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NanoCare Plus SilverGold® Can Eliminate *Enterococcus faecalis* from Dentinal Tubules

NanoCare Plus SilverGold® może eliminować *Enterococcus faecalis* z kanalików zębinowych

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A – concept, **B** – data collection, **C** – statistics, **D** – data interpretation, **E** – writing/editing the text, **F** – compiling the bibliography

Abstract

Background. Nanotechnology is one of the most promising fields in generating new applications in today's world. One of its branches is nanomedicine which is nothing else but applicatied nanotechnology in medicine. The most prominent nanoproduct is nanosilver. Due to its strong antibacterial activity, there are trials to use nanosilver as root canal disinfection agent.
Objectives. NanoCare Plus Silver Gold® (NanoCare) (Dental NanoTechnology, Poland) contains nanosilver particles within an alcoholic solution. In this study, the antimicrobial properties of NanoCare within dentinal tubules were evaluated with the *Haapasalo*-Model.
Material and Methods. 52 bovine roots were trimmed to a length of 8 mm, an outer diameter of 6 mm, and a root lumen of 1.3 mm. Smear layer was removed and the specimen autoclaved. Sterility of specimen was checked (3 randomly chosen specimen). *Enterococcus faecalis* ATCC 29212 was inoculated within the specimens for 24 h. 10 teeth (group 1) were immediately evaluated as described below. The teeth of 3 further groups (n = 13 each) received an intracanal dressing with saline (group 2), NanoCare (group 3), or calcium hydroxide Biopulp (Chema – Elektromet, Rzeszów, Poland) (group 4) for 1 week. Dentin powder samples (0.2 to 0.4 mm from the root canal surface) were collected, weighed, mixed with sterile saline solution and plated on agar plates. After 24 h of incubation, CFU were counted and related to 1 mg of dentin powder. Data was analyzed statistically using PASW 18.0 ($\alpha = 0.05$).
Results. Sterility of specimen, bacterial growth (mean CFU 3×10^6 per mg dentin powder in group without dressing), and potential bacterial survival (with saline dressing; mean CFU 9×10^4 per mg dentin powder) were assured. Zero CFU could be found with NanoCare. Calcium hydroxide allowed some bacteria to survive (mean CFU 5.7×10^3 per mg dentin powder). Differences between groups were statistically significant (*t*-test, $p < 0.001$).
Conclusions. NanoCare showed promising antimicrobial properties as an intracanal medicament. Further tests have to be carried out on this product regarding an application as a final irrigation (**Dent. Med. Probl. 2013, 50, 4, 418–423**).

Key words: *Enterococcus faecalis*, NanoCare Plus Silver Gold®, nanomedicine, nanoparticles, nanosilver.

Streszczenie

Wprowadzenie. Nanotechnologia jest jedną z najbardziej obiecujących dziedzin mających zastosowanie w dzisiejszym świecie. Jedną z jej gałęzi jest nanomedycyna, która jest niczym innym jak zastosowaniem nanotechnologii w medycynie. Nanocząsteczką, której poświęca się ostatnio najwięcej uwagi jest cząsteczka nanosrebra. Dzięki swoim bardzo silnym właściwościom przeciwbakteryjnym pojawiają się próby wykorzystania jej jako środka wspomagającego odkażanie kanału korzeniowego.

Cel pracy. NanoCare Plus Silver Gold® (NanoCare) (Dental NanoTechnology, Polska) jest nowym preparatem – alkoholowym roztworem cząsteczek nanosrebra. Celem pracy jest zbadanie jego działania antyseptycznego na drobnoustroje znajdujące się w kanalikach zębinowych. W pracy wykorzystano własną modyfikację modelu Haapasalo.

