Using Silver Nanoparticles to Combat Harmful Bacteria

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Abstract - The consistent and unnecessary misprescription and overuse of antibiotic drugs has led to an increasing incidence of antibiotic resistance in bacteria and other microbes, which can diminish the efficacy of these treatments in cases ranging from common infections to life versus death situations. This dilemma has given rise to the development of new methods of antibacterial treatment, including the use of silver nanoparticles to kill or inhibit reproduction of bacteria in the body. Silver nanoparticles can serve as an effective remedy to the problem of antibiotic resistance in bacteria.

The antibacterial effects of silver have been known since antiquity, with usage gradually evolving over time. However, researchers have recently discovered that by scaling the size of individual silver particles down to the nanoscale—a size comparable to that of biological units—the silver’s antibacterial effects rise significantly due to an increased surface area to mass ratio.

Once introduced to bacteria, silver nanoparticles bind to the bacterial membranes and silver ions infiltrate the cell, inhibiting essential protein synthesis and cell reproduction. One of the main advantages of silver nanoparticle treatment is the difficulty microbes have developing resistance to it. This is extremely important, as the main problem in repeated use of antibiotics, such as penicillin, is that bacterial colonies eventually become resistant to these treatments, causing them to no longer be effective.

Key Words - Antibiotic Resistance, Bacteria, Chemical Engineering, Nanotechnology, Misprescription, Silver Ions, Silver Nanoparticles.

An Overview of Antibiotic Resistance in Bacteria (1)

The Problems of Inappropriate Antibiotic Use (1A)

Perhaps one of the greatest breakthroughs in medical history was Sir Alexander Fleming’s 1928 discovery of the Penicillin molecule, the first natural and widely-used antibiotic. Penicillin is derived from penicillium mold, which secretes an antibiotic substance to kill any threatening bacteria. Fleming and the chemists that he commissioned were able to isolate the specific bactericidal molecule, allowing it to be utilized and mass produced by the 1940’s [1].

Antibiotics have saved countless lives since their discovery, essentially eliminating one of the most prevalent causes of human mortality in history: death from infection. The discovery, however, held more latent consequences which would not reveal themselves until many years later.

One of the most pressing concerns facing the modern scientific community is the problem of growing antibiotic resistance in bacteria caused by persistent, inappropriate use of antibiotics [2]. A species of microbe becomes resistant to a particular antibiotic drug when the drug is administered to a patient, usually in insufficient doses or for too short a period of time. This causes the majority, but not all, of bacteria to die, leaving some able to resist the treatment and pass on their resistant genes to the next generation of bacteria [3]. This gradually developed resistance is a problem, as it eventually makes some types of antibacterial treatments ineffective, forcing doctors to prescribe heavier dosages for longer periods of time, which will not only drive up expense for the patient, but also allow the microbes to develop further resistance. Once these drugs are no longer effective to combat certain infections, scientists are forced to go through the long and extremely expensive process of developing new antibiotics—antibiotics which will face the same threat of developed resistance.

All resistant bacterial strains begin in a single host, in whom a resistant bacterium or resistant bacteria have survived an antibiotic treatment. This perceived isolation—starting with a single host—causes many patients to turn a blind eye to the problem, assuming it will not affect them, since they personally use antibiotics correctly. However, the bacteria are not quarantined to one host; in as little as a cough or even touch, the resistant bacteria can spread to new hosts or remain in wait, on inanimate objects, to infect a host at a later time [4]. This situation can turn a once trivial problem into a worldwide one as seen in one of the Center for Disease Control and Prevention’s brochures— “Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these [antibiotic resistant] infections” [3]. Lee Ventola expresses the gravity of this situation, stating, “Methicillin-Resistant Staphylococcus Aureus (MRSA) [a type of resistant bacteria] kills more Americans each year than HIV/AIDS, Parkinson’s disease, emphysema, and homicide combined” [2]. What once was a miracle drug, is now turning into an obsolete technology; we
must act quickly in order to preserve the efficacy of one of medicine’s greatest discoveries.

The main setting in which this problem manifests itself is hospitals. With the constant turnover of patients, each having any number of diseases or infections, bacteria often remain in hospitals, lying in wait for a new host. Even with hospitals’ stringent disinfection processes, some bacteria survive, becoming the start of super-resistant strains of bacteria. Patients, whose immune systems are already compromised due to treatments or preexisting illnesses, are easily infected by these strains with sometimes fatal results, since the bacteria in question are resistant to even the most heavy-duty antibiotics [5]. These fears of super-resistant bacteria materialized in May of 2016 when doctors diagnosed the first person in the US with an E. coli infection that was resistant to colistin—an extremely powerful, last-resort drug [6]. In early 2017, our fears were further solidified when a woman died from an infection caused by a strain of bacteria found to be resistant to all twenty-six available antibiotics in the United States [7]. With the very real possibility of pan-resistant bacterial strains—strains resistant to every known antibiotic—becoming widespread, we are now in a race to develop a solution or alternative treatment.

