HELLENIC REPUBLIC

National and Kapodistrian University of Athens

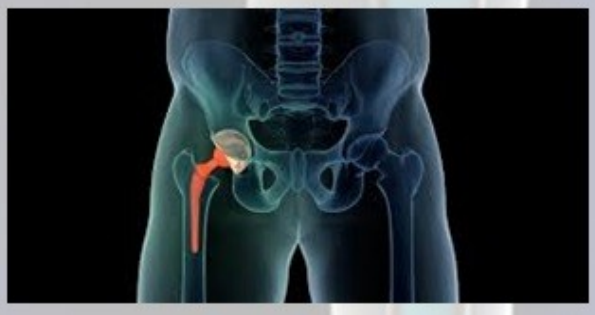
EST. 1837

School of Health Sciences
School of Medicine and Department of Pharmacy

Interdisciplinary M.Sc course in Nanomedicine

2018-2019

APPLIED NANOTECHNOLOGY IN PERIPROSTHETIC INFECTIONS IN ORTHOPAEDICS



Tsipouras Loukas Medical Laboratory Technologist

Supervisor:
Olga Savvidou: Associate Professor of Orthopaedics, National & Kapodistrian University of Athens
Co-Supervisors:
Efstathios P. Efstathopoulos: Professor, Medical Radiation Physics National and Kapodistrian University of Athens
I.K. Triantafyllopoulos: Assistant Professor of Orthopaedics, National & Kapodistrian University of Athens

SEPTEMBER 2019

Table of contents

Abbreviations

ADA (Alginate dialdehyde)
AMPs (Anti-microbial peptides)
ATRP (Atom transfer radical polymerization)
CHI (Chitosan)
CMC (Carboxymethyl cellulose)
CuAAC (Cu Catalyzed Azide-Alkyne Cycloaddition)
DAC® (Defensive Active Coating)
DI water (Deionized water)
DMP (Direct metal printing method)
EPD (Electrophoretic deposition)
EPS (Extracellular polymeric substance)
HCM (Hydrothermic chemistry mode)
LBL (Layer-by-layer self-assembly)
LC0024 (5- (4-bromophenyl) -N-cyclopentyl-1-octyl-1H-imidazol-2-amine)
LPS (Lipopolysaccharides)
MgF_2 (Magnesium fluoride)
MIC (Minimal inhibitory concentration)
MRSA (Methicillin-Resistant Staphylococcus aureus)
NaAlg (Sodium alginate)
PDA-HA-LF (Polydopamine-assisted hydroxyapatite and lactoferrin multilayer structure)
PEO (Plasma Electrolytic Oxidation)
PIIID (Plasma immersion ion implantation and deposition)
PNIPAM (Poly(N-isopropylacrylamide))
PJI (Periprosthetic joint infection)
PLA (Polylactic acid)
PMMA (Polymethylmethacrylates)
PVD (Physical vapor deposition)
RMS (Reactive magnetron sputtering)
ROS (Reactive oxygen species)
SH (Sulfur-Hydrogen)
TGF-β (transforming growth factor-β)
THA (Total hip arthroplasty)
Ti (Titanium)
VEGF (Vascular endothelial growth factor)

1. Abstract

The aim of this dissertation is to review coating types that use nanotechnology to inhibit infection and allow faster osteointegration of total joint arthroplasties. Number total joint arthroplasties are constantly increasing over the years due to the increase of the general population life expectancy. However, as the number of implants increases, the number of revisions due to implant failure because of loosening or infection. Periprosthetic joint infection (PJI) and biofilm formation by resistant bacteria such as Staphylococcus aureus or Staphylococcus epidermidis is a common cause of failure. To inhibit biofilm formation and increase implant osteointegration, nanotechnology applications improve the surface coating of the implants in order to make them more biocompatible. There are mainly three coating types with different beneficial properties each. Passive coating helps faster osteointegration, active coating helps biofilm formation inhibition, and intraoperative hydrogel use is an anti-bacterial attachment for a short period of time. To perform these coatings, laboratory techniques were used with high technology machines to form the implant surface in nanoscale. Forming an implant surface or coating it with nanoparticles is intended to make the implant more biocompatible and increase the implant's life expectancy within the human body. The aim of this dissertation is to review the current nanotechnology applications in vivo, in vitro or in situ regarding total arthroplasty implants, as well as the future perspectives that occur from these experimental applications.

2. Introduction

Nowadays nanotechnology has transformed orthopaedics through recent advances in bone tissue engineering, implant materials and surface, diagnosis and therapeutics. One of the most frequent challenge in orthopaedic practice is the management of an infected implant and the repair of the damaged bone. Recently published data show that Periprosthetic Joint Infection (PJI), have an incidence of 0.75 to 1.24% for primary total hip arthroplasty (THA) that becomes higher in 1-2 years and increase to 1.6 to 3.96%, and even more as the years pass, up to 11 to 30%. (1) Multiple surgical revisions for the treatment of PJI is one of the most common reason for increase morbidity especially in older people, up to 3.25% in the first month postoperative. This rate rises to 8% within 5 years after a revision surgery. (1)

More than three decades ago, Antony Gristina, MD, Orthopedic Surgeon introduced the term “race for the surface”. By this, phrase he wanted to describe the “race” that microorganisms make against the osteocells of the patient’s, to occupy more space in the surface of the implant inserted in the patient’s body. Bacteria can adhere to the biofilm formed on implant surface. Biofilm is a well organized structure of bacteria through physical, chemical, and biological cell to implant interactions. The most common bacteria are Staphylococcus aureus and Staphylococcus epidermidis, in an incidence of 80%. (2) The percentage is nearly 53% for facultative anaerobic, Gram-positive bacterium, typically found in skin flora. The impact of these microbial strains on implants has serious consequences, as it can lead to increased mortality, especially in older patients.

Moreover, multidrug - resistant strains, such as MRSA (Methicillin-Resistant Staphylococcus aureus) is another reason for increase number of chronic osteomyelitis, multiple revisions procedures and finally in some cases even death. (2) The maturation of a biofilm by those Gram positive bacteria is completed in less than 24 hours after insertion of the implant. (3) The financial burden on the National Health System for a

revision of an infected implant, can be up to 5 times more than the index procedure initial surgical operation. (4)

Another major problem in orthopaedic practice after insertion of a total joint arthroplasty (total knee or total hip arthroplasty) is the satisfactory osteointegration of the implants. Osteointegration is the direct structural and functional connection between living bone and the surface of a load-bearing artificial implant. One important factor for bone integration is the bone tissue health and the surface of the implant at nanometer level. In particular, the bone tissue should be amorphous at a level of 20 to 50 nanometers. If osteointegration was not achieved, aseptic loosening of the implants happens. (4) The incidence of aseptic loosening after total joint arthroplasties is 11–30%. (1) There are some factors that can lead to osteointegration failure. Such conditions may occur due to the low biocompatibility of the implant, the design and the shape of the surface of the implant, the quality of the bone and the surgical technique. (4)

Taking into consideration that loosening may implicate with infection of the bone around the implants, the orthopaedic surgeon must treat not only the loosening of the implant but also the infection, with major implications for the patient's morbidity and mortality.

In order to "fight" biofilm formation and to promote osteointegration between implant and bone, nanotechnology appears to be a powerful tool in the near future. Nanotechnology can alter or final finishing the orthopaedic implant surface by 3 ways. Two of these ways are passive and active modification of the implant surface. The third one has to deal with antimicrobial agents placed intraoperative. The prevention of biofilm formation results also in faster osteointegration of the implant. Among the factors that influence osteointegration are the properties of the implant material, the physicochemical properties of its surface or its geometry, the implantation technique, and the preparation of the tissue that will accept it. (5)

Considering all the above and taking into account the increasing number of total joint arthroplasties in the future, it is imperative to find a solution. Research regarding tissue engineering, scaffolds, biomaterials and nanoparticles or the use of stem cells is emerging. (4) Nanotechnology may give answers to these difficult problems. The applications of nanotechnology regarding the management of PJI are analyzed in this dissertation.

3. Biofilm and osteointegration

a) Bacterial adhesion and biofilm formation

Biofilm formation occurs as a result of a series of physicochemical and biological processes. Staphylococcus aureus has the ability to adhere to both eukaryotic cells and abiotic surfaces because of certain cell surface proteins. The ability of bacteria to link directly on abiotic surfaces and after that, cell to cell bacteria interaction creates the coherence of biofilm. (2) Biofilm formation is divided into three stages. (Figure 1) (3)

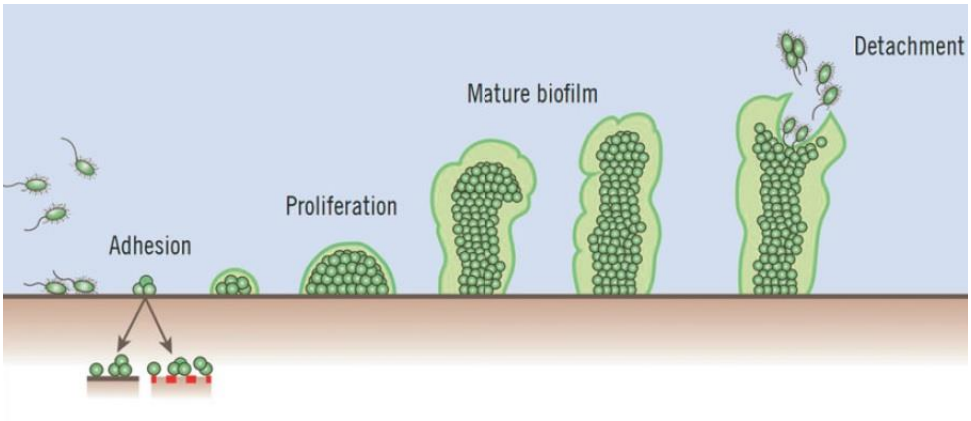


Figure 1 Biofilm formation from bacterial adhesion to detachment stage. (K.-D. Kóhn et al Management of Periprosthetic Joint Infection © Springer-Verlag GmbH Germany 2018)

In particular, the first stage is the bacterial attachment to the surface, which then develops into a small colony. As a result, the permanent maturation of the biofilm will lead to the detachment of certain bacterial cells.

More specifically, regarding the initial bacterial attachment, the surface treatment at macromolecular level, as well as the environmental conditions that the implant has been exposed, play an important role. The two most pathogenic strains of Staphylococcus (aureus and epidermidis) are capable of binding primary biofilm forming agents due to

the existence of proteins in their cellular membrane, such as fibrinogen and fibronectin that are readily found in the extracellular environment (Figure2). Extracellular matrix proteins adhere to the implant surface and may be the binding target of Staphylococcus strains pathogens. At this stage of adhesion on the implant surface, reversible Van der Waals interactions and steric and electrostatic interactions develop. Bacterial adhesion becomes more robust due to different and stronger bonds which have developed, like hydrogen bonds and ionic and hydrophobic interactions. (2) Next, the stage of maturation involves an extracellular polymeric substance (EPS) consisting of extracellular DNA, proteins, lipids, lipopolysaccharides (LPS) and exopolysaccharides. These EPS components contribute to the stability of the microbial colonies. As a result of these secretions, bacteria strains continue to adhere to this structure by forming micro-colonies. This formation structurally simulates hydrogel that acts like a boundary between the microbial colony and the extracellular environment. In the final stage of biofilm maturation, diodes are formed between bacterial cells. These feed every part of the colony with nutrients, water and signaling molecules. Signal molecules begin the colony spreading when nutrients end up with aggregates or individual cells are degraded from the original colony in order to infect other implant sites. A bacterium found in biofilm in comparison with a single bacterium can exhibit antibiotic resistance by 500 to 5000 times more. (2) It is worth noting that biofilm itself activates the immunogenic response by producing antibodies. However it is not affected by their activity over time. (2) Low penetration of antimicrobial agents as well as the biofilm barrier around the microbial colony contributes to the resistance of the bacteria to antimicrobial agents. Low antibiotic efficacy may result from the chemical composition change of biofilm microbubble. This chemical change allows bacteria to adapt by mutating their genome. (2) However, it has been recently found that resistant strains of Staphylococcus aureus form within the synovial fluid composts to which they freely adhere, showing strong structural cohesion like that of a biofilm and infect the perimplant area. (6)

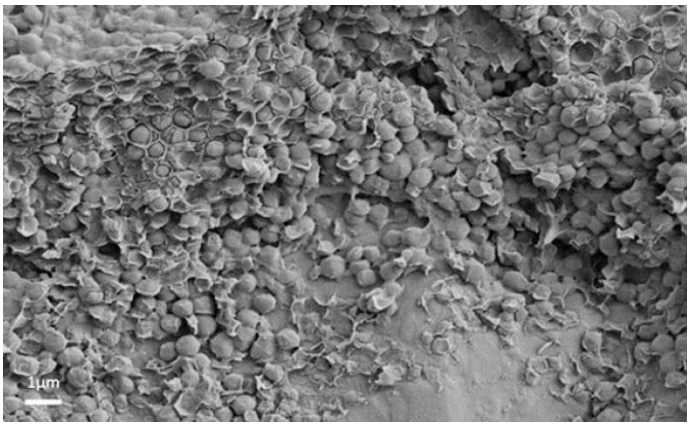


Figure 2 Staphylococcus aureus biofilm development on titanium surface as seen in scanning electron microscopy (SEM). (David Armbruster et al B. Li, T. Webster (eds.), Orthopedic Biomaterials © Springer International Publishing AG, part of Springer Nature 2018)

b) Implant surface osteointegration

The battle against biofilm creation on implant surfaces, according to researcher Arciola and his associates, is targeted at four areas. Initially, the implant surface should be turned into a surface that prevents the adhesion of bacteria, next it could be enriched with antimicrobial agents. A third option could be combined with the two above mentioned cases and finally, formulation could include agents that affect osteogeneration together with antimicrobial coatings. By following this kind of strategy, you can develop "smart implants". (7)

Indicatively, it is the implant material, the treatment it has undergone to aid faster integration, resilience and elasticity in loads, and ultimately resistance to corrosion. The best alternative material to stainless steel is titanium (Ti). (4) Titanium is used extensively in total arthroplasty, because it exhibits better biocompatibility, greater resistance to loads with reduced elasticity and is not permanently eroded. It has been observed that it causes smaller macrophage cells presence compared to stainless steel when inflammation occurs. (4) The most popular titanium alloy in orthopaedic applications is Ti-

6AL-4V. This kind of implant should prevent the creation of fibrous tissues perimetrically. At the same time, surface alternation promotes the osteoblasts activity in order to increase the likelihood of its life span within the patient's body. These procedures are made by active implant coating with materials like hydroxyapatite, silicotitanate or stemm cells. (4)

Corrosion occurs when osteoclasts affect the biomaterial surface, causing the generation of metallic ions. This reaction causes local inflammation that leads to fibrous tissue formation which prevents osteoblast action. Cytotoxicity of metal ions acts in a way that prevents new bone formation. These local reactions lead to aseptic implant loosening. (4)

The osteointegration process is largely influenced by the surface material pores diameter ranging between 150μm and 600μm and the surface energy as positive charge gives hydrophilic implant properties. Implant hydrophillicity attracts proteins more rapidly and thus, accelerates osteoblasts action. (8) Also, surface electric charge can be directly affected by the surface roughness, the composition of the implant alloy, sterilization, even by the handling during the surgical operation. An additional factor that affects osteointegration and survival rate is the implant shape. (4)

4. Nanocoating for biofilm inhibition

In order to stop microbial adherence on implant surfaces, different techniques have been developed in experimental stage or already in the market. Biofilm inhibition has been restrained and divided into three major categories. These are passive, active surface finishing / modification (Figure 3) and intraoperative antibacterial local carriers or coating.

