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Investigating the Efficacy and Safety of Silver Nanoparticles in Treating Oral Biofilms

Abstract

Silver nanoparticles provide a novel method for fighting the growth of dental biofilms, which primarily consist of the *streptococcus mutans* and *candida albicans* bacteria. These biofilms lead to a number of harmful oral maladies, particularly periodontal or gum disease which tend to greater affect older Americans. This study synthesized the applications of silver nanoparticles as a means of breaking up these biofilms in humans. Many of these biofilms and their associated effects exist in species other than human but those cases are beyond the scope of this study. As part of the synthesis of the applications for silver nanoparticles, this study evaluated their efficacy in fighting such bacteria in addition to any known problems with their toxicity. This study was done through a systematic literature review concluding in a focused summary of findings. Silver nanoparticles were found to be effective against both strains of bacteria. No issues of toxicity to human cells were found in clinical studies wherein patients used appliances infused with silver nanoparticles. Future research should be conducted to determine whether silver nanoparticles can be applied safely outside of an appliance.

Keywords: *Streptococcus mutans*, *Candida albicans*, biofilm, periodontal disease, silver nanoparticles, human, denture, denture base liner

Gap

Through analysis of the results of previous experimentation with silver nanoparticles regarding their toxicity to *S. mutans* and *C. albicans* and their integration into the denture and denture base liner, this study aims to produce a focused summary of findings that will determine whether silver nanoparticles are a safe and effective treatment method for oral biofilms.

Introduction

In the United States today, 47% of adults experience some degree of periodontal disease, growing to 70% for 65 years old and over as the human body ages and weakens (Eke, et al., 2012). Periodontal disease, commonly known as gum disease, is an infection of the gum tissue caused by the buildup and hardening of plaque biofilms on the teeth that leads to bleeding, soreness, and tooth loss (NIH, 2018). The issue of periodontal disease is becoming more prominent as the population ages in the United States. In addition, an increased rate obesity and high sugar diets have also been linked to heightened risk of developing periodontal disease due to an increased sugar content in the body that gives harmful bacteria more fuel to grow and reproduce (Corrêa et al., 2015).

Figure 1: *Presence of bacterial biofilms on the teeth surface (European Federation of Periodontology, 2016)*

Periodontal disease, as shown in the figure above, is one of a group of health complications arising from the formation of oral bacterial biofilms including oral cancer and gingivitis. Biofilms consist of microorganisms in which cells adhere to each other and often to a surface (Wady et al., 2012). In this study, analysis was focused on two types of bacteria which naturally exist in the human mouth and body: *streptococcus mutans* and *candida albicans*. These bacteria are the primary culprits for the growth of biofilms that decay teeth and gums leading to the bacterial biofilm related issues mentioned earlier. They cling to both human and synthetic surfaces, such as the base of denture liners, in the mouth (Monteiro et al., 2012). After attaching to a surface, the biofilms grow by feeding on sugars and using it as fuel for growth and cellular reproduction. The biofilm spreads across the mouth through reproduction and can begin to break down the teeth and the area that surrounds them, damaging the enamel and creating cavities (Corrêa et al., 2015).

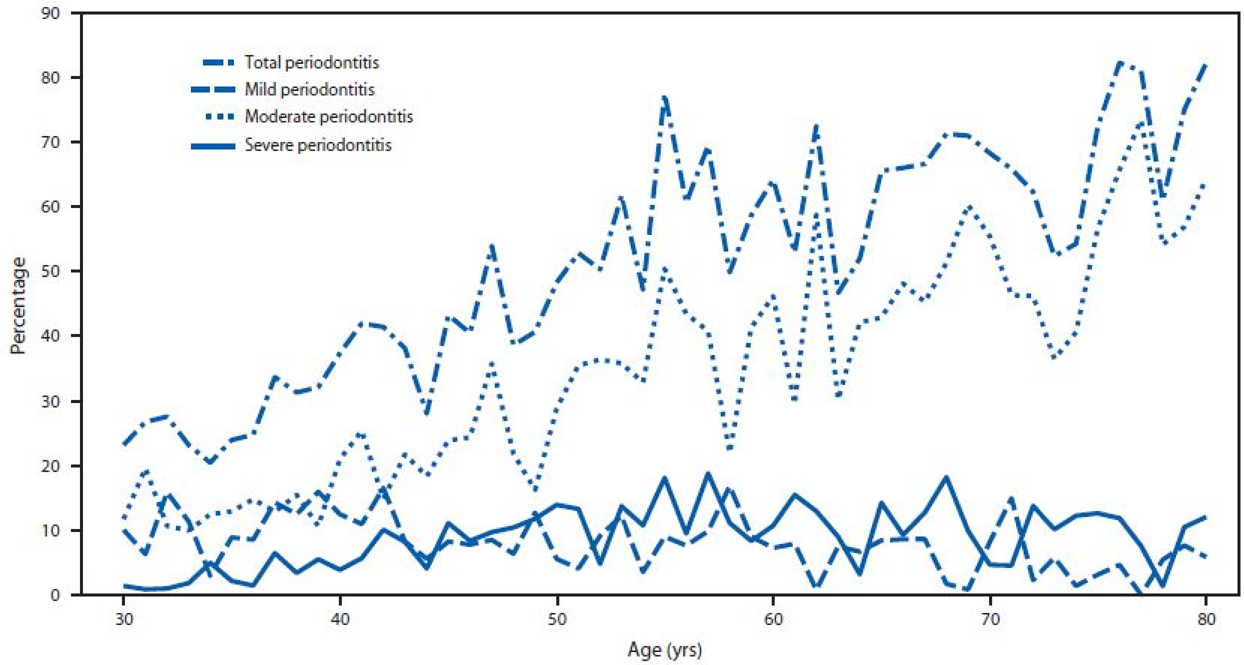

Figure 2: *Rate and type of periodontal disease, or periodontitis, present amongst age subsets of the adult population in the United States (CDC, 2010).*

