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Concluding remark

Combating implant infections. Remarks by a women's team

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*An International Cooperative Group**

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ABSTRACT: Research on implant infections requires cooperative efforts and integration between basic and clinical expertises. An international group of women scientists is acting together in this field. The main research topics of the participants of this group are described. Formation of bacterial biofilms, antibiotic resistance and production of virulence factors like adhesins and toxins are investigated. New biomaterials, coatings and drugs designed to inhibit microbial adhesion are evaluated, and infection-resistant biomaterials are under study, such as a novel heparinizable polycarbonate-urethane (Bionate) or incorporation of diamino-diamide-diol (PIME) to reduce bacterial attachment. The correlation between biofilm production and the accessory-gene-regulator (agr) is investigated in Staphylococcus aureus*. The ability to form biofilm has also been shown to be one of the important virulence factors of* Enterococcus faecalis*, favouring colonization of inert and biological surfaces. The study of* quorum sensing *has led to the discovery of a* quorum sensing *inhibitor termed RIP that suppresses staphylococcal biofilm and infections. The immune response and the local defence mechanisms of the host against implant-associated infections, activation and infiltration of immunocompetent cells into the sites of infection have been studied in patients with implant-associated osteomyelitis. Production of monoclonal antibodies (mAbs) as possible vaccines against the staphylococcal collagen-binding MSCRAMMs is in progress. (Int J Artif Organs 2008; 31: 858-64)*

KEY WORDS: Molecular epidemiology, Implant infections, Biofilm, Quorum sensing*, Host immune response, Infection-resistant biomaterials*

INTRODUCTION

Research on implant infections requires cooperative efforts and integration between basic and clinical expertises. An international group of women scientists acting together in this field and their lines of research are briefly presented here.

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Orthopedic Institute, Bologna, Italy.

Her main research topics concern the study of the etiopathogenesis of implant-associated infections, the development of new methods for the characterization of virulence factors (1), such as slime (2), adhesins (3, 4), antibiotic resistance traits (5, 6) and toxins (7) of bacteria responsible for orthopedic infections, the evaluation of new biomaterials and of coatings and drugs designed to inhibit microbial adhesion, and the assessment of infection-resistant biomaterials (8-10). In her laboratory, a strain library has been created where the bacterial clinical isolates, responsible for periprosthetic and surgical infections diagnosed and treated at the Rizzoli Orthopedic Institute, are stored (11). The strain collection currently en-

lists over 1800 isolates.

Recent research is focused on molecular epidemiology of implant infections (12).

In the recent Arciola's researches, new molecular techniques to profile bacterial genomes and to share bacteria responsible for infections into groups derived from a common ancestor have beenapplied to the study of microbial collections of bacteria from implant infections (13).

Besides a precise knowledge of the pathogenesis and epidemiology of implant infections, the goal of these studies is also the design of infection-resistant biomaterials or in the introduction of new preventive or therapeutic measures as virulence gene silencing strategies.

Naomi Balaban, PhD, is a Faculty member at Tufts University, Massachusetts, USA.

Dr. Balaban discovered that virulence in *Staphylococcus aureus* is autoinduced by molecules secreted by the bacteria (14). Autocrine regulation in bacteria is now a well-accepted phenomenon termed *quorum sensing*, and is a subject of interest throughout the world. Dr. Balaban also discovered a *quorum sensing* inhibitor termed RIP that suppresses staphylococcal biofilms and infections (15). She has used multiple device-associated infections models to show that the *quorum sensing* inhibitor RIP can prevent or treat any type of staphylococcal infection, including those involving antibiotic resistant strains like MRSA (reviewed in this issue of IJAO). Her studies thus pave the path for clinical development of *quorum sensing* inhibitors as therapeutics for biofilm diseases and device associated infections (16), currently affecting millions around the world.

Lucilla Baldassarri, PhD, is Research Manager at the Department of Infectious, Parasitic and Immunomediated Diseases, National Institute of Health, Rome, Italy. She has collaborated for a long time with Arciola's team on biofilm production by *Staphylococcus aureus* and *S. epidermidis* and the role of biofilm and of its genetic control as virulence markers in implant and catheter infections (17). Her research interest in the field of implant infections is specifically focused on the virulence factors of *Enterococcus faecalis* and on the pathogenesis of infections caused by enterococci.