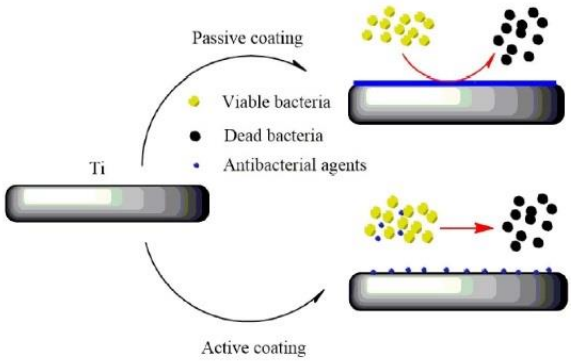


*Figure 3 R*epresentation of passive and active antibacterial Ti coating. Passive coating kills bacteria upon contact, while active coating kills bacteria by releasing antibacterial agents. *(Sheng et al.* Approaches based on passive and active antibacterial coating on titanium to achieve antibacterial activity. *Society for biomaterials 2018)*

a) Passive implant surface coating

In particular, passive surface finishing / modification (Figure 4) based on formation on the implant surface by chemical agents use or by modifying it without pharmaceutical active substance use. (3) Thanks to technological advances, we are able to form nanotubes in vitro on titanium implant surface in nanometer scale, which causes reduction of up to 90% of gram positive bacterial colonization capacity. The creation of nanotubes could promote also the function of osteoblasts. (9) Surface roughness, hydrophilicity, surface energy and conductivity, play an important role in the bacterial colonization of implant surfaces. An example is the change of the crystal structure of the

surface with scaffolds or in a different case, a polymer coating that reduces the adhesion of bacterial cell surface proteins. The main disadvantage of these coatings is the effect on the osteointegration process. Recent research reveals that increasing surface roughness in nanometer levels has an impact on bacterial adhesion on an implant surface. Unfortunately, there is no technique that can be applied to any biomaterial surface which will act on any kind of bacteria that may adhere. One drawback is that placing a coating on an implant surface may damage the osteointegration, leading to premature implant rejection. (3)

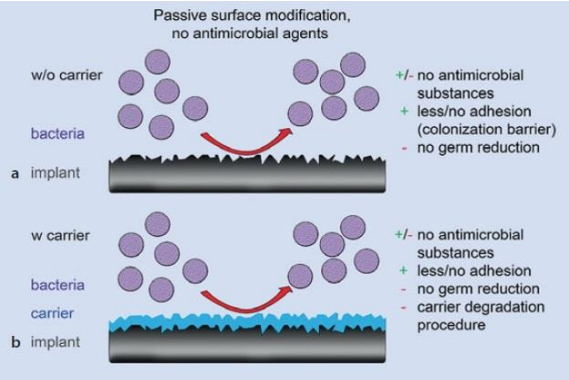


Figure 4 Mechanism of action on passive surface modification against bacteria. (K.-D. Kóhn et al Management of Periprosthetic Joint Infection © Springer-Verlag GmbH Germany 2018)

b) Active implant surface coating

In the case of active implant surface modification (Figure 5), we try to place agents on the surface which are gradually released. The goal of this modification is to reduce the bacterial adhesion on the implant. Agents which activate the implant surface are antibiotics, antiseptics, metallic ions or other organic and inorganic substances. (3) The application of these agents affects the surface electric charge and changes it, so that the implant can be able to cope with bacterial colonization. One major drawback of this application is the difficulty in predicting the long-term elution of a pharmaceutical active substance. Further inhibitors for the spread of this coating type are their implosion at the

time of the implant application, as well as the resistance of certain pathogenic bacteria to certain metals used in these coating techniques. (3)

Active surface finishing / modification can be divided into two subcategories. In the first category we classify techniques that when the bacteria try to attach to the surface of the implant, the contact kills them. In the second, we classify antibiotic coating techniques that emerge implant surface antibiotic coating. This antibiotic coating must have a concentration higher than the minimal inhibitory concentration (MIC) against the most common pathogenic bacteria. Those two subclasses have a double effect. First, they protect the implant from colonization, while giving time to the patient's immune system to respond promptly against pathogenic bacteria. (3)

The resilience of these coatings is divided into degradable and non-degradable applications. This means that the implant coatings have been tested using a variety of metals such as silver, zinc, copper. Also non-metallic elements such as selenium, iodine and organic substances such as antibiotics, contaminating peptides, chitosan and any possible combination of all the above agents. (3)

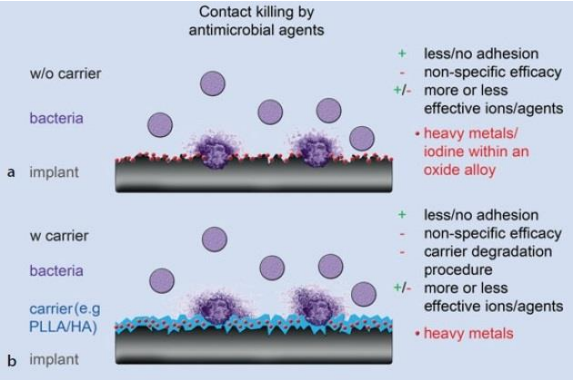


Figure 5 Mechanism of action on active coating with PLLA (Poly-L-lactacide Acid) and HA hydroxyapatite. surface modification against bacteria. (K.-D. Kóhn et al Management of Periprosthetic Joint Infection © Springer-Verlag GmbH Germany 2018)

c) Intraoperative implant surface coating

The third category of bacterial biofilm inhibitors includes intraoperative antibacterial local carriers or coatings (Figure 6). In this category, antibacterial agents or coatings are applied during the operation, as opposed to prior coating techniques. The greatest advantage of this technique is that it can be applied to any existing biomaterial, against any pathogenic bacteria. (3) The idea for antibacterial protection of implants intraoperative is not new. In 1970s several surgeons used orthopedic cement enriched with antibiotics like polymethylmethacrylates (PMMA). From time to time, other applications have been tested, like collagen sponges or most recently, orthopaedic cement enriched with calcium phosphate and antibiotics. The applications mentioned above are designed to protect the implants from biofilm formation. However they present some disadvantages related to high cost, potential inhibition of osteointegration and lack of plenty in vivo, in vitro clinical data. An Italian pharmaceutical factory NOVAGENIT® SRL (Mezzolombardo Trento), introduced a fast-absorbing hydrogel, suitable for any kind of implant based on hyaluronic acid and polylactic acid (PLA). DAC® (Defensive Active Coating) designed for short-term release of a drug substance (Vancomycin 5% or Gentamicin 3,2%) in order to combat bacteria trying to adhere to an implant surface. (3)

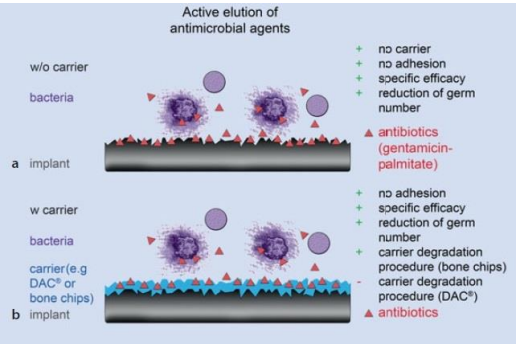


Figure 6 Intraoperative coatings that elute antimicrobial agents. (K.-D. Kóhn et al Management of Periprosthetic Joint Infection © Springer-Verlag GmbH Germany 2018)

5. Techniques of nanocoating applications

Titanium alloy (Ti) is widely used in orthopaedic implants, and more specifically in total hip arthroplasty. Different techniques across the world attempt to modify orthopaedic implant surface, using passive or active coating, to inhibit bacterial adhesion and colonization. The main objective of research is the prevention of biofilm formation on titanium implant surfaces. Different methods prevent bacterial adhesion by placing certain antibacterial agents on the implant surface, as active coating. Implant surface appropriate modify using passive coating. Titanium surface formation is done by a variety of techniques. Many trials have been made to turn the implant surface bioactive, with antibiotics, anti-microbial peptides (AMPs) and the release of inorganic ions. (10) Surface nanoparticles attempt to mimic their biological environment by simultaneously acting against infection and inflammation. (10) However, the cytotoxicity of these coatings is still under research. (11)

In order to cover titanium implant surfaces, different coating techniques have been developed. These techniques include : 1) Plasma spray coating, 2) Plasma immersion ion implantation and deposition (PIII & amp D), 3) Physical vapor deposition (PVD), 4) Electrospinning, 5) Sintering between two metals, 6) Plasma Electrolytic Oxidation (PEO), 7) Magnetron sputtering, 8) Atom transfer radical polymerization (ATRP), 9) Layer by layer deposition and 10) Click chemistry.

Plasma spray coating

The application of this technique requires high temperature and the coating materials are in powder form. As a result, the high temperature causes the coating materials to melt, which are then sprayed onto the surface we want to cover. (12)

Plasma immersion ion implantation and deposition (PIII & amp D)

This is the most widespread technique for creating admirable coatings with the use of electric discharge in a vacuum. Plasma is generated to aid the deposition of positively charged ions on any surface material. As a result, an ion-oxide film with continuous interactions is produced. (12)

Physical vapor deposition (PVD)

It takes place in a high vacuum chamber where the metal is evaporated and then its vapors deposit on the surface of the material to be coated. (12)

Electrospinning

Electrospinning use high potential electric field (Figure 7), consists of syringes that absorb the material to be coated. This coating procedure produces ultra-fine fibers with a diameter in the nanoscale range and their deposition on the surface material. (13)

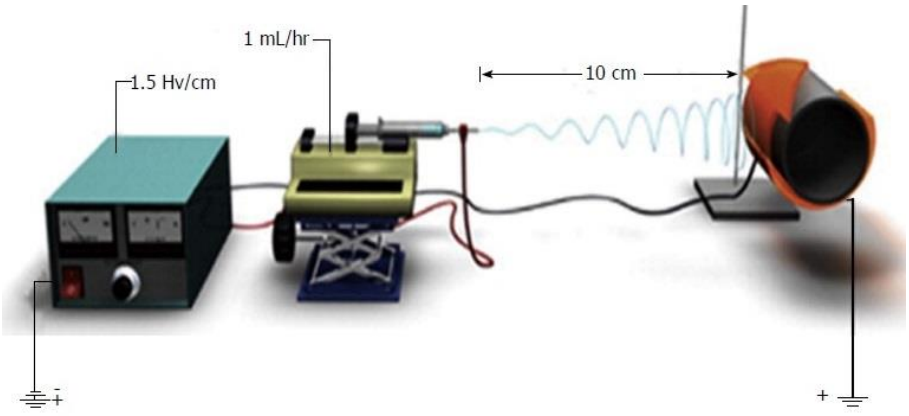


Figure 7 Charging electrospining apparatus spinning a PLGA Poly (lactic-co-glycolic-acid on implant surface. (Adam EM Eltorai et al. World Journal Orthopaedics 2016 June 18 © 2016 Baishideng Publishing Group Inc. All rights reserved*)*

Sintering between two metals

Sintering between two metals, usually silver and titanium. The process takes place in atmospheric pressure, creating porous surfaces. The nanoparticles formed may have a diameter varying from 10nm to 75nm. (14)

Plasma Electrolytic Oxidation (PEO)

Plasma electrolytic oxidation (PEO) is a process that uses high voltage to generate oxide film coatings on metal surfaces. It is characterized by plasma arcs that move rapidly on the metal surface. Surface coating is the result of multiple surface discharges due to plasma thermo-chemical interactions. In particular, the voltage regulation changes the surface microstructure, converting it from a metal alloy to a ceramic coating. This process offers greater protection against corrosion and is similar to anodization. (15)

Magnetron sputtering

The magnetron sputtering technique (Figure 8) allows the deposition of metal oxides such as zinc oxide, indium tin oxide, tin oxide at low temperatures. This technique can be applied for large-scale production. Magnetron sputtering uses a combination of electric and magnetic fields for coating. The magnetic field is adjusted, so that electrons but not ions are strongly affected during the coating process. (16)

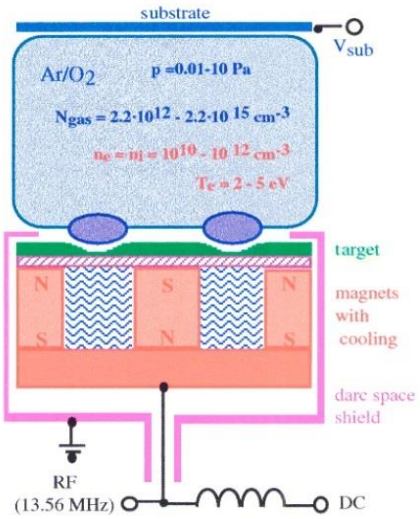


Figure 8 Magnetron sputtering configuration. (Klaus Ellmer Hahn Magnetron sputtering of transparent conductive zinc oxide: relation between the sputtering parameters and the electronic properties Journal Physics. D: Applied Physics 33 2000)

Atom transfer radical polymerization (ATRP)

The name originates from the atom transfer step. This step plays a crucial role in the reaction that creates uniform polymer chains. The ATRP mechanism is based on the reversible oxidation reaction of two materials in the presence of a catalyst and a material in intermediate phase serving as a ligand in the reaction. The reaction is conducted at a controlled rate to produce polymer chains at a constant rate as it occurs in conventional polymerization. (17)

Layer by layer deposition

This deposition technique involves the application of a two-component electrostatic force with opposite electric charges on the material surface. (18)

Click chemistry

The CuAAC (Cu Catalyzed Azide-Alkyne Cycloaddition) click chemistry method is catalyzed by Cu. It has been developed to make bioactive molecules stick to titanium

surface with specific orientation that allows them to have antimicrobial activity. At the same time, it protects the activity of molecules during the implant coating process. (19)

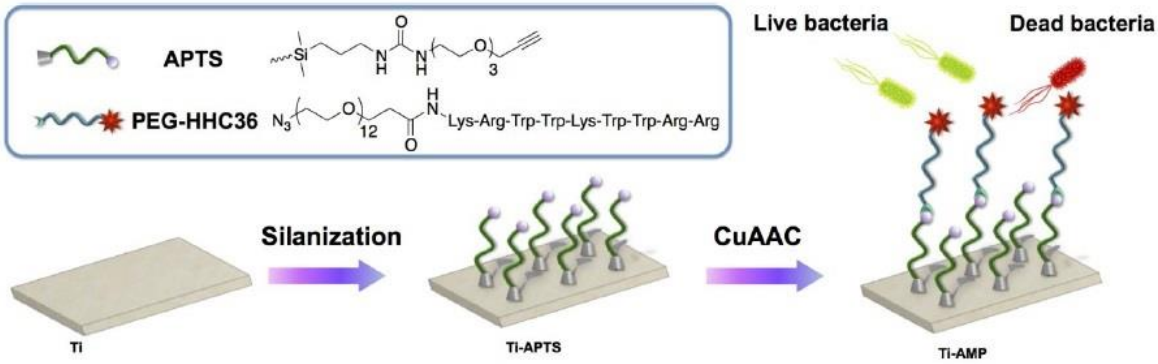


Figure 9 CuAAC click chemistry on titanium surface. (Junjian Chen et al. Antimicrobial Titanium Surface via Click-Immobilization of Peptide and Its in Vitro/Vivo Activity ACS Biomaterials Science and Engineering 2018)

6. Nanomaterials for surface antibacterial coatings

Scientific groups modify titanium surfaces and perform experiments to test the physicochemical surface properties (i.e. chemical composition and hydrophilicity). This approach inhibits bacterial adhesion and biofilm formation. The great advantage of this method is that no antibacterial agents are released from the implant surface that could damage bone marrow mesenchymal cells. An additional advantage is that there is no mutation of bacteria to resistant strains. In contrast, when bacteria adhere on the implant surface, the smooth surface could not kill them in vivo. This is due to body plasma protein coat implant, so bacterial colonization can be accelerated. (11)

Passive surface mimicking natural antibacterial textures

In one surface modification effort a nanotropic composition was made to damage bacterial cell membrane. In particular, the pathogenic bacterium Staphylococcus epidermidis .The inspiration for the formation of a surface with antibacterial properties came from nature, by observing lotus leaves and nanostructured shark skin. Those natural textures are difficult to transfer on hard composition materials like titanium. The dragonfly feathers texture acts against Gram- negative strains of Pseudomonas aeruginosa and Gram-positive Staphylococcus aureus due to their spike formation, but at the same time promotes staggered action by fibroblasts. Nanotube formation observed in these cases exhibits superhydrophobic properties against Staphylococcus epidermidis adhesion.