Finding effective methods for killing and inhibiting the reproduction of the *s.mutans* and *c. albicans* bacteria is an increasingly important issue as the population of the United States on average becomes older due to the younger generation having fewer children. As depicted in the graph above, the rate of periodontal disease, or periodontitis, increases with age, especially in its moderate form (CDC, 2010). This leads to an increased average burden on younger people to support their older, often retired counterparts (Ortman et al., 2014). Finding more effective treatment methods will decrease the overall healthcare cost of treating issues that stem from oral decay (Oldenburg, 2014).

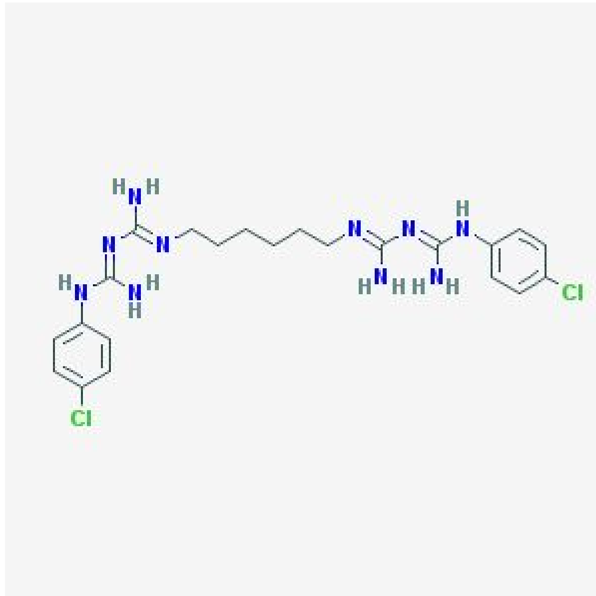

Figure 3: *Molecular structure of chlorhexidine solution, a common antiseptic that has been used to kill oral bacteria (NIH, 2018).*

Currently, oral antimicrobial solutions, primarily chlorhexidine, are used to kill *s.mutans* and *c.albicans*. Chlorhexidine, as shown in the above depiction, is a biguanide compound that is used as an antiseptic or antibacterial agent. It has a positive charge and thus bonds to the negatively charged cell membrane, destroying the cell membrane and causing a leakage of cell organelles, killing the cell (NIH, 2018). However, chlorhexidine doesn't have as great a bactericidal ability as other materials and chemicals that can be used for treatment (Besinis et al., 2012).

Silver nanoparticles, a form of nano-biomaterial, have been demonstrated to be more effective at killing *s.mutans* and *c.albicans* in addition to other potentially harmful bacteria beyond the scope of this study, namely *enterococcus faecalis* and *lactobacillus* (Corrêa et al., 2015). Silver nanoparticles (AgNPs) are an important group of nanomaterials, or miniscule synthetic materials that compose a subset of nanotechnology, in the dental practice. Their microscopic size provides them with a great antimicrobial effect allowing them to be included in many nano-biomaterials, or nanomaterials with a biological component or usage, looking for these properties (Targino et al., 2013).

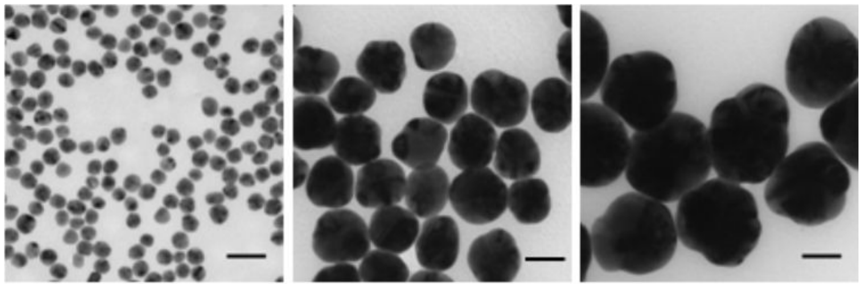

Figure 4: Silver nanoparticles seen at different magnitudes using transmission electron microscopy (Oldenburg, 2010).

Silver nanoparticles are created in a variety of sizes as shown in the above image created with the aid of transmission electron microscopy (TEM), in which electrons are breamed through an object to produce an image. The smaller nanoparticles have a greater overall surface area, allowing them to excrete more silver ions into bacterial cells; however, the difference in efficacy between the nanoparticles is negligible in comparison to the increase in efficacy between chlorhexidine solution and silver nanoparticles (Oldenburg, 2010).

Silver nanoparticles kill bacteria in a method similar to that of chlorhexidine solution, however they have been demonstrated to greater decrease the population of *s.mutans* and *c.albicans* in in vitro experimentation (Nam, 2011). Silver nanoparticles clump around the cell membranes of bacteria but instead of destroying the membrane, as with chlorhexidine solution, silver nanoparticles excrete silver ions into the cell through the membrane. These silver ions then collect around cell organelles where they are reduced and then able to clump together again. Reduction is the process of gaining an electron; in this case, the cell organelles donate electrons to the positively charged silver ions that pass onto them from the membrane. The clumps of silver nanoparticles prevent vital cell functions from occurring, such as the movement of organelles, DNA replication, and cell division. This inhibits cell reproduction in addition to killing the cells. Although silver nanoparticles are not a 100 percent effective antimicrobial agent, they are much more effective than current treatment methods (Targino et al., 2013).