Enterococcus faecalis is responsible for the majority of enterococcal infections, and *Enterococcus faecium* accounts for about 20 per cent of them. The presence of implants represents the most relevant risk factor for the development of enterococcal infections. In the pathogenesis of enterococcal implant infections, the ability to form biofilm has recently been shown to be one of the important virulence factors of *Enterococcus faecalis*, encouraging colonization of inert and biological surfaces, while protecting bacterial colonies against antimicrobial substances, and mediating adhesion and invasion of host cells and survival within professional phagocytes (18). Biofilm formation has been shown to be particularly important in the development of prosthetic valve enterococcal endocarditis and stent occlusion. Enterococci are also able to express other surface factors that may support colonization of both inert and biological surfaces, and that may be involved in the invasion of, and survival within, the host cell (19, 20).

Katharina Fromm, is Professor at the University of Fribourg, Department of Chemistry.

Her distinctive research interests are on direct chemical modification of implant surfaces with bactericidal agents in order to provide a new generation of self-protective implants (21). This may be an efficient solution for defeating bacterial adhesion and biofilm formation and should be an important step in the fight against infections. Silver coordination polymer compounds combined with antibiotic derivatives are currently being tested in micro-biological studies for their biocompatibility in leading to soft- and hard-tissue integration and vascularization.

There is an increasing interest in the coordination chemistry silver(I) ions in the context of coordination polymer networks and their polymorphs, as well as in their biological and pharmaceutical activity (22). The main ligand used is the ethanediyl bis(isonicotinate) ligand (L) because (i) it has a flexible backbone; (ii) it contains O- and N-donor atoms to allow coordination to a "soft" cation via the nitrogen atoms, and a hard cation via the oxygen atoms, and, most importantly in this context here, (iii) it is biocompatible (23).

A study of silver(I) coordination polymer compounds [AgL](NO_3) deposited on metal surfaces as nano-structured coating, characterized by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM), showed good *in vitro* performances of antimicrobial activity against *S. sanguinis* and *S. aureus*.

Gertrud Maria Hänsch and **Ursula Obst**. Gertrud Maria Hänsch is Professor at the University of Heidelberg

Medical School (Germany), Department of Immunology and Professor Ursula Obst's research activity is at the Department of Environmental Microbiology, Institute for Technical Chemistry Water Technology and Geotechnology, Forschungszentrum Karlsruhe, Germany.

They are mainly interested in the immune response and the local defence mechanisms of the host against implant-associated infections. They make use of two approaches:

(i) In patients with implant-associated osteomyelitis, activation and infiltration of immunocompetent cells into the sites of infection (in collaboration with the Trauma Center Ludwigshafen, Germany) are being studied. Massive infiltration of leukocytes into the site of infection, particularly of polymorphonuclear neutrophils (PMN), have been found. These cells are highly activated – as shown by the up-regulation of activation-associated receptors. The cells were also primed for enhanced oxygen syntheses. Despite the PMN influx, bacterial biofilm persisted, suggesting that bacteria in biofilms escape the PMN. Moreover, by releasing the cytotoxic and proteolytic activities, the highly activated PMN contribute to local tissue damage as "collateral damage". Moreover, activation and infiltration of T lymphocytes have been found and phenotypic and functional characterization of these cells is in progress.

(ii) The interaction of polymorphonuclear neutrophils (PMN) with bacterial biofilms is studied *in vitro*. Phagocytosis of *S. aureus* biofilms by PMN is under investigation with the aim of identifying basic mechanisms, means to enhance the functional activity of the PMN, and the means used by the biofilm to defend itself against the PMN attack.

The interaction of PMN with *Pseudomonas aeruginosa*, especially effects of *quorum-sensing* molecules and of the extracellular polymer substance on PMN, is a further field of investigation (24-30).

