaste at 57.34% concentration (8.6% nano-HAp) Varnish using nano-XIM Care-paste, a revolutionary nano-hydroxyapatite formula approved by the SCCS in Europe. Prebiotics and L-Arginine have been added to aid in microbiome support and plaque reduction, and it is sweetened with Xylitol and Monkfruit. Fluidinova's nanoXIM.CarePowder, micro-hydroxyapatite has also been added to improve bioavailability and efficacy. The product is supplied in tubes with 1.41oz (40g) of the easy-to-dispense varnish.

As of March 2023, the SCCS in Europe made its final decision regarding nano-HAp public, stating that based on the data provided, hydroxyapatite (nano) is safe when used at concentrations up to 10% in toothpaste, and up to 0.465% in mouthwash.

This safety evaluation only applies to the hydroxyapatite (nano) with the following characteristics:
-rod shaped particles of which at least 95.8% (in particle number) have an aspect ratio less than 3
-the remaining 4.2% have an aspect ratio not exceeding 4.9
- the particles are not coated or surface modified.

The upper limit of 10% nano-HAp for toothpastes corresponds to 64.5% nanoXIM.CarePaste and the 0.465% nano-HAp for mouthwashes corresponds to 3% nanoXIM.CarePaste, given that nanoXIM.CarePaste contains 15.5% nano-HAp in its composition.

This opinion and safe use conclusion currently only applies to nano-hydroxyapatite with the characteristics of nanoXIM.carePaste product referred to in the studies and manufactured by FLUIDINOVA.

SUMMARY OF ADVANTAGES

- The only nano-HAp varnish available in US with approved SCCS-sourcing
- 15.5% nano-HAp nanoXIM.CarePaste at 57.34% concentration (8.6% total nano-HAp)
- Contains micro-HAp nanoXIM.CarePowder for added efficacy
- Contains prebiotics for oral microbiome nourishment
- Contains xylitol for microbiome support and pleasant flavor
- No need to clean teeth or dry tooth surface prior to application
- Easy sweeping-motion application technique
- Fast chair time with quick use of gloved finger, brush, or cotton swab.
- Incorporates with saliva for optimal access to hard-to-reach spots
- Relieves sensitivity, remineralizes, and whitens
- Safe to use with restorations of all types, including metal or ceramic orthodontic brackets
- Over 30 years of combined clinical experience driving the formulation

PROCEDURE

Fygg's nano-hydroxyapatite varnish is a replacement for conventional fluoride varnishes. Fygg is quick and easy to apply because there's no need to dry tooth surfaces before application and it can be applied with an easy sweeping motion over teeth. It is unnecessary to individually paint each tooth surface separately. The ease of Fygg's nano-hydroxyapatite varnish formula and dispensing system allows it to be placed on a gauze or the back of a gloved hand for quick application and workflow.

1. Fygg nano-hydroxyapatite Varnish can be easily applied to tooth surfaces, with or without a proper dental prophylaxis.
2. Convenient dosing card included with product to ensure product is not wasted or used in excess.
3. Apply using a sweeping motion and a gloved finger, brush or cotton swab. It is not necessary to completely cover all tooth surfaces as it will quickly dissolve into the patient's saliva.
4. After application, instruct the patient to avoid eating or drinking for 30 minutes. Patients may feel and see a very thin coating on the teeth for a short period of time after the application.
5. For maximum efficacy after application, please instruct the patient to follow all of the post-application instructions above.

Shake or massage the tube for 10 seconds before each use. Dispense appropriate amount of varnish based on the patient's dentition.

Use a brush, cotton swab, or gloved finger to apply, aiming for an application time of 30 seconds, using sweeping strokes. Can be applied directly to areas of light plaque and moisture.

Instruct patients to have nothing to eat or drink for 30 minutes after application for optimal benefits. Floss between teeth if the patient has incipient interproximal lesions. Smile because you just helped another human thrive ;)

PATIENT INSTRUCTIONS

To obtain optimal results and efficacy, we suggest the following after your varnish application:

- Nothing to eat or drink for 30 minutes.
- Avoid hot beverages and alcohol, including antiseptic mouthwashes for the remainder of the day.
- Do not brush or floss your teeth for three hours.
- You may feel a thin coating of varnish on your teeth immediately after the procedure. Our varnish is free of carrier agents like hexanes and propylene glycol and any similar derivatives that many traditional varnishes use, making it safe to swallow and for the need to brush off after the 30 minute period.

The power of our varnish lies in its ability to nourish your saliva, boosting its natural remineralization abilities and filling in microporosities and demineralization areas directly on your tooth surfaces.

FAQ

What advantages does Fygg's nano-hydroxyapatite (nano-HAp) varnish have over a traditional fluoride varnish?

Fygg's nano-hydroxyapatite varnish was created for professionals seeking the safest, most effective, biomimetic, and oral microbiome-supportive dental care for their patients of all ages. Fygg varnish can be a beneficial addition to your dental practice, allowing patients aware of fluorid's toxicity to maintain optimal oral health without the risks to neurodevelopment, the endocrine system, and to immune health.

More and more, patients are looking for alternatives to fluoride based products and therapies. Fygg nano-HAp varnishto its science-backed properties and clean ingredients, while still maintaining comparable efficacy in terms of remineralization and desensitization.

Fygg nano-HAp uses 15.5% nano XIM at 57.34% concentration (8.6% nano-HAp), using what is currently the only form of nano-hydroxyapatite (nanoXIM.CarePaste manufactured by Fluidinova) presently approved by the SCCS in Europe for safety and efficacy.

Is Fygg nano-hydroxyapatite effective for remineralization and sensitivity?

Yes! The appropriate concentrations and types of nano-hydroxyapatite in Fygg varnish can be as effective in supporting remineralization, reducing tooth demineralization, and helping with sensitivity as fluoride varnishes. Several studies have shown that nano-hydroxyapatite has similar or better efficacy as fluoride in remineralizing early carious lesions, preventing dental caries formation, as well as helping with sensitivity.

How does it help to support the oral microbiome of a patient?

With the presence of a prebiotic and L-Arginine, Fygg nano-hydroxyapatite actively nourishes your patient's oral microbiome, not destroying it as many other products do. The prebiotic fiber in the formula will feed and nourish the beneficial bacteria in the mouth. L-arginine has shown to elevate dental plaque pH, inhibit biofilm formation, and destabilize biofilm structure. Also, with the elimination of unnecessary oral microbiome disruptors, emulsifiers, surfactants, artificial flavors and sweeteners, hexanes and propylene glycol derivatives, and excessive essential oils, Fygg supports commensal and beneficial bacteria, allowing them to thrive!

Is it safer than fluoride?

Many dental professionals consider hydroxyapatite varnish to be safer than fluoride varnish due to the recent concern that fluoride has neurotoxic effects. This fluoride-free varnish can benefit pregnant women, developing infants, and young children. Fluoride can be absorbed via the stomach and/or the oral tissues, elevating the concentration of fluoride in the brain and, within minutes, crosses the blood-brain barrier, causing permanent cognitive harm to children. We also were able to remove hexane and propylene glycol derivatives from our varnish, making it a much cleaner choice for all patients and providers.

Is nano-hydroxyapatite safe?

Nano-hydroxyapatite (nano-HAp) of a certain shape, size, concentration, and sourced from a specific source has been extensively studied and proven safe for dental use. It also has been in use in Japan for over 40 years without any noted health issues.

How is it applied?

Fygg nano-hydroxyapatite varnish is applied similarly to fluoride varnish. The varnish is painted onto the tooth surface using a brush, a cotton swab, or a gloved finger, typically after a dental cleaning. The process is quick and easy, and patients greatly prefer the subtle taste and texture of Fygg varnish compared to the strong, astringent taste of fluoride varnishes.

Who applies it?

Fygg's professional strength nano-hydroxyapatite varnish is applied by a dental professional, such as a dentist or dental hygienist. Applications can occur during a routine dental check-up, following a cleaning appointment, or at a specific frequency as determined by the dental team to help with sensitivity, demineralization, and remineralization.

How often is it applied?

Most dental professionals will apply it twice yearly based on common recall frequencies, but Fygg nano-hydroxyapatite varnish can be applied at a higher frequency, depending on a patient's needs, as you can not over-apply the product. Custom trays can be made for home use based on the dental professional's diagnosis and discretion.

Can patients use this at home?

Patients with extensive decay, especially smooth surface or root caries, or chronic tooth sensitivity may benefit from at-home use in custom trays that they wear at night, but this should be recommended based on a dental professional's diagnosis and discretion. Currently, patients can only purchase Fygg's nano-hydroxyapatite varnish from a dental professional.

Does insurance pay for it?

Hydroxyapatite varnish may vary depending on your specific insurance plan. It is recommended to check with your insurance provider for details on coverage and reimbursement. We are working hard behind the scenes to have hydroxyapatite varnishes as a covered insurance benefit soon!

Is it safe if swallowed?

Fygg nano-hydroxyapatite varnish is developed as a safer alternative to fluoride varnish, and studies have shown that our version of nano-hydroxyapatite is safe when swallowed. Unlike fluoride, there is no need for a Poison Control label on Fygg varnish.

How does it taste compared to fluoride varnishes?

Our varnish has far less of an undesirable aftertaste and texture due to removing unnecessary and potentially harmful chemicals like hexanes and propylene glycol derivatives. We have many flavors coming soon to appeal to all patient preferences! You will find a lot fewer tears, nausea, and vomiting (aka no "fluoride face!") after the application of Fygg nano-hydroxyapatite varnish.

Can I use this on my adult patients? Pediatric patients?

Yes, Fygg nano-hydroxyapatite varnish can promote remineralization, prevent tooth demineralization, help with sensitivity, and brighten and whiten teeth in patients of all ages.

INGREDIENTS

Vanilla Vibes Varnish

nano-hydroxyapatite, Glycerin, Xylitol, Hydrated Silica, Xanthan Gum, *Vanilla Planifolia Fruit (Vanilla) Extract, Gluconolactone, Potassium Sorbate, Monk Fruit (Siraitia grosvenorii) Extract Powder, L-Arginine, Polydextrose Non GMO (Litesse Powder)
*Organic

coming soon!

Mild Mint Varnish

nano-hydroxyapatite, Glycerin, non-GMO Xylitol, Hydrated Silica, Xanthan Gum, Creamy Mint Flavor, Gluconolactone, Potassium Sorbate, Monk Fruit (Siraitia grosvenorii) Extract Powder, L-Arginine, Polydextrose non-GMO (Litesse Powder)

Fygg Toothpaste

Mild Mint Toothpaste

Aqua/Water, Xylitol, nano-hydroxyapatite, Hydrated Silica, Bentonite Clay, Creamy Mint Flavor, Potassium Sorbate, Siraitia grosvenorii (Monk Fruit) Extract, Gluconolactone, Matcha Flavor, L-Arginine, Hydroxyapatite, Polydextrose (Litesse) Powder

Vanilla Vibes Kids Toothpaste

Aqua/Water, Xylitol, nano-hydroxyapatite, Hydrated Silica, Bentonite Clay, Vanilla Planifolia Fruit Extract (Vanilla) Extract, Potassium Sorbate, Siraitia grosvenorii (Monk Fruit) Extract, Gluconolactone, Matcha Flavor, L-Arginine, Hydroxyapatite, Polydextrose (Litesse) Powder

coming soon!

Coconut Cream Toothpaste

Aqua/Water, Xylitol, nano-hydroxyapatite, Hydrated Silica, Bentonite Clay, Coconut Cream Flavor, Potassium Sorbate, Siraitia grosvenorii (Monk Fruit) Extract, Gluconolactone, Matcha Flavor, L-Arginine, Hydroxyapatite, Polydextrose (Litesse) Powder

coming soon!

