

Silver Nanotechnology – the Future in Caries Therapy? A Report of Two Cases

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ABSTRACT

Introduction: Dental decays remain the most common and rampant biofilm-dependent oral disorders. Influencing the delicate dynamic between demineralization and remineralization is a big challenge in clinical practice, and nanotechnology is considered a viable solution. The therapeutic management of caries, which includes nanotechnology, has two big approaches, an antibacterial one and a remineralizing one. Silver is recognized to display a powerful toxicity to a large variety of micro-organisms, thus silver-based composites have been widely used in several bactericidal applications. **Case report:** We present our attempts and results in using silver nanoparticle solutions on a 14-year-old and a 34-year-old patient with dental decays and no previous dental pain. One of the cases was treated with chlorhexidine 2% and the other with Nanocare Plus, as antimicrobial agents. In both cases we recorded decreased values of the bacterial burden in comparison with the initial values. **Conclusion:** This two-case presentation compared the antibacterial effect of two antibacterial solutions, providing useful information regarding novel therapies for dental caries, but further research in this domain is needed.

Keywords: silver nanoparticles, antibacterial activity, caries therapy, dental biomaterials

INTRODUCTION

Dental decays seem to continue being the most common and rampant biofilm-dependent oral diseases, resulting in the destruction of teeth due to the acidic attack of cariogenic micro-organisms such as *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp.* The bacteria appear to be attached to each other and to the dental surface (oral biofilm or plaque), forming a mass of microorganism cells.^{1,2}

The dynamic of caries progression consists of a discrepancy in the physiological balance between the mineral ions and the dental plaque. This imbalance consists of demineralization and remineralization processes, thus a pH smaller than 5.5, caused by bacterial acids, can cause dental demineralization (with loss of calcium and phosphate), and the remineralization process starts after removing the biofilm.^{2,3}

The amount of gained ions through remineralization is smaller than the number of lost ions, resulting in a small mineral loss. When the demineralization

exceeds the ability of oral fluids to repair the mineral loss, decay or carious lesions appear as the first signs seen in clinical practice.^{1,4}

Influencing this delicate dynamic between demineralization and remineralization is a big challenge in clinical practice, and the latest research in this domain seems to consider nanotechnology as a viable solution.

Nanotechnology consists in the use of functional materials, devices, and systems with nanometric size (1–100 nm). This technology is experiencing a rapid growth, with a lot of applications in dentistry such as bio-nanointerfaces (adhesive resin-dentin interface, dental implant-bone interface); remineralization — reversing incipient decays and periodontal diseases; diagnostic nanodentistry — diagnosis of oral cancers, biochips; therapeutic nanodentistry — root canal disinfection, dentin hypersensitivity, tissue engineering.^{5,6}

The strategies for dental caries management, which include nanotechnology, have two big approaches, an antibacterial one and a remineralizing one. Silver is recognized to display a powerful toxicity to a large range of bacterial agents. Thus, silver-based amalgams have widely been used in many bactericidal mixtures.^{1,7,8} Such mixtures include inorganic composites with a slow release of silver particles, used as preservatives in some products, e.g. the silica gel microsphere-composed compounds, which contain a silver thiosulfate complex, providing a long-term antibacterial protection to plastics.⁷

Several studies have shown that silver particles interact with the thiol groups of vital enzymes, inactivating them. Furthermore, these particles inhibit the DNA replication of bacteria, when it remains in contact with silver ions. Other studies have demonstrated evident structural modifications in the bacterial membrane and the development of electron-dense granules formed by silver and sulfur.^{5,7}

Metal particles of nanometric size can display greater physical properties than the ions of silver or the bulk material, such as an increased catalytic activity and a proper penetration of the bacteria. The bactericidal characteristic of silver nanoparticles is dimension-dependent: nanoparticles between 1 and 10 nm attach better to the surface of the cell membrane, leading to disturbed main functions such as permeability and respiration. They are perhaps interacting with sulfur- and phosphorus-containing composites, such as DNA, and release the silver ions, having a great contribution to the bactericidal effect.⁷

An important step in the development of argentic nanoparticle (AgNP)-containing materials is their characterization. Several studies have analyzed the dispersion of silver nanoparticles (NAg) with transmission electron microscopy (TEM). This technique allows researchers to

visualize particle size and its spread into the tested material. Using this technique, silver particles of 3 nm are clearly visible and well dispersed throughout the polymer matrix, and very small particles ranging from 2 to 5 nm can penetrate the dentinal tubules, possibly inactivating the residual bacteria within.^{9–11}