The problem of resistance poses a serious threat to humanity’s future, as one of the world’s most important medicines is on the road to becoming obsolete. This could reverse almost a century of progress, leaving no way to treat what we currently view as easily preventable infections. It is urgent that society makes a serious effort to find a solution to the threat of antibiotic resistant bacteria.

**INAPPROPRIATE USE OF ANTIBIOTICS—MISPRESRIPTION, OVERUSE, AND MISUSE (1B)**

The three main catalysts for this spreading problem of antibiotic resistance are misprescription, overuse, and misuse. Although these problems manifest themselves in different ways, they all lead to the same result: developed antibiotic resistance in bacterial species.

Misprescription is the medical prescription of antibiotics to patients who do not necessarily need them. Doctors will often unnecessarily prescribe antibiotics in order to please patients and speed up healing processes. In a study conducted by the *Journal of the American Medical Association*, researchers found that approximately thirty percent of antibiotic prescriptions in the United States are unnecessary [8]. This is not to say that the antibiotics did not help in treating the patients’ illnesses, but many patients would have healed by simply allowing their immune systems to combat the bacteria. For example, antibiotics may be given to patients to treat viruses, such as the flu, which will not respond to the treatment. While the administration of antibiotics typically expedites recovery by making the patient less susceptible to bacterial infection during the illness, it has little to no effect on the main virus causing the patient’s symptoms. Instead, these prescriptions give any bacterial strains present the opportunity to encounter the antibiotics and develop resistance. Since the bacterial strains are not specifically targeted with a tailored, lethal prescription, they will often survive and develop resistance. In countries where antibiotics are available over-the-counter, the problem of “self-misprescription” also arises; someone with a common viral cold may unnecessarily take antibiotics, opening doors to the development of antibiotic resistance.

The next threat to the future efficacy of antibiotics is their overuse—widespread, unchecked use of antibiotics—including problems with over-the-counter and broad-spectrum antibiotics. Over-the-counter antibiotics essentially allow people to “self-prescribe” antibiotics. With a lack of intense medical training, many people will “self-prescribe” the antibiotics too often, for the wrong amount of time, or in the wrong dosage. This inappropriate prescription of antibiotics gives bacteria many opportunities to develop resistance. Additionally, broad-spectrum antibiotics are often given to animals, such as poultry or livestock, in small quantities to make the animals grow faster [9]. Although this saves money by increasing the rate of food production, this practice has global health consequences which must be taken into account. Broad-spectrum antibiotics are intended to work for a wide range of treatments, but have a higher likelihood of creating resistance due to widespread use. Consequently, narrow-spectrum antibiotics are preferred because they target more specific pathogens, decreasing their frequency of use and thereby decreasing the chances of developed resistance. All of these cases of overuse encourage the development of antibiotic resistance by increasing how frequently bacterial strains encounter the antibiotics—similar to the problem of misprescription.

A third catalyst for creating bacterial resistance to antibiotics is misuse or failure to follow the necessary prescription. Antibiotics must be taken at a certain dosage for a certain amount of time in order to be effective. However, patients often halt medication once they begin to “feel better.” This failure to completely see the treatment through allows the “injured, but not killed” bacterial strains to survive. By failing to ensure the complete death of the strain, resistant stragglers may pass on their resistant genes to the next generation of bacteria. Over time the injured strain will regrow into a new, resistant strain of bacteria. The bacteria can also survive if the dosage given is lower than the effective dose. The less concentrated drug may affect the bacteria, but not enough to kill them, or it may not affect the bacteria at all. This problem is the same as the first; the treatment wounds the bacterial strains but does not completely kill them, allowing them to regrow stronger than before.

These three problems provide avenues for bacterial strains to develop antibiotics resistance through the mechanisms that will be discussed in the following section.
HOW BACTERIA DEVELOP RESISTANCE (1C)

The general method by which bacteria develop resistance is relatively simple. The process begins with the reproduction of bacteria. Every time a bacterium reproduces, there is a small possibility of a genetic mutation in the new bacterium’s DNA. Even though genetic mutations are only ten times more likely to occur in the DNA of E. coli than in that of humans, the short life cycle and high reproductive rate of bacteria amplifies this number significantly [10,11]. In many cases, these genetic mutations are deleterious to the bacterium’s health, but in some cases, they may benefit the bacterium giving them antibiotic resistant genes (AR genes) [12]. Bacteria possessing these AR genes exhibit antibiotic resistance; after antibiotics are administered to a colony of bacteria, the vast majority of individual organisms are targeted and killed. The AR gene possessing bacteria, however, are naturally resistant to the antibiotics, which allows them to survive.

