



# INHIBITORY EFFECTS OF CHITOSAN COATING AGAINST BIOFILM FORMATION ON METAL IMPLANTS

Franja Šulek<sup>1</sup>, Ingrid Milošev<sup>1,2</sup>

<sup>1</sup>Valdoltra Orthopaedic Hospital, Research Department, Jadranska c.31, SI-6280 Ankaran

<sup>2</sup>Jožef Stefan Institute, Department of Physical and Organic Chemistry, Jamova c.37, SI-1000, Ljubljana

**Abstract** — An effective approach to combat bacteria adhesion onto metallic implants surface is to functionalize the biomaterial surface such that bacterial growth could be impaired or the bacteria are killed upon contact with the surfaces. In the recent years, a majority of the research in material science has been devoted to modification or functionalization of implant surfaces with composite coatings with bactericidal capability such as polymeric coatings. For instance, chitosan (CH) is a polycationic polysaccharide which antibacterial properties and osteoblast function-enhancing nature has received substantial interest. The main goal of our study was to evaluate the effect of different chitosan-coated metals, routinely used in orthopaedics, on the survival of *Staphylococcus epidermidis* and *Staphylococcus aureus*. The results clearly showed that survival of attached bacteria onto metals functionalized with chitosan was lower when compared to bacterial survival determined on the surface of unmodified metals. Moreover, chitosan coating caused bacterial cells to lose their regular spherical shape. Thus, the results proved that chitosan could be used as alternative material for the preparation of antimicrobial coatings for implants.

**Index Terms** — TRANS2CARE, bacterial adhesion, orthopedic metals, chitosan coating, antibacterial

## 1 BACKGROUND

The ability of bacteria to form thin biofilm layer upon adhesion on metal surfaces is a widespread problem involving many technological areas [1]. Thus, the surface of human implants used in orthopedics also represents an ideal environment for biofilm formation, which leads to bacterial-related

inflammation process in the human body and consequently to septic loosening of the endoprosthesis [2]. With advancements in surface technology techniques and tribochemistry there are new solutions available for the preparation of material surfaces so to be less susceptible to bacterial adhesion and other superficial damages [3].

Fabrication of chitosan (CH) coating for orthopedic implants represents a potential tool to overcome the problem of prosthetic joint bacterial infections. Chitosan is a widely distributed nontoxic biopolymer which possesses antibacterial properties due to the presence of free amino groups in the polymeric chain [4]. Additionally, it has the ability to stimulate the proliferation of the osteoblasts which indeed facilitates the osteointegration process [4]. In this regard, the requirements of the bioactive chitosan coating to be antibacterial and osteoinductive are thus fulfilled. Therefore, chitosan coating may represent an effective approach to combat bacterial adhesion onto implant surface and at the same time it may allow a more proper integration of the implant inside the body due to faster osteointegration process.

## 2 OBJECTIVES

Owing to its high biocompatibility, nontoxicity and antimicrobial properties, chitosan is regarded as a new generation of antibacterial agents and has a great potential to be utilized in antibacterial surface coatings for medical materials. Therefore, our primary objective is to design and prepare an »intelligent« surface for the orthopedic implants and evaluate its bioactive properties; as a) the resistance to biocorrosion, b) the effect of the prosthesis surface to adhesion and cohesion in the periprosthetic tissue and c) the ability of the modified surface to stimulate bone cells proliferation during the bone integration process.

To broaden chitosan`s antimicrobial capability, our second objective in collaboration with University of Udine (PP8) aims at enriching the chitosan coating with antimicrobial peptides – AMPs. It means that a selected antimicrobial peptide is introduced by immobilization in the polymerized chitosan matrix and consequently the resulting biomaterial is tested again for its antimicrobial efficacy. Antimicrobial peptides represent valuable candidates for orthopedic applications due to their efficacy against *Staphylococcus* species, the most frequently found pathogens on implant surfaces [5].

## 3 APPROACH & METHODS

**General approach:** comprises the preparation of chitosan coating deposited on different metals and evaluation of its antibacterial activity, as follows: 1. Pretreatment of metals surface. 2. Deposition of chitosan onto metals. 3. Surface characterization of chitosan coating. 4. Determination of antibacterial activity of chitosan coating against bacteria, *S. aureus* and *S. epidermidis*.