Chocolate Swirl Kids Toothpaste

Aqua/Water, Xylitol, nano-hydroxyapatite, Hydrated Silica, Bentonite Clay, Cocoa Flavor, Potassium Sorbate, Siraitia grosvenorii (Monk Fruit) Extract, Gluconolactone, Vanilla Extract, L-Arginine, Hydroxyapatite, Polydextrose (Litesse) Powder

nanoXIM CarePaste

WHITE PAPER

ENAMEL REMINERALIZATION

Effectiveness of nanoXIM•CarePaste on enamel remineralization

Enamel remineralization

The **mineralized tissues** found **in the human body are composed mostly of hydroxyapatite (HAp)**, a natural calcium phosphate ceramic that is abundant in bone and dentin (70%), and enamel (97%). **Tooth enamel is** the hardest tissue in the human body and is **made up of** building blocks of **HAp nanocrystals**, 40 nm in size. The tissue is acellular and, unlike bone, **cannot be repaired naturally** [1]. Therefore, the regeneration of the enamel surface represents a significant challenge.

Demineralization occurs when the mineral content of the teeth begins to wear away. This process can happen with the ingestion of acidic foods and drinks, but can also occur due to the metabolization of starches and sugars by oral bacteria [2, 3]. Consequently, the nano-hydroxyapatite ions are removed from its structure, destroying the enamel crystals. If demineralization continues over time, it can result in the formation of dental caries [4]. The latter represents a serious health problem that affects a significant portion of the world's population. Particularly, in the United States, only 10% of adolescents and young adults are caries-free. Caries continue throughout adulthood and more than 95% of adults suffer from caries on enamel and root surfaces [5].

These facts highlight the importance of remineralizing agents in dental hygiene. The chemical and structural similarity of nano-hydroxyapatite (nHAp) to enamel HAp makes it an excellent remineralizing agent. It deposits on the enamel surface and strongly binds to it, creating a new homogeneous surface layer (Figure 1) [6].

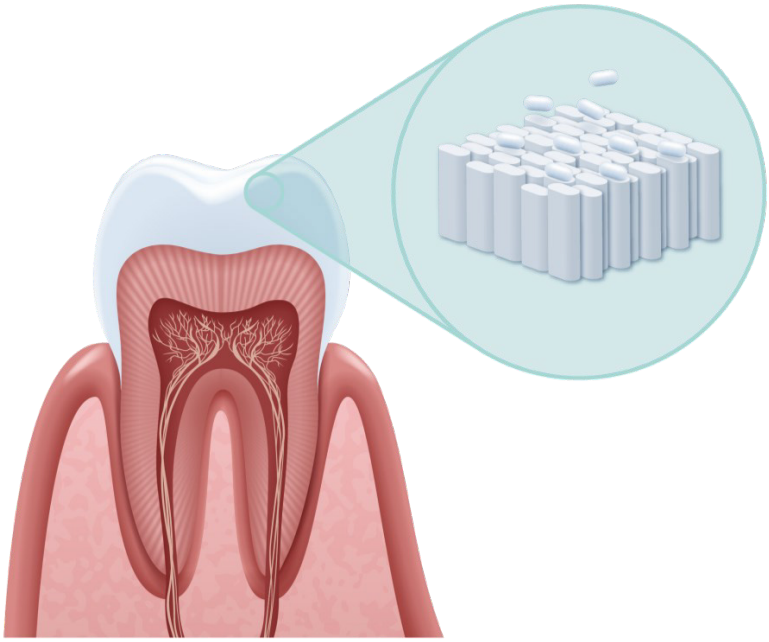
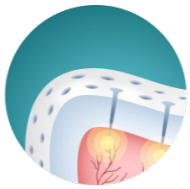


Figure 1: Nano-hydroxyapatite rod-shaped particles deposition on the enamel surface. A strong connection between the nanoparticles and the enamel is formed, originating a new remineralized layer.

nanoXIM•CarePaste

The nanoXIM•CarePaste is a nano-hydroxyapatite (nHAp) ingredient produced and marketed by FLUIDINOVA. This synthetic water-based suspension ingredient has been **specifically developed for oral care applications**, such as toothpastes, gels, mouthwashes, dental floss, and other oral care products (personal and professional use). Nano-hydroxyapatite is a calcium phosphate material widely accepted in dentistry and medicine due to its exceptional biocompatibility and bioactivity. Its excellent performance is related to its nanometer size, being very similar to natural teeth and bone. nanoXIM•CarePaste contains high-purity nanoparticles under 100 nm in size, being much smaller than the dentin tubules. Therefore, they can be easily integrated inside the tubules, blocking them and **reducing the pain associated with dental hypersensitivity**. In addition, nanoXIM•CarePaste is able to bind to the dentin apatite and tooth enamel. Consequently, a new apatite layer is formed, **remineralizing the enamel, protecting the tooth surface**, and **restoring its natural whiteness**.

Mode of action



1.

Dental hypersensitivity, a short and sharp pain, prevents us from drinking hot coffee, ice cream, or even an orange juice without feeling pain. The action of certain food and drinks (hot, cold, acidic) are considered aggressions to our teeth, resulting in the exposure of dentin tubules and the underlying nerves to the external environment (the dentin loses its protective covering).

2.

HAp has a great potential in the treatment of dental hypersensitivity, as it can be incorporated inside the dentin tubules. Consequently, these become sealed and pain is reduced.

3.

As a result, a new layer is formed, remineralizing the tooth enamel and protecting the tooth surface, preventing the appearance of new cavities and making it resistant to acid attacks of our favourite meals.

4.

The deposition of HAp on the enamel surface improves its smoothness for better light reflection, and consequently brighter and whiter teeth.

The effectiveness of nanoXIM•CarePaste has been confirmed in numerous studies

Study 1

The goal of this in vitro study was to evaluate the potential of VITIS Whitening toothpaste (Dentaid S.L., Spain) containing 3% of nanoXIM•CarePaste in the remineralization of the enamel and dentin [7].

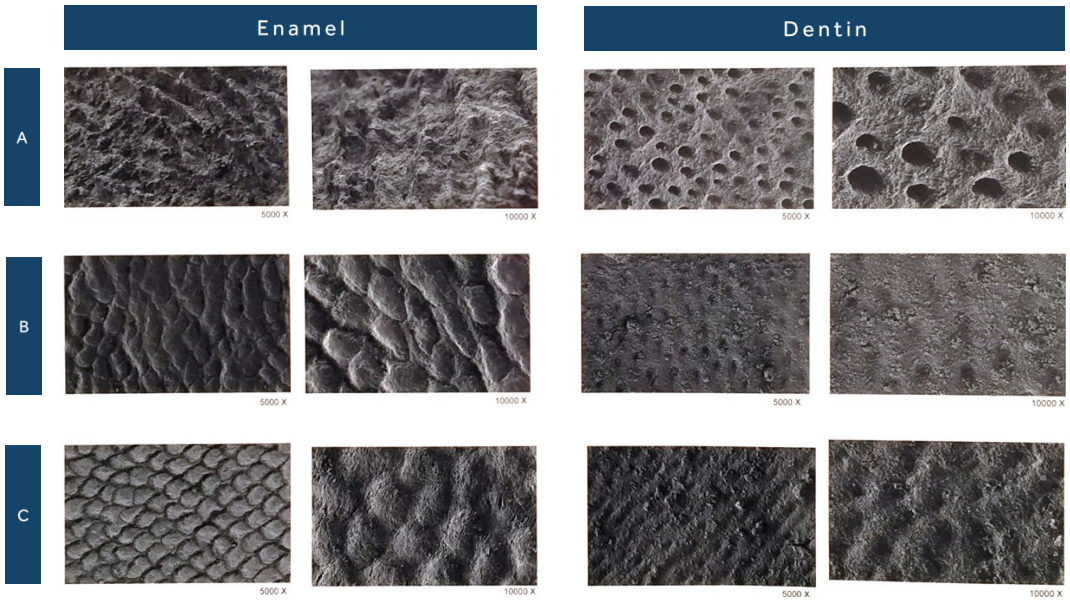


Figure 2: A: demineralized enamel and dentine with orthophosphoric acid at 37%;
B: remineralized enamel and dentine after four days of treatment with nHAp toothpaste, twice a day for 4 minutes;
C: after treatment, specimens were placed in contact with Coca-Cola® (pH = 2.52) for four minutes, twice a day for four days.

- ✓ A four-day treatment with VITIS Whitening toothpaste (containing 3% nanoXIM•CarePaste) effectively remineralized the enamel and dentin after their previously demineralization with orthophosphoric acid;
- ✓ The remineralized effect was maintained even after a four-day treatment with Coca-Cola®.

Study 2

In the present study, it was compared the efficiency of four toothpastes in inhibiting demineralization adjacent to orthodontic brackets *in vitro*. The tested toothpastes were Aclaim (6.5% nanoXIM•CarePaste, Group Pharmaceuticals, India), Apagard (nano-hydroxyapatite, Sangi, Japan), Clinpro Tooth Crème (Tricalcium phosphate and fluoride, 3M ESPE) and Colgate Total (Fluoride, Colgate-Palmolive Company, India). For that purpose, stainless steel brackets were applied on healthy maxillary first premolars. After thirty-one days of daily treatment with each toothpaste, the brackets were removed and the teeth were observed under a polarized light microscope. The depths of demineralized enamel were measured in three different sites of gingival demineralized area: gingival margin, middle third and occlusal margin [8].

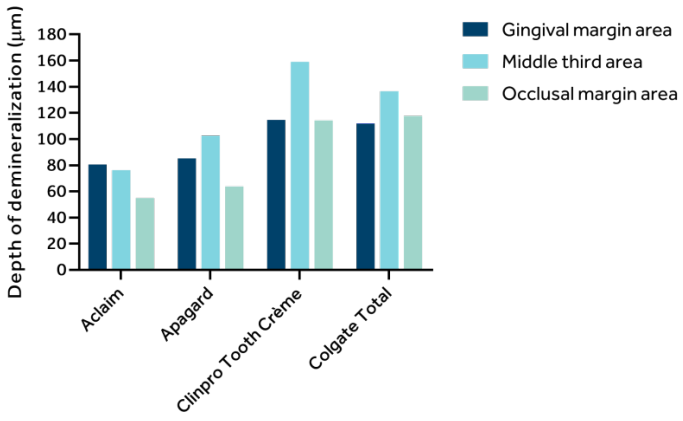


Figure 3: Site-specific comparison of depth of demineralization of each toothpaste tested. Lower values represent less demineralization.

- ✓ The Clinpro Tooth Crème and Colgate Total toothpastes were the less successful to inhibit enamel demineralization, in opposition to nano-hydroxyapatite-containing toothpastes;
- ✓ Comparing the two toothpastes that contain nano-hydroxyapatite (Aclaim and Apagard), Aclaim containing nanoXIM•CarePaste was more effective at reducing demineralized areas;
- ✓ The daily application of a toothpaste with nanoXIM•CarePaste provided higher protection against enamel demineralization in orthodontics.

Conclusion

The studies stated in this document evidence the **success of nanoXIM•CarePaste as an oral care ingredient**, demonstrating **excellent performance in enamel remineralization**.

nanoXIM•CarePaste achieves a higher rate of enamel remineralization than other commercial brands, and remains on the teeth even after washing. Moreover, research demonstrates the ability of nanoXIM•CarePaste to create a new and restored tooth surface.

References:

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2. Kanzow, et al. Quintessence international, 2016.
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8. Singh, et al. The Journal of Indian Orthodontic Society, 2017.

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nanoXIM CarePaste

WHITE PAPER

DENTAL HYPERSENSITIVITY

Effectiveness of nanoXIM•CarePaste on dental hypersensitivity prevention

Dental hypersensitivity

Dental hypersensitivity is a major and common dental condition that **occurs in approximately 57% of adults**. Among individuals with periodontal disease, the prevalence can be as high as 98% [1]. This condition is considered a relevant clinical problem. It is characterized by a short and sharp pain that arises from exposed dentin in response to chemical, thermal, tactile or osmotic stimuli [2, 3]. Essentially, dentin may become exposed by the removal of enamel or cementum as a result of friction, abrasion or erosion (caused by the action of acidic foods and sugary drinks). Consequently, the dentin tubules are unprotected, providing a direct connection between the internal pulp of the tooth and the external environment [3]. The contact of the pulp with the external stimuli triggers the nerves (Figure 1), causing intense pain that is a considerable concern for patients.