The problem of lingering resistant bacteria is typically solved by subsequent administrations of the antibiotic, or by using different types of antibiotics synergistically. However, patients sometimes fail to finish the antibiotics prescribed to them once they begin to heal, or the dosage prescribed by the doctor is simply too small, which allows some bacteria to survive and pass on their AR genes to subsequent generations. Even worse, some countries fail to regulate the antibiotic market, allowing citizens to buy antibiotics over-the-counter without a prescription, essentially self-medicating even when it is unnecessary [1]. This can lead to artificial selection of even more resistant bacteria, worsening the problem.

Once a bacterium has an AR gene, there are three main biological mechanisms which allow the bacterium to pass it on to others. The first mechanism, with which people are most familiar, is the same one that allows humans to pass on their genes to one another: reproduction. Bacteria perform asexual reproduction, during which one cell splits into two exact copies of the original cell, duplicating the original DNA—containing the mutation—into both new cells. The second method is through bacterial conjugation, wherein bacteria in direct contact with one another can transfer genetic material back and forth. Bacteria have rings of DNA called plasmids, which can be copied and given to other bacteria to transfer genetic material. During bacterial conjugation, one bacterium extends a tube-like appendage to another, pulling the neighboring bacterium into contact with itself and allowing it to directly transfer DNA plasmids to the recipient [13]. The plasmids are extremely important to bacterial survival, as they allow a colony to quickly adapt to any environmental changes or threats without going through the process of reproduction. The third way that bacteria can pass their resistive genes on to others is through transformation. During the process of cell transformation, a cell with desirable DNA dies via cell lysis; the cell membrane is broken down and a fluid known as lysate is released into the surrounding environment. Lysate contains the contents of the dead cell, including any DNA which may be useful to other cells. Through transformation, living cells can uptake this genetic material, incorporating it into their own genetic codes [14]. This helps the colony develop resistance, as it gives the bacteria yet another means to pass their resistant genes on to others.

The development of resistance can occur through multiple mechanisms and can happen in isolated populations as well as throughout entire bacterial species. Society must make a conscious effort to reduce or eliminate these problems of antibiotic abuse; however, even if these problems are completely eradicated, existing resistance will still remain and must be dealt with through alternative methods.

THE FLAWS OF PREVIOUSLY EXPLORED SOLUTIONS (2)

Many proposed solutions to the problem of antibiotic resistance do not suggest the development of new treatments, but rather a change in the current practices of antibiotic use. As misprescription is one of the main contributing factors to the development of antibiotic resistance in bacteria, many scientists propose that we strictly regulate the prescription of antibiotics, especially discontinuing the availability of over-the-counter antibiotics and broad-spectrum antibiotics. However, this action will not solve the problem of antibiotic resistance on its own; it can only slow its progress. In the past, the main solution to the emergence of antibiotic resistance has been to develop new antibiotics or to alter old ones to give them different or stronger antibiotic properties. However, this is like fighting a losing battle as it is very costly and time consuming to constantly develop new antibiotics. Lee Ventola, MS, a medical writer, expresses in her paper, The Antibiotic Resistance Crisis Part 2: Management Strategies and New Agents, this dilemma, “although 20 new classes of antibiotics derived from natural substances were identified from 1940 to 1980, this pace could not be sustained” [15]. An additional problem with this approach is that the similar base structure of the developed antibiotics allows the bacteria, who are already resistant to the old drugs—those with the parent structure—to quickly gain resistance to the new drugs. It takes roughly two years for a strain of bacteria to become resistant to a new antibiotic and on average it takes twelve years for a new drug to be developed and released [16,17]. The time deficit between the development of a new drug and the development of resistance to that drug highlights the need for new approaches to countering antibiotic resistance.
thinner membrane supplemented by a thin yet strong “cell wall”. Because the two types of cells have extremely different cell membranes, the permeability of these membranes also differs greatly; gram-negative membranes are generally much more difficult to penetrate. As many gram-negative bacteria have never seen gram-positive treatments—since they cannot normally enter their cells—they will have not yet developed resistance to them; this allows the gram-positive antibiotics to easily kill the gram-negative bacteria, if they can breach the tough bacterial defenses. However, both of these solutions have the same problems—the bacteria can simply reduce their membrane permeability or develop resistance to these treatments in the same way they develop resistance to the original treatments.