**Methods:** Chitosan coating was prepared on different type of metals such as commercially pure titanium (Ti), titanium alloy (Ti-6Al-4V) and stainless steel (ASTM F139, 18Cr-14Ni-2.5Mo). Prior to surface chemical modification with chitosan the metal samples were mechanically ground using SiC grinding paper and etched by sulfochromic acid under specified experimental conditions. Then, the metals were immersed directly into chitosan solution and left 24 h to form a uniform chitosan layer on the surface. Afterwards, the metals were additionally rinsed with a selected crosslinker. The resulting deposited chitosan coating was characterized by Fourier infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). The chitosan-coated metals were then subjected to antibacterial tests. The antibacterial capability of chitosan coating was determined against two reference strains, *S.*

epidermidis ATCC 12228 and *S. aureus* ATCC 25923, using a sensitive fluorescence-based antibacterial activity assay. The process of surface functionalization of an artificial hip replacement with chitosan is symbolically depicted in Fig. 1.

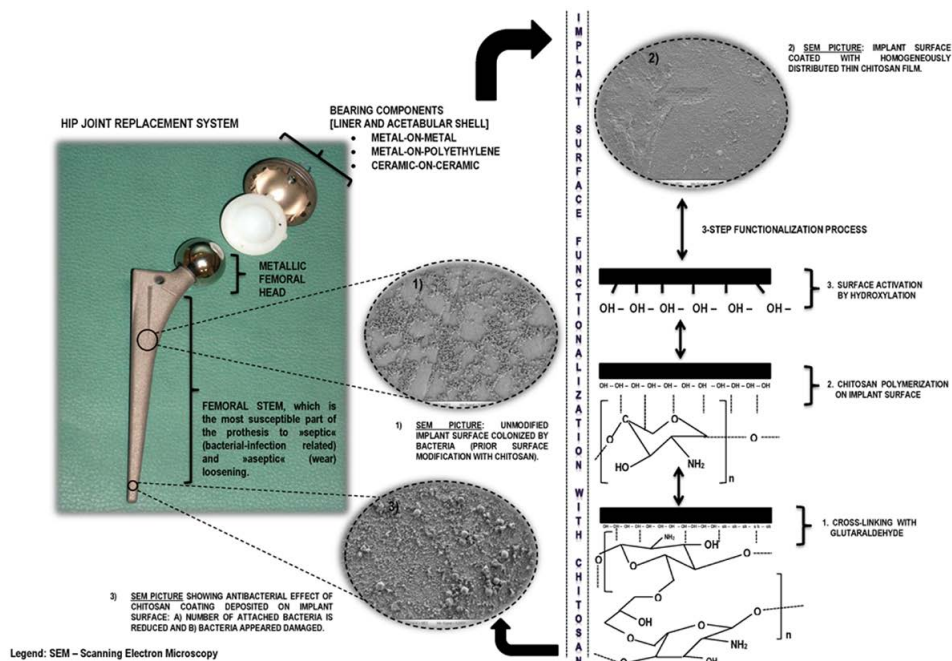


Figure 1: Flowsheet development of chitosan coating on implant surface

## 4 RESULTS

The present study emphasises the killing effect of chitosan-based coating against two *Staphylococcus* species. The coating, prepared by layer-by-layer chemical deposition, was used to cover the surface of orthopaedic metal components, mainly made of titanium and its alloy, and stainless steel. The inhibitory effect of chitosan coating caused bacterial cells to lose their regular spherical shape (Fig.2b). By close inspection of chitosan-coated metal surface, we can observe that upon direct contact with chitosan bacterial cells shrank. The size of the damaged bacterial cell was only 60 % of the size of the undamaged bacterial cell, respectively.

In parallel, the survival of bacteria attached onto chitosan-coated metal surfaces was quantitatively measured by a fast fluorescence test. Indeed, the results showed that the number of adhered bacteria onto chitosan-modified metals was substantially lower in comparison to the number of bacteria attached onto unmodified metal surfaces, showing the antibacterial capability of chitosan.

The chitosan coating was further characterized. Fourier infrared spectroscopy was used to determine the typical absorption tapes for chitosan which proved the binding of chitosan on metal surface, therefore, at 1100 cm<sup>-1</sup>, 1550 cm<sup>-1</sup> and 3350 cm<sup>-1</sup>. Scanning electron microscopy showed morphological properties of the chitosan coating, which is mesoporous and amorphous.

