New generation of nanozyme based-tooth bleaching gel with dual effect: tooth whitening and enamel microhardness improving

Samira Jafari (samiraa.jafari1362@gmail.com)
Kermanshah University of Medical Sciences, Iran, Islamic Republic Of
Mohsen Meimanatabadi
Hamedan University of Medical Sciences

Article

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Abstract

Teeth discoloration is one of prevalent aesthetic concerns resulted from intrinsic/ extrinsic reasons. Bleaching process can be a valuable treatment for patients with severe discolorations, which can cause an immediate change in their teeth color. According to literature review, use of commercial products owing to their high concentration of hydrogen peroxide can lead to lessen the enamel microhardness. Regarding these issues, the purpose of this present scenario is the design of novel three-components dental bleaching systems based on cerium oxide (CeO_2) nanozyme with dual impact, whitening of teeth as well as enhancement of the enamel microhardness. This system composed of three sections: whitening gel (i.e. gel includes H_2O_2), the activator gel (i.e. gel includes CeO_2 nanozyme) and supplemental gel (i.e. gel includes hydroxyapatite). Base on in vitro and clinical studies, the synthesized whitening system not only is able to bleach the stained teeth but also possesses the microhardness improvement ability. Interestingly, the clinical case reports in current study reveal the remarkable change of tooth color by using the prepared in-office whitening dental gel.

1. Introduction

Aesthetic dentistry is one of the most popular approaches in the dentistry fields with purpose of designing a beautiful smile to improve the facial esthetic as well as promote the quality of social life. In addition to shape, size and teeth alignment, color of teeth can be considerate as a determinant parameter in the formation of a beautiful smile [1].

Abnormal color of teeth whether due to congenital situations or to consumption of foods, smoking, colored beverage (e.g. coffee, tea, red wine and etc.,), some of drug (e.g., doxycycline, tetracycline, fluoride, and etc.,) results in an increase the public demand to form the whiter and beautiful smile [2].

Accordance to literature review, optimization of teeth color using dental bleaching gel based on hydrogen/carbamid peroxide is one of the common dental aesthetic strategies at affordable cost manner, which is performed both in-office (with high concentration of hydrogen peroxide, 35–38%) and at home (with low concentration of hydrogen peroxide by dentist's prescription, 5–22%) [3]. It is documented that in office-bleaching gels is the more efficient rather than at- home ones owning to high concentration of peroxide content and also their activation's with laser light. Generally, 10% carbamide peroxide is applied overnight in custom-fitted trays as the "gold standard" for teeth bleaching (Figure S1). Reluctance of some patients to use the whitening trays as well as time-consuming process of whitening are the common reasons to desire the in-office systems, which is employed along with a laser light [4]. This trajectory, for the first time was introduced by Abbot in 1918. To date, in this field, various commercial products have been presented in the cosmetic dental market [5]. Action mechanism of these systems include the penetrating of bleaching materials into enamel nanopores with diameter of 2–6 nm and subsequently contact of enamel with these agents could be lead to produce the free radicals; these free radicals are able to obliterate the teeth stains through oxidizing of the colored organic molecules [6].

Nanozymes, nanostructured artificial enzyme, are a group of nanoparticulate materials which are able to mimic the catalytic activations of natural enzymes. During recent decade, a wide variety of nanozymes have been reported to apply in the different fields, particularly in the biomedicine era [7].

Despite the growing use of bleaching gels specially in the dental clinics, their negative impacts on the enamel microhardness have been presented in several reports [8–10]. Consequently, design of an efficient bleaching gel with capability of improvement of color tooth as well as the increase of enamel microhardness is a challenging issue for researchers in the field of aesthetic dentistry.

Considering these issues, this scenario proposes a novel generation of bleaching systems comprised of three separate segments: 1) gel contains bleaching agent (i.e., hydrogen peroxide), 2) activator gel (i.e., cerium oxide nanozyme) and 3) supplemental gel (i.e., hydroxyapatite and sodium fluoride).

2. Materials And Methods

2.1. Materials

Calcium hydroxide, cerium nitrate and hydrogen peroxide were purchased from Sigma (Germany). Mouse Fetal Fibroblast was commercially purchased from the national cell bank of Iran (Tehran, Iran). The extracted teeth were obtained from the Meimanat Clinic, (Tehran, Iran). Other chemicals were selected from the analytical grade/best grade.

2.2. Synthesis of cerium oxide nanozyme

Similar to HAp nanoparticles synthesis, CeO_2 nanozymes were created through precipitation approach. In this regard, the diluted ammonia (1M) was added dropwise into cerium nitrate hexahydrate (1 M), until the pH of the mixture reaches to 10. After filtration, the produced precipitates were washed with distilled water and the dried for 2 hours in an oven, 110 °C. In the final stage, the resulted powders of CeO_2 heat treated about 700 °C for 2 hours in a conventional furnace.