Maybe one of the most promising solutions yet is the idea of pathogen specific antibiotics. These super-regulated antibiotics would not be used nearly as widely as today’s antibiotics. This would deny the bacteria many opportunities to develop resistance to these antibiotics, giving them only small windows of time during the few treatments. However, the main downfall of this solution is the cost and time required. In addition, it is nearly impossible to create an antibiotic for every strain of bacteria; even the meager 30,000 species of bacteria we have isolated in pure culture and named is a staggering number [21]. Besides the prospect of designing each of these new antibiotics, the cost for such an endeavor would be monumental.

Due to the high costs, extensive development times, and the lack of sustainability in the previously explored solutions, we are left to continue the search for a new solution to this widespread problem; to do so, scientists are revisiting a solution that has been around for millennia.

**SILVER AS AN ANTIBIOTIC (3)**

### ANTIBACTERIAL PROPERTIES OF SILVER (3A)

The antimicrobial properties of silver have been known since antiquity, when the ancient Romans first invented “silverware” by using silver containers to store water. They noticed that by putting water in silver containers, it became safer to drink compared to water stored by other means, especially for long periods of time. This discovery transcended throughout history, with wealthy individuals from every society utilizing silver’s antimicrobial effects in their dining utensils. Silver was first used widely in medicine during World War I, where it was put in wound dressings to combat infection. In contemporary medicine, silver is also commonly used to fight infections, similarly implemented in wound dressings as well as coatings on medical devices such as catheters. Silver is also woven into fabrics to improve hygiene by inhibiting bacterial growth [22]. The main property of silver that makes it so effective at combating bacteria is its tendency to ionize in solution.

Additionally, The Pew Charitable Trusts points out that no new antibiotics have been discovered in the past 30 years [18]. This dry spell has prompted scientists and engineers to look for new solutions to the problem. One possible solution is the use of beta-lactamase inhibitors. These are additional molecules administered along with the penicillin-class (beta-lactam) antibiotics that inhibit the function of the bacterial enzymes—beta-lactamases—which break down the antibiotic. These inhibitor molecules “distract” the beta-lactamases, occupying them and leaving the antibiotics free to kill the bacterial cells before the enzymes dismantle the antibiotics. However, one of the major downsides of this proposed solution is that in order for a beta-lactamase inhibitor to bind to the enzyme’s active site to inhibit it, it must resemble the original target molecule. Thus, many beta-lactamase inhibitors are beta-lactams themselves; this allows the bacterial cells to eventually develop resistance to the inhibitors as well [19].

Other explored options include combating the various defenses of the bacterial cells—membrane permeability, enzymes, and efflux pumps. One method uses “decoy” molecules to trick efflux pumps (proteins in the cell membrane that pump unwelcome material out of the cell) into pumping the wrong thing—the “decoys”—out of the cell [20]. These “decoy” molecules are usually fragments of the actual antibiotic. The efflux pump will bind to these pieces thinking they are the actual antibiotic and pump them out. This gives the actual antibiotic time to destroy the bacterium. Another solution is to treat the resistant bacteria with gram-positive treatments. While gram-positive bacteria have a thicker but more flexible cell membrane, gram-negative bacteria have a

**FIGURE 1 [18]**

*Shows the number of new antibiotics discovered during each decade*

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Silver will begin to dissolve and ionize when exposed to water, bodily fluids, or organic tissue. The ions given off by silver are known to have a relatively low toxicity to human cells while adversely affecting bacteria and fungi. By interacting with bacterial cell membranes, the silver is able to inhibit reproduction of harmful bacteria [23]. The ions deposit themselves into the cell walls and vacuoles of bacteria, damaging cell structures including the cell envelope, cytoplasmic membrane, and the membrane’s contents. Once inside the cell, silver ions bind to DNA and RNA molecules, causing them to condense. This makes it more difficult for ribosomes to transcribe or read the DNA and RNA, a process necessary to protein synthesis and cell division [24]. Because of these properties, silver is a practical antimicrobial agent which has been utilized for millennia, and will likely continue to be developed and utilized in years to come.