Elisabeth Presterl is Professor at the Medical University of Vienna, Department of Medicine I, Division of Infectious Diseases and Tropical Medicine. In her experience, during more than 15 years of infectious diseases consultancy service – particularly dealing with nosocomial infections – infections of implanted devices, fixation material or prostheses are still the most wearisome to treat. To find out why these kinds of infections do not respond to conventional antimicrobial treatment despite obvious susceptibility to the administered antimicrobial agents, the concept of microbial biofilms emerged into medical microbiology. When testing the antimicrobial susceptibility of viridians streptococci isolated in patients with endocarditis on established biofilms, the results showed that a dose of at least 32- to 64-fold the MIC of the respective isolate is needed to reduce the biofilm density and eradicate the bacteria within streptococcal biofilms (31). Staphylococcal biofilms are by far more resistant to antibiotics. However, alcoholic biocides are bactericidal on biofilms formed by *S. epidermidis* isolates of patients with implant infections and catheter-related bacteremia within very short incubation time (32). The next goal is to evaluate effects of standard and newer antibiotics on biofilms of *S. epidermidis* clinical isolates and to find other supplemental measures to enhance the efficacy of the antibiotic treatment. During the course of her work, Presterl's group has become larger because of interdisciplinary cooperation with orthopedic and trauma surgeons, the otorhinologists (focusing on dynamic Candida biofilms of laryngeal protheses) and the dental medical school.

Stefania Stefani is Professor of Microbiology at the University of Catania, Department of Microbiological and Gynecological Sciences. Since 2001, she and her research group (Viviana Cafiso and Taschia Bertuccio) have added the study on biofilm production in *Staphylococcus* spp. to their topics of research. At the beginning, they started studying *S .epidermidis* by demonstrating that the *ica*RADBC was present in only 45% of the strains and that these showed a significantly higher percentage of resistance to the major antibiotics tested than the corresponding *ica*-negative strains. They also demonstrated that a co-transcription of at least *ica*AD genes was necessary for biofilm production (33).

Subsequently, the correlation between biofilm production and the accessory-gene-regulator (*agr*) was investigated in *Staphylococcus aureus* strains isolated from CVC infections. They demonstrated that inactivation of the *agr* system in *agr*-II and IB-variant is linked to the production of a large amount of biofilm in all strains, which was due to the lack of expression of *ica*R and *rsb*U and a consequent early *ica*A and *atl* expression, whereas the peptidoglycan-hydrolase *dlt*A, responsible for the cell wall turnover was expressed later (34, 35).

In an attempt to look at possible anti-biofilm therapies, a study was started of serratio-peptidase (SPEP) as a biofilm inhibitor. In preliminary results, it was observed

that the treatment with this enzyme affects biofilm growth and protein expression in different Gram-positive bacterial species including *Staphylococcus* spp. (36).

Investigation into this important aspect of the biology of microorganisms (above all *S. aureus,* but also *P. aeruginosa*) is continuing in Stefani's laboratory. The study of the involvement of different metabolic and regulatory genes by measuring the level of expression in different environmental conditions is currently in progress.

Joanna Verran, is Professor at the Manchester Metropolitan University, School of Biology, Chemistry and Health Science, UK. Her research group is diverse and active, currently comprising besides Professor Verran, two postdoctoral researchers, and six PhD students.

The research at MMU focuses on the interactions occurring between microorganisms and inert surfaces, many of which find use in implants. Other applications include hygienic food contact surfaces, and external surfaces in the hospital environment, where potential exists for cross-infection. Verran's group is able to fabricate surfaces with defined topography and with metal and alloy coatings, produced via plasma vapor deposition.

A review of surfaces used in the food industry (37) includes issues of interest in implant infections, by focusing on the effect of surface topography and chemistry, and the presence of organic material on the retention and survival of microorganisms on these surfaces.

Recent work had provided interesting focus on the survival of microorganisms on stainless steel and copper surfaces with potential use in the hospital environment, subjected to repeated soiling and cleaning cycles. While stainless steel exerted no antimicrobial effect, it remained relatively easy to clean: copper was antimicrobial, but tarnished rapidly, and lost some antimicrobial properties due to its reactivity with soil and cleaning formulations. Implications are discussed in the publication (38).

A different, but very relevant aspect focuses on denture plaque. Microorganisms isolated from this important but neglected biofilm include those associated with denture stomatitis, oral malodor, and some opportunist pathogens more commonly associated with systemic disease. Thus denture hygiene and plaque control are important aspects to maintenance of oral and general health of this increasing elderly population (39), and the characteristics of the polymers used to fabricate these prostheses might be modified to improve overall health status.

Livia Visai, PhD, is Research Manager at the University of Pavia, Department of Biochemistry, Italy.