2.3. Synthesis of hydroxyapatite nanoparticles

Synthesis of the hydroxyapatite (HAp) nanoparticles conducted through wet precipitation approach, as was described in our previous report, with a calcium/phosphate molar ratio of 1.67 [11]. In brief, orthophosphoric acid solution (100 ml, 0.6 M) was added dropwise into calcium hydroxide solution (100 ml, 2 M) to create a milky solution under vigorous stirring at ambient condition within 24 hours; pH was adjusted on 11. After that, the free ions were removed through washing of the collected precipitation with distilled water. In the next stage, the obtained cake, first was dried under 100 °C in an oven, overnight. In the end, the generated HAp was sintered for 2 hours in the conventional furnace, at 1000 °C.

2.4. Characterization of the synthesized nanoparticles

2.4.1. Fourier transform infrared spectroscopy (FTIR)

The recognition of functional groups in CeO_2 and HAp NPs were conducted using an FT-IR spectrophotometer (Shimadzu 43000, Shimadzu) viathe KBr disk method. The recorded spectra with resolution of 2 cm^{-1} were in the range of 4000 to 400 cm^{-1}.

2.4.2. Morphology analysis with SEM

The surface morphology evaluation of the synthesized nanoparticles was executed through a scanning electron microscopy (SEM, MIRA3, TESCAN, USA), at 15 kV. Prior to examination, the nanoparticles were mounted on aluminum stubs and then coated with a thin layer of gold by a sputter apparatus.

2.4.3. Cytotoxicity assay

Mouse Fetal Fibroblast (MFF)is used to evaluate the in vitro toxicity of HAp nanoparticles and CeO_2 nanozymes by using MTT assay. The studied cells were cultured in DMEM-high glucose medium supplemented with 10% of fetal bovine serum (FBS) and 1% of penicillin-streptomycin solution in incubator with a humidified atmosphere of 5% CO_2 and 37ºC for 24 h before seeding in 96-well culture plates at the density of 5×10^5 per well. Then, the cells were treated at different concentrations (0, 20, 40, 80, 160 and 200 µg/ml) of CeO_2 nanoparticles, PBS as solvent of nanoparticles and a blank (empty wells, no cells) to measure the background for tow incubation time of 24 and 48 hours.

At each interval, the medium was removed and the wells were washed twice with PBS, then, each well was replenished with fresh culture medium with 10 µl of MTT (5 mg/ml) The culture plates were incubated for 4 hours at 37 ºC. Subsequently, the culture solution was removed and 100 ml of DMSO was added to each well and plate kept at room temperature in dark for 1 to 2 hours until intracellular purple formazan crystals became visible under the microscope. Subsequently, the absorbance of cells was measured by Microplate reader scanning spectrophotometer Model 680 (Microplate reader, BioTek *Synergy™ H1*) at 570 nm. The experiments were measured in triplicate.

Relative Cell viability was calculated using the equation below

$$\%Relative\ cell\ viability = \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}}\right) \times 100$$

Where Abs_{sample} is the absorbance of the cells incubated with nanoparticles and $Abs_{control}$ is the absorbance of the cells incubated with PBS and wells treated with MTT without the presence of any cell as a blank.

2.5. Designing of three-component dental bleaching system

In the current project, a three-component dental bleaching system was designed that not only could dental stains but also could improve the enamel microhardeness. Briefly, the mentioned system includes

3 gels with different performance:

1. Gel 1: this gel contains the hydrogen peroxide (15%), carbomer 941 (2% w/w), menthol (0.2% w/w), saccharin (0.1% w/w).
2. Gel 2: this gel composed from nanozyme CeO_2 (0.2% w/w), carbomer 941 (2% w/w), menthol (0.2% w/w), saccharin (0.1% w/w).
3. Gel 3: this gel comprises from hydroxyapatite (0.2% w/w), sodium fluoride (0.5% w/w) carbomer 941 (2% w/w), menthol (0.2% w/w), saccharin (0.1% w/w).

It is noteworthy that the gel 1 as a whitening agent, gel 2 as an activator agent and gel 3 as a supplemental gel were considered.

2.6. Sample preparation and staining procedure

The 18 human extracted teeth were randomly divided into two groups of 9 teeth, which teeth of each group were separately stored into coffee and tea for 72 hours (Figure S2).

Accordance with a standard protocol for artificial staining, the staining solutions were prepared as follow:

1. Coffee solution: 12 g coffee in 200 mL boiling water
2. Tea solution: 2 g tea in 100 mL boiling water

Then, the prepared teeth were immersed into the filtered solutions over a 3 days-period at room temperature; coffee and tea solutions were renewed daily. After the embedding period, the teeth were washed and placed into distilled water until required time. To evaluate staining, various approaches are reported in the literature [12]; in this study, we used a standard Vita shade guide (Vita Zahnfabrik, Bad Säckingen, Germany), which the shade tabs were arranged in a numeric value based on the manufacturer suggestion (B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4). After artificial staining of teeth, only teeth with shade C4 were selected for bleaching process (14 teeth).

To evaluate the effectiveness of the prepared gel, first, the teeth were brushed and washed with distilled water before applying of bleaching gel onto them to avoid the false-positive results. In the following, the dried teeth mounted separately in plaster, so that the root of each tooth was placed in a plaster cast and the crown of the tooth was visible at the plaster outside. Besides, pumice powder accompanied with brush was applied onto the teeth surface to verify the gel performance (Figure S3).