In addition to being applied to bacterial cells alone, silver nanoparticles are able to be applied as part of more complex treatments. Silver nanoparticles are able to be incorporated with irreversible hydrocolloid impression materials. Irreversible hydrocolloid impression materials are hydrosols of alginic acid which form calcium alginate through an irreversible chemical reaction. These materials are used to form dental impressions to use for positive reproduction, or casting of the mouth (Ginjupalli et al., 2016). Silver nanoparticles are also able to be used in tissue conditioners at the denture base to clean the area before casting of the dentures using irreversible hydrocolloid impression materials. The tissue conditioners help to clean the area before impressions are made. Incorporating the tissue conditioners with silver nanoparticles increases their bactericidal potency (Nam et al., 2011). Finally, silver nanoparticles can be implanted into the denture base liner, the piece of the dentures that makes contact with the gums to prevent the growth of biofilms there (Chladek et al., 2013).

Despite their great advantages over current treatment methods, there remains much debate among scientists as to whether silver nanoparticles are a safe method of treatment for bacterial inside the human body. Their toxic properties could allow them to damage and kill human cells, possible leading to issues for those who are undergoing treatment. Scientists are especially worried about treatment over the long term, under which circumstances it is harder for doctors or dentists to keep close track of the activity of the silver nanoparticles. Great concern arose from the recent publication of an article detailing the harmful effects that silver nanoparticles had in vitro against human and rat embryonic neural stem cells (Liu et al., 2015).

This study used a systematic review of literature with focused findings to determine whether silver nanoparticles can both effectively and safely kill and inhibit oral bacterial biofilms of the *s.mutans* and *c.albicans* variety over the short and long term. The use of silver nanoparticles is considered long term if they are in place for more than four months, as this is the conventional minimum time of wearing an oral appliance such as a removable retainer before replacement (Farhadian et al., 2016). The study analyzed data from in vitro testing of silver nanoparticles and materials which incorporate them against the two bacterial strains. In addition, clinical testing of materials incorporating silver nanoparticles was analyzed. Based on results from previous experimentation and scientific knowledge surrounding silver nanoparticles and their relationship to bacteria and the human body, this study aimed to determine whether they are viable for long term usage in oral applications, specifically those related to dentures and as cleaning solutions.

Background

As shown in the silver pot below, silver has been used in medicine for centuries. In ancient Rome and Egypt, silver was believed by the people to carry health benefits in addition to being a sign of wealth. People used it to store food and drinks. Silver did not give off the harmful effects of lead, which was also commonly used for kitchenware at that time. As time moved on, silver began to be used to produce utensils such as the notorious silver spoon, which became symbols of health and prestige. As the world entered the nineteenth century, research began on the applications of silver in medicine. Silver colloids were researched, although they ended up being eclipsed by pharmaceuticals. Research into silver continued however and silver nitrates egan to be used for sterilization. More recently, silver has found its place in bandaging, acupuncture, suturing, water purification, and dentistry. In dentistry, silver has been used to make Amalgam fillings for over a century (Colloidal Silver Information, 2011). Today, researchers are finding novel applications for silver to fight growing issues with oral health as the population becomes generally older.

Figure 5: *Early use of silver for food storage (Indian Express, 2017).*

In 2010, the Center for Disease Control and Prevention came out with a study outlining the instances of periodontal disease, or periodontitis, among adult United States citizens over the age of 30. The study collected data from thousands of participants who were examined by dental hygienists who classified their periodontal disease as mild, moderate, or severe. The study found that the rate of periodontal disease to be increasing in the United States. Forty-seven percent of those over thirty years of age were found to have some form of periodontal disease, with those cases increasing with age. This study spawned increased interest in finding more effective methods of preventing and treating periodontal disease (Eke et al., 2012). One such of these new methods of treatment was the use of silver, specifically silver nanoparticles, in treating oral biofilms more effectively than the traditional chlorhexidine solution.

An article looked at another application for silver as an antimicrobial material by using silver diamine fluoride against enterococcus faecalis biofilms. Silver diamine fluoride was applied to shaped radicular dentin and was examined using low-pressure scanning electron microscopy. It was found that the silver diamine fluoride was effective at removing biofilm from the root dentin surface (Hiraishi et al., 2010).

An experiment from 2011 sought to find an antimicrobial solution to the problem of bacterial buildup on the supporting tissue of dentures. They put three strain bacteria alongside GC Soft-Liner tissue from Tokyo in well culture plates and then added silver particles and observed the results. They found that the bactericidal and fungicidal properties of the silver particles were enough to inhibit the three reference strains tested: *s.aureus*, *s.mutans*, *and c.albicans* (Nam et al., 2011).

In 2011, a study similar to the one previously mentioned sought to modify denture liner materials to prevent mucosal infections through the addition of silver nanoparticles. They present the results of silicone soft lining materials that were created with the addition of silver nanoparticles They found the resulting antifungal efficacy of the composites to be somewhat effective in killing the candida albicans (Chladek et al.). In 2012, these scientists continued their research on denture lining composites when they conducted an experiment to look into the effect of silver nanoparticles on denture lining composites and evaluated changes in sorption, solubility, hardness and tensile bond strength. The samples were evaluated with the two-way ANOVA and Newman-Keuls post hoc tests or the chi-square Pearson test at the $p < 0.05$ level. It was found that an increase on the amount of silver nanoparticles decreased both hardness and bond strength while increasing the solubility and sorption (Chladek et al.).