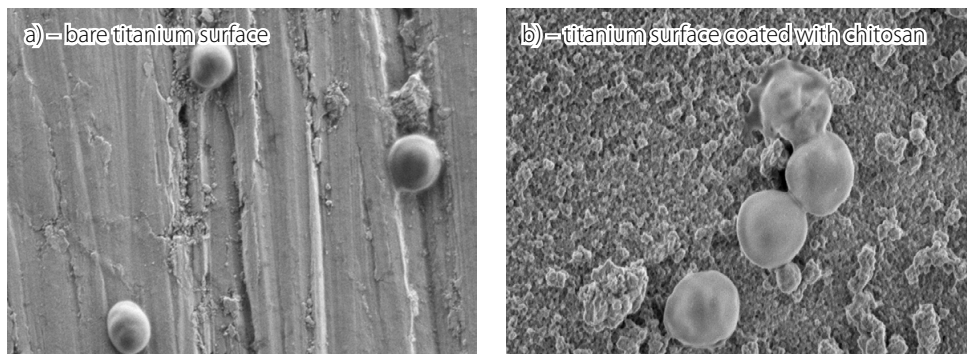


Figure 2: SEM images of titanium surface with adherent bacteria before (a) and after (b) metal surface functionalization with chitosan. Irregularly shaped bacterial cells indicate the biocide effect upon their contact with chitosan (2b). Magnification: 90000x.

## 5 POTENTIAL NEW PRODUCTS & SERVICES

Product: in collaboration with University of Udine (PP8) we aim to create an affordable and easy to use antibacterial coating for orthopedic implants with optimal antibacterial duration. Indeed, high-quality composite coating based on chitosan biopolymer with incorporated antimicrobial peptides (AMPs) with reinforced antibacterial and osteoinductive capability would gain applicability mainly in the domain of orthopedic implants. If the product would result highly effective against various bacterial pathogens and other ambient microbes, the product will be properly functionally adjusted to extend its use to other medical surfaces, such as surgical devices and other easy contaminated medical surfaces.

## 6 CURRENT COLLABORATIONS

### 6.1 With other researchers

Within Trans2Care consortium:

- Dr. Francesca D'Este, Dr. Barbara Skerlavaj (University of Udine)
- Prof. Andrej Cör (University of Primorska, Faculty of Health Sciences)
- Prof. Alessandro Tossi (University of Trieste)

Collaboration with Jožef Stefan Institute, Dept. of Physical and Organic Chemistry, headed by Prof. Ingrid Milošev, for technical assistance in FT-IR and SEM technique.

### 6.2 With hospitals

Collaboration with »Institute of Public Health Koper«, Dept. of Medical microbiology headed by Martina Kavčič, M.D., for the generous donation of clinical samples such as bacterial isolates and synovial fluid.

## 7 CONTACT OR COLLABORATIONS NEEDED

Future collaborations with research groups specialized in bone and biomaterials studies are welcome as well as contacts with biomedical companies in the field of orthopaedics.

## 8 COMMUNICATION TOOLS

Our scientific results obtained up to now have been successfully disseminated at various international Italy-Slovenia conferences, within them the most important contributions are:

Chemistry towards Biology, Trieste, September 2013:

- ŠULEK, Franja, MILOŠEV, Ingrid. Inhibition of bacteria onto chitosan-based coated metals. V: Chemistry towards biology. [Trieste: Università degli studi di Trieste], 2013, str. [33].
- ŠULEK, Franja, MILOŠEV, Ingrid, D'ESTE, Francesca, SKERLAVAJ, Barbara. Trans2care approach towards prevention of biofilm information : antimicrobial coatings. V: Chemistry towards biology. [Trieste: Università degli studi di Trieste], 2013, str. [96].
- NanotechItaly 2013, Venice, November 2013:
- ŠULEK, Franja, MILOŠEV, Ingrid, D'ESTE, Francesca, SKERLAVAJ, Barbara. Bio-molecular coating for orthopaedic implants with a strong antibacterial capability. V: Key enabling technologies for responsible innovation : [conference handbook]. [Roma]: Agra editrice, 2013, str. 86.

## 9 FUNDS NEEDED

**9.1 For basic research (investigation of biological mechanisms): 50000€**

**9.2 For applied research (solutions for real-world problems): 150000€**

**9.3 For pilot & demonstrator activities (to develop a prototype): 150000€**

## 10 CONCLUSION

Prevention of implant-related bacterial contamination is becoming an emerging field in orthopaedics that directs the future efforts towards the development of new biomaterials with enhanced bactericidal capabilities for orthopaedic applications. The present study emphasises the killing effect of chitosan-based coating against two types of Staphylococcus bacteria. The coating, prepared by immersion method, was used to cover the surface of orthopaedic metal components of titanium (Ti), titanium alloy (Ti-4V-6Al) and stainless steel (ASTM F139, 18Cr-14Ni-2.5Mo). The results showed that the number of adhered bacteria onto chitosan-modified metals was substantially lower in comparison to the number of bacteria attached onto unmodified metal surfaces, showing the bactericidal capability of chitosan. In the near future, the chitosan coating will be additionally functionalized with antimicrobial peptides (AMPs) to further exploit the antibacterial effect of the resulting composite coating against clinical pathogens.

## ACKNOWLEDGEMENT

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