Silver Nanoparticles Bacteriostatic Effect over Dentures Infected with *Candida Albicans*

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Oral candidiasis is a frequent and common affection of subjects with immunity deficiencies, children and subjects that are wearing dentures. Acrylic resin involved in making the dentures is a material that can maintain a favourable environment for candida albicans. In case of denture wearing subjects, antifungic drugs, local and general treatment cure the oral candidiasis only in association with a new denture or with the disinfection of the old one. Silver was used for a long time in dentistry and is known for its beneficial properties. Silver nanoparticles have a strong bacteriostatic effect. The purpose of this study is to spot the bacteriostatic effect over dentures infested by candida albicans.

Key words: dentures, acrylic resin, oral candida, silver nanoparticles

Candida albicans is the major etiologic factor for oral *stomatitis*. The ability to eat, pain or difficulty swallowing, feeling that food gets stuck in the throat or mid-chest area, difficulties in talking and also sleep can be affected due to sore mouth. Stomatitis can occur on tongue, lips, cheeks, palate and gums. Candida infection is not limited to the mouth and can cause diaper rash in infants or vaginal yeast infections in women. Patients with immunity disorders like AIDS, cancer, diabetes, antibiotic therapy for long periods of time and strong antibiotics, and other similar diseases, may suffer this type of infections. In case of denture-wearing patients the oral mucosa covered by dentures may present erythematous inflammation. Elder individuals have a weakened immune system as well as children.

Beyond *Candida albicans* other species of candida such as *Candida glabrata, Candida tropicalis, Candisa krusei, Candida parapsilosis and Candida dubliniensis* have been associated with denture stomatitis [1-4]. Elder persons are suffering higher rates of infection diseases than young persons. The most common inflammatory condition of oral mucosa in different populations and worldwide is denture stomatitis and is associated with *Candida albincans* [5-9]. *Candida albicans* is an opportunistic microorganism that changes its status from commensal to pathogenic when has favourable conditions that were already mentioned. The immune response is responsible for controlling the Candida related infection of oral mucosa. *Neutrophils* are antifungal cells that are able to destroy the pathogen microorganism through phagocytosis and to produce reactive oxygen [10]. *Neutrophils* have a key role in the hosts self defence mechanism against localized *Candida albicans* infections [11].

Neutrophils are participating to acute response against pathogen microorganisms, their activity in the oral cavity occurs at any time and is more intense than it is in the other tissues. Alteration in the number of these cells and a vicious function of them predispose individuals to specific oral diseases [12]. Defence line represented by neutrophils from aged individuals showed different behaviours to different stimulus [13].

Many treatments were proposed for Oral Candida associated to denture stomatitis. The specific literature and the studies are mentioning a low number of antifungal agents. The therapy may induce side effects, resistance or recurrence of the infection [14-17]. Global programs have been implemented for dealing with candida resistance to antifungal drugs. One program in 2006-2007 is SENTRY focused on fungus resistance to amphotericin B give reports on candida susceptibility to candida-type antifungals [18,19].

New therapeutical strategies focused on natural products can generate positive results. Essential oils are promising positive results for oral infections. The natural oils are a complex mixture of volatile compounds obtained from plants such as *terpenoids.* The properties are anti-oxidative and antimicrobial against of a wide range of pathogens including *Candida albicans* and *dermatophytes*. Natural deviants with bacteriostatic effect against *oral candida* are *carvacol, farmesol, geraniol, linalool, menthol, menthone, terpinen-4-ol, a-terpineol, eugenol (phenyl-propanoid) and phenethyl alcohol (tyrosol)* [1, 20-22].