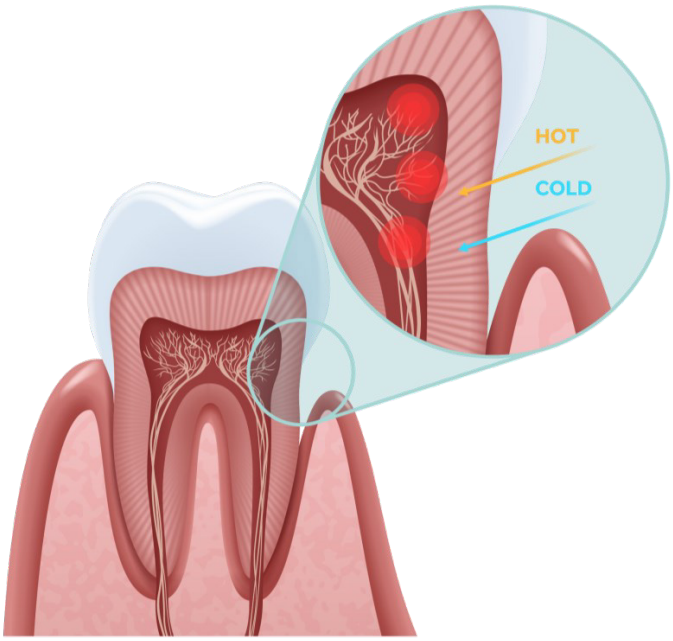

Figure 1: Dental hypersensitivity as a result of exposed dentin tubules in response to hot and cold stimuli.

Dental hypersensitivity is usually treated by applying desensitizing toothpastes composed mainly of strontium chloride or potassium nitrate. However, these products do not simulate the natural composition and structure of dentin and enamel [4]. **The mineralized tissues found in the human body are composed mostly of hydroxyapatite (HAp),** a natural calcium phosphate ceramic that is abundant in bone and dentin (70%), and enamel (97%). Tooth enamel is the hardest tissue in the human body and is made up of building blocks of HAp nanocrystals, 40 nm in size. The tissue is acellular and, unlike bone, cannot be repaired naturally [4]. Therefore, the regeneration of the enamel surface represents a significant challenge.

nanoXIM•CarePaste

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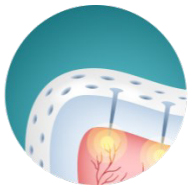
Mode of action



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2.

HAp has a great potential in the treatment of dental hypersensitivity, as it can be incorporated inside the dentin tubules. Consequently, these become sealed and pain is reduced.

3.

As a result, a new layer is formed, remineralizing the tooth enamel and protecting the tooth surface, preventing the appearance of new cavities and making it resistant to acid attacks of our favourite meals.

4.

The deposition of HAp on the enamel surface improves its smoothness for better light reflection, and consequently brighter and whiter teeth.

The effectiveness of nanoXIM•CarePaste has been confirmed in numerous studies

Study 1

The purpose of this *in vitro* study was to evaluate the effect of three different desensitizing agents: 6.5% nanoXIM•CarePaste (Aclaim Toothpaste, Group Pharmaceuticals, Ltd., India), 5% NovaMin (Shy NM toothpaste, Group Pharmaceuticals, Ltd., India) and 8% Pro-Argin™ (Colgate sensitive pro-relief toothpaste, Colgate-Palmolive India Ltd., India) [5].

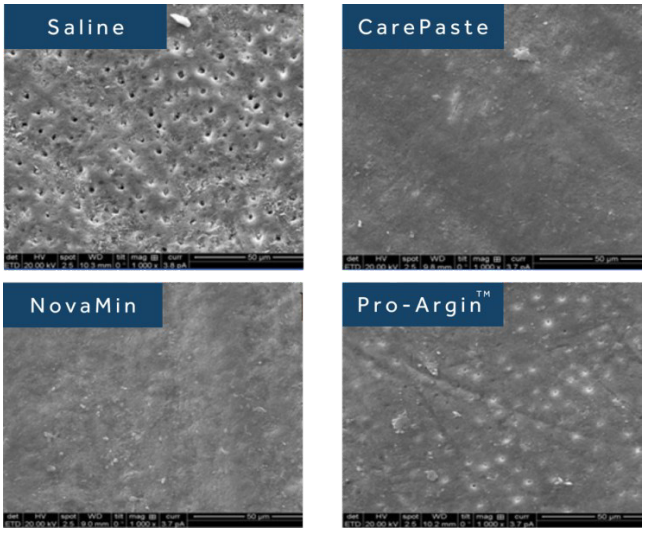

Figure 2: Scanning Electron Microscopy images of dentin discs treated daily with saline solution (negative control), nanoXIM•CarePaste, NovaMin and Pro-Argin™ for two minutes, for seven days.

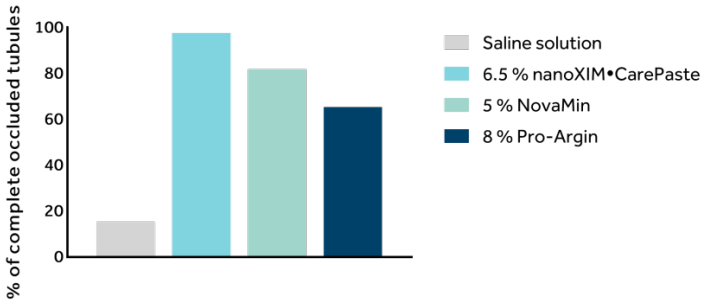

Figure 3: Percentage of completely occluded dentin tubules after seven days of treatment (two minutes daily treatment) with the different desensitizing agents.

- ✓ nanoXIM•CarePaste was the most effective desensitizing agent of this study, in comparison with NovaMin and Pro-Argin™ ingredients, showing almost complete tubule occlusion during the treatment period;
- ✓ A two-minute daily treatment for seven days with nanoXIM•CarePaste allowed 98% of completely occluded dentin tubules, in opposition to NovaMin and Pro-Argin™, with 82% and 65%, respectively.

Study 2

An *in vivo* clinical study of forty-five patients was performed to test the efficacy of Aclaim toothpaste (Group Pharmaceuticals, Ltd., India) containing 6.5% nanoXIM•CarePaste, Sensodent-K toothpaste (potassium nitrate) and a propolis dentifrices in controlling dental hypersensitivity [6].

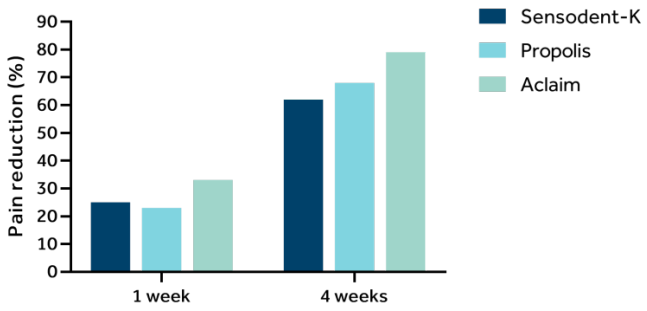

Figure 4: Reduction of dental hypersensitivity after treatment with Sensodent-K (potassium nitrate), Propolis and Aclaim (nanoXIM•CarePaste) during one and four weeks.

- ✓ Immediately after one week of treatment, a pain reduction of approximately 35% was observed among the patients treated with the nanoXIM•CarePaste. In opposition, the dentifrices containing potassium nitrate and propolis only decreased the hypersensitivity by 25%;
- ✓ The dentifrice containing nanoXIM•CarePaste was the most efficient in reducing dental hypersensitivity, allowing superior pain relief after one and four weeks of treatment with a final pain reduction of up to 80%.

Study 3

In this *in vivo* study, thirty patients diagnosed with dental hypersensitivity were treated with a commercially available toothpaste (Aclaim, Group Pharmaceuticals, Ltd., India) containing 6.5% nanoXIM•CarePaste. The patients were instructed to use the toothpaste twice a day and the treatment was performed for six months, with follow-up observations at one and three months [7].

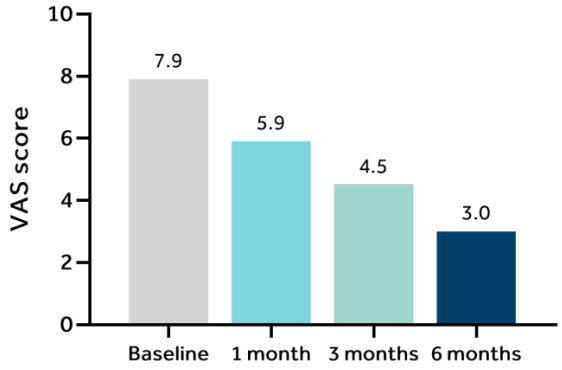

Figure 5: Hypersensitivity mean scores measured at baseline, one, three and six months after treatment, with Aclaim toothpaste (containing 6.5% nanoXIM•CarePaste).

- ✓ After one month of treatment, the patients treated with the Aclaim toothpaste containing nanoXIM•CarePaste experienced a pain reduction of 26%;
- ✓ An average hypersensitivity reduction of 62% was obtained after six months of treatment;
- ✓ On average, the regular use of a toothpaste containing nanoXIM•CarePaste allowed to reduce in six months from severe and intense pain to a mild level of pain.

Conclusion

The studies stated in this document evidence the **success of nanoXIM•CarePaste** as an oral care ingredient, demonstrating **excellent performance in treating dental hypersensitivity**, with successful dentin tubule occlusion and pain relief.

The nanoXIM•CarePaste achieves a higher rate of dental tubule occlusion than other commercial brands and remains on the teeth even after washing. Moreover, research demonstrates the ability of nanoXIM•CarePaste to create a new and restored tooth surface.

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FLUIDINOVA

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nanoXIM CarePaste

WHITE PAPER

WHITENING

Effectiveness of nanoXIM•CarePaste on restoring dental natural whiteness

Whitening

Smile aesthetics has gained increasing importance over the years. People value the appearance of their teeth and want to achieve the perfect white smile [1]. This not only suggests good dental health, but it is also considered an ideal beauty in most cultures. For that reason, dental aesthetics can have a huge impact on confidence, self-esteem and social acceptance [2]. Therefore, consumers are looking for products that will make their **teeth whiter and brighter**, with a healthier appearance. However, a significant number of whitening products can damage tooth enamel due to the presence of highly abrasive ingredients [3]. Clinicians and formulators have been trying to find alternatives for effective whitening ingredients that do not cause tooth wear. **One of the most effective is nano-hydroxyapatite**. The latter effectively binds to tooth enamel, creating a new and uniform mineralized layer. The new white and opaque layer formed on the tooth surface blocks incoming light and reflects white light. This results in a tooth-whitening effect (Figure 1) [4].