In 2014, a study explored the ability of several nanotechnology solutions to replace the traditional chlorhexidine disinfectant. Changes to the *streptococcus* mutans bacteria were assessed using the minimum inhibitory concentration, or MIC, fluorescent staining to determine dead and live cells, and measurements of lactate. Of the several nanomaterials tested against the bacteria it was concluded that the silver-containing materials, AgNPs and AgNO3, were the most successful at eliminating the bacteria (Besinis et al., 2014).

An experiment from 2014 attempted to find a solution to the problem of recurrent caries, which are partly due to a lack of antibacterial properties on the composite resins used to fill and treat them. The scientists prepared synthetic tablets: one control, one with a zinc oxide composite, and one with a silver composite. Then they placed streptococcus mutans and lactobacillus bacteria above them and found that the two composites exhibited high bactericidal properties compared to the control (Kasraei et al., 2014).

A study undertaken by several Brazil-based researchers investigated the activity that silver-nanoparticle treated mixtures have against *streptococcus mutans*, the most common cause of dental caries. They analyzed the minimum inhibitory concentration, or MIC, using spectrophotometric microdilution method, or SMM, and turbidity. They found that silver-containing composites are a much more effective method for treating oral biofilm than the current chlorhexidine solution (Santos et al., 2014). Another study analyzed the impact of silver nanoparticles against candida albicans, the most common fungal pathogen found in the human body. To do this, the researchers performed various tests on various tests on candida albicans to determine the ultrastructural distribution of the nanoparticles using transmission electron microscopy (TEM) and energy dispersive spectroscopy (EDS). From this it was determined that while most particles remain outside the cell, silver ions are released into the cell and are made into nanoparticles by reduction from organic cellular molecules (Vazquez-Munoz et al., 2014). In addition, other researchers analyzed the antimicrobial ability of silver nanoparticles as an endodontic irrigant against enterococcus mutans. They inoculated dentin sections with enterococcus faecalis and then irrigated them with silver nanoparticles and analyzed the materials with confocal laser scanning microscopy combined with viability staining. Bacterial biofilms were shown to be eliminated through the use of silver nanoparticles during root canal disinfection (Wu et al., 2014).

A major study came out in 2015 discussing the harmful effects of silver nanoparticles against the embryonic neural stem cells of rats and humans. The cells were placed in vitro with silver nanoparticles. The cells were unable to reproduce normally and died from exposure to silver nanoparticles (Lui et al., 2015). This study sparked controversy in the scientific community about whether silver nanoparticles could be used safely.

In a secondary data analysis in 2015, several researchers compiled the results of previous experiments to describe several potential uses for silver nanoparticles, or AgNPs. They found that the silver nanoparticles were effective in killing and inhibiting several types of the most common carie, or cavity, causing bacteria. In addition, the researchers used Transmission Electron Microscopy (TEM) to classify the silver particles by dispersion and size (Correa et al., 2015).

Researchers in 2016 investigated the potential of silver nanoparticles to fight the streptococcus mutans bacteria that forms easily on conventional Hawley orthodontic retainers through a clinical trial. They infused some retainer acrylic plates with silver nanoparticles and some without and then analyzed the impact they had on the amount of colony forming streptococcus units after one and seven weeks. They found that silver nanoparticles were highly effective at reducing the amount of *streptococcus mutans* colony-forming units (Farhadian et al., 2016). Another study in investigated the properties and antimicrobial activity of irreversible hydrocolloid impression materials, which are used to make the castings for Hawley retainers incorporated with silver nanoparticles. Their method involved using the disk diffusion method on 2 conventionally used irreversible hydrocolloid impression materials to determine gel strength, permanent deformation, flow, and gelation time using disk diffusion. They found that silver nanoparticles do not negatively affect the properties of irreversible hydrocolloid impression materials (Ginjupalli et al., 2016). A study by those same authors attempted to find the antimicrobial effect of silver nanoparticles being used as a final endodontic irrigation agent. To find this, they inoculated uni-radicular extracted dental organs with enterococcus faecalis and then irrigated some of them with a dispersion of silver nanoparticles. They found 10 nm silver nanoparticles to be most effective at killing the enterococcus faecalis (Gonzalez-Luna et al., 2016).

Bovine serum albumin and chitosan were coated with AgNPs and tested against streptococcus mutans and its four serotypes. The purpose was to coat organic compounds in silver nanoparticles and then determine if the silver remained just as effective. Ultimately, both of the coated organic compounds demonstrated high inhibition towards the bacteria and the smaller the coated particle, the more potent (Martinez-Robles et al., 2016).

A few years later, a study meant to evaluate the antifungal activity and cytotoxicity of silver containing denture base material containing silver nanoparticles. The denture base material was evaluated based on its cytotoxic ability to kill colony forming candida albicans cells. It was found that the base material containing silver nanoparticles was observed to have antifungal abilities but no cytotoxic activity (Kurt et al., 2017).

Purpose

The purpose of this study is to evaluate the potential of silver nanoparticles to be applied safely and effectively both alone and as part of other materials via killing and preventing the reproduction of *s.mutans* and *c.albicans* in the human mouth. The results of this study provide benefits to the oral health of many Americans, particularly older Americans who are at an increased risk of oral diseases.

Research Questions and Hypotheses

Overarching Question: Are silver nanoparticles and materials which incorporate them safe and effective methods of killing and inhibiting the reproduction of *streptococcus mutans* and *candida albicans* bacterial biofilms in the human mouth?

Silver Nanoparticle Efficacy in Treating Oral Bacterial Biofilms

Question: Are silver nanoparticles and materials which incorporate them effective treatment methods for bacterial biofilms in the human mouth?

Null Hypothesis: Silver nanoparticles and materials which incorporate them are not an effective method of treating oral bacterial biofilms.