ENHANCING SILVER’S ANTIBIOTIC POTENTIAL USING NANO TECHNOLOGY (3B)

By decreasing the size of a silver particle down to the nanoscale, not only does biocompatibility increase because of smaller size, but its surface chemistry is also changed drastically, unlocking a myriad of properties which previously could not be utilized [25]. In a June 2015 study of silver nanoparticles’ effect on bacteria, Swiss chemists Benjamin Le Ouay and Francesco Stellacci found that the particles’ surface properties “influence both physical (aggregation, affinity for bacterial membrane, etc.) and chemical (dissolution, passivation, etc.) phenomena.” [26]. By decreasing aggregation, or the clumping together of particles, the surface area to mass ratio of the silver is increased. This allows for easier and faster dissolution of silver ions into the surrounding solution, and consequently a greater degree of reactivity. Since these ions are the active inhibitor of bacterial growth and activity, it is essential that as many of these be released as possible in order to have the highest efficacy. Additionally, large silver particles can be less effective as an antibacterial due to the high likelihood of interference from salts. However, using particles of silver on the nanoscale can overcome these limitations [25].

Aggregation is also detrimental to silver’s use as an antibiotic because it can cause the particles to precipitate out of their colloidal form, creating large chunks of silver solute rather than an evenly distributed colloid [26]. Silver nanoparticles are advantageous compared to other forms of silver because they can be deposited in thin films on the surfaces of medical devices. This helps preserve the sterility of an instrument by preventing bacteria and other microbes from growing on its surface [27]. By keeping medical devices as sterile as possible, the risk of infection following a procedure is reduced greatly.

Silver nanoparticles are particularly useful due to their high degree of ionization. It is the ions not the actual silver particles that provide the antibacterial properties; the nanoparticles simply provide an exceptional source of the ions [28]. Le Ouay and Stellacci state that, “One nanoparticle immobilized close to a bacterium (or even internalized) is able to release several tens of thousands of silver atoms in its vicinity, producing a locally high concentration of antibacterial ions;” this is known as the Trojan horse effect [26]. When introduced to water, a polar molecule, the crystalline silver particles will dissociate into individual silver cations, or positively-charged ions. These cations then congregate on a bacterium’s cell membrane due to the matching properties of their surface chemistries, while the negatively-charged silver nanoparticles rip holes in the membrane due to their high reactivity. By binding to a bacterium’s membrane, the nanoparticles will make additional perforations in it, allowing the cell’s contents to flow out into the extracellular fluid, killing the bacterium. The chemical processes by which this occurs will be discussed further in section 4A. In addition, silver cations can infiltrate the cell through proteins in the cell membrane. The ions subsequently act on the cell’s DNA and RNA in a way which makes it more difficult for the cell to synthesize proteins necessary for reproduction and survival. The specific biological implications which allow this to occur will be discussed in section 4B. Section 4C will discuss an interesting effect—the “zombie effect”—which leads to a higher efficacy of silver nanoparticle treatment. Additionally, it is known that gold nanoparticles showed no effect when tested against a variety of bacteria, signifying that it is specifically silver nanoparticles that inhibit bacterial growth [25].

SURFACE INTERACTIONS (4A)

The exact method by which silver nanoparticles interact with the membranes of bacteria is not known, however, there are many speculations for possible mechanisms. Silver gains charge, when reduced to the nanoscale, which plays an integral part in the destruction of the bacterial cell membrane.

FIGURE 2 [27]
Shows different sizes of silver nanoparticles (20 nm, 60nm, 100nm diameters)

THE MECHANISMS OF SILVER NANOPARTICLES (4)
One method through which silver nanoparticles may destroy cell membranes is the negative charge they possess [25]. This negative charge is called a free radical—an unpaired valence electron. Although free radicals are suspected to have carcinogenic effects, the low dosage of silver required alleviates this concern in silver nanoparticle treatment [26]. This will be further discussed in section 7 on the advantages and disadvantages of silver nanoparticle treatment. In a study, Kim et al. showed that the addition of negatively-charged silver nanoparticles to an agar plate of E. coli resulted in the inhibited growth of the bacteria. When NAC—a compound which reduces free radicals—was added, the inhibitory effect of the silver ions was significantly decreased as seen in Figure 3. When NAC alone was added to the agar plate there was no change in growth inhibition; this implies that the decreased efficacy of silver nanoparticles when NAC is added is solely due to the reduction of free radicals and is not intrinsic of the NAC itself [25].

![Graph showing the effect that NAC has on silver’s antimicrobial properties](image)

**FIGURE 3 [25]**

*Shows the effect that of NAC has on silver’s antimicrobial properties*

The actual means by which the free radicals interfere with the cell membrane is yet unknown, but it is most likely due to the property of free radicals to be extremely reactive. A stable valence shell usually means there are eight electrons in the outermost shell of orbitals for an atom. In an atom with a free radical, this outer shell only has one electron. The easiest way for that atom to reach a stable state is for it to lose the electron through bonding. This is most likely what happens with negatively-charged silver nanoparticles. Their extremely reactive free radical takes any chance it gets to bind, ripping apart anything it needs to, including the bacterial membranes. Pits and pores, in turn, form in the membrane, leading to cell death.

