Available online at www.sciencedirect.com

ScienceDirect

Procedia Materials Science 8 (2015) 346 – 350

Procedia Materials Science

www.elsevier.com/locate/procedia

International Congress of Science and Technology of Metallurgy and Materials, SAM - CONAMET 2013

Bacterial Migration Cell

Mario Spector[a]*, Leandro Peretti[a,c], Favio Vincitorio[b], Luciano Iglesias[a]

[a]*Laboratorio de Materiales. Depto. Electromecánica. UTN Facultad Reg. Paraná. Almafuerte 1033 Paraná E. Ríos Argentina.*
[b]*Laboratorio de Física. Depto Ciencias Básicas. UTN Facultad Reg. Paraná. Almafuerte 1033 Paraná E. Ríos Argentina.*
[c]*Instituto de Desarrollo Tecnológico para la Industria Química (UNL-CONICET), Güemes 3450, Santa Fe, Santa Fe, Argentina.*

Abstract

Infected prostheses are usually removed from patients when this infection is difficult or impossible to treat. In this context, an unprecedented physical phenomenon, "bacterial cell migration", by which bacteria from an infected plate is migrated to a clean one has been observed and studied at the UTN FRP Materials Laboratory. This transference is obtained when two facing plates with definite chemical and metallurgic properties, one colonized with a microorganism and the other sterile, are faced to each other in an electrically conductive liquid. For the purposes of the present study, two different cultures have been used: yeast and Staphylococcus aureus, which is one of the bacterium that causes most hospital infections. It is expected that with the implementation of this method, infected prostheses in patients can be completely cleaned in the near future.

Selection and peer-review under responsibility of the scientific committee of SAM - CONAMET 2013

Prostheses, infection, bacteria, bacterial migration, prostheses disinfections.

1. Introduction

In the coming years, the treatment of infections will be a great challenge due to the impossibility of treating them with antibiotics (Marenco et al, 2008; Ramos, 2008) that can specifically kill the ever-mutating germs. Infections caused by bacteria colonization in a prosthesis can become very serious and incur economic as well as human loss (García-Pont et al, 2006) In many cases, it is necessary to remove the infected prosthesis so as to combat the infection to finally re-introduce the piece in a new surgical intervention. In order to evaluate an alternative to

* Corresponding author. Tel.:+54-343- 424-3054; fax: +54-343- 424-3054.
E-mail address: mariospector@frp.utn.edu.ar / somitor@arnet.com.ar.

Selection and peer-review under responsibility of the scientific committee of SAM - CONAMET 2013
doi:10.1016/j.mspro.2015.04.083

surgery, the present work studies disinfection of colonized prostheses of different metallic surfaces by means of non-conventional disinfection procedures.

The use of an electromagnetic field to remove bacteria has previously been reported in the literature. Giladi et al. (2008) make reference to the possibility of inhibiting microbial growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with an alternating electric field. Pickering et al. (2003) describes the way to increase the antibiotic efficacy with the application of an electromagnetic field on orthopedic implants. The bio-electric effect described by Khoury et al. (1992) shows how a bacterial biofilm increases antibiotic susceptibility in the presence of an electric field of a current density of 15 $\mu A/cm^2$.

In the present study, by analyzing the influence of different metals and its relationship with bacterial inoculation it could be systematically observed that some materials are more likely to be colonized than others. These observations pointed to the hypothesis that it might be possible to prevent inoculation, or even remove the bacteria from a colonized specimen. The aim of this work was to examine methods for separating bacteria from a metal sample, in which case it would be possible to disinfect prostheses without removing it from the patient. The assumption made was that if the bacteria (and yeast) in aqueous medium have a negative electrical charge, it will be attracted to a prostheses having a positive charge and a much greater mass. This could explain why some bacteria colonize some metals and other metals are difficult to inoculate.

The results of this work promoted the development of a new disinfection procedure, "bacterial cell migration", which will be introduced below. This phenomenon is described from empirical and experimental observations and a mathematical formulation together with the intervening variables are still to be developed. The tests were carried out in vitro but the knowledge gained may lead to new treatment techniques of infections. This represents an advance in scientific knowledge of metal prostheses, especially of stainless steel pieces.

2. Methodology

Two types of microorganisms were used: a) commercial yeast (*Sacaromise spp*) at the Materials Laboratory at UTN FRP; and b) *S. aureus* at the Laboratory of Microbiology, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral to validate the results obtained with yeast.

2.1 Tests with yeast

The use of yeasts has the advantage of being safe for human health and easily visible in a metallographic microscope as shown in Figure 2. Furthermore, as their behavior can be extrapolated to bacteria, they allow the possibility of drawing valid conclusions.

A sterile atmosphere was guaranteed working between Bunsen burners and using glassware sterilized by dry heat for 120 minutes at 270 °C. For the development of yeasts, pasteurized grape juice was used as a culture medium. Four grams of yeast were placed in an Erlenmeyer flask and 200 mL of culture medium was added. Then different metallic specimens from different types of materials, such as copper, stainless steel, aluminum, magnesium and titanium were placed in the culture. After yeasts colonized the metal, the pieces were observed in a metallographic microscope and the number of adhered yeasts was recorded.