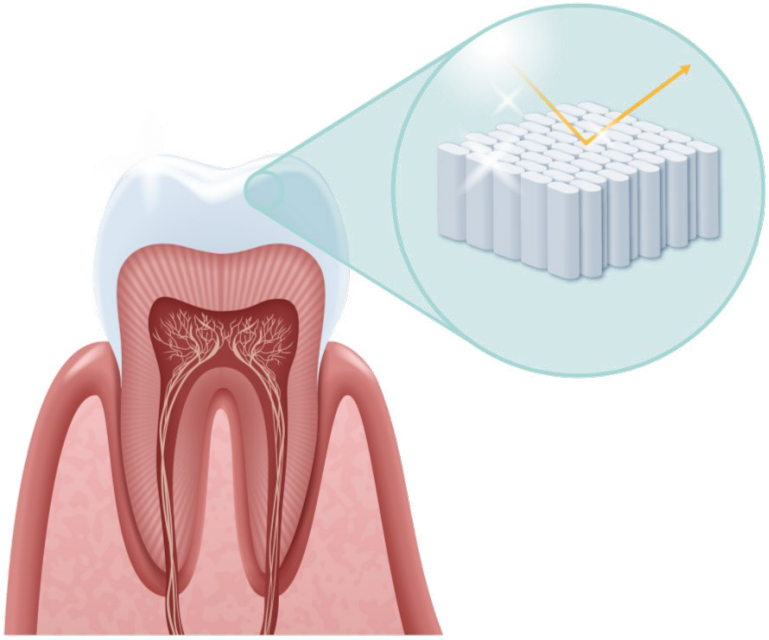
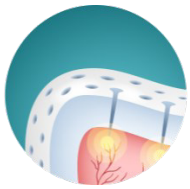


Figure 1: Nano-hydroxyapatite contributes to the formation of a uniform and smooth enamel layer. This new enamel layer is whiter, not only due to the remineralization but also related with its ability to reflect light, resulting in a tooth-whitening effect.

nanoXIM•CarePaste

The nanoXIM•CarePaste is a nano-hydroxyapatite (nHAp) ingredient produced and marketed by FLUIDINOVA. This synthetic water-based suspension ingredient has been **specifically developed for oral care applications**, such as toothpastes, gels, mouthwashes, dental floss, and other oral care products (personal and professional use). Nano-hydroxyapatite is a calcium phosphate material widely accepted in dentistry and medicine due to its exceptional biocompatibility and bioactivity. Its excellent performance is related to its nanometer size, being very similar to natural teeth and bone. nanoXIM•CarePaste contains high-purity nanoparticles under 100 nm in size, being much smaller than the dentin tubules. Therefore, they can be easily integrated inside the tubules, blocking them and **reducing the pain associated with dental hypersensitivity**. In addition, nanoXIM•CarePaste is able to bind to the dentin apatite and tooth enamel. Consequently, a new apatite layer is formed, **remineralizing the enamel, protecting the tooth surface, and restoring its natural whiteness.**

Mode of action



1.

Dental hypersensitivity, a short and sharp pain, prevents us from drinking hot coffee, ice cream, or even an orange juice without feeling pain. The action of certain food and drinks (hot, cold, acidic) are considered aggressions to our teeth, resulting in the exposure of dentin tubules and the underlying nerves to the external environment (the dentin loses its protective covering).

2.

HAp has a great potential in the treatment of dental hypersensitivity, as it can be incorporated inside the dentin tubules. Consequently, these become sealed and pain is reduced.

3.

As a result, a new layer is formed, remineralizing the tooth enamel and protecting the tooth surface, preventing the appearance of new cavities and making it resistant to acid attacks of our favourite meals.

4.

The deposition of HAp on the enamel surface improves its smoothness for better light reflection, and consequently brighter and whiter teeth.

The effectiveness of nanoXIM•CarePaste has been confirmed in numerous studies

Study 1

The purpose of this study was to investigate the tooth-whitening effects of mouthrinses containing different sizes of hydroxyapatite (HAp) particles after prolonged application time and compare them with a commercial whitening mouthrinse. For that, fifty bovine incisors were stained and randomly distributed into five groups: the HAp groups with 3 µm, 200 nm and 50 nm particle size (nanoXIM•CarePaste), the commercial whitening mouthrinse group and the distilled water group. The teeth underwent prolonged mouthrinse applications that were equivalent to simulated three and six-month mouth rinsing applications. Tooth color was measured and calculated before and after mouth rinsing [5].

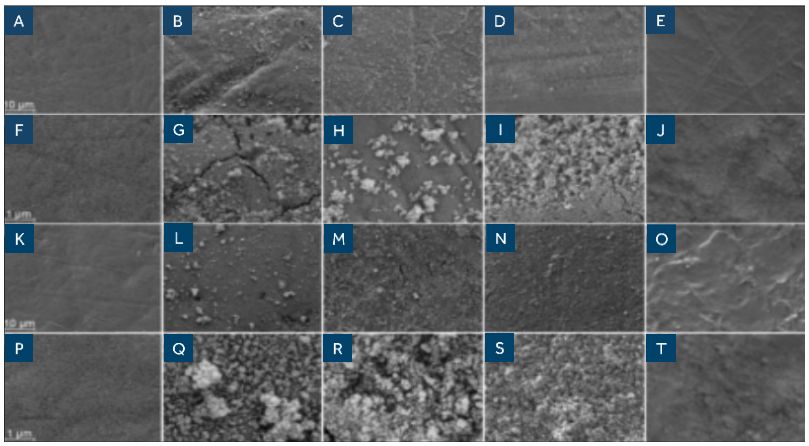


Figure 20: The mouthrinse-treated enamel surfaces were visualized at 1,000× and 10,000× after applications equivalent to three and six months.

A and F: untreated enamel surface at 1,000×.

K and P: untreated enamel surface at 10,000×.

B-E: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 1,000× after applications equivalent to three months.

G-J: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 10,000× after applications equivalent to three months.

L-O: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 1,000× after applications equivalent to six months.

Q-T: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 10,000× after applications equivalent to six months.

- ✓ The whitening effect of HAp mouthrinses after the prolonged application time was confirmed.
- ✓ The HAp mouthrinses exhibited similar tooth-whitening effects to the commercial whitening mouthrinse. It was also observed that the tooth-whitening performance of HAp was dependent on the particle size and application time;
- ✓ The HAp 50 nm particles (nanoXIM•CarePaste) showed better tooth-whitening performance after a longer period of mouth rinsing than the microsized HAp particles.

Study 2

In this study, it was evaluated the whitening efficacy of VITIS Whitening toothpaste (Dentaid S.L., Spain) that contains 3% of nanoXIM•CarePaste [6].

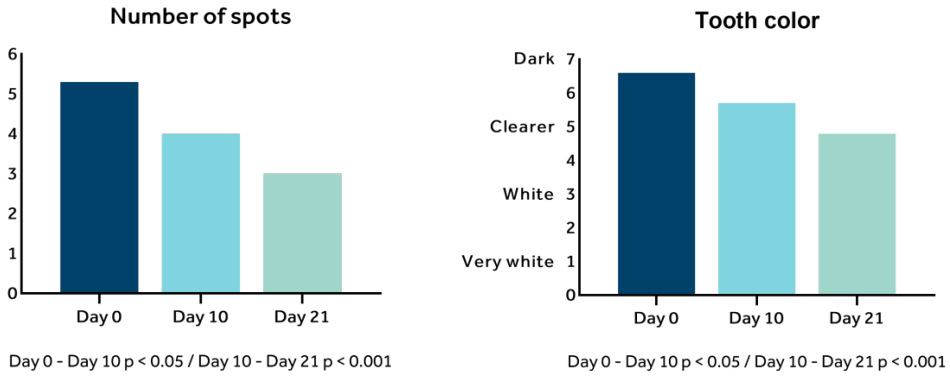


Figure 21: Whitening effect of VITIS Whitening toothpaste (containing 3% nanoXIM•CarePaste). A reduced number of dental stains and whiter teeth are achieved after ten and twenty-one days of use.

- ✓ A 24% reduction in the number of dental staining was observed in 65% of patients after ten days of use;
- ✓ A 38% reduction in the number of dental staining was observed in 75% of patients after twenty-one days of use;
- ✓ After ten days of use, it was noticed teeth whitening in 45% of patients;

Conclusion

The studies stated in this document evidence the **success of nanoXIM•CarePaste as an oral care ingredient**, demonstrating **excellent performance in restoring the teeth's natural whiteness**. Whitening performance was also found to be dependent on particle size, with nano-sized HAp particles being the most effective.

Moreover, research demonstrates the ability of nanoXIM•CarePaste to create a new and restored tooth surface.

References:

1. Alkahtani, et al. Journal of Dentistry, 2020.
2. Dudea, et al. Journal of Dentistry, 2012.
3. Schemehorn, et al. Journal of Clinical Dentistry, 2011.
4. Limeback, et al. Dentistry Journal, 2023.
5. Shang, et al. American Journal of Dentistry, 2021.
6. Dentaid SL. Vitis Whitening Brochure, 2015.

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Technical Data Sheet

Mod.068.11
Revision date: 18/04/2023

PRODUCT DESCRIPTION

nanoXIM•CarePaste is a synthetic nano-hydroxyapatite aqueous suspension for cosmetic applications, manufactured by FLUIDINOVA, S.A.

nanoXIM CarePaste

Identification

Trade name	nanoXIM•CarePaste
SKU	504102
INCI name	Hydroxyapatite (nano)
IUPAC name	Pentacalcium hydroxide triphosphate
CAS number	12167-74-7
EC number	235-330-6
Synonyms	Hydroxyapatite (CAS No.1306-06-5)
Chemical formula	$Ca_{10}(PO_4)_6(OH)_2$
REACH ID number	01-2119490075-38-0021

General properties

	Unit	Value
Density	g/cm³	1,13 ± 0,02
Particle size	nm	< 100

Particle type	rod-shaped
Shelf life	3 years
Physical appearance	White aqueous suspension

PRODUCT SPECIFICATIONS

	Unit	Specifications
Hydroxyapatite identification		Conforms to structure
Solids	wt%	20,0 ± 1,0
Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$	wt%	15,5 ± 0,5
Potassium Chloride, KCl	wt%	4,5 ± 0,5
Water, H_2O	wt%	80,0 ± 1,0
pH @ 25°C		10,0 ± 0,5
Total heavy metals (as Pb)	ppm	≤ 20
Total Aerobic Mesophilic Microorganisms (bacteria, yeast & mold)	cfu/g	≤ 100

USES AND APPLICATIONS

nanoXIM•CarePaste is recommended for cosmetic products. In oral care formulations is mainly used in toothpastes, mouthwashes, foams, gels and products for whitening, remineralization and sensitivity treatment.
This products can be easily incorporated in water-based formulas, simply by replacing part of the water in the formulation.
For toothpastes, typical incorporation range is 3 to 15%, with a maximum of 64.5%.
For mouthwashes, typical incorporation range is 1 to 3%, maximum.

PACKAGE

Standard packing available in 20 kg food-grade HDPE containers. Large quantities are supplied in 20 kg containers packed on pallets, preferably in multiples of 240 kg up to a maximum of 720 kg per pallet. Other options may be available upon request.

Minimum Order Quantity: 1kg

COMPANY CERTIFICATION/REGISTRATIONS

ISO 9001:2015
https://www.fluidinova.com/docs/iso_9001_certificate.pdf

REACH

All products sold by FLUIDINOVA are compliant with REACH regulatory requirements. All substances of the FLUIDINOVA portfolio have been registered under REACH or are exempt from REACH registration.
To fully understand the obligations that you as our customer have under REACH, we recommend that you consult the ECHA website.

STORAGE, SAFETY AND HANDLING

To ensure the quality of the product, keep it in a closed container at room temperature in a clean and dry place. Do not freeze.
For more details about product safety and handling information, please see the product Safety Data Sheet (SDS).
Shake before use to ensure homogeneity.

PRODUCT SEALS

VEGAN

NATRUE Approved

FLUIDINOVA, S.A.
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www.fluidinova.com | nanoxim@fluidinova.com | T: +351 220 119 746

FLUIDINOVA
THE NANO-HYDROXYAPATITE SPECIALIST

STATEMENTS

Absence of restricted substances: This product does not contain and it is not derived from the known substances listed in Annex II of REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products.

Allergen: This product does not contains and is not derived from the known allergens listed in Annex II regarding SUBSTANCES OR PRODUCTS CAUSING ALLERGIES OR INTOLERANCES of the REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Gluten-free and Lactose-free

Animal Non-Testing: This product has not been subject to any animal testing by or on behalf of FLUIDINOVA S.A.
This product complies with the animal test restrictions of the Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, in particular with the article 18 of chapter V of this regulation.

Animal and vegetable derivatives: This product does not contain and it is not derived from any animal or vegetable source.
Therefore, any animal or vegetable based material is present in its composition.
This product is fully synthetic produced by wet chemical precipitation using purified water and inorganic chemical reactants.