The now pitted and perforated membrane leaves the bacterium open to a quick death. It is no longer silver that causes the death of the bacterium, but death by simple diffusion. The cytoplasm of the punctured bacterium rushes out into the extracellular fluid bringing with it key intracellular components of the bacterium. The lysed bacterium soon dies.

Additionally, Kim et al. tested the effects of silver nanoparticles on both E. coli, a gram-negative strain of bacteria, and S. aureus, a gram-positive strain of bacteria. The silver nanoparticles showed a much lower efficacy rate when used in the agar plates containing S. aureus. They concluded that this may be linked to the difference in membrane structure, as gram-positive bacteria lack the peptidoglycan layer that the gram-negative bacteria possess; however, they decided more research would need to be conducted [25]. If this is true, that silver nanoparticle efficacy is linked to the presence of a peptidoglycan layer in the cell membrane, silver nanoparticles may be an even better solution than previously thought. We will discuss this concept further in section 7A on the advantages of silver nanoparticle treatment.

**INTRACELLULAR EFFECTS (4B)**

Apart from the effect on the cell membrane, silver nanoparticles can wreak havoc inside of the bacterial cells as well. When the nanosilver breaks down it also forms Ag⁺ ions which exhibit different mechanisms than the negatively-charged silver nanoparticles. In a study by Le Ouay and Stellacci, they explain this effect saying that Ag⁺ ions bind to the negatively-charged components inside of the cell [26]. DNA, RNA, and various peptides—linked amino acids—are all negatively-charged. When the Ag⁺ ions bind with the peptides inside of the bacteria, they inhibit their function. This limited function stops the bacteria from carrying out cellular functions necessary for survival. This lack of cellular function eventually leads to cell death because of the deficiency of necessary cell products and the neglect of important tasks such as communication within the cell.

In addition to stopping the function of proteins, Ag⁺ ions stop the synthesis of proteins as well. The presence of Ag⁺ ions inside the cell causes the negatively-charged bacterial DNA and RNA to condense due to binding with the positively-charged cations. This is extremely detrimental to the bacterium as its genetic code is now essentially “locked”. The affected bacterium can no longer copy the necessary DNA to synthesize vital proteins and, even if the DNA is copied, the RNA is also affected by the Ag⁺ ions. This results in RNA that cannot cleanly synthesize the necessary proteins. In addition, this condensed DNA results in a “complete blockage of the S phase [of bacterial replication]” says Park in *Toxicology in Vitro*’s article “Silver nanoparticles induce cytotoxicity by Trojan-horse type mechanism” [29]. In the bacterial cell cycle, the bacterium starts by growing in size. Following this, it goes through the S phase, or synthesis phase, where it copies its DNA so it can give the new copies to its daughter cells. Finally, it goes through binary fission where it pinches apart into two new bacteria cells [30]. However, when
Ag⁺ is present and the DNA condenses, the bacterium cannot copy its DNA. This halts the cell cycle, preventing the bacterium from reproducing. The short life cycle of bacteria and this inability to create a new generation on top of the lack of necessary proteins, leads to a short-lived colony of infertile and incapable bacteria that will quickly die off. The indiscriminate binding of Ag⁺ ions to anything negatively-charged inside of the bacterial cell leads to a quick death of the bacteria colony.

**THE “ZOMBIE” EFFECT—POSTMORTEM DOUBLE AGENTS (4C)**

An interesting side effect of killing bacteria with silver nanoparticles is the so called “zombie effect” [31]. Once the bacteria die, they absorb Ag⁺ particles and slowly release them later as the external Ag⁺ concentration changes. This can lead to dead bacteria cells killing healthy bacteria in their vicinity. This observed effect can boost the efficacy of silver nanoparticle treatment further by allowing the bacteria-killing Ag⁺ ions to remain in the body for longer, giving them more time to interact with the bacteria. These silver ions in the dead “zombie” bacteria can be seen in Figure 4 from *Science Magazine’s* article “Silver turns bacteria into deadly zombies” [32].