In vitro studies examined two different surface modifications by Mr Yunil Cao's scientific team (2018). The team used the same surface treatment technique in different time frames. The surface of the first implant was treated for two hours. The nanotube formation had a sharp-edged shape (Figure 10). The second implant surface received an additional hour treatment by machined nanoparticles. Bacterial colonization and biofilm formation was observed within six days after the initial infection. In contrast, pocket type

surface suspended biofilm formation six times. It managed to kill colonic bacteria by compressing and leading them to the sharp edges formed. As a consequence, the bacterial cell membrane was destroyed by this formation. The second surface formation was more effective compared to the first. It managed to destroy a larger number of bacteria and prevent biofilm formation for a longer period of time. These studies of titanium surface modification require further investigation about mechanical loads application, to ascertain whether these structures are maintained as well as the assessment after their introduction into animal models. (20)

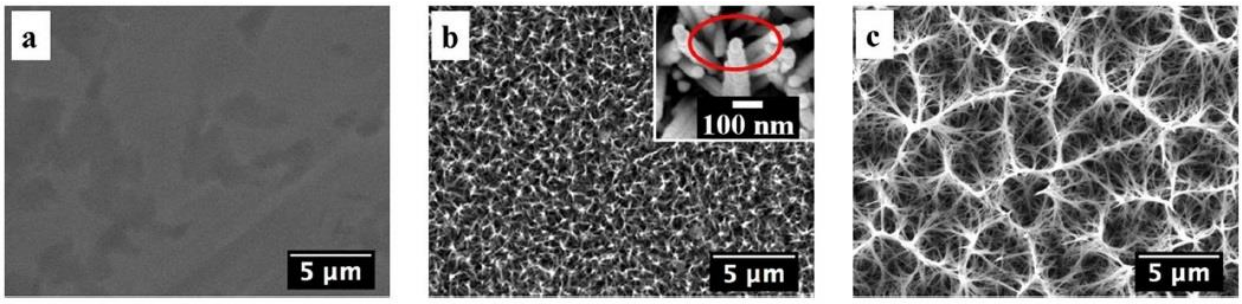


Figure 10 a) polished titanium b) spear type The inset at 100.000X magnification c) pocket type SEM images at magnification of 8000X. The inset shows a SEM. (Yunyi Cao et al. Nanostructured titanium surfaces exhibit recalcitrance towards Staphylococcus epidermidis biofilm formation. Scientific Reports 2018)

Besides the formation on titanium surfaces, some other materials have been explored in this direction. Ceramic biomaterials such as zirconia, alumina and hydroxyapatite have been used predominantly in artificial joints. The degree of degradation in these applications is quite low and due to their physicochemical surface properties, they can exhibit bacterial colonization resistance. One major disadvantage, however, is the lack of bibliographical references about their use in orthopaedic applications. In a comparative in vitro study by Mrs. Rita Sorrentino (2017) between ceramic biomaterials and chromium molybdenum, decreased bacterial adhesion and reduced biofilm formation was observed on the ceramic surfaces, by pathogenic bacteria strains investigated (Staphylococcus aureus, epidermidis) for inhibitory effects. This comparison was made in order to propose

the use of bioceramics in total hip arthroplasty to replace infected implants. (21)

Active surface antibacterial coatings

Some scientific teams approach the titanium coating challenge by experimenting with hydroxyl group coatings such as -OH, NaOH and NH_2, silane acting as a biomolecule linker between titanium and pharmaceutical molecules and antibiotics such as vancomycin, bacitracin or antimicrobial peptides in local delivery for sustained release pharmakokinetics. Chitosan has been used on negatively charged surfaces preventing bacterial proliferation. Hydroxyapatite combined with antibiotics inhibits biofilm formation, but combined with silver promotes osteointegration. We should not omit to mention the formation attempt on titanium surfaces enriched with nanotubes carrying antibiotics or nanoparticles of silver, gold, zinc. The purpose of this configuration is the simultaneous antibacterial and osteogenic action by orthopaedic implants. In addition, experiments have been carried out with successive coatings of charged polymers at nanoscale level leading in electrostatic interactions with the surface formation. (11)

Silver nanoparticles

When further exploring the literature related to titanium coatings, we can distinguish applications with silver nanoparticles that enhance local antimicrobial action without the disadvantages of systemic antibacterial or antibiotic therapy. (22) The action of silver against any species of bacteria, virus, protozoa or fungus is well-known. The mechanism of action of silver (Figure 11) has been explored in laboratory. It is based on releasing bioactive ions from a surface that has been coated. These ions are attached to different cellular structures such as cell walls containing peptidoglycan, bacterial proteins or bacterial DNA. Initially, ions break through the cell membrane, causing the cell to lose its consistency. As a result, the cell contents are exposed in extracellular environment. At a second stage, ions interact with SH-linked (Sulfur-Hydrogen). SHs located in bacterial

proteins and enzymes that are vital in bacterial cell survival (respiration, permeability). The effect of silver ions on the DNA of bacterial cells may damage the reproductive capacity. Additional toxicology has been observed due to production of free oxygen radicals. In combination all these effects on sulphide bonds (SH) may cause free oxygen radicals. A key factor in the ion extraction is the size of nanoparticles in which they are released. Silver nanoparticles below 10nm are much more active, since there is a larger active surface. The size of nanoparticles is more important compared to the use of larger mass of nanoparticles. (23)

Silver nanoparticles effectiveness of less than 20mg / L has been found to be very high in particularly small doses without simultaneous cytotoxicity or resistance to bacterial strains resistance, compared to ionic silver. It is important to note that silver does not causes point mutations in bacterial genome like antibiotics do, due to simultaneous action at several levels. This property helps to tackle the ever-increasing antibiotics use leading in resistant bacteria strains.

Apart from the beneficial action of the silver nanoparticles, there are some side effects such as argyria, clotting in kidneys and liver. Many silver forms already used in clinical trials have not been observed to be highly toxic or cause serious health side-effects. Silver nanoparticles toxicity depends on size, surface shape, charge, shape, dosage, ion release capacity and time remaining within the body. As an example, spherical nanoparticles are less toxic compared to nanoscale wire. Negatively charged nanoparticles are less toxic. Each type of nanoparticle is affected by factors within a biological environment. For human osteoblasts, it has been found that concentration of more than 10 mg / g is toxic. In conclusion, the smaller a silver nanoparticle is, the more toxic it is, due to the faster release of ions, which in turn is due to the surface-to-volume ratio, while parameters such as gender, age, general health of the patient and time of exposure affect the degree of toxicity. In the kidney and liver the concentration increases more rapidly after entry into the body compared to the brain and the pulmonary pulp, where accumulation increases

after prolonged presence in the body. (24)

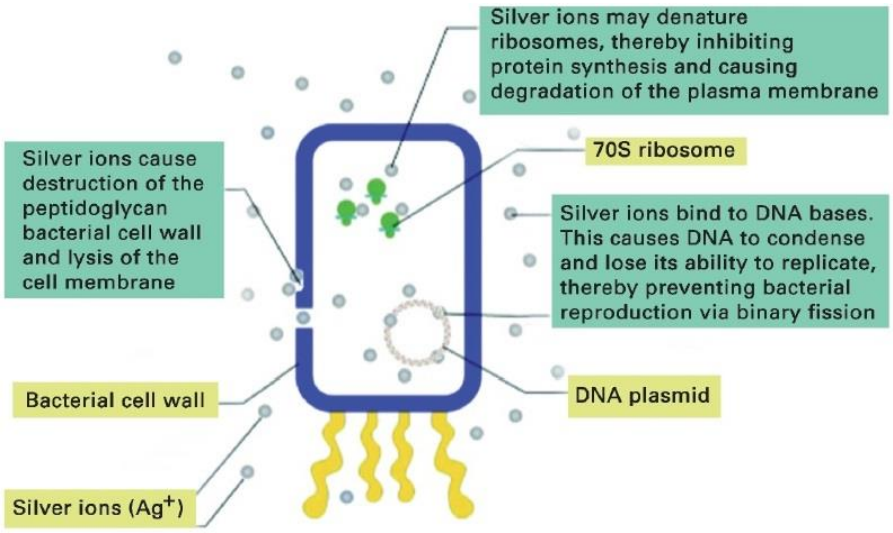


Figure 11 Action mechanism of silver nanoparticles. (S. A. Brennan et al. Silver nanoparticles and their orthopaedic applications. The bone & joint journal 2015)

Silver nanoparticles film coating

A scientific team headed by Mrs Sarah Goderecci investigated silver-coated film surface formation on titanium using the reactive magnetron sputtering (RMS) technique in order to inhibit the primary bacteria attachment or pre-emergence bacteria biofilm. The RMS technique produced AgO and Ag_2O or mixtures of these oxides, which are inherently effective against Staphylococcus aureus. Specific oxides are applied to a plurality of surfaces and materials. The method we report produce nanoparticle sizes with 100nm diameter and 60nm height. Although different metals have been studied for their antimicrobial properties, silver nanoparticles are those that exhibit broad antibacterial and antiviral spectrum. Since we know silver dissolution rate over time, we can calculate the silver ion extraction from known nanoparticle dimensions. In this particular study, the rate was $4x10^{16}$ ions / cm^2. Changing dimensions also results in changing the ion yield rate, so greater amount of silver nanoparticles can increase bactericidal activity. Different concentrations of silver ions were studied in a wide range of Gram positive and negative

bacteria to test simultaneous efficacy of both bacterial classes. It has been suggested that a silver concentration close to 52 mg / ml is ideal, but should be explored more in vivo environment since many studies have shown silver cytotoxicity. In vitro environmental cytotoxicity occurred only in a small portion of the culture. As the experiment was conducted in ambient temperature, ion release should further be explored within body temperature, so we can have a more accurate image of the antimicrobial and cytotoxic silver film properties on titanium surface. (25)

Silver nanoparticles and antibiotics combination coating

In recent years, in vitro and in vivo trials have been made on the use of silver nanoparticles as titanium coating. Different coating ways, in combination with other materials or antibiotics, in order to inhibit the biofilm formation by simultaneous osteoblasts activation. In this study of Mr Jiaxing Wang, silver nanoparticles were incorporated into titanium oxide TiO_2 nanotubes with the help of the technical PIII technique (Figure 12). Titanium oxide nanotubes have been used as "storage area" for antibacterial agents due to their excellent biocompatibility in order to provide short and long lasting protection. Vancomycin placement in titanium nanotubes was done by the vacuum extraction and lyophilization technique. This coating protects the implant in a small period of time through the release of antibiotics at the peripheral implant region. Antibiotic delivery was made in two stages. A large amount of antibiotics were released in a 24-hour period and in a second period of 28 days, gradually diminished without simultaneous diffusion of silver ions in 28 days. Silver nanoparticles help by releasing ions that destroy bacterial cell the when it touches the implant. These antibacterial actions offer long-lasting antibacterial protection. The test was made both in vitro and in vivo rabbit models. The two-dimensional surface modification (active, passive coating) with antibiotic and silver nanoparticle has resulted in implant protection during perioperative period while promoting further the fibroblasts action. (6)

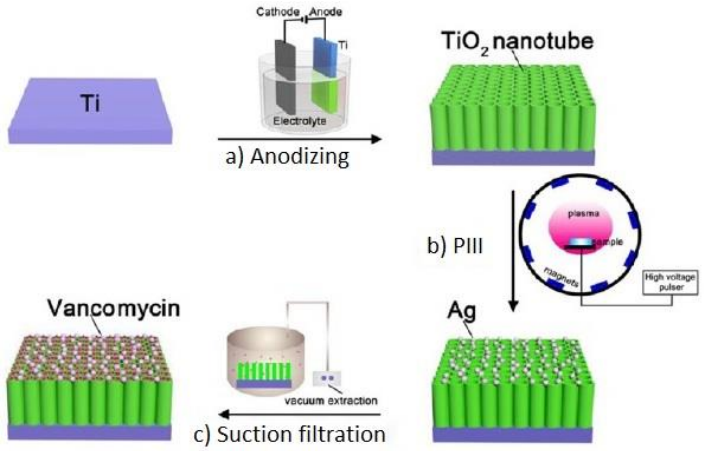


Figure 12 a)anodic acidation to form nanotubes b) PIII to embed silver nanoparticles in nanotubes c) vacuum extraction to load vancomycin. (Jiaxing Wang et al. Antibacterial Surface Design of Titanium-Based Biomaterials for Enhanced Bacteria-Killing and Cell-Assisting Functions Against Periprosthetic Joint Infection ACS Applied Materials and Interfaces 2016)