Then, the colonized specimens were used for assembling a cell, whose operation is described in Figure 1. Plate 1, which was colonized by a microorganism, was immersed in a sterile saline bath, along with another sterile plate (2) of a similar surface area and more electropositive. Then, both plates were externally connected causing a short. The electronegative plate easily accepted electrons functioning as anode (+) and the electropositive plate donated electrons functioning as cathode (-).Thus, yeasts (and bacterial) migration began from 1 to 2.

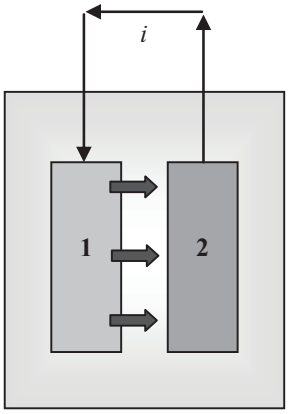

Fig 1. Functional diagram of the bacterial cell migration. Plate 1 colonized with bacteria or yeast and plate 2 is sterile.

2.2 Tests with S. aureus

In tests carried out in the Laboratory of Microbiology (FBCB-UNL) the colonization of the specimens was performed as described for yeast. Then, successive rinses were carried out and counts of Colony Forming Units (CFU) were performed to determine viable bacteria remaining in the samples.

These assays were done as follows: two samples of 316L stainless steel, one with metalworking defects and another perfectly polished, were placed in an Erlenmeyer flask with a culture of *S. aureus* and incubated for 24 hours. After that, the samples were removed and rinsed with sterile saline. Then, they were placed in contact with other metal more electropositive than steel in a solution of the antibiotic cefazolin (Northia) at a concentration of 32 ug/ml, equivalent to the amount used in patients, for 7 hours. Then the specimens were removed and successive rinses were performed, determining in each one the CFU/mL. Figure 2 shows a stainless steel plate inoculated with *S. aureus*.

a **b**

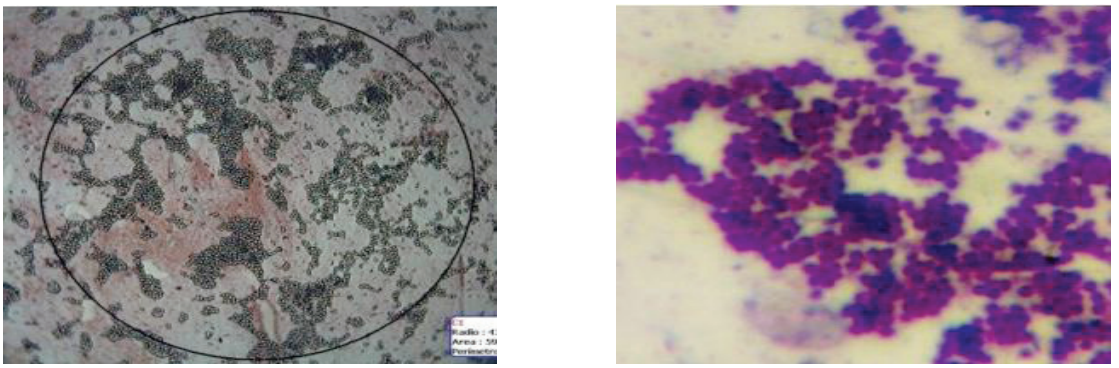

Fig 2. (a) Copper plate colonized with yeasts; (b) Steel plate colonized with *S. aureus*

3. Results and Discussion

2.1 Tests with yeast

It was observed that yeast adhered faster and in greater quantity to the more electronegative metal, and as it descended in the electronegativity scale the amount of adhered yeast was lower and the adhesion time required was increased. Then, it could be stated that that the driving force for the interaction between microorganisms and the

surface of metals is the electric charge, since yeasts are negative and are electrically attracted to the electropositive metal.

This led to a new hypothesis: considering that 316L stainless steel is easily inoculated and more electronegative than Al, Mg or Zn, if the cell is formed between the inoculated stainless steel and another metal (more electronegative) it will generate a stream of negative to positive charge, but if both plates are connected directly the current is reversed. This means that if the plate is inoculated by being electronegative, then it should eject the yeast (or bacteria) when it reverses its load.

After the experiments were carried out it was possible to state the following principle: "Given a sterile, electropositive plate facing a yeast or bacteria inoculated electronegative plate at a specified distance, immersed in an electrically conductive liquid, connected externally creating a short circuit and generating a potential difference between the two, the microorganisms in the contaminated surface will be expelled to the sterile surface plate."

It was observed that the system developed works well and that if plate 1 has no defects it will be completely clean after a characteristic time. If biofilm developed, it was removed during the event or transformed into a dry clay (Figure 3).

