

ICBWH2025.

INTERNATIONAL CONGRESS ON BIOMATERIALS FOR WOUND HEALING



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FOREWORD

Dear Colleagues, Distinguished Speakers, Participants, and Supporters,

It is my distinct honor, on behalf of the Organizing Committee, to welcome you to the International Congress on Biomaterials for Wound Healing (ICBWH-2025), taking place September 2–4, 2025, at the Alev Alatlı Conference Hall, Alanya Alaaddin Keykubat University, in the breathtaking region of Alanya, Antalya, Türkiye.

This esteemed congress is a collaborative achievement of Necmettin Erbakan University and Alanya Alaaddin Keykubat University, and represents a convergence of minds from academia, clinical practice, and industry, all united by a shared mission: to explore, exchange, and advance the rapidly evolving field of biomaterials in wound healing.

Over the course of three stimulating days, participants will engage with a rich scientific program featuring keynote presentations, invited lectures, oral and poster sessions, panel discussions, and networking opportunities. Topics span a wide spectrum—from advanced wound dressings, biocompatible materials, 3D printing technologies, and scaffold fabrication, to infection control, personalized medicine, and regulatory frameworks, as well as the integration of tissue engineering, regenerative medicine, nanotechnology, and AI-driven wound healing solutions.

Importantly, ICBWH-2025 also serves as the final conference of the HE-Twinning project REGENEU (Project No. 101079123), funded by the European Union. This highlights our commitment not only to scientific excellence but also to fostering collaborative innovation across borders.

I extend my profound gratitude to all invited speakers, session chairs, committee members, sponsors, and volunteers whose dedication has been instrumental in bringing this congress to fruition. Your expertise and enthusiasm ensure that ICBWH-2025 will be a highly impactful and memorable event.

Thank you for joining ICBWH-2025. May the exchanges here pave the way for meaningful collaborations and groundbreaking innovations that benefit both research and patient outcomes.

Warm regards,

Prof. Dr. Gökhan KARS

Chair, ICBWH-2025

On behalf of the Organizing Committee



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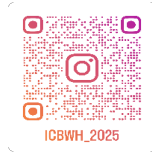
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ACADEMIC REPRESENTATIVE FOR CONGRESS RECOGNITION AND VALIDATION

In the realization of the International Congress on Biomaterials for Wound Healing (ICBWH-2025), organized in cooperation by Necmettin Erbakan University and Alanya Alaaddin Keykubat University, to be held in Alanya, Türkiye, on September 2-4, 2025, Prof. Dr. Meltem Demirel Kars has been officially assigned as the Academic Representative for congress recognition and validation.

This assignment has been approved by the Dean's Office of the Faculty of Engineering, Necmettin Erbakan University, with the Dean's authorization dated February 14, 2025 and numbered E-10419229-900-640993.



INTERNATIONAL CONGRESS ON BIOMATERIALS FOR WOUND HEALING (ICBWH)

Date: 2-4 September 2025 (Tue-Thu)

Venue: Alev Alatlı Conference Hall, Alanya Alaaddin Keykubat University (ALKU), Alanya, Antalya, TÜRKİYE.

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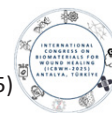
Congress Scientific Program

Day 1: Tuesday, 2 September 2025

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| 08.30 - 16.00 | Registration |
| 09.00 - 10.00 | Opening ceremony |
| 10.00 - 11.00 | Keynote Speech Dr. Meltem Demirel Kars (Necmettin Erbakan University, Türkiye) <i>Healing Wounds, Building Futures: The Transformative Journey of the REGENEU Biomaterials Project</i> |
| 11.00 - 11.20 | Tea/Coffee Break |
| 11.20 - 11.50 | Invited Speech 1 Dr. Conor Buckley (Trinity College Dublin, Ireland) <i>Engineering Regenerative Solutions: Harnessing Naturally Derived Biomaterials for Tissue Repair</i> |
| 11.50 - 12.10 | Speech 1 Dr. Tobias Weigel (Fraunhofer Institute for Silicate Research, Germany) <i>Physiological and Synthetic Stromal Scaffolds for Animal-Free Tissue Models</i> |
| 12.10 - 12.30 | Speech 2 Michael B. Keogh (Royal College of Surgeons in Ireland, Kingdom of Bahrain) <i>Enhanced Biological Effects of Non-Thermal Plasma Treating Collagen GAG Scaffolds</i> |
| 12.30 - 13.30 | Lunch |



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| 13.30 - 14.00 | Invited Speech 2 Dr. Sofia Dembski (Fraunhofer Institute for Silicate Research, Germany) <i>Inorganic Phosphate-based Supra particles: New Approaches for Bone Regeneration and Drug Delivery</i> |
| 14.00 - 14.20 | Speech 3 Zülal Mızrak (Marmara University, Türkiye) <i>The Effects of Pullulan on Cell Proliferation and The Wnt Pathway During Wound Healing in Zebrafish Embryos</i> |
| 14.20 - 14.40 | Speech 4 Marko Dobricic (Royal College of Surgeons in Ireland, Ireland) <i>Local Delivery of Mirna-31 Mimics Via RNA-Activated Scaffolds Enhances ECM Deposition, Angiogenesis, and Neurite Outgrowth for Diabetic Wound Repair</i> |
| 14.40 - 15.00 | Speech 5 Fazilet Canatan Ergün (Necmettin Erbakan University, Türkiye) <i>Application of PCL/Gel Fiber Functionalized with LL37-Loaded CSNP in A 2D Scratch Model for Supporting Wound Healing</i> |
| 15.00 - 15.20 | Tea/Coffee Break |
| 15.20 - 15.50 | Invited Speech 3 Dr. Fergal O'Brien (Royal College of Surgeons in Ireland, Ireland) <i>Biomaterial Scaffolds for The Delivery of Gene Therapeutics for Enhanced Wound Repair</i> |
| 15.50 - 16.10 | Speech 6 Matthew McGrath (Royal College of Surgeons in Ireland, Ireland) <i>Development of a Biomimetic Multi-Layered Functionalised Antimicrobial Biomaterial Scaffold for Healing of Complex Wounds</i> |
| 16.10 - 16.30 | Speech 7 Juan Carlos Palomeque Chávez (Royal College of Surgeons in Ireland, Ireland) <i>A Multi-Faceted Mirna-Activated Scaffold as An Immuno-Modulatory Platform for Chronic Wound Healing Applications</i> |

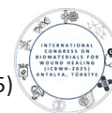


Day 2: Wednesday, 3 September 2025

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| 09.00 - 09.30 | Invited Speech 4 Dr. Bilsev Ince (Prof. Dr. Bilsev Ince Aesthetic Surgery Clinic, Türkiye) <i>Wound Care and Current Treatment Approaches</i> |
| 09.30 - 09.50 | Speech 8 Dr. Jörn Probst (Fraunhofer Institute for Silicate Research, Germany) <i>Renacer® Fiber Fleeces for Chronic Wound Regeneration</i> |
| 09.50 - 10.10 | Speech 9 Katja Nadler (Fraunhofer Institute for Silicate Research, Germany) <i>Sol-Gel Derived Renacer® Fiber Fleeces as A Fully Resorbable Drug Delivery System for Local Post-Operative Glioblastoma Treatment</i> |
| 10.10 - 10.40 | Invited Speech 5 Dr. Atıf Emre Demet (Necmettin Erbakan University, Türkiye) <i>European Patent Applications in Biotechnology: Mapping Innovation Pathways</i> |
| 10.40 - 11.00 | Tea/Coffee Break |
| 11.00 - 11.30 | Invited Speech 6 Anke Wixmerten (University of Basel, Switzerland) <i>Overcoming Obstacles: Key Challenges for Manufacturers of Combined ATMPs</i> |
| 11.30 - 11.50 | Speech 10 Mihraç Görünmek (Istanbul Medeniyet University, Türkiye) <i>Preliminary Investigation of Mycosporine-like Amino Acids from Antarctic Klebsormidium sp. ASYA17 for Advanced Wound Healing Applications</i> |
| 11.50 - 12.10 | Speech 11 Juan Carlos Palomeque Chávez (Royal College of Surgeons in Ireland, Ireland) <i>Development of a Mirna-29b-Activated Scaffold for The Inhibition of Fibrosis During Wound Healing</i> |
| 12.10 - 12.30 | Speech 12 Şeref Akay (Alanya Alaaddin Keykubat University, Türkiye) <i>Lipid Based Multifunctional Drug Delivery Systems for Implant Infections</i> |
| 12.30 - 13.30 | Lunch |
| 13.30 - 14.00 | Invited Speech 7 Dr. Oliver Pullig (University Hospital of Würzburg, Germany) <i>Regulatory Challenges in Advanced Therapies: Navigating Innovation in Medical and Medicinal Products</i> |



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| 14.00 - 14.20 | Speech 13 Dr. Sedef Akçaalan (Necmettin Erbakan University, Türkiye) <i>Sinapic Acid Stimulates Keratinocyte-Driven Wound Healing Via Regulation of Key Migratory and Adhesion Related Genes</i> |
| 14.20 - 14.40 | Speech 14 Julia Burke (Royal College of Surgeons in Ireland, Ireland) <i>Development of a Next Generation Electroconductive Biomaterial for Peripheral Nerve Regeneration</i> |
| 14.40 - 15.10 | Invited Speech 8 Mustafa Ersöz (Selçuk University, Türkiye) <i>Strengthening Research Excellence and Capacity Building in Widening Countries through Marie Skłodowska-Curie Actions</i> |
| 15.10 - 16.10 | Poster Session & Tea/Coffee Break |
| 15.30 - 17.00 | REGENEU Project Management Meeting (For REGENEU project members) |
| 18.30 | GALA DINNER |