Mr Zhang Yuan's research team tried to make active titanium surface in vitro. They tried to place silver nanoparticles into 70nm diameter titanium oxide nanotubes coated with chitosan (CHI) and dialdehyde alginate (ADA). They formed titanium nanotubes using ultraviolet radiation and nanoparticles 20-40nm in diameter, through electrochemical deposition into titanium oxide nanotubes. Layer-by-layer self-assembly (LBL) was applied to place 10 layers in consequence. This technique makes titanium more biocompatible with extra antibacterial properties. Layer-by-layer self-organization is made by electrostatic forces and covalent bonds between amino group ($-NH_2$) of chitosan (polycation) and aldehydes of the dialdehyde alginate (ADA) (polyanion). This reaction stabilizes the coating materials further via silver-ion controlled release at a low release rate, in order to reduce its toxicity. This happens by the simultaneous activation of the osteoblasts. Gradual release prevents bacteria from adhering to the implant surface. This method demonstrated in vitro (Figure 13), can help the growth of osteoblasts while reducing bacterial colonization on the implant surface by controlled silver ions release. (26)

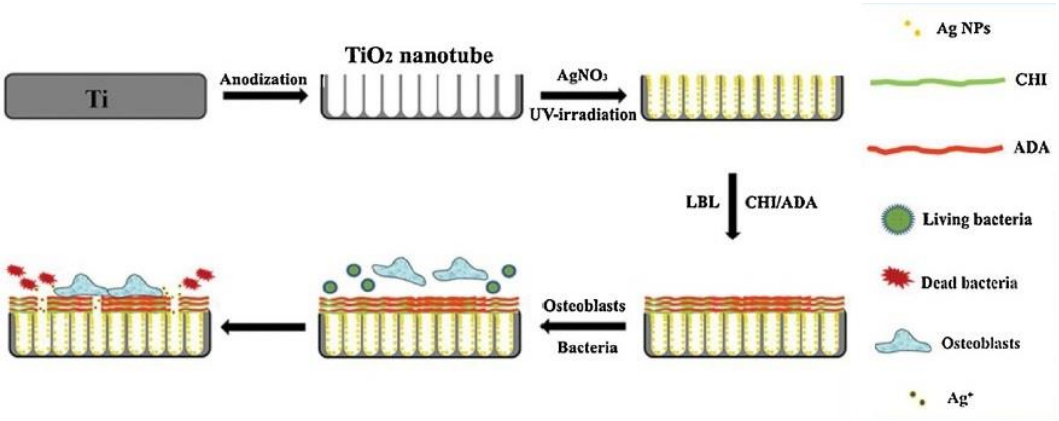


Figure 13 Image of nanotubes construction covered with Chitosan/ADA layer by layer films. (Zhang Yuan et al. Construction of Ag-incorporated coating on Ti substrates for inhibited bacterial growth and enhanced osteoblast response. Colloids and Surfaces B: Biointerfaces 171 2018)

Mr Zhanpeng Zeng's team tested on animal models the use of titanium oxide nanoparticles enriched with silver oxide nanoparticles against Staphylococcus aureus infection. Silver nanoparticles were deposited in nanotubes by using the pulsed electrophoretic deposition method. It turned out that the coating with these characteristics fared well against Staphylococcus aureus. The broad antibacterial spectrum of the silver nanoparticles and the nanotube formation allowed a slow release rate. (27)

The application of silver nanoparticles by another scientific group headed by Mr Jiaxing Wang proved in vitro that a modified surface may interact with different strains of Staphylococcus (epidermidis, MRSA). Furthermore, bacteria already adherent or placentally, also inhibited the gene expression that helps in biofilm formation. Genes like icaA, icaR for Staphylococcus epidermidis or fnbA, fnbB for MRSA. The implant coating process tested in this study was the hydrothermic chemistry mode (HCM). This method has the advantage over other methods, because it can adjust the surface morphology according to the researcher's requirements. As a consequence, the rate of silver nanoparticles loading, used in this study, can be controlled. Correspondingly, the PEO was used to form the surface of the titanium before it was coated. This method forms porous

of similar porosity on the titanium surface and the coating promotes biocompatibility and bioactivity. In previous bibliographic references, this pretreatment with silver nanoparticles is necessary for the gradual and not abrupt ions release, thus reducing their cytotoxicity. Surface deposition of silver can change the surface's electrical charge when the implant is in a bacterial environment. Through this deposition, the implants surface acquires bactericidal properties with a greater dose of silver nanoparticles, thus having greater antimicrobial action. The effect of this coating type on biofilm formation is due to the ions cell destructive properties, since it can enter the cytoplasm by destroying more intracellular constructs and expressing genes, like icaA, icaR for Staphylococcus epidermidis or fnbA, fnbB for MRSA, found from experimental study measurements. Finally, measurements showed that fibroblast activity increased due to biofilm inhibition by silver nanoparticles. The two types of bacteria were scarred by giving incurable results about orthopaedic applications. (28)

Silver nanoparticles with chitosan and vancomycyn combination coating

More recent attempts of Mr Croes' research team were made on titanium-coated surface with a combination of chitosan and silver nanoparticles in different concentrations of chitosan, with vancomycin in vitro and in vivo. At the in vitro part of the study, the group focused on the implant's behavior in terms of osteoblast and neutrophil cholesterol compatibility and ability to kill bacteria. The in vivo part of the study included an assessment of the osteogenic, immunogenic and antibacterial titanium coating's action. The manufacturing of titanium implants was done by using the direct metal printing method (DMP). The coating of enriched chitosan gel with silver nanoparticles or vancomycin was made by the Electrophoretic deposition (EPD) technique. Antibiotic implants were used as a means of control for antibacterial capability of the formed surface. The bacterial strain investigated in vitro was Staphylococcus aureus in two different time points (after 1 day and 7 days). It was observed in vitro that this coating

method with enriched chitosan – vancomycin, completely neutralized the Staphylococcus aureus. Chitosan and nanoparticle-coated silver implants also reduced the Staphylococcus aureus adhesion against bacterial cells, which they believe are bactericidal, although the high silver nanoparticles concentration has damaging osteoblasts action. It was discovered that the in vivo the placement of coated implants in tibial rats model after 28 days, the chitosan and silver nanoparticle coated implants did not cause particular osteogenesis change, neither did it change the appearance of inflammation. They find that, under appropriate conditions, the osteogenic capacity of the organ that received an implant can overcome the cytotoxic action of silver nanoparticles on osteoblasts. Usually this happens when there is no increased microbial load. In a different case, silver nanoparticles can exacerbate an already existing inflammation, because they have strong effect on neutrophil cells, the organism's first line of action against inflammation. (29)

Vanadium nanoparticles

Over the past few years, research teams have been dealing with metallic nanoparticles other than silver nanoparticles. Scientists have begun to investigate the effects of vanadium as a nanoparticle both in vitro and in vivo (Figure 14). Vanadium can be found in the human body, mainly in bones and cartilage, as a tetravalent or pentavalent ion. It is a vital trace element due to its multiple actions as a bioantioxidant. As a growth factor and an antioxidant, it helps accelerate osteogenicity. Vanadium compounds of different valency and hemopoietic process play an important role in processes such as sugar metabolism, due to a similar effect to insulin, or osteogenesis. Also vanadium contribution to chondrogenesis is of major importance. It inhibits the production of cholesterol and is involved in the creation of hypertension. (30)

In addition to silver nanoparticles, some scientific groups such as Mr Jiaxing Wang's, tested in vitro the use of vanadium nanoparticle films. Researchers study the resistance to Staphylococcus aureus and erythrocytes. The coating of a Quartz glass was done by the

magnetron sputtering technique to investigate how these different vanadium nanoparticles (V_2O_3, VO_2, V_2O_5) 50nm in diameter, helped blood flow erythrocytes to combat bacteria that move inside the veins. These nanoparticles were selected to offer additional protection to a biomaterial from pre-implantation infection. The cytotoxicity of vanadium is due to the concentration in mitochondria on cells and is dose-dependent in biological systems. The mechanism of vanadium action works against the cellular disruption of cellular electron translocation. As a result, it damages the membrane functionality and increases the intracellular production of free radicals. This suggests that vanadium with different valency, interferes with the regulation of the intracellular oxidative activity. Measurements done by scientists showed that the release of vanadium ions with different vigor from coating films was continuous. Those films with micro valence (V_2O_3, VO_2) are more compatible compared to cells with increase valence (V_2O_5) that exhibit strong antibacterial activity against Staphylococcus aureus. (31)

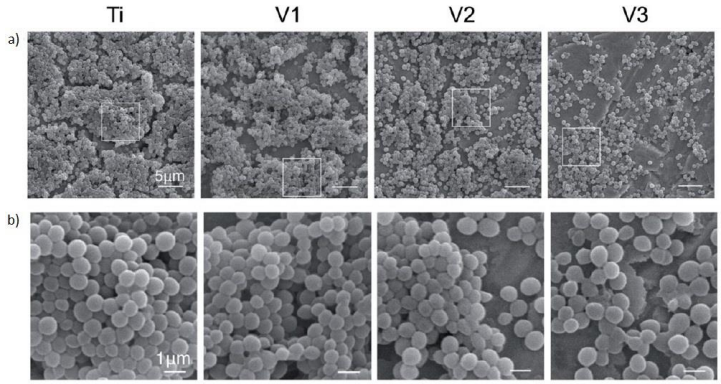


Figure 14 In vitro evaluation of vanadium nanoparticles a)SEM images b) magnification of white box in a) image. (Jinxiao Guo et al. Nano vanadium dioxide films deposited on biomedical titanium: a novel approach for simultaneously enhanced osteogenic and antibacterial effects Artificial Cells, Nanomedicine, and Biotechnology An International Journal 2018)

Another research team, headed by Mr. Jinxiao Guo, tested vanadium nanoparticles in vitro and in vivo conditions for future use in hip replacement patients without the use of orthopaedic cement. The use of vanadium dioxide VO_2 was investigated as an osteogenic

factor and as an antimicrobial agent against resistant strains of MRSA. The nature of vanadium dioxide promotes osteogenicity with concurrent action against bacteria, because of controlled ions release. The ions in turn induce a controlled reactive oxygen species reaction in bone marrow mesenchymal cells of rat model, by increasing the osteointegration degree. Corresponding controlled ions release against bacteria destroys them without increasing the toxicity of the patient's cells. Vanadium dioxide coating on a titanium surface was done by using the magnetron sputtering technique. The analysis of results obtained from in vitro and in vivo conditions. The vanadium dioxide coating can have beneficial properties both in osteointegration and in MRSA inflammation treatment (Figure 15). (30)

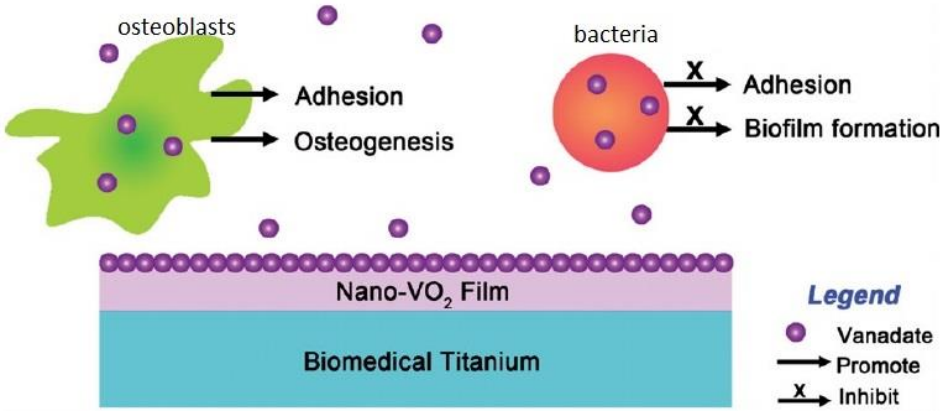


Figure 15 Schematic illustration of VO_2 action in inflammation and osteogenesis The VO_2-modified surface possesses good antibacterial ability. *(Jinxiao Guo et al. Nano vanadium dioxide films deposited on biomedical titanium: a novel approach for simultaneously enhanced osteogenic and antibacterial effects Artificial Cells, Nanomedicine, and Biotechnology An International Journal 2018)*

Zinc nanoparticles

Another metal that can be found in the human body as a trace element has raised the interest of research teams over the past few years. This is zinc that we find primarily in the bone structure. (32) Zinc significantly affects the bone development and it can modulate the bone metabolism. In an in vitro research it has been reported as necessary

to increase osteoblasts activity due to increased cell division. Furthermore, it promotes alkaline phosphatase activation, through collagen synthesis and protein expression, but also inhibits the osteoclasts function. (33) In addition to its significant effect on osteogenesis, zinc also is involved in a variety of biological processes, such as hundreds enzymes and proteins operation. Through the DNA synthesis regulation, it affects the cell division and it appears to be involved in the hormone regulation mechanism as well. The zinc oxide has been studied as a broad spectrum antibacterial agent. It increases the reactive oxygen species (ROS) production that impacts the direct bacterial cell membrane structure. In nanoparticle form, it adheres to cell membrane by affecting its functionality. Due to its valence, zinc occupies a position in the cell membrane surface proteins. It significantly affects the bacteria metabolism and eventually leads them to death. Finally, in the field of biomaterials, there has been synergistic research with hydroxyapatite or as an additive to metallic materials. (32) But scientists should be aware about gradual release and high concentrations of zinc, as it may be toxic. (33)

Researcher L. Sopchenski dealt with titanium coating by zinc nanoparticles to assess in vitro ion release for cytotoxicity and bactericidal activity against the Staphylococcus aureus strains. The coating technique applied was PEO (Figure 16). After the coating, no changes in crystallinity and on the titanium surface were observed, despite the fact that the titanium was coated with zinc. The measurements showed that zinc ions were released even after 28 days in DI water. The greatest antibacterial effect was observed after six-hour incubation with a sluggish course in the first 24 hours and full balance (between dead and live bacteria) after 7 days. Zinc oxide coating appears to not have been cytotoxic against adipose derived stem cells. Also, it has protected the metallic surface from bacterial colonization in vitro. The investigation of the same device at in vivo model is necessary. (32)

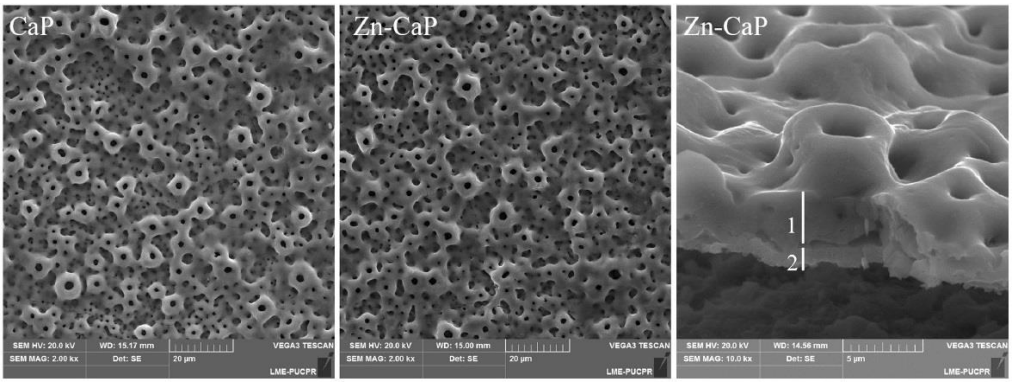


Figure 16 Sem images of CaP and Zn-CaP coatings and cross-sectional image of the Zn-CaP coating. *1)Outmost porus layer 2)inner dense layer obtained by PEO. (L. Sopchenski et al. Bactericidal activity and cytotoxicity of a zinc doped PEO titanium coating. Thin Solid Films 660 2018)*