2.7. Bleaching process

To scrutinize the whitening efficacy of the synthesized gel in comparison with commercial bleaching gel, the prepared teeth were divided into four experimental groups, as follows:

Group 1) the teeth bleached with the prepared bleaching gel

Group 2) the teeth bleached with the prepared bleaching gel accompanied with supplemental gel

Group 3) the teeth bleached with the bleaching gel BOOST (Germany, 35% hydrogen peroxide)

Group 4) the teeth bleached with the bleaching gel FGM (Brazil, 35% hydrogen peroxide)

It should be noted, which in this study for more accurate assessment, the crown thickness, for each tooth, was vertically sectioned in half; after cutting of each tooth into two equal halves of A and B, the teeth were mounted in acrylic. Then, bleaching gels were separately applied to only one half of each tooth and the other half was considered as the control.

2.8. Microhardness assessment

Fourteen days post-bleaching, a microhardness tester (Kentron Microhardness tester, Torsion Ballance Company, Clifton, NJ, USA) was employed to determine

Knoop microhardness (KHN; kg/mm2), at a load of 200 g with the indentation time of 15 seconds.

2.9. Ethical aspects

For the clinical studies, all methods are performed according to the guidelines approved by Kermanshah University of Medical Sciences. The current project has succeeded in receiving the code of ethics with the number 1399.946.56 from Kermanshah University of Medical Sciences. All the ethical issues have been monitored during the project in accordance with the existing protocols.

2.10. Statistical analysis

The reported data in this study, are expressed as mean ± standard deviation (SD). The statistical differences between values was assessed through on way ANOVA with P-value> 0.05.

3. Results And Discussion

3.1. Fourier transform infrared spectroscopy (FTIR)

The functional groups in the HAp and CeO_2 nanozyme were recognized through FTIR analysis, as observed in Figure 1 and 2, respectively. Characteristic absorption peaks were recorded for HAp at around 1043.61 cm^{-1} (phosphate groups), 3642.97 cm^{-1} (hydroxyl groups) and 1474.15 cm^{-1} (carbonyl groups).

In the CeO_2 spectrum, absorbance bands at 3410 and 500 cm^{-1} observed, which the former clarified the presence of O-H group and the latter Ce-O group.

3.2. Morphology of nanoparticles

Morphology of HAp nanoparticles and CeO_2 nanozymes are presented in Figures 3 (A and B, respectively). As can be seen in Figure 6-A, SEM image of HAp nanoparticles depicted the agglomerates of the flaky crystals and SEM image of 6-B, confirmed a nanoscale sized globular structure for CeO_2 nanozymes.

3.3. Cytotoxicity assay

Figure 4 and 5 depicts the viability of MFF cell line upon treatment with various concentrations of HAp nanoparticles and CeO_2 nanozymes, respectively. In previous work, we reported a dose- and time-dependent cytotoxicity for the treated cell lines with HAp nanoparticles [11]. Indeed, HAp nanoparticles displayed a significant viability for the incubation times of 24 and 48 hours at lower concentrations (10–100 µg g/ml). Although, with increasing the HAp concentration (> 100 µg/ml), their cell viability was decreased that confirmed a dose-dependent cytotoxicity. Regarding these obtained results, we hypothesized that HAp and CeO_2 nanozymes could be an appropriate biomaterial for the dental applications.

3.4. Effect of bleaching gel on the teeth color changes

The color changes of the bleached teeth with the selected commercial (BOOST and FGM brands) and synthesized gels are presented in Table S1. As shown, shade value scale of treated teeth with coffee and tea were A3. Although, after bleaching process with the studied gels this value altered to lighter type in Vita shade tabs.

Based on the obtained data, it found that out of 8 specimens, the whitening efficacy of synthesized gel was similar to BOOST brand in the 5 specimens; in the 1 specimen was better than BOOST brand whilst in the 2 specimens showed weaker whitening effects compared to BOOST brand. Besides, the whitening effect of the synthesized gel was better than FGM brand in all treated specimens.

3.5. Effect of bleaching gel on the enamel microhardness

Vickers assay was employed to evaluate the enamel microhardness of the treated teeth with the commercial and the prepared bleaching gel. In this regard, the obtained results are summarized in Table 1. In agreement with the calculated data, among of the selected bleaching gels, the prepared gel along with supplemental gel appeared significantly increase in the enamel microhardness whereas BOOST brand displayed decrease in the microhardness.

Table 1. The effect of commercial gel and prepared gel on the enamel microhardness.