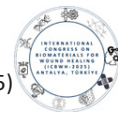


Day 3: Thursday, 4 September 2025

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| 09.00 - 09.30 | Invited Speech 9 Dr. Aylin Şendemir (Ege University, Türkiye) <i>In vitro Evaluation of the Regenerative Effects of Piezoelectric Nanofibrous Scaffolds on Spinal Cord Injury</i> |
| 09.30 - 09.50 | Speech 15 Maria Paula Morales-González (University of La Sabana, Colombia) <i>Hemostatic and Wound Healing Non-Isocyanate-Polyhydroxyurethanes (Niphus) Dressings</i> |
| 09.50 - 10.10 | Speech 16 Dr. Emre Fatih Ediz (Necmettin Erbakan University, Türkiye) <i>Development and Characterization of PLA/Gelatin-Based Biocompatible Nanosponges Enriched with Bioactive Agents</i> |
| 10.10 - 10.30 | Invited Speech 10 Dr. Fatih Kaleci (Necmettin Erbakan University, Türkiye) <i>Global Biomaterials Research (1980–2025): A Comprehensive Bibliometric and Visualization Analysis</i> |
| 10.30 - 10.50 | Tea/Coffee Break |
| 10.50 - 11.20 | Invited Speech 11 Dr. Alexandra Margarida Pinto Marques (University of Minho, Portugal) <i>Dermal Extracellular Matrix in Wound Healing: Applications and Therapeutic Potential</i> |
| 11.20 - 11.40 | Speech 17 Dr. Elif Didem Örs Demet (Necmettin Erbakan University, Türkiye) <i>Enhancing Browning of 3T3-L1 Cells Using Liposomal Naringenin and Berberine</i> |
| 11.40 - 12.00 | Speech 18 Dr. Pelin Ilhan (PA Biotechnology Trade Industry Incorporation, Türkiye) <i>Development and Pre-Validation of a New In Vitro Skin Irritation Test Kit for Safety Assessment</i> |
| 12.00 - 12.15 | Speech 19 Ümran Ünüvar (Necmettin Erbakan University, Türkiye) <i>Comparative Evaluation of Chemically and Green Synthesized Gold Nanoparticles: Antioxidant Properties and Wound-Healing Effects</i> |



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| 12.15 - 12.30 | Speech 20 Canan Sevinç Şaşmaz (Necmettin Erbakan University, Türkiye) <i>Peganum Harmala-Mediated Zinc Oxide Nanoparticles with Antibio- film Potential Against Staphylococcus Aureus: Implications for Wound Healing Applications</i> |
| 12.30 - 13.30 | Lunch |
| 13.30 - 14.30 | Closing ceremony & Poster Awards |



POSTER PRESENTATIONS

1. Sümeyye Kozan, *IN-VITRO EVALUATION OF BIOCOMPATIBILITY OF CBD ON KERATINOCYTE CELLS.*
2. Melike Tuncer, *PREPARATION OF STARCH-BASED AEROGELS VIA FREEZE-THAWING AS A POTENTIAL DRUG RELEASE SYSTEM*
3. Besna Dalmış, *COMPARISON OF PHB PRODUCTION EFFICIENCIES OF *Cereibacter sphaeroides* AND *Cupriavidus necator* USING FOUR DIFFERENT CARBON SOURCES*
4. Nur Banu Soylu, *NATURAL CAROTENOID EXTRACTION FROM *Cereibacter sphaeroides* O.U.001 CULTIVATED UNDER CARBON DIOXIDE FIXATION CONDITIONS*
5. Tuğba Baş, *OPTIMIZING POLYHYDROXYBUTYRATE BIOSYNTHESIS IN *Cereibacter sphaeroides* AND *Rhodopseudomonas palustris* VIA GROWTH-INDUCTION APPROACH*
6. Innocent Manga, *BIOCONVERSION OF ACETIC ACID TO PHB BY *Cereibacter sphaeroides* AND *Rhodopseudomonas palustris*: YIELD OPTIMIZATION and STRUCTURAL CHARACTERIZATION*
7. Beyza Nur Sayaner Taşçı, *BIOCOMPATIBLE AND ANTIMICROBIAL PHB-BASED NANO-FIBROUS DRESSINGS FOR WOUND HEALING APPLICATION*



HEALING WOUNDS, BUILDING FUTURES: THE TRANSFORMATIVE JOURNEY OF THE REGENEU BIOMATERIALS PROJECT

Meltem Demirel KARS

Necmettin Erbakan University, Department of Biomedical Engineering, Konya, Türkiye

Corresponding author: mdkars@erbakan.edu.tr

Biomaterials have emerged as a cornerstone of regenerative medicine, offering innovative solutions for tissue repair, wound healing, and enhanced quality of life. Globally, the biomaterials market is projected to grow steadily over the next fifteen years. The REGENEU project funded under the HORIZON-WIDERA-2021-ACCESS-03-01 Twinning initiative aims to elevate the scientific excellence and innovation capacity of Necmettin Erbakan University (NEU) in Konya, Türkiye. By building targeted capabilities in functionalized biofiber research for wound healing and tissue regeneration, REGENEU serves as a gateway for NEU to actively engage in Europe's biomaterials research ecosystem. The project is implemented through strategic collaborations with leading European institutions: Fraunhofer ISC (Germany), University Hospital Würzburg (Germany), Trinity College Dublin (Ireland), and the Royal College of Surgeons in Ireland.

Scientifically, the REGENEU project focuses on advancing electrospun nanofiber scaffolds designed for bioactivity, biocompatibility, and clinical potential. One recent publication demonstrates the use of biodegradable PCL/collagen fibers loaded with herbal oils resulting in scaffolds with high wettability, mechanical strength, antioxidant activity, and over 92% cell viability. These functional biomaterials serve as promising wound dressings, combining structural support with enhanced healing properties. Another study highlights the integration of antimicrobial peptide into PHB/collagen nanofibers, significantly reducing bacterial viability and promoting in vitro wound closure showcasing dual regenerative and antimicrobial action an essential requirement in chronic wound care.

Over the whole project period since its launch in October 2022, REGENEU has executed a diverse set of activities aligned with its objectives. Key actions include establishment of the project website and visual identity; delivery virtual courses and online seminars; implementation of on-site training schools in Ireland, Germany and Türkiye for early-stage researchers and postdocs; and administrative capacity building via project support office training events. Consortium-wide project management meetings have been held to ensure monitoring and coordination. Scientific productivity has also accelerated. NEU initiated research projects aligned to REGENEU themes



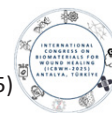
currently in progress. A collaborative PhD project between NEU and Fraunhofer ISC directly contributes to the development of functional biofibers. Furthermore, project outcomes have been disseminated through peer-reviewed open-access journal publications and international oral conference presentations, increasing visibility within the scientific community.

Ultimately, REGENEU represents a transformative journey not only in enhancing NEU's scientific capacity and networking capabilities but also in positioning Turkey as a future contributor to Europe's innovation driven biomaterials sector. By bridging knowledge gaps, nurturing talent, and fostering collaborative research, REGENEU builds sustainable capacity for healing wounds and building biomedical futures.

Keywords: Biomedical Innovation, Capacity Building, Functional Biofibers, International Collaboration, REGENEU

Research Area: Biomaterials for Wound Healing

Acknowledgements: This study was supported by the European Union Horizon Europe REGENEU project (Project No: 101079123).



ENGINEERING REGENERATIVE SOLUTIONS: HARNESSING NATURALLY DERIVED BIOMATERIALS FOR TISSUE REPAIR

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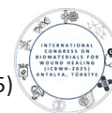
The repair and regeneration of damaged tissues remains a critical challenge in modern medicine, with increasing demand for therapies that can restore native tissue function and reduce reliance on donor grafts. Naturally derived biomaterials, particularly those based on extracellular matrix (ECM) components, offer unique biological and physicochemical properties that make them highly attractive for regenerative medicine applications. This talk will explore advances in the design, fabrication, and translation of ECM-based biomaterials for tissue repair, drawing on recent research into scalable, reproducible, and clinically relevant biofabrication strategies. ECM biomaterials inherently provide bioactive cues, offering both structural support and biochemical signals that facilitate cell adhesion, drive proliferation, and influence cellular phenotype. By harnessing tissue-specific ECM sourced from animal or human origin, these materials can be tailored to replicate or mimic the microenvironment of the target tissue, enhancing integration and functional recovery. Processing approaches such as decellularisation, freeze-drying and bioprinting can be optimised to preserve bioactivity while enabling customisable architectures for diverse applications including orthopaedic, peripheral nerve repair and wound healing. Recent work from our group has developed various hydrogel and bioink formulations and case studies will highlight their application in developing injectable bioadhesives for minimally invasive orthopaedic repair, bioactive and antimicrobial materials for wound healing and nerve guidance conduits for peripheral nerve regeneration. Emerging trends, including the integration of ECM biomaterials with advanced manufacturing methods such as 3D bioprinting and stimuli-responsive systems, open new opportunities for personalised regenerative therapies. These innovations promise not only to accelerate healing but also to reduce complications, improve patient outcomes, and expand the scope of minimally invasive treatments. By harnessing the intrinsic properties of naturally de-



rived biomaterials combined with engineering precision, these advanced biomaterials represent a transformative platform for the next generation of regenerative therapies. This talk will provide insights into their scientific foundations, translational progress, and future potential in addressing unmet clinical needs across multiple tissue types.

Keywords: Biomaterials, Tissue Engineering, ECM, Hydrogels, Regeneration

Research Area: Innovative Biomaterials for Wound Healing, Bioactive and Natural Biomaterials



INORGANIC PHOSPHATE-BASED SUPRAPARTICLES: NEW APPROACHES FOR BONE REGENERATION AND DRUG DELIVERY

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Supraparticles are hierarchically structured microparticles made up of nanoscale building blocks. They are typically formed through controlled self-assembly or aggregation processes. This architecture enables the integration of nanoscale functionalities with the practical handling advantages of microscale materials. In biomedical applications, supraparticles provide a robust platform for the targeted delivery of therapeutic agents, including antibiotics and metal ions. They can also be incorporated into medical coatings via high-temperature processes such as flame spraying, which causes conventional drug carriers to fail. Their tunable porosity, degradation behavior, and release kinetics make supraparticles highly adaptable for implant surface engineering and localized drug delivery.

