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Article

Silver nanoparticle-coated orthopedic screws lead to greater calcium precipitation

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SUMMARY
Orthopedic implants are commonly used for treating major bone injuries and offer enormous benefits to the healing process. However, these implants can face issues such as patient rejection, infection, and a lack of integration into surrounding bone which could lead to inflammation and implant loosening. To overcome these limitations, we hypothesized that stainless steel orthopedic screws coated with environmentally friendly silver nanoparticles would enhance acellular calcium precipitation, thus promoting the integration of the implant into surrounding bone. We synthesized environmentally friendly silver nanoparticles then coated stainless steel orthopedic screws with the 633 nm silver nanoparticles. Afterwards, we placed the screws into a calcium solution that models the soluble calcium surrounding an implant in the body. We observed that screws coated with silver nanoparticles and uncoated screws visibly accumulated calcium when left in the beaker overnight, as demonstrated by scanning electron microscopy (SEM). This indicates that if placed in the human body, prominent calcium precipitation would occur, aiding implant integration into bone. Upon further evaluation, we found that SEM images of screws after being left in the calcium solution revealed a more nanorough surface for the screws which were coated with silver nanoparticles beforehand, indicating more calcium formation. We also used energy dispersive spectroscopy (EDS) to confirm that calcium accumulated on the screw surface. In summary, this study suggests that the silver nanoparticle-coated stainless-steel screws should be further investigated for improved orthopedic implant efficacy.

INTRODUCTION
Orthopedic implants are medical devices that are commonly used to support bone healing. They are usually made of metals like stainless steel or titanium and have uses from joint replacement to dental applications (1-3). For example, it is estimated that 800,000 people receive knee replacements in the United States every year (2). Moreover, the orthopedic implant market was valued at $44.94 billion in 2024 (4). However, even with extensive resources in the field, not all implantations are successful.

Implant failure is surprisingly common, affecting approximately 10% of patients who receive an implant (5). This can be caused by infection or even the patient having a negative immune response to the implant, resulting in swelling and pain (5-6). Orthopedic implant failure can also occur if the implant loosens due to insufficient attachment to bone (7). There are various consequences of implant failure: joint instability, decreased mobility, bone damage, and risk of infection (7). Since implant failure affects so many people and has terrible consequences, it is crucial that research is done to prevent devices from failing.

Promoting initial bone growth on an orthopedic implant has shown promise towards reducing implant failure and allowing an implant to attach to bone (8). This is a viable solution because if bone can be stimulated to grow, the body can attach to the orthopedic implant more easily and reduce loosening. If bone grows and attaches quickly, there will be a higher chance of implant success. Faster initial bone growth can be achieved through the use of nanoparticle coatings (9). Nanoparticles are defined as particles measuring between 1 and 100 nanometers (nm) with further categories measuring between 100 and 2500 nm (10). These small sizes give nanoparticles different properties than larger materials. One of these properties includes a larger surface area when compared to the same material in bulk (10). This increased surface area increases the space available for contact with outside materials, including calcium precipitation and bone (10). With more space available to attach to, bone growth could be increased on a nanoparticle surface compared to a traditional one. Coating a medical implant with nanoparticles could implement attractive higher surface areas and energy to an orthopedic implant.

Silver nanoparticles are promising because they can be made in a lab using non-hazardous substances (11). It is important to use more environmentally friendly ways of making nanoparticles because the alternative (of combining chemicals like silver nitrate, sodium borohydride, and sodium chloride) leads to the release of toxic waste into the environment (12). Instead, chamomile tea can be used to synthesize the nanoparticles in a process called green synthesis (12). Chamomile tea contains phytochemicals, including flavonoids and terpenoids, that act as capping and reducing agents to reduce silver ions to silver nanoparticles (12). Being a metal, silver nanoparticles can easily coat a metal surface of orthopedic devices (13). Silver nanoparticles also have the benefit of being antibacterial, making them frequently used in medical applications such as drug delivery, as well as in the textile industry (14). These particles can be integrated into the body without being harmful and could have the potential to provide health benefits, from their ability to kill a variety of pathogens, compared to the traditional stainless steel of many orthopedic implants (14-15).