Type of bleaching gel	Microhardness values (before bleaching)	Microhardness values (after bleaching)	% Microhardness changes	% Mean changes of microhardness
Prepared gel	329	315	-4.25	-3.23
Prepared gel	418	405	-3.11	
Prepared gel	340	332	-2.35	
Prepared gel with supplemental gel	330	372	+12.72	12.78
Prepared gel with supplemental gel	291	353	+4.34	
Prepared gel with supplemental gel	276	288	+21.3	
Boost	360	342	-5	-6.21
Boost	362	351	-3.03	
Boost	292	261	-10.61	
FGM	361	354	-3.60	-5.82
FGM	323	308	-4.64	
FGM	347	315	-9.22	

4. Case Reports

4.1. First volunteer

The volunteers included 2 females (27 years old 32 years old) that were dissatisfied with the front teeth appearance regarding to their wide smile. Prior to participate in the current study, volunteers were informed of benefits and probable risks involved in the prepared bleaching gel. After signing of informed consent form by participations, dental bleaching process was performed on their teeth. Prior to bleaching treatment, original color of teeth was registered as B1 using Vita Classical Shade Guide (Vita Zahnfabric, Bad Säckingen, Germany). Then, the pumice powder was employed to wipe the organic materials from the surface crown. Since peroxide compounds can cause the soft tissue irritation, therefore it should be noted for sealing of the soft tissue prior to bleaching process through light-curing soft composite resin (Smartblock, SBI nv., Herzele, Belgium) as a gum protector at marginal border. In the following, the bleaching process was conducted placing of the prepared gel on the teeth surface with irradiation of laser light (Brand and country), for 20 seconds for each tooth. The teeth photographs of first volunteer (a 32-year-old woman), pre- and post-bleaching are displayed in Figure 6, respectively. As observed in Figure 6

(C and D photographs), the teeth color in this participant significantly improved and recorded as A1 using Vita Classical Shade Guide.

2. Second volunteer

Similar to first volunteer, all pre-bleaching steps were performed for the second candidate, as mentioned-above. The pre- and post-bleaching photographs of the second volunteer are displayed in Figure 7. In accordance with this figure, the original color of teeth in this volunteer was recorded as A35, whilst after single bleaching, her teeth color changed to A1 using Vita Classical Shade Guide. It is note-worthy that in the none of the two volunteers, sensitivity or pain sensation were observed (Informed Consent for Tooth Whitening Treatment for both of volunteers are presented in supplementary file S5 and S6).

5. Conclusion

In this study, CeO_2 nanozyme was benefited to design generate the activator gel for designing the *de novo* bleaching system. It is likely that addition of supplemental gel including HAp nanoparticles in the prepared bleaching system was responsible for enhancement of the enamel microhardness. Besides, three-components whitening system, which created I this study, during in-office dental bleaching has without any deleterious effects on bleaching effectiveness.

Declarations

Conflict of interest

No.

Acknowledgement

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

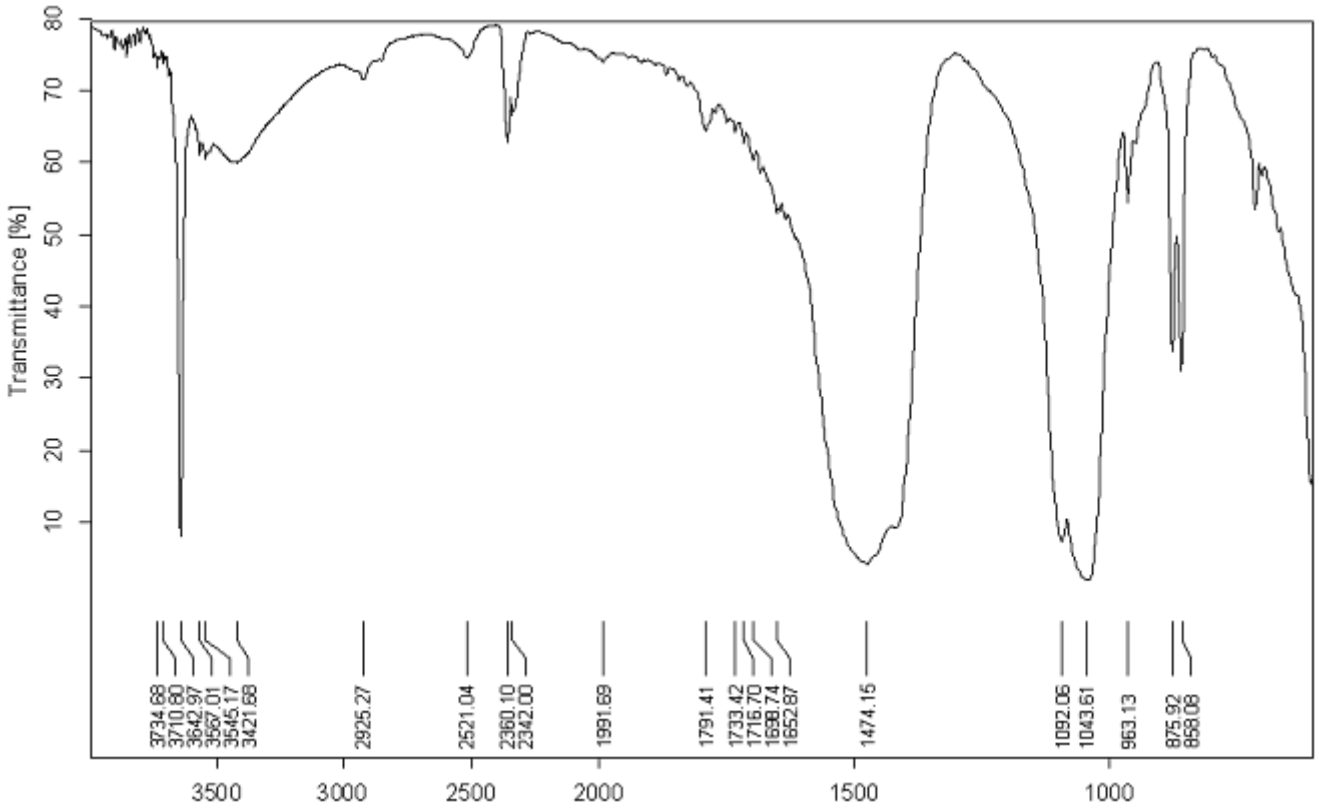

Figure 1

FT-IR spectra of the HAp nanoparticles.