Materiał i metody. 52 zęby bydlęce zostały opracowane tak, by uzyskać „bloczki zębinowe" identycznych rozmiarów, wynoszących odpowiednio: długość – 8 mm, średnica zewnętrzna – 6 mm, średnica kanału korzeniowego – 1,3 mm. Po usunięciu warstwy mazistej próbki wysterylizowano w autoklawie. Na 3 losowo wybranych próbkach została przeprowadzona kontrola procesu sterylizacji. Pozostałych 49 próbek poddano 24-godzinnej inkubacji w bulionie zawierającym zawiesinę *Enterococcus faecalis* ATCC 29212. Po inkubacji dziesięć losowo wybranych próbek (grupa 1) zbadano w celu potwierdzenia zakażenia. Pozostałe „bloczki zębinowe" podzielono losowo na 3 równe grupy (n = 13) i wprowadzono do światła kanału na okres jednego tygodnia odpowiednio: 0,9% roztwór NaCl (grupa 2), NanoCare Plus Silver Gold (grupa 3), wodorotlenek wapnia (grupa 4). Po 7 dniach z każdego z „bloczków" pobrano próbki „wiórków zębinowych" ze ściany kanału korzeniowego (z głębokości 0,2–0,4 mm), które następnie zważono i posiano na pożywce agarowej. Po 24-godzinnej inkubacji dla każdej z próbek została obliczona liczba CFU przypadająca na jednostkę masy. Dane poddano analizie z użyciem PASW 18.0 ($\alpha = 0,05$).

Wyniki. Potwierdzono jałowość próbek po procesie sterylizacji oraz zwiększenie liczby bakterii po 24-godzinnej inkubacji sterylnych próbek w bulionie z zawiesiną *E. faecalis* (grupa 1) – CFU 3×10^6/1 mg zębiny. W grupie 2 (0,9% NaCl) liczba CFU wynosiła 9×10^4/mg zębiny. W grupie 3 (NanoCare Plus Silver Gold®) liczba CFU wynosiła 0. W grupie 4 (wodorotlenek wapnia) liczba CFU wynosiła $5,7 \times 10^3$. Różnice były istotne statystycznie (*t*-test, $p < 0,001$).

Wnioski. NanoCare wydaje się obiecującym antyseptykiem, który może mieć zastosowanie jako opatrunek wewnątrzkanałowy podczas leczenia endodontycznego. Należy przeprowadzić dalsze badania pozwalające stwierdzić, czy preparat ten może być wykorzystany jako ostatni środek płuczący podczas chemomechanicznego opracowania kanału (**Dent. Med. Probl. 2013, 50, 4, 418–423**).

Słowa kluczowe: *Enterococcus faecalis*, NanoCare Plus Silver Gold®, nanomedycyna, nanocząsteczki, nanosrebro.

Nanotechnology is one of the most promising fields in generating new applications in technology, everyday life and medicine. The early genesis of the concept of nanomedicine sprang from the visionary idea that tiny nanorobots and related machines could be designed, manufactured, and introduced into the human body to perform cellular repairs at the molecular level [1]. Nanomedicine is the process of diagnosing, treating, and preventing disease and traumatic injury, relieving pain, and preserving and improving human health, using molecular tools and molecular knowledge of the human body [1]. In short, nanomedicine is the application of nanotechnology to medicine [1, 2].

The most prominent nanoproduct is nanosilver. Nanosilver particles are generally smaller than 100 nm and contain 20–15.000 silver atoms. At nanoscale, silver exhibits remarkably unusual physical, chemical, and biological properties [3]. Due to its strong antibacterial activity, nanosilver coatings are used on various textiles but as well as coatings on certain implants. Furthermore, nanosilver is used for treatment of wounds and burns or as a contraceptive and marketed as water disinfectant and room spray [4].

Recently, a new disinfecting agent for use within dentistry has been introduced to the market: NanoCare Plus Silver Gold® (NanoCare) (Dental Nanotechnology, Katowice, Poland) containing silver nanoparticles and small amount of gold nanoparticles.

The aim of the study was to evaluate the antibacterial activity of NanoCare against *Enterococcus faecalis* within dentinal tubules using the experimental model described by Haapasalo and Ørstavik [5]. NanoCare was to be compared with saline (positive control) and calcium hydroxide (comparison group). The null hypothesis tested was that there is no difference in antibacterial activity between groups.