**FIGURE 4 [32]**
Shows silver ions (white specks) inside dead bacterial cell

**MANUFACTURING SILVER NANOPARTICLES (5)**

Silver nanoparticles are formed by mixing a 1:3 ratio of 1.0 x 10⁻³ M Silver Nitrate (AgNO₃) and 2.0 x10⁻³ M Sodium Borohydride (NaBH₄), each in aqueous solutions of triply-distilled water. As a consequence of this reaction, dissociated silver ions cluster together to form a translucent, yellow solution. If the solution begins to darken, this is a sign that nanoparticles in solution are decreasing, as they have a specific light absorption spectrum of ~390 nm, which causes the solution to remain yellow. This absorption spectrum method can also be used to determine the shape of the nanoparticles, as subtle differences produce different absorption spectra [25]. The solution must be stirred repeatedly until it is able to hold its yellow pigment for an extended period of time. A rotary vacuum evaporator can be used to increase the concentration for various tests of the solution. Once the solution reaches the desired concentration, the nanoparticles can be precipitated out of solution and collected as a powder [25]. By stirring the solution with a zinc rod specifically, the aggregation of Ag⁺ ions is induced, causing them to precipitate out of solution, since zinc breaks the charge balance between individual nanoparticles. This is preferred to other aggregation-inducing methods, as it does not involve adding any extra substance to solution which may affect subsequent portions of the experiment. As far as chemical manufacturing processes are concerned, this one is relatively simple, and can likely be adopted on a larger scale to increase manufacturing capacity.

**FIGURE 5 [25]**
(A) Shows a scalar picture of silver nanoparticles while (B) displays a histogram of their size distribution

**ETHICAL IMPLICATIONS AND SUSTAINABILITY (6)**

There exists a myriad of factors to be considered when preparing to bring a product to the medical market. While silver nanoparticles’ use as an antibiotic is still in the early stages of development and has not been widely tested for humans, we are still able to comment on some of the major concerns when considering widespread adoption of the technology. One of the main concerns is the availability of silver. Should the treatment be used as a global replacement for existing antibiotics, how much silver would have to be mined to do so, and at what cost to the consumer? After all, silver is a rare and expensive metal. This, of course, depends on the dosage needed for effective treatment. Would a patient need to be prescribed less, the same amount, or more silver when compared to the amount of antibiotic currently prescribed to treat the same afflictions? Le Ouay and Stellacci state that silver nanoparticles “still possess a very high activity against a broad range of microbes and parasites, even when low doses are used (full growth inhibition of bacteria
Daniel Zunino
Daniel Lutz

can occur at only a few mg/ml),” suggesting that the required dosage of silver nanoparticles may be smaller than the current dosage of antibiotics [26]. However, this is still unsure and these are questions that need to be answered during the later stages of development. Despite the high cost of silver, we speculate that the cost of the treatment for consumers would be similar to the cost of existing antibiotics, as the current drugs are already very expensive.

Additionally, we must consider any harmful effects that this may have on the patient undergoing treatment. Many scientists have voiced concerns about the toxicity of silver in the body. Logically, if silver nanoparticles attack and kill bacteria, there should not be anything to stop them from attacking and killing human cells. However, other medical uses of silver such as in wound dressings or to coat medical instruments have exhibited little to no negative health effects, since they are in small, often single-use doses [22]. Humans have also, as discussed in section 3A, used silver as an antimicrobial in dining utensils since antiquity, ingesting its Ag⁺ ions as a consequence. The main side effect associated with excessive silver consumption is a condition known as argyria, which causes one to exhibit grayish-blue lips or skin when exposed to large amounts of silver ions. This condition itself is not known to negatively affect health in any way [33]. Assuming that the drugs would be delivered orally, we can examine the Agency for Toxic Substances and Disease Registry Toxicological Profile for Silver, which details any health effects that silver may have on humans. According to this report, no studies have shown that oral exposure has caused death or any cardiovascular, immunological, developmental, reproductive, genotoxic, or carcinogenic effects. There is evidence that ingestion of silver can leave silver-containing granules in the central nervous system, but there is nothing to support that these have any neurotoxic effects [33]. Although these observations deal with silver as a whole rather than its nanoparticles specifically, it can still be assumed that silver nanoparticles will most likely yield similar results, since they function in the body similarly. Obviously further research would need to be conducted to confirm this, but existing evidence does support the theory that nanoparticles would have similar effects to those previously observed.