Zinc, as coating on titanium surface implants at in vivo conditions, has been the subject of Mr. Guodong Jin's study. He studied zinc behavior as a promoting osteogenesis factor in an animal model of rats. For covering titanium, the scientific team used the PIII, without changing the surface roughness, microstructure and wettability. Additional properties resulting from the experiment's measurements are quicker response of osteoblastic cells, mineralization of extracellular matrix in animal model and collagen production. In addition to the above properties, zinc exhibits significant anti-bactericidal effect against Staphylococcus aureus, compared to titanium without any coating. It also appears to play a role in the alkaline phosphatase activity. Incidentally, the PIII helped in the control of zinc ions release. This property does not cause any implant cytotoxicity in the patient's body while retaining its antibacterial and osteogenesis properties. (33)

Boron nanoparticles

In searching for new materials that could offer antibacterial protection, scientists have turned their attention to a trace element that has been used up to today as a food preservative. Boron has been recently identified as a pharmaceutical substance that acts against a wide range of Gram positive and negative bacteria. It prevents biofilm formation

and acts against fungi. The mechanism of action against bacteria is not fully understood and is considered to be non-specialist. In all the studies up to today, research has shown that it changes the functionality of bacterial membrane. This happens because boron reacts on phospholipids, glycoproteins and lipopolysaccharides. Boric acid can be produced by various reactions. It affects enzymes and metabolism, because it can penetrate the cell membrane. Boron's effect on biofilm is due to block gene signals from bacteria that begin the biofilm formation. Finally, boron takes part in many biochemical processes within the human body. It promotes the expression of growth factors that are involved in the healing processes (VEGF and TGF-β). It also suppresses certain enzymes action that is involved in the inflammation progress. (34)

Researchers, headed by Mr. L. Sopchenski, applied the PEO technique to deposit boron on a titanium surface without altering the roughness, morphology and surface crystallinity. An evaluation against Staphylococcus aureus and Pseudomonas aeruginosa was done by comparing the boron coated titanium surface to the clear titanium surface in vitro (Figure 17). The results showed that the boron coated titanium inhibited the biofilm synthesis by preventing the potential growth of infection. Boron expands the research for new trace minerals that are less toxic and more effective against bacteria. This study demonstrated for the first time that boron is less cytotoxic and equally bactericidal, compared to well-known trace elements used in titanium coating. (34)

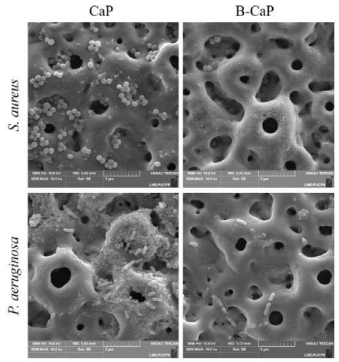


Figure 17 Boron coating against Staphylococcus aureus and Pseudomonas aeruginosa in 24 hour evaluation SEM images of the B-CaP TiO2 coating. (L. Sopchenski et al. Bioactive and antibacterial boron doped TiO2 coating obtained by PEO. Applied Surface Science 458 2018)

Magnesium nanoparticles

In addition to trace elements that have been reported so far, researchers have started to explore magnesium as an antimicrobial agent in recent years and also as an accelerant of osteointegration, due to its effects to bone metabolic processes. This is due to the presence of magnesium ions. The antibacterial capability of magnesium is not clear yet and we will need further studies to this direction in order to clarify the mode of action for use in orthopaedic applications. (35)

Mr. Geyong Guo's research team has coated titanium surface with a magnesium fluoride (MgF_2) nanoparticle film. The scientific team used the magnetron sputtering technique at different nanoparticle diameters (50nm, 100nm, 150nm). The aim was the in vivo and in vitro study of the antimicrobial ability of the MgF_2 nanoparticles against Staphylococcus aureus and Staphylococcus epidermidis. In vitro measurements showed a remarkable inhibition ability of biofilm formation, as opposed to the inhibition ability of anti-planktonic bacteria. Ionic effect occurred only up to 12 hours after incubation. Biofilm formation inhibition is probably due to bacteria that adhere on the implant surface, but also to bacterial necrosis that were killed when they came in contact with the coated implant surface. In both cases, the reaction against bacteria was proportional to the nanoparticle diameter. In the osteomyelitis rat animal model the performance against active infection was remarkable. Cytotoxicity of different nanoparticle diameters was not apparent in this study, but there was a large difference between in vivo and in vitro effects, possibly due to the macrophage cells antibacterial aid present in animal models. Finally, research has shown that although there have been bactericidal effect, more research on the mechanism of action of magnesium for orthopaedic applications is needed. (35)

A team of researchers headed by Mr. D. Sivaraj combined hydroxyapatite-bound magnesium nanoparticles located in single-wall carbon nanotubes in order to protect stainless steel implants from erosion and to test its antibacterial protection in vitro. The

surface coating was carried out by the spray pyrolysis deposition technique, which was developed specifically for this type of coating (Figure 18). This composition reduces the bone mineral absorption while increasing bone density, thanks to a similar growth factor in the early stages of osteogenesis. Carbon nanotubes make the implant surface more hydrophilic. As a consequence, this effect improves osteointegration. Antibacterial evaluation was performed against Staphylococcus aureus strain. Experimental measurements showed that the coating contributes to the corrosion and anti-corrosion properties of stainless steel. The chances of using this biomaterial property in orthopaedic applications are increasing. (36)

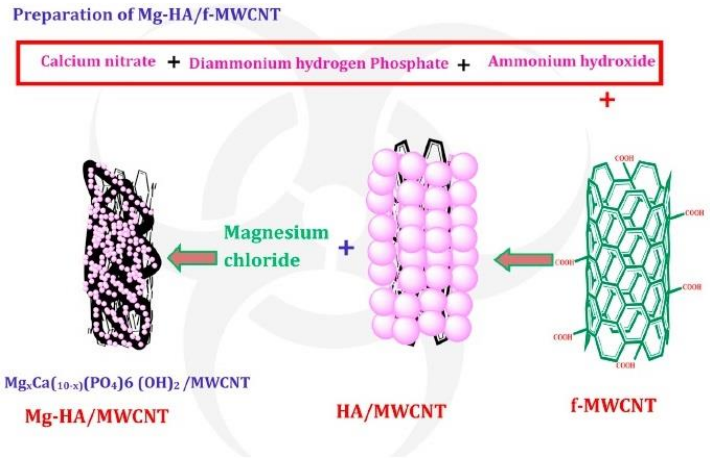


Figure 18 Preparation of multiwall carbon nanotubes with Mg/HA nanoparticles. (D. Sivaraj et al. Substantial effect of magnesium incorporation on hydroxyapatite carbon nanotubes coatings on metallic implant surfaces for better anticorrosive protection and antibacterial ability. Journal of Analytical and Applied Pyrolysis 2018)

Lactoferrin

Recently, the research into implant antibacterial protection with simultaneous stimulation of osteoblasts for faster osteointegration has begun to turn its attention to proteins. For example, lactoferrin is an iron-binding glycoprotein that promotes the differentiation of cells to the osteoblasts pathway. Their use of an overlay showed antimicrobial activity. This is due to the bacterial adhesion inhibition. In this way

lactoferrin indirectly affects the biofilm formation and, also, it seems to help the immune system response. (37)

A few months earlier, Mr. Tingting Shen's research team dealt with a titanium surface coating with polydopamine-assisted hydroxyapatite and lactoferrin multilayer structure (PDA-HA-LF). The scientific team assessed its antibacterial capacity in vitro against Staphylococcus aureus strains. The coating was done with the spin-assisted LBL. This way lactoferrin and carboxymethyl cellulose (CMC) creates a stable mattress structure. This structure is expanded in many different layers. CMC is chosen as a natural polymeric anion because of its ability to retain moisture and to create strong crosslinks between its molecules. Hydroxyapatite is added to improve the synthesis of organic and inorganic compounds on the coated titanium surface. Dopamine addition increases the layers' adhesion on the titanium surface (Figure 19). The results obtained from this synthesis showed that the lactoferrin antimicrobial activity reduced significantly the Staphylococcus aureus adhesion ability on titanium. In this form, lactoferin exhibited significant antibacterial activity. In any case, more research is needed to set the appropriate lactoferrin concentration that maximizes the results against bacteria and osteogenesis promotion. (37)

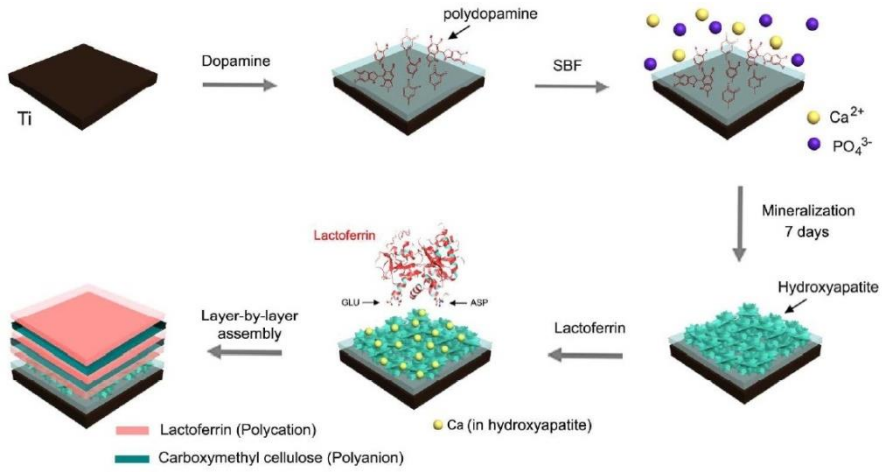


Figure 19 Fabrication of PDA-HA-LF on Titanium with layer by layer technique. (Tingting Shen et al. Polydopamine-assisted Hydroxyapatite and Lactoferrin Multilayer on Titanium for Regulating Bone Balance and Enhancing Antibacterial Property. ACS Biomaterial Science and Engineering 2018)

Catechol

The research for more biocompatible materials so that they can be used as orthopaedic implant coating has made scientists search the action of certain organic compounds, such as catechol. The catechol structure exhibits a high tissue adhesion ability and excellent biocompatibility. In past years, attempts have been made to modify chemical catechol by combining it with polymeric materials, such as polyethylene glycol, hyaluronic acid or chitosan and alginate. Recently, attempts were made with modified catechol layers on a titanium surface, which showed high antioxidant activity in a laboratory environment, while in vivo conditions osteogenesis was promoted. (38)

Considering all the above, a scientific team headed by Mr. Zhang Yuan, tested a functional catechol coating in multiple layers formation on a modified titanium implant surface with dioxide nanotubes. The functional catechol was synthesized from three different components (dopamine, hyaluronic acid and chitosan). Antibacterial-enriched titanium dioxide nanotubes were used as vancomycin stores. This formation changed the titanium surface hydrophilicity by producing antibacterial implant properties. An additional functional catechol layers coating caused increased gene expression associated with osteoblast adhesion. In vivo results from this experimental structure showed increased osteoblasts adhesion and bone formation in infected microbial environments. (38)

Antimicrobial peptides (AMP)

Combating biofilm formation using antimicrobial peptides (AMP) is a new approach which uses biological agents for implant coating. (39) Morphologically they are short chains with binary or cationic character. (40) AMP's exhibit a broad antimicrobial spectrum and antifungal activity. (39) They carry positive charge that interferes with the cell membrane lipids negative electrical charge to dissipate it. When AMP's enter the cytoplasm they interact with proteins that are involved in cell metabolism. (40) They

manage to deal with resistant bacterial strains because of the easy cell membrane penetration ability. This ability has the disadvantage of the cytotoxic effect increase. (39) One more disadvantage is the AMP‘s degradation by proteases when they enter the patient’s body. (40)

A new approach to active implant coating was made by Mr. Janjian Hen's research team. The idea to place AMPs as titanium coating against Staphylococcus aureus strains was done with the help of "CuAAC click chemistry". Azido-AMPs (PEG-HHC36: N3-PEG12-KRWWKWWRR) placed on a titanium surface by Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC) as an implant coat. HHC36 peptide was selected for the broad antimicrobial spectrum and low susceptibility bacterial resistance. This peptide was coupled with silane as a linking agent to the titanium surface and with an alkynyl group (alkynyl-PEG-triethoxysilane, abbreviated APT5) to induce its properties. Coating assessment was done both in vitro and in an osteomyelitis rabbit model. The results in vitro showed stable antimicrobial activity, almost without any cytotoxicity. In the animal model, the antimicrobial response within the first seven days was good, after having managed to kill 78% of the Staphylococcus aureus strains. In fact, this kind of coating is very demanding in terms of handling technique. (19)

An attempt to cover titanium with antimicrobial peptides was done by Mr Zhiyuan Liu's research team through acid etching and layer by layer assembly techniques. Acid etching was used for creating nanotubes in different diameters on a titanium surface. Lbl was used to assembly layers of lactoferrin (bovine origin) and sodium alginate (NaAlg). In order to cross-link NaAlg with lactoferrin, they used calcium as the linker. This final formulation was evaluated in vitro against Staphylococcus aureus bacteria. Results showed that the antibacterial agent release depends on the nanotube diameter, as well as on its rate of rapid release. This kind of coating is affected by rapid release, so antibacterial action cannot last long. (18)

Mr. Jiezhao Zhan's scientific team was aware of the drawbacks of using AMPs when they enter the patient's body. In order to overcome this obstacle, they synthetized an active surface that acts against bacteria in room and body temperatures (Figure 20). To achieve this, a polydopamine substrate composition was set on the implant surface, and after that an ATP polymer (ATPP), polymer (pNIPAM) was added. This technique of coating placed AMPs on an implant surface by click chemistry reaction. This composition imparts increased biocompatibility when an implant enters in body temperature in comparison to an implant having the surface of the mono AMPs. The evaluation of the antimicrobial activity was performed in vitro and in vivo in a rabbit animal model. Results showed how antimicrobial activity was, prior to implantation and under storage, and also the handling conditions during implantation surgery. After surgery there was a positive effect on biocompatibility, apart from the antimicrobial activity. (39)

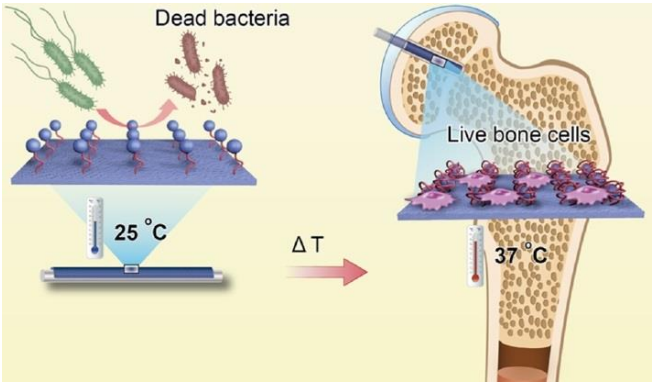


Figure 20 Temperature sensitive antimicrobial peptides (AMP) coating. (Jiezhao Zhan et al. Temperature-Controlled Reversible Exposure and Hiding of Antimicrobial Peptides on an Implant for Killing Bacteria at Room Temperature and Improving Biocompatibility in Vivo. ACS Applied Materials and Interfaces 2018)