Considering the above, we hypothesized that stainless

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steel orthopedic screws coated with silver nanoparticles would improve acellular calcium deposition. The calcium deposition on an implant surface is more likely to integrate into juxtaposed bone. In this study, we found that calcium will precipitate on orthopedic screws when left in a model calcium containing solution overnight regardless of if they were coated with nanoparticles or not. However, we also found that the screws coated with silver nanoparticles before being placed in a calcium-containing solution had a rougher surface after being left in the solution than the same screws without a nanoparticle coating, providing evidence of greater calcium precipitation. While more experimentation is needed, this study highlights the positive role that a silver nanoparticle coating can have to promote orthopedic implant integration into surrounding bone.

RESULTS

Silver Nanoparticle Synthesis by Silver Wound Gel

The first part of this study investigated environmentally friendly ways to make silver nanoparticles to serve as a coating on orthopedic implants. Experiments were conducted to make silver nanoparticles from chamomile tea and a commercially available CVSHealth™ silver wound healing gel (**Figure 1**). This process was a fully green method, as chamomile tea and CVSHealth™ silver wound gel are not considered damaging to the environment and are readily available to purchase (since they are made by large brands and are widely distributed). Two brands of chamomile tea were investigated (Tazo® and Benner Tea Co®) and were separately boiled in distilled water while adding equal amounts of CVSHealthTM silver wound gel to both. We expose both tea mixtures to UV light/sunlight and a small amount of silver nanoparticles visibly formed at the bottom of the beakers. Using dynamic light scattering (DLS) to measure nanoparticle size, we found that this method resulted in average nanoparticle sizes of 351nm for the Tazo® brand of tea and 425nm for the Benner Tea Co® brand of tea (**Figure 1**). However, the standard deviations for these two brands differed greatly. According to these results, the Benner Tea Co® brand of tea gave a more consistent result and was therefore chosen as the brand of tea used for further trials to make silver nanoparticles. There was not a significant difference in the average nanoparticle size between the two brands of tea (p = 0.340). However, one drawback was that this method produced silver nanoparticle yield too low to be calculated.

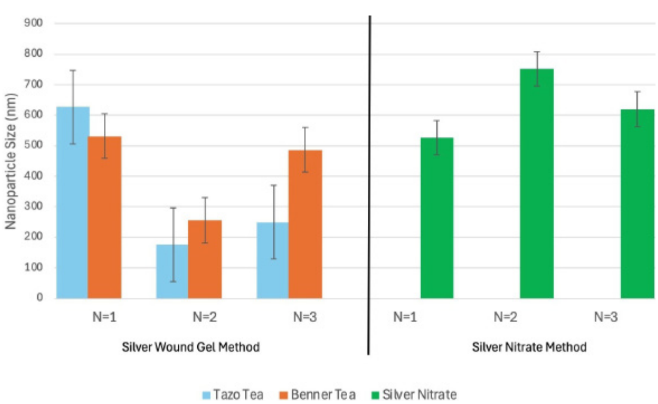

Figure 1: Silver nanoparticle sizes produced by silver wound gel method and silver nitrate method. Bar graph showing the sizes of nanoparticles produced by two different methods in three trials (n=3). Silver wound gel was combined with either Tazo® or Benner Tea Co® and exposed to sunlight. Silver nitrate was combined with Tazo® tea and left covered overnight. Samples were run though dynamic light scattering (DLS) which calculated the sizes of the nanoparticles.

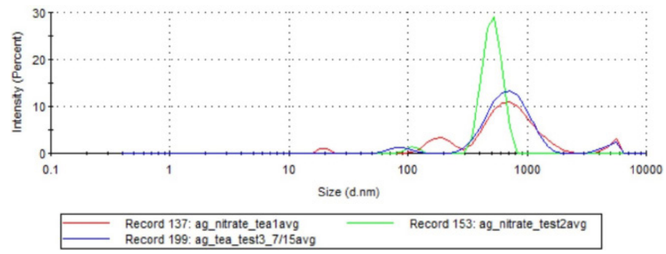

Figure 2: Silver nanoparticle sizes produced by the silver nitrate method. Line graph with the average sizes of silver nanoparticles produced by silver nitrate. Silver nitrate was combined with Tazo® tea and left covered overnight. Samples were run through dynamic light scattering (DLS) which calculated the sizes of the nanoparticles. Each colored line represents an independent nanoparticle synthesis.