The nano-materials science is present in medical domain and in dentistry. The medical uses of silver include its incorporation into wound dressings, creams, and as an antibiotic coating on medical devices. Wound dressings containing *silver sulfadiazine* or *silver nanomaterials* may be used on external infections but there is little evidence to support such use [23]. There is tentative evidence that silver coatings on urinary catheters and endotracheal breathing tubes may reduce the incidence of catheter-related urinary tract infections and ventilator-associated pneumonia [24, 25]. Silver ion (Ag^+) is bioactive and in sufficient concentration readily kills bacteria *in vitro*. Silver exhibits low toxicity in the human body and silver nanoparticles are used as an antimicrobial substance.

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The nanoparticle science can be considered a new path in science and is continuously developing. If the dimension of a material is characterised by nanometric scale the materials physical and chemical properties are considerable modifying. The most spectacular phenomena is the colour and the brightness of colloidal nanoparticle. These two aspects are the result of electromagnetic interactions between the light incidence and conduction electrons from metallic nanoparticles. Light incidence is connecting with oscillation frequency of conductive electrons in noble metallic nanoparticle and produces the surface plasmonic resonance (SRP). The result is the intense absorption string in visible spectrum. In case of metallic nanoparticle the SRP is located on the nanoparticle's surface and are named Localised Surface Plamotic Rezonnt (LSPR).

Due to chemical, electronical and plasmonic exceptional properties the metallic nanoparticles are very attractive for biomedical purposes such as: molecular identification molecular diagnostic, antibacterial action, drug transport and cancer therapy. The antibacterial activity of silver nanoparticles over Gram-negative and Gram-positive bacteria was studied in many scientific papers. The toxicity mechanism is not completely explained. Synthesis of nanosized particles with antibacterial properties is of great interest in the development of new pharmaceutical products. Silver nanoparticles (Ag NPs) have inhibitory and bactericidal effects. Nanosized silver (Ag NPs) can be prepared by chemical reduction from aqueous solutions of silver nitrate, containing a mixture of hydrazine hydrate and sodium citrate as reductants and sodium dodecyl sulfate as a stabilizer. Ag NPs show agglomerates of grains with a narrow size distribution (from 40 to 60 nm), whereas the radii of the individual particles are between 10 and 20 nm. Kirby-Bauer method can measure the antibacterial activity. The results showed reasonable bactericidal activity against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus [26].

Many studies suggest that the silver nanoparticles are penetrating the cellular membrane and the result is the cell's death [27]. Other hypothesis sustain that antimicrobial mechanism is produced by free radicals that destroy the cell [28]. Another mechanism suggest that antimicrobial activity of nanoparticles is activated by the released ions. A general accepted hypothesis is that the antibacterial effect of silver nanoparticles depends on particle's dimension and shape and stability in the environment.

A new concept based on gold and silver nanoparticles with strong bacteriostatic effects is already used in dentistry. These materials find their applicability in root canals in the final stage of root canal treatment. Silver and also gold were used in dentistry for many years. The disadvantage was the predisposition of metals to corrosion and the loss of physical and chemical properties. In present the silver nanoparticles are successfully used in the procedure of tooth root canal system cleaning during final rinsing of canals, immediately before embedding of tooth filling. The NANOCARE PLUS (nano-silver) liquid is used during the last rinsing, cleaning the surface of canals off bacteria and organic remains. In addition, the fluid leaves the layer of nanoparticles of silver on the canals surface, which has a strong bacteriostatic effect and prevents the re-colonization of canal system by bacteria and fungi. Low surface tension of the carrier substance allows nanoparticles to get to the smallest fissures of dental structure. As a result, even bacteria residing in the fissures in spore form after treatment have no chances for growth and development. Gold and silver nanoparticles do not undergo corrosion process, and their minimum concentration in the liquid allows the avoidance of any problems during the final canals filling followed by tooth filling [29].

Experimental part

The present in vitro study involved 37 subjects. A total of 74 dentures, that belong to the candidate subjectswere micro-biologically investigated. A total of 23 subjectswere selected for this study. The selected subjectsthat participated to this study were infested with candida albicans. The infection was confirmed by micro-biological analyses. Form the selected group of 23, only 5 subjects had evident and severe symptoms of denture stomatitis (table 1).