The focus of this study was to develop an innovative drug delivery strategy using Cu-doped calcium phosphate (CaP) and magnesium phosphate (MgP) supraparticles for bone implant coatings. The goal was to improve osteointegration and provide long-term antibacterial protection independently of conventional antibiotics. Nano-sized mesoporous CaP or MgP particles were prepared via a modified Pechini sol-gel method and processed into supraparticles through spray drying. Integrating Cu-ions during spray drying allowed for precise control of antibacterial functionality. A key innovation of this study was the use of porous supraparticles as multifunctional carriers embedded in ultrathin (approximately 30 μm) β -tricalcium phosphate (β -TCP) coatings on titanium (Ti) implants via high-velocity suspension flame spraying (HVSFS). These supraparticles served as structural components and drug delivery systems capable of sustained ion release.

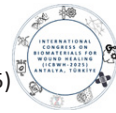
We characterized the newly developed particles and coatings using standard methods such as dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray diffraction analysis (XRD). The particles and coatings were also successfully evaluated in vitro and in vivo. In vitro studies focused on biocompatibility, using cell viability and proliferation assays to evaluate potential Cu ion cytotoxicity. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was also evaluated. Initial animal experiments with New Zealand white rabbits demonstrated promising results for this new coating modification approach.



In general, the supraparticle-based drug delivery approach provides a highly versatile platform for implant surface modification. This approach enables the controlled, localized release of therapeutic agents and paves the way for the incorporation of sensitive bioactive compounds into high-temperature coating processes, marking a significant advancement in orthopedic implant technology.

Keywords: Supraparticles, Bone Implant Coatings

Research Area: Bioactive and Natural Biomaterials



BIOMATERIAL SCAFFOLDS FOR THE DELIVERY OF GENE THERAPEUTICS FOR ENHANCED WOUND REPAIR

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The once-in-a-century COVID-19 pandemic was conquered using revolutionary messenger RNA (mRNA) treatments. This has given new momentum to gene therapy research for a myriad of applications. The field of regenerative medicine is well placed to be a beneficiary whereby, for example, gene therapy might be a valuable tool to avoid the limitations of local delivery of growth factors. While non-viral vectors (NVV) are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated NVV gene therapy approach for regenerative applications. Utilising plasmid DNA and mRNA encoding therapeutic proteins, these gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also can be engineered to direct autologous host cells to take up specific genes and then produce therapeutic proteins in a sustained but eventually transient fashion. Similarly, we have demonstrated how scaffold-mediated delivery of siRNA and miRNA can be used to silence specific genes associated with reduced repair or pathological states. This presentation will provide an overview of ongoing research in our lab in this area with a particular focus on gene-activated biomaterials for promoting nerve and wound repair.

Keywords: Biomaterial, Scaffold, Gene Delivery

Research Area: Biomaterials for Wound Healing, Nanotechnology and Nanomaterials in Wound Healing



WOUND CARE AND CURRENT TREATMENT APPROACHES

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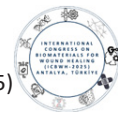
A wound is the disruption of tissue integrity due to a pathological event. Wound healing is the tissue response that attempts to return injured tissue to a normal state. The wound healing process consists of inflammation, cell proliferation, matrix deposition and matrix remodeling stages. The wound healing process, which begins with the formation of a wound, ends in approximately 1 year. In the treatment approach, before wound closure surgery, management of infection, elimination of factors causing chronic wound, preparation of the wound bed and revascularization if necessary should be performed. In this presentation, we aim to present current approach options used in wound treatment.

A chronic wound can be defined as a wound that does not heal completely within 6-8 weeks or shows no signs of healing within 4 weeks. The primary goal of treating chronic wounds is to convert them into acute wounds. The first step in wound care is to assess the wound and select appropriate wound care products. First and foremost, wound care products are used to support the wound's healing environment. Wound healing will not be possible without addressing the underlying causes. It's important to remember that there is no miracle wound care product that will heal all wounds.

For optimal wound healing, the wound should be free of necrotic tissue, a moist environment should be provided, a balanced exudate should be provided, the wound area should be healthy, contamination should be prevented, infection should be eliminated. Many different options are being tried and used in wound management, such as debridement, topical antimicrobial agents, antiseptics, antibacterial dressings, skin-equivalent dressings, growth factors, topical negative pressure, hyperbaric oxygen therapy, and stem cell therapy. Recently, Mesenchymal Stem Cell-Derived Exosome Therapy and PRP have come to the fore in wound healing treatment. It's important to remember that there is no miracle wound care product that will heal all wounds.

It is very important to be knowledgeable about current treatment approaches in wound treatment and to use the appropriate wound care product at the appropriate time.

Keywords: Wound, Wound Healing, Wound Treatment



EUROPEAN PATENT APPLICATIONS IN BIOTECHNOLOGY: MAPPING INNOVATION PATHWAYS

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This study maps biotechnology innovation pathways by analyzing patent applications filed at the European Patent Office (EPO). The purpose is to monitor the European patent applications in biotechnology and to establish a transparent, reproducible baseline for tracking who is innovating, how activity in biotechnology field evolves over time, and where strengths concentrate across Europe. The analysis uses the patent data retrieved from the EPO, particularly for the biotechnology technology field, over the period of 2015–2024 for applications and grants, and country shares by origin.

Methods focus on descriptive patent indicators such as yearly application and grant counts, and country of origin composition. Trend measures include absolute and compound growth, and the ratio of grants issued per 100 applications in the same year. It is defined using the “Biotechnology” technology field, anchored in International Patent Classification (IPC) classes including C07G, C07K, C12M, C12N, C12P, C12Q, C12R, and C12S, explicitly excluding A61K to segregate pharmaceutical formulations from core biotechnological processes and materials.

Basic findings indicate sustained activity across core biotechnology process classes, with notable intensity around genetic and cell-based technologies (e.g., C12N) and molecular diagnostics and assay platforms (e.g., C12Q). Applicant portfolios suggest concentration among diversified life-science companies and major biopharma, alongside growing participation from universities, spin-offs, and public research organizations. It also shows sustained expansion of biotechnology filings at the EPO. Applications rose from 5,724 in 2015 to 8,479 in 2024, with an increase of 48% and a compound annual growth rate of 4.46% reaching a series high in 2024. Growth was steady both before and after 2019. In contrast, the number of grants issued per year fell from 2,658 (2015) to 1,835 (2024), which means lowering from 46% to 22% for the same years, respectively.

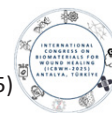


The 2024 country composition highlights Europe's continued centrality alongside strong extra-European participation. EPO member states collectively account for 41% of biotechnology applications, led by Germany (9.6%), France (5.2%), Switzerland (5.1%), the United Kingdom (4.1%), the Netherlands (3.2%), Denmark (3.2%), Belgium (2.7%), and other EPO states (7.9%). The United States is the largest single country contributor at 36%, followed by China (7.6%), Japan (5.9%), the Republic of Korea (3.7%), and other origins (6.0%).

Discussion connects these patterns to translational dynamics and policy: Europe remains the largest filing bloc with diverse national strengths, while global players, especially the United States and China, are deeply engaged in the EPO route. The decoupling of applications and grants underscores the importance of monitoring throughput as well as inflows. Hence, mapping innovation pathways in biotechnology offers robust indicators for benchmarking institutions, guiding R&D and IP strategy, and prioritizing support for high-impact sub-domains.

Keywords: Biotechnology, European Patent Office, Patent Landscape, IPC

Research Area: Intellectual Property & Innovation Management, Biotechnology



OVERCOMING OBSTACLES: KEY CHALLENGES FOR MANUFACTURERS OF COMBINED ATMPs

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The development of products combining medical devices and medicinal products is associated with several challenges due to the multifaceted regulatory environment, complex pre-clinical development and demanding manufacturing processes and testing procedures. A key aspect is the classification of the product to determine the regulatory path to be followed during development and later approval procedure for the market. For ATMPs intended for the European market the Committee of Advanced Therapies (CAT) can provide classification advice, to clarify the applicable regulatory framework. Combined ATMPs often require dual pathways to be followed for the medical device part as well as for the cellular part of the product. While each part must be assessed individually to demonstrate compliance with the applicable regulations and directives, also the combined product must be assessed. Here, mainly the interaction between the cellular part and the medical device is evaluated and the influence they may have on each other and the intended therapeutic effect must be investigated. Therefore, safety and efficacy, assessed in animal models, must use the combined product to study the effects on the body. These may be different from the effects due to each single component. Animal studies pose an additional challenge, as often homologous models must be used, meaning, that the combined ATMP is prepared from the animal cells in the same way as for the human product. While this eliminates rejection reactions, this approach has the drawback, that not the real product for humans is assessed and potential risks may not be found. Moreover, potency may not be possible to be tested in these homologous models. The key challenge in the manufacturing of combined ATMPs is the development of relevant release criteria, especially for potency, identity and purity, including the related assays. Often surrogate markers, such as molecules produced by the cells, have to be used for identity markers or potency tests since functional tests can often only be performed in vivo. However, the use of surrogate markers must be well justified and correlation between potency markers and intended biological effect must be demonstrated. An additional challenge in the release testing is the limited shelf-life of ATMPs, which may range from a couple of hours to a few days. Due to the challenges associated with combined ATMP development, authorities have established tools such as scientific advice meetings to support compliance with regulatory requirements for these individual innovative products.