Alternate Hypothesis: Silver nanoparticles and materials which incorporate them are an effective method of treating oral bacterial biofilms.

Silver Nanoparticle Safety in Treating Oral Bacterial Biofilms

Question: Are silver nanoparticles and materials which incorporate them safe treatment methods for bacterial biofilms in the human mouth?

Null Hypothesis: Silver nanoparticles and materials which incorporate them are not an safe method of treating oral bacterial biofilms.

Alternate Hypothesis: Silver nanoparticles and materials which incorporate them are a safe method of treating oral bacterial biofilms.

Data Collection Method

The study was done through a systematic literature review to collect data from sources published and peer reviewed between 2005 and the present which relay information on oral and dental applications of silver nanoparticles, toxicity experiments, bactericidal testing against oral bacteria, reports of issues with usage, and other information that proved helpful in relaying well-supported results related to these topics.

Articles were chosen from online databases including the Public Library of Science, EBSCOhost, Google Scholar, and Science Direct. Criteria for the use of an article in this study's systematic review was based on the available data within the article, reputation of the authors, and whether the information within the article fit within the scope of the systematic literature review and would contribute to the focused summary of findings at the end of the review. Keywords for article searches included: silver nanoparticles, efficacy, safety, applications, toxicity, human, *S. mutans*, *C. albicans*, biofilms, denture, denture base liner, and chlorhexidine.

Data points were taken from the articles and used to create data tables and graphs. Data points taken from the articles included concentrations of silver nanoparticles versus concentration of *S. mutans* and *C. albicans*. The analyzed clinical studies were also investigated to determine whether any negative impacts to the humans were noted.

Results

Silver nanoparticle efficacy was determined based on the presence of bacteria after treatment. If colony forming units, or cells that are viable for reproduction, are significantly reduced to the point where biofilms are unable to form, the treatment is considered very effective. Silver nanoparticles were placed at very low concentrations in denture liners with *C. albicans*. The clinical testing was incremented according to silver nanoparticle concentration. The number of colony-forming units after treatment was measured and standard deviations were also calculated (p=0.000122).

Silver Nanoparticles (ppm)	Colony Forming Units of *C. albicans* per mL	Standard Deviation
0 (Control)	1430	220
10	960	160
20	870	40
40	790	40
80	740	60
120	600	70
200	550	70

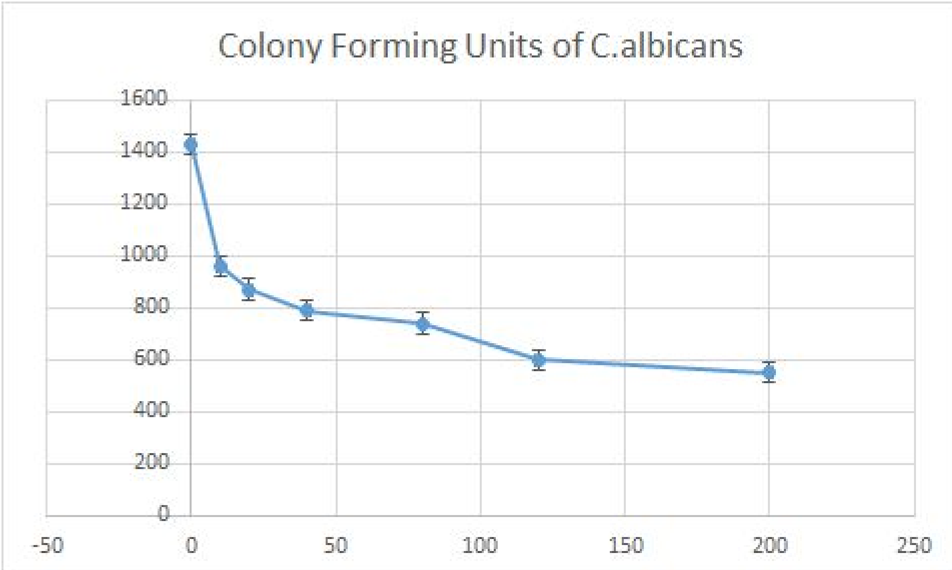

Figure 6: *Clinical testing of silver nanoparticles in a denture liner against C. albicans. Evaluated by silver nanoparticle concentration (p=0.000122).*

The results of clinical testing demonstrate a significant correlation between increased silver nanoparticle concentration and decreased concentration of *C. albicans* (Chladek et al., 2011; Munoz et al., 2014). Silver nanoparticles were only measured in ppm against *C. albicans*. It is estimated that due to their similar properties, *S. mutans* colony forming units would demonstrate similar results although future side by side testing is required to confirm this.

No issues with toxicity wo the human mouth were reported during clinical testing. Participants continued to appear healthy after the testing (Chladek et al., 2011; Munoz et al., 2014). In additional clinical trials, no signs of damage to human cells surrounding the silver-nanoparticle infused materials were found. Clinical trials checked for toxic effects up to the seven week period when the study concluded and no negative effects to human cells or saliva were reported (Farhadian et al., 2015).

Silver nanoparticles were placed into tissue conditioner discs and tested in vitro against both *S. mutans* and *C. albicans*. The concentration of silver nanoparticles was calculated as a percentage of the mass of the tissue conditioner discs. The amount of the two separate bacterial strains was measured after 24 and 72 hours.