Table 1
THE LOT OF SUBJECTS

1	Clinical Condition	Number of patients
2	Total investigated Subjects	34
3	Subjects -negative to candida albicans infection	9
4	Subjects -positive to candida albicans infection	23
5	Subjects with severe symptoms of denture stomatitis	5
6	Subjects without severe symtoms of denture stomatitis	18

The microbiologic analyse dedicated to identifying the fungal micro-organisms also spotted other pathogen organisms like serratia odorifera and citrobacter freundii. The sampling of microbiologic environment present on the denture was made with dedicated probes with gel transporting medium. Before sampling the dentures were not washed with any antimicrobial or disinfected substance. Just immediately after the denture was removed from the oral cavity the samples were assayed from the mucosal surface and the oral surface of the denture (fig.1).

No antifungal or antimicrobial substance was used by the subject or over the denture. All 37 subjects were instructed not to quit to their everyday usual habits. The oral cavity and the dentures were not cleaned with tooth paste, mouth water or any other antiseptic substances. The first lot of samples were taken immediately after the denture was removed from the oral cavity. Samples were taken from the mucosal and oral face of the superior and inferior dentures.

Samples were sent to the microbiologic laboratory for micro-biological investigation. The dentures with candida albicans positive results were included in the study. From the total of 37 individuals, 23 subjects had a positive micro-biological diagnostic to candida albicans. The negative micro-biologic analysis of dentures negative to candida albicans were excluded from this study.

Nanocare+ is a complex pharmaceutical substance with a long-lasting bacteriostatic effect designed for final

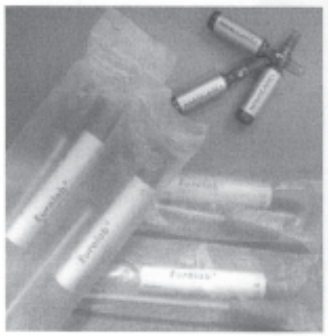

Fig.1 Nano-silver ampoules and sampling kit

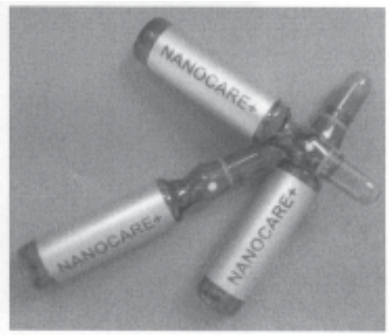

Fig.2. Nano-silver ampoules

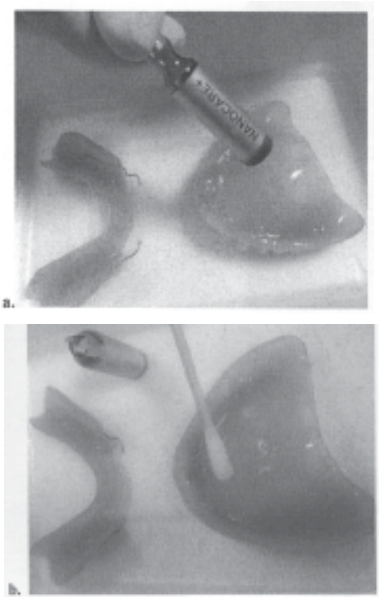

Fig.3 (a,b) Tester samples of maxillary denture

rinsing of the root canals during endodontic treatment based on silver nanoparticles. It is efficient over a large number of germs and bacteria.

The silver nanoparticles are presented as a liquid solution and it has been prepared based on the existing experience with pharmaceuticals containing nanoparticles. It is well known that silver pegs in root canals prevented the infection development although the bacteria were present. The nano-silver liquid would supplement current procedures of cleansing the root canals due to bacteriostatic effects. It is proven that in the root canal nano-silver impedes re-colonisation of bacteria in the root canal system (fig. 2) [28].

