

Low Temperature IPD AgO Bacterial Static / Bactericidal Coatings for Medical Applications

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Low temperature Ionic Plasma Deposition (IPD) of thin film silver and silver oxide has been proven to provide predictable infection control for short term bacterial-stasis implantable devices, to long term (>60 day) bacterial toxicity for wound care products, indwelling and percutaneous medical devices and surgical tools. IPD deposits large area thin films (>18,000 in²/hour) of silver and silver oxide. IPD silver surface engineering has successfully treated Polypropylene, PVC, 440 stainless steel, PTFE, Al₂O₃, and Ti6Al4V, to name a few.

Introduction

Silver and silver oxide have been known to show bacterial stasis and bactericidal properties for thousands of years. Romans in ancient times used silver coins to purify water. In the mid 19th century, J. Marion Sims developed silver surgical sutures. In 1881, Carl Crede used diluted silver nitrate to wash the eyes of newborns to prevent blindness from gonorrhea. It was Albert Barns who developed Argyrol, a silver colloid medicine, which dominated wound care in the early 20th century. In the mid 20th century, penicillin and other types of antibiotics were discovered and the use of silver declined. With the recent surge of antibiotic resistant bacteria, silver and silver oxide have reemerged as a preferred method of fighting infection. [1]

Silver Oxide

Metallic silver, silver oxides, and silver salts show great antimicrobial properties and have been shown to control infection by killing bacteria and viruses at wound sites. Silver is able to block infection by preventing the transportation of electrons in microbes and by impairing cell replication through interaction with DNA. The reactivity of the ionic form with a variety of electron donating functional groups that contain reactive entities such as oxygen, sulfur, or nitrogen. Electron donating functional groups in biological systems are many and varied, including groups such as phosphates, hydroxyl, carboxylates, thiol, imidizoles, amines, and indoles. Microbial macromolecules are richly endowed with these functional groups that, when bound by a silver ion, may become inactivated and dysfunctional resulting in the death of the microorganism. Ionic silver is known to disrupt microbial cell wall, cell membrane, electron transport, metabolic and anabolic enzymes, and nucleic acid function. Silver can also damage receptors on cells by binding metabolically ineffective compounds to cell pathways. To achieve these results, silver ions must be released continuously due to this binding. In addition to the bacterial static / bactericidal properties, silver encourages epithelial growth via stimulation of increased wound calcium.

To date, no pathogens have been able to survive contact with silver and there have been no reports of allergic reactions by patients. [2]

One of the most daunting problems with a silver oxide type of antimicrobial coating is the inability to apply it to a surface without flaking, peeling, or sloughing off the substrate. Ionic Plasma Deposition (IPD) technology by Nexxion Corporation is the only commercial method that infuses silver and silver oxide into the surface of plastic medical device materials, providing outstanding adhesion and custom engineered surfaces. Furthermore, the surface exhibits excellent antimicrobial characteristics. The advantages offered by IPD have significant applications towards reducing device and hospital acquired infections. Releasing an overwhelming level of silver ions for an extended period of time can cause localized cell death, or necrosis. This problem caused St. Jude Medical to withdraw a sewn-in silver heart valve cuff from the market in 2001, when it was theorized the valve cuff prevented proper healing. [3] Recent work with IPD coatings on plastics has shown ion release that is effective and safe, with no resulting necrosis.

Biofilms

Microbiologists have traditionally based their knowledge of bacteria on the behavior of single cell bacteria. These so-called "planktonic" or free-floating specimens have been used to determine how well antibiotics and disinfectants kill bacteria. In the 1990's researchers found that bacteria communicate with each other through chemical signals called homoserine lactones (HSLs) when forming biofilms. Any bacterial colony can transform itself into a biofilm once it attaches to a hard surface in a moist environment. At least 40 other types of bacteria produce HSLs, suggesting that the natural messenger is widespread among microbes.

Medical devices and implants are outstanding surfaces for primary bacterial adherence and biofilm formation because devices, implants, and catheters provide hard surfaces in warm, moist, nutrient-rich environments. Biofilms, once formed, are very difficult to eradicate. It takes 1,500 times

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more of an antimicrobial agent to kill a biofilm than a planktonic bacteria. [4]

In nature, 99% of bacteria aggregate as biofilms; that is, complex colonies composed of billions of bacteria that pool their resources to resist being killed by antimicrobial agents. Medical devices offer ideal surfaces for primary bacterial adherence and biofilm formation because once in the body they provide a hard surface in warm, moist nutrient environments. Since biofilm microorganisms are difficult to eradicate with antibiotic therapy, acute local and blood infections often develop. With the increased use of biomedical implants and plastic catheters, hospital-acquired infections have become prevalent in recent years. Bacterial colonization of indwelling devices can be a prelude to both systemic infection and malfunction of the device.