BSE/TSE: This products is made from inorganic calcium and phosphate salts and water, in a dedicated plant. The ingredients and the sources of these ingredients are inorganic and not of animal or vegetable origin. There are no additives or extracts of the brain, spinal cord, bone marrow, or eyes of any animal. The product does not contain, nor is it derived from any category A, B or C materials as defined in the Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMA/410/01 rev.3) (2011/C 73/01). Bovine Spongiform Encephalopathy (BSE) / Transmissible Spongiform Encephalopathy (TSE) should not be a concern associated with the use of this product.

CMR substances: This product does not contains any carcinogenic, mutagenic or toxic for reproduction (CMR) substances classified as category 1A, 1B and 2 according to Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

Dyes free: This product is dye free. No dyes are used at any stage of the production process.

GMO: This product is not genetically modified or not derived from a genetically modified organism as defined by the EC regulations:
- Directive 2001/18/EC on the deliberate release of GMOs into the environment
- Regulation (EC) 1829/2003 on genetically modified food and feed
- Regulation (EC) 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms.

HALAL: This product sole manufactured by FLUIDINOVA is compliant with all the HALAL requirements.
- Do not contain any ingredient of animal origin;
- No process aid of animal origin is used in the production process;
- Do not contain any ingredient of vegetable origin;
- Do not contain alcohol and alcohol has not been used in the manufacturing process;
- The equipment used for the manufacturing, filling and packing is dedicated for these products and is not used for any other process related with products of animal origin or containing ingredients of animal source;
- During the manufacturing, storage and handling, the product is completely fully separated from foods and alcohol;
- The ingredients used to produce these products are inorganic and purified water.

Ionization: This product has not been subject to any irradiation or ionization procedure at any stage of its manufacturing or product final storage.

KOSHER: This product sole manufactured by FLUIDINOVA is compliant with all the KOSHER requirements
- Do not contain any ingredient of animal origin;
- No process aid of animal origin is used in the production process;
- Do not contain any ingredient of vegetable origin;
- Do not contain alcohol and alcohol has not been used in the manufacturing process;
- The equipment used for the manufacturing, filling and packing is dedicated for these products and is not used for any other process related with products of animal origin or containing ingredients of animal source;
- During the manufacturing, storage and handling, the product is completely fully separated from foods and alcohol;
- The ingredients used to produce these products are inorganic and purified water.

Nano-safety and SCCS Final Opinion approval: SCCS considers hydroxyapatite (nano) safe when used at concentrations up to 10% in toothpaste (i.e. 64.5% nanoXIM•CarePaste), and up to 0.465% in mouthwash (i.e. 3% nanoXIM•CarePaste).
The SCCS safety evaluation only applies to the hydroxyapatite (nano) with the characteristics of nanoXIM•CarePaste:
- composed of rod-shaped particles of which at least 95.8% (in particle number) have an aspect ratio less than 3, and the remaining 4.2% have an aspect ratio not exceeding 4.9;
- the particles are not coated or surface modified.
This Opinion is not applicable to hydroxyapatite (nano) composed of needle-shaped particles. The nanoXIM•CarePaste does not contains needle-shaped particles.
The Final Opinion SCCS/1648/22 (https://health.ec.europa.eu/publications/hydroxyapatite-nano-0_en) and safe use conclusions will only apply to the nano-hydroxyapatite with the characteristics of nanoXIM.CarePaste product referred in the studies and manufactured only by FLUIDINOVA. For more information, visit https://fluidinova.com/nano-safety.

NATRUE: This product is approved by The International Natural and Organic Cosmetics Association (NATRUE) as a NATRUE approved raw materials, classified as 100% Nature-identical substances, being suitable for cosmetic formulations, including, but not limited, to oral care products.

Origin: This product is fully synthetic, produced at our site in Maia, Portugal (COO).

Parabens free: This product is a parabens free.

Palm Oil: This product do not contain any palm oil source and are considered completely palm oil free. It is not used any vegetable source components or raw materials in its manufacturing process. This product is inorganic and synthetic, being manufactured, packaged and stored in a dedicated equipment and facilities.

Pesticides: This product comply with Regulation 2005/396/EC on maximum level of pesticides in or on food and feed and animal origin and its amendments.

Prop. 65: This product do not contain any of the chemicals listed on California's Safe Drinking Water & Toxic Enforcement Act of 1986, commonly known as Proposition 65 (Prop 65).

Residual Solvents: This product is a fully synthetic inorganic product and does not contains nor it is derived from any solvent source. This product is never in contact with any solvent at any stage of its manufacturing or storage. No residual solvents are present in this product.

SLS/SLES: This product is free from Sulphate (aka Sulfate), SLS, SLES.

SVHC: This product do not contain any of the REACH Substances of Very High Concern (SVHC), according to the Candidate list published by ECHA (European Chemical Agency).

Vegan: This product sole manufactured by FLUIDINOVA is compliant with all the VEGAN requirements.
- Do not contain any ingredient of animal origin;
- No process aid of animal origin is used in the production process;
- Do not contain any ingredient of vegetable origin;
- Do not contain alcohol and alcohol has not been used in the manufacturing process;
- Do not contain known animal-derived GMOs or genes in the ingredients or finished product manufacture;
- Do not involve any animal testing of ingredients or finished product;
- The equipment used for the manufacturing, filling and packing is dedicated for these products and it is not used for any other processes related with products of animal or vegetable origin nor containing ingredients of animal or vegetable source;
- The ingredients used to produce these products are inorganic salts and purified water.

MISCELLANEOUS

Although the information set forth herein are presented in good faith and believed to be correct as of the date hereof, the persons receiving the same will make their own determination as to its suitability for their purposes prior to use.
Additionally, it is the user's responsibility to verify, in every case, the local legislation related to the use of the product.
The product specification limits are subject to change. Please contact FLUIDINOVA for the most current data sheet.

FLUIDINOVA
THE NANO-HYDROXYAPATITE SPECIALIST

nanoXIM
CarePowder

MICRO-HYDROXYAPATITE

THE FUTURE OF ORAL CARE: AN ALTERNATIVE TO FLUORIDE

- To enhance enamel remineralization
- To prevent dental hypersensitivity
- To restore natural whiteness

INNOVATIVE ORAL CARE

FLUIDINOVA

THE NANO-HYDROXYAPATITE SPECIALIST

ABOUT US

FLUIDINOVA was founded in 2005 in Portugal as a specialized manufacturer of synthetic nano-hydroxyapatite and tricalcium phosphate materials, which are commercialized worldwide for different applications (e.g., oral care, biomaterials, 3D printing, food-supplements, biotech, catalysts, etc), under the brand name nanoXIM®.

nanoXIM
CARE POWDER

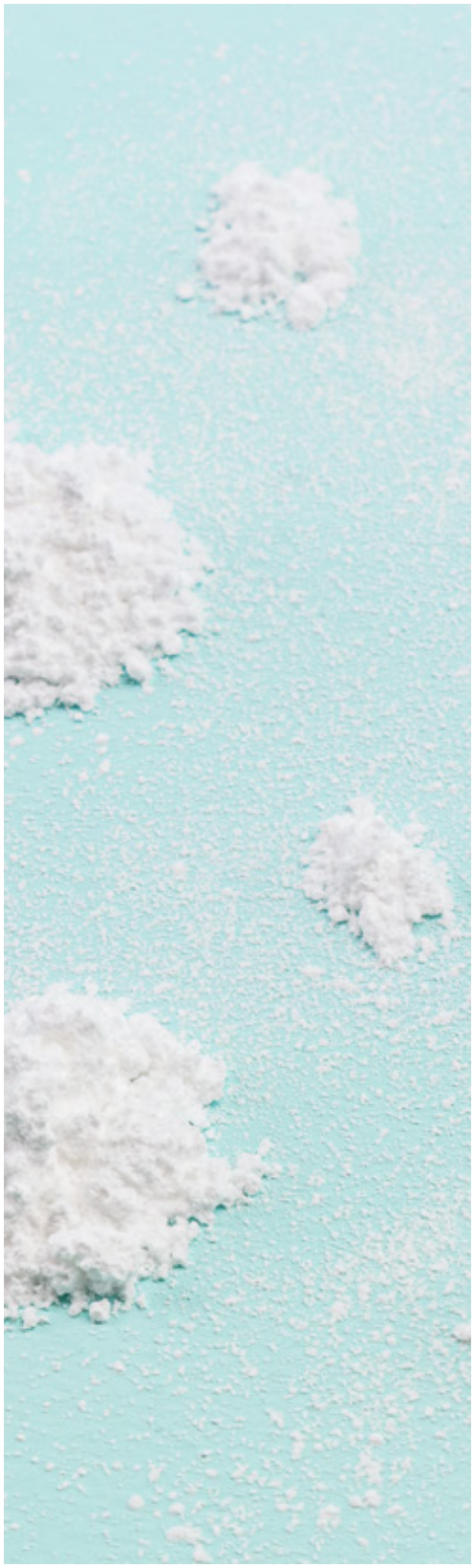
Can I effectively develop a fluoride-free oral care product?

YES, you can!

An alternative to fluoride is Hydroxyapatite – a 100% safe and non-toxic ingredient to oral care products and routine.

The nanoXIM•CarePowder is a micro-hydroxyapatite ingredient produced and marketed by FLUIDINOVA.

Its excellent performance is related to its high similarity to natural teeth.



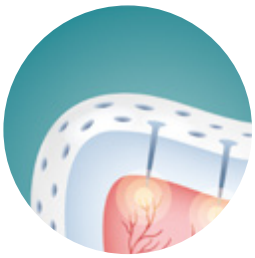
BENEFITS

- Pain reduction
- Smooth and protected tooth surface
- Dental hypersensitivity prevention
- Enamel remineralization
- Cavity prevention
- Restored natural whiteness

What is Hydroxyapatite?

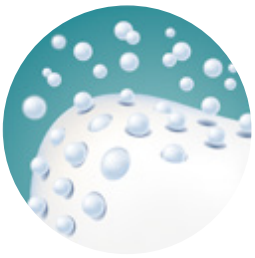
Hydroxyapatite (HAp) is a form of calcium phosphate that makes up to 97% of tooth enamel and 70% of dentin. Since it is a key component of teeth, it is biocompatible. Our body recognizes HAp as a familiar compound.

Thus, products that incorporate HAp simulate the natural composition and structure of teeth, which is why they work effectively.



1.

Dental hypersensitivity, a short and sharp pain, prevents us from drinking hot coffee, ice cream, or even an orange juice without feeling pain. The action of certain foods and drinks (hot, cold, acidic) are considered aggressions to our teeth, resulting in the exposure of dentin tubules and the underlying nerves to the external environment (the dentin loses its protective covering).



2.

HAp has a great potential in the treatment of dental hypersensitivity, as it can be incorporated inside the dentin tubules. Consequently, these become sealed and pain is reduced.

3.

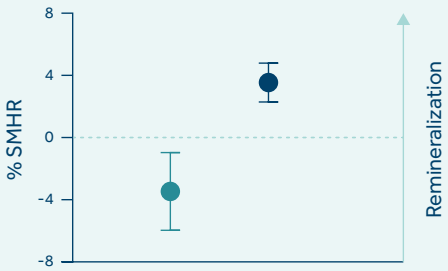
As a result, a new layer is formed, remineralizing the tooth enamel and protecting the tooth surface, preventing the appearance of new cavities and making it resistant to acid attacks of our favourite meals.

4.

The deposition of HAp on the enamel surface improves its smoothness for better light reflection, and consequently brighter and whiter teeth.

The effectiveness of nanoXIM·CarePowder on enamel remineralization was confirmed in the following study:

The enamel remineralization potential of a 5 % hydroxyapatite nanoXIM·CarePowder toothpaste tablet was evaluated by treating demineralized enamel lesions during 2 minutes, twice a day for 20 days. A similar toothpaste tablet without hydroxyapatite was used as a control. Both toothpaste tablets were fluoride-free.