Silver Nanoparticle Synthesis by Silver Nitrate

A second method for making silver nanoparticles was tested to see if it would produce a higher yield. This experiment used Tazo® chamomile tea boiled in distilled water, filtered, and combined with a silver nitrate solution. There were greater quantities of Tazo® tea on hand which is why we used it instead of Benner Tea Co®. We considered silver nitrate to be a better source of silver than CVSHealthTM silver wound gel because silver nitrate has a higher silver concentration with a concentration of 63.50% silver by mass compared to the 1.4% silver in CVSHealthTM silver wound gel. We used DLS on the three trials of silver nanoparticles produced using silver nitrate, which showed an average silver nanoparticle size of 633 nm (**Figure 2**). We excluded outliers, that were vastly too large and most likely due to tea components that had not been filtered out, from the calculation. We found that the results were more consistent for the silver nitrate method of synthesis. The three trials using silver nitrate had consistent results with the DLS graph of their particle sizes possessing consistent overlapping peaks (**Figure 2**). This method was also able to produce a much greater quantity of silver nanoparticles than the first method using the CVSHealthTM silver wound healing gel. We used centrifugation and found that around 2.1 grams of silver nanoparticles were produced during each trial.

Calcium Precipitation

After the successful synthesis of silver nanoparticles was accomplished, stainless-steel orthopedic screws were coated in the separate silver nanoparticles. Silver nanoparticle coatings were formed since the silver nanoparticles visibly gathered and were attracted to the metallic screw.

The next step was to determine if the silver nanoparticle coated screws promoted calcium precipitation. After being left overnight in a calcium-containing solution, white calcium growth could be visibly observed on screws both exposed and not exposed to silver nanoparticles. There was no observable difference to the eye of calcium growth between the nano-coated screws and uncoated screws (**Figure 3**). Neither could a difference in mass between the screws be calculated. When we ran energy dispersive spectroscopy (EDS) on the sample,

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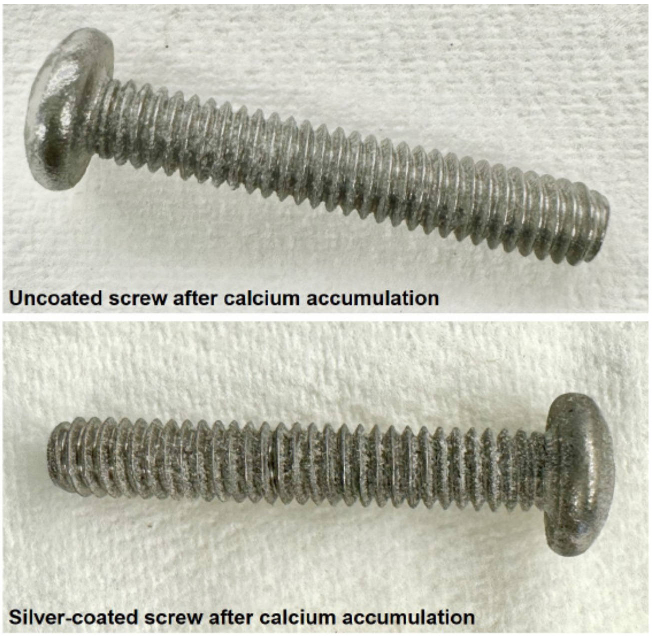

Figure 3: Uncoated and silver nanoparticle-coated screws after being left in calcium overnight. Pictures of orthopedic screws with calcium accumulation. Top: Uncoated screw after calcium accumulation.

calcium was one of the main elements present (**Figure 4**), showing that calcium truly accumulated on the screws.

