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Conference Report

Eco-Friendly Antimicrobial Strategies to Fight Chronic Wound Infections Associated with Multidrug-Resistant Pathogens for the Development of Innovative Medical Systems (SCIAMI) †

Simonetta D'Ercole

Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy; simonetta.dercole@unich.it

† Presented at the Conference SCIAMI 2024, Chieti, Italy, 7–8 June 2024.

Abstract: The SCIAMI International Workshop was held in Chieti, Italy, at University G. d'Annunzio, Chieti-Pescara, from 7 to 8 June 2024. It was organized by Simonetta D'Ercole and was funded by the European Union–Next Generation EU, "MUR-Fondo Promozione e Sviluppo–UdA, SCIAMI, Eco-friendly antimicrobial Strategies to fight Chronic wound Infections Associated with Multidrug resistant pathogens for the development of Innovative medical systems". This conference report is an abstract collection from different sessions of SCIAMI.

Keywords: antimicrobial strategies; multidrug resistant pathogens; chronic wound

1. Introduction

Antimicrobial resistance (AMR) is one of the major public health problems of our time and is considered the leading cause of mortality by the World Health Organization (WHO), which encourages the search for new strategies to overcome this issue. In particular, multidrug-resistant (MDR) chronic wound pathogens compromise wound healing, affecting billions of people worldwide and impacting the quality of life of patients. In a chronic wound, poly-microbial biofilms play a key role in pathogenesis and increase the resistance/tolerance phenomenon, suggesting the need for new solutions. "Eco-friendly antimicrobial Strategies to fight Chronic-wound Infections Associated with Multidrug-resistant pathogens for the development of Innovative medical systems" (SCIAMI) was a two-day event (Figure 1) dedicated to chronic wound infections, aiming to provide cutting-edge knowledge on the worrying phenomenon of antimicrobial resistance and the development of new strategies, advanced technologies, and clinical therapies, under the One-Health umbrella.

The development of new antimicrobial agents with novel mechanisms of action, and/or restoring antibiotic efficacy against the main human and animal chronic wound pathogens, is the basis of new strategies to combat chronic wound infections.

In line with the "One Health" approach, during the SCIAMI International Workshop, new, non-antibiotic, eco-sustainable strategies to fight the spread of AMR and corresponding methods were proposed. In particular, the antibacterial activity of graphene oxide, resveratrol, natural compounds, such as carvacrol, curcumin, and rutin, many forms of food waste such as hazelnut shells and saffron petals, enzymes derived from microbial and bacteriophage sources, mucoadhesive polymers, nanoclays, and novel technologies (LEDs and CMFs) have been reported.

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Figure 1. Conference SCIAMI 2024 (https://microbiolab.it/congress/).

The SCIAMI International Workshop was held in Chieti, Italy, at Università G. d'Annunzio, Chieti-Pescara, from 7 to 8 June 2024.

The Chair of the Organizing Committee was Prof. Simonetta D'Ercole. The members of the Organizing Committee were Morena Petrini, Emanuela Di Campli, and Paola Di Fermo. The Chair of the Scientific Committee was Luigina Cellini and the members were Mara Di Giulio and Silvia Di Lodovico (www.microbiolab.it).

2. Focus on Chronic Wound Infections

2.1. Innovative Strategies for Facing the Silent Pandemic

Concepción González Bello [1]

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.

concepcion.gonzalez.bello@usc.es

The ability of antibiotics to cure bacterial infectious diseases is now at serious risk due to the consequences of broad-spectrum antibiotic use on microbiome stability and pathogen resistance [1]. The disappointing returns from this approach have triggered a big shift in the antibiotic discovery perspective in recent years, with attention turning to precision antibacterials and thus compounds with focused and well-defined activities, to address infections without damaging microbiomes or incentivizing resistance. This also includes the implementation of novel vectorization approaches directed to improve the internalization of antibacterial agents into deadly Gram-negative pathogens through precise and well-defined mechanisms [2]. Merging novel antibiotic classes, clinically unexploited targets, and new mechanisms of action seems to be an excellent combination to mitigate the risk of pre-existing resistance. Herein, we present our recent efforts to develop new antimicrobial agents with a new mechanism of action, by selectively disabling clinically unexploited bacterial targets that have vital roles in bacterial pathogens' viability [3–5] (Figure 2). In addition, our efforts to restore antibiotic efficacy in deathly pathogens will be discussed [6,7].

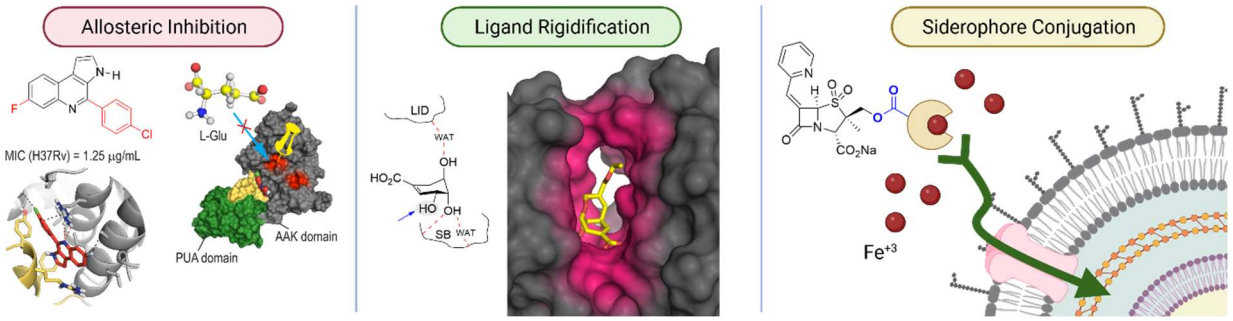

Figure 2. New antimicrobial agents with a new mechanism of action.

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2.2. It Is Not the Hole in the Patient—But the Whole Patient. Biofilm Is a Key Factor in Wounds

Trine Rolighed Thomsen [1,2], Yijuan Xu [1,2], Camilla Jessen [2], Kim Tanja Hejsenvak Nørgaard [1], Jan Lorenzen [2], Ida Clement Thaarup [3], Thomas Bjarnsholt [3,4], Klaus Kirketerp-Møller [5]

[1] Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark.

[2] The Danish Technological Institute, Aarhus C, Denmark.

[3] Costerton Biofilm Center, Institute of Immunology and Microbiology, University of Copenhagen, Denmark.

[4] Department of Clinical Microbiology, Copenhagen University Hospital, Copenhagen, Denmark

[5] Copenhagen Wound Healing Center, Bispebjerg University Hospital, Copenhagen, Denmark.

Chronic wounds are a burden to society, the healthcare system, and the individuals affected. Microbes are known to exist as biofilms in wounds and contribute to the development of the 'chronicity' of such wounds. They are generally difficult to eradicate and prevent despite treatment. Many wound care products and strategies are available today; however, only very few wound care products have been evaluated for their antibiofilm effect. In addition, it must now be time to combine such treatment with more holistic approaches, looking at the whole patient.

The aim of this study was to explore whether new in vitro and ex vivo models can be used to evaluate antimicrobial products and whether they can ultimately be combined with digital tools for individuals with diabetic wounds.

Two different methodologies were utilized. Novel in vitro and ex vivo porcine models employing Staphylococcus aureus, Pseudomonas aeruginosa, and Candida were applied to test antimicrobial products. The Wound App, which was developed, co-designed, and tested by citizens in collaboration with healthcare professionals, companies, and researchers as part of the project HealthD360, was applied as well. A citizen registers a wound, takes photos, and reports the wound size, pain, inflammation, and wound fluid daily. A data plugin collects the numbers of steps, activity, and other 24/7 data from Apple Health and Google Fit devices.