0 % nanoXIM·CarePowder: -3.57 ± 0.62

5 % nanoXIM·CarePowder: 3.22 ± 0.30

Percentage of surface microhardness recovery (SMHR) for the two toothpaste tablet (0% and 5 % nanoXIM·CarePowder), after 20 days of treatment. Higher SMHR represents a superior enamel remineralization capacity.

This study showed that nanoXIM·CarePowder is an effective remineralizing agent when incorporated in toothpaste tablets – with a 6.8 % outperformance of SMHR, compared to a toothpaste tablet without nanoXIM·CarePowder.

Reference: Therametric Technologies, *In Vitro* Enamel Remineralization Testing Using a pH Cycling Model (2022).

FEATURES

- Synthetic fine white powder
- Small size microparticles
- High purity
- Biocompatible
- Vegan
- Safe if accidentally swallowed

nanoXIM•CarePowder is the recommended ingredient for dry formulations.

It is incorporated in tooth powders, toothpaste tablets and chewing gums.

Tooth Powders

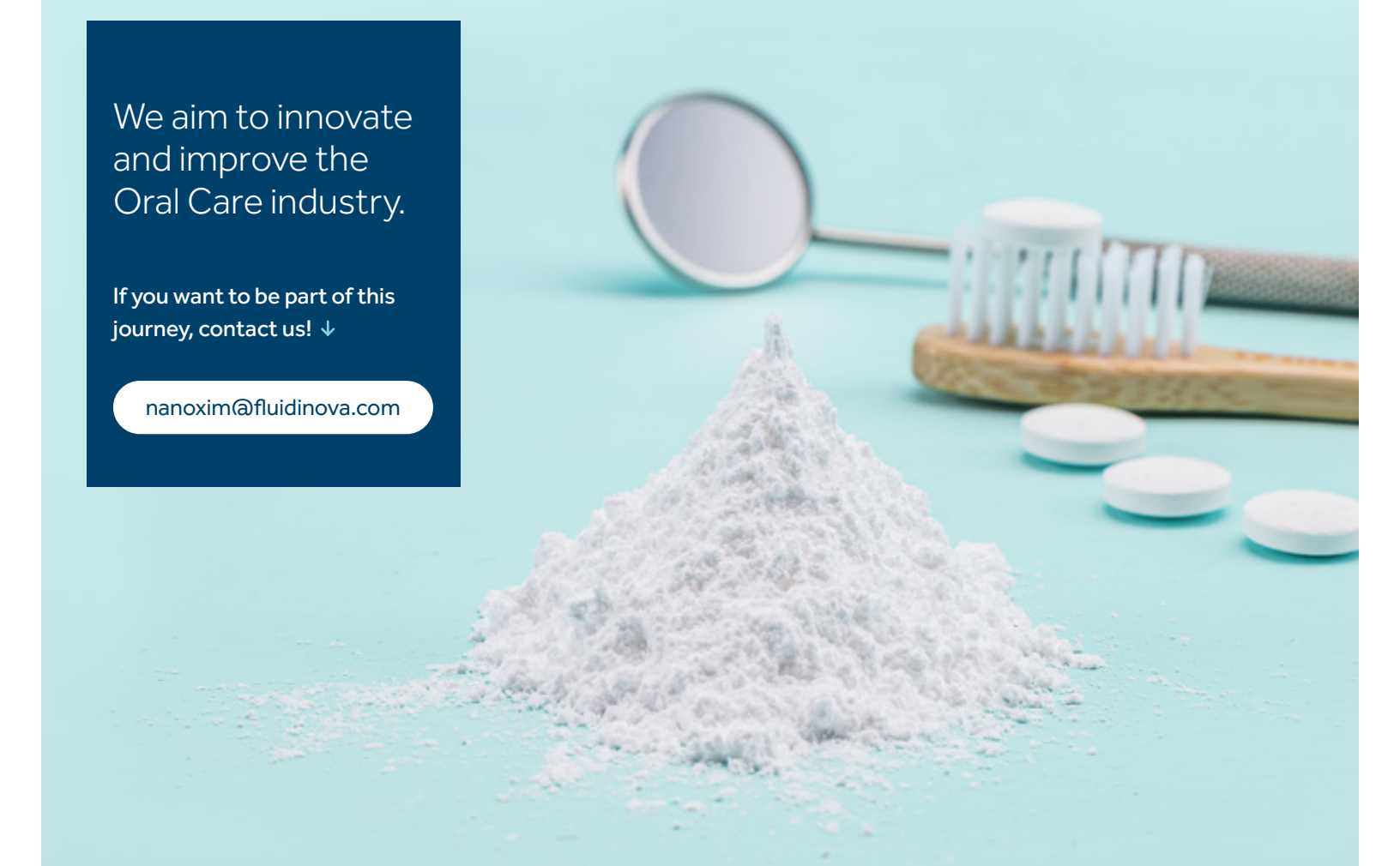
Toothpaste Tablets

Chewing Gums

We aim to innovate and improve the Oral Care industry.

If you want to be part of this journey, contact us! ↓

nanoxim@fluidinova.com



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FLUIDINOVA
THE NANO-HYDROXYAPATITE SPECIALIST

VEGAN
NATRUE Approved

List of publications in Oral Care

nanoXIM•CarePaste

1. R. Shang, D. Kaisarly, K.-H. Kunzelmann, “Tooth whitening with an experimental toothpaste containing hydroxyapatite nanoparticles”, BMC Oral Health 22(331), (2022). **LINK**

2. R. Shang, “Non-oxidative Tooth Whitening Effect of Hydroxyapatite on Bovine Enamel”, PhD thesis in Dentistry, Faculty of Medicine Ludwig-Maximilians-Universität München (2022). **LINK**

3. F. Tekrawala, H. Shah, A. Singh, J. Pariyani, “Prospective, Single-Centre, Non-Randomized, Open-Label, Single-Arm, Post Marketing Study to Evaluate Safety and Efficacy of DENTE91® Mom Toothpaste and DENTE91® Mom Mouthwash in Pregnant Females”, Paripex - Indian Journal of Research, 11(12), p. 161 (2022). **LINK**

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List updated on 28/02/2023.

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Biomimetic tooth-whitening effect of hydroxyapatite-containing mouthrinses after long-term simulated oral rinsing

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ABSTRACT: Purpose: To investigate the tooth-whitening effects of mouthrinses containing different sizes of hydroxyapatite (HAP) particles after prolonged application time and compare them with a commercial whitening mouthrinse. **Methods:** 50 bovine incisors were stained and randomly distributed into five groups: the HAP groups with 3 μm, 200 nm and 50 nm particle size, the commercial whitening mouthrinse group and the distilled water group. The teeth underwent prolonged mouthrinse applications that were equivalent to simulated 3- and 6-month mouthrinsing. Tooth color was measured and calculated before and after mouthrinsing. The group and application time effects were analyzed with a nonparametric analysis of longitudinal data using the nparLD package in R and ANOVA-type statistic was reported. Pairwise Wilcoxon rank-sum tests with BH correction were performed to compare the tooth color changes of individual groups. The mouthrinse-treated enamel was observed by SEM. **Results:** The whitening effect of HAP mouthrinses after the prolonged application time was confirmed. The HAP mouthrinses exhibited similar whitening effects to the commercial mouthrinses. The particle size and application time could significantly affect the whitening performance of HAP mouthrinses. The 50 nm HAP group exhibited significantly higher △E values than the 3 μm group after the 6-month-equivalent application ($P= 0.024$). A longer period of application increased significantly the △E and △L values ($P< 0.05$). The HAP-treated enamel surfaces were entirely covered with HAP after the 6-month-equivalent application. (*Am J Dent* 2021;34:307-312).

CLINICAL SIGNIFICANCE: The HAP nanoparticles showed better tooth-whitening performance after a longer period of mouthrinsing than the microsized HAP particles. This should be taken into consideration by dental manufacturers for optimizing the particle size for their HAP-containing products. To achieve a better outcome in tooth-whitening, the patients should apply the mouthrinse regularly for an extended period of time.

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Introduction

The portrayal of perfect white smiles in the media has increased the public self-awareness of discolored teeth. The desire and demand for whiter teeth have led to a growing need for oral whitening products. In response, over-the-counter (OTC) bleaching products soon became popular when they were introduced in the market at the beginning of the 2000s.[1] Hydrogen peroxide, the active agent in the OTC bleaching products, has potential adverse effects such as post-bleaching hypersensitivity, gingival irritation, alteration in enamel micro-hardness, and even genotoxicity and carcinogenicity.[2-4] Concerning those adverse effects, the EU Council Directive 2011/84/EU stated that only the products containing up to 0.1% of hydrogen peroxide were safe and could be sold as cosmetics to consumers without the supervision of dentists on 31 October 2012. However, this concentration might be too low to have a satisfying tooth-whitening effect.[5] For safety reasons, as well as the change of regulatory framework for the OTC bleaching products, non-peroxide alternatives are in urgent need.

Hydroxyapatite (HAP) is chemically equivalent to human tooth enamel and is widely used as a biomimetic material in various areas of dentistry because of its outstanding biocompatibility, e.g., in the treatment of dentin hypersensitivity, remineralization and repair of early caries, and prevention of periodontal diseases.[6-8] In the past decade, HAP has also been proven to be a promising tooth-whitening alternative by a series of laboratory and clinical studies[9-14] as it has no irritation effect on oral mucosa and does not change the enamel micro-structure during the whitening process.

The whitening mechanism of HAP dental products is that HAP particles could adhere seamlessly to the enamel surface and form a thin white layer,[15] which could increase light reflection and thereby make the tooth appear brighter.[11,16] Considering that smaller particles could exhibit stronger attachment and bonding ability to enamel due to their higher surface charges and larger contact area,[17] it is reasonable to assume that particle size might influence the HAP tooth-whitening effect. Indeed, this was confirmed by a study[10] showing that the HAP particles with a particle size smaller than 1 μm could lead to better whitening performance than the ones that were 1- 10 μm in size. However, it was reported[9] in a different study that the particle size of HAP had no influence on the whitening ability. So far, there is no consensus on the influence of HAP particle size on its whitening effect.

The use of a tooth whitening mouthrinse usually involves a daily mouth rinsing process and a long period of application. However, previous studies focused either on different application approaches where the HAP solutions were applied to the tooth surface with a cotton pellet[9,10] or on the instant whitening effect of HAP by applying it to the enamel with the application duration ranging from 1 to 9 minutes.[11,12,18] Although these studies confirmed the feasibility of the tooth-whitening effectiveness of HAP dental products, none of them investigated the whitening effect of HAP in a condition that is comparable to the real clinical condition. Therefore, studies that take a further step towards clinical reality and investigate a clinically relevant application protocol are needed.

To bridge this gap, the current study investigated the tooth-

whitening effects of mouthrinses containing different sizes of HAP particles after prolonged application time and compared them with a commercial peroxide-free whitening mouthrinse. The prolonged mouthrinse applications were equivalent to simulated 3- and 6-month mouthrinsing. Hopefully, these findings could provide information on the whitening effect of HAP mouthrinses after an extended (3-6 months) period of daily self-application and help the dental manufacturers make a rational decision on optimizing the HAP particle size in their products. The null hypothesis states that the HAP particle size does not influence the whitening effect of HAP mouthrinses.

Material and Methods

HAP suspension preparation – Mouthrinses, containing different particle sizes of HAP were prepared. The HAP materials used in the current study were provided by the companies Fluidinova and Budenheim free of charge. The median particle sizes of the HAP materials, according to the manufacturers' descriptions, are 3 μm, 200 nm and 50 nm, respectively. Each HAP material was mixed into water[a] to form a HAP suspension with a concentration of 10 wt%. The HAP mouthrinses were freshly made before every use.

Specimen preparation - A total of 50 extracted bovine incisors were randomly assigned to five groups (n=10 for every group): 3 μm HAP group, 200 nm HAP group, 50 nm HAP group, commercial whitening mouthrinse group (Listerine Advanced White[b]) and distilled water group.