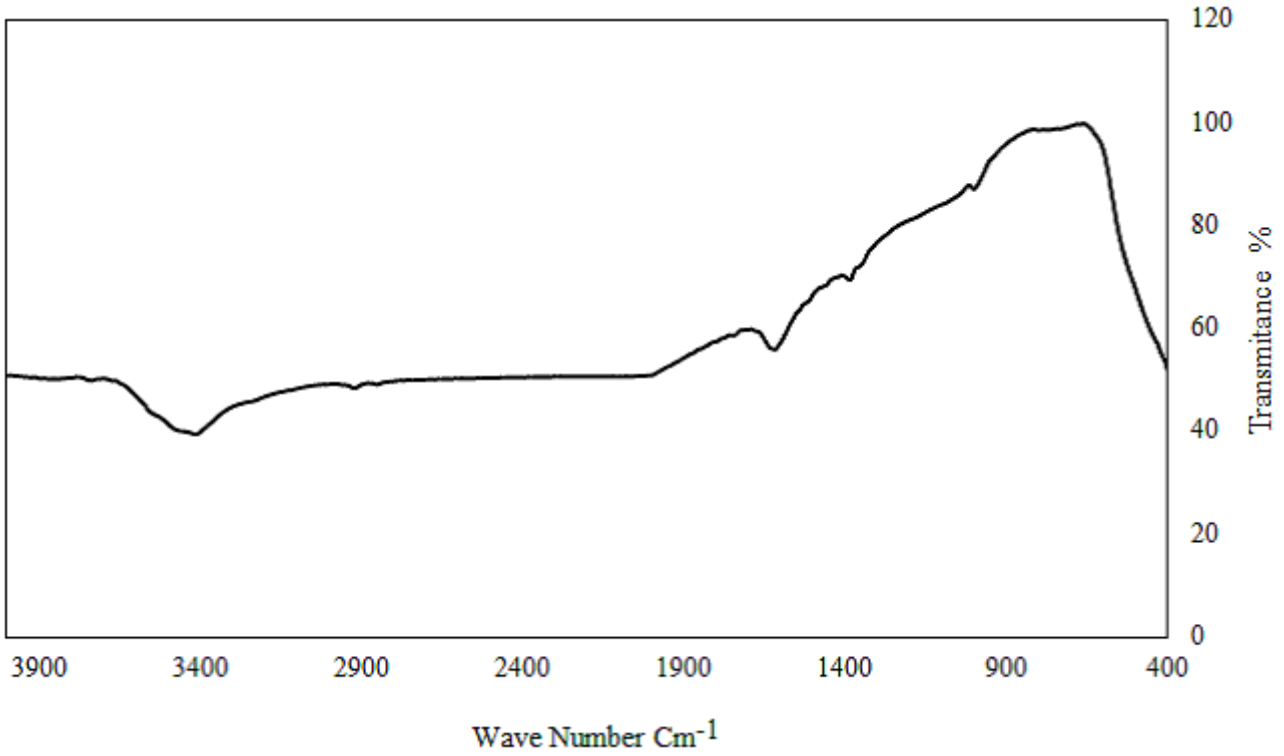

Figure 2

FT-IR spectra of the CeO_2 nanozyme.