Another important characteristic analyzed in studies is the minimum inhibitory concentration (MIC) of AgNPs. The MIC can be defined as the lowest concentration of an antimicrobial solution at which 90% of growth is observed in the medium. It was reported that different AgNP sizes have different MICs, the highest one being recorded in the case of nanoparticles of 98 nm ($320.63 \pm 172.83 \mu\text{g/mL}$). This certifies that the MIC is directly proportional to particles size.⁹

The form of incorporation of AgNPs in dental materials can be different, depending on the type of material. In most composite resin and adhesive systems a monomer is added (usually 2-tert-butylaminoethylmethacrylate) to improve the solubility of silver in the resin solution. Dental implants, instead, are soaked in AgNO₃ solutions, rinsed with deionized water and irradiated with UV light.⁹

Since most articles in the literature are based on *in vitro* studies, there is a slight lack of knowledge in the clinical part of dentistry. Clinical trials and *in vivo* experiments are needed to follow-up and show evidence regarding the benefits of silver nanoparticles, which were demonstrated in *in vitro* experiments.

Tooth decay is one of the most common chronic diseases worldwide, its complications being one of the causes of temporary or permanent tooth loss. Most clinicians choose the mechanical treatment as therapy, which in many cases leads to the opening of the pulp chamber due to the depth of the carious lesions. This is the reason why there have been attempts of trying chlorhexidine 2% solutions in the depth of the mechanical-treated cavity, in order to stop bacterial multiplication and to avoid an unexpected pulp chamber opening. It has been proved that this substance has some influence on the cavity microbial flora, but it is not completely efficient.¹⁰

In the current literature, chlorhexidine is described as the most effective solution, capable of inactivating bacteria in dentin tubules, with a major advantage compared to alexidine, cetrimide, garlic extracts, or different solutions based on calcium hydroxide, used especially in endodontics. Of course, chlorhexidine is not capable to destroy all viable microorganisms, but it is able to reduce the bacterial microflora.^{11–13} Some studies have shown that chlorhexidine can be used in therapy as well, due to its ability to improve the adhesion of resins to dentin.¹³

Nanocare Plus (Dental Nanotechnology, Katowice, Poland) contains a concentration of 50 ppm silver and 1 ppm gold. This product is known to have a bacteriostatic effect within the oral cavity, being used in fixed restorations for its prophylactic and bacteriostatic effects, and because it facilitates the adhesion of cement to dental structures. Of course, it is used in periodontology as well, for minimizing the number of bacteria in the gingival sulcus. The product was created based on the idea that gold and silver particles have been scientifically proven to have a bacteriostatic effect when used in the final irrigation of root canals, after the mechanical treatment. Thus, Nanocare Plus was created for the final, chemo-mechanical phase of an endodontic treatment, before tridimensional root canal filling. The bacteriostatic effect consists in its ability to penetrate the dentinal tubules, due to the nanoscale dimension of the particles and the small superficial tension.¹⁴

The DIAGNOdent device (KaVo Dental, Lake Zurich, IL, USA) is a laser fluorescent light tool for the assessment of dental caries, and it is used in addition to the visual examination of cavities. DIAGNOdent uses a laser light at a wavelength of 655 nm, creating fluorescence in some bacterial components such as porphyrin, and the intensity of the fluorescence light is measured. Thus, the values shown by DIAGNOdent guide the treatment plan (Table 1 and Table 2).^{12,13}

In this article, we present our attempts and results in using silver nanoparticles solution in two cases of dental decays, a superficial decay and a deep carious lesion, in comparison with chlorhexidine.

CASE REPORT

We have chosen two different cases of carious lesions, in two subjects, aged 14 and 34 years. Case 1 is presented in Figure 1 and case 2 in Figure 2. Two different substances

with antimicrobial effect were used, chlorhexidine and Nanocare Plus, and the initial DIAGNOdent values were evaluated to see a decrease or consistency in the bacterial burden, as compared to the initial evaluation. We used Gluco Chex, the liquid form (Cerkamed, Stalowa Wola, Poland) with a concentration of 2%, and Nanocare Plus mixture, as bactericidal solutions.

None of the cases presented past, spontaneous, aching episodes or periapical pathology, and the vital tests were normal in both cases. To confirm the diagnosis of carious lesion, retroalveolar or bitewing radiographies were acquired. The only noticeable difference between the two cases was the initial value captured by the DIAGNOdent pen, which was 20 and 25, respectively.

The first step was to draw up the patients' form, a complete consultation of the oral cavity and professional oral sanitation. One of the cases was treated with chlorhexidine 2% and the other one with Nanocare Plus.