Keywords: Combined ATMP, Tissue Engineering, Regulatory Affairs, GMP



REGULATORY CHALLENGES IN ADVANCED THERAPIES: NAVIGATING INNOVATION IN MEDICAL AND MEDICINAL PRODUCTS

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Novel therapeutic approaches employing innovative biomaterials are increasingly recognized as highly promising, particularly in clinical areas where conventional pharmacological treatments reach their limitations or are not feasible. The introduction of materials with novel properties in combination with advanced manufacturing technologies, including additive manufacturing and 3D printing, is expanding the scope of therapeutic options. These innovations not only enable new treatment modalities but also allow for patient-specific solutions that were previously unattainable. The international research community has responded with intense academic activity, accompanied by the establishment of dedicated funding programs that specifically aim to foster such material-based innovations in medicine.

While encouraging results are being generated in research and development, the translation of these concepts into clinical practice remains a long and demanding pathway. A successful transfer requires more than proof of feasibility: it depends on demonstrating clear comparability or superiority over existing therapeutic approaches, as well as the successful execution of rigorous clinical evaluation in human subjects. As many of these technologies involve Class III medical devices with long-term residence in the human body, regulatory and manufacturing requirements are particularly stringent. This includes comprehensive quality management systems, validated aseptic production processes, and carefully designed clinical trials. Above all, patient safety remains the guiding principle throughout development and implementation.

Regulatory authorities are therefore faced with the complex dual mandate of ensuring robust safety standards while simultaneously fostering innovation and avoiding excessive bureaucratic barriers. To meet this challenge, the European Union has implemented several harmonization measures designed to shorten timelines and reduce redundancies in the approval process without compromising quality or safety. A central milestone in this regard is the Clinical Trials Information System (CTIS), which has been mandatory since January 2023 for the submission, authorization, and management of all clinical trials within the EU. CTIS harmonizes not only the initial



approval process but also amendments, reporting requirements, and monitoring activities, thus increasing transparency and efficiency across member states.

These developments represent crucial steps toward ensuring that innovative biomaterial-based combination products can be made available to patients within clinically relevant timeframes. By aligning scientific progress, regulatory oversight, and clinical application, the pathway from bench to bedside can be accelerated, ultimately broadening the spectrum of therapeutic options and improving patient outcomes in areas of high unmet medical need.

Keywords: Regulatory Affairs, ATMP, Clinical Trial, Combination Products

Research Area: Regulatory Affairs



STRENGTHENING RESEARCH EXCELLENCE AND CAPACITY BUILDING IN WIDENING COUNTRIES THROUGH MARIE SKŁODOWSKA-CURIE ACTIONS

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The Marie Skłodowska-Curie Actions (MSCA) under Horizon Europe provide a unique framework for fostering international, interdisciplinary, and inter-sectoral research collaborations through researcher mobility, advanced training, and knowledge exchange. These actions are designed to enhance the career prospects of researchers at all stages, while addressing global challenges through cutting-edge science and innovation. MSCA projects are implemented through structured secondments, joint research activities, and targeted training modules that build complementary skills and promote innovation-driven outcomes.

In the context of **widening countries**—regions with lower participation rates in EU research programmes—MSCA offers a strategic opportunity to strengthen research capacity, integrate national institutions into high-performing European networks, and bridge the innovation divide. By hosting secondments and training events in widening countries, projects can transfer advanced methodologies, establish sustainable research infrastructures, and foster long-term partnerships with leading institutions from across the EU and associated countries.

The MSCA framework deliver a comprehensive programme of research, training, and dissemination activities that will:

- Enable bidirectional mobility between widening and non-widening partners, facilitating the exchange of expertise and best practices.
- Develop research excellence in emerging scientific fields by leveraging complementary expertise and advanced infrastructure.
- Provide interdisciplinary and cross-sectoral training that combines scientific excellence with transferable skills such as entrepreneurship, intellectual property management, and science communication.



- Enhance the visibility and competitiveness of widening country institutions within the European Research Area (ERA) by increasing participation in collaborative R&I projects and strengthening links to industry.
- Foster an inclusive and sustainable talent, encouraging early-career researchers from widening countries to engage in international research careers while addressing local and regional innovation needs.



IN VITRO EVALUATION OF THE REGENERATIVE EFFECTS OF PIEZOELECTRIC NANOFIBROUS SCAFFOLDS ON SPINAL CORD INJURY

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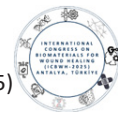
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Spinal cord injury (SCI) is a debilitating condition with huge long-term personal, social and economic costs. Patients with SCI experience serious clinical problems, such as partial or complete paralysis, spasticity, pain, loss of bowel/bladder/sexual function, and difficulty in breathing. Ongoing research for SCI comprises pharmacological therapies and cell transplantation. The combination of several treatment strategies makes partial recovery possible, but there is no extensive treatment for regeneration.

This work investigates the potential of a novel, functionalized biomaterial-based treatment to induce neuronal regeneration by use of electrospun piezoelectric nanofibrous scaffolds on an *in vitro* model of SCI. Piezoelectric materials generate local electric fields in response to mechanical stresses, and this effect is used to stimulate neuronal regeneration. After SCI, the inflammation observed in the injury zone creates an inhibitory environment for neurons and myelin formation. This inflammatory environment inhibits neuronal regeneration, and is the main cause of limited recovery.

A three-dimensional (3D) *in vitro* SCI model was produced using neuronal, astrocytic and oligodendrocytic cell lines embedded in an alginate hydrogel loaded by bacterial cellulose (BC) nanofibers. While alginate hydrogel simulated the mechanical properties of the spinal cord extracellular matrix, BC nanofibers provided nodes for cell attachment. The seeding ratio and the co-culture medium was optimized in order for all three cell types to show their phenotype characteristics. The injury was simulated



both by a mechanical cut using a punch, and addition of lipopolysaccharides (LPS) to induce inflammatory response. The glial inflammation (gliosis) was characterized by increased expression of glial fibrillary acidic protein (GFAP) and reduced expression of myelin oligodendrocyte glycoprotein (MOG).

Four types of scaffolds from polyvinylidene fluoride (PVDF), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), cellulose acetate (CA) and polycaprolactone (PCL) were produced by electrospinning. PCL was used as a biocompatible, but non-piezoelectric control. Fiber alignment was achieved using a drum collector. The nanofiber scaffolds were tested for increased neurite formation and length on 3D SCI models.

It was shown that the nanofibrous piezoelectric fibers induced a positive effect on neurite formation and elongation – despite the existence of the inflammatory microenvironment. The nanofiber morphology provided sites for neural and neurite attachment. The results propose that piezoelectric nanofibrous scaffolds have potential therapeutic effects on spinal cord regeneration.

This work was conducted within PIECRISCI project founded by the European Union's Horizon 2020 Research & Innovation Programme within M-ERA.NET, funded by TÜBİTAK Grant# 122N440.

Keywords: Spinal Cord Injury (SCI), *In Vitro* Model, Piezoelectric Fibers, Electrospinning

Research Area: Wound Healing, *In Vitro* Model, Regenerative Medicine



GLOBAL BIOMATERIALS RESEARCH (1980–2025): A COMPREHENSIVE BIBLIOMETRIC AND VISUALIZATION ANALYSIS

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This study provides an extensive bibliometric and visualization analysis of global biomaterials research spanning the period from 1980 to 2025, offering a longitudinal perspective on the evolution and transformation of the field over more than four decades. Data were retrieved from the Web of Science Core Collection using the keywords biomaterial, biocompatibility, and biomaterials, ensuring comprehensive coverage of the literature across multiple disciplines including materials science, biomedical engineering, chemistry, and life sciences. A total of approximately 230,000 publications were analyzed to explore temporal publication trends, geographical distribution of research output, leading authors, influential institutions, high-impact journals, collaboration networks, and thematic research hotspots.

Bibliometric indicators such as annual publication growth rates, citation performance, h-index values, authorship patterns, and international co-authorship ratios were calculated to evaluate both productivity and scientific influence. Science mapping techniques, implemented via VOSviewer and Bibliometrix, were used to construct visualizations of co-authorship networks, country and institutional collaboration maps, keyword co-occurrence structures, and thematic evolution pathways. These visualizations facilitated the identification of emerging research fronts and the shifting thematic structure of the field over time.

The findings indicate a continuous and accelerating growth in biomaterials-related publications, with marked expansion after 2000, driven by rapid advancements in nanotechnology, regenerative medicine, tissue engineering, and smart material design. The United States, China, and Germany emerged as the most prolific contributors, collectively accounting for a significant proportion of global output, while top institutions exhibited high levels of international collaboration, particularly in large-scale multidisciplinary projects. Citation analysis revealed that research in the areas of bioactive scaffolds, nanostructured surfaces, and stimuli-responsive materials has garnered substantial scholarly attention in recent years. Keyword clustering demonstrated a clear thematic shift from foundational studies on material biocompatibility and mechanical properties toward multifunctional nanomaterials, 3D-printed scaffolds, drug delivery systems, and bioinspired materials for precision medicine.



By mapping four decades of global biomaterials research, this study not only documents the historical development of the field but also provides actionable insights into current research landscapes and future trajectories. The results can serve as a strategic resource for scientists, policymakers, funding agencies, and industry stakeholders seeking to prioritize investments, foster international collaboration, and accelerate innovation in biomaterials science.

Keywords: Biomaterials, Bibliometrics, Visualization Analysis, General Information, Research Trends

Research Area: Biomaterials for Wound Healing, Future Directions and Emerging Technologies



DERMAL EXTRACELLULAR MATRIX IN WOUND HEALING: APPLICATIONS AND THERAPEUTIC POTENTIAL

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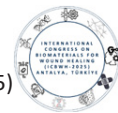
The extracellular matrix (ECM) is a highly complex and dynamic structure that has long inspired the development of bio-instructive materials for tissue engineering and regenerative medicine. Beyond providing a physical scaffold for cell adhesion and biomechanics, the ECM exerts a regulatory role by orchestrating key physiological and pathological processes through the presentation and storage of soluble biochemical cues. This dual function, as both a structural and signalling entity, positions the ECM as a central determinant of tissue homeostasis, repair, and disease. Importantly, its bioresponsive nature has also brought increasing attention to the ECM as a potential therapeutic target, highlighting the need for advanced strategies that replicate its molecular and biomechanical complexity in order to uncover disease mechanisms and enable the discovery of novel interventions.