Stable co-existence of *P. aeruginosa*, *S. aureus*, and Candida was confirmed in the models and some test results will be presented. Inflammation and pain level registered on a daily/weekly basis was positively correlated to the wound size of patients using the Wound App and negatively correlated with activity level.

The in vitro and ex vivo models combined with a citizen app collecting 24/7 data show great potential for evaluating antibiofilm products and other interventions in the future.

2.3. Biocatalytic Dispersion of Biofilms: Enzymes on the Battlefield with Wound Bacterial Infections

Radosław Drozd [1] and Magdalena Chareza [1]

[1] Department of Microbiology and Biotechnology, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin, Piastów 45, 70-311 Szczecin, Poland

Bacterial biofilms present a formidable challenge in effectively managing chronic wounds of various origins, frequently resulting in severe and even life-threatening complications that may necessitate amputations or lead to sepsis. The resilience of these infections to traditional antibiotic treatments arises from the complex structure of the biofilm matrix, which is abundant in diverse exopolymers (EPS) including proteins, polysaccharides, lipids, and nucleic acids. This matrix forms a robust barrier that shields bacterial cells from environmental stressors such as UV radiation, antibiotics, and disinfectants. Consequently, the effectiveness of most antibiotics against biofilms in chronic infections is limited, underscoring the need to explore innovative therapeutic approaches.

A promising approach to enhance biofilm disruption involves the use of highly specific enzymes derived from microbial and bacteriophage sources, including those involved in EPS synthesis pathways. Enzymes offer several advantages, including high biocompatibility, minimal cytotoxicity, and reduced propensity for resistance development compared to antibiotics. Furthermore, advancements in protein engineering enable the design of enzymes with enhanced stability, specificity and reduced immunogenicity, broadening their therapeutic potential in diseases where effective biofilm eradication is paramount.

By harnessing the unique properties of enzymes, we can pave the way for more effective treatments targeting bacterial biofilms in chronic wounds, ultimately improving patient outcomes and reducing the burden of associated complications in treating difficult-to-heal wounds.

3. Focus on Ecofriendly Strategies

3.1. Nanoclays in Wound Healing Formulations

César Viseras

Department of Pharmacy and Pharmaceutical Technology, University of Granada

Nanoclays have been studied in depth as useful materials for drug delivery. Several active compounds have been loaded onto these inorganic components to increase solubility, improve stability, reduce toxicity and enhance bioavailability, with a consequent increase in therapeutic response. Together, nanoclay–polymer nanocomposites have the potential to provide predictable, precise, and reproducible patterns of controlled release and site-specific delivery, as with nanoparticulate systems. Polymer–clay nanocomposites with highly specific biopharmaceutical properties are promising biomedical nanomaterials, showing high biocompatibility and mucoadhesion. If required, the compatibility of such clays with organic biopolymers can be improved through functionalization with cationic surfactants such as alkylammonium (organoclays), reducing the surface energy of the inorganic host and providing new functional groups that can react with the polymer matrix. Moreover, nanoclays are well-known ingredients of skin formulations once they have met safety and stability requirements. Our group has paid particular attention to the potential biomedical applications of nanoclays in skin therapeutics. With these premises, the presentation will focus on the potential of nanoclays as useful in wound healing formulations and tissue engineering scaffolds and, in particular, their use as bioink ingredients for THE 3D printing and 3D bioprinting of scaffolds for tissue engineering and regenerative medicine.

3.2. Bio-Derived Products for Innovative Wound Healing Products

Cinzia Pagano and Luana Perioli

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia.

The treatment of chronic wounds, characterized by long healing times or non-healing, is very challenging. These wounds are often characterized by inflammation events associated with infections, and often, the use of conventional antibiotics is very limited due to the growing problem of antibiotic resistance. The treatment currently available for chronic wounds is still not satisfactory and the search for new, more efficacious formulations is ongoing.

Recent EU Regulation 2055/2023 poses serious concerns about the use of many excipients (non-biodegradable synthetic and semi-synthetic polymers), employed for many years in health products, currently classified as microplastics. They represent a serious problem for human and environmental health as people are exposed every day to microplastics present in healthcare products, foods, and in the air, with consequent increased incidence of many pathologies.

Thus, the determination of valuable substitutes of currently used polymers as well as the determination of bioactive molecules that come from the environment is a field worthy of investigation. Moreover, many forms of food waste represent a valuable source of both biopolymers and active ingredients, suggesting a valuable reuse of materials known to be useless.

The present talk will show the peculiarities of some environmentally sustainable formulations developed (emulgel and patches) using naturally derived products. In particular, the virtuous valorization of waste such as hazelnut shells and saffron petals as well as abundant natural sources as flaxseeds, barley, and corn starch will be presented.

4. Topic 1: AMR and Chronic Wound Pathogens

4.1. Oral Presentation

Prevalence and Antimicrobial Resistance Profiles of Bacteria Isolated from the Wounds of Stray Dogs and Stray Cats Before and After Sterile Physiological Solution Washing

Francesca Paola Nocera [1], Francesca Pizzano [1], Rossana Schena [1], Emine Ipek [1], Cristina Di Palma [1], Silvia Cappiello [2], Marina Pompameo [2], Barbara Lamagna [1], Luisa De Martino [1,3]

[1] Department of Veterinary Medicine and Animal Production, University of Naples "Federico II" Via F. Delpino 1, 80137 Naples, Italy;

[2] Veterinary Hospital, ASL Napoli 1 Centro, via M. Rocco di Torrepadula 13, 80145 Naples, Italy;

[3] Task Force on Microbiome Studies, University of Naples "Federico II, Naples, Italy.

francescapaola.nocera@unina.it

Contaminated and infected wounds occur very frequently in veterinary medicine [8,9]. Sterile physiological solution is the most common cleansing method used by hospitals because it is a non-toxic isotonic solution [10]. Stray cats (n. 16) and dogs (n. 15) presenting traumatic or polytraumatic injuries, sampled under deep sedation for surgical debridement of the wound, were selected. The administration of antimicrobial treatment before sampling represented an exclusion criterion of this study. The first objective of this study was the comparison of the diversity of bacterial cultures obtained from swabs performed before and after physiological solution washing. Precisely, the swabs were firstly incubated in enrichment broth (BHI) for 24 h at 37 °C and then plated using different culture agar media for the isolation of Gram-positive and Gram-negative bacteria. Then, species identification was carried out through proteomic analysis (MALDI TOF MS). Antimicrobial susceptibility testing, the second objective, was performed by using 18 and 16 antibiotics for Gram-positive and

Gram-negative bacteria, respectively. The number of bacteria isolated from 61.3% (19 out of 31) of the small animals was found to be the same before and after physiological solution washing. The most common canine bacterial strain was *Staphylococcus pseudintermedius* (15 strains), of which 80% (12 out of 15) were phenotypically resistant to oxacillin, penicillin, and cefoxitin, genotypically confirmed by their positivity to the *mec*A gene detected using conventional PCR. A wider variety of bacterial species of both Gram-positive and Gram-negative bacteria was observed in feline wound samples. In total, 75 bacterial isolates were recovered, and several multidrug-resistant (MDR) strains (40%) were detected. Based on the obtained results, it is possible to hypothesize that (*i*) the viable microbial community composition of wounds tends not to change in relation to physiological solution washing and (*ii*) stray cats and dogs could be considered as reservoirs of MDR bacteria and thus potential indicators of environmental health.