Nosocomial Infections

Within the medical device arena there has been a significant drive to reduce nosocomial infections, which are infections that originate or occur in a hospital or hospital-like setting. Clinically significant nosocomial infections affect approximately two million Americans or 10% of hospital patients each year; resulting in about 20,000 deaths per year [5]

The key pathogens associated with nosocomial infections are primarily: *Enterococcus* spp., *Escherichia coli*, *Pseudomonas* spp., and *Staph. aureus*. The sites of nosocomial infections, in order from most to least common, are urinary tract, surgical wounds, respiratory tract, skin (especially burns), blood (bacteremia), gastrointestinal tract, and central nervous system. [6]

In the hospital setting, microorganisms acquire antibiotic resistance and are not easily treated once under way. The key to preventing their growth is to prevent them from attaching to the surface of the medical device. Even fast-mutating pathogens cannot withstand silver's antimicrobial properties which attack the cell in several different ways simultaneously. Silver is therefore uniquely suited to prevent the spread of medical device related infection.

Most hospital-acquired bloodstream infections are associated with use of an intravascular device, such as central venous catheters. Catheter-associated bloodstream infections occur more often in intensive care unit (ICU) patients than in ward patients. The mortality rate attributable to bloodstream infections in surgical ICUs has been estimated to be 35%. ICU-acquired bloodstream infections account for an estimated \$40,000 increase in costs per survivor and an estimated \$6,000 increase in hospital costs. [7]

There are two critically important factors regarding implant infection: 1) the introduction of bacteria during implant surgery, and 2) transdermal openings following surgery.

Transdermal devices are a prime location for infections. As the device separates from the skin, a fissure forms between the skin and device, allowing bacterial contamination.

Nosocomial infections are the result of three factors occurring in tandem: 1) high prevalence of pathogens, 2) high prevalence of compromised hosts, and 3) efficient mechanisms of transmission from patient to patient, also known as chains of transmission. These three factors lead not just to a higher likelihood of transmission of pathogens within hospitals, but also to an evolution of enhanced disease-causing potential among microorganisms present within hospitals.

Sterilization Treatments

Most medical devices are exposed to some form of sterilization. Testing to date shows that certain methods of product sterilization will affect the color of the IPD silver and silver oxide surfaces but they do not change the effectiveness of the antimicrobial action. There are only two methods proven to not affect the efficacy of the silver and silver oxide coatings: gamma radiation and ethylene oxide (EtO). The surface of an EtO or ozone treated device will brown or darken slightly as the very top layer of the pure silver component of the surface is exposed to high levels of reactive oxygen.

Experimental Introduction

The purpose of this research is to test the efficacy of the IPD silver oxide on various substrates. To do this, several tests have been performed on three different substrates: Polypropylene, Polyurethane, and Collagen. four different coatings thicknesses were used to get a range of efficacy: 0 nm (control), 50 nm, 100 nm, and 200 nm. Finally, three main experiments were performed on a representative sample group to test the properties of the coatings: serial dilution, histology and elution.

This paper only covers representative material and coating samples. The results of these tests are reported here with the understanding that more complete tests have been completed. Results are consistent across all materials and coating thickness tested.

Methods

Sample Preparation

Samples of varying materials were prepared with three levels of Ag/AgO coatings produced by the IPD method (See Table 1).

Sample Serial Dilution Testing

Serial dilution testing is a technique that allows an accurate measure of the amount of bacteria per given volume. When compared to a control sample, it provides a quantitative measure of the efficacy of an AgO coating.

Sample Number	Substrate Material	Substrate Form	Coating Thickness (nm)
1	Polypropylene	Sheet	50
2	Polypropylene	Sheet	100
3	Polypropylene	Sheet	200
4	Polyurethane	Catheter	50
5	Polyurethane	Catheter	100
6	Polyurethane	Catheter	200
7	Collagen	Sheet	50
8	Collagen	Sheet	100
9	Collagen	Sheet	200

Table 1. Silver oxide parameters and sample number assignment.