In our work, we focus on dermal ECM as a paradigm for studying and harnessing these multifaceted roles. On one side, we aim to engineer next-generation regenerative templates capable of guiding effective cutaneous wound healing by leveraging the instructive properties of native ECM. On the other, we employ dermal ECM as the foundation for in vitro disease models that faithfully recapitulate the pathophysiology of human skin disorders. By capturing disease-relevant ECM alterations, these models allow us to dissect ECM-driven mechanisms underlying skin diseases, while simultaneously providing a platform for therapeutic target identification and testing.

Together, these complementary approaches establish the dermal ECM not only as a source of inspiration for the design of regenerative biomaterials but also as a powerful tool for modelling disease, deepening our understanding of ECM-driven biology, and advancing the translation of ECM-based therapeutic strategies.

Keywords: Extracellular Matrix, Biomaterials, Wound Healing, Therapeutic Target, Skin Diseases

Research Area: Biomaterials for Wound Healing, Mechanisms of Action: Cellular and Molecular Aspects



PHYSIOLOGICAL AND SYNTHETIC STROMAL SCAFFOLDS FOR ANIMAL-FREE TISSUE MODELS

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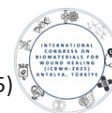
Stromal tissues are essential components of engineered soft tissues intended as alternatives to animal testing or advanced biological implants. Due to a lack of synthetic or recombinant options, the reliance is on animal-derived materials like collagen or decellularized tissues. However, these biological scaffolds raise ethical issues, suffer from variability between batches and result in an aversion for the development of standardized, validated and approved preclinical test protocols. The challenge in developing suitable synthetic 3D scaffold materials is to meet requirements such as biomimetic structure, high porosity and bioactivity. To overcome this issue, we developed a highly porous fibrous scaffold suitable for a range of stromal and epithelial tissues without animal or human-derived extracellular matrix (ECM). Scaffolds were produced by electrospinning with polyamide as an exemplary polymer. Porosity was controlled by adding NaCl porogen particles in specified proportions and sizes. The resulting scaffolds were characterized for structural (SEM, LSM) and micro-mechanical properties (nanoindentation). For biologization, 3D scaffolds were seeded with human stromal tissue cells from the target tissues (fibroblasts or mesenchymal stromal cells, MSCs). After 1-4 weeks of cell migration, proliferation, differentiation and matrix synthesis, the resulting human ECM tissues were characterized. Different size distributions of NaCl porogen particles up to 500 µm were tested in scaffold fabrication and evaluated by the structural properties, stability and 3D cellular interactions. Mechanical properties of the scaffolds at the cellular level, with a Young's modulus of 3 kPa, were comparable to traditional collagen gels. During biologization, fibroblasts migrated through the whole scaffold and induced a remodeling process including dynamic rearrangement of fibers as well as secreting tissue-specific ECM components. Additionally, MSCs were able to differentiate within the 3D structure (e.g. into adipogenic/osteogenic cells), transforming the synthetic scaffold into various biologically



applicable tissue types. The biologized scaffolds were suitable for combination with human epithelial cells to create physiological and functional full-thickness models e.g. skin, airways and intestine. The described modular approach, to start with physiological structured synthetic open meshed scaffolds followed by the transformation of an organ specific human stromal tissue, provides multiple options in applications. Goal is to facilitate the replacement of animal-derived materials, enhance tissue complexity and human-specific experimental readouts for human in vitro wound models, uptake studies, drug testings and infections.

Keywords: Physiological Scaffold, Stromal Tissue, Electrospinning

Research Area: Tissue Engineering and Regenerative Medicine, Scaffold Fabrication Techniques for Wound Healing.



ENHANCED BIOLOGICAL EFFECTS OF NON-THERMAL PLASMA TREATING COLLAGEN GAG SCAFFOLDS

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Background: Tissue-engineered scaffolds are designed to stimulate and promote wound healing. They act as an anchor on the wound bed and can release drugs or molecules as required to reinstate a physiological state both anatomically and functionally. Collagen being biocompatible, biodegradable, low immunogenic making it an ideal candidate biomimetic biomaterials, however, it is not structurally resistant and require crosslinkers like EDAC or glutaraldehyde copolymerization. Some of these chemical crosslinking agents can be cytotoxic or even carcinogenic and may not be suitable for clinical use in tissue engineering. Non-thermal plasma (NTP) generated by ionized gas has been shown to improve mechanical and biological properties of biomaterial without any chemical toxicity. The aim of the study is to understand the effect of NTP using nitrogen plasma on collagen scaffold, and the modifications done to the structure and functionality of the biomaterial. Methodology included: CG scaffolds NTP treated with nitrogen plasma for 2 and 5 minutes @100mTorr and compared with non-treated controls. Mechanical properties were assessed by mechanical testing, SEM imaging while chemical properties assed by FT-IR, enzymatic degradation & wettability via contact angle. Bio-compatibility was assessed by attachment (histology, metabolic activity and cell number) and differentiation studies (5x10⁴ adipose

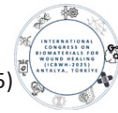


derived stem cells seeded on 5mm diameter constructs and cultured in osteogenic media for 28 days; alizarin red, osteogenic gene expression, osteocalcin, RUNX2, ALP, BSP). The results following NTP treatment show changes in surface modification and chemical properties at 2 and 5 mins. We show that NTP treatment enhanced the mechanical stiffness and pore size of the scaffolds. Scaffold pore size increased over treatment and time non-treated ($31\mu\text{m}$), 2-min treatment ($42\mu\text{m}$) and 5min ($86\mu\text{m}$), this was supported by the Alician blue histology. However, FT-IR spectra reveals that the chemical composition of the scaffolds remains highly conserved after treatment. Degradation rates were similar to each other with non-treated and 2 mins, similar and 5 min slightly higher. Wetability and hydrophilicity increased with treatment with untreated contact angles of 106.7° , 2min NTP 85.9° and 5 min 91.2° . There was a significant increase in cell numbers were noted both on 2min plasma treated ($p=0.001$), and 5 min plasma treated scaffolds ($p=0.02$) versus control. Similar trends were noted in metabolic activity. Osteogenic induction was greatest after 2-minute treatment with greatest levels of alizarin red staining for calcium and highest osteogenic markers Osteocalcin expression at day 21.

The results of this study show that NTP plasma treatment typically used to sterilize materials can also modify positively the biocompatibility of collagen GAG sponges and may be a preferred choice of crosslinking to traditional methods.

Keywords: Plasma Treatment, Mechanical Stiffness, Stem Cell, Osteogenesis

Research Area: Advanced Wound Dressings and Coating, Tissue Engineering and Regenerative Medicine



THE EFFECTS OF PULLULAN ON CELL PROLIFERATION AND THE WNT PATHWAY DURING WOUND HEALING IN ZEBRAFISH EMBRYOS

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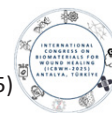
Wound healing is a complex, sequential series involving the interaction of various cellular and biochemical mechanisms that occurs in response to disruption of tissue integrity. WNT signal transduction system is a complex cascade that mainly controls cell proliferation. Wounding initiates Wnt signaling, which is involved in all subsequent phases of the healing process. As a member of the WNT gene family, WNT10A has been reported to be essential for controlling fibrogenic factors like collagen and for many morpho/organogenesis processes. The primary function of wound dressings is to protect the damaged tissue and promote accelerated healing. Polysaccharide polymers have been utilised in the production of wound dressings. Pullulan is a non-toxic, water-soluble natural polysaccharide molecule that is produced by a fungus called *Aureobasidium pullulans*. It is also regarded as an antioxidant, thereby providing protection against environmental damage. It has been demonstrated that pullulan hydrogels have the capacity to accelerate the healing process of wounds. Due to its genetic similarity to humans, the zebrafish (*Danio rerio*) has become a model organism for several aspects of human disease and development in recent years. Similar mechanisms are used by zebrafish and mammalian embryonic wounds to heal their epidermis. The objective of this study was to evaluate the effects of pullulan on zebrafish embryos following tail amputation focusing on Wnt pathway, cell proliferation and oxidant-antioxidant status. In the experiment, zebrafish embryos were anaesthetised and underwent amputation of the posterior caudal fin using a scalpel



at 72 hours post-fertilisation (hpf). One group of embryos was exposed to pullulan after amputation for a period of up to 120 hpf, while a control group received no such treatment. The developmental parameters and mortality rates of the embryos were monitored and documented daily up to 120 hpf. The expression of genes associated with cell proliferation and WNT pathway was analysed by RT-PCR to evaluate the recovery effects of pullulan. The antioxidant effects of pullulan were evaluated through the measurement of oxidant-antioxidant system parameters by spectrophotometric analysis. There were significant alterations in the oxidant parameters and the gene expression in the embryos treated with pullulan compared to the tail amputee group. The results of our study showed that zebrafish embryos are a suitable model for wound healing research and that the WNT pathway, cell proliferation and oxidant-antioxidant system may be potential mechanisms that may be effective in the regulation of the effects of pullulan on wound healing.