Both patients have benefited from local anesthesia, followed by tooth isolation with rubber dam, for a better control of saliva and for minimizing the contamination of the mechanical-prepared cavity. The teeth were prepared with a "pear-shaped" bur (active side length 3 mm) ISO 008, 010 and 012 to perform the primary access and the initial cavity. Afterwards, we performed the excision of the infected dentin, at a smaller speed, with round bur number 3, 4 or 5 (ISO 012, 014, 016), depending on the dimension of the cavity.

After preparation, we measured the initial bacterial fluorescence with DIAGNOdent, recording the maximal value that was displayed on the device screen.

The next step consisted in the application of the bacteriostatic substances: in the first case, Nanocare Plus was applied with a single-use syringe (concentration of 50 ppm Ag and 1 ppm Au), and in the second case we applied chlorhexidine Gluco Chex 2%. Both solutions were left on the dentin for two minutes.

TABLE 1. Caries located in fissures and on plain surfaces. Explaining the values and the recommended therapies

| DIAGNOdent pen's values | Diagnosis, therapy |
|-------------------------|--|
| 0–12 | Normal prophylactic therapy (e.g. fluorinated tooth-paste) |
| 13–24 | Intense prophylactic therapy (e.g. fluorization, KaVo HealOzone) |
| >25 | Minimally invasive restorative therapy. Restorative and intense prophylactic materials (e.g. KaVo HealOzone, RONDOflex, SONICflex). In case of deep lesions, classic restoration, taking into account the diagnosis and the evaluation of risks. |

TABLE 2. Proximal caries lesions. Explaining the values and the recommended therapies

| DIAGNOdent pen's values | Diagnosis, therapy |
|-------------------------|--|
| 0–7 | Normal prophylactic therapy (e.g. fluorinated tooth-paste) |
| 8–15 | Intense prophylactic therapy (e.g. fluorization, KaVo HealOzone) |
| >16 | Minimally invasive restorative therapy. Restorative and intense prophylactic materials (e.g. KaVo HealOzone, RONDOflex, SONICflex). In case of deep lesions, classic restoration, taking into account the diagnosis and the evaluation of risks. |



FIGURE 1. Class I cavities on the occlusal surface of the second, inferior premolar. Initial moment value = 20. Moment values after applying Nanocare Plus = 19.

After two minutes of waiting, the solutions were rinsed out and the cavity was dried with a dental air-water syringe from the dental unit. The DIAGNOdent pen was used again, to measure new values of the bacterial flora in the treated cavities.

The DIAGNOdent pen recorded changes in the values following the therapeutic bacteriostatic intervention in both cases (Table 3). Decreased values were registered after treatment, in comparison with the initial values, in both cases. By performing a comparative analysis of the two cases, a slightly greater efficiency can be observed in the case in which chlorhexidine 2% was used, with a difference of 4 points in variation values.

DISCUSSION

There are three important directions in dental caries management: prevention, control, and treatment.¹⁵ A high, specific precision is required in detecting small and deep carious lesions, and a correct treatment plan is needed, with the use of antimicrobial substances as adjuvants, to help in fighting or stopping the progression of the bacterial flora through the dentinal tubule, thus compromising dental vitality.

In this study, we have registered lower values of fluorescence captured by the DIAGNOdent pen after using the bacteriostatic solutions, one of them decreasing under the

TABLE 3. Comparison of initial and final values, measured with the DIAGNOdent pen

| Case 1 – Nanocare plus | | | Case 2 – Chlorhexidine | | |
|------------------------|-------|-----------|------------------------|-------|-----------|
| Initial | Final | Variation | Initial | Final | Variation |
| 20 | 19 | 1 | 25 | 20 | 5 |



FIGURE 2. Class III cavity, on a central, upper incisor. Initial moment value = 25. Moment values after applying chlorhexidine = 20.

base value of 20 and the other one decreasing to the base value of a healthy dentin.

This slight difference may have appeared due to the differences in the cavity depth, in the thickness of the remained dentin, the latter being, probably, a major factor. We think that in cases with a thick infected dentin layer, no bacteriostatic substance can penetrate the entire layer and inactivate the remaining bacteria.