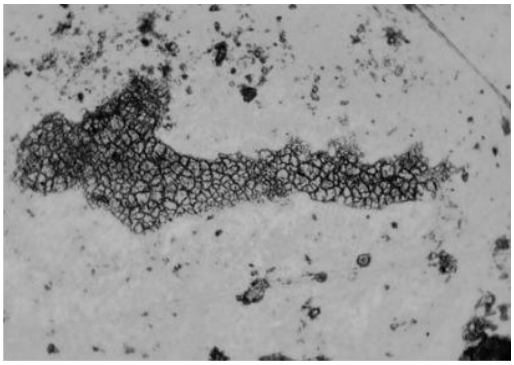

Fig 3. Biofilm turned into dry clay.

2.2 Tests with S. aureus

Similar results were obtained when samples were colonized with *S. aureus*, as the use of the migration cell allowed total elimination of bacteria. When working with a sample with mechanical defects it was observed that there was a reduction of CFU / mL through successive rinses but after 4 rinses CFU could not be determined. When working with the polished sample, CFU could not be determined from the first rinse, indicating that the bacteria were quickly expelled.

The bacterial migration cell is a principle that can be applied and even extrapolated to other types of interactions. However, these are preliminary results and the role played by all the variables must be studied in depth.

Migration is achieved more efficiently when the surfaces are smoother and have fewer defects. As described in a previous study (Spector, 2013) defects mechanically lock microorganisms and make the migration difficult. At a certain time, the cleaned surface or the amount that migrates is proportional to time and to the potential difference generated between the metals.

Other authors have studied the issue of surface adhesion of bacteria. Arnold and Bailey (2000) refer to the surface morphology of the steel and its relationship with the inoculation of bacteria, concluding that more polished surfaces the fewer bacteria they absorbed.

The aim of this work was to verify the influence of the different materials on bacterial inoculation. Unlike the conclusions in Na and Friedman's (1998) work on bacterial adhesion mechanisms to surfaces, the results of this study point to the colonization as a function of the electronegativity of the material.

In our study, we observed there is more bacterial adhesion to highly electronegative metals. Then, we propose the idea of a cell with a highly electropositive plate in a conductive medium (sterile saline) and short-circuited to change the polarity of the plates. This effect produces a migration of bacteria from the colonized plate towards the other plate, cleaning the former in the process.

4. Conclusions

In this paper, bacterial migration was experimentally studied by means of bacterial cell migration, an unprecedented system developed at the UTN FRP Materials Laboratory. The results showed that it is possible to migrate bacteria from a yeast or bacteria colonized plate to a sterile one.

Given that the legislation of the behavior of this phenomenon in properly studied and analyzed, the use of the system developed could bring a solution to many prosthetic infection problems as well as provide practical applications of medical significance. Further studies to corroborate the efficacy of the principle outlined could lead to the disinfection of prostheses without their removal from the patient. As well as this, a system to prevent contamination during surgery could be devised.

Acknowledgements

The authors would like to acknowledge the support of the Government of the Province of Entre Rios for funding this project, Universidad Tecnológica Nacional Regional Paraná for its logistics support and infrastructure, and Dr. Maria Cristina Lura for providing the strain of *S. aureus*.

References

Arnold, J.W. and Bailey, G.W., 2000. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study, Poultry Science, Vol. 79, p. 1839-.1845

García-Pont J, Blanch-Falp J, Coll-Colell R, 2006, Infección de prótesis articulares: estudio prospectivo en 5 Hospitales de Cataluña, Enfermedades infecciosas y Microbiologia clínica, Vol 24. Num. 03.

Giladi M., Porat Y., Blatt A., Wasserman Y., Kirson E., Dekel E. and Palti Y., 2008. Microbial Growth Inhibition by Alternating Electric Fields, Antimicrobial Agents Chemotherapy, Vol. 52, p. 3517-3522

Khoury A.E., Lam K., Ellis B. and Costerton JW, 1992. Prevention and control of bacterial infection associated with medical devices, ASAIO Journal, Vol. 38, p. 174-178.

Marenco J.L., Corzo J, 2008. Infección de prótesis articular. ¿Cuándo sospecharla? Reumatología Clínica, Vol 4, p. 7-12

Na, Y.H. and Friedman, R.J., 1998. Concise review of mechanisms of bacterial adhesion to biomaterial surface. Journal of Biomedical Materials Research, Vol. 43, p. 338-348.

Pickering S., Bayston R. and Scammel B., 2003. Electromagnetic augmentation of antibiotic efficacy in infection of orthopedic implants, The journal of Bone and Joint Surgery, Vol. 85, p. 588-93.

Ramos A, 2008. Infecciones en el paciente inmunodeficiente. ¿Qué papel tienen los fármacos?, Reumatología clínica, Vol. 4 Núm.Extra.3 p.1-6

Spector M., Peretti L., Salas F., Romero G., Iglesias L., 2013. Conducción Bacterial en Prótesis, Anales SAM/CONAMET, 2013.