Keywords: Wound Healing, Pullulan, Cell Proliferation, Wnt Pathway, Zebrafish Embryos

Research Area: Biocompatible Materials



LOCAL DELIVERY OF MIRNA-31 MIMICS VIA RNA-ACTIVATED SCAFFOLDS ENHANCES ECM DEPOSITION, ANGIOGENESIS, AND NEURITE OUTGROWTH FOR DIABETIC WOUND REPAIR

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Complex wounds, such as diabetic foot ulcers, represent a significant healthcare challenge due to impaired tissue regeneration, increased risk of infection, and debilitating outcomes. Standard treatments, such as wound debridement and autologous tissue replacement, often fail to achieve rapid and complete wound closure, partly due to challenges including impaired tissue regeneration, prolonged inflammation, and susceptibility to infection. These limitations emphasise the need for innovative,



minimally invasive approaches that not only promote tissue and vascular repair but also facilitate neurogenic regeneration. In collaboration with Queen's University Belfast, this project explored the local delivery of RALA nanoparticles carrying microRNA-31 (miR-31) mimics, designed to enhance endogenous miR-31 levels and activate key pro-regenerative pathways. MiR-31 has been shown to regulate the expression of HIF-1 α , a transcription factor critical for angiogenesis, fibroblast migration, and extracellular matrix (ECM) remodelling under hypoxic wound conditions. Delivered via collagen-based scaffolds that mimic skin composition, these gene-activated biomaterials aim to promote vascularisation, matrix deposition, and neurogenic support, offering a next-generation alternative to current wound therapies.

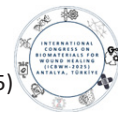
First, the complexed miRNA-nanoparticles were incorporated into a biomimetic collagen-chondroitin sulphate scaffold developed for wound repair and their potential for angiogenesis and improved ECM deposition evaluated. Functional assays, such as endothelial tube formation assays and RT-qPCR, were performed to test the efficacy of miR-31 in driving angiogenic behaviour and ECM deposition. Our results demonstrated that the scaffold-based delivery of miR-31 significantly increases angiogenic behaviour and induces overexpression of ECM components, including collagen I and IV, particularly at day 7 post-treatment. Next, we used the chorioallantoic membrane (CAM) assay. This assay utilises a blood vessel-rich membrane from a developing chick embryo, allowing for the evaluation of scaffold integration and blood vessel growth. As a result, miR-31 gene-activated scaffolds exhibited strong biocompatibility, promoting biomaterial-membrane integration and blood vessel formation. In addition, cells showed pronounced infiltration within the miRNA-activated scaffold compared to non-functionalised ones. Finally, miR31-activated scaffolds displayed enhanced neuroregenerative effects, as evidenced by improved neurite outgrowth from chick embryo dorsal root ganglia *ex vivo* models. This suggests a relatively unexplored role of gene therapy in the context of wound healing.

These findings draw attention to the potential of miR-31 gene-activated scaffolds as a promising strategy for treating non chronic wounds. Future work will focus on *in vivo* validation in diabetic wound models to maximise repair outcomes. This clinically relevant approach represents an essential step toward minimally invasive, personalised therapies in regenerative medicine.

Keywords: Tissue Engineering, Gene delivery, Wound healing, Nanoparticles, Biomaterials

Research Area: Tissue Engineering, Gene delivery

Funding: Higher Education Authority on behalf of the Department of Further and Higher Education, Research Innovation and Science through the North South Research Programme.



APPLICATION OF PCL/GEL FIBER FUNCTIONALIZED WITH LL37 LOADED CSNP IN A 2D SCRATCH MODEL FOR SUPPORTING WOUND HEALING

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This study aims to develop and evaluate a chitosan nanoparticle (CSNP) based antimicrobial delivery system encapsulating LL37 peptide to promote wound healing through a fiber mat platform. CSNPs were synthesized via ionic gelation and loaded with two concentrations of LL37 peptide (7.5 µg/mL and 15 µg/mL). The physicochemical characteristics of the nanoparticles including average particle size, polydispersity index (PDI), and zeta potential (ZP) were determined. The optimal CSNPs were then embedded into a polycaprolactone/gelatin (PCL/Gel) fiber matrix produced via electrospinning using HFIP as a solvent. The resulting mats exhibited a uniform fiber morphology with an average diameter of 510.3±227.62 nm, as confirmed by FE-SEM analysis. To evaluate the in vitro wound healing effect, a scratch assay was performed using 2D keratinocyte cultures. Experimental groups included: control (only cells), blank CSNP-PCL/Gel mats, 7.5-LL37-CSNP-PCL/Gel mats, and 15-LL37-CSNP-PCL/Gel mats. A consistent scratch wound was introduced after 24 hours of incubation, and fiber mats were placed over the wound area. Scratch closure was observed microscopically on days 1, 7, and 14, and closure percentages were quantified using ImageJ software. The results demonstrated that LL37-loaded mats significantly enhanced wound closure compared to control and blank groups, particularly during early healing stages. On day 1, the 7.5-LL37-CSNP-PCL/Gel group achieved over 65% closure, while the 15-LL37-CSNP-PCL/Gel group yielded slightly higher values. By day 7, all groups showed >90% closure, yet LL37 groups maintained superior performance through day 14. These findings align with LL37's known effects on epithelial migration, angiogenesis, and inflammation modulation. This study uniquely demonstrates that LL37-loaded CSNPs embedded in PCL/Gel fibers provide both controlled release and localized delivery, enhancing peptide stability and therapeutic efficacy. The system supports early, and late-stage wound repair and represents a promising approach for advanced wound dressing design. The authors acknowledge the support of REGENEU project (no:101079123) funded by Horizon Europe.

Keywords: Chitosan Nanoparticles, LL37, Electrospinning, Wound Healing, Controlled Release

Research Area: Biomaterials For Wound Healing, Nanotechnology And Nanomaterials In Wound Healing



DEVELOPMENT OF A BIOMIMETIC MULTI-LAYERED FUNCTIONALISED ANTIMICROBIAL BIOMATERIAL SCAFFOLD FOR HEALING OF COMPLEX WOUNDS

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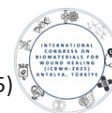
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Chronic wounds fail to progress through normal healing stages, significantly impacting patients' quality of life and place heavy burdens on healthcare systems. Impaired angiogenesis and nerve repair are key contributors, leaving wounds open and vulnerable to infection. Biomaterials, particularly collagen-glycosaminoglycan (collagen-GAG) scaffolds, show promise in promoting wound healing but traditionally support cellular infiltration without addressing underlying pathologies. Functionalisation incorporating additional ECM molecules targeting impaired angiogenesis and neurogenesis could enhance healing. This project aimed to develop a biomimetic bi-layered scaffold mimicking native skin, combining an antimicrobial epidermal collagen/chitosan film with a pro-angiogenic, neurogenic functionalised dermal collagen-GAG scaffold to promote complex wound healing.

The bi-layered scaffold was created by lyophilising a type I collagen/chondroitin-6-sulfate slurry onto a collagen/chitosan film. The film's composition and scaffold crosslinking (non-crosslinked, dehydrothermal, carbodiimide EDAC) were optimised to enhance mechanical properties, interlayer adhesion, antimicrobial properties, and vascular cell proliferation. For *in vivo* validation of the bi-layered scaffold, a healthy adult rat model was used (10-12 month old, Sprague Dawley rats, 10mm-splinted wounds). The dermal scaffold was then functionalised *in vitro* with fibronectin, collagen



IV, or laminin-1 to improve angiogenic/neurogenic potential. Antimicrobial efficacy was tested against *Staphylococcus aureus*, while re-epithelialisation was assessed using human epidermal keratinocytes (HaCaTs). Scaffold vascularisation potential was evaluated by culturing human umbilical vein endothelial cells (HUVECs) and human dermal fibroblasts (HDFs). Neurogenic properties were assessed using mouse motor neuron-like cells (NSC-34s) and injured *ex vivo* dorsal root ganglia (DRGs).

EDAC crosslinking improved scaffold mechanical strength and interlayer adhesion. All collagen/chitosan films exhibited antimicrobial activity and supported keratinocyte metabolic activity, regardless of crosslinking method. Crosslinked scaffolds demonstrated enhanced vascular cell proliferation. *In vivo*, the bi-layered scaffold supported angiogenesis and re-epithelialisation. Laminin-1 functionalisation of the collagen-GAG layer significantly increased vascular endothelial growth factor (VEGF) production by HDFs over seven days. HUVECs showed enhanced coverage and tubular structure formation on these scaffolds. Additionally, all ECM-functionalised scaffolds supported axon outgrowth from injured DRGs.

A functionalised, antimicrobial, biomimetic scaffold was successfully developed for chronic wound healing. The collagen/chitosan film showed potential as an antimicrobial protective epidermal layer, inhibiting *S. aureus* growth and supported keratinocyte proliferation, while the scaffold successfully supported *in vivo* healing. Laminin-1 enhanced angiogenic and neurogenic potential, promoting angiogenic growth factor secretion, vascular structure formation, and axon regrowth. These results highlight the bi-layered scaffold's potential as a comprehensive biomaterial solution for chronic wound repair.

Keywords: Scaffolds, Angiogenesis, Nerve Repair, Antimicrobial

Research Area: Biomaterials For Wound Healing, Tissue Engineering And Regenerative Medicine

Funding: Research Ireland, AMBER Centre, Grant No.: SFI/12/RC/2278_P2.



A MULTI-FACETED MIRNA-ACTIVATED SCAFFOLD AS AN IMMUNO MODULATORY PLATFORM FOR CHRONIC WOUND HEALING APPLICATIONS

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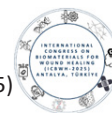
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The wound healing inflammatory response is facilitated by M1 polarised macrophages, whereas the subsequent inflammation resolution and promotion of vascularisation are mediated by M2 macrophages. However, this balance is genetically dysregulated in chronic wounds, perpetuating the inflammatory state, reducing vascularisation, and impairing nerve regeneration. While collagen-based (CG) scaffolds are promising for wound repair, additional functionalisation might overcome this state and promote healing. MicroRNAs (miRNAs) regulate gene expression in many biological processes, including inflammation, angiogenesis, and neurogenesis in wound heal-



ing. MiRNA-155 inhibition has shown anti-inflammatory, angiogenic, and neurogenic outcomes *in vivo*. Thus, this study focused on functionalising this CG scaffold with microRNA-155 inhibitor nanoparticles as a reparative platform in chronic wounds by promoting anti-inflammatory, pro-angiogenic, and pro-neurogenic processes.