Percentage of silver nanoparticles in conditioner	Mean CFU count +/- SD for *S. mutans* at 24hr	Mean CFU count +/- SD for *S. mutans* at 72hr	Mean CFU count +/- SD for *C. albicans* at 24hr	Mean CFU count +/- SD for *C. albicans* at 72hr
0	1.2*10^7+/-(3*10^6)	3.5*10^6+/-(4*10^5)	4.3*10^7+/-(9*10^6)	5.2*10^7+/-(9*10^6)
0.1	3.6*10^3+/-(6*10^2)	5.4*10^2+/-(4*10^2)	2.6*10^5+/-(9*10^4)	5.5*10^4+/-(9*10^3)
0.5	30+/-75	7+/-11	2.2*10^2+/-(3*10^2)	1.2*10^2+/-75
1.0	0	0	10+/-25	20+/-34
2.0	0	0	0	0
3.0	0	0	0	0

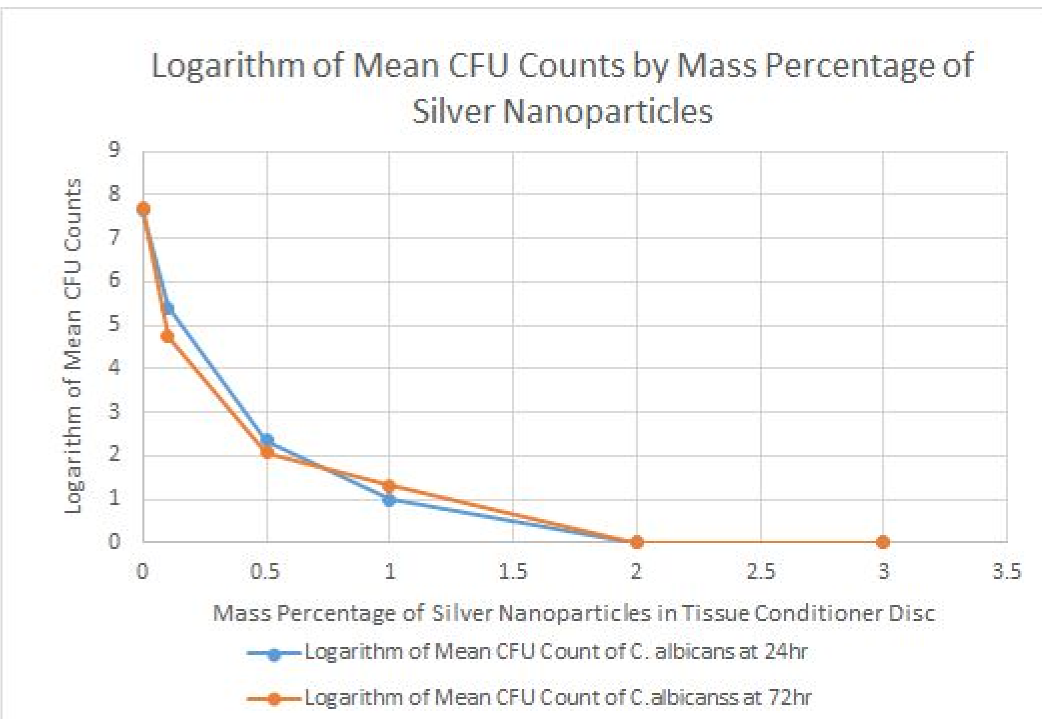

Figure 7: *In vitro testing of silver nanoparticles in tissue conditioner against S. mutans. Evaluated by mass percentage and duration of use.*

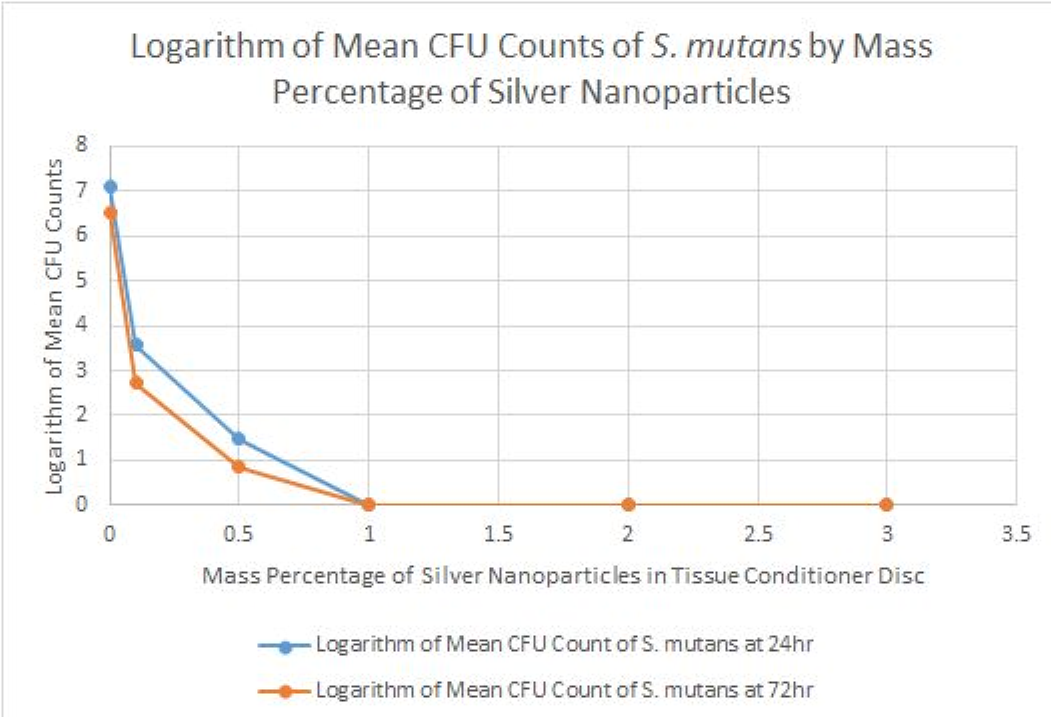

Figure 8: *In vitro testing of silver nanoparticles in tissue conditioner against C. albicans. Evaluated by mass percentage and duration of use.*

Silver nanoparticles maintained their potency after 72 and 24 hours (Nam et al., 2009). This demonstrates that their properties are maintained. Silver nanoparticles were charted against colony forming unit populations of the two bacterial strains by logarithm due to the intense change in bacterial concentration. Results show the efficacy of silver nanoparticles in vitro and applied in tissue conditioner (Nam et al., 2009).