In order to more clearly discern differences between the uncoated and silver nanoparticle-coated screws after calcium precipitation, we used SEM (scanning electron microscopy). The surface of a screw that was not exposed to anything appeared flat, which was expected (**Figure 5**). The surfaces of the screws placed in the calcium-containing solution appeared rugged and had obvious deposits on them (**Figure 5**). Comparing the SEM results for an uncoated screw and a silver nanoparticle-coated screw left in calcium, results visually showed that the nano-coated screw had a rougher surface. Moreover, brighter SEM images indicate more electron excitation which was apparent for the silver nanoparticle-coated compared to uncoated screws. Such information collectively implies that the screws coated in silver nanoparticles possessed increased calcium precipitation. Using Fiji software, we found the grey scale pixel values for each SEM image (**Figure 6**). The maximum RGB (Red Green Blue) increased from the control screw to the one left in calcium and increased again for the silver nanoparticle-coated screw left in calcium. Having a low maximum RGB value as well as lower standard deviation means that the colors present in the SEM image were less and more uniform, which could be expected of a screw that was not exposed to calcium or nanoparticles. A maximum RGB value and higher standard deviation indicates that there was material present on the screw surface that caused different coloring in the SEM image, which was observed for both screws left in calcium but was greater for the screw that was also exposed to the silver nanoparticles. Looking at the maximum RGB value of the SEM images, the screw with nanoparticles and calcium growth had the highest RGB value, which corresponds to the color white that was caused by significant calcium precipitation on the screw. The control screw had a maximum RGB value that corresponds to grey, accurately representing that there was no calcium present to create a white color on the screw surface. The screw with only calcium had a value between the other two screws meaning that there was calcium present but not as intense as the silver nanoparticle-coated screw. Hence, although requiring more experiments, orthopedic screws coated in silver nanoparticles may promote calcium precipitation.

DISCUSSION

The goal of this study was to find a way to solve issues associated with orthopedic implants, such as inflammation and implant loosening caused by patient rejection, infection, and a lack of integration into surrounding bone. We hypothesized that coating stainless steel orthopedic screws with environmentally friendly silver nanoparticles would enhance acellular calcium precipitation, thus promoting the integration of the implant to surrounding bone. When we left orthopedic stainless-steel screws in a calcium-containing solution, which mimics the solution surrounding an orthopedic implant after implantation, we observed calcium precipitation on the screw surfaces. We also observed more calcium precipitation on the silver nanoparticle-coated orthopedic screws than uncoated screws. We achieved the goal of being environmentally friendly by using silver nitrate and chamomile tea, which is less harmful than making silver nanoparticles using solely harmful chemicals. Although requiring more experiments, such results provide promising data that after implantation, more calcium could form on silver nanoparticle-coated screws and thus integrate faster and more effectively into surrounding bone.

Further experiments that should be carried out include

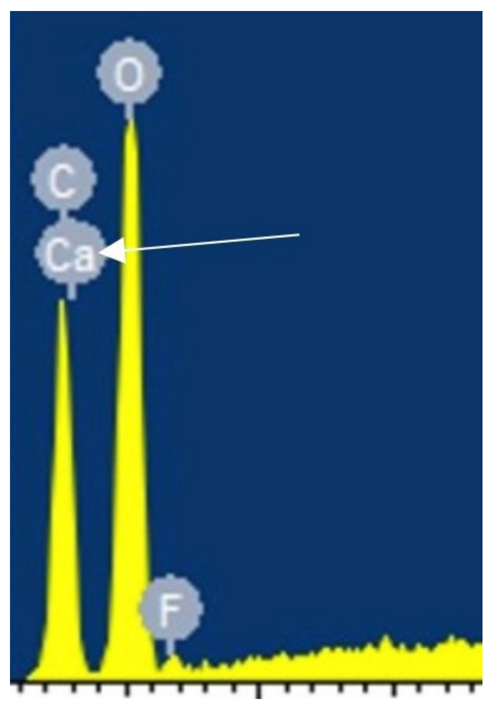

Figure 4: EDS of screw surface after calcium precipitation. This shows an energy dispersive spectroscopy (EDS) graph with elements present on the surface of a stainless-steel screw after being left in a calcium solution overnight. F = fluorine, O = oxygen, C = carbon, and Ca = calcium.