Material and Methods

For the experiment, the model of Haapasalo and Ørstavik [5] was used with our own modification. 52 freshly extracted intact single-rooted permanent bovine mandibular teeth were used. After extraction the teeth were stored in 0.5% NaOCl overnight for removal of organic tissue debris. After this procedure, teeth were rinsed with saline and dimensions were standardized. The coronal and apical parts were cut off by means of a cylinder-shaped diamond bur (Dentsply Maillefer, Ballaigues, Switzerland) mounted on a high-speed handpiece (KaVo, Biberach, Germany) under water cooling. In this way, "dentinal tubes" (middle root segments) were obtained – each one with the length of 8 mm. Following that, root canals were prepared for the standaridization of the root lumina using Largo® burs (Dentsply Maillefer) up to size #4 (1.3 mm). Canals with an initial diameter greater than a size #3 Largo (1.1 mm) were discarded. Root cementum was removed with large grit (brick-coloured) SofLex® disc (3M ESPE, Seefeld, Germany) and the outer surface of

each "dentinal tube" was prepared in such a way as to obtain the outer diameter of 6 mm. Eventually, dentinal tubes with 8 mm of length, 1.3 mm of internal diameter and 6 mm of external diameter were acquired (Fig. 1).

The smear layer was removed by rinsing in 17% EDTA for 10 min and in 5.25% NaOCl for 10 min. The tubes were sterilized by being autoclaved at 131°C for 30 min. Then, 3 randomly selected specimens were transferred to 3 viols containing 10 mL of sterile Brain-Heart Infusion® (BHI) broth (Graso, Starogard Gdański, Poland) to confirm sterility. A change of optical density of BHI broth (measured by a turbidity meter) would result in incomplete sterilization. After 24 h a change in optical density was not noticed.

A suspension of *Enterococcus faecalis* ATCC 29212 in sterile BHI broth was prepared by gradually adding the pure culture of *E. faecalis* to a flask containing 100 mL of sterile BHI until the density of 0.5 according to McFarland scale (1.5×10^8 bacteria/mL) was obtained. The sterilized "dentinal tubes" were transferred to the flask and remained there for 24 h at 37°C. Following the contamination period, specimens were removed from the flask under aseptic conditions, rinsed in sterile distilled water and dried with sterile paper points and sterile cellulose wadding. In order to confirm the contamination of the dentinal tubes, 10 specimens were randomly selected (Group 1) and the dentine samples were obtained and cultured in the way described below.

After that the 39 specimens left were divided randomly into 3 groups as follows:

– Group 2 (13 specimens, control group): sterile saline,

– Group 3 (13 specimens): NanoCare,

– Group 4 (13 specimens, comparison group): calcium hydroxide Biopulp® (Chema-Elektromet, Rzeszów, Poland).

Following the placement of the agents inside the dentin tubes, they were sealed with sticky wax and placed into 3 flasks (according to groups) containing 100 mL of saline each and incubated at 37°C for one week.

Following this, specimens were removed from the flasks and dried with sterile cellulose wadding. The canals were rinsed with sterile saline to remove the majority of the agents and dried with sterile paper points. To remove all the medicament remains, the canals were equipped with a Largo bur #5 mounted to an endodontic handpiece TR ZX® (Morita, Japan).

Then, dentinal chips were obtained by preparing the canals with a Largo bur #6. The chips from each canal were collected with individual sterile aluminium foils with known weights. The obtained dentinal chips were dried, the aluminium foils with dentinal chips were weighed again, and the weight of the chips was calculated.