Silver nanoparticles placed into tissue conditioner samples in vitro were much more potent than those in clinical trials due to their much higher relative concentrations. A 0.1 percent by mass tissue conditioner still has a much greater silver nanoparticle concentration than does 200 ppm of silver nanoparticles in a denture liner, with that difference growing even more apparent with 1 percent by mass tissue conditioner discs.

Discussion

Silver nanoparticles are clearly an effective means of fighting biofilms and stopping the reproduction of colony forming units. Silver nanoparticles effectively prevent reproduction of *S. mutans* and *C. albicans*.

In addition, the data demonstrates that silver nanoparticles kill more bacteria when the concentration of silver nanoparticles is increased. When silver nanoparticles were applied in vitro to *C. albicans* and *S. mutans* colonies, *S. mutans* was demonstrated to be slightly more responsive to the presence of the silver for reasons that are not entirely understood.

The bactericidal capacity of silver nanoparticles in a number of settings has been analyzed, demonstrating that silver nanoparticles are effective at killing a number of bacteria native to the human mouth and stopping their growth. The AgNPs(silver nanoparticles) were effective at killing and stopping the reproduction of *S. mutans* and *C. albicans*. With an increased number of silver nanoparticles present in an environment, the bactericidal capacity increases, but with diminishing returns. AgNPs can be successfully used as denture base liner cleaners and as a temporary agent to eliminate biofilms growing in the mouth. There is no conclusive work to demonstrate that AgNPs would not cause harm to the mouth if present alone for an extended period of time. The efficacy of silver nanoparticles was far stronger than that of the traditional treatment method of using chlorhexidine solution to kill bacteria.

In clinical trials, the dental materials infused with silver nanoparticles did not exhibit any negative effects on the human body over the one or seven week period so it can be safely assumed that these materials can be used long term into and past the four month marker for normal usage of retainers.

It should be noted that the silver nanoparticle infused denture liners demonstrated a much weaker bactericidal effect against the colony forming units than did the simple in vitro testing. This can be explained by the fact that the contact of silver nanoparticles on the denture liners was much less with the biofilms than that of the ppm of silver nanoparticles in vitro due to the increased dispersion throughout the in vitro solution compared to the small contact between the denture liner and biofilms in the mouth..

Positive Social Change Implication

This study provides the information necessary to benefit the health of Americans suffering from ora health problems caused by *S. mutans* and *C. albicans* bacterial biofilms. By relaying information on the efficacy and safety of silver nanoparticles as antimicrobial agents, this study informs that silver nanoparticles can be safely applied long term as part of oral devices such as dentures and denture liners. In addition, this study gives a solid foundation for future research into inserting silver nanoparticles alone into the mouth and research into their continued efficacy over time when not used as part of a larger device.

Conclusion

Silver nanoparticles have been demonstrated to be an effective means of killing *s.mutans* and *c.albicans* in the human mouth clinically and in in vitro trials. They have been shown to kill colony-forming units (CFUs) of these two bacterial strains, making them effective at inhibiting reproduction of these bacteria, thus greatly decreasing the creation of oral bacterial biofilms. Silver nanoparticles have been incorporated into removable retainers that were used for clinical trials and successfully prevented the growth of biofilms. While experiments have shown that silver nanoparticles can cause harm to human cells, the harm is negligible given that there were no notable negative impacts in any clinical trials of silver nanoparticle-containing materials.

Further Work

Further research needs to be conducted to determine whether silver nanoparticles can be safely placed in the human mouth alone over the long term . Also, assuming that further work concludes that silver nanoparticles can be safely implanted alone over the long term, work should be done to determine whether their efficacy decreases over time as the particles run short of ions to release through the bacterial membrane.

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References

Kurt, A., Erkose-Genc, G., Uzun, M., Emrence, Z., Ustek, D., & Isik-Ozkol, G. (2017). The antifungal activity and cytotoxicity of silver containing denture base material. *Nigerian Journal of Clinical Practice, 20*(3), 290. doi:10.4103/1119-3077.181362

Corrêa, J. M., Mori, M., Sanches, H. L., Cruz, A. D. D., Poiate, E., & Poiate, I. A. V. P. (2015). Silver nanoparticles in dental biomaterials. *International journal of biomaterials*, *2015*.