4.2. Poster

4.2.1. A Multi-Pronged Approach for an Effective Program in the Management of AMR in Chronic Wounds

Michael Balzani [1,2], Valentina Crocetta [1], and Anna Janowicz [1,3]

[1] Scuola di Specializzazione in Microbiologia e Virologia Clinica, Università "G. d'Annunzio" Chieti-Pescara, Chieti, Italy

[2] Azienda Ospedaliero Universitaria delle Marche–SOD Medicina Trasfusionale, Ancona, Italy

[3] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, Teramo, Italy

Chronic wounds pose a significant public health threat due to their susceptibility to persistent infections with bacterial and fungal strains, leading to an increased risk of the development of antimicrobial resistance (AMR). Inappropriate or incomplete initial antibiotic therapy can lead to the selection of AMR strains, and the chronic nature of the wound, often requiring treatment in a healthcare environment, augments the probability of acquiring healthcare-associated infections with multidrug-resistant (MDR) strains. Additionally, chronic wounds frequently harbor bacteria growing in biofilms that are protected from antimicrobials and the immune system. This combined effect of AMR and biofilm formation renders chronic wound infections increasingly challenging to manage. While the exploration of alternative treatment methods is vital to ensure effective wound management, combating AMR in chronic wounds must be grounded in an effective and comprehensive antimicrobial stewardship (AMS) program.

Herein, we present AMS strategies in wound care as the foundations to prevent or decrease the emergence and spread of AMR. These strategies are based on clearly defined objectives targeting both clinical personnel and patients and include the prevention of the infection and early intervention, correct antimicrobial prescribing and drug dose optimization based on early diagnosis, use of alternative therapies, and, importantly, the effective collaboration and thorough education of all of the involved personnel. Continuous surveillance and data-driven adjustments to local protocols are paramount to ensuring optimal wound management and minimizing the emergence of resistant pathogens. Moreover, sharing knowledge across healthcare disciplines, along with industry support, is crucial for identifying successful AMS strategies and establishing the most sustainable methods for chronic wound management. While the best practices described herein are not exhaustive, they will provide an important starting point to introducing and managing a well-structured and effective AMS program on a local scale.

4.2.2. Emerging Resistance to Florfenicol in *Actinobacillus pleuropneumoniae* Isolates from an Italian Pig Herd

Sonia N. Coccitto [3], Marzia Cinthi [1], Francesca R. Massacci [2], Elisa Albini [2], Lucilla Cucco [2], Martina Ustulin [4], Marta Paniccià [2], Denis Vio [4], Eleonora Giovanetti [1], Chiara F. Magistrali [2,5], Andrea Brenciani [3]

[1] Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

[2] Department of Research and Development, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Perugia, Italy

[3] Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy

[4] Department of Microbiology, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy

[5] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Italy

Actinobacillus pleuropneumoniae is the major contributor to swine respiratory disease, which causes considerable economic losses worldwide. To date, sick pigs are treated with the use of florfenicol. However, reports on florfenicol-resistance in *A. pleuropneumoniae* are increasing worldwide. Florfenicol resistance is generally associated with the presence of the *floR* gene, which is located both on plasmids or Integrative and Conjugative Elements. In this study, we identified for the first time in Italy two isolates of *A. pleuropneumoniae* from a pig farm, resistant to florfenicol due to the presence of the *floR* gene.

A. pleuropneumoniae B2176 and 2177 strains were isolated from diseased pigs in 2022 and 2023, respectively. Susceptibility tests showed that B2176 had a florfenicol intermediate profile, while B2177 was resistant. Both strains harbored the *floR* gene. Genome analysis (Illumina approach) revealed that the *floR* gene was located on a new 5588 bp plasmid, named pAp-floR, with high-level nucleotide identity with the pMVSCS1 plasmid, devoid of *floR*, described in a porcine *Mannheimia varigena*. The pAp-floR plasmid was also compared with six other *floR* plasmids previously identified in *A. pleuropneumoniae*, showing meaningful differences both in antibiotic resistance gene content and the plasmid backbone.

Stability assays also demonstrated that in B2176 and B2177 strains, the *floR* gene was stably conserved even in the absence of selective pressure, suggesting that its presence does not affect the fitness cost.

B2176 and B2177 were further compared to 35 *A. pleuropneumoniae* genomes from the NCBI database. Both clustered with the FEMO genome originating from a porcine strain in Switzerland.

Given the increase in florfenicol resistance associated with the *floR* gene and its stable persistence in the *A. pleuropneumoniae* population, it is necessary to constantly monitor resistance in pathogenic bacteria and to continue along the path already undertaken for the prudent use of antibiotics in livestock farming.

4.2.3. Unveiling the Peri-Implantitis Microbiome and Resistome Through Shotgun Metagenomic Sequencing

Lucinda J. Bessa [1,2], Carolina Pires [2], Ricardo Alves [1,2,3]

[1] Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Almada, Portugal;

[2] Instituto Universitário Egas Moniz (IUEM), Egas Moniz School of Health & Science, Almada, Portugal;

[3] Clinical Research Unit (CRU), CiiEM, Egas Moniz School of Health & Science, Almada, Portugal.

lbessa@egasmoniz.edu.pt

Peri-implantitis (PI) is an immune-mediated biofilm pathology that can ultimately result in the loss of dental implants, thus representing a major challenge in dental care. To gain deeper insights into this complex condition, advanced methodologies such as shotgun metagenomic sequencing are imperative. These techniques allow for a comprehensive exploration of microbial communities and their functional dysbiosis in PI.

This study aimed to investigate the taxonomic diversity and abundance and identify antibiotic resistance genes (ARGs) within subgingival biofilm samples collected from patients with both healthy and PI-affected dental implants. Following approval from the Institutional Ethics Committee, a cohort of 20 patients was recruited from the Egas Moniz Dental Clinic. Subsequently, subgingival biofilm samples were obtained from each patient, and total microbial DNA was extracted utilizing the DNeasy PowerSoil Pro Kit (Qiagen). These DNA samples underwent shotgun metagenomic sequencing analysis, followed by comprehensive bioinformatics processing. Taxonomic profiling (diversity and abundance) was generated using MetaPhlAn2. HUMAnN2 was used to provide information regarding the functional pathways present in the metagenomes. ARGs were identified using the CARD, ResFinder, and AMRFinderPLus databases.

A comprehensive analysis is underway to identify differences in genomic architecture and functionality between microbial biofilms from implants affected by peri-implantitis (PI) and those from healthy implants. The expected outcomes will contribute to a deeper understanding of the pathogenesis of PI, paving the way for improved diagnostic and therapeutic strategies.