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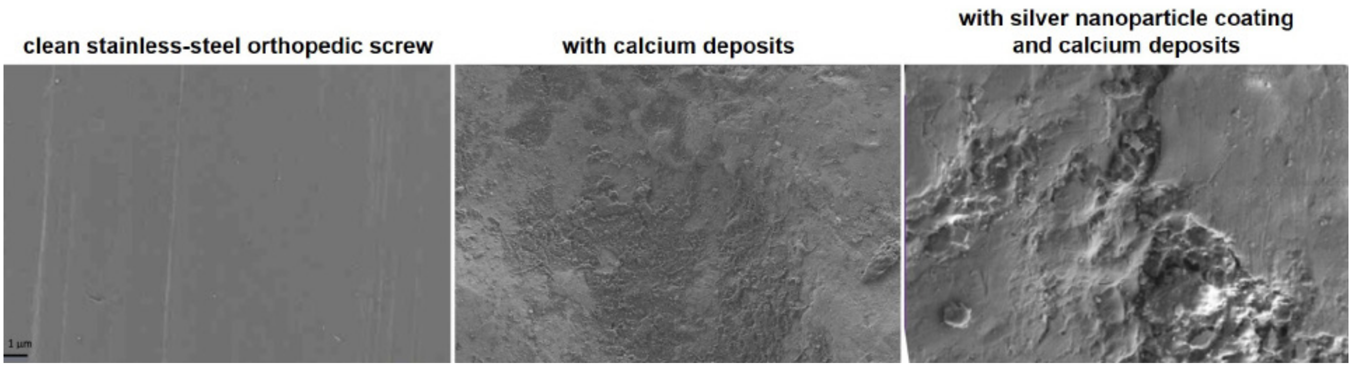

Figure 5: SEM of orthopedic screws with or without calcium deposits and silver nanoparticles. Three scanning electron microscopy (SEM) images at one micrometer of stainless-steel orthopedic screws. From left to right: clean stainless-steel orthopedic screw with no roughness on the screw surface; stainless-steel orthopedic screw with calcium deposits and slight roughness; stainless-steel orthopedic screw with calcium deposits and silver nanoparticles present and roughness. Magnification = 25,000X.

testing different types of nanoparticle-coated surfaces. This could also involve testing non-metal nanoparticles. One limitation of the present study, which we conducted at room temperature, that could be resolved by further experimentation is placing the screws and solutions into a body-temperature environment instead, where calcium precipitation may be more pronounced. This is important to consider because the purpose of the orthopedic implants is to help a patient by being present in the body. Similarly, to produce more accurate results, testing these screws with living cells is also something that should be studied in the future. Doing this could provide insights into how the orthopedic implants will attach to living parts in the body. Another major limitation was that the rougher appearance in the SEM image of the screw left in the calcium containing solution with a nanoparticle coating may appear rougher because of the silver nanoparticles, although EDS confirmed calcium presence and not silver presence. Taking an SEM picture of an orthopedic screw with only silver nanoparticles should be investigated in further studies. A closer more extensive analysis of specific areas of the orthopedic screws to determine which chemicals are present in the textured areas can be used to indicate if calcium was truly accumulating at higher rates on the orthopedic screws coated with silver nanoparticles.

Despite these limitations, SEM images of the coated and uncoated screws revealed that there may be a relationship between a silver nanoparticle coating and increased calcium precipitation which could increase binding to juxtaposed bone. If applied to orthopedic screws to be implanted in patients, these screws could result in greater bone growth and have better attachment to the patient's own bone, providing stability and fixation and preventing orthopedic implant loosening. If screws have greater attachment to bone, they are more likely to be successful as supports. Healthy tissue would be able to grow with the implant and the patient will have more successful healing. Beyond only improving bone attachment, silver also has the benefit of killing bacteria. Therefore, the use of silver can prevent yet another health concern. Additional health issues are prevented by the use of a safer method of producing the silver nanoparticles. Unlike fully harmful chemical-based methods, using chamomile tea to produce nanoparticles will expose patients to a lower level of hazard. Considering that implantations are common today and increasing every year, as well as the issues that arise from them, this application of nanotechnology can have a large-scale impact in helping injured people have a swift recovery without the common complications orthopedic implants pose.