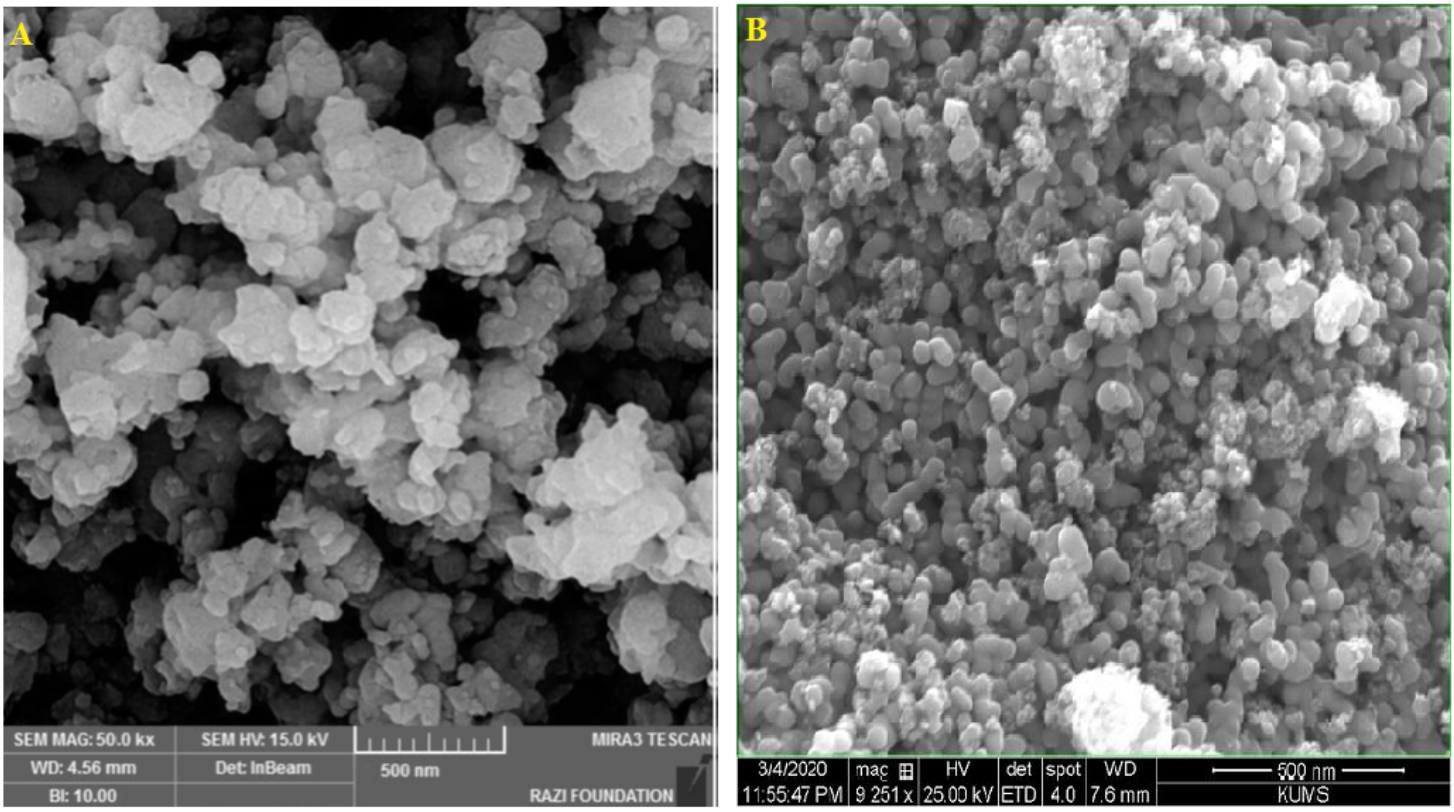

Figure 3

FE-SEM images of HAp nanoparticles (A) and the CeO_2 nanozyme (B).

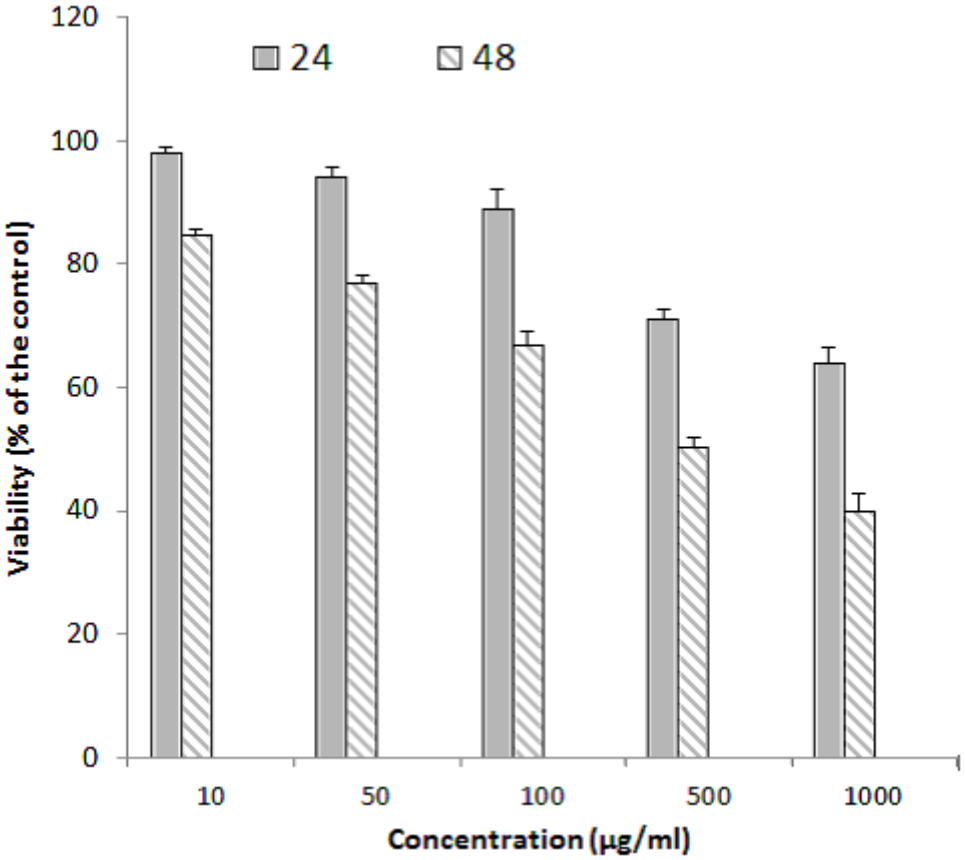

Figure 4

Viability of and the treated MFF cell line with HAp nanoparticles after 24 and 48 h. Data are presented as the average of four replications (± standard deviation).