Prior to treatment, the teeth were scaled with sound-driven oscillating instruments and carefully polished to remove the unwanted connective tissue. After thorough cleaning under running tap water, the teeth were stained artificially. The staining solution was prepared by dissolving six grams of instant coffee (Nescafe Espresso[c]) in 200 ml distilled, boiling water. The coffee solution was then centrifuged at 2,000×g/minute for 10 minutes (ROTIXA/A[d]). The supernatant after centrifugation was carefully selected, in which the samples were incubated for 3 days at 37°C. This incubation time simulated 3 months of coffee consumption.[19]

After removing the samples from the staining solution, the samples were polished with a polishing paste and cleaned with ultrasound for 5 minutes followed by blotting with an absorbent paper towel. Each sample was then embedded in a 3D-printed sample holder using a self-cured polyester material (Technovit 4000[e]). The sample holder had a measuring window aligned to the middle third of the tooth's labial surface.

Mouthrinsing simulation - Samples were mounted on the inner wall of a glass beaker, which contained the mouthrinse being tested. The mouthrinse was continuously stirred at the speed of 100 rpm to simulate the oral rinsing process. The glass beaker was sealed to avoid evaporation. Continuous exposure to mouthrinse for 12 hours is equivalent to 1 year of daily application (for 1 minute twice a day).[20] Therefore, in the present study, the samples were exposed to the mouthrinse regime for 3 and 6 hours to simulate the 3- and 6-month daily uses of mouthrinses. After the mouthrinsing process, the samples were gently rinsed with distilled water.

Color measurement - Sample color was measured with a spectrophotometer (Color Eye 7000[f]). To eliminate the interference of ambient light, the color measurement was performed in darkness. Before each measurement, the spectrophotometer was calibrated with a ceramic calibration tile. The color assessment was defined by Commission Internationale de l'Eclairage (CIE) L*a*b* color system, where L* is the axis of lightness from black (0) to white (100), a* is the axis from green (−) to red (+), and b* is the axis from blue (−) to yellow (+). The color of each sample was measured before and after the simulated mouthrinsing. The average color changes (△E values) were calculated using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where △L, △a, and △b are the differences in lightness, greenness-redness, and blueness-yellowness between before and after oral rinsing. The repositioning and reproducibility of color measurement were strictly controlled with the help of a 3D-printed position locator. The enamel surface was blotted with a soft paper towel to remove the excess liquid and kept moist before the color measurement.

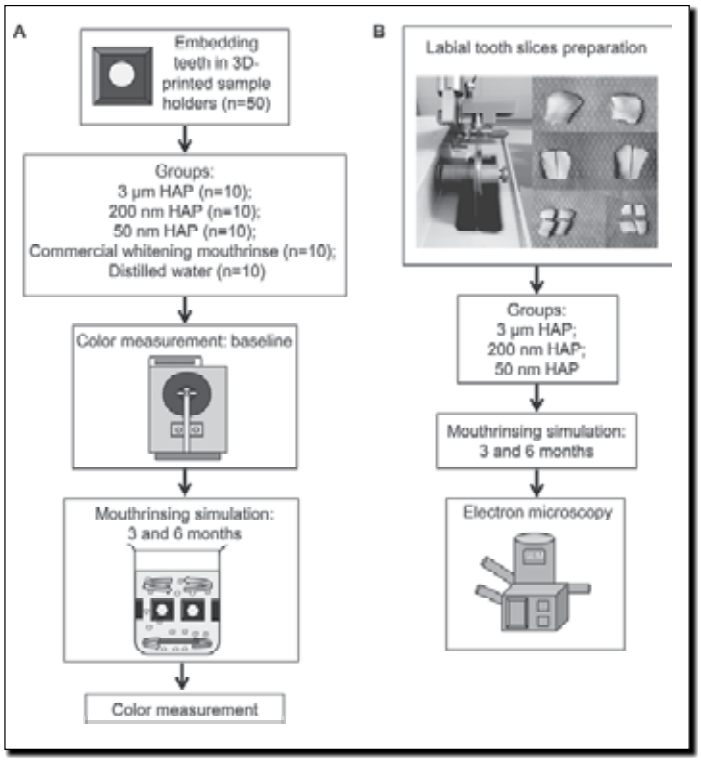

Fig. 1. The workflow of the present study. **A:** color assessment; **B:** SEM evaluation.

SEM evaluation - Bovine incisors were randomly selected, and were firstly cut along the median line into two parts. Only the labial tooth slices were chosen and randomly assigned to the three HAP groups. After the 3-month-equivalent and 6-month-equivalent mouthrinsing, the enamel surfaces were observed by field-emission scanning electron microscopy (FE-SEM, Supra 55vp[g]). After dehydration in increasing concentrations of ethanol, the specimens were sputter-coated with a 25 nm Au film using Au plasma (8*10-2 Pa, 20 mA, argon) in a vacuum evaporator (Polaron SC7620 Mini Sputter Coater[h]). Subsequently, the enamel surfaces were examined at the magnification of 1,000× and 10,000×. The workflow of the present study is presented in Fig. 1.

Statistical analysis - The data were analyzed in R. A non-parametric analysis of longitudinal data using the nparLD

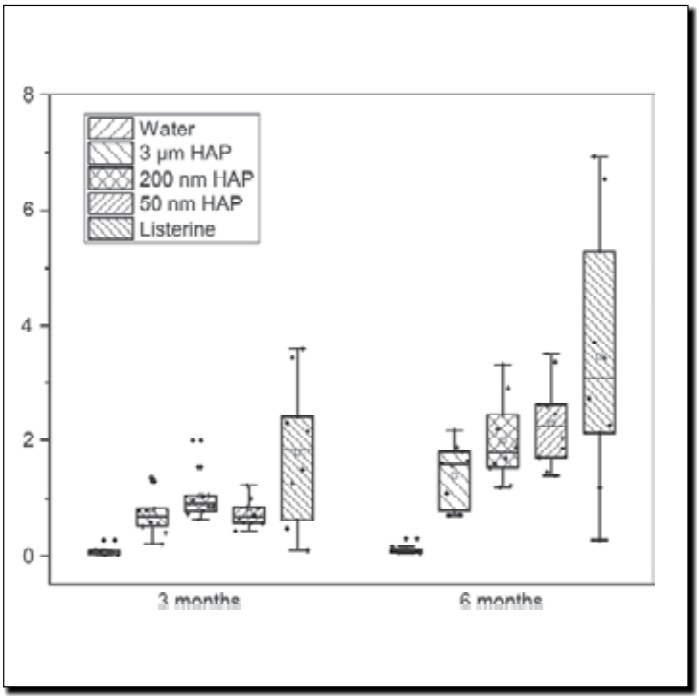

Fig. 2. The median and 95% confidence interval of △E values after 3- and 6-month-equivalent application.

package with the F1-LD-F1 design was performed to determine the group effects, the time effects and their interactions. When an interaction between the two main effects was found, a Kruskal-Wallis test was applied to confirm the group effect. Pairwise Wilcoxon rank-sum tests with BH correction were performed to compare the tooth color changes of individual groups. Paired Samples Wilcoxon tests were used to compare the color changes between the 3-month-equivalent and 6-month-equivalent applications. Statistical significance was set for all tests at $P< 0.05$.

Results

Color assessment - The ANOVA-type statistic revealed significant main effects of group and application time for the △E, △L, △a and △b values ($P< 0.00001$). Significant interactions were found between the two main effects for the △E and △L values ($P< 0.01$). The Kruskal-Wallis test confirmed the group effect on the △E and △L values ($P< 0.01$).

The △E values of each group were shown in Fig. 2. The commercial mouthrinse group exhibited the highest △E median values after the 3-month-equivalent (1.83) and the 6-month-equivalent (3.07) HAP applications. In contrast, the water group showed the lowest △E median values (0.05 and 0.08).

After the 3-month-equivalent HAP application, the △E median values of the 3 µm, 200 nm and 50 nm HAP groups were 0.67, 0.91 and 0.67, respectively. No significant differences in the △E values were found between individual HAP groups. After the 6-month-equivalent HAP application, the △E values of each HAP group increased significantly to 1.59 ($P= 0.027$), 1.79 ($P= 0.002$) and 2.25 ($P= 0.002$), respectively. In addition, the 50 nm HAP group exhibited significantly higher △E values than the 3 µm group ($P= 0.024$).

The tooth color changes of the HAP groups and commercial mouthrinse group were produced by the increased L* values and decreased a* and b* values. The median values and 95% confidence interval of the △L, △a, and △b values of each group were summarized in the Table. After the 6-month-equivalent HAP application, the △L values of HAP groups were significantly higher than those after the 3-month-equivalent application ($P< 0.05$). After the 6-month-equivalent application, the commercial mouthrinse group showed significantly higher △L values than the 3 µm HAP group ($P< 0.05$) but not the other two HAP groups.

Table. The color changes in L*, a* and b* axes (expressed as ΔL, Δa, and Δb values) at 3- and 6-month-equivalent applications. Results are shown as median value (95% confidence interval).

Groups	3 months	6 months
ΔL		
3 µm HAP	0.50 (-0.48, 0.68)a	1.07 (0.12, 1.92)b*
200 nm HAP	0.40 (-0.22, 1.21)a	1.38 (0.44, 3.27)ab*
50 nm HAP	0.34 (-0.43, 0.81)a	1.52 (-1.24, 3.43)ab*
Water	-0.01 (-0.06, 0.08)b	0.03 (-0.13, 0.08)c
Commercial mouthrinse	1.12 (-0.26, 2.49)a	2.25 (0.07, 4.36)a*
Δa		
3 µm HAP	-0.06 (-0.25, 0.38)a	-0.01 (-0.5, 0.52)ab
200 nm HAP	-0.16 (-0.31, 0.20)a	-0.45 (-0.63, -0.08)ab*
50 nm HAP	-0.07 (-0.30, 0.06)a	-0.21 (-0.65, 0.16)ab
Water	0.00 (-0.05, 0.04)a	0.01 (-0.07, 0.10)a
Commercial mouthrinse	-0.14 (-1.70, 0.06)a	-0.23 (-1.28, 0.07)b
Δb		
3 µm HAP	-0.35 (-1.18, 1.14)b	-0.86 (-1.49, 0.30)b*
200 nm HAP	-0.85 (-1.71, -0.12)c	-1.13 (-2.40, 0.31)b
50 nm HAP	-0.53 (-1.16, 0.08)c	-1.09 (-2.28, 1.12)b
Water	0.02 (-0.04, 0.27)a	0.03 (-0.07, 0.30)a
Commercial mouthrinse	-1.03 (-3.02, -0.09)c	-1.67 (-5.26, 0.33)b*

Lowercase letters indicate the differences between different groups with the same application time.
* indicates the statistical differences of the same group between the 3- and 6-month-equivalent application.
Superscripts of individual letters indicate groups that statistically differed from any other group ($P< 0.05$).
Shared superscripts suggest that groups showed no statistical differences ($P> 0.05$).
From a to c, the median value is decreased.

No significant difference in the △a values was found between the individual HAP group and commercial mouthrinse group. After the 3-month-equivalent application, the 3 µm HAP group exhibited significantly lower △b values than the other two HAP groups ($P< 0.05$).

SEM evaluation - After the 3-month-equivalent application, HAP agglomerates were identified on the enamel surfaces in all HAP groups (Figs, 3B-D, G-I). In the 3 µm group, most of the adhered HAP agglomerates were less than 1 µm (Figs. 3B and G), which were smaller than the median particle size (3 µm). The size of HAP agglomerates in the 200 nm HAP was similar to those in the 3 µm group (Figs. 3C and H). The 50 nm group exhibited a uniform size of HAP agglomerates, which connected with each other and distributed evenly on enamel (Figs. 3D and I). The enamel surfaces of the three HAP groups were not totally covered by HAP agglomerates. The enamel of the commercial mouthrinse group (Figs. 3E and J) seemed to have similar surface morphology to the untreated enamel (Figs. 3A and F).