To start the serial dilution, a standard must be established. This is done by using a 0.5 McFarland standard in a spectrophotometer at 625 nm wavelength. The standard is calibrated to read between 0.08 and 0.1 which gives a standardized count of 1.5×10^8 cfu/mL.

Once this standard (control) is calibrated, the test samples can be prepared and the standard is used to inoculate the other tubes. Once the sterile TSB tubes are inoculated, the one square inch samples are placed in the freshly inoculated tubes. A control without a sample is also inoculated.

These are allowed to grow for the various times (usually 1, 3, 5, 7, 10, 14, 21 and 28 days). At the allotted days, a specific, measured amount of the TSB is extracted (usually 0.9 mL per 50 mL), and diluted into 10 mL of DI water. This dilution is continued until it has been diluted to 10^{-7} . All the dilutions (10^{-1} to 10^{-7}) are plated out and the colonies are counted (see Figure 1 for example of plates).

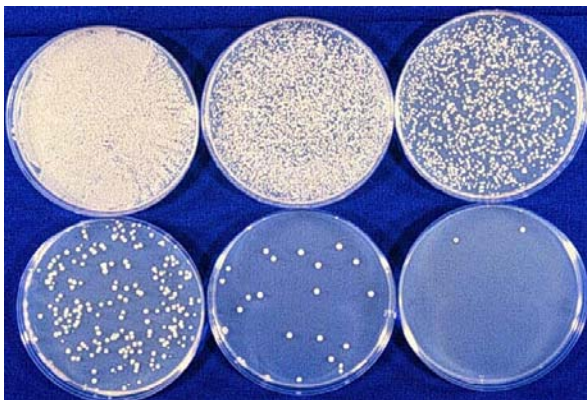


Figure 1. Example of a serial dilution series. Top left shows a plate with a log reduction of 1, bottom right shows a log reduction of 6. In this case, the bottom middle plate is the correct log reduction.

Sample Histology Testing

A representative sample of case materials were sent to two testing facilities immersed in 10% formalin solution. Chip numbers noted on both the container and the shipping bag were transcribed to a spreadsheet. The last three number - letter combination and the date were used to identify each case uniquely. In cases in which a case number was duplicated, the treatment group was added to the slide number and within the reporting spreadsheet for record keeping purposes only.

Standardized toxicological pathology criteria and nomenclature for the rabbit were used to categorize microscopic tissue changes. [8][9] The tissues were evaluated without knowledge of the specific pharmacologic activity or formulation of the test articles. Each tissue specimen was trimmed such that a two to three mm tissue margin remained from the edge of the sample patch. Adhesions, both fibrotic and visceral, were left intact as much as possible.

Each sample specimen was cut in cross-section at three to four mm serial increments. Typically two to three cross-sections were placed in series within standard microtomy cassettes. Occasionally, a single large cross-section was placed in a single cassette.

Cassettes were submitted for paraffin embedment and histologic processing. Sections cut at four to five microns were affixed to glass slides and stained with Hematoxylin & Eosin (H&E).

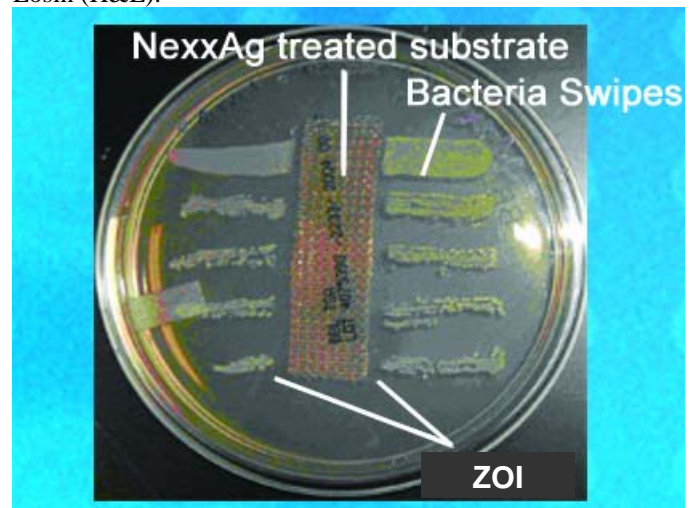


Figure 2. Sample of Zone of Inhibition (ZOI) testing.