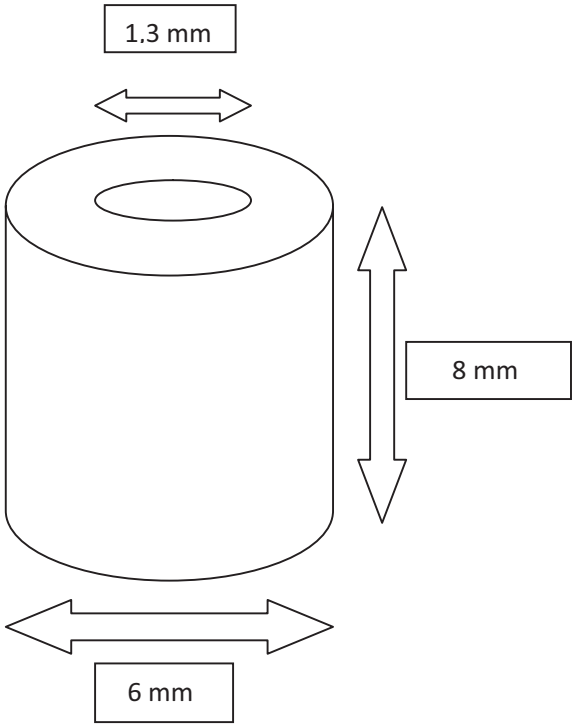

Fig. 1. Appearance and dimension of a prepared dentine test specimen

Ryc. 1. Wygląd i wymiary próbki – bloczka zębinowego

Dentinal chips from each root were then placed into viols containing 2 mL of sterile saline and disintegration was performed (5 Hz, 30 s). Following that, the suspensions composed of dentine with *E. faecalis* in sterile saline were cultured on selective agar plates Enterococosel® (Graso, Starogard Gdański, Poland) and incubated for 24 h at 37°C.

Dentinal chips from the tubes used as controls of inoculation were obtained and cultured in the same way. The only difference was that all these procedures were performed immediately after the end of the inoculation phase.

After 24 h the colony forming units (CFU) were counted. CFUs were then related to 1 mg of collected dentin chips.

Data was analyzed statistically using PASW 18.0 (SPSS Inc., Chicago, Ill; Kolmogorov-Smirnov-test, *t*-test; $\alpha = 0.05$).

Results

1 mg of contaminated dentine initially contained an average of 3×10^6 CFU of *E. faecalis* (group 1, Table 1). The possible survival of bacteria during the one-week incubation period (average:

Table 1. Results of different specimens: Pre – initial status without medication (group 1), Saline – physiological saline solution (group 2), NPSG – NanoCare Silver Gold (group 3), $Ca(OH)_2$ – calcium hydroxide suspension (group 4). Numbers of CFU are given, related to 1 mg of dentin powder

Tabela 1. Wyniki CFU uzyskane dla poszczególnych próbek: Pre – stan wyjściowy, po zakażeniu (grupa 1); Saline – fizjologiczny roztwór soli (grupa 2), NCSG – NanoCare Plus Silver Gold (grupa 3), $Ca(OH)_2$ – wodorotlenek wapnia (grupa 4). Liczba CFU jest podana w przeliczeniu na 1 mg suchej masy wiórków zębinowych

Sample number	Pre	Saline	NPSG	$Ca(OH)_2$
1	4.1×10^6	90×10^3	0	11.4×10^3
2	2.6×10^6	90×10^3	0	5.0×10^3
3	3.5×10^6	88×10^3	0	4.3×10^3
4	2.7×10^6	86×10^3	0	2.7×10^3
5	2.1×10^6	74×10^3	0	7.7×10^3
6	4.0×10^6	112×10^3	0	2.4×10^3
7	2.1×10^6	75×10^3	0	6.5×10^3
8	3.2×10^6	121×10^3	0	7.3×10^3
9	3.0×10^6	104×10^3	0	5.8×10^3
10	2.7×10^6	100×10^3	0	7.3×10^3
11		86×10^3	0	4.2×10^3
12		75×10^3	0	6.7×10^3
13		69×10^3	0	2.7×10^3
Average	3.0×10^6	90×10^3	0	5.7×10^3

9×10^4 CFU) was documented by the control group with sterile saline (group 2, Table 1). No CFU were found after one-week incubation with NanoCare. Calcium hydroxide allowed some bacteria to survive (average: 5.7×10^3 CFU). The differences between groups 2, 3 and 4 were statistically significant (*t*-test, $p < 0.001$).