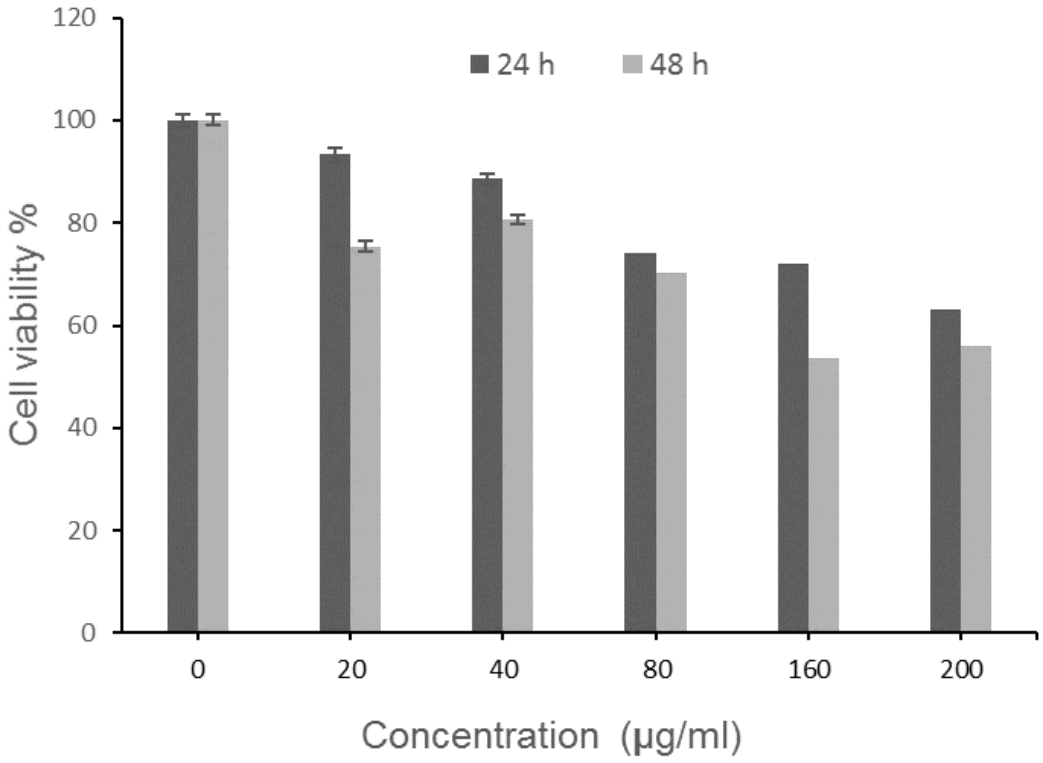

Figure 5

Viability of and the treated MFF cell line with CeO_2 nanozymes after 24 and 48 h. Data are presented as the average of four replications (± standard deviation).