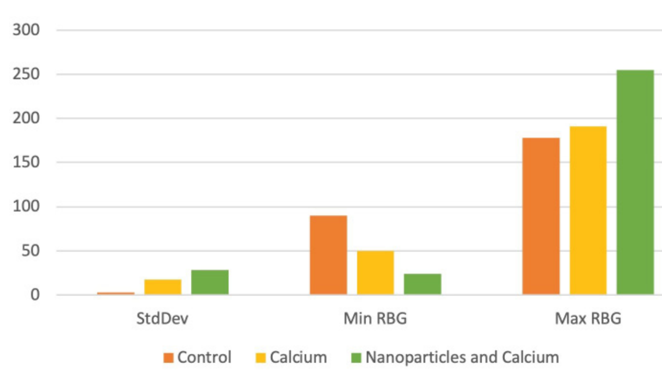

Figure 6: Grey scale pixel (y-axis) evaluation for the SEM screw images. Bar graph with values for standard deviation (StdDev), minimum (Min) RGB (Red Green Blue), and maximum (Max) RGB calculated by analysis of SEM images by Fiji software.

MATERIALS AND METHODS

Synthesis of Silver Nanoparticles by the Silver Wound Gel Method

Two beakers of 150 ml of distilled water were left on a hot plate until boiling. Once boiling, one chamomile tea bag was added to each beaker and removed after three minutes. This was done for two different brands of tea (Tazo® and Benner Tea Co®). Five grams of CVSHealth™ silver wound gel were measured out and added to the beaker. The beakers were left on a stirring plate until the gel was well combined. The beakers were left in the sunlight for 40 minutes. Each beaker was analyzed to see if the brand of tea led to a difference in the results.

Statistics

A graph was made using the DLS values for nanoparticle size of the two experiments using Tazo® and Benner Tea Co® chamomile tea. Average values and standard deviations were calculated for each experiment. Analysis of the SEM images was done by utilizing Fiji software. The images were analyzed on a greyscale and measured including standard deviation, minimum RGB, and maximum RGB.

Synthesis of Silver Nanoparticles by the Silver Nitrate Method

To make silver nanoparticles by the silver nitrate method, 4.5 grams of chamomile tea were taken out of the tea bags and soaked in 50 ml of distilled water. The beaker was placed on a hot plate and heated to 100ºC. Using a filter, the tea leaves were separated out. The remaining tea solution was added in equal parts to a silver nitrate solution which was made by adding 0.85 grams of silver nitrate (Sigma) combined with 25 ml of distilled water. Then, 15 ml of the tea solution were added to 15 ml of the silver nitrate solution. This beaker was left sealed and in the dark overnight. The next morning, DLS was used to determine the sizes of the nanoparticles created. To find the quantity of nanoparticles produced, the contents of the beaker were poured into centrifuge tubes. They were centrifuged at 4900RPM for six minutes. The excess liquid in the tubes was poured out and the mass of the filled centrifuge tubes were compared to the mass of empty centrifuge tubes to get 2.1 grams of silver nanoparticles.

Orthopedic Screw Coating Procedure

To ensure a silver nanoparticle coating, 316 L stainless-steel screws were left in a solution of nanometer silver nanoparticles for 10 minutes. They were removed from the nanoparticle solution and dried before being added to an empty beaker. Another beaker had an uncoated screw placed in it. Then, 50 ml of a calcium nitrate solution was added to the beakers followed by 50 ml of sodium carbonate. These beakers were covered and left to precipitate overnight. The screws were removed the next morning and the accumulation of calcium on them was visually observed. Energy dispersive spectroscopy (EDS) was utilized to determine if calcium was present on the screw surface. To compare the screws, scanning electron microscopy (SEM) helped to visualize the screw surface.

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