Fatty acids

The fatty acids physical properties contribute to the antibiotic or antiseptic agent ionic binding. As a result, fatty acids have increased adhesion on the implants surface. Antibiotics are suspended inside the fatty acids until the formation of ionic bonds

between them. Additionally, in combination with the hydrophobicity property, fatty acids constitute as an appropriate implant coating. Once the coated biomaterial is implanted in the patient's body, ionic bonds are dissolved and antibacterial agents are released locally around the implant. (41)

In an effort to tackle periprosthetic infection, Dr. Klemens Vertesich's scientific team investigated in vitro the application of fatty acids as biodegradable antibiotic carriers on a titanium surface. The final goal of the study was the evaluation of this coating against bacteria. The coating techniques used in this study was elution and spray gun techniques (Figure 21). The antibiotics that were combined with the fatty acids were gentamicin-palmitate, laurate octenidine, vancomycin eluted in trilaurine. The antibiotic effect on Staphylococcus aureus and epidermidis strains showed that the antibacterial action lasted for two to seven days in vitro conditions. (41)

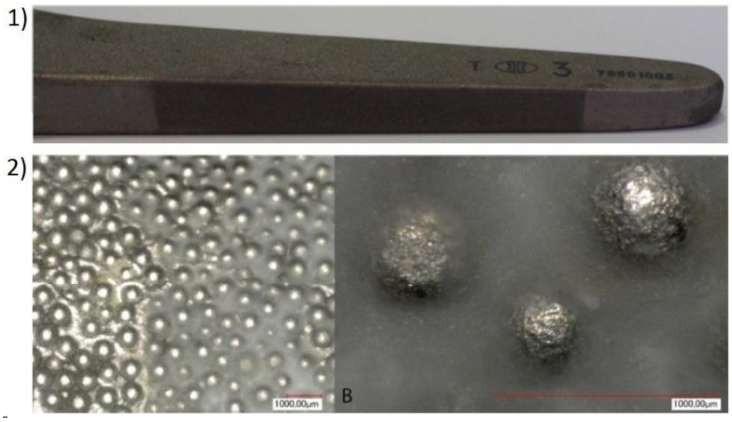


Figure 21 1) Spraying technique coating, dark gray coated area, light gray uncoated area 2) Close up of megaprosthetic area. (Klemens Vertesich et al. Accessible Agent-Fatty Acid Coatings of Titanium Prostheses for Local Prevention and Treatment of Anti-Microbial Infections. Journal of Surgery 2018)

Chitosan and Gallium Ga^{3+}

Chitosan as a natural polymer is one of the most commonly used in tissue engineering materials for cellular development, cartilage or artificial skin substrates. Furthermore, it is used as drug carrier, since it exhibits high biocompatibility properties and degradation.

Chitosan toxicity is particularly low. (42) Chitosan acts as an antibacterial agent because it has a positive charge that reacts with the negative charge of the bacterial cell membrane. This interaction leads to cell membrane permeability disorder. (43)

Gallium is a metal with similar physical properties to iron in Ga^{3+} form. It has similar radius, electric charge and electronegativity with Fe^{3+}. These properties make gallium enter the bacterium cell and manage to affect the iron metabolism by stopping the oxidation process, since Gallium is not oxidized further than Ga^{3+}. Finally, without being toxic to osteoclasts, it inhibits their bone adhesion. (42)

Apart from using the antimicrobial substance deposition to make a titanium surface active against the bacterial infection, Mr A. Ghalayani Esfahani's team tried to combine metal nanoparticles with natural polymer. In particular, they investigated in vitro synergistic action of Gallium with chitosan against Staphylococcus aureus strains. The coating technique they used was EPD. They also tested the effect of coating intended for orthopaedic implantation in an electromagnetic field. The research revealed that the combination of chitosan gallium inhibited the biofilm formation of Staphylococcus aureus by 80%. The external electromagnetic field application appeared to enhance even more the bactericidal effect of Gallium. (42)

Anti-biofilm formation coating

So far, we have read about scientific groups attempting to make an active titanium surface by different ways of depositing materials. Mrs Elien Peeters's team focused on the creation of an anti-biofilm that could combat the Staphylococcus aureus strains biofilm. A synthesis of anti-biofilm by 5- (4-bromophenyl) -N-cyclopentyl-1-octyl-1H-imidazol-2-amine (briefly LC0024) was covalently bound to titanium. Each of the LC0024 components act on a different spectrum of bacteria. Overall, the synthesis has a broad and strong antibacterial spectrum of Gram positive bacteria and fungi. A great advantage of LC0024 is the minimal effect on osteoblasts viability. In addition to the antimicrobial assessment in vivo, a mouse animal model helped in vivo assessment. Laboratory results showed

biofilm inhibition formation of up to 96%, while in an animal model it was raised up to 47% without affecting the osteoblasts cell population in both tests. It is worth to underline that this type of coating prevents orthopaedic implant infection. (44)

Nanomaterial for intraoperative surface coatings

A third way to deal with orthopaedic implant infection is the intraoperative placement of biomaterial around an orthopaedic implant. Besides well-known applications of orthopaedic cement, we will concentrate our interest on new forms of biodegradable biomaterial. (45)

Bioadhesive hydrogel enzymes coating in preclinical stage

The development of an injectable, bioadhesive hydrogel that contains antimicrobial enzymes and adheres to bone exposed surfaces in order to inhibit orthopaedic infections growth. A team of researchers led by Nancy E. and Peter C developed a hydrogel structure that degraded by protease when the lysostaphin enzyme acts against Staphylococcus aureus. The antibacterial activity of the enzyme, in combination with a prophylactic antibiotic administration, lasted over two weeks in a mouse animal model. This way demonstrated that hydrogel can efficiently be utilized during an orthopaedic restorative surgery and can be effective against Staphylococcus aureus strains. (45)

Novagenit® Defensive Antibacterial Coating (DAC) ® hydrogel coating

Novagenit® has placed in the European market the hydrogel Defensive Antibacterial Coating (DAC) ® since 2015 (Figure 22). It's a combination of hyaluronic acid with polylactic acid and the antibiotic substance is added by choice. It is biodegradable and it is placed intraoperatively around the orthopaedic implant. It releases the antibiotic for 96 hours against bacteria and to stop biofilm formation. The DAC® activity is demonstrated not only by in vitro and in vivo studies but also in case studies. (46) The DAC® hydrogel is designed to cover orthopaedic dental and maxillo-surgical implants. This hydrogel

reduces the bacterial adhesion and biofilm formation, as demonstrated in vitro. Besides the hydrogel basic formulation, it can be enriched with additional antibacterial agents. The concentration of antibiotic drugs ranges between 2-10%. The antibiotics are released in a dose-dependent and time-dependent manner locally up to 72 hours, at a much higher concentration than the MIC of a drug. Studies on vancomycin-enriched hydrogels in vivo (animal models) have shown that it is safe and effective against implant infection even without a systemic antibiotic administration. The hydrogel's enrichment with antibiotics did not affect the osteointegration, as shown in extensive clinical studies performed at patients with total hip arthroplasty restoration. There have been no side effects of the hydrogel's application at implants over a 1.5-year period, nor any negative effects on osteointegration. Surely it is necessary to investigate the effects against infection at longer intervals. (26)

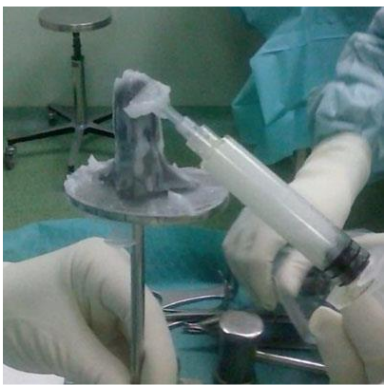


Figure 22 Intraoperative application of hydrogel Defensive Antibacterial Coating (DAC)® on implant. (K.-D. Kóhn et al Management of Periprosthetic Joint Infection © Springer-Verlag GmbH Germany 2018)

7. Preclinical patents and final product in the market

Many ideas that have been developed to fight bacterial infections in orthopaedic implant surgical operation have been researched in laboratory scale. Only few of them overcome certain difficulties and issues discussed extensively in previous chapters. Until now three solutions have managed to find a way to get patent number from the authorities of the United States of America. In Europe, since 2015, an Italian company named Novagenit® has released an antibacterial gel named DAC® (Defensive Active Coating) for use on orthopaedic implants during surgical operation.

U.S. Patent No. 9,999,706 B2 Implantable devices having antibacterial properties and multifunctional surfaces

More specifically, U.S. Patent No. 9,999,706 B2 and the title "Implantable devices having antibacterial properties and multifunctional surfaces" refer to implantable devices designed to improve both osteointegration and antibacterial activity. These properties were avoided by conversion of the surface of the material. (47)

U.S. Patent 2018/0243556 A1 Prophylactic bactericidal medical device

The second embodiment US 2018/0243556 A1 and distinctive title "Prophylactic bactericidal medical device" refers to an orthopaedic implant that can inhibit bacterial infection. Bacterial infection is inhibited by releasing metal ions from the metal nanoparticles deposited on the surface of the implant. The toxic metallic ions are produced by an external supply of energy from a device outside the patient's body. (Figures 23-25) (48)

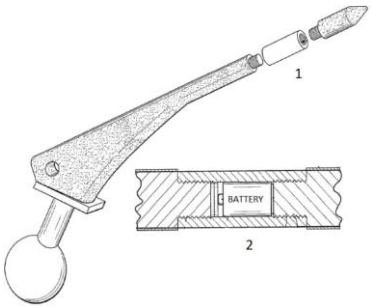


Figure 23 1) Metal coated hip implant in three pieces. The middle piece has a power source inside 2) Power source for current flow for metal ions production. (Fuller et al. Prophylactic bactericidal medical device. United States Patent Application Publication Pub. No. : US 2018 / 0243556 A1 2018)

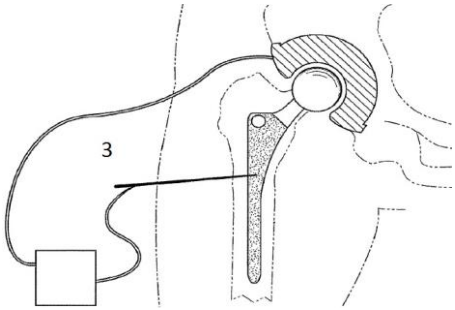


Figure 24 3)External power supply in order to make current flow for metal ions production. (Fuller et al. Prophylactic bactericidal medical device. United States Patent Application Publication Pub. No. : US 2018 / 0243556 A1 2018)

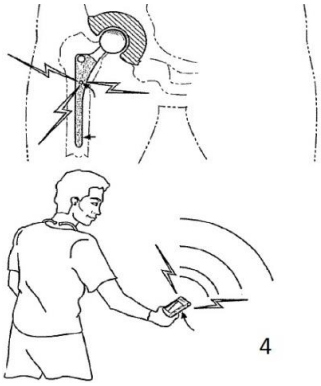


Figure 25 4)External wireless power supply in order to make current flow for metal ions production. (Fuller et al. Prophylactic bactericidal medical device. United States Patent Application Publication Pub. No. : US 2018 / 0243556 A1 2018)

U.S. Patent 10, 207, 030 B2 Implantable devices for bone of joint defects

The third biomaterial with patent numbers US 10, 207, 030 B2 and distinctive title "Implantable devices for bone of joint defects" refers to an implant that inhibits bacterial leaching by coating biodegradable material. The biodegradable material has deposited a drug, natural or synthetic bone particles, and a soluble polymeric homogeneous distribution. (Figure 26) (49)

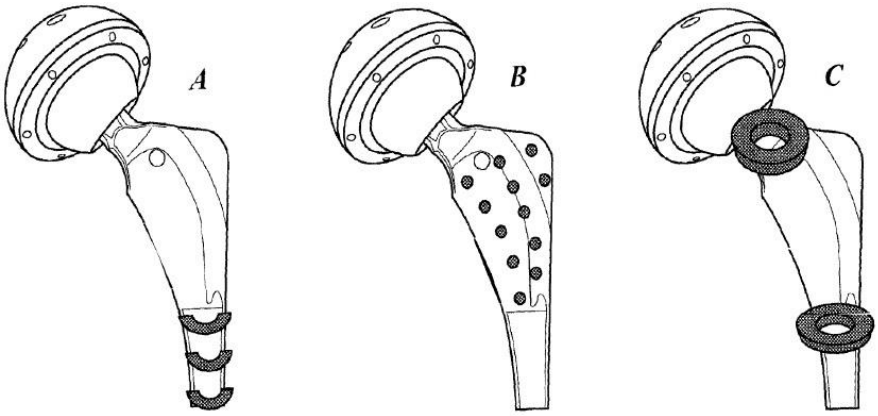


Figure 26 a)Stripes of molten polymer, bone substitute and antibiotic painted on the stem b) Macro /micro spots with molten polymer substitute and antibiotic c) molten polymer antibiotic rings above and below the porous metal. (Brooks et al. Implantable devices for bone of joint defects. United States Patent Application Patent No . : US 10 , 207 , 030 B2 2019)

Novagenit® Defensive Antibacterial Coating (DAC) ®

The European application for antibacterial coating is marketed on the world market since 2015. It is in the form of dust and produced by NOVAGENIT®. The reduction of the powder forms a hydrogel composed of hyaluronic acid and PLA. It is called DAC® (Defensive Active Coating) and as an option, surgeons can introduce a vancomycin or gentamicin solution at different concentrations from 2% to 10% to enhance the action of hydrogel up to 72 hours. (50)

8. Future perspectives in surface biomaterials

Surface modifications play an important role in metal or polymeric implants. In both types of biomaterials, the difference in mechanical strength in comparison to the natural bone can lead to a loose connection or even a crack. Other factors for implant rejection may be infection, inflammation, misallocation of weight. Thus, biomaterial surface properties that come in contact with the cells of the body determine implant longevity and biocompatibility. When this interaction is successful, it reduces the likelihood for a revision surgery. A primary factor limiting the implant's life is the lack of attention in the mechanism of cellular recognition by cellular surface proteins. It is important that osteoblasts proliferate, adhere and rapidly differentiate from cells that do not deposit calcium. In recent studies orthopaedic research has focused on understanding cellular recognition and biomaterial surface improvement to maximize these effects. (51)

Future applications that could deliver better results in all three contingency categories have been proposed by different research teams. Below we review some of these examples in each category.