Nanoparticles were complexed with miRNA-155 inhibitor and the non-viral GAG-binding enhanced transduction (GET) peptide before soak-loading onto lyophilized CG scaffolds to form miRNA-activated scaffolds. The effect of miRNA delivery on macrophage polarisation from non-polarised (M0) and pro-inflammatory (M1) phenotypes was assessed using THP-1 monocytic cells. Expression of key inflammatory and angiogenic markers, including TNF- α and VEGF, was examined by PCR and ELISA. Cell phenotype was assessed by microscopy through pro-inflammatory (CD80) and anti-inflammatory (CD206) marker staining. Macrophage polarisation effect on angiogenesis was evaluated through macrophage-endothelial cell paracrine signalling interactions in migration and tube formation assays. Additionally, pro-neurogenic outcomes were assessed in an *ex vivo* model of axonal injury with dorsal root ganglia (DRG).

MiRNA-155 inhibitor-activated scaffolds increased the expression of anti-inflammatory and angiogenic markers from M0 and M1 macrophages over 7 days. Reduced circularity (associated with anti-inflammatory phenotypes), increased CD206+ expression, and reduced CD80+ macrophage numbers were observed on M0 and M1-seeded miRNA-activated scaffolds. Endothelial cell migration and vascular-like structure organisation was enhanced when exposed to the conditioned media from miRNA-activated scaffolds. Furthermore, average neurite extension was increased on DRGs seeded on miRNA-activated scaffolds.

In this work, we show that miRNA-155 inhibitor-activated scaffolds enhance an anti-inflammatory M2 phenotype confirmed through gene expression and surface markers. We show that the secretome from these macrophage-seeded scaffolds enhance pro-angiogenic processes in endothelial cells, essential for the vascularisation of chronic wounds. We finally confirm that the scaffolds support neurogenic processes that further benefit the process of wound healing. Taken together, this data indicates that miRNA-activated scaffolds enable pro-regenerative processes key for the resolution of chronic wounds.

Keywords: Gene Delivery, RNA interference, Collagen, Chronic Wounds, Inflammation

Research Area: Biomaterials for Wound Healing, Tissue Engineering and Regenerative Medicine

Acknowledgements: Research Ireland / Advanced Materials and BioEngineering (AMBER) Research Grant: SFI/12/RC/2278_P2



RENACER® FIBER FLEECES FOR CHRONIC WOUND REGENERATION

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Fibrous materials are used extensively in biomedical applications. Besides nano- and sub- μ -fiber scaffolds that are often used to mimic the extracellular matrix, also μ -fibrous non-wovens, wovens, or knitted fabrics are established, especially in wound healing applications.

Also, in the field of biotechnology, environmental aspects are also becoming increasingly important in the development of new biomaterial solutions. Here, naturally occurring organic polymers like proteins or polysaccharides are mostly used to replace non-degrading, synthetic polymers. With the development of RENACER® fibers, the authors are pursuing a strategy using a resorbable, inorganic, material that is 100% dissolved into natural and bioactive ortho-silicic acid (oSA). In parallel, the synthesis of the material can be carried out using green solvents.

In recent years, research has also proven bioactive effects of oSA in tissue regeneration. Thus, next to the green synthesis and microplastic-free biodegradation, the bioactive effect on regeneration on wounded skin will be presented.

RENACER® fibers were obtained via dry spinning techniques using bi-ethanol. In detail, electrospinning techniques were used in the fabrication of sub- μ -fibers and pressure spinning techniques for the production of μ -fibers. All of the resulting fibers showed an amorphous structure (powder x-ray diffraction, XRD) and adjustable fiber diameters and mesh sizes, characterized by scanning electron microscopy (SEM) and optical coherence tomography (OCT). Full fiber dissolution was proven, using a USP4-dissolution device and oSA was identified subsequently as the degradation product based on DIN ISO 38405-21 for detection of dissolved silicic acids. In-vitro (geno)toxicity assessment of the fibrous materials by cell counts, WST-1, lactate dehydrogenase release and comet assays showed neither (geno)toxic effects in direct material contact nor when testing oSA



saturated cell culture media.

3D Cell culture experiments with human primary cells have shown regenerating properties – especially in boosting the extracellular matrix production in skin regeneration. oSA-releasing wound patches applied to wounded 3D full skin models have proven a regeneration of the epidermal and dermal layer.

The RENACER® fiber platform not only opens up the possibility of generating eco-friendly materials of the future, but at the same time activates the regenerative forces of the body directly by the release of oSA.

Keywords: Wound Care, Fleece Technology, Fibers, Bioresorbable, Inorganic

Research Area: Wound Management



SOL-GEL DERIVED RENACER[®] FIBER FLEECES AS A FULLY RESORBABLE DRUG DELIVERY SYSTEM FOR LOCAL POST-OPERATIVE GLIOBLASTOMA TREATMENT

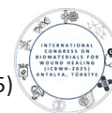
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Background: The most aggressive brain tumor of adults (glioblastoma-GBM) poses significant treatment challenges due to its invasive growth and high recurrence rates arising from the resection walls after GBM extirpation. Current post-operative systemic chemotherapy is inadequate due to drug limitations for oral administration and dose restrictions, and it remains associated with a low life expectancy. This study aims to reduce the recurrence rate by replacing systemic with local chemotherapy by applying a biocompatible and degradable, drug-loaded silica gel fleece (RENACER[®]) to the resection walls.

Material and Methods: Pressure-spun μ -fibers were combined with electrospun sub- μ -fibers to form a nonwoven fabric and loaded with different active pharmaceutical ingredients (APIs). A hydrophilic API was incorporated into the μ -fibers, while a water-insoluble API was embedded in the sub- μ -fibers. Both fiber systems were produced using a sol-gel process from the liquid sol-gel precursor tetraethoxysilane. The fiber systems were characterized by microscopy, gravimetric degradation in PBS, NMR, flexibility, and *in vitro* cytotoxicity testing based on DIN EN ISO 10993-5.



Feasibility and applicability were subsequently tested using a dynamic adhesion test *ex vivo* on porcine brain tissue and *in vitro* efficacy on GBM cell lines was verified in real-time using the xCELLigence system. Drug release was quantified utilizing HPLC.

Results: The structure-property relationships of the unloaded fibers were optimized by synthesis parameters, including flexibility (0 to 24 mm diameter at break), degradation time in Si(OH)₄ (7 - 35 days), and the cross-linking and silica binding structures observed in the ²⁹Si spectrum. Both the unloaded μ -fibers ($46.1 \pm 5.1 \mu\text{m}$) and sub- μ -fibers ($0.9 \pm 0.2 \mu\text{m}$) demonstrated no cytotoxicity in direct and indirect contact assays, with cell viability exceeding 80 %. In drug-loaded fiber systems, both APIs were completely released from the individual fiber systems and their combination, and their effectiveness against GBM cells was demonstrated through significant reductions in cell count and viability in real-time monitoring. Additionally, a sufficient affinity of the fleeces to *ex vivo* porcine brain tissue was proven.

Discussion and Conclusion: Our RENACER® drug delivery system for post-operative GBM therapy not only combines tissue adhesive properties and absorption in non-toxic orthosilicic acid but also enables the dual release of two different drugs. Following *in vitro* efficacy testing and general tissue applicability, an *in vivo* study is now planned.

Keywords: Brain Tumor, Local Chemotherapy, Fibrous Platform Technology, Drug Delivery, Inorganic

Research Area: Personalized Medicine and Patient-Specific Treatments



PRELIMINARY INVESTIGATION OF MYCOSPORINE-LIKE AMINO ACIDS FROM ANTARCTIC *KLEBSORMIDIUM* SP. ASYA17 FOR ADVANCED WOUND HEALING APPLICATIONS

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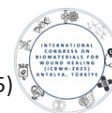
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Mycosporine-like amino acids (MAAs) are small, water-soluble secondary metabolites produced by various microalgae and cyanobacteria, primarily recognized for their UV-absorbing and antioxidant properties. While their application has been extensively studied in photoprotection and cosmeceuticals, their potential use in wound healing remains largely unexplored. This study aims to evaluate the wound healing properties of MAAs isolated from an Antarctic strain of *Klebsormidium* sp. ASYA17 is an extremophilic microalga adapted to harsh environmental conditions, including intense UV radiation, desiccation, and sub-zero temperatures. MAAs were extracted from freeze-dried biomass using methanolic extraction followed by fractionation with semi-preparative HPLC. The purified compounds were characterized using LC-MS/MS and ¹HNMR, confirming the presence of several UV-absorbing MAA structures, including shinorine and porphyra-334 analogs. Antioxidant capacity was evaluated using DPPH and ABTS radical scavenging assays, demonstrating vigorous radical quenching activity comparable to that of standard antioxidants, such as ascorbic acid.

To assess wound healing relevance, in vitro scratch assays were performed on HaCaT keratinocyte monolayers. Treatment with MAA-enriched fractions resulted in a statistically significant increase in cell migration and proliferation rates compared to untreated controls ($p < 0.01$), indicating a proregenerative effect. No cytotoxicity was observed in MTT assays across a wide concentration range (5–100 µg/mL). These results suggest that MAAs not only mitigate oxidative stress but may also promote epidermal regeneration. Given their dual function as antioxidants and potential cellular stimulants, MAAs from *Klebsormidium* sp. ASYA17 represents a promising class of natural biocompounds for advanced wound dressing formulations. Future work will focus on incorporating them into biopolymeric hydrogel matrices, stability testing, and in vivo wound closure models in animal systems to validate their clinical relevance.



