

Can 2% Hydrogen Peroxide-Silver be an Effective Natural Disinfectant in the Dental Office?

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Abstract

Background and aim: Dentists and healthcare workers are commonly in contact with microorganisms present in blood and saliva which may potentially transfer infectious diseases. Use of methods to remove and or decrease the number of contaminants on instruments and tabletops are of paramount importance. Important properties of a good disinfectant aside from the ability to kill microbes include being safe, noncorrosive or damaging to instruments, odorless, colorless, economical and eco-friendly. This study was done to assess effectiveness and longevity of 2% hydrogen peroxide-silver for disinfection.

Materials and methods: Using sterilized physiological serum a spore suspension of *Bacillus subtilis* of 1×10^8 cfu/ml was prepared on McFarland standard media. A sterilized capped tube was used for preparation of the suspension; 2 ml of a newly opened bottle of 2% hydrogen peroxide silver was poured in the test tube using a sterilized pipette and the tube was recapped. Then, 1 ml of the spore suspension was added to the test tube (test group). On the first day eight cultures were taken (at 30', 60', 120', 180', 240', 300', 360' and 420') and repeated twice each time. Also the same number of cultures was taken from the suspension at all the aforementioned time points without addition of hydrogen peroxide silver (control group). Then the samples were transferred to the culture medium after the required time points elapsed. All cultures were placed in the incubator for 24 hours at 37°C and assessed for growth. This procedure was the repeated using the same batch of 2% hydrogen peroxide silver until it was no longer effective on the organism.

Results: The batch of 2% hydrogen peroxide silver re-opened daily was effective on *Bacillus subtilis* up to 4 days of repeated daily use after contact for 180 min. There was no effect on day 4. All controls showed viability and growth of *Bacillus subtilis*.

Conclusion: Instruments should be soaked in 2% hydrogen peroxide –silver for at least 3 hours before being sterilized in an autoclave; 2% hydrogen peroxide silver will not disinfect after 3 days of use. A fresh bottle should be used daily for greater guarantee of disinfection.

Keywords: 2% Hydrogen peroxide-silver; *Bacillus Subtilis*; Disinfection

Introduction

One of the concerns among healthcare providers (HCP) is daily contact with microorganisms in blood and saliva of patients. Continuous contact with micro-organisms has considerably increased the incidence of specific infectious diseases among HCP as compared to the normal average of the society [1]. A study revealed that 14 to 28 percent of general dentists, 13 percent of their assistants and 17 percent of their staff have been exposed to Hepatitis B [2] and annually more than 200 HCP in the USA die from immediate and or chronic effects of infection by Hepatitis B virus from their work environment [3]. Blood and saliva contain considerable types of viruses, bacteria and other pathogens that may cause diseases such as flu, fever blisters, pneumonia, tuberculosis, hepatitis and AIDS. These issues indicate the importance of infection control methods in HCP [1,4]. Applying a method that removes and or decreases the number of microorganisms on reusable instruments before autoclaving is very useful in preventing cross infection because in many offices reusable instruments are still hand-washed before processing [1]. The reusable instruments with possibility of being disinfected and sterilized should be disinfected and then sterilized to remove the microbes and spores [5-7]. We found no study available regarding effective-time span of Nano-Silver disinfectant which is an H₂O₂-based solution with silver ions (Nano-trade Company, Czech Republic). So, we decided to study the effect

of this solution on spores of *Bacillus subtilis* to see if 2% hydrogen peroxide silver can be an effective disinfectant in the dental setting. The null hypothesis was that NANOSIL is not an effective disinfectant for the dental setting.

Material and Methods

Nano-Silver disinfectant an H₂O₂-based solution (Nano-trade Company, Czech Republic) was used in this study. *Bacillus subtilis* (BS) spores ATCC6638 KD were purchased as spores with culture medium (Browne co, UK, certification by FDA and biologic test standards number 11138). A spore suspension with normal opacity including 1×10^8 CFU/ML was prepared by using sterilized physiological serum according to McFarland 0.5 standard. Then a sterilized sealed

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tube was used for preparing the suspensions; 2 ml of a newly opened bottle of 2% Nano-Silver disinfectant liquid was poured in the tube by sterilized sampler and then the Nano-Silver bottle was recapped. Then 1 ml of the spore suspension was prepared with the same standard opacity and added to the tube.

On the first day, samples were taken from the tube after 30', 60', 120', 180', 240', 300', 360', and 420'(minutes).

On day two, 15 samples were taken from the tubes containing Nano-Silver (from the bottle opened the day before) added to BS and incubated for 24 h.

On day three, 9 samples were taken from the tubes containing Nano-Silver (from the bottle opened the day before) added to BS and incubated for 24 h.

