In vitro Determination of the Chromatic Effect of a Silver Nanoparticles Solution Linked to the Gantrez S-97 Copolymer on Tooth Enamel

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Silver nanoparticles (NNPs), alone or in combination with the bioadhesive Gantrez S-97, have demonstrated their efficacy against Streptococcus mutans; however, it is not known if this combination changes the color of teeth. The aim of this work was to measure the color changes occurring after the use of a Gantrez-NNP combination on enamel tooth blocks. Two study groups were randomly formed: enamel blocks brushed with a) the Gantrez-NNP combination and b) conventional toothpaste, for 1 minute once daily for 4 weeks, then rinsed with distilled water and placed in thymol solution. Color changes in the enamel blocks were measured using a Minolta colorimeter CR300. Analysis of mixed models was performed with R 2.10.1 at a 95% confidence level, using the nonlinear mixed effects (NLME) package. The results showed that there were no color changes over time, only a high luminosity equal in both groups. Our study showed that the use of the Gantrez-NNP combination is safe with respect to dental esthetics in the control of S. mutans.

Keywords*: nanoparticles, silver, tooth enamel, Gantrez S-97, chromatic effect.*
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INTRODUCTION

Chemical control of dental bacterial plaque for prophylactic or therapeutic purposes using substances such as chlorhexidine is widely used today.[1] Several silver-based compounds have been shown to have coagulant protein, antienzymatic, and bacteriostatic activity that prevents dextran clumping, inactivates sucrose plaque, and displays antibacterial activity against *S. mutans*—the main caries-causing agent. Silver diamine fluoride is a particularly excellent cariostatic, remineralizing, and bactericidal agent.[2] However, one of its disadvantages is that it produces tooth surface pigmentation, especially in porous enamel.[3,4] Previous studies have shown that silver nanoparticles (NNPs) alone or linked to the bioadhesive Gantrez S-97 are bactericidal against *S. mutans,*[5,6] although it is unknown whether their direct application causes tooth-color changes. The purpose of this study was to measure color changes occurring after the use of a Gantrez-NNP combination on enamel tooth blocks.

METHOD

Design

This was an experimental *in vitro* study, performed at the Universidad Autónoma de San Luis Potosi (UASLP).

Preparation of enamel samples

The enamel blocks were obtained from premolars and third molars extracted for orthodontic purposes, without caries, fractures, or defects. They were maintained in a 0.1% thymol solution which was changed every 2 days to keep them hydrated and avoid demineralization.[7] Two study groups were randomly formed: Group A, enamel blocks brushed with the Gantrez-NNP combination (n = 30), and group B, enamel blocks brushed with conventional toothpaste (n = 30). Brushing was performed on the vestibular

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and lingual (or palatal) surfaces for 1 minute once daily for 4 weeks. Subsequently they were rinsed with distilled water and replaced in the thymol solution.

Manufacturing of silver nanoparticles-Gantrez S-97

The laboratory of Science Materials elaborated the NNPs. The procedure involved reactions of colloidal solutions with oversaturation of salts and the creation of a precipitate by homogeneous or heterogeneous nucleation. After nucleation, the NNPs' growth took place by diffusion. The concentration gradient, temperature changes, agitation, and surfactants allowed modification of the size increment. We produced monodispersed, nanoagglomerated particles of uniform size. First, we prepared a solution of silver with sodium tetrahydroborate ($NaBH_4$) included as a reducing agent. The ratio of silver to the reducing agent was greater than 1. After the reaction, we added a stabilizer for controlling size and uniformity. For the experiment, the mixture was prepared with 98 µg/mL of silver nanoparticles (10^{-3} mol) with Gantrez S-97 2%, suspended in distilled water. The size of the nanoparticles was between 40 nm and 80 nm.

Color measurement

Color changes in the enamel blocks were measured with a Minolta colorimeter CR300 (Chromameter, Minolta, Osaka, Japan). The measurements reflected an enamel surface color. The measuring head has an 8-mm-diameter opening, uses diffused light, and has a viewing angle of 0 degrees. Whiteness of the enamel was expressed in L*, a*, and b* color space measurements. It allows identification of color stimuli in 3-dimensional spaces. The ***L***- axis is the brightness, and it ranges from 0 (black) to 100 (white). The *a*-coordinate defines the point deviation toward red if $a > 0$ and toward the green if $a < 0$, with values of −500 to +500. Similarly, the *b*-coordinate defines the deviation toward yellow if $b > 0$ and toward the blue if $b < 0$, with values of −200 to +200. For 1 month, triple baseline and weekly measurements were made of the enamel samples treated with both the Gantrez-NNP combination and with conventional toothpaste.