Passive surface coating with Magnesium and Hydroxyapatite

Research teams such as Mrs Nancy C. Andrés have turned their attention to creating new materials with antibacterial properties. They are tested in vitro with nanoparticles creation by biocompatible material of Magnesium Mg^{2+} and hydroxyapatite HA. Incorporation into the implant surface happens in order to inhibit pathogenic bacteria by altering the surface morphology. This attempt seems to have many advantages over the administration of antibiotic drugs to combat resistant bacteria. For example, reduced cost of implant manufacture with rapid and prolonged antibacterial action without an expiration date. Also, it can act against a wide range of bacteria by simultaneously activating the immune system. This will help cytocine and AMPs production. The new material evaluated on Staphylococcus aureus strains. Experimental measurements

showed considerable decrease in population when exposed to Mg^{2+} + -HA nanoparticles due to different roughness topography and surface electrostatic change behavior. (52)

Active surface coating by external source activation

In the second category, active surface formation with nanoparticle deposition on materials has been proposed to promote surface activation by an external source of energy. For example, photodynamic therapy could be used in collaboration with a photosensitive coating. This could inhibit the biofilm formation and destroy bacteria attachment on the implant surface and activate more neutral cell function. A concept like this has several limitations to overtake. Some of them are the correct settings of wavelength and frequency light, implant placement and accessibility to this area. Another idea for external body activation is the exploitation of the magnetic properties of the metallic nanoparticles that have been deposited on the implant surface. In this case, with the use of a magnetic field, metal ions could concentrate on infected sites. This action is considered necessary to destroy the biofilm that has been created, while allowing the antibiotics to act in the infected area. One last proposition for external surface activation is by ultrasound or laser waves (Figure 27). This energy transaction could disturb mechanically bacterial attachment and biofilm structure. The need of covering the whole implant (not a part of it) with the device that produces these waves is the main limitation. (46) As for the formation of nanotubes on the surface of the orthopedic implant, this could be of a protein nature attached to an aptamer. This final modification could be based on alumina or carbon patterns. (43)

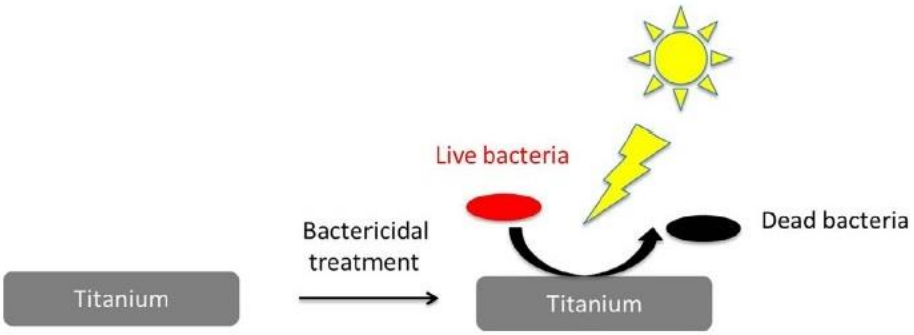


Figure 27 External light source for surface activation against bacteria. (H. Chouirfa et al. Review of titanium surface modification techniques and coatings for antibacterial applications. Acta Biomaterialia 2018)

Intraoperative surface coating with antibacterial proteins

The third category of active implant surface formation happens during surgery. It has been proposed to exploit the properties of marine organisms, to secrete proteins with antibacterial ability which inhibits biofilm formation. (46)

For each of the above nanotechnology applications, and also for those to be explored in the future, we will have to take into account the biological consequences. (51)

9. Discussion

Prosthetic joint infection is nowadays more and more common. Due to the increasing patients life expectancy there is an increasing trend in total hip arthroplasty surgery. Within 5 years, approximately 30% of these patients may have a revision surgery. (1) In 80% of these cases, the implant was rejected due to infection from Staphylococcus aureus and epidermidis. (2) A bacteria resistant biofilm on titanium surface can be formed in less than 24 hours. (3) A consequence of biofilm development is the loss of implant support due to loose osteointegration. Bone surface at nanoscale level is amorphous at 20-50nm. (4) With the help of nanotechnology techniques, we are called upon to increase titanium biocompatibility that is most commonly used in total joint arthroplasty. Increasing biocompatibility is achieved by passive, active and intraoperative coating. With passive coating, scientists try to process titanium surface to increase the capacity of osteointegration without coating any additional material to titanium surface. Just to change the titanium surface's physicochemical properties. Active coating refers to some additional organic or inorganic material that will be coating the titanium surface in order to inhibit biofilm formation. This kind of coating helps osteoblasts to adhere to the titanium surface faster. (11) The intraoperative application of orthopaedic cement or hydrogel needs a rapid release of antibiotic drug in the implantation area, in order to destroy certain bacteria that have infected the surgical field. (45)

Scientific teams, as the team of researcher Mr M. Yunil Cao came up with a similar conclusion trying to inhibit bacterial surface attachment by changing the titanium surface electrical charge. (20) More emphasis has been given by scientists on the active titanium coating by various techniques such as a) Plasma spray coating, b) PIII & amp D, c) PVD, d) Electrospinning, (e) Sintering with two metals, (f) PEO, (h) Magnetron sputtering, i) ATRP, j) Lbl deposition, k) Click chemistry. Many different minerals such as silver, vanadium, zinc, boron, magnesium and organic substances such as lactoferrin, catechol, antimicrobial peptides, lipids and chitosan have also been studied. The effort of Mrs.

Goderecci's research team with silver coating as a film on titanium surface showed dose-dependent antibacterial activity of the silver in vitro. (25) Silver nanoparticles placed in titanium nanotubes studied by Mr Jiaxing Wang's research team, resulted in more implant protection from bacteria and at the same time it increased fibroblast activity found in vivo and in vitro models. (6) Another in vitro attempt by Mr Zhang Yuan's research team with silver nanoparticles combined with chitosan in an Lbl layout has been shown to favor osteoblast attachment while at the same time the bacterial colony formation was reduced. (26) A more extensive study was done by Mr Croes on synthesized chitosan, vancomycin and silver nanoparticles in vitro and in vivo models. The resulting data showed that high silver nanoparticle concentrations may damage osteoblast adhesion. (29) Respectively, in a recent study in vitro with vanadium nanoparticles coating done by Mr Jiaxing Wang's research team has shown a dependency between vanadium valence and nanoparticle toxicity. (31) Regarding the boron nanoparticles coating in titanium surface, the scientific team of Mr. L. Sopchenski showed that boron has the least toxic effect compared to any other metals that are used for titanium surface coating in our days. (34) Experimental observations in vitro and in vivo models with magnesium nanoparticles as titanium surface coating did not allow Mr. Geyong Guo's research team to have clear results. It is probable that macrophages of the immune system played major role in the final measurements. (35) The research team of Mr D. Sivaraj used a combination of hydroxyapatite, magnesium nanoparticles inside carbon nanotubes for stainless steel implant coating giving excellent corrosion properties to stainless steel surface. (36)

Beside the inorganic experiments, there are reports on experiments with organic substances such as lactoferrin. Researchers headed by Mr. Tingting Shen's showed that lactoferrin was able to help with osteogenesis and with the inhibition of pathogenic bacteria in vitro. (37) Coating with carbon nanotubes and catechol was investigated by Mr. Zhang Yuan on in vivo model. This combination had a beneficial effect on the

osteogenesis process even on infected bone. (38) Another way of inhibiting biofilm formation is the AMPs coating applied by Mr. Janjian Hen's in vitro and in vivo model. In each case, the results were intriguing, since the AMPs resisted up to 78% Staphylococcus aureus strains in vivo. (19) An AMP with carbon nanotubes combination was investigated by Mr Zhiyuan Liu's in vitro with poor results, since very rapid AMPs were released without long lasting antimicrobial action. (18) Mr. Jiezhao Zhan's research with thermo-responsive AMPs as coating had great results. It managed to achieve antimicrobial action before implantation, and to increase implant biocompatibility after implantation. Coating evaluation was done in vivo and in vitro model. (39) A research directed by Dr. Klemens Vertesich evaluated in vitro a lipid coating as a biodegradable antibiotic carrier for the treatment of bacterial infection. The duration of the antibiotic release was two to seven days. (41) The coating with nanoparticles of Gallium combined with chitosan is a promising application of nanotechnology. The research team of Mr. A. Ghalayani Esfahani found in vitro that this combination managed to inhibit the growth of Staphylococcus aureus biofilm by 80%. The results were further amplified when the implant was placed in an electromagnetic field. (42) Mrs Elien Peeters's team dedicated itself to creating an organic antibiofilm that would inhibit bacterial biofilm. A synthesis of anti-biofilm by LC0024 inhibited the biofilm formation by 96% in vitro and 47% in vivo, without affecting the attachment of osteoblasts. (44) The approach of scientists to inhibit infection intraoperative led the research team of Nancy E. and Peter C to create an injectable biodegradable hydrogel containing antimicrobial enzymes. The purpose of this synthesis was to inhibit intraoperative implant infection. Antibiotic drug release was up to two weeks as evaluated in an in vivo rat model. The only product in the market since 2015 is the Defensive Antibacterial Coating (DAC) ® by Novagenit®, a biodegradable hydrogel of hyaluronic acid with PLA and antibiotic drug. It is an intraoperative hydrogel that is placed around the implant and releases antibiotic drug locally. (46)

10. General conclusion

This dissertation reviews different coating techniques and nanomaterials. With most of them in preclinical studies phase. Passive coating favors osteointegration. Active coating acts against infection, while intraoperative coating techniques aim to biofilm inhibition in a short term of time, immediately after the injection. In the last years, there has been extensive research on the active implant coating. Scientific groups are constantly increasing their interest at this coating type. Until now, there is no active coating implant that has been into mass production. Several limitations during the in vitro study phase should be taken seriously into consideration, until the next experimental test in animal model. Hydrogels are already in the European market with very encouraging results as antibiotic carriers. In the future, in order to come up with realistic conclusions about active implant coating, research into animal models should be extended to simulate those patients group that where most commonly involved in total arthroplasty. As an example, an animal model with chronic use of osteoporosis medication would be suitable for study. At least, extensive investigations should be made concerning the strength of active coating at the intraoperative implant handling. Finally, the combination between passive coating and intraoparative hydrogel looks ideal for revision periprosthetic joint arthroplasty surgery. This combination provides short-term treatment of bacterial infection and long term condition for successful osteointegration, thanks to the fact that the implant surface treatment is coated with an antibiotic biodegradable hydrogel.

A remarkable observation about all the scientific studies reviewed is the lack of long-term comprehensive analysis of the side effects of using any kind of nanoparticles as titanium coating. This is because several applications in the in vitro study phase already show increased toxicity as well as in vivo studies lasting a short time so that safe conclusions can be drawn regarding the toxicity of the nanoparticles used. Finally, we should realize that the use of nanotechnology is a very new tool in orthopedics to tackle

infections that need to be investigated more in depth for any side effects caused by nanoparticles.

11. References

1 Ashley E. Levack & Erika L. Cyphert & Mathias P. Bostrom & Christopher J. Hernandez & Horst A. von Recum & Alberto V. Carli
Current Options and Emerging Biomaterials for Periprosthetic Joint Infection
Current Rheumatology Reports (2018) 20: 33
DOI: https://doi.org/10.1007/s11926-018-0742-4

2 W.F. Oliveira, P.M.S. Silva, R.C.S. Silva, G.M.M. Silva, G. Machado, L.C.B.B. Coelho, M.T.S. Correia
Staphylococcus aureus and Staphylococcus epidermidis infections on implants
Review Journal of Hospital Infection 98 (2018) 111e117
DOI: 10.1016/j.jhin.2017.11.008.

3 K. - D. Kóhn (Ed.)
Management of Periprosthetic Joint Infection © Springer-Verlag GmbH Germany 2018
DOI: 10.1007/978-3-662-54469-3_ 9

4 Dragos Apostu, Ondine Lucaciu, Cristian Berce, Dan Lucaciu and Dan Cosma
Current methods of preventing aseptic loosening and improving osseointegration of titanium implants in cementless total hip arthroplasty: a review
Journal of International Medical Research 2018, Vol. 46(6) 2104–2119
DOI: 10.1177/0300060517732697 journals.sagepub.com/home/imr

5 Furqan A. Shah, Peter Thomsen, Anders Palmquist
Osseointegration and current interpretations of the bone-implant interface
Review article Acta Biomaterialia 84 (2019) 1–15
DOI: https://doi.org/10.1016/j.actbio.2018.11.018

6 Jiaxing Wang, Jinhua Li, Shi Qian, Geyong Guo, Qiaojie Wang, Jin Tang, Hao Shen, Xuanyong Liu, Xianlong Zhang, and Paul K. Chu
Antibacterial Surface Design of Titanium-Based Biomaterials for Enhanced Bacteria-Killing and Cell-Assisting Functions Against Periprosthetic Joint Infection
ACS Appl. Mater. Interfaces 2016 © American Chemical Society A
DOI: 10.1021/acsami.6b02803

7 Rimondinia, Sarita R. Shah, Alexander M. Tatara, Rena N. D'Souza, Antonios G. Mikos and F. Kurtis Kasper
Evolving strategies for preventing biofilm on implantable materials
Materials Today Volume 16 Number 5 May 2013
DOI: http://dx.doi.org/10.1016/j.mattod.2013.05.003

8 Silvia Spriano, Seiji Yamaguchi, Francesco Baino, Sara Ferraris
A critical review of multifunctional titanium surfaces: New frontiers for improving osseointegration and host response, avoiding bacteria contamination
Acta Biomaterialia 79 (2018) 1–22
DOI: https://doi.org/10.1016/j.actbio.2018.08.013

9 David Armbruster
Anti-Infection Technologies for Orthopedic Implants: Materials and Considerations for Commercial Development
© Springer International Publishing AG, part of Springer Nature 2018 219
B. Li, T. Webster (eds.), Orthopedic Biomaterials,
DOI: https://doi.org/10.1007/978-3-319-89542-0_11

10 Laila Damiati, Marcus G Eales, Angela H Nobbs, Bo Su, Penelope M Tsimbouri, Manuel Salmeron-Sanchez and Matthew J Dalby
Impact of surface topography and coating on osteogenesis and bacterial attachment on titanium implants
Journal of Tissue Engineering Volume 9: 1–16 © The Author(s) 2018
DOI: 10.1177/2041731418790694 journals.sagepub.com/home/tej

11 Sheng Qin, Kaihang Xu, Binen Nie, Fang Ji, Hao Zhang
Approaches based on passive and active antibacterial coating on titanium to achieve antibacterial activity
Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/jbm.a.36413

12 H. Chouirfa, H. Bouloussa, V. Migonney, C. Falentin-Daudré
Review of titanium surface modification techniques and coatings for antibacterial applications
Acta Biomaterialia 83 (2019) 37–54
DOI: https://doi.org/10.1016/j.actbio.2018.10.036

13 Adam EM Eltorai, Jack Haglin, Sudheesha Perera, Bielinsky A Brea, Roy Ruttiman, Dioscaris R Garcia, Christopher T Born, Alan H Daniels
Antimicrobial technology in orthopedic and spinal implants
WJO|www.wjgnet.com 361 June 18, 2016|Volume 7|Issue 6| World J Orthop 2016 June 18; 7(6): 361-369
ISSN 2218-5836 (online) © 2016 Baishideng Publishing Group Inc. All rights reserved.
DOI: 10.5312/wjo.v7.i6.361