Sample Zone of Inhibition Testing

The fastest, least expensive test to check efficacy is the Zone of Inhibition test (ZOI). This is an easy, 24 hour test that indicates if a sample has any antimicrobial activity. This test is not a quantitative test, and only provides enough information to indicate if a serial dilution test is warranted. This test provides no information regarding tissue re-growth or necrosis. An example of a ZOI plate can be seen in Figure 2.

Sample Elution Testing

Elution testing was performed to determine the silver elution profile of one square inch samples of coated Polypropylene. Silver elution testing provides a quantitative method for determining the amount of silver released from the test article over a specified period of time. The testing was completed per the current FDA Good Laboratory Practice (GLP) Standards, 21 CFR, Part 58. Each test article was extracted in USP 0.9% Sodium Chloride (NaCl) for injection at a temperature of 37° +/- 1° C for silver elution analysis by Inductively Coupled Plasma (ICP) Spectroscopy. Each test article was separately placed in 10 mL of USP 0.9% NaCl for a specified period of time. The time period analyzed during this study included 15 min., 30 min., 1 hr, 2 hr, 4 hr, 8 hr, 24 hr, days 2-7, day 10, day 15, day 20, day 25, and day 30. At each time point, the fluid surrounding the sample was decanted into a clean glass container and fresh NaCl was added to the sample container. The decanted liquid was brought to a total volume of 50 mL with deionized water, then acid digested and examined by ICP for silver content.

Results

Serial Dilution Results

The antimicrobial coating used on the various implant surfaces was approximately 0.2 microns, which is considered very thin in the coating industry. The coating was a proprietary construction of silver, silver oxide and other elements. The coating results in serial dilution tests shows an 8-log reduction in bacteria count during the first week and maintains a 3-log reduction past 14 days (Figure 3). The efficacy duration of the coating can be “tuned” to achieve 3, 7, 14, and 21-day efficacy depending on the requirements of the device and application. For example, there was a measured log reduction of bacteria counts 14 days on the Polypropylene, using serial dilution tests. After 14 days, the samples showed antimicrobial effectiveness well above the 3-log reduction threshold requirement.

Silver Oxide Log Reduction Test (February 05, S. aureus)

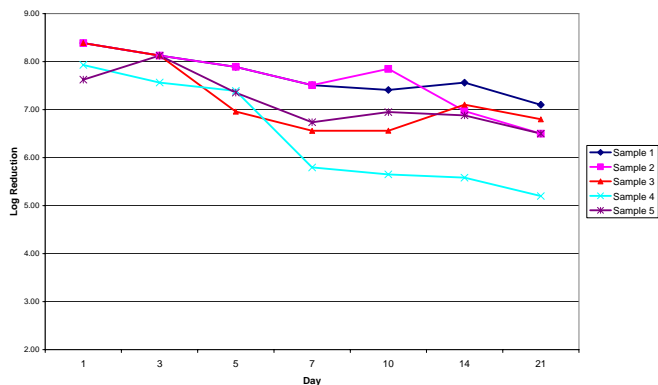


Figure 3. Example of a 21 day serial dilution test and associated log reduction results.

Histology Results –Polypropylene

Untreated (control) material Nine-Day Observations

The visceral surface of the Polypropylene lamina was overlaid by a fibrotic response comprised of spindle shaped cells (myofibroblasts) within a matrix containing newly formed collagen. Vascular elements were often observed. Macrophages were noted at the host-material interface. The presence of adhesions usually resulted in a thickened fibrotic response often with an increased abundance of vascular elements.

The response at the Polypropylene surface and within the Polypropylene was similar in character to that at the visceral surface. Macrophages with occasional giant cells were noted at the host-material interface. Abundant new collagen formation was noted within the Polypropylene that followed the architecture of the Polypropylene including encircling the monofilament elements. Within the response, vascular elements as well as occasional PMNs and lymphocytes were noted. The monofilament interface was comprised off macrophages with occasional giant cells.

Twenty-Two Day observations

A modestly thick fibrotic response was noted on the visceral surface. Spindle-shaped cells (fibroblasts) were noted between the collagen fibers that comprised a bulk of the newly formed matrix. Macrophages and occasional giant cells were noted at the host-material interface.

At the Polypropylene surface, a modestly thick fibrotic response was noted that was continuous with the fibrotic response within the Polypropylene as well as that surrounding each monofilament. Macrophages and giant cells were noted at the host-material interface both at the surface of the Polypropylene as well as at the surface of the PPL1 monofilament. The fibrotic response in turn was continuous with a fibrotic response on the surface of the underlying muscle.

