



# Reduced Bacterial Adhesion to **nanoVIS Ti™** **Surface Technology**

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## Introduction

The purpose of this study was to look at the ability of the nanosurface to resist bacterial colonization with blood proteins and bacteria having an unrestricted food source. Infections on medical devices are a growing problem<sup>1-2</sup>. Antimicrobial resistance in bacterial communities makes this problem even worse. A patient getting an infection can mean a painful, slow, and costly recovery. This may result in implant removal or revision surgeries with no guarantee that the new implant will remain infection-free. The industry is trying multiple approaches from antimicrobial coatings to new electromagnetic therapies to treat infected implants<sup>3-4</sup>. Nanovis utilizes the inherent properties of nanostructured surface technology to inhibit bacterial attachment and proliferation on implant surfaces<sup>5-6</sup>.

Using tunable nanostructured titanium has significant advantages in recruiting the host tissue to integrate the implant, including vascular integration, bone growth, and soft tissue attachment<sup>7-11</sup>. The best long-term defense against infection that could occur is a well-integrated implant. For early infections, it is a question of how the bacteria in any open wound can attach to the surface to protect the growing biofilm from the host immune system. The nanoVIS Ti™ Surface Technology has nanotube structures made of titanium dioxide with an average outer diameter of 70 nanometers, Figure 1. This is about 1/10<sup>th</sup> the size of the bacteria.

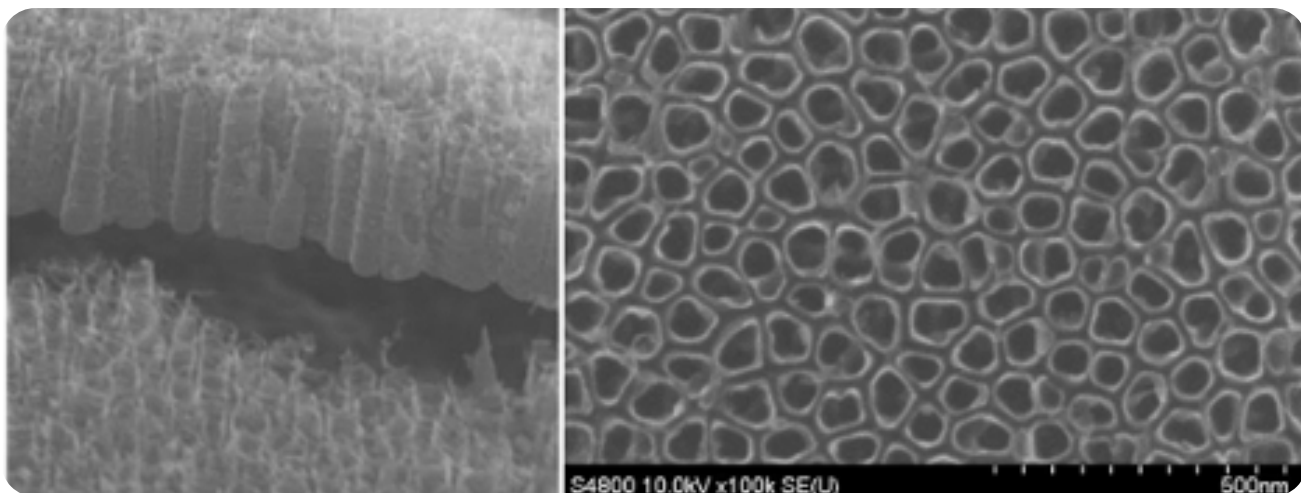


Figure 1 - Scanning Electron Microscope images of the nanoVIS Ti™ Surface Technology. Left image - The side profile of the nanotube surface shows the aspect ratio of the nanofeatures at 50,000x magnification. Right Image - 100,000x magnification of the surface from a top-down perspective.

## Methods

Samples consisted of shot peened and electropolished titanium alloy Grade 23. The nanoVIS Ti™ Surface Technology has 70 nm nanotubes anodized into the surface. Samples were sterilized via ethylene oxide gas sterilization following standard procedures. Samples were placed into 12 well plates and 2 ml of DMEM + 10% FBS were added to each well. Bacteria: *Escherichia coli* ATCC 33694, MRSA ATCC 43300, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus aureus* ATCC 14222, and *Pseudomonas aeruginosa* ATCC 10145. Cultures were counted using a Beckman Coulter Counter and separately seeded onto the substrates at an appropriate volume to equal  $1 \times 10^6$  CFU. Bacteria were allowed to grow in an incubator (37°C, humidified, and 5% CO<sub>2</sub>) for 24 hours. At the end of that time, media was removed, and bacteria were lifted from the substrates through sonication and soaking in 2mL of a 0.05% trypsin/0.02% EDTA solution for 5 minutes. The supernatant was collected and assessed for bacteria numbers using a Coulter Counter. Measurements were taken in triplicate and averaged. Nine samples of each surface were used to generate the data. Results are reported as  $10^6$  CFU/cm<sup>2</sup>.