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5. Topic 2: In Vitro/In Vivo Chronic Wound Models

5.1. Poster

5.1.1. Lubbock Chronic Wound Biofilm (LCWB) Model: An In Vitro *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* Dual-Species Biofilm

Silvia Di Lodovico [1], Francesca Paola Nocera [2], Valeria De Pasquale [2], Morena Pinti [1], Sara D'Arcangelo [1], Morena Petrini [3], Mara Di Giulio [1], Simonetta D'Ercole [3]

[1] Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;

[2] Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy;

[3] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" of Chieti, Italy.

silvia.dilodovico@unich.it

The increase in *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* multidrug-resistant strains associated with dog wounds represents an important challenge for chronic wound treatment. This pressing problem is strongly connected with the dynamic zoonosis that needs to be monitored to fight the antimicrobial resistance/tolerance phenomenon. In particular, poly-microbial wound biofilms are the main cause of traditional treatment failure. These considerations strongly underline the study of a suitable in vitro canine chronic wound model. The aim of this study was to create a canine wound infection model, Lubbock Chronic Wound Biofilm (LCWB), with a focus on *S. pseudintermedius,* drawing inspiration from the established human model involving *S. aureus*. Methicillin-resistant *S. pseudintermedius* 115 (MRSP) and resistant *Pseudomonas aeruginosa* 700 strains, isolated from dog wounds, were used to set up the LCWB at 24, 48, and 72 h. The LCWBs were evaluated in terms of volume, weight, and microbial CFU/mg. The microbial spatial distribution in the LCWBs was assessed using SEM and CLSM imaging. The best incubation

time for LCWB production in terms of volume (3.38 $cm^3 \pm 0.13$), weight (0.86 gr $\pm$ 0.02), and CFU/mg (up to 7.05×10^6 CFU/mg $\pm$ 2.89×10^5) was 48 h. The SEM and CLSM images showed major viable microbial colonization at 48 h with a non-mixed bacteria with the prevalence of MRSP on the surface and *P. aeruginosa* in the depth of the wound. The obtained findings demonstrate the capability of *S. pseudintermedius* to grow together with *P. aeruginosa* in the LCWB model, representing a suitable model to reproduce an in vitro animal chronic wound. This is a suitable in vitro model that mimics the microbial distribution and in vivo environment like that in a dog chronic wound. These characteristics make the LCWB appropriate for the evaluation of new strategies before translation in vivo with an animal model.

The study was funded by the European Union–Next Generation EU, National Recovery and Resilience Plan (PNRR), Mission 4 "Education and research"—Component 2 "From research to business" of the National Recovery and Resilience Plan (PNRR). A heparan sulfate proteoglycan binding protein and Light-Emitting Diode (LED)/Complex Electromagnetic Field (CMF) technologies as innovative eco-sustainable strategies to counteract chronic wound infections associated with *Staphylococcus pseudintermedius*-resistant strains: An interdisciplinary approach to animal–human health. P20224AEAC.

5.1.2. Graphene Oxide and Led: Eco-Sustainable Strategies to Counteract Poly-Microbial Colonization of Chronic Wounds

Simonetta D'Ercole [1], Silvia Di Lodovico [2], Emma Pace [2], Firas Diban [2], Mara Di Giulio [2], Morena Petrini [1]

[1] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, 66100, Chieti, Italy

[2] Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, 66100, Chieti, Italy.

simonetta.dercole@unich.it

In a chronic wound, poly-microbial biofilms play a pivotal role in pathogenesis, impairing cutaneous healing. The Lubbock Chronic Wound Biofilm (LCWB) model, which mimics the microbial distribution and environment of a real chronic wound, represents a suitable tool for novel antimicrobial proposals. Innovative non-antibiotic compounds such as graphene oxide (GO) and light-emitting diodes (LEDs) may represent a valid strategy for managing chronic wound infections related to resistant pathogens.

This study aimed to evaluate a 630 nm LED and 880 nm LED's ability to enhance GO's antimicrobial activity against *Staphylococcus aureus*- and *Pseudomonas aeruginosa*-resistant strains in a dual-species biofilm in the LCWB model.

The effect of a 630 nm LED, alone or plus 5-aminolevulinic acid (ALAD)-mediated photodynamic therapy (PDT) (ALAD-PDT), or an 880 nm LED on action of GO (50 mg/L) was evaluated by determining the CFU/mg reduction and employing live/dead analysis, scanning electron microscope observation, and a reactive oxygen species assay.

Among the LCWBs, the best effect was obtained with GO irradiated with ALAD-PDT, with percentages of CFU/mg reduction up to 78.96% $\pm$ 0.21 and 95.17% $\pm$ 2.56 for *S. aureus* and *P. aeruginosa*, respectively. The microscope images showed a reduction in cell number and viability when treated with GO + ALAD-PDT. In addition, increased ROS production was detected. No differences were recorded when GO was irradiated with an 880 nm LED versus GO alone. The obtained results suggest that treatment with GO irradiated with ALAD-PDT represents a valid, sustainable strategy to counteract the poly-microbial colonization of chronic wounds.

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gies to fight Chronic wound Infections Associated with Multidrug resistant pathogens for the development of Innovative medical systems"

6. Topic 3: Development of Innovative Strategies

6.1. Oral Presentation

6.1.1. Design and Characterization of Clay–Carvacrol Nanoemulsions for Wound Healing

Anna Imbriano [a], Fatima García-Villén [b], Federica Rinaldi [c], Luana Perioli [a], Cinzia Pagano [a], Giuseppina Sandri [d], Carlotta Marianecci [c], Cesar Viseras [b], Maria Carafa [c].

[a] Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, Perugia, 06123 Italy.

[b] Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Granada, Campus of Cartuja, 18071 s/n, Granada, Spain.

[c] Department of Drug Chemistry and Technology, University of Rome "Sapienza", Piazzale A. Moro 5, 00185, Rome, Italy.

[d] Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100, Pavia, Italy.

Herein, the present research focuses on the design of a new pharmaceutical product with potentiated anti-inflammatory, antibacterial, and tissue regeneration activity useful in wound healing. Carvacrol (CRV) is one of the main components of Origanum, Thymus, and Satureja Montana essential oils and exhibits promising properties in wound treatment. However, high lipophilicity, rapid oxidation, and inadequate retention time at the site of action limit CRV's therapeutic application. A possible technological strategy to overcome these disadvantages could involve the preparation of CRV-based oil in water nanoemulsion (O/W NE), in which CRV itself is used both as the oil phase and as the active compound. CRV-NE left uncoated or coated with Chitosan were prepared and deeply characterized. In particular, stability studies were carried out to monitor some parameters such as dimensions, ζ-Potential, pH, and CRV amount at different temperatures over 90 days. Furthermore, the size was evaluated through DLS analyses and compared with data obtained through TEM observations. Secondly, two clay minerals (a montmorillonite and a sepiolite, VHS and PS9, respectively) were added to the NE, with a semisolid formulation being obtained. The rheological properties of the formulations were also studied. Finally, the biocompatibility of CRV-loaded nanoemulsions, as well as their antioxidant and anti-inflammatory properties, was proven in vitro with fibroblasts and macrophages.

6.1.2. Potential Antibacterial and Wound Healing Activity of *Juniperus oxycedrus* L. ssp. Essential Oil Microneedles

Basem Battah [1], Lama Shbibe [2], Osama Ahmad [3], Chadi Soukkarieh [2], Luigi Rosati [4], Teresa Chianese [4], Ryan Donnelly [5], Matthew Gavino Donadu [6,7].

[1] Department of Biochemistry and Microbiology, Faculty of Pharmacy, Antioch Syrian Private University, Maaret Siadnaya, Syria

[2] Faculty of science, Damascus university, Damascus, Syria.

[3] Faculty of medicine, Syrian Private University (SPU), Daraa international highway, Syria.

[4] Department of Biology, University Federico II, Via Cintia 21, 80126 Napoli, Italy.

[5] Medical Biology Centre, School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK.

[6] Scuola di Specializzazione in Farmacia Ospedaliera, Department of Medicine, Surgery and Pharmacy.

[7] Hospital Pharmacy, Giovanni Paolo II Hospital, ASL Gallura, 07026 Olbia, Italy.

