



TOWARDS NOVEL STRATEGIES TO PREVENT PROSTHETIC JOINT INFECTION: THE POTENTIAL OF CATIONIC ANTIMICROBIAL PEPTIDES

Francesca D'Este^{1*}, Debora Oro¹, Alessandro Tossi², Margherita Zanetti¹,
Barbara Skerlavaj^{1§}

¹ Dept. Medical and Biological Sciences, University of Udine, Ple Kolbe 4, 33100 Udine

² Dept. Life Sciences, University of Trieste, via Giorgieri 5, 34127 Trieste

* francesca.deste@uniud.it, §barbara.skerlavaj@uniud.it

Abstract — The prevention of implant-associated infection, one of the leading causes of arthroplasty failure, remains a major challenge in orthopaedic surgery. The main strategies currently pursued are based on surface modifications to render implants less susceptible to bacterial colonization. The antimicrobial peptides of the innate immunity (AMPs) are considered a promising source of novel anti-infective agents and are receiving increasing attention in the field of medical devices due to their proven anti-biofilm properties. Our group has selected three AMPs derived from natural sequences, that are highly effective *in vitro* against prosthetic joint pathogens, also including antibiotic-resistant clinical isolates. These peptides maintain their antimicrobial activity under conditions relevant to the joint environment and are safe to osteoblast cells under similar conditions, which makes them suitable for orthopaedic applications. Current studies aimed at evaluating their anti-inflammatory and osteogenic potential will allow to identify the best candidate to be used for the development of novel AMP-based antibacterial coatings for arthroplasty implants.

Index Terms — TRANS2CARE, prosthetic joint infection, antimicrobial peptides, biomaterials, arthroplasty

1 BACKGROUND

Infection represents a potentially devastating complication of total joint arthroplasty, resulting in substantial morbidity and disability with huge economic and social costs [1]. Most infections are caused by *Staphylococcus* spp and often involve multidrug-resistant strains. Biofilm formation on the implant surface is almost universally associated with these infections, which poses relevant challenges to clinical management and urges development of effective methods to prevent bacterial colonization of prosthetic implants [1].

The antimicrobial peptides (AMPs) (Figure 1) are small protein components of the innate immune system produced in the phagocytic and the epithelial cells of mammals [2]. These molecules are considered promising alternatives to conventional antibiotics owing to a potent and broad-spectrum antimicrobial activity also against multi-drug resistant strains, capacity to inhibit biofilm formation and low propensity to induce selection of resistant mutants [3,4]. Importantly, immobilized AMPs have been shown to retain antimicrobial activity [5]. Additionally, some AMPs may inhibit inflammatory responses [6] and promote bone formation [7,8], which makes this class of compounds attractive candidates for orthopaedic applications.