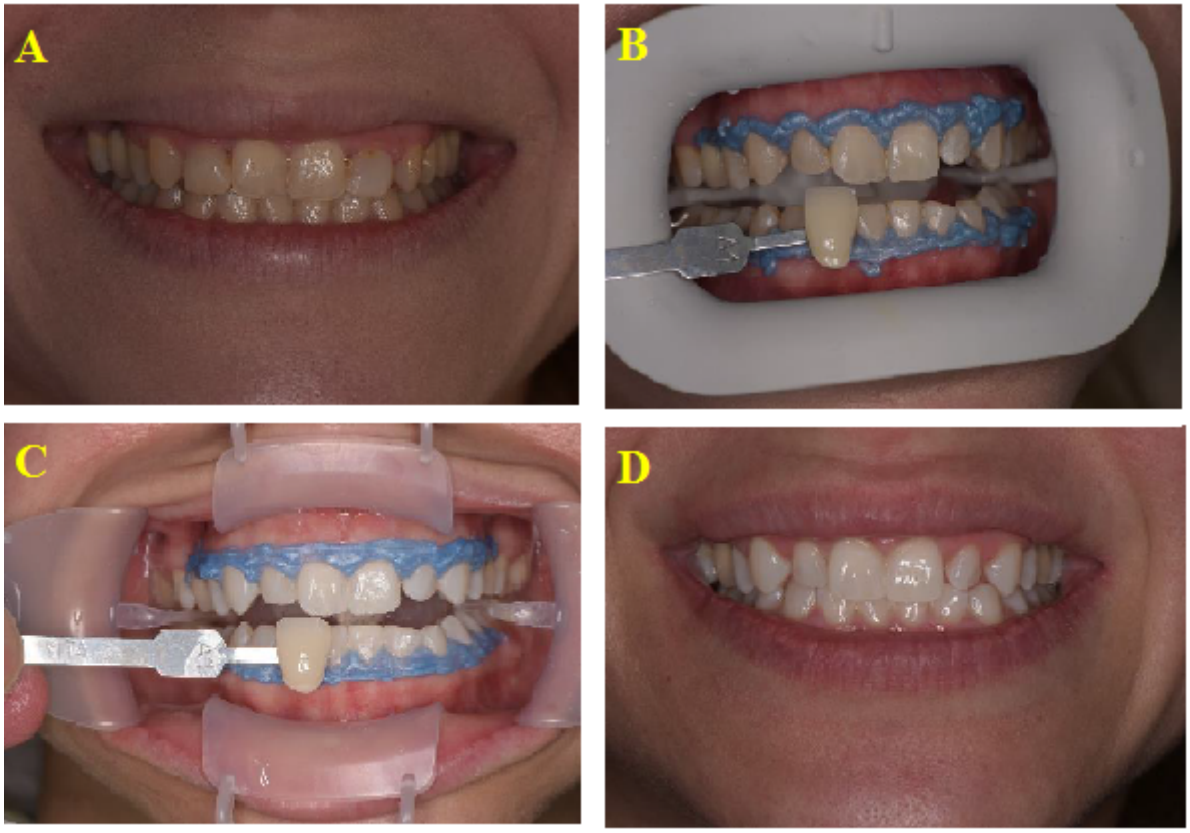

Figure 6

Photographs of the first volunteer. A) The teeth color in the pre-bleaching. B) The change color of teeth in the single post-bleaching. C) The change color of teeth in the double bleaching. D) The final change color of teeth after removing the gum protector.

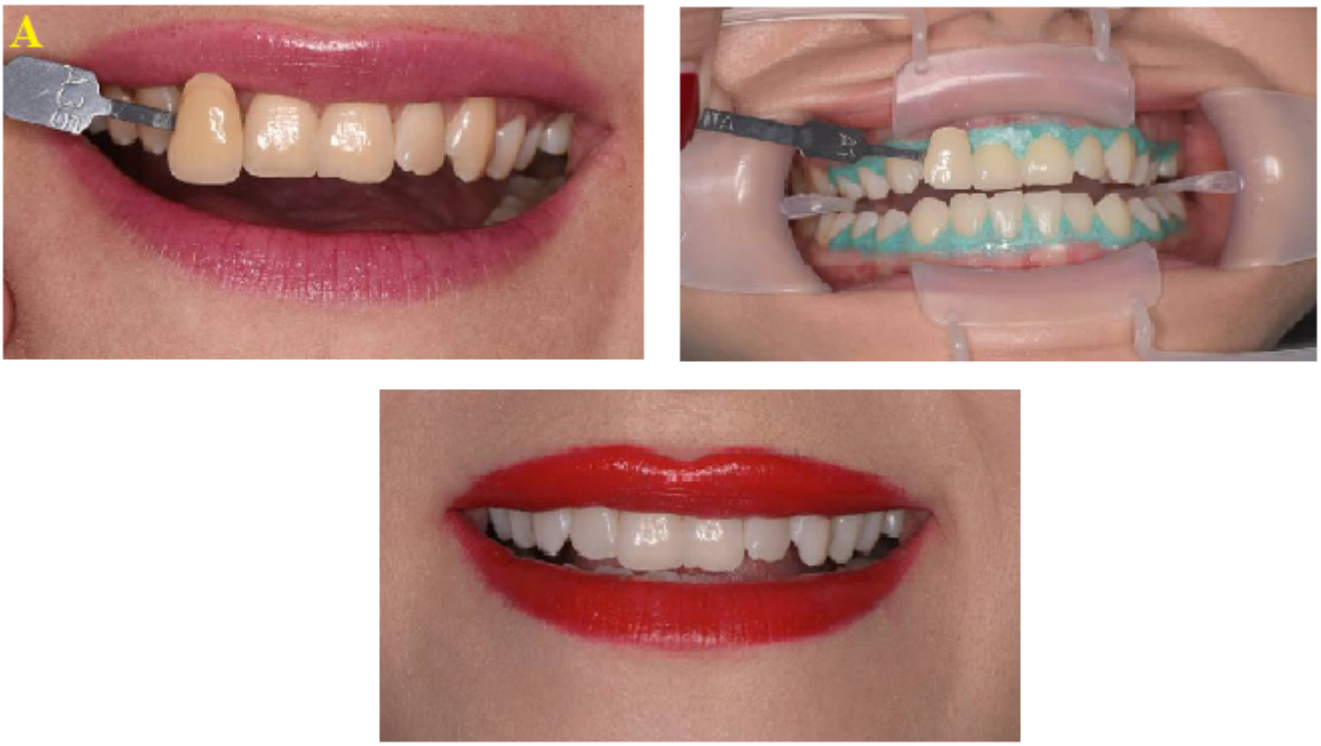

Figure 7

Photographs of the second volunteer. A) The teeth color in the pre-bleaching. B) The change color of teeth in the post-bleaching. C) The final change color of teeth after removing the gum protector.

Supplementary Files

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- supplementaryN.docx