Her research interest has been focused on staphylococci as important causes of nosocomial and medical-device-related infections. In order to prevent or treat bacterial infections of implanted devices, three strategies have been investigated: a) production of monoclonal antibodies (mAbs) against the staphylococcal collagen-binding MSCRAMM (CNA) as a possible vaccine; b) development of the synthesis of a novel heparinizable polycarbonate-urethane (Bionate) incorporating a tailor-made diamino-diamide-diol (PIME) to reduce bacterial attachment; c) photodynamic treatment (PDT) for the inactivation of bacteria in biofilms. In the first case, mAbs raised against the minimal ligand binding domain, CNA-(151–318), were able to inhibit collagen binding to intact *S. aureus* cells and also to interfere with the attachment of bacteria to collagen substrates. Some of the mAbs were also able to detach bacteria that had adhered to a collagen substrate in a preincubation, raising the possibility that these mAbs may be used as therapeutic agents (40). In the second study, a new synthesized Bionate-PIME was developed to be used for intravascular catheters. *In vitro* bacterial interaction tests showed a decrease in colonization of staphylococcal strains on the heparinized Bionate-PIME surfaces, confirming that preadsorbed heparin plays a role in mediating the biomaterial surface/bacterial cells interactions (41). In the last approach, the effect of PDT of toluidine blue O (TBO) on the viability and structure of biofilms of staphylococcal strains was investigated. Significant inactivation of cells was observed when staphylococcal biofilms were exposed to TBO and laser simultaneously. The results suggest that PDT may be a useful approach for the inactivation of staphylococcal biofilms already adhering to solid surfaces of medical implants (42).

CONCLUSIONS

An international team of women scientists is engaged in a cooperative task: combating implant infections. The team gathers numerous, complementary areas of expertise and is animated by a spirit of collaboration among the research groups. Collections of hospital and environmental bacterial strains coming from different countries, an exchange of methods and technologies, and the sharing of information on the scientific progress of each group will be the targets of the cooperative team.

The women scientists participating in the cooperative team have also forged friendships and the opportunity to periodically collaborate in contributing to the issue “Focus on Implant Infections” in the *International Journal of Artificial Organs* provides a common forum of their scientific progress.

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Conflict of interest statement
The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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REFERENCES