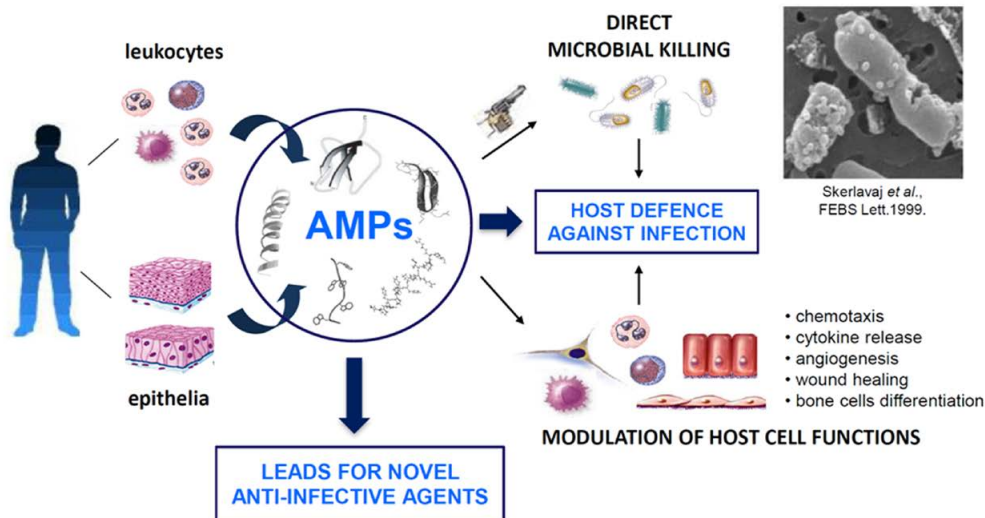


Figure 1: Biological properties and therapeutic potential of AMPs.

2 OBJECTIVES

In the framework of the Trans2Care project, we are currently investigating the antimicrobial and host cell -directed activities of selected natural AMPs and *in silico* designed variants in the context of prosthetic joint infection, with the aim of identifying promising candidates for application in arthroplasty.

Our final goal, in collaboration with the Research Department of Valdoltra Orthopaedic Hospital (Ankaran, Slo; PP11, Trans2Care), is to exploit these peptides for the development of novel infection-resistant coatings for arthroplasty implants.

3 APPROACH & METHODS

3.1 General approach

To assess their potential for the development of infection-resistant orthopaedic biomaterials, AMPs are evaluated for:

1. *In vitro* antimicrobial activity against prosthetic joint pathogens;
2. Stability in biological fluids (e.g., human serum, synovial fluid);
3. Toxicity towards osteoblast and blood cells;
4. Anti-inflammatory activity (neutralization of proinflammatory bacterial components, i.e., lipopolysaccharide - LPS and lipoteichoic acid - LTA);
5. Osteogenic potential (osteoblast proliferation and differentiation).

3.2 Methods

1. MIC-MBC determination, growth kinetics and anti-biofilm assays on reference *S. aureus* and *S. epidermidis* strains and clinical isolates from orthopaedic infections. Assays are performed both in standard media and under conditions relevant to the *in vivo* environment (i.e., in the presence of human serum, hyaluronic acid, synovial fluid).
2. Liquid chromatography – mass spectrometry.
3. Hemolysis tests; LDH (lactate dehydrogenase) release and MTT assays on osteoblast (MG-63, Saos-2) and macrophage (RAW 264.7) cell lines.
4. Inhibition of LPS/LTA-induced NO release (Griess test) and cytokine induction (qPCR) in RAW 264.7 cells.
5. MTT assay; ALP (alkaline phosphatase) activity assay and qPCR analysis of osteoblast differentiation markers in MG-63 cells.

4 RESULTS

This study focuses on five cationic AMPs derived from natural sequences. All of these peptides display good *in vitro* activity against pathogens involved in prosthetic joint infections, including reference biofilm-forming *S. aureus* and *S. epidermidis* strains and clinical isolates from prosthetic joint infections. Their MIC values against these pathogens are comparable to those of cefazolin, the antibiotic of choice in perioperative prophylaxis in orthopaedic surgery. All peptides inhibit biofilm formation on abiotic surfaces at MIC values.

Three AMPs in particular appear to hold promise for orthopaedic applications as they are safe to osteoblast cells at microbicidal concentrations and maintain their antimicrobial activity also in the presence of hyaluronic acid and in synovial fluid.

Preliminary data indicate that the activity of the latter peptides is affected to a different extent by the presence of human serum. The stability of these AMPs in human serum and in synovial fluid is currently under investigation. Further studies aimed at evaluating their effects on osteoblast functions and on

macrophage inflammatory responses will allow to better define their potential for the development of innovative biomaterials for arthroplasty.