Previous experimental or in situ studies have shown the use of the DIAGNOdent pen for detecting demineralization and remineralization; one of these studies was both experimental and in situ.^{14,15}

Another study carried out by Bahrololoomi *et al.* showed that the DIAGNOdent device has a good registration of demineralization, but a weak registration of remineralization. That is the reason for which they considered it not to be useful in detecting remineralization.¹⁵

In the two presented cases, the DIAGNOdent succeeded in recording the pathological values of the infected dentin and revealed a slight bacteriostatic effect of the two solutions, after a short time of action (two minutes). Of course, other factors should also be taken into consideration:

- the type of the study – in vitro or in vivo. It is well known that vital tooth and dentin present different clinical behaviors and absorption rates compared to an extracted tooth.
- the type and localization of the tooth, and the depth of dentin (initial morphology);
- the type of carious lesion: incipient or advanced;
- the localization of the carious lesion: occlusal, proximal, or on smooth surfaces;

- the extent of the infected dentin reminiscent before the DIAGNOdent diagnosis;
- the degree of dental dehydration.

Further studies are required, with a higher number of cases, which test other antibacterial agents, such as the FOTO-SAN solution created by CMS Dental A/S, to achieve a validated comparative analysis. Additionally, the cases should include various types of carious lesions, incipient and deep ones, with different locations, different types of teeth with different degrees of carious progression, in different patients, in comparison with teeth from the same patient.

Moreover, several studies are necessary to determine the continuous release of silver nanoparticles through dentinal tubules and their long-term effects and possible toxicity above the remaining dentin, under the layer of AgNP-containing dental materials, or under the AgNP solution-treated dentin.

We encourage researchers to carry out further studies and clarify the best ways of using silver nanoparticles in dentistry and its negative effects, if they exist.

CONCLUSION

This two-case presentation succeeded in comparing the antibacterial effect of the two solutions, collecting some useful information, possibly to be used for further research in this area. The decrease of bacterial fluorescence was higher in the case of chlorhexidine than in the case of Nanocare Plus. The two cases had intermediate carious lesions, in which the infected dentin could be completely removed, and the DIAGNOdent values were under the diagnostic value of 20.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this article.

REFERENCES

1. Melo MAS, Guedes SFF, Xu HHK, Rodrigues LKA. Nanotechnology-based restorative materials for dental caries management. *Trends Biotechnol.* 2013;31:10.1016/j.tibtech.2013.05.010.
2. Fejerskov O, Kidd E. Dental caries: the disease and its clinical management, 2nd edition, UK: Blackwell Munksgaard, 2008. p. 93-99.
3. Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? *Braz Oral Res.* 2009;23:23-30.
4. Fontana M, Young DA, Wolff MS, Pitts NB, Longbottom C. Defining dental caries for 2010 and beyond. *Dent Clin North Am.* 2010;54:423-440.
5. Idorași L, Negruțiu ML, Sinescu C, et al. Impact of nanotechnology in dentistry; a systematic review. *Bioactive Materials.* 2016 [in press].
6. Hannig M, Hannig C. Nanotechnology and its role in caries therapy. *Advances in Dental Research.* 2012;24:53-57.
7. Morones M, Elechiguerra J, Camacho A, et al. The bactericidal effect of silver nanoparticles. *Nanotechnology.* 2005;16:2346-2353.
8. Nomiya K, Yoshizawa A, Tsukagoshi K, et al. Synthesis and structural characterization of silver(I), aluminium(III) and cobalt(II) complexes with 4-isopropyltropolone (hinokitiol) showing noteworthy biological activities. Action of silver(I)-oxygen bonding complexes on the antimicrobial activities. *J Inorg Biochem.* 2004;98:46-60.
9. Liu T, Song X, Guo Z, Dong Y, Dong Y, Guo N, Chang X. Prolonged antibacterial effect of silver nanocomposites with different structures. *Colloids Surf B Biointerfaces.* 2014;116:793-796.
10. Borges FM, de Melo MA, Lima JP, Zanin IC, Rodrigues LK. Antimicrobial effect of chlorhexidine digluconate in dentin: In vitro and in situ study. *J Conserv Dent.* 2012;15:22-26.
11. Nanotec Nanocare Plus. Available at: <http://www.nanotecendo.com/nanotec-nanocare-plus/>
12. Rechmann P, Charland D, Beate M. T. Rechmann et al. Performance of laser fluorescence devices and visual examination for the detection of occlusal caries in permanent molars. *J Biomed Opt.* 2012;17:036006.
13. Kouchaji C. Comparison between a laser fluorescence device and visual examination in the detection of occlusal caries in children. *Saudi Dent J.* 2012;24:169-174.
14. DIAGNOcam, Caries detection - Seeing is believing. Available at: <http://www.kavo.com/en/small-devices/diagnocam>
15. Bahrololoomi Z, Musavi SA, Kabudan M. In vitro evaluation of the efficacy of laser fluorescence (DIAGNOdent) to detect demineralization and remineralization of smooth enamel lesions. *J Conserv Dent.* 2013;16:362-366.