It is still difficult to treat infected wounds with essential oils (EOs). Many attempts have been made to improve the convenience of this application. In order to address certain

drugs' poor skin penetration, limited bioavailability, and poor absorption and distribution, microneedles (MNDs) have recently been proposed as a smart dermal delivery method. Our study's objective was to assess the ability of microneedles (MNDs) filled with juniper essential oil to cure wounds infected with bacteria. Juniper essential oil (EO) was placed into Polyvinylpyrrolidone (PVP) microneedles that were made using a gel-filled mold process. Two bacterial strains, clinically isolated *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*), were introduced into rat wounds using in vivo models, and the effects of juniper EO MNDs were examined. The results demonstrated that the application of juniper MNDs improved wound healing, with noticeable improvements observed as early as the third day post infection. By the sixth day, the treated wounds had greatly shrunk in comparison with the untreated wounds. A full recovery was observed on the twelfth day following infection. Additionally, our cytotoxicity data demonstrated that juniper EO MNDs had a cytotoxic effect on epithelial cells, which accounts for the rats' quicker wound healing. According to our research, MNDs infused with juniper EO offer an innovative approach to essential oil delivery with minimal invasion. MNDs supplied with juniper EO showed notable antibacterial activity in the treatment and healing of bacterial strain-infected wounds.

6.1.3. Resveratrol Derivatives for Chronic Wounds: A Promising New Treatment Option

Emira D'Amico [1] and Barbara De Filippis [2]

[1] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Via dei Vestini 31, 66013 Chieti, Italy;

[2] Department of Pharmacy, University "G. d'Annunzio", Via dei Vestini 31, 66013 Chieti, Italy.

barbara.defilippis@unich.it

Chronic wounds are a healthcare challenge, and researchers are constantly exploring new therapeutic strategies to improve healing. Natural phenols offer a potential alternative or adjunctive therapy to conventional treatments, showing promising beneficial efficacy and safety, low immunogenicity, and low toxicity. These characteristics make phenols a promising natural source to be employed in the development of plant-based therapeutics, with wide application use.

Resveratrol (RSV) is a polyphenolic phytoalexin that exhibits several biological activities such as antioxidant, anti-inflammatory, and antibacterial. In vitro and in vivo studies suggest RSV's potential to promote wound healing through various mechanisms, such as stimulating cell migration, increasing collagen production, promoting blood vessel formation, and reducing inflammation.

Herein, we present the synthesis of and biological studies on a first set of sulfonamide derivatives of RSV containing the stilbene core bound by a sulfonamide bridge to a lipophilic portion. Human gingival fibroblasts (hGFs), endothelial cells (Huvecs), and human osteoblasts (hOBs) were treated with RSV derivatives, and cytocompatibility, morphology, and gene expression were evaluated. The results suggested that these compounds could have beneficial effects on wound healing and overcome the unfavorable pharmacokinetic profile of the parent compound. RSV derivatives induced cell proliferation and upregulation of the expression of COL1, eNOS, and OCN genes. Furthermore, observations made using an optical microscope showed a preserved cell morphology and an increase in cell density. In conclusion, in this study, we identified molecules that could represent a promising approach for the development of new therapeutic strategies for chronic wounds. Further research is needed to fully explore their potential and establish their role in wound healing. New structure–activity relationship studies are in progress.

6.1.4. Cytotoxic Investigation of a Photodynamic Protocol Based on 5% Delta Aminolaevulinic Acid and Red LED Irradiation in the Treatment of Chronic Wounds

Morena Petrini and Tania Vanessa Pierfelice

University G. d'Annunzio of Chieti-Pescara, Department of Medical, Oral and Biotechnological Science

morena.petrini@unich.it

This study aimed to verify the absence of cytotoxic effects on human fibroblasts through photodynamic therapy, based on the use of a gel containing 5% delta aminolaevulinic acid (ALAD) for 45 min followed by irradiation with a 630 nm LED (PDT) for 7 min. This protocol has recently been proposed as a promising treatment for chronic wounds because of its antibacterial and antifungal activity as shown in vitro. Another advantage of this treatment is the lack of side effects and invasiveness. The therapy is painless, so no precautions, like anesthesia, are necessary for the patients. The treatment has been tested on human gingival fibroblasts (hGFs) because this protocol is already used to treat chronic periodontitis. The untreated cells were considered as the control (CTRL). Cells subjected to the photodynamic protocol ALAD were grouped and tested. Viability assays, morphological analyses (optical, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM)), and assays for reactive oxygen species (ROS) and collagen production were performed. All samples were treated in triplicate, and statistical analyses were performed with software, utilizing one-way ANOVA followed by Tukey's post hoc multiple comparisons analysis. Values of $p < 0.05$ were considered statistically significant.

The results showed that ALAD did not affect cell viability and did not cause morphological alterations in cells subjected to the treatment with respect to the controls. CLSM showed an increase in the thickening of actin filaments in the test group. ROS production was augmented only at 0 h and 3 h, while collagen appeared to be enhanced 7 days after treatment with respect to the controls.

In conclusion, the tested treatment is not only not cytotoxic to fibroblasts but also seems to have a positive effect on collagen production.

6.1.5. Exploring New Antimicrobial Technologies in Pediatric Endodontics

Luca De Gregoris, Simonetta D'Ercole, and Domenico Tripodi

Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Italy

Antibiotic resistance has become one of the major threats to public health worldwide. Therefore, it represents an important driver of increased morbidity and mortality rates, resulting in growing healthcare costs. It has become peremptory to develop treatment options as an alternative to antibiotic therapy for combating antibiotic-resistant pathogens. In this report, we explore a new technology, photodynamic therapy (PDT), in treating one of the most frequent causes of antibiotic prescription in dentistry: endodontic infections.

PDT is an emerging therapeutic modality that uses light energy to activate photosensitizing agents that selectively kill microorganisms. It is a promising alternative to conventional antimicrobial therapies, especially in the context of rising antimicrobial resistance and the need for novel treatment strategies to treat dental diseases.

PDT involves the use of a photosensitizing agent, which is a molecule that absorbs light energy and transfers it to oxygen molecules in the surrounding environment.

It represents an excellent alternative strategy to fight antibiotic resistance; the development of resistance to a photodynamic protocol presents difficulties because in microbial cells, the action of singlet oxygen and free radicals, produced during PDT, acts on different cellular structures.

Another crucial piece of knowledge is the identification of microbial pathogens. Over the years, with advances in analytical accuracy and sensitivity, the list of candidate endodontic pathogens has consequently expanded. A census of the microbial species detected in different types of endodontic infections revealed approximately 500 different species, most of which were bacteria but also fungi and archaea. The use of 5-aminolevulinic acid (ALA) gel in conjunction with red LED irradiation has demonstrated powerful antibacterial activity against pathogens commonly implicated in endodontic infections, including *Enterococcus faecalis*. In this report, we explore the potential of this new antimicrobial technology in one of its possible fields of application: pediatric dentistry.

6.2. Poster

6.2.1. In Vitro and In Vivo Activities of Carbon Dots (CD-NH_2) Against Bacteria and Fungi Associated with Chronic Wound Infections

Virginia Giusti [a], Alessandro Camilli [b], Fabrizio Vetica [b], F. Leonelli [b], A. Valletta [a], G. Simonetti [a].

[a] Department of Environmental Biology, Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy

[b] Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy.

The prevalent fungal and bacterial species identified in diabetic foot ulcers are *Candida albicans* and *Staphylococcus aureus*, found in 47% and 95% of diabetic foot ulcers, respectively. Notably, *S. aureus* exhibits a strong adhesion capacity to the surface of *C. albicans* in biofilms. This study aims to investigate the antimicrobial activity of an emerging class of carbon nanoparticles, known as carbon nanodots (CDs), of less than 10 nm in diameter in size. They possess a range of intriguing properties such as biocompatibility and low toxicity. The effectiveness of CDs also originates from the diverse array of functional groups found on their surface.