Silver oxide sample numbers one, two, and three Nine-Day Observations

Coating both the visceral and the Polypropylene surface with silver demonstrated a significant impact on the host response at both the cellular and extra-cellular levels. On the visceral surface, an increased number of macrophages and giant cells were noted which frequently demonstrated silver particles and particulate inclusions at nine days. There was a reduced number of myofibroblasts and a reduction in the amount of newly formed collagen. At the sample number three level, within the Polypropylene, areas of necrotic cellular debris were noted rather than the well-organized fibrotic matrix noted with the ‘control’ sample. At the sample number two level, the Polypropylene response was noted to be a sparsely cellular fibrin matrix with focal areas of tissue debris and an absence of fibrotic (collagen) elements. Of particular note is the change in the character of the macrophage response to that

epithelioid in character with an accompanying shift toward formation of giant cells suggesting response ‘chronicity.’

Silver oxide sample number one Twenty-Two Day observations

A relatively ‘typical’ fibrotic lamina in comparison to the untreated control was observed on the visceral surface of the Polypropylene in terms of thickness and fibrotic character.

Within the visceral response, macrophages containing black precipitated particulates were observed. These particulates were brightly refringent when viewed with plane polarized light. The observed macrophages containing particulate debris were sparse in numbers and scattered in their distribution within the fibrotic lamina. Macrophages lined the host-material interface. Particulate debris was not observed within these macrophages.

Within the Polypropylene surface, a fibrotic response ‘typical’ when compared to the ‘control’ group in terms of character and thickness was observed. The fibrotic response also surrounded the PPL monofilaments and was continuous with the face of the underlying muscle. Occasional macrophages containing refractile precipitates were observed within the Polypropylene response.

Silver oxide sample number two Twenty-Two Day observations

A ‘typical’ fibrotic lamina in terms of both character and thickness was observed at the visceral surface of the Polypropylene lamina. In a manner similar to the sample number one group, macrophages containing black precipitate that was refractile when viewed with plane polarized light was observed. Macrophages containing precipitate debris appeared more numerous than in the sample number one group with clusters of macrophages on the ‘surface’ of the visceral fibrotic response. Macrophages and giant cells containing precipitate debris were also noted within the host response at the material surface.

Observations within the Polypropylene were similar to those noted at the visceral surface. A ‘typical’ fibrotic response was noted at the Polypropylene surface. Occasional macrophages containing refractile debris were noted within the relatively less dense response between the PPL monofilaments. Only sparse debris laden macrophages were noted at the Polypropylene surface.

Silver oxide sample number three Twenty-Two Day observations

A fibrotic lamina similar in thickness to that noted within the control group was observed at both the visceral and Polypropylene surfaces. However, a marked distinctive lamina of giant cells containing black particles that were refractile was noted within the fibrotic lamina on the visceral surface of the Polypropylene. Giant cells containing black particles with

a similar appearance to those observed within the visceral response were noted within the response at the Polypropylene surface as well as adjacent to the fibrotic response at the Polypropylene interface. The macrophages and giant cells at the host-material interface often contained either refractile particulates or black precipitate particles. The presence of giant cells and macrophages containing refractile debris was often associated with clusters of macrophages and giant cells organized to suggest a lamina appearance as compared to a sparse and scattered appearance noted within the ‘lower-dose’ groups. The host response surrounding the PPL monofilaments as well as the response of the underlying muscle were similar to that observed in the control group.

Histology Results – Polyurethane

All polyurethane specimens had similar lesions, which are characterized by active/chronic inflammation in the subcutaneous tissue and also along the catheter tract in the muscle. There was considerable variation in the plane of section through the individual specimens, which made comparing the severity of the inflammatory reactions among the eight specimens difficult.

Polymorphonuclear cells mixed with a few mononuclear cells comprised the inflammatory infiltrate in the subcutaneous tissue, whereas mononuclear cells with a few multinucleated cells predominated in the catheter tracts in the muscle. A few basophilic structures that may be individual bacteria were observed, but no colonies of bacteria were recognized. **There was no biofilm formation observed at any of the catheter sites. There was no necrosis observed.**

The character and severity of the inflammatory infiltrates observed suggested the presence of some type of irritant. There were no nesting polymorphonuclear cells or microabscesses and bacterial colonization, which are usually observed with bacterial infected lesions. Although there was no foreign material observed on the hemoatoxylin and eosin stained sections, some type of irritating material appears to have been present. It is possible this irritant was the silver particles coating the catheters; however, no silver particles were noticed microscopically within the tissue (see Figure 4).