14 Joseph Salvatore
Plasma-Sprayed and Sintered Silver-Based Antimicrobial Coatings for Industrial and

Biomedical Application by Joseph Salvatore A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of Master of Applied Science in Biomedical Engineering Carleton University Ottawa, Ontario © 2018
DOI: https://doi.org/10.22215/etd/2018-13224

15 A.L. Yerokhin, X. Nie, A. Leyland, A. Matthews, S.J. Dowey
Review Plasma electrolysis for surface engineering
Surface and Coatings Technology 122 (1999) 73–93
© 1999 Elsevier Science S.A. All rights reserved. PII: S0257-8972 (99) 00441-7
DOI: https://doi.org/10.1016/S0257-8972(99)00441-7

16 Klaus Ellmer Hahn
Review article Magnetron sputtering of transparent conductive zinc oxide: relation between the sputtering parameters and the electronic properties
Journal of Physics D: Applied Physics, Volume 33, Number 4 Year 2000
DOI: https://doi.org/10.1088/0022-3727/33/4/201

17 Krzysztof Matyjaszewski and Jianhui Xia
Atom Transfer Radical Polymerization
Chem. Rev. 2001 © 2001 American Chemical Society Published on Web 09/12/2001
DOI: 10.1021/cr940534g

18 Zhiyuan Liu, Mou Zhong, Yuhua Sun, Junhong Chen, Bo Feng
The preparation, cytocompatibility and antimicrobial property of micro/nano structural titanium loading alginate and antimicrobial peptide
Zhiyuan Liu et al 2018 Mater. Res. Express in press
DOI: https://doi.org/10.1088/2053-1591/aab28a

19 Junjian Chen, Yuchen Zhu, Menghua Xiong, Guansong Hu, Jiezhao Zhan, Tianjie Li, Lin Wang, and Yingjun Wang
Antimicrobial Titanium Surface via Click-Immobilization of Peptide and Its in Vitro/Vivo Activity
ACS Biomater. Sci. Eng., Just Accepted Manuscript Publication Date (Web): 07 Dec 2018
DOI: 10.1021/acsbiomaterials.8b01046

20 Yunyi Cao, Bo Su, Subash Chinnaraj, Saikat Jana, Leon Bowen, Sam Charlton, Pengfei Duan, Nicholas S. Jakubovics & Jinju Chen
Nanostructured titanium surfaces exhibit recalcitrance towards Staphylococcus epidermidis biofilm formation
Scientific reports (2018) 8:1071
DOI:10.1038/s41598-018-19484-x

21 Rita Sorrentino, Andrea Cochisa, Barbara Azzimontia, Carlos Caravaca, Jerome Chevalier, Meinhard Kuntz, Alessandro Alan Porporati, Robert Michael Streicher, Reduced bacterial adhesion on ceramics used for arthroplasty applications
Journal of the European Ceramic Society 38 (2018) 963–970
DOI: https://doi.org/10.1016/j.jeurceramsoc.2017.10.008

22 S. Ferraris, A. Venturello, M. Miola, A. Cochis, L. Rimondini, S. Spriano
Antibacterial and bioactive nanostructured titanium surfacesfor bone integration
Applied Surface Science 2014 0169-4332/© 2014 Elsevier B.V. All rights reserved.
DOI: http://dx.doi.org/10.1016/j.apsusc.2014.05.056

23 S. A. Brennan, C. Ní Fhoghlú, B. M. Devitt, F. J. O'Mahony, D. Brabazon, A. Walsh Silver nanoparticles and their orthopaedic Applications
©2015 The British Editorial Society of Bone & Joint Surgery
DOI: 10.1302/0301-620X.97B5

24 Jiri Gallo, Ales Panacek, Robert Prucek, Eva Kriegova, Sarka Hradilova, Martin Hobza and Martin Holinka
Review Silver Nanocoating Technology in the Prevention of Prosthetic Joint Infection
Materials 2016, 9, 337;
DOI: 10.3390/ma9050337

25 Sarah S. Goderecci, Eric Kaiser, Michael Yanakas, Zachary Norris, Jeffrey Scaturro, Robert Oszust, Clarence D. Medina, Fallon Waechter, Min Heon, Robert R. Krchnavek, Lei Yu, Samuel E. Lofland, Renee M. Demarest, Gregory A. Caputo and Jeffrey D. Hettinger
Article Silver Oxide Coatings with High Silver-Ion Elution Rates and Characterization of Bactericidal Activity
Molecules 2017, 22, 1487;
DOI: 10.3390/molecules22091487

26 Zhang Yuan, Peng Liu, Yansha Hao, Yao Ding, Kaiyong Cai
Construction of Ag-incorporated coating on Ti substrates for inhibited bacterial growth and enhanced osteoblast response
Colloids and Surfaces B: Biointerfaces Available online 29 July 2018
DOI: https://doi.org/10.1016/j.colsurfb.2018.07.064

27 Zhanpeng Zeng, Xian He, Benqian Tan, Caifeng Dai, Weijie Zheng
Original Article Titanium oxide nanotubes embedded with silver dioxide nanoparticles for staphylococcus aureus infections after prosthetic joint replacement in animal models
Int J Clin Exp Med 2018;11(7):7392-7399
www.ijcem.com /ISSN:1940-5901/IJCEM0076966

28 Jiaxing Wang, Jinhua Li, Geyong Guo, Qiaojie Wang, Jin Tang, Yaochao Zhao,Hui Qin, Tuerhongjiang Wahafu, Hao Shen, Xuanyong Liu & Xianlong Zhang
Silver-nanoparticles-modified biomaterial surface resistant to staphylococcus: new insight into the antimicrobial action of silver
Scientific Reports 6:32699 Published: 07 September 2016
DOI: 10.1038/srep32699

29 M. Croes, S. Bakhshandeh, I.A.J. van Hengel, K. Lietaert, K.P.M. van Kessel, B. Pouran, B.C.H. van der Wal, H.C. Vogely, W. Van Hecke, A.C. Fluit, C.H.E. Boel, J. Alblas, A.A. Zadpoor, H. Weinans, S. Amin Yavari
Antibacterial and immunogenic behavior of silver coatings on additively manufactured porous titanium
Acta Biomaterialia 1742-7061/ 2018 Acta Materialia Inc.
DOI: https://doi.org/10.1016/j.actbio.2018.09.051

30 Jinxiao Guo, Huaijuan Zhou, Jiaxing Wang, Wei Liu, Mengqi Cheng, Xiaochun Peng, Hui Qin, Jianfeng Wei, Ping Jin, Jinhua Li & Xianlong Zhang
Nano vanadium dioxide films deposited on biomedical titanium: a novel approach for simultaneously enhanced osteogenic and antibacterial effects
Artificial Cells, Nanomedicine, and Biotechnology An International Journal 2018
DOI: 10.1080/21691401.2018.1452020

31 Jiaxing Wang, Huaijuan Zhou, Geyong Guo, Tao Cheng, Xiaochun Peng, Xin Mao Jinhua Li, Xianlong Zhang
A functionalized surface modification with vanadium nanoparticles of various valences against implant-associated bloodstream infection
International Journal of Nanomedicine 2017
DOI: http://dx.doi.org/10.2147/IJN.S129459

32 L. Sopchenski, K. Popatb, P. Soaresa
Bactericidal activity and cytotoxicity of a zinc doped PEO titanium coating
Thin Solid Films 2018
DOI: https://doi.org/10.1016/j.tsf.2018.05.055

33 Guodong Jin, Huiliang Cao, Yuqin Qiao, Fanhao Meng, Hongqin Zhu, Xuanyong Liu
Osteogenic activity and antibacterial effect of zinc ion implanted titanium
Colloids and Surfaces B: Biointerfaces 0927-7765/© 2014 Elsevier B.V. All rights reserved
DOI: http://dx.doi.org/10.1016/j.colsurfb.2014.02.025

34 L. Sopchenski, S. Cogo, M.F. Dias-Ntipanyj, S. Elifio-Espósito, K.C. Popat, P. Soares
Bioactive and antibacterial boron doped TiO2 coating obtained by PEO

Applied Surface Science 0169-4332/ © 2018 Elsevier B.V. All rights reserved
DOI: https://doi.org/10.1016/j.apsusc.2018.07.049

35 Geyong Guo, Huaijuan Zhou, Qiaojie Wang, Jiaxing Wang, Jiaqi Tan, Jinhua Li, Ping Jin, and Hao Shen
Nano-layered magnesium fluoride reservoirs on biomaterials surface strengthen polymorphonuclear leukocytes resistant to bacterial pathogens
Nanoscale 2016
DOI: 10.1039/C6NR07729C

36 D. Sivaraj, K. Vijayalakshmi
Substantial effect of magnesium incorporation on hydroxyapatite/carbon nanotubes coatings on metallic implant surfaces for better anticorrosive protection and antibacterial ability
Journal of Analytical and Applied Pyrolysis 0165-2370/ © 2018 Elsevier B.V. All rights reserved
DOI: https://doi.org/10.1016/j.jaap.2018.09.027

37 Tingting Shen, Weihu Yang, Xinkun Shen, Weizhen Chen, Bailong Tao, Xiaoqing Yang, Jianping Yuan, Peng Liu, and Kaiyong Cai
Polydopamine-assisted Hydroxyapatite and Lactoferrin Multilayer on Titanium for Regulating Bone Balance and Enhancing Antibacterial Property
ACS Biomater. Sci. Eng., Just Accepted Manuscript Publication Date (Web): 14 Aug 2018
DOI: 10.1021/acsbiomaterials.8b00791

38 Zhang Yuan, Suzhou Huang, Shaoxiong Lan, Haizhou Xiong, Bailong Tao, Yao Ding, Yisi Liu, Peng Liu and Kaiyong Cai
Surface engineering of titanium implants with enzyme-triggered antibacterial properties and enhanced osseointegration in vivo
Journal of Materials Chemistry B 2018
DOI: 10.1039/c8tb01918e

39 Jiezhao Zhan, Lin Wang, Yuchen Zhu, Huichang Gao, Yunhua Chen, Junjian Chen, Yongguang Jia, Jingcai He, Zhou Fang, Ye Zhu, Chuanbin Mao, Li Ren, and Yingjun Wang
Temperature-Controlled Reversible Exposure and Hiding of Antimicrobial Peptides on an Implant for Killing Bacteria at Room Temperature and Improving Biocompatibility in Vivo
ACS Appl. Mater. Interfaces 2018
DOI: 10.1021/acsami.8b14534

40 Minqi Wang, Tingting Tang
Surface treatment strategies to combat implant-related infection from the beginning
Journal of Orthopaedic Translation 2018

DOI: https://doi.org/10.1016/j.jot.2018.09.001

41 Klemens Vertesich, Thomas Mayrhofer, Reinhard Windhager, Klaus-Dieter Kóhn
Accessible Agent-Fatty Acid Coatings of Titanium Prostheses for Local Prevention and Treatment of Anti-Microbial Infections
Journal of Surgery 2018
DOI: 10.11648/j.js.20180606.11

42 Ghalayani Esfahani, Lazazzera, L. Draghi, S. Far, R. Chiesa, L. De Nardo and F. Billi
Bactericidal activity of gallium-doped chitosan coatings against staphylococcal infection
Journal of Applied Microbiology 2018
DOI: 10.1111/jam.14133

43 Debmalya Ganguly, Reza Shahbazian-Yassar, Tolou Shokuhfar
Recent Advances in Nanotubes for Orthopedic Implants
Journal of Nanotechnology and Smart Materials 2014

44 Elien Peeters, Geert Hooyberghs, Stijn Robijns, Ami De Weerdt, So_na Kucharíková, Hélène Tournu, Annabel Braem, Katerina _Ceh, Gregor Majdi, Tanja Špani, Estera Pogorevc, Birgit Claes, Barbara Dovgan, Lenart Girandon, Frédéric Impellizzeri, Martin Erdtmann, Annika Krona, Jef Vleugels, Mirjam Fröhlich, Jordi Garcia-Forgas, Katrijn De Brucker, Bruno P.A. Cammue, Karin Thevissen, Patrick Van Dijck, Jozef Vanderleyden, Erik Van der Eycken, Hans P. Steenackers
An antibiofilm coating of 5-aryl-2-aminoimidazole covalently attached to a titanium surface
J Biomed Mater Res B Part B. 2018 1–12 Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/jbm.b.34283

45 Ankur Singh Nancy E. and Peter C
Bactericide hydrogel prevents orthopedic implant infection
Science Translational Medicine 30 May 2018: Vol. 10, Issue 443, eaau0463
DOI: 10.1126/scitranslmed.aau0463

46 D. A. George, V. Gant, F. S. Haddad
The management of periprosthetic infections in the future
©2015 The British Editorial Society of Bone & Joint Surgery
DOI: 10.1302/0301-620X.97B9

47 Daniele Bollati, Brescia (IT) ; Marco Morra , Concesio (IT) ; Clara Cassinelli , Concesio (IT) ; Giovanna Cascardo , Concesio (IT)
Implantable devices having antibacterial properties and multifunctional surfaces

United States Patent Bollati et al. (10) Patent No.: US 9,999,706 B2 (45)
Date of Patent: Jun. 19, 2018

48 Thomas A. Fuller, State College, PA (US); Richard A. Wysk, Raleigh, NC (US); Wayne J. Sebastianelli, Boalsburg, PA (US)
Aionx Antimicrobial Technologies, Inc., Boalsburg, PA (US)
Prophylactic bactericidal medical device
United States Patent Fuller et al. (43) (12) Patent Application Publication (10) Pub. No.: US 2018 / 0243556 A1
Pub. Date: Aug. 30, 2018

49 Amanda Elaine Brooks, South Jordan, UT (US); David W. Grainger, Salt Lake City, UT (US); Kristofer D. Sinclair, Salt Lake City, UT (US); Benjamin Delbert Brooks, South Jordan, UT (US)
Implantable devices for bone of joint defects
University of Utah Research Foundation, Salt Lake City, UT (US)
United States Patent Brooks et al. (10) Patent No.: US 10,207,030 B2 (45)
Date of Patent: Feb. 19, 2019

50 C. L. Romano, H. Tsuchiya, I. Morelli, A. G. Battaglia, L. Drago
Antibacterial coating of implants: are we missing something?
Bone Joint Res 2019
DOI: 10.1302/2046-3758.85.BJR-2018-0316

51 Parchi, Vittorio, Andreani, Piolanti, Cirillo, Iemma, Hampel and Lisanti
How Nanotechnology can Really Improve the Future of Orthopedic Implants and Scaffolds for Bone and Cartilage Defects
Parchi et al., J Nanomedine Biotherapeutic Discov 2013, 3:2
DOI: http://dx.doi.org/10.4172/2155-983X.1000114

52 Nancy C. Andrés, Juan Manuel Sieben, Monica Baldini, Carlos Hernan Rodriguez, Angela Famiglietti, and Paula V. Messina
Electroactive Mg2+- Hydroxyapatite Nanostructured Networks against Drug - Resistance Bone Infection Strains
ACS Appl. Mater. Interfaces, Just Accepted Manuscript Publication Date (Web): 25 May 2018
DOI: 10.1021/acsami.8b0605