After the 6-month-equivalent application, the enamel surfaces of all HAP groups were completely covered by thin layers of HAP (Figs. 3L-N, Q-S). Some of the HAP agglomerates of the 3 µm group grew larger and reached the

American Journal of Dentistry, Vol. 34, No. 6, December, 2021

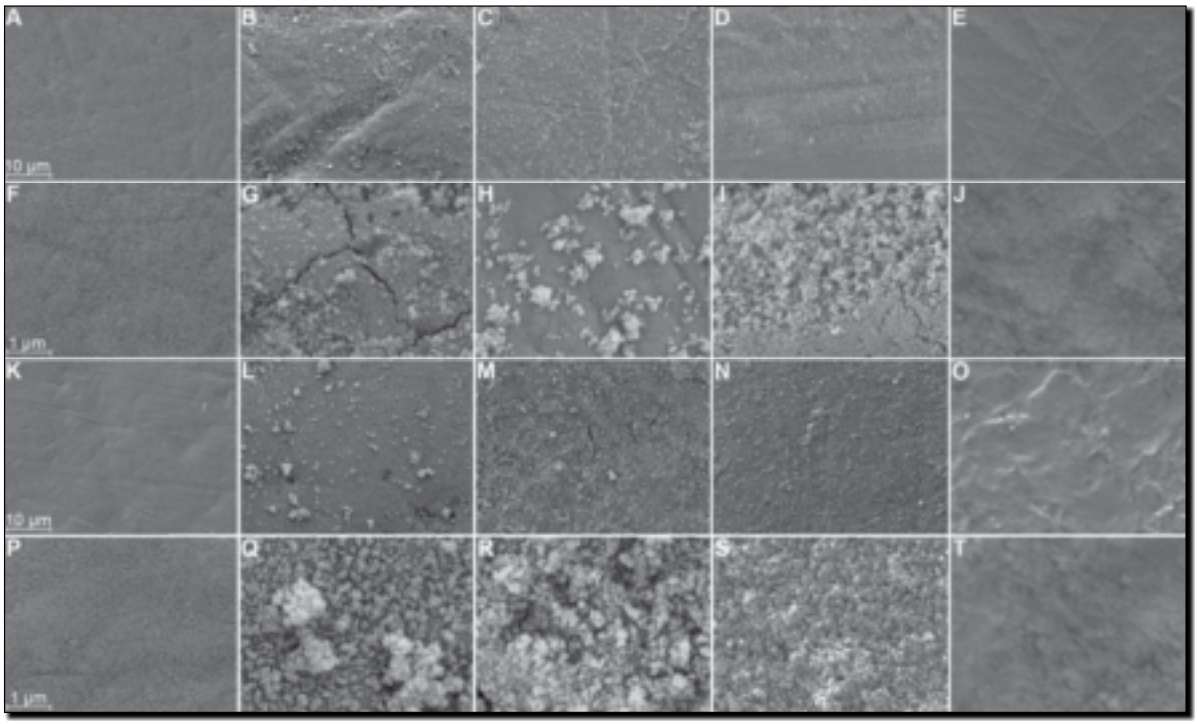

Fig. 3. The mouthrinse-treated enamel surfaces were visualized at 1,000× and 10,000× after the 3- and 6-month-equivalent applications.
A and F: untreated enamel surface at 1,000×.
K and P: untreated enamel surface at 10,000×.
B-E: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 1,000× after the 3-month-equivalent application.
G-J: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 10,000× after the 3-month-equivalent application.
L-O: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 1,000× after the 6-month-equivalent application.
Q-T: the enamel of the 3 µm, 200 nm, 50 nm HAP groups and the commercial mouthrinse group at 10,000× after the 6-month-equivalent application.

size of up to 3 µm in length (Figs. 3L and Q), which made the enamel surface seem to be rougher than the other two HAP groups. The HAP film of the 50 nm group seemed to be the least rough (Figs. 3N and S). No adhesion was observed on the enamel of the commercial mouthrinse group (Figs. 3O and T).

Discussion

HAP is a widely used biomimetic material in OTC dental products for the treatment of dental hypersensitivity and enamel demineralization. In their slogans, the "tooth-whitening effect" is often mentioned, but no studies, so far, have examined the whitening effect of HAP after prolonged application time. Here we show that the HAP mouthrinses exhibited similar tooth-whitening effects to the commercial mouthrinse throughout the observation period. We also found that the particle size and application term could significantly affect the whitening effect.

A strength of the present study is that we took a further step towards clinical reality. We applied HAP mouthrinses by the simulated mouthrinsing, which enabled us to evaluate the whitening effect of HAP mouthrinses by self-application. Second, we used pure HAP aqueous suspensions as the mouthrinses. This allowed us to investigate the whitening effect of HAP without interference from other mouthrinse additives, such as flavors, preservatives, and rheology modifiers, which may have an impact on the HAP whitening effect. Third, the vital tooth- and measurement-related interferences factors were strictly controlled. The labial middle third of the tooth was chosen for our research, which represented the best tooth color.[21] As the native tooth surface is multilayered and exhibits color transitions in all directions,[22] a reproducible positioning is crucial for accurate color measurements. We created a 3D-printed repositioning system to achieve precise repositioning. On the other hand, the study also had limitations. As with any other in vitro study, we could not reveal the influence of the complicated and non-predictable oral environment (such as variations of temperature, pH value, or nutritional habits, etc.) on the whitening effect of HAP.

The △E values after the 3- and 6-month-equivalent applications did not statistically differ between the commercial mouthrinse and individual HAP mouthrinse. Increased L* values and decreased a* and b* were observed in all HAP groups and the commercial mouthrinse group, indicating that both HAP and commercial mouthrinses could change the tooth color to a bright, green and blue tone. The HAP mouthrinses had lower median △E values but relatively narrower 95% confidence intervals than the commercial one, indicating the HAP mouthrinses had a relatively stable whitening effect. This finding may be explained by the different whitening mechanisms. According to the manufacturer, the commercial mouthrinse in the current study is non-abrasive and contains polyphosphate, which can dissolve the pigment molecules and prevent their deposition on enamel surfaces.[23,24] Therefore, the whitening performance might vary from tooth to tooth as each tooth has a different amount of pigment. Instead of dissolving pigments, the whitening effect of HAP products is caused by the adhered HAP particles, which could attach firmly to enamel

surfaces and change the light propagation, making the tooth appear whiter.[10,25]

Understanding the relationship between HAP particle size and adhesion behavior is crucial for optimizing the tooth-whitening performance of HAP whitening products. Fabritius-Vilpoux et al[26] found that the attachment of HAP to enamel surfaces relied largely on the ratio between the adhesive forces and the mass of HAP particles and that large particles tended to fall off when the sample was being washed. This finding was confirmed in the current study. After the 3-month-equivalent application, the sizes of the adhered HAP agglomerates in the 3 μm group (Figs. 3B and G) were smaller than 1 μm which suggested that smaller particles attached more strongly to the tooth surfaces compared with larger ones. The sizes of the HAP agglomerates in the 200 nm group (Figs. 3C and G) were similar to that in the 3 μm group after the 3-month-equivalent application, which might be the reason for the non-significance in the △E values. After the 6-month-equivalent application, the agglomerates in the 3 μm group grew larger (Figs. 3L and Q) and made the HAP-treated enamel surface rougher than the 50 nm group (Figs. 3N and S). In response, significantly higher △E values were found in the 50 nm HAP group compared with the 3 μm one. We assumed that the difference in the roughness of the adhered HAP layers could affect the light propagation on or through the tooth tissue and thereby change the tooth color appearance. However, no significant difference in the △E values was found between the 200 nm and 50 nm HAP groups. This result is consistent with a previous study, in which the researchers reported that the HAP particles with the length of 60-100 nm and 100-200 nm had similar whitening abilities.[27]

In the present study, prolonged application times led to increased △E and △L values of all HAP groups. The researchers of the previous studies, which focused on the HAP instant whitening effect, believed that once the enamel surface was covered with the maximal adhering HAP load, reapplication would not result in any further color changes.[10,12] They reported the maximal HAP adherence could be achieved within three cycles of application. In their studies, the HAP materials were mechanically rubbed on the teeth with the application term ranging from 30 seconds to 3 minutes. In contrast to these studies, we did not observe the maximal HAP adhering load. This suggested a different adhesion behavior for HAP when it was applied in a hydrodynamic environment induced by the simulated oral rinsing.

After the 6-month-equivalent HAP application, the △E values of the 3 μm, 200 nm and 50 nm HAP groups, compared with the values from the 3-month-equivalent application, increased significantly to 1.59, 1.79 and 2.25, respectively. Given that the △E values of higher than 1.7 could be detected by observers,[28] the tooth color changes in the 200 nm and 50 nm group after the 6-month-equivalent application were considered visually perceptible. Comparing to the tooth color changes caused by prolonged HAP applications, the previous 9-minute short-term approaches showed similar △E values, ranging from 0.91 to 2.20.9.[10] It is worth mentioning that in these studies the teeth were air-dried before the color measurement. Considering that tooth dehydration could lead to a decrease in enamel translucency and an enhancement in luminosity and therefore result in a false whiter appearance of a tooth,[29] the tooth color changes in these previous in vitro studies might be overestimated. In our study, we kept the teeth moist before the color measurement and conducted the measurement as quickly as possible to avoid the undesirable influence of dehydration[30]

The increase in the L* values has been proven to be due to the enhanced light reflection at the HAP-adhered tooth surface.[11,31] The SEM images showed that the enamel surfaces of all HAP groups were entirely covered by adhered HAP layers after the 6-month-equivalent mouthrinsing, whereas the enamel surfaces were not completely covered after the 3-month-equivalent HAP application. We assumed that more light was reflected from the 6-month-equivalent HAP-treated enamel surface and therefore made the tooth appear brighter.

The adhered HAP layers observed in the present study might protect the tooth surface from acidic attacks as the layers would interact initially with the acid.[10] From a chemical point of view, the adhered HAP layers would be dissolved in the acidic environment, resulting in the reduction of the whitening effect. However, researchers observed the HAP particles could adhere to enamel even in an acidic solution,[15] which suggested that the acidic condition might not affect the whitening ability of HAP materials. Studies, which are designed to evaluate the HAP whitening effect with complicated pH value changes in the oral environment, are needed in the future. To further augment the whitening effect of HAP mouthrinse, non-oxidizing, blue-toned agents (such as blue covarine) could be combined, which might provide teeth a yellow to blue hue color shift.[32]

In summary, this study confirmed for the first time the tooth-whitening effect of prolonged application of HAP mouthrinses. After the 3- and 6-month-equivalent mouthrinsing, the HAP mouthrinses exhibited similar tooth-whitening effects to the commercial whitening mouthrinse. It was also noticed that the tooth-whitening performance of HAP was dependent on the particle size and application time. After the 6-month-equivalent application, the 50 nm HAP mouthrinse showed significantly higher color changes than the 3 μm one. This finding should be taken into consideration by dental manufacturers for their HAP containing dental products. To achieve a better outcome in tooth-whitening, the patients should apply the mouthrinse regularly for a longer period of time.

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b. Johnson & Johnson, Neuss, Germany.
c. Nestle AG, Munich, Germany.
d. Hettlich, Tuttlingen, Germany.
e. Kulzer Technik, Wehrheim, Germany.
f. Gretag MacBeth, X-Rite, Neu-Isenburg, Germany.
g. Zeiss, Oberkochen, Germany.
h. Quorum Technologies, East Sussex, UK.

Acknowledgements: To Dr. Y. Chen for his help on statistical data analysis and to the companies Fluidinova and Budenheim for providing the HAP materials free of charge, and to Mrs. G. Dachs and Mrs. E. Koebele for their assistance with the SEM evaluation.

Disclosure statement: Dr. Shang and Dr. Kunzelmann declared no conflict of interest. The current study was supported by the Department of Conservative Dentistry and Periodontology, Ludwig-Maximilians-University of Munich, Germany.

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