CD-NH_2, with its charged surfaces, has been evaluated against *S. aureus* and *C. albicans* biofilm, either as individual species or as a poly-microbial biofilm, through in vitro and in vivo studies. CD-NH_2 demonstrated significant antibiofilm activity, reducing *S. aureus* and *C. albicans* biofilm formation by more than 50%. Fluorescence microscopy observations revealed the adhesion of CD-NH2 to cells and their likely uptake. The in vivo efficacy of CD-NH_2 was evaluated using *Galleria mellonella* larvae. Larvae infected solely with *S. aureus* or co-infected with *C. albicans* exhibited a substantial reduction in mortality compared to untreated and non-CD-NH_2-treated larvae. The promising activity of CD-NH_2 may be attributed to the presence of amines. Nonetheless, further investigations are warranted to explore the potential application of CD-NH_2 as an antimicrobial agent against *C. albicans* and *S. aureus* infections in chronic wounds.

6.2.2. Promising Strategies Based on Natural Compounds and Conventional Antibiotics Entrapped into Nanocarriers for the Treatment of Wound Infections

Giovanna Ginestra [1], Loredana Ferreri [2], Giuseppe Granata [2], Grazia M.L. Consoli [2], Ludovica Maugeri [3], Salvatore Petralia [3], Benedetta Galletta [1,4], Maria Fernanda Taviano [1] and Antonia Nostro [1]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;

[2] Institute of Biomolecular Chemistry (ICB-CNR) Catania, Italy;

[3] Department of Drug and Health Sciences, University of Catania, Italy; [4] Foundation "Prof. Antonio Imbesi", University of Messina, Italy

Novel antimicrobial strategies and more affordable treatments to fight antibiotic-resistant and biofilm-associated wound infections need to be developed. Topical treatment often represents a challenge due to limited drug availability at the site of infection owing

to different physicochemical properties, such as instability in different biological environments, low solubility, or poor distribution. The entrapment of antimicrobial drugs in nanocarriers is an appealing strategy to improve their efficacy, provide more selective local delivery, and reduce systemic side effects. Due to their size, nanocarriers can also enhance interaction with the bacterial surface and favour cell uptake. The present study aimed to determine the antibacterial activity of natural compounds with high hydrophobicity (carvacrol and curcumin) and a conventional antibiotic (ciprofloxacin) and the advantage of their delivery and co-delivery via loading into nanocarriers. Two classes of nanocarriers based on carbon-nanodots and calixarene derivatives were prepared and their physicochemical properties were evaluated. Drug loading into the nanocarriers was performed according to eco-friendly approaches. Antibacterial activity was evaluated through broth microdilution against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VREfm), multidrug-resistant *Acinetobacter baumannii*, ESBLs producing *Escherichia coli*, and VIM-2 producing *Pseudomonas aeruginosa*. The carbon nanodots/carvacrol nanosystem and the micellar sulfonate–calixarene co-loading curcumin and ciprofloxacin improved the solubility and stability of the selected bioactive compounds. Conclusions: Furthermore, the drug-loaded nanocarriers demonstrated antibacterial activity comparable to or even better than that of the individual bioactive compounds. Our findings suggest that these novel nanosystems are promising candidates for the topical treatment of antibiotic-resistant wound infections.

Keywords: wound infections; biofilm formation; carvacrol; curcumin

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6.2.3. Chronic Wound-Derived Pathogens Are Affected by Methylglyoxal Application Alone and in Combination with Novel Technologies

Firas Diban [1], Paola Di Fermo [2], Silvia Di Lodovico [1], Mara Di Giulio [1], Simonetta D'Ercole [2], Luigina Cellini [1]

[1] Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;

[2] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" of Chieti, Italy.

firas.diban@unich.it

Evolving antimicrobial resistance represents a serious issue facing the global public health system and requires the development of new effective methods to counteract it. Chronic wounds provide a suitable environment to enhance antimicrobial tolerance/resistance traits through several mechanisms. Several studies have evaluated the antimicrobial action of novel technologies against resistant pathogens. This work aimed to evaluate the antimicrobial and anti-virulence effect of methylglyoxal-MGO and light emitting diodes (LEDs) (630 nm) and complex magnetic fields (CMFs) in single/combined application against pathogens isolated from chronic wounds. The single (MGO, LEDs, and CMFs) and combined (MGO + LED and MGO + CMFs) treatments were evaluated regarding the antimicrobial growth (MIC, OD_{600}, and synergism), cell membrane permeability and fluidity, and motility of *Pseudomonas aeruginosa*. Standardized broth cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* were used in the experiments. The results showed the antimicrobial activity of MGO, alone, against the bacterial strains (MIC = 64–256 μg/mL) and *C. albicans* (MIC = 4096 μg/mL) with a reduction in OD_{600} after LED and CMF application. The combinations MGO + LED and MGO + CMFs exhibited synergistic activity, and the best FIC index value (0.001) was recorded with the MGO + CMFs combination against *C. albicans*. The single/combined treatments showed

antimicrobial activity and reduced the Colony Forming Units (CFU)/mL of each microbial strain (species-dependent reduction), and the highest reduction was 83.5% of *C. albicans* after CMF treatment. The single/combined treatments induced a variable change in membrane permeability and fluidity. LED application increased the membrane permeability of *P. aeruginosa*, while MGO + LED increased the permeability of *S. aureus*. Finally, the single/combined treatments affected *P. aeruginosa* soft swimming, swarming, and twitching motility in which the LEDs caused the greatest reduction among the treatments. In conclusion, it is important to take into consideration the action of MGO, LEDs, and CMFs as a possible non-antibiotic method to use against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* pathogenic infections.

6.2.4. Sulfobutylether-β-Cyclodextrin Inclusion Complex Improves the Activity of Rutin Against Resistant Strains

Federica De Gaetano [1], Martina Pastorello [1], Venerando Pistarà [2], Antonio Rescifina [2], Fatima Margani [3], Vincenzina Barbera [3], Cinzia Anna Ventura [1], Andreana Marino [1].

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy.

[2] Department of Pharmaceutical and Health Sciences, University of Catania, Viale Andrea Doria 6, 95125, Catania, Italy.

[3] Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Via Mancinelli 7, 20131 Milano, Italy.

Alternative strategies to conventional antibiotics are needed to fight resistant pathogens. Flavonoids, bioactive metabolites from plants, could be promising agents to treat, alone or in combination with antibiotics, infections caused by resistant and biofilm-producing bacteria. However, their clinical employment is underexploited due to their low water solubility. Rutin (RTN) is a flavonoid with anti-inflammatory, anticancer, antioxidant, and antibacterial activity. Cyclodextrins (CDs), cyclic oligosaccharides that are able to interact with hydrophobic molecules to form inclusion complexes, could be used to overcome the low water solubility of rutin in order to increase its bioavailability and improve its stability. The aim of this study was to design a liquid formulation based on a rutin/sulfobutylether-β-cyclodextrin (RTN/SBE-β-CD) inclusion complex for treating bacterial infections. A deep physical–chemical characterization of the RTN/SBE-β-CD inclusion complex showed the formation of a stable complex with higher water solubility. The antibacterial activity of the RTN/SBE-β-CD inclusion complex, compared to both free RTN and SBE-β-CD, was assayed against *S. aureus* and *P. aeruginosa* strains. The maximum RTN concentration tested was 1250 μg/mL as a soluble RTN/SBE-β-CD inclusion complex, 150 μg/mL for free RTN (maximum solubility). The complexed RTN showed bactericidal activity against *S. aureus* ATCC 6538, *S. aureus* ATCC 43300 (MRSA), and *P. aeruginosa* ATCC 9027 and bacteriostatic activity against *P. aeruginosa* DSM 102273, a resistant strain. Moreover, the inclusion complex significantly decreased biofilm biomass and cell viability at 0.5 MIC against *S. aureus* ATCC 6538 and *S. aureus* MRSA. Free RTN showed bacteriostatic activity against *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027 and no activity against the resistant strains. The results suggest that SBE-β-CD could be a suitable carrier for RTN, permitting the realization of liquid formulations with antibacterial properties and representing a good starting point for successive studies such as those on the combinatorial activity of RTN with conventional antibiotics.