1. Campoccia D, Speziale P, An YH, Del Pozo JL, Ceresa L, Pegreffi F, Montanaro L, Arciola CR. Innovative methods of rapid bacterial quantification and applicability in diagnostics and in implant materials assessment. Int J Artif Organs 2007; 30: 842-51.
2. Arciola CR, Baldassarri L, Montanaro L. Presence of icaA and icaD genes and slime production in a collection of staphylococcal strains from catheter-associated infections. J Clin Microbiol 2001; 39: 2151-6.
3. Bustanji Y, Arciola CR, Conti M, Mandello E, Montanaro L, Samorí B. Dynamics of the interaction between a fibronectin molecule and a living bacterium under mechanical force. Proc Natl Acad Sci U S A 2003 Nov 11; 100: 13292-7.
4. Arciola CR, Bustanji Y, Conti M, Campoccia D, Baldassarri L, Samorì B, Montanaro L. *Staphylococcus epidermidis*-fibronectin binding and its inhibition by heparin. Biomaterials 2003; 24: 3013-9.
5. Campoccia D, Montanaro L, Baldassarri L, An YH, Arciola CR. Antibiotic resistance in *Staphylococcus aureus* and *Staphylococcus epidermidis* clinical isolates from implant orthopedic infections. Int J Artif Organs 2005; 28: 1186-91.
6. Arciola CR, Campoccia D, An YH, Baldassarri L, Pirini V, Donati ME, Pegreffi F, Montanaro L. Prevalence and antibiotic resistance of 15 minor staphylococcal species colonizing orthopedic implants. Int J Artif Organs 2006; 29: 395-401.
7. Arciola CR, Baldassarri L, Von Eiff C, Campoccia D, Ravaioli S, Pirini V, Becker K, Montanaro L. Prevalence of genes encoding for staphylococcal leukocidal toxins among clinical isolates of *Staphylococcus aureus* from implant orthopedic infections. Int J Artif Organs 2007; 30: 792-7.
8. Legeay G, Poncin-Epaillard F, Arciola CR. New surfaces with hydrophilic/hydrophobic characteristics in relation to (no)bioadhesion. Int J Artif Organs 2006; 29: 453-61.
9. Petrini P, Arciola CR, Pezzali I, Bozzini S, Montanaro L, Tanzi MC, Speziale P, Visai L. Antibacterial activity of zinc modified titanium oxide surface. Int J Artif Organs 2006; 29: 434-42.
10. Campoccia D, Montanaro L, Agheli H, Sutherland DS, Pirini V, Donati ME, Arciola CR. Study of *Staphylococcus aureus* adhesion on a novel nanostructured surface by chemiluminometry. Int J Artif Organs 2006; 29: 622-9.
11. von Eiff C, Arciola CR, Montanaro L, Becker K, Campoccia D. Emerging *Staphylococcus* species as new pathogens in implant infections. Int J Artif Organs 2006; 29: 360-7.
12. Montanaro L, Campoccia D, Arciola CR. Advancements in molecular epidemiology of implant infections and future perspectives. Biomaterials 2007; 28: 5155-68.
13. Campoccia D, Baldassarri L, Pirini V, Ravaioli S, Montanaro L, Arciola CR. Molecular epidemiology of *Staphylococcus aureus* from implant orthopaedic infections: Ribotypes, agr polymorphism, leukocidal toxins and antibiotic resistance. Biomaterials 2008; 29: 4108-16.
14. Balaban N and Novick RP. Autocrine regulation of toxin synthesis by *Staphylococcus aureus*. Proc Natl Acad Sci USA 1995; 92: 1619-1623.
15. Balaban N, Goldkorn T, Nhan RT, Dang LB, Scott S, Ridgley RM, Rasooly A, Wright SC, Larrick JW, Rasooly R, Carlson JR. Autoinducer of virulence as a target for vaccine and therapy against *Staphylococcus aureus*. Science 1998; 280: 438-40.
16. Balaban N (ed). Control of Biofilm Infections by Signal Manipulation. New York: Springer-Verlag; 2008.
17. Arciola CR, Baldassarri L, Montanaro L. In catheter infec-