AN ANALYSIS OF THE ADVANTAGES AND DISADVANTAGES OF SILVER NANO PARTICLE TREATMENT (7)

ADVANTAGES OF SILVER NANO PARTICLE TREATMENT (7A)

The main advantage of silver nanoparticles over existing antibiotic drugs is that nanoparticles have a far lower inclination to induce bacterial resistance. This is because the nanoparticles interfere with bacterial processes in multiple ways, as discussed in sections 4A and 4B. Because the Ag⁺ ions indiscriminately attack the bacterial cell components, a bacterium is far less likely to have a gene mutation that will defend against all of the nanoparticles’ effects, and it will be unable to pass on any partially resistant genes as it will be killed by other mechanisms. Additionally, because silver nanoparticles interfere physically with the bacteria, instead of chemically; it is much harder for the bacteria to defend against the silver’s attacks than it is for them to stop the usual chemical attacks. This means that resistance will be significantly less likely to develop, solving a major problem in the antibiotic realm.

Furthermore, the way in which silver nanoparticles interact with the bacterial cell membrane provides significant advantages over existing antibiotic treatments. The tendency of nanoparticles to affect a given cell is largely reliant upon the properties of the cell’s membrane, specifically the peptidoglycan layer. This peptidoglycan layer is a polymer of sugars and amino acids which are meant to provide a protective coating around the cell membrane. Evidence supports the assertion that the tendency of nanoparticles to interact with bacteria relies largely on the thickness of this layer, and cannot happen without its presence [25]. This is advantageous to humans, as the peptidoglycan layer is only present in gram-negative bacterial species and not mammalian cells. Because of this, the nanoparticles will most likely not interact with human cells, and therefore be safe to use within the human body.

DISADVANTAGES OF SILVER NANO PARTICLE TREATMENT (7B)

One of the more widespread negative consequences of silver nanoparticle treatment is the potential release of these particles into the environment. Should nanosilver be adopted for widespread use, the potential release of it into the environment will increase greatly. Once deposited into waterways through drainage systems, the extremely small particles make their way into soil and groundwater, and eventually into plants, fish, insects, and other organism that thrive in and around waterways. Additionally, the nanoparticles can make their way up the food chain as these smaller organisms are eaten by predators. Silver, as shown in its Toxicology Profile can have negative health effects in small organisms, as they must ingest less of it to be affected. These health effects, exhibited in rats, include slowed neural functioning as well as the possibility of death if a large enough volume is ingested [33]. Should these nanoparticles weaken or kill enough of these organisms, there could be significant consequences for consumers higher on the food chain as well as the environment as a whole. Additionally, these effects raise concerns about potential negative health effects that silver may have on humans. Although studies support the assertion that silver has no negative effects on human health, it is still recommended that further research be conducted to confirm this. Studies must be conducted to research how the
body processes silver—retaining or liberating it—and what effects possible build ups may have, if any, in the body.

Another concern for the use of silver as a bactericidal agent within the human body is the effect that anions can have on its effectiveness. Chlorine is present in the body due to the dissociation of salts such as sodium chloride, or the table salt that most people ingest every day. Chlorine and other anions such as sulfides can reduce the efficacy of nanoparticles due to their tendency to ionically bond with silver cations, the main agent in bacterial inhibition, and precipitate out of solution due to the new compound’s low solubility [26]. When silver cations bond with anions, they are neutralized, and are therefore no longer effective against bacteria, which can be seen in Figure 6. This is a major concern that must be addressed before silver nanoparticle treatment becomes widespread.

**SILVER NANOPARTICLES—AN ANSWER TO ANTIBIOTIC RESISTANCE (8)**

After further research into the possibility of silver nanoparticle therapy as a replacement for existing antibiotic drugs, we conclude that this is a viable solution which is worth further exploration. As a result of growing bacterial resistance to antibiotics, these drugs will soon become ineffective unless something is done to either halt the development of this resistance or to create a new treatment which will not induce resistance. Silver, which has been used as an antimicrobial since antiquity, is a promising solution to this problem. In nanoparticle form, silver’s antimicrobial properties are greatly increased, with the nanoparticles displaying multiple different mechanisms that inhibit bacterial growth. Additionally, these nanoparticles are far less likely to lead to resistance due to the diverse means by which they interact with bacteria. Although silver nanoparticle technology is still in its early stages of antibiotic development, it is a viable solution to the ever-growing problem of antibiotic resistance.

**SOURCES**

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http://www.nature.com/scitable/definition/conjugation-prokaryotes-290


http://emerald.tufts.edu/med/apua/about_issue/what_can_be_done.shtml


https://micro.cornell.edu/research/epulopiscium/binary-fission-and-other-forms-reproduction-bacteria
http://www.nature.com/articles/srep09555

ADDITIONAL SOURCES

http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html
http://www.aiche.org/about/code-ethics
https://www.nspe.org/resources/ethics/code-ethics
https://microbewiki.kenyon.edu/index.php/Silver_as_an_Antimicrobial_Agent
http://www.nature.com/articles/srep07161

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