6.2.5. A Heparan Sulfate Binding Protein Able to Counteract Chronic Wound Infections

Valeria De Pasquale [1], Silvia Di Lodovico [2], Mariangela Ciampa [3], Federica Rossin [1], Chiara Fiorentino [3], Melania Scarcella [3], Francesca Paola Nocera [1], Giovanni Della Valle [1], Luigina Cellini [2], Luisa De Martino [1], Simona Tafuri [1].

[1] Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Italy;

[2] Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy;

[3] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Italy.

valeria.depasquale@unina.it

Currently, there is an urgent need to develop new therapeutic strategies to combat chronic wound infections caused by bacteria with antibiotic resistance. Innovative strategies that interfere with bacterial adherence and the colonization of host cells, representing the critical initial steps for the infection process, may provide the basis for the development of new molecules able to fight infections and overcome antimicrobial resistance. Recently, we produced a recombinant protein (NK1), a natural variant of hepatocyte growth factor, able to bind with high-affinity heparan and dermatan sulfate chains of proteoglycans (PGs) present on the cell surface and extracellular matrix of target cells. PGs are involved not only in bacterial adherence and colonization but can also participate in other steps of infection pathogenesis. Indeed, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* are able to induce the release of PG ectodomains from the cell surface, thus inhibiting innate immune mechanisms. Since bacterial pathogens utilize a variety of mechanisms to subvert PGs to sustain infection, the developing PG-based antimicrobial therapies may be of great benefit. In this study, we explored the molecular mechanisms by which the recombinant NK1 affects cellular processes using a normal human epithelial cell line heavily decorated by heparan sulfate proteoglycans. Our results showed that cell exposure to NK1 induces c-MET phosphorylation and activation of downstream signaling pathways including ERK1/2, Akt, c-Src, p125FAK, SMAD2/3, and STAT3. The activation of these signaling pathways correlates with NK1's ability to enhance cell proliferation, motility, scattering, and mainly wound healing. Studies are in progress to evaluate the efficacy of NK1 to counteract chronic wound infections associated with *Staphylococcus pseudintermedius* in the Lubbock Chronic Wound Biofilm model.

The study was funded by the European Union–Next Generation EU, Piano Nazionale di Ripresa e Resilienza, Missione 4 "Istruzione e ricerca"—Componente 2 "Dalla ricerca all'impresa" del Piano Nazionale di Ripresa e Resilienza (PNRR). A heparan sulfate proteoglycan binding protein and Light-Emitting Diode (LED)/Complex Electromagnetic Field (CMF) technologies as innovative eco-sustainable strategies to counteract chronic wound infections associated with *Staphylococcus pseudintermedius*-resistant strains: An interdisciplinary approach to animal–human health. P20224AEAC (Linea SUD Under 40, Macrosettore LS6).

6.2.6. The Impact of Complex Magnetic Fields (CMFs) on Human Gingival Fibroblasts—Critical Players in Chronic Wound Healing

D'Amico Emira [1], Petrini Morena [1], Pierfelice Tania Vanessa [1], Cinquini Chiara [2], Iezzi Giovanna [1]

[1] Department of Medical Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy.

[2] Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy.

The fight against resistant microorganisms represents a current problem. Thus, Complex Magnetic Fields (CMFs) have emerged as a promising, eco-friendly, and sustainable technology to counteract infections. Recent research has already shown CMFs' effectiveness against pathogens, including *Candida albicans* and both Gram-positive and Gram-negative bacteria. Thus, this study aimed to evaluate the response of human gingival fibroblasts (hGFs) to this treatment. hGFs play a crucial role in wound healing; therefore, understand-

ing how CMFs interact with these cells is vital to ensuring the overall safety and efficacy of this innovative approach. hGFs were exposed to two CMF programs: STRESS (A) and ANTIBACTERIAL (B). The control (CTR) was represented by the non-exposed cells. The analysis was performed to evaluate their viability, morphology, wound healing properties, ROS, and collagen production.

CMFs induced no adverse effects on the viability of hGFs at 24 h. Typical cell morphology was observed with toluidine blue staining at 24 h, and SEM observations showed the presence of elongated and spindle-shaped cytoplasmic extensions and lamellipodia. A full scratch closure was observed after the CMFs' exposition at 24 and 48 h. Treatment B induced major ROS levels at 3 and 24 h and statistically increased collagen production compared to the control and program A ($p < 0.0001$).

In conclusion, the study revealed positive outcomes from CMF treatment: no cytotoxicity, enhanced migration, and a boost in collagen production. These findings are highly encouraging. The lack of cytotoxicity indicates that CMFs are unlikely to harm healthy tissues during treatment. Furthermore, the increased migration suggests that CMFs might promote wound healing, a crucial factor in infection recovery. Finally, the increased collagen production by CMFs strengthens the extracellular matrix, a critical component for tissue repair and preventing future infections.

6.2.7. Advancing Chronic Wound Treatment: Effects of MGO, LED, and CMF Technologies on Dermal Fibroblasts

Tania Vanessa Pierfelice [1], Morena Petrini [1], Emira D'Amico [1], Giovanna Iezzi [1]

[1] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Via dei Vestini 31, 66013-Italy.

tania.pierfelice@unich.it

Chronic wounds can significantly impact patients' quality of life and healthcare costs. Wound management focuses on balancing infection control and the progression of the healing process.

This study aimed to explore different treatments for chronic wounds, highlighting the role of emerging technologies. Methylglyoxal (MGO) displays antimicrobial properties. Light-emitting diodes (LEDs) and complex magnetic fields (CMFs) have shown promise in reducing inflammation. To achieve this aim, this study investigated the effect of these treatments, individually and in combination, on human dermal fibroblasts (NHDFs), which play a crucial role in chronic wounds.

NHDFs were cultured with different concentrations (16–11,000 μg/mL) of MGO to detect the optimal concentration. Treated cells were divided into five TEST groups (MGO-16 μg/mL, LED17 minutes, CMFs-antibacterial program, MGO + LED, and MGO + CMFs), whereas untreated cells were considered the control (CTRL) group. Cell viability, changes in cell shape, and cell attachment were evaluated at 24 h using the MTT assay, toluidine blue staining, and CLSM and SEM analysis. The ability of cells to close wounds was assessed at 24 h and 48 h using the scratch assay. Collagen production, a key component of wound healing, was assessed and measured after 7 days with picrosirius red staining. Statistics were considered significant for p-values ≤ 0.05.

The optimal dose (16 μg/mL) of MGO was determined. The results showed that all of the treatments affect the viability, morphology, and adhesion of NHDFs compared to the untreated ones. The wound area (%) decreased as cell migration (μm/h) progressed over time in the presence of all treatments, with the best results achieved when CMFs were used alone or in combination with MGO. Collagen production was significantly augmented by applying LED alone or combined with MGO.