5 POTENTIAL NEW PRODUCTS & SERVICES

In collaboration with the Research Department of Valdoltra Hospital, the AMP with the best properties will be exploited to develop novel protective coatings for titanium implants. The idea is to incorporate the peptide into a chitosan film deposited on the titanium surface. The ultimate goal is to obtain infection-resistant biomaterials possibly also endowed with anti-inflammatory and osteoinductive properties. Such coatings may be suitable for joint prostheses and for various orthopaedic, maxillofacial and dental applications.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Prof. A. Tossi (University of Trieste - LP, Trans2Care);
Dr. F. Šulek, Prof. I. Milošev (Valdoltra Orthopaedic Hospital - PP11, Trans2Care).

6.2 With SMEs

Preliminary contacts with a company in the field of orthopaedic devices.

6.3 With hospitals

Ongoing collaborations with Valdoltra Orthopaedic Hospital (PP11, Trans2Care), with the University Hospital "S. Maria della Misericordia" of Udine and the Institute of Public Health Koper (Slo) for the supply of clinical samples, e.g., bacterial isolates, synovial fluid.

7 CONTACTS OR COLLABORATIONS NEEDED

Research groups specialized in bone and biomaterials studies: for sharing of knowledge-expertise-facilities (e.g., animal experimental models); joint grant applications.

Biomedical companies in the orthopaedic and biomaterials fields: for technical and/or financial support; joint grant applications.

8 COMMUNICATION TOOLS

8.1 Conference contributions and presentations

Conference contributions:

- Šulek F, Milošev I, D'Este F, Skerlavaj B. Trans2Care approach towards prevention of biofilm formation: antimicrobial coatings. 6th Central European Conference "Chemistry towards Biology", Trieste (Italy), 10-13.09.2013.
- Oro D, D'Este F, Skerlavaj B, Tossi A, Zanetti M. Towards novel strategies to prevent prosthetic joint infection: the potential of α -helical antimicrobial peptides. 6th Central European Conference "Chemistry towards Biology", Trieste (Italy), 10-13.09.2013.

- Šulek F, Milošev I, D'Este F, Skerlavaj B. Bio-molecular coating for orthopaedic implants with a strong antibacterial capability. "NanotechItaly 2013 – key enabling technologies for responsible innovation", Venice-Mestre (Italy), 27-29.11.2013.

Oral presentations - the results of the present study have been presented at:

- Third Scientific Day of Valdoltra Orthopaedic Hospital (Ankaran, Slo, 30.11.2012)
<http://www.trans2care.eu/NewsData.aspx?IdNews=56&ViewType=Old&IdType=390>,
- Workshop "Orthopaedic diseases: Diagnostics, treatments and research" (Ankaran, Slo, 18.10.2013)
<http://www.trans2care.eu/NewsData.aspx?IdNews=89&ViewType=Old&IdType=390>.

8.2 Additional information

Trans2Care project:
www.trans2care.eu

University of Udine:
www.uniud.it
www.trans2care.eu/PartnerData.aspx?section=377.424&IdPartner=93

Authors:
[linkedin.com/in/francescadeste](https://www.linkedin.com/in/francescadeste)
[researchgate.net/researcher/39862473_Barbara_Skerlavaj/](https://www.researchgate.net/researcher/39862473_Barbara_Skerlavaj/)
it.linkedin.com/pub/debora-oro/77/550/317

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): 22.000€, personnel costs included.

9.2 For applied research (solutions for real-world problems): 22.000€, personnel costs included.

9.2 For pilot & demonstrator activities (to develop a prototype): 22.000€, personnel costs included.

10 CONCLUSION

In the present study we have selected three promising AMP candidates for orthopaedic applications. Current efforts are aimed at incorporating one of these peptides in a chitosan antibacterial coating synthesized at the Valdoltra Hospital. Such functionalization of the coating may allow to enhance and broaden its antibacterial capability while minimising the risk of raising pathogen resistance. Moreover, given the proven multifunctionality of AMPs and their ability to interact with the host cells, we have in the pipeline the possibility to test additional properties of the AMPs under study that could be advantageous in the context of prosthetic joint infection.

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