González-Luna, P., Martínez-Castañón, G., Zavala-Alonso, N., Patiño-Marin, N., Niño-Martínez, N., Morán-Martínez, J., & Ramírez-González, J. (2016). Bactericide Effect of Silver Nanoparticles as a Final Irrigation Agent in Endodontics on Enterococcus faecalis: An Ex Vivo Study. *Journal of Nanomaterials, 2016*, 1-7. doi:10.1155/2016/7597295

Ginjupalli, K., Alla, R. K., Tellapragada, C., Gupta, L., & Perampalli, N. U. (2016). Antimicrobial activity and properties of irreversible hydrocolloid impression materials incorporated with silver nanoparticles. *The Journal of Prosthetic Dentistry, 115*(6), 722-728. doi:10.1016/j.prosdent.2015.11.006

Farhadian, N., Mashoof, R. U., Khanizadeh, S., Ghaderi, E., Farhadian, M., & Miresmaeili, A. (2016). Streptococcus mutans counts in patients wearing removable retainers with silver nanoparticles vs those wearing conventional retainers: A randomized clinical trial. *American Journal of Orthodontics and Dentofacial Orthopedics, 149*(2), 155-160. doi:10.1016/j.ajodo.2015.07.031

Vazquez-Muñoz, R., Avalos-Borja, M., & Castro-Longoria, E. (2014). Ultrastructural Analysis of Candida albicans When Exposed to Silver Nanoparticles. *PLoS ONE, 9*(10), 1-10. doi:10.1371/journal.pone.0108876

Santos, V. E., Targino, A. G. R., Flores, M. A. P., Pessoa, H. D. L. F., Galembeck, A., & Rosenblatt, A. (2014). Antimicrobial activity of silver nanoparticles in treating dental caries. *Revista da Faculdade de Odontologia-UPF*, *18*(3).

Martínez-Robles, Á. M., Loyola-Rodríguez, J. P., Zavala-Alonso, N. V., Martinez-Martinez, R. E., Ruiz, F., Lara-Castro, R. H., ... & Espinosa-Cristóbal, L. F. (2016). Antimicrobial Properties of Biofunctionalized Silver Nanoparticles on Clinical Isolates of Streptococcus mutans and Its Serotypes. *Nanomaterials*, *6*(7), 136.

Kasraei, S., Sami, L., Hendi, S., AliKhani, M. Y., Rezaei-Soufi, L., & Khamverdi, Z. (2014). Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. *Restorative dentistry & endodontics*, *39*(2), 109-114.

Wu, D., Fan, W., Kishen, A., Gutmann, J. L., & Fan, B. (2014). Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against Enterococcus faecalis Biofilm. *Journal of Endodontics, 40*(2), 285-290. doi:10.1016/j.joen.2013.08.022

Besinis, A., De Peralta, T., & Handy, R. D. (2014). The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays. *Nanotoxicology*, *8*(1), 1-16.

Chladek, G., Kasperski, J., Barszczewska-Rybarek, I., & Żmudzki, J. (2012). Sorption, Solubility, Bond Strength and Hardness of Denture Soft Lining Incorporated with Silver Nanoparticles. *International Journal of Molecular Sciences, 14*(1), 563-574. doi:10.3390/ijms14010563

Chladek, G., Mertas, A., Barszczewska-Rybarek, I., Nalewajek, T., Żmudzki, J., Król, W., & Łukaszczyk, J. (2011). Antifungal Activity of Denture Soft Lining Material Modified by Silver Nanoparticles—A Pilot Study. *International Journal of Molecular Sciences, 12*(12), 4735-4744. doi:10.3390/ijms12074735

Nam, K. Y. (2011). In vitro antimicrobial effect of the tissue conditioner containing silver nanoparticles. *The journal of advanced prosthodontics*, *3*(1), 20-24.

Hiraishi, N., Yiu, C. K., King, N. M., Tagami, J., & Tay, F. R. (2010). Antimicrobial Efficacy of 3.8% Silver Diamine Fluoride and Its Effect on Root Dentin. *Journal of Endodontics, 36*(6), 1026-1029. doi:10.1016/j.joen.2010.02.029

Eke, P.I., Dye, B.A., Wei, L. (2012). Prevalence of Periodontitis in Adults in the United States: 2009 and 2010. *Journal of Dental Research*, vol. 91, no. 10, 2012, pp. 914–920., doi:10.1177/0022034512457373.

Ortman, Jennifer M., Velkoff, Victoria A., Hogan, Howard (2014) "An Aging Nation: The Older Population in the United States."*Current Population Reports*, May 2014, pp. 1–28., www.census.gov/prod/2014pubs/p25-1140.pdf.

Oldenburg, S.J. (2014). Silver Nanoparticles: Properties and Applications. Retrieved from https://www.sigmaaldrich.com/technical-documents/articles/materials-science/nanomaterials/silver-nanoparticles.html

Monteiro, D. R., Silva, S., Negri, M., Gorup, L. F., De Camargo, E. R., Oliveira, R., . . . Henriques, M. (2012). Silver nanoparticles: Influence of stabilizing agent and diameter on antifungal activity against Candida albicans and Candida glabrata biofilms. *Letters in Applied Microbiology, 54*(5), 383-391. doi:10.1111/j.1472-765x.2012.03219.x

Wady, A. F., Machado, A. L., Zucolotto, V., Zamperini, C. A., Berni, E., & Vergani, C. E. (2012). Evaluation of Candida albicans adhesion and biofilm formation on a denture base acrylic resin containing silver nanoparticles. *Journal of Applied Microbiology, 112*(6), 1163-1172. doi:10.1111/j.1365-2672.2012.05293.x

Gum Disease. (n.d.). Retrieved January 15, 2018, from https://www.nidcr.nih.gov/health-info/gum-disease/more-info

Chlorhexidine. (n.d.). Retrieved January 15, 2018, from https://pubchem.ncbi.nlm.nih.gov/compound/chlorhexidine#section=Top

General Medical History of Silver. (2011, March 02). Retrieved February 16, 2018, from https://colloidalsilverinformation.wordpress.com/general-medical-history-of-colloidal-silver/

Liu, F., Mahmood, M., Xu, Y., Watanabe, F., Biris, A. S., Hansen, D. K., . . . Wang, C. (2015). Effects of silver nanoparticles on human and rat embryonic neural stem cells. *Frontiers in Neuroscience, 9*. doi:10.3389/fnins.2015.00115