This study suggests that MGO, CMFs, and LEDs positively impact cells involved in wound healing, and future research could explore combining them for even more significant benefits.

6.2.8. Antibacterial and Antibiofilm Activity of Novel Nanofiber Bandages Formulated with *Juniperus communis* Essential Oil Targeting Antibiotic-Resistant Bacterial Strains

Mohannad Abu Haweeleh [1], Ahmad Ryad Hamdan [1], Menatalla Metwally Said [1], Maryam Hassiba [1], Mahmoud Elgamal [1], Robin Augustine [2], Susu M. Zughaier [1], Matthew G. Donadu [3,4]

[1] College of Medicine, Qatar University, Doha, Qatar.

[2] Department of Chemical Engineering, University of Massachusetts, Lowell, Massachusetts 01854, USA

[3] Scuola di Specializzazione in Farmacia Ospedaliera, Department of Medicine, Surgery and Pharmacy, University of Sassari, 07100 Sassari, Italy.

[4] Hospital Pharmacy, Giovanni Paolo II Hospital, ASL Gallura, 07026 Olbia.

Abdominal surgeries have the highest rate of surgical site infections and account for 3–20% of all surgical procedures. The alarming increase in antimicrobial resistance (AMR) threatens the current armament of antibiotics. This global threat to public health has revived the search for plant-derived antimicrobial therapeutics. *Juniperus communis*, known as Juniper, has been used in folk medicine for centuries. This study aims to examine the antibacterial and antibiofilm activity of polycaprolactone (PLC) nanofiber bandages formulated with essential oil extracted from *Juniperus communis* against clinically relevant ESKAPE bacterial pathogens that commonly cause nosocomial infections.

Bacterial growth curves and bactericidal assays were performed on antibiotic-sensitive- and resistant clinical bacterial strains collectively known as ESKAPE pathogens. The antibiofilm activity of Juniper essential oil was tested against MRSA and *E. coli* individually in a 96-well plate and incubated overnight to allow for biofilm formation and stained using crystal violet. The antibacterial activity of a PLC-based nanofiber membrane formulated with various doses (2%, 4%, 6%, and 8%) of Juniper oil was tested using the disc diffusion method. The PLC membranes were placed on agar plates inoculated with MRSA and incubated overnight.

Juniper essential oil dilutions of 50, 25, and 12.5 μL/mL demonstrated dose-dependent antibacterial activity against the tested strains and significantly reduced growth after 24 h. Of note, the antibacterial activity of Juniper essential oil against *Pseudomonas aeruginosa* was observed at higher doses (50 μL/mL). Juniper oil also exerted antibiofilm activity against MRSA and *E. coli* strains that are resistant to antibiotics. Additionally, PLC nanofiber membranes formulated with 8% Juniper oil inhibited MRSA growth, as demonstrated by a large zone of inhibition on an agar plate.

Juniper essential oil has antibacterial activity against ESKAPE pathogens. The potential application of Juniper oil formulated in PLC nanofabric bandages can be used in wound dressing to treat surgical site infections or skin ulcers.

6.2.9. Innovative Therapeutic Approach to Counteract Persistent *Helicobacter pylori* Infections: The Role of Resveratrol Derivatives

Morena Pinti [1], Paola Di Fermo [2], Barbara De FilippisS [1], Firas Diban [1], Silvia Di Lodovico [1], Emanuela Di Campli [1], Luigina Cellini [1], Mara Di Giulio [1]

[1] Department of Pharmacy, University of "G. d'Annunzio" Chieti-Pescara, Italy;

[2] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Italy.

morena.pinti@phd.unich.it

Helicobacter pylori infection is closely related to gastric and duodenal ulcers and carcinomas. The World Health Organization has urged *H. pylori* eradication to reduce gastric cancer incidence worldwide. Pharmacological treatment is particularly challenging due to the widespread antibiotic resistance of *H. pylori* strains to common antimicrobial drugs. Over recent years, resveratrol (RSV) has attracted significant attention for its biological multi-activities (anti-inflammatory, anti-carcinogenic, and antimicrobial activity). In our recent work, we demonstrated the antibacterial and anti-virulence effects (biofilm reduction and swarming motility inhibition) of RSV and newly synthesized RSV–phenol derivatives (RSV-3 and 4), with higher bioavailability, alone and combined with levofloxacin-LVX against resistant clinical strains of *H. pylori*. In this work, we explore in depth the mechanisms of action of RSV derivatives in terms of antibiofilm action, modulation of *H. pylori* virulence gene expression, and modification of the fluidity and permeability of the cell membrane.

The best RSV derivatives significantly reduced biofilm formation, affected bacterial membrane permeability, and the gene expression of the main *H. pylori* virulence factors.

The alarming phenomenon of multidrug resistance in *H. pylori* underlines the need to search for novel strategies to improve eradication rates. Overall, our results underline the anti-*H. pylori* effect of RSV derivatives, representing interesting candidates for innovative therapeutic schemes to tackle *H. pylori* antibiotic resistance.

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6.2.10. Staphylococci Skin Strain Modulation by Pomegranate Peel Extract: An Eco-Sustainable Approach

Sara D'Arcangelo [1], Paola Di Fermo [2], Emanuela Di Campli [1], Morena Pinti [1], Anna Janowicz [3,4], Mara Di Giulio [1]

[1] Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;

[2] Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy;

[3] Scuola di Specializzazione in Microbiologia e Virologia Clinica, Università "G. d'Annunzio" Chieti-Pescara, Chieti, Italy;

[4] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, Teramo, Italy.

sara.darcangelo@unich.it

Skin dysbiosis is a condition characterized by the loss of homeostasis between cutaneous commensals and opportunistic pathogens like *Staphylococcus aureus*, leading to skin alterations often difficult to treat due to antimicrobial resistance and biofilm production.

A new therapeutic strategy is represented by the eco-sustainable use of natural compounds, in particular food waste, according to the One Health approach. Pomegranate peel, which represents 50% of the fruit itself, could represent a valid strategy for the balance of the skin microbiota thanks to its multiple properties such as antimicrobial and wound healing activities.

The aim of this study was to evaluate the species-specific ability of pomegranate peel extract (PPE) to affect different skin strains isolated from healthy subjects and subjects with skin alterations.

Extraction was performed through two green methodologies, using n-butane and Dimethyl Ether (DME) solvents, and characterization was performed through HPLC analysis.

PPE's antimicrobial action was evaluated against both reference strains and the main isolated skin strains. The ability of PPE in DME to counteract biofilm formation was evaluated against the mono- and dual-species biofilm of S. epidermidis and *S. aureus*, through biomass quantification and CFU/mL determination after 3 and 24 h. The extract's toxicity was evaluated by using the *Galleria mellonella* model.

Catechin, quercetin, vanillic, and gallic acid are the main compounds found in PPE in DME and showed MIC values ranging from 1 to 128 mg/mL and species-specific anti-adhesive activity against the mono-species biofilm of *S. aureus* DLS 69 at sub-MIC concentrations, without affecting *S. epidermidis* DAS 31. In dual-species biofilm, there was an improvement in *S. epidermidis* DAS 31 adhesion.

The results show that PPE could be considered as a valuable non-toxic strategy for restoring cutaneous microbial homeostasis in skin dysbiosis thanks to its capability to act species specifically against strains responsible for skin dysbiosis, protecting beneficial commensal bacteria.

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