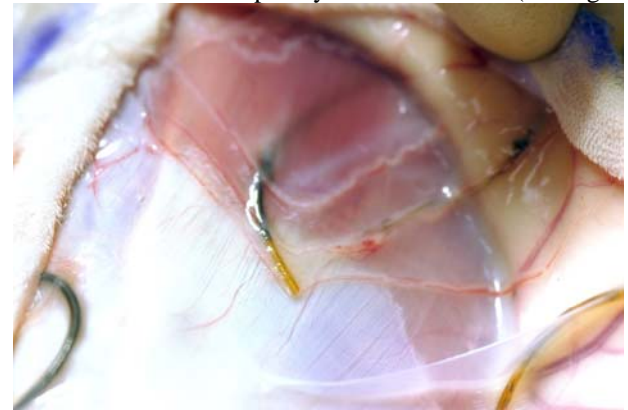


Figure 4. Catheter track photo in an implantable

In addition, after seven days, the transdermal site was challenged with an inoculant (Staph. aureus and E. coli) at the 10^3 level. After two days (day nine) of incubation, no infection or inflammation was noted.

Zone of Inhibition Results

The ZOI test is good only for an initial confirmation of antimicrobial activity and the relative potency that coating. All samples exhibited approximately the same ZOI for this test. It must be noted that the zone of antimicrobial inhibition can be designed to range from one to ten millimeters.

Elution Results

A total of twenty test articles were evaluated for the Polypropylene sample. Two samples were taken from a total of ten different samples for both the test groups. The testing was performed in duplicate using inductively ICP to determine the amount of silver present at each time point. The values were then averaged for a total of ten reported values for each test group. The elution values are given as mg/sample, which in this case is mg/square inch.

The samples all exhibited a consistent behavior over the first 24 hours in the NaCl solution. There was a slight peak around the four hour time point, and then the values level off around the 24 hour time point.

All of the samples were again very consistent in their behavior. The values were fairly stable from day 1 through day 5; the values then peaked around the 6 day time point and then leveled off from day 7 through day 30.

Collagen Results

EBM (Engineered Biological Matrix) is an acellular dermal tissue matrix. It is derived from fetal bovine skin that is mechanically and chemically processed to remove the epidermis and cells, and cell components of the dermis. At the same time, the manufacturing process preserves the extracellular matrix components, namely the collagen fibers.

EBM was treated with IPD AgO in a preliminary experiment. It was found to have no noticeable change in its mechanical properties (tensile strength, elastic modulus, DSC). Further, a sub-Q implantation study in small animals has shown that the product retains its biocompatibility; no inflammatory response was observed.

Conclusions

Conclusions - Polyurethane

The silver/silver oxide coated antimicrobial catheters prevented the formation of bacteria, bacteria colonies and biofilms. The antimicrobial results were consistent across all implant sites, and the antimicrobial coating remained effective even following a microbial challenge at seven days with E. coli and S. aureus. There was no necrosis observed. The lesions were consistent with a foreign body reaction in the muscle, with a more acute inflammatory reaction in the

subcutaneous tissue. It should be noted these are preliminary results and at the time of this test, no control (uncoated) sample was tested.

Conclusions - Polypropylene

The silver/silver oxide coated antimicrobial Polypropylene also prevented the formation of bacteria, bacteria colonies and biofilms. The antimicrobial coating remained effective for up to 21 days as tested in vitro. In the healing study, there was no necrosis observed and tissue in-growth at 28 days was equal to the control (uncoated) sample. The lesions were consistent of a foreign body reaction in the muscle, with a more acute inflammatory reaction in the subcutaneous tissue.

The average elution for the coated Polypropylene samples over all time points is approximately 0.005 mg per square inch (0.0048 mg/sq inch). The samples show a fairly consistent silver elution over the entire length the study with slight peaks noted at the 4 hour time point and after 6 days in saline solution. Using the elution values and an approximate total silver value of 1.05 mg per sq inch (obtained from outside testing) for the Polypropylene.

Conclusions - Collagen

There are no formal conclusions from the collagen testing as at the time of publication, only proof of concept tests have been completed.

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