tions by *Staphylococcus epidermidis* the intercellular adhesion (ica) locus is a molecular marker of the virulent slime-producing strains. J Biomed Mater Res 2002; 59: 557-62.
18. Baldassarri L, Creti R, Montanaro L, Orefici G, Arciola CR. Pathogenesis of implant infections by enterococci. Int J Artif Organs 2005; 28: 1101-9.
19. Creti R, Imperi M, Bertuccini L, Fabretti F, Orefici G, Di Rosa R, Baldassarri L. Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. J Med Microbiol 2004; 53 (Pt 1): 13-20.
20. Baldassarri L, Creti R, Recchia S, Pataracchia M, Alfarone G, Orefici G, Campoccia D, Montanaro L, Arciola CR. Virulence factors in enterococcal infections of orthopedic devices. Int J Artif Organs 2006; 29: 402-6.
21. Fromm KM. Coordination Compounds. In: Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc.
22. Fromm KM, Gueneau ED. Structures of Alkali and Alkaline Earth Metal Clusters with Oxygen Donor Ligands. Polyhedron 2004; 23; 1479-1504.
23. Robin AY, Fromm KM. Coordination Polymer Networks with O- and N-Donors: What they are, why and how they are made. Coord Chem Rev 2006; in press.
24. Wagner C, Kondella K, Bernschneider T, Heppert V, Wentzensen A, Hänsch GM. Post-Traumatic osteitis: analysis of inflammatory cells recruited into the site of infection. Shock 2003; 20: 503-10.
25. Wagner C, Kaksa A, Müller W, Denefleh B, Heppert V, Wentzensen A, Hänsch GM. Polymorphonuclear neutrophils in posttraumatic osteomyelitis: cells recovered from the inflamed site lack chemotactic activitiy, but generate superoxides. Shock 2004; 22: 108-15.
26. Wagner C, Obst U, Hänsch GM. Implant-associated posttraumatic osteomyelitis: collateral damage by local host defence? Int J Artif Organs 2005; 28: 1172-80.
27. Wagner C, Iking-Konert C, Hug F, Stegmaier S, Heppert V, Wentzensen A, Hänsch GM. Cellular inflammatory response to persistent localized *Staphylococcus aureus* infection: phenotypical and functional characterization of polymorphonuclear neutrophils (PMN). Clin Exp Immunol 2005; 143: 70-77.
28. Wagner C, Heck D, Lautenschläger K, Iking-Konert C, Heppert V, Wentzensen A, Hänsch GM. T-lymphocytes in implant-associated posttraumatic osteomyelitis: identification of cytotoxic T-effector cells at the site of infection. Shock 2006; 25: 241-246.
29. Zimmermann S, Wagner C, Müller W, Brenner-Weiss G, Hug F, Prior B, Obst U, Hänsch GM. Induction of neutrophil chemotaxis by the *quorum*-sensing molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL). Infect Immun 2006; 74: 5687-92.
30. Wagner C, Zimmermann S, Brenner-Weiss G, Hug F, Prior B, Obst U, Hänsch GM. The *quorum*-sensing molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL) enhances the host defence by activating polymorphonuclear neutrophils (PMN). Anal Bioanal Chem 2007; 386: 481-7.
31. Presterl E, Grisold AJ, Reichmann S, et al. Viridans streptococci in endocarditis and neutropenic sepsis: biofilm formation and effects of antibiotics. J Antimicrob Chemother 2005; 55: 45-50.
32. Presterl E, Suchomel M, Eder M, et al. Effects of alcohols, povidone-iodine and hydrogen peroxide on biofilms of *Staphylococcus epidermidis*. J Antimicrob Chemother 2007; 60: 417-20
33. Cafiso V, Bertuccio T, Santagati M, Campanile F, Amicosante G, Perilli MG, Selan L, Artini M, Nicoletti G, Stefani S. Presence of the ica operon in clinical isolates of *Staphylococcus epidermidis* and its role in biofilm production. Clin Microbiol Infect 2004; 12: 1081-8.
34. Viviana Cafiso, Taschia Bertuccio, Maria Santagati, Vanessa Demelio, Daniela Spina, Giuseppe Nicoletti and Stefania Stefani. agr-Genotyping and transcriptional analysis of biofilm-producing *Staphylococcus aureus*. FEMS Immunol Med Microbiol 200; 1: 220-7.
35. Cafiso Viviana, Bertuccio Taschia, Demelio Vanessa, Spina Daniela, Vizzari Giuseppe, Purrello Simona and Stefania Stefani. Differential gene expression in biofilm forming agr- I and II *Staphylococcus aureus*. 43rd ESCMID 2007.
36. Artini M, Papa R, Scoarughi G, Cellini A, Cafiso V, Stefani S, Selan L. How can Different Proteases (Metallo-Proteases and Serin-Proteases) Influence Biofilm Formation in Various *Staphylococcus* Strains? A Correlation with Bacterial Genetic Background. 108th ASM. Boston (USA) 2008.
37. Verran J, Airey P, Packer A, Whitehead KA. Microbial retention on open food contact surfaces, and implications for food contamination. Adv Appl Microbiol 2008; 64: 223-46.
38. Airey P, Verran J. Potential use of copper as a hygienic surface: problems associated with cumulative soiling and cleaning. J Hosp Infect 2007; 67: 272-8.
39. Coulthwaite L, Verran J. Potential pathogenic aspects of denture plaque. Br J Biomed Sci 2007; 64: 180-9.
40. Visai L, Xu Y, Casolini F, Rindi S, Höök M, Speziale P. Monoclonal Antibodies to CNA, a Collagen-binding Microbial Surface Component Recognizing Adhesive Matrix Molecules, Detach *Staphylococcus aureus* from a Collagen Substrate. J Biol Chem 2000; 275: 39837-45.
41. De Nardo L, Fare S, Di Matteo V, Cipolla E, Saino E, Visai L, Speziale P, Tanzi MC. New heparinizable modified poly(carbonate urethane) surfaces diminishing bacterial colonization. J Mater Sci Mater Med 2007; 18: 2109-15.
42. Sharma M, Visai L, Bagheri F, Cristiani I, Gupta PK, Speziale P. Toluidine Blue-Mediated Photodynamic Effects on Staphylococcal Biofilms. Antimicrob Agents Chemother 2008; 52: 299-305.