Nano-silver liquid solution was poured over the mucosal face of the upper and lower jaw's denture (fig. 3a) and any other method or disinfection solution was avoided. The nano-silver ampoule was poured on the centre of palatal area. Deposition of nano-silver liquid in the centre of denture palatal area is the best option because this area has a positive profile. From this area, due to the curved shape of the palatal surface, the liquid will flow toward peripheral margins of the denture. All the palatal mucosal surface of the denture will be washed by the nano-silver liquid which will finally arrive in the area that corresponds to the edentulous crest. The edentulous crest area of the denture is a negative one. After washing the mucosal palatal face of the denture, the nano-silver liquid will be cumulated in the negative profile of the denture represented by the negative area of the dentures – edentulous crests. From this area the nano-silver solution was spread over the entire mucosal surface of the denture. The nano-silver solution wasn't diluted.

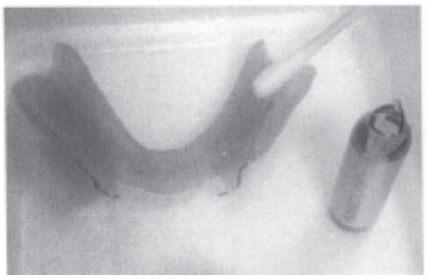

Fig.4. Tester sample of jaw denture

The same protocol was used for the dentures that rehabilitate the functionality of the lower jaw. In case of the dentures that restore the functionality of the lower jaw the nano-silver liquid was deposited on the mucosal surface in the area of the anterior lower incisors. Because of the curved shape of the mandibular residual crest the liquid was guided into the molar distal area and peripheral margins of the dentures.

For each denture were used three ampoules. Before applying the protocol it was taken another samples from the denture surfaces just immediately after it was removed from the oral cavity. Though the subjects were already selected the laboratory analysis was repeated just to confirm that the previous microbiologic analyse did not change and the oral pathology is still maintained. The nano-silver solution from the first ampoule was maintained for 5 min. After 5 min two samples with sampling kit were assayed. From each denture were taken two samples after 5 min. One sample was taken from the area where the nano-silver liquid was laid –centre of palatal area (maxillary denture) and area of anterior ridge (mandibular denture). The second sample was taken from a distant area where the nano-silver solution was present due to the pouring properties and low superficial tension of the liquid: incisors anterior ridge (maxillary denture) and molar lateral ridge (mandibular denture). One tester sample for the maxillary dentures was preleased from the palatal area where the nano-silver liquid was first poured and the second one was taken from the anterior (incisors area) edentulous ridge area (fig.3b). For the jaw dentures one tester sample was taken from the anterior ridge area and the second sample was preleased from the lateral-distal (molar area) area of the ridge (fig.4). The first lot of samples was taken at 5 min after the nano-silver solution had washed the mucosal surface of the dentures.

The second nano-silver ampoule was poured over the denture and over the liquid from the first ampoule. After ten minutes another sampling was taken, following the same protocol of sample taking. Following the same protocol, the third ampoule was poured in the same conditions. Respecting the same conditions and protocol, the last sampling was taken after 15 min and sent to the laboratory.

The samples were divided in four groups. The first group was composed by tester samples preleased before nano-

Fig.5. Nano-silver treatment after 10 min

silver treatment; the second group was composed from tester samples preleased after 5 min of nano-silver action over the denture mucosal surface. The third group was composed from tester samples preleased after 10 minutes of nano-silver action over the denture mucosal surface (fig.5).

The fourth group was composed from tester samples preleased after 15 minutes of nano-silver action over the denture mucosal surface.

All the tester samples were preleased with the same kit and sent to the microbiologic laboratory. The microbiological analysis was made after the same parameters and in the same conditions as the ones performed for the selection of the subjects . The samples from the oral cavity were taken with sterile plugging and suspended in Stuart transport environment. All the hygienic conditions were respected not to contaminate or over-contaminate the pledges. Each pledge was inoculated on blood agar 5%, *Sabouraud, Chapman* and *Mac Conkey* culture medium and incubated at 37 ^{0}C for 24 h. After 24 h the isolated germs were identified after Gram coloration, morphology, and bio-chemical tests. For fungus identification were used Candifast. The fungus colonies were well developed, white with clear and well defined margins.