Statistical analysis

The analysis was performed with *R* 2.10.1 at a 95% confidence level.[8] An initial analysis with exploratory and descriptive statistics and graphs was performed. Testing of mixed models was performed using the nonlinear mixed effects (NLME) package.[9] The maximum model that included the explanatory variables and interactions of interest for each response variable was defined. The maximum models were simplified by serial elimination of the explanatory variable with the largest probability that was not significant until all explanatory variables in the model were significant. The final model compliance with the prerequisites of residual normality and homogeneous residual treatment variances was evaluated graphically. Finally, the multiple determination coefficients for each model were calculated.

RESULTS

The mean baseline values for *L* with and without brushing with the Gantrez-NNP combination were 67.02 (SD = 3.99) and 70.48 (SD = 3.40), respectively. One month after brushing, there was an increase of about 6 units *per* group, which is equivalent to 6% of the full scale (L = 72.97733, L = 76.17167, P = NS). Figure 1 shows the means obtained in the 5 measurements.

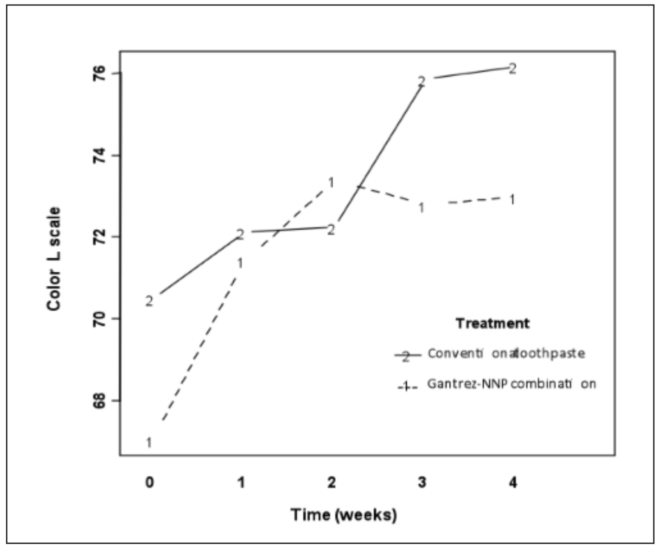

Figure 1. Basal and weekly brushing L values with the Gantrez-NNP combination (1) and conventional toothpaste (2).

The Gantrez-NNP group mean baseline value for *a* was −0.81 (SD = 0.66) and −0.51 (SD = 0.77) for the control group which shows a very slight tendency toward red in the first group and green in the second group. The bivariate analysis did not detect significant differences between the groups where minor changes were 1% of the full scale (a = −0.88 and a = −0495, Figure 2).

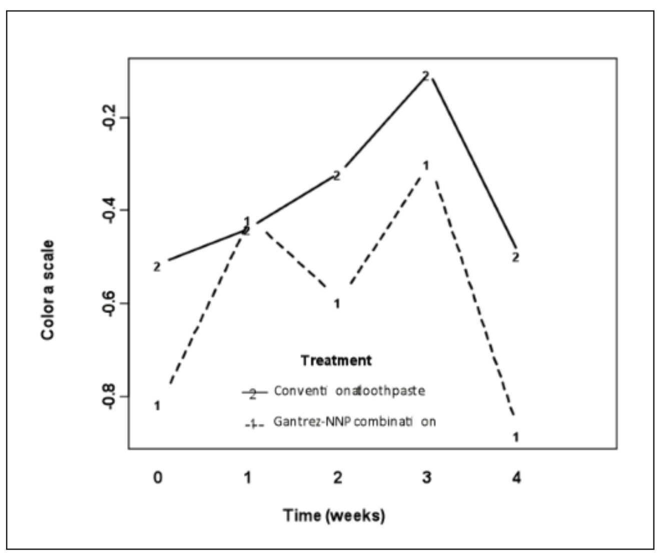

Figure 2: Basal and weekly a values for teeth brushed with the Gantrez-NNP combination (1) and conventional toothpaste (2).