On day four, 6 samples were taken from the tubes containing Nano-Silver (from the bottle opened the day before) added to BS; and incubated on agar for 24 h.

For sampling, a standard loop was flamed for 10 seconds and then the samples were prepared after the loop cooled. Each culture sample was repeated twice. A sample from the suspension was taken at each of the aforementioned time-points and then transferred to the nutrient agar (NA) immediately (without contact with Nano-Silver) for controls. Culture was done using 1 cc of the spore-contaminated solution added to nutrient agar using linear technique. Then the cultures were placed in the incubator for 24 hours at 37 degrees Centigrade. Along with each series, a NA plate was placed inside the incubator and when no growth was observed, agar sterility was assured. On day two, the procedure was repeated in the same fashion except that samples were taken from the tube starting at the time it took Nano-Silver to kill the spores on day one. This procedure was repeated for 5 days,

Results

On the first day, in the 24 samples taken from the tubes containing Nano-silver and bs, no growth was seen on agar in the samples which had 180' of contact or more.

On day two, in the 15 samples taken from the tubes containing Nano-silver (from the bottle opened the day before) added to bs no growth was seen on agar in the samples which had 180' of contact or more.

On day three, in the 9 samples taken from the tubes containing Nano-silver (from the bottle opened the day before) added to bs no growth was seen on agar in the samples which had 180' of contact.

On day four, in the 6 samples taken from the tubes containing Nano-silver (from the bottle opened the day before) added to bs for 180', growth was seen on agar 24 h later. Growth was observed in all control samples indicating the viability of BS. The growth of the control spores showed that the spores were viable; the case spores were matched with controls showed no- growth up to the fourth day. In other words, samples taken on the fourth day showed growth in all samples after 180 minutes of contact with the solution.

Discussion

Applying methods that remove and or decrease the number of microorganisms on reusable instruments before autoclaving is very useful in preventing cross infection because in many offices reusable instruments are still hand-washed before processing [1]. The reusable instruments with possibility of being disinfected and sterilized should

be disinfected and then sterilized to remove the microbes and spores. This spore is the most resistant form of the microbe. Bacterial spores are among the most resistant of all living cells to biocides [8].

Although some studies have assessed similar solutions [9-16], we found no study available regarding shelf-life of Nano-Silver disinfectant which is an H₂O₂-based solution with silver ions (Nano-trade Company, Czech Republic) [7]. So, we decided to study the effect of this solution on spores of *Bacillus subtilis* to see if 2% hydrogen peroxide silver can be an effective disinfectant in the dental office. This solution is for disinfection and not for sterilization. We sought to assess the shelf life of this new solution. To assess how long it can be used comparing with the other solution in the market. Because this solution is natural with no color and does not ruin the dental equipment. It is noncorrosive and is odorless contrary to sodium hypochlorite. It does not stain instruments. However it is very volatile. Every day new brands of disinfectants with different properties enter the market; consumers have no idea about their real effectiveness or properties compared to the previous products. Nano-Silver disinfectant an H₂O₂-based solution with silver ions is a new product for use in dentistry. Important properties of a good disinfectant aside from the ability to kill microbes includes being safe, noncorrosive, should not cause discoloration or damage to instruments and being economical and eco-friendly. This study was done to assess effectiveness and longevity of 2% hydrogen peroxide-silver for disinfection. Nano silver is a newly marketed product and the producer claims it has a high level of disinfection without mentioning its shelf life. As Nano-Silver disinfectant is an H₂O₂-based solution its effectiveness is based on releasing oxygen and thus short-lived. Silver has strong antibacterial effects. Silver does not let bacteria thrive in the environment and reaction with SH group creates oxidative enzymes. Silver is known to exhibit a strong toxicity to a wide range of micro-organisms. Bactericidal effect of silver ions on micro-organisms is well known, but the mechanism is only partially understood [17]. A proposed theory describes that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them and has extensive use in many bactericidal applications [17]

The cultures were taken at 30' to 420' on day 1 because the exact time of effectiveness on the BS spore was unknown. Lack of growth of BS spores after contact with Nano-Silver on day one was 180'. As this time period of three hours is a long wait for instrument disinfection this is one of the limitations of this product; additionally, as the disinfection is ineffective after 3 days a new bottle must be opened daily to play it safe.

Conclusion

Instruments should be soaked in 2% hydrogen peroxide-silver for at least 3 hours before being sterilized in an autoclave; 2% hydrogen peroxide silver will not disinfect after 4 days of use. Thus this refutes the null hypothesis provided a fresh bottle is used daily for greater guarantee of disinfection in a shorter time period.

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