Discussion

Statistical analysis showed significant differences for the different intracanal dressings used. Thus, the null hypothesis was rejected.

Antibacterial activity can be tested by various methods. However, a test of the zones of inhibition within agar plates or other direct contact tests would have been trivial because the potential antibacterial properties of nanosilver are beyond doubt, as shown by a considerable number of already published studies on this material [4, 6, 7]. The property of an antimicrobial agent to disinfect inside dentinal tubules is a far more interesting topic within endodontology that has been recently raised in endodontic studies [3, 8–11]. For this aspect, a suitable test was first described by Haapasalo and Ørstavik [5]. This test was applied within the present study with only minimal variation.

When particles have at least 1 dimension, which is less than 100 nm, they are named nanoparticles. Upon reaching nanoscale, like other nanomaterials, silver particles exhibit remarkably unusual physicochemical properties and biological activities [3, 12–14]. Silver nanoparticles are emerging as one of the fastest growing product categories in the nanotechnology industry.

Applications of engineered silver nanoparticles (nanosilver), especially in the healthcare sector, have been and are being deeply explored. Silver nanoparticles have been synthesized through an array of methods, e.g. spark discharging, electrochemical reduction, solution irradiating and cryochemical synthesis, to name a few [15–17]. As is the case with all nanomaterials, the principle characteristic of silver nanoparticles is their ultra small size. Ultra small particle size leads to ultra large surface area per mass where a large proportion of atoms are in immediate contact with environment and readily available for reaction. Unique interactions with bacteria and virus have been demonstrated for silver nanoparticles [18–20]. The remarkably strong anti-microbial activity is a most important characteristic and major direction for development of nanosilver products. A wide category of products has already been available on the market. In medical arena, there are wound dressings, contraceptive devices, surgical instruments and bone prostheses all coated or embedded with nanosilver [21–24]. In daily life, consumers may have nanosilver containing room sprays, laundry detergents, water purificants and wall paint [8, 21, 25]. Silver nanoparticles are also incorporated into textiles for manufacture of clothing, underwear and socks [26].

Our studies showed that nanosilver has great potential for antimicrobial activity and is very effective against *Enterococcus faecalis*, which is in agreement with other studies [9, 11]. The possible contact time to *E. faecalis* in this study was 1 week. Due to its properties, NanoCare may also serve as a kind of impregnation with a substatial effect, similar to chlorhexidine, when used as a final irrigation. However, this application has to be examined in further studies.

The use of the described material in medicine and dentistry has just started and will continue. Crede introduced l% silver nitrate as an eye solution for the prevention of *Gonococcal ophthalmia neonatorum*, which is perhaps the first scientifically documented medical use of silver. Irreversible

pigmentation of the skin and/or the eye, i.e. argyria or argyrosis, due to silver deposition, may develop after prolonged exposure to silver or silver compounds [27, 28]. In endodontics, possible discoloration may interfere with the growing esthetic demands of the patients. Thus, this aspect needs further investigation.

Moreover, there are only few studies that recognize the toxicity of nanosilver [4, 8, 23]. As silver nanoparticles are and will be more and more widespread in medicine and related application toxicological and environmental issues need to be raised. When used as an intracanal dressing, NanoCare may get into contact to human tissue if accidentally pressed beyond the apex or by diffusion out of the "impregnated" dental tissues. A recently published study has shown only limited reaction of tissues to nanosilver, but comparable with 2.5% sodium hypochlorite [8]. Thus, toxicological aspects of NanoCare should also be a matter of further research.

Within the present study NanoCare showed very promising potential as an intracanal dressing between visits. Further research on NanoCare should be undertaken regarding discoloration, its possible toxicity in the case of accidental delivery to the periapical tissues, and also on its potential as a last irrigation before drying of the root canal.

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