Results and discussions

Oral micro-flora depends by age, and organism status. The mouth is sterile at birth but is soon contaminated with flora from birth canal. Infant's oral cavity will acquire organisms from mother and environment. The micro-organisms are streptococcal and staphylococcal species, Lactobacilli, bacillus, *neisseria* and Yeasts. *Streptococcus salivarius* is the most common and and forms the pioneer community with *Staphylococcus albus*.

The eruptions of deciduous teeth provide a new attachment surface and turns *Streptococcus sanguins* and *muttans* as regular inhabitants of oral cavity. The anaerobes are few in number due to absence of deep gingival crevice. Adults have an increased number of *Bacteroides* and *Spirochetes* with maturity of dental plaque. As the teeth are lost availed sites for microbial colonization decreases and several species diminish disproportionately in numbers. Edentulous persons harbour few *Spirochetes* or *Bacteroides* but carriage of *Yeast* increases. *S sanguins* and *mutans* disappear (table 2).

The bio-chemical analysis for the first group, the control group confirmed the presence of candida albicans and other germs that were not considered in this study. The second group, treated with nano-silver particles for five minutes were free fungus, candida albicans and other germs. The micro-biological tests of the third group treated with nanosilver particles for 10 min registered negative results for candida albicans. The samples taken from the centre of palatal area and anterior edentulous anterior crest of mucosal face of maxillary denture were negative to candida albicans. The samples taken of the mandibular denture were also negative to candida albicans.

Table 2
MICRO-FLORA OF DENTAL PLAQUE

Bacterii	*Frecquency (%)*
***Streptococcus* sp.**	88%
Streptococcus mutans	50%
Streptococcus sanguis	63%
Streptococcus oralis	75%
Streptococcus anginosus	63%
Streptococcus salivarius	38%
***Staphylococus* sp.**	100%
Staphylococcus aureus	88%
Staphylococcus epidermidis	13%
Bacili gram positive	100%
***Actinomyces* sp.**	88%
Actinomyces israelii	63%
Actinomyces naeslundii	63%
Actinomyces Odontolitycus	25%
***Propionibacterium* sp.**	50%
***Veillonella* sp.**	100%
Bacili gram negative	38%
Fungus	**63%**

The results for the fourth group, treated with nanosilver particles for 15 min were also negative to candida albicans and other germs [30- 33].

Conclusions

Subjects diagnosed with *oral candia* have to follow a two direction treatment. Beyond the general, systemic treatment the association of the oral mucosa and local treatment of oral cavity is needed. Because there are a few species of candida and the most common are causing invasive fungal infections, it is part of the protocol to identify the specific specie of candida. Identification the dedicated drug or treatment can be done only after the bio-chemical tests of the sample. Though the diagnostic of *candida* can be obtained without bio-chemical testes the identification of the candida specie is not possible. Specific anti-fungus drugs like Fluconazole, Voriconazole, Amphotericin B Deoxycholate have to be associated with local treatment of oral cavity and with the removal of the denture until the bio-chemical testes are negative. Otherwise the denture will maintain the infection in the oral cavity. The alternative

of a new denture can be avoided by using the disinfecting substances like silver nanoparticles.

The bio-chemical tests were negative after nano-silver particles treatment. The association of systemic treatment with nano-silver disinfections of dentures can be a treatment protocol for oral candida. Bio-chemical tests showed that only 5 min of nano-silver treatment are needed for obtaining a fungus free denture.

In the limitations of this study, nano-silver particles find their applicability in clinical treatment of oral canadida. The association of the systemic treatment with 5 minutes nano-silver particle disinfection of the denture assure the complete remission of oral candida.

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