## 24 Hour Bacterial Adhesion Assay

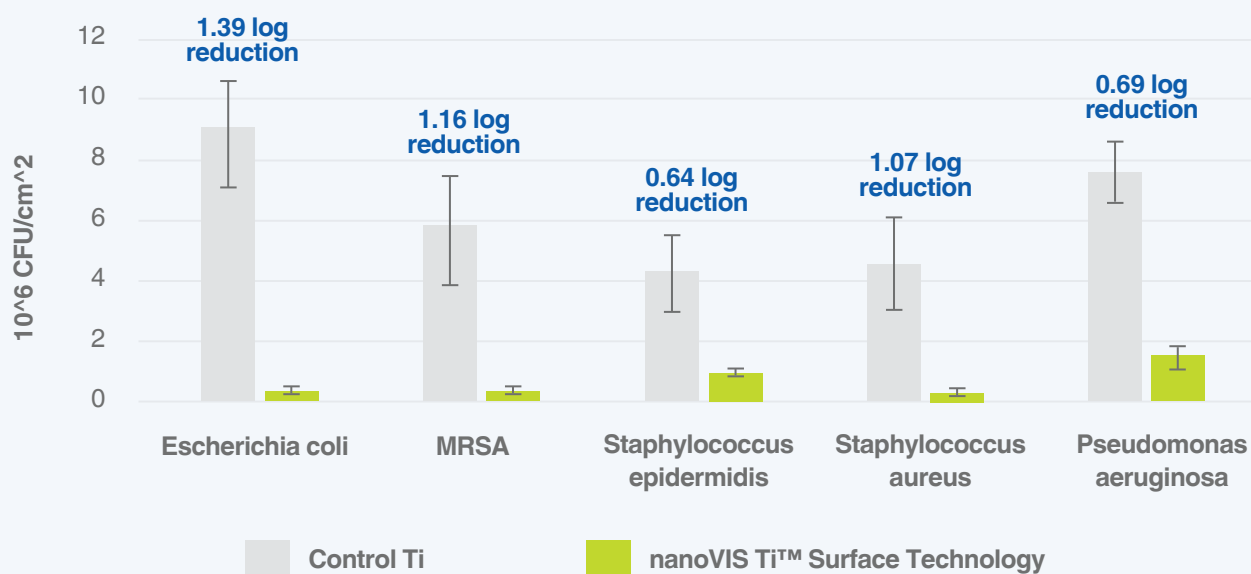


Figure 2 - Reduction in bacterial adhesion to nanoVIS Ti™ Surface Technology

## Results

At 37°C and in the presence of growth media and FBS, allowing the bacteria to rapidly proliferate. Even with rapidly proliferating bacteria, there was limited attachment to the nanoVIS Ti™ Surface Technology, Figure 2. *Escherichia coli*, MRSA, and *Staphylococcus aureus* all showed at least a 1-log reduction in bacterial adhesion. The nanoVIS Ti™ Surface Technology achieved a 0.64 and 0.69 log reduction on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, respectively.

## Discussion

Bacteria prefer a flat surface on the nanoscale with nooks and crannies that are the size of the bacteria or slightly larger<sup>12</sup>. This allows them some shelter from the immune system and limits the ability of antibiotic drugs to access the bacteria. Bacteria have stiff membranes and cell walls to preserve the structure and function of the bacteria. Because the bacteria are so rigid and much larger than the nanotubes, they cannot access all the surface area of the nanotube surface. The high aspect ratio of the nanotubes and the short distance between nano features effectively reduces the available surface area for bacteria to adhere, acting as a bed of nails for the bacteria—they can only interact with the tips of the nanotube “nails”<sup>6</sup>. However, human cells have much more fluid membranes that can better conform to and interact with the surface area available on the nanotubes<sup>6</sup>.

## Conclusion

Reductions in bacterial attachment *in vitro* demonstrate that bacteria have a more difficult environment to establish a biofilm on the nanoVIS Ti™ Surface Technology. The ability to reduce bacterial attachment along with reduced inflammatory markers, improve vascular on-growth, and improve bone tissue attachment making the nanoVIS Ti™ Surface Technology an essential part of next-generation medical implants.

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