Baseline values of *b* were 8.7 (SD = 3.11) and 10.76 (SD = 3.28) with a tendency toward yellow, with a similar decrease in both groups at the end of the brushing (P = NS) of approximately 2 units, which is equivalent to 1% of the full scale (b = 6.29 and b = 8.12).

To study the process of color change and the influence of the variables time, treatment group, initial tooth-color values, and their interactions, repeated measures analysis was performed (Table 1). The baseline value of ***L***, brushing week, toothpaste type, and the brushing week toothpaste interaction were significant for the variable ***L***. The increase in brightness was higher in the group treated with Gantrez-NNP combination.

Table 1. Repeated measures analysis evaluation of the factors that determine enamel block color change

	Value	Std. Error	*P*-value
Intercept	23.274	4.3	< .0001
Treatment	–2.858	0.7	.0005
Time	0.543	0.1	.0037
Basal *L* Value	0.713	0.1	< .0001
Interaction Treatment:Time	0.993	0.230	< .0001
R^2= 0.57			
Intercept	–0.1200	0.06925	.0848
Basal *a* Value	0.4681	0.07061	< .0001
R^2 = 0.529			
Intercept	1.8009	0.4663	.0002
Time	–0.4898	0.0532	< .000001
Basal *b* Value	0.7509	0.0424	< .000001
R^2 = 0.716			

For variable *a*, only the initial value was significant, and both the baseline value and the week were significant for variable *b*.

The multiple R^2 values, which are effect-size measures and which evaluate the proportion of the explained variation in the response variable in the model, were L = 0.57 (57%), a = 0.52 (52%), and b = 0.71 (71%).

DISCUSSION

The modern approach of dentistry for tooth protection includes early lesion detection, individual caries risk analysis, use of chemical agents with specific antimicrobial activity against *S. mutans*, and management of established lesions with smaller repairs and adhesive materials that can be repaired rather than replaced in case of injury recurrence.[10,11]

Chlorhexidine, fluoride, xylitol, and triclosan are commonly used antimicrobial agents in preventive dentistry, but the adverse effects of their prolonged use include dental pigmentation, palate alterations, and reversible mucosa sloughing, and as such they are used only for short periods of time.[12-16] Triclosan shows low adhesion to the tooth surface, which implies rapid elimination from the application sites; its possible cariostatic action has not been systematically studied in humans.[17-20] Both silver diamine fluoride and silver nitrate have been shown to have a bactericidal effect against *S. mutans*. Its use is limited because of dental pigmentation caused by high concentrations of silver precipitation, which can also cause pulp irritation by high silver ion penetration of the dentin.[21,22]

Color evaluation in dentistry includes both subjective measurement (color guides, maps, color, and photographic records) and objective measurement (colorimeters, spectrophotometers).[23-27] Subjective measurement has multiple sources of variability including lighting, eye fatigue, and age. The reported observer reproducibility ranges from 26% to 70%. Therefore, to evaluate changes of color, it is necessary to use a method that is not only sensitive to small changes in color, but is consistent and accurate, because traditional methods of evaluation require subjective visual grading. Objective measurements are more accurate; reported observer reproducibility for the methods used range from 83.3% to 95%.[28-30] In this study, changes in color were evaluated using a Minolta Chroma Meter CR300 (Chromameter, Minolta, Osaka, Japan). This device is an excellent tool to assess gradual changes in the shade of teeth because evaluations are not affected by the above factors and they can accurately, quantitatively, and reliably express color changes.[31,32] Light-surface reflectance is expressed in L*, a*, and b* color space measurements established by the Comission Internationale de L'Éclairage,[33] and they are related to human color perception in all 3 color dimensions: L* values represent color gradients from white to black; a* values represent green to red; and b* values, blue to yellow. For all color measurements in this study, a Chroma Meter was used; we observed a quantitative interpretation of color changes. After 4 weeks of brushing, we found no tooth discoloration and only minimal changes occurring in both variable *a* (from green to red) and *b* (blue to yellow) and an increase in the brightness or whiteness of the teeth brushed with the Gantrez-NNP combination.

CONCLUSIONS

Our study showed that use of the Gantrez-NNP combination is safe with respect to dental esthetics in the control of *S. mutans*. Further studies are needed to assess adherence, toxicity, and the administration vehicle before including it in everyday clinical practice.

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