This study expands the functional scope of MAAs beyond photoprotection and proposes their use in regenerative medicine, especially for chronic or UV-compromised wounds.

Keywords: Mycosporine-Like Amino Acids, *Klebsormidium* Sp., Wound Healing, Antioxidant, Skin Regeneration

Research Area: Bioactive and Natural Biomaterials, Biomaterials for Wound Healing



DEVELOPMENT OF A MIRNA-29B-ACTIVATED SCAFFOLD FOR THE INHIBITION OF FIBROSIS DURING WOUND HEALING

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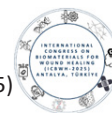
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Wound healing involves coordinated responses ending with matrix remodelling, where dermal fibroblasts migrate, proliferate, and reconstruct extracellular matrix (ECM) to restore skin integrity. However, fibroblast overactivation, associated with excessive collagen type I (Col I) deposition, can escalate to a fibrotic response, forming pathological scars. MicroRNAs (miRNAs) regulate gene expression in most biological processes, including wound remodelling. Specifically, miRNA-29b regulates the overproduction of Col I in dermal fibroblasts. Collagen-GAG (CG) scaffolds have been shown to promote tissue repair and remodelling, while facilitating the sustained release of bioactive factors. Thus, we propose the gene-activation of CG scaffolds with miRNA-29b nanoparticles to direct anti-fibrotic gene expression during remodelling, reducing the likelihood of scarring.

MiRNA-29b nanoparticles were synthesised *via* electrostatic complexation of miRNA-29b mimic and the non-viral GAG-binding enhanced transduction (GET) pep-



tide. Human dermal fibroblasts (hDF) were treated with TGF- β 1 to induce a fibrotic phenotype before miRNA-29b treatment. Analysis of Col I gene expression and deposition was assessed through PCR and histology, while expression of the pro-fibrotic marker α -SMA was characterised by fluorescent microscopy. Assessment of functional anti-fibrotic outcomes were also carried out *via* a collagen gel contraction assay. Consequently, scaffolds were soak-loaded with miRNA-29b nanoparticles (CG-29b) and seeded with TGF- β 1-treated hDFs. Analysis of gene expression, biocompatibility, and α -SMA staining were carried out as before to assess the reduction in fibrotic response.

miRNA-29b mimic-treated hDFs displayed reduced Col I expression and deposition without compromising cell viability. Similar results were obtained when quantifying α -SMA expression through fluorescent microscopy. Moreover, gel contraction was significantly reduced in miRNA-29b-treated hDFs. Crucially, Col I and α -SMA expression were also reduced in hDFs seeded on CG-29b scaffolds without inflicting a detrimental effect on the cells as confirmed by cell viability assay, gene expression, and image analysis.

Here, we demonstrate the formulation of anti-fibrotic miRNA-29b nanoparticles capable of inhibiting the overexpression of pro-fibrotic markers. Furthermore, incorporation of this nanoparticle system within the CG scaffolds resulted in the formation of a promising platform for the control of fibrosis during wound healing. This combination also mitigated the overexpression of pro-fibrotic markers in TGF- β 1-treated hDFs while maintaining cell viability. Taken together, this data indicates that anti-fibrotic responses can be elicited in hDFs through the delivery of miRNA-29b mimic nanoparticles, making this platform a promising alternative for the treatment of fibrotic pathologies such as keloids or hypertrophic scars.

Keywords: Gene Delivery, MicroRNA, Collagen, Fibrosis, Scaffolds

Research Area: Biomaterials for Wound Healing, Tissue Engineering and Regenerative Medicine

Acknowledgements: Research Ireland / Advanced Materials and BioEngineering (AMBER) Research Grant: SFI/12/RC/2278_P2



LIPID BASED MULTIFUNCTIONAL DRUG DELIVERY SYSTEMS FOR IMPLANT INFECTIONS

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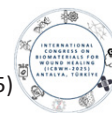
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Combating infections and effective wound management is crucial to prevent complications and ensure the recovery in clinical settings. In orthopedics, implant infections are widespread due to the comfortable surface of implant, which facilitates bacteria adhesion and biofilm formation. However, infections significantly impair the healing process and delay the recovery period. These infections are persistent infections due to the capability of bacteria to adhere and form biofilms on implant surfaces. Systemic antibiotic prophylaxis and revision surgery are the major treatment for preventing orthopedic implant infections. However, these treatments often failed due to inadequate local concentrations of antibiotic levels around implants and risk of re-infection after revision surgery. The restrictions have driven research into developing alternative strategies for managing orthopedic implant infections and improving healing process. Antibacterial coatings based on amphiphilic lipid self-assembly are promising, owing to their biocompatibility and capacity to load various drug components. Here, we developed Monoolein (MO) based drug delivery depot loaded with Vancomycin and Tobramycin as an antibacterial local drug delivery system for orthopedic implants. MO is a food-grade lipid that forms liquid crystalline structures, which contains water channels separated by a lipid bilayer, when hydrated with excess water. This internal structure enables the integration of both hydrophilic and hydrophobic drugs in a single carrier as a multifunctional drug delivery system. The produced drug depots were coated on Titanium substrates and tested against two *Staphylococcus aureus* strains (SH1000 and ATCC 29213). To obtain the most efficient system; antibiotic concentration (50 to 1000 µg/ml), antibiotic loading method (pre-loading vs hydration loading) and surface coating method (spin coating vs dip



coating) were optimized in this study. Results demonstrated that MO liquid crystalline drug depots loaded with antibiotics efficiently inhibiting the bacterial growth. Our findings demonstrated that these liquid crystalline phases may offer a promising strategy of preventing infections in both open and closed wounds associated with orthopedic surgery.

Keywords: antibacterial coating, drug delivery, infections, self-assembly

Research Area: Antibacterial coatings, Drug delivery.

Acknowledgements: The study is funded by the European Union (Grant No: 101107704).



SINAPIC ACID STIMULATES KERATINOCYTE-DRIVEN WOUND HEALING VIA REGULATION OF KEY MIGRATORY AND ADHESION RELATED GENES

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Sinapic acid (SA), a naturally occurring hydroxycinnamic acid with well-documented antioxidant and anti-inflammatory properties, has emerged as a promising candidate in regenerative medicine due to its biological activity in various tissue systems. Despite its therapeutic potential, the keratinocyte-derived mechanisms of SA on wound healing remain poorly understood at the molecular level. The present study aimed to evaluate the pro-healing effects of SA on human keratinocyte behavior using the HaCaT cell line as an *in vitro* model. To determine the appropriate SA treatment dose, cell viability assays were performed for time (24 and 48 h) and dose (0–2500 μ M) manner. Based on cytotoxicity results, 10 μ M SA was selected for downstream analyses, as it was found to be non-toxic and biologically active. Wound healing capacity was assessed via scratch assay and gene expression profiling was conducted using quantitative real-time PCR (qRT-PCR) for key regulators involved in cell adhesion, migration, and epithelial-mesenchymal transition (EMT) including CTNNA1 (α E-catenin), CTNNB1 (β -catenin), ILK (Integrin-linked kinase), OCLN (Occludin), MMP7 (Matrix Metalloproteinase 7), MMP9 (Matrix Metalloproteinase 9), ZEB1 (Zinc Finger E-Box Binding Homeobox 1), and ZEB2 (Zinc Finger E-Box Binding Homeobox 2).

SA treatment has been shown to significantly stimulate keratinocyte migration at >55% ratio of wound closure observed at 48 h, indicating a clear stimulatory effect on re-epithelialization. Gene expression analysis revealed that SA induced a marked upregulation of CTNNA1, CTNNB1, OCLN, MMP7, and ZEB2, suggesting enhanced cell-cell adhesion, tight junction stabilization, and matrix remodeling. These molecular alterations are likely to contribute to improved cellular coordination during wound closure. In contrast, ILK, MMP9, and ZEB1 displayed non-significant changes.



These findings provide novel insights into the wound healing potential of sinapic acid at the cellular and molecular level. By promoting keratinocyte migration and upregulating key genes involved in adhesion and extracellular matrix regulation, SA may serve as a valuable bioactive agent for the development of advanced wound healing therapies. Further investigations, including *in vivo* studies and formulation-based applications, are warranted to validate its clinical utility in skin tissue repair and regeneration.

Keywords: Keratinocyte, Sinapic acid, Wound healing

Research Area: Mechanisms of Action: Cellular and Molecular Aspects, Bioactive and Natural Biomaterials



DEVELOPMENT OF A NEXT GENERATION ELECTROCONDUCTIVE BIOMATERIAL FOR PERIPHERAL NERVE REGENERATION

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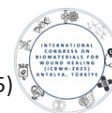
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Current biomaterial-based strategies for peripheral nerve regeneration are insufficient for repairing injuries over 3 cm. This is often due to the lack of support for electrical impulses to assist the natural nerve healing process. Patients are then left with a significant loss of nerve function. Prior work in our labs using pristine graphene to electrically stimulate central nervous tissue in 2D showed promise in accelerating its regenerative capacity. This study now aims to build upon this work to utilize pristine graphene for peripheral nerve regeneration by engineering a clinically relevant, collagen-based nerve guidance conduit (NGC) with electroconductive properties. The specific aims were to 1) determine the optimal concentration of graphene to integrate in the collagen-based NGC, 2) evaluate the optimal method of graphene incorporation for the 3D NGC, and 3) evaluate the enhanced 3D NGC biocompatibility *ex vivo* with applied electrical stimulation.

10-50 vol% graphene/collagen 2D films were investigated for suitable conductivity, electrochemical measurements, and initial biocompatibility using S42 Schwann cells. The highest performing graphene concentration was determined and incorporated into the core scaffold of the NGC and characterised using a variety of criteria, such as reproducibility and degradation rate. Finally, 3D biocompatibility and gene



expression of the optimized collagen/graphene NGC was assessed using explanted adult rat dorsal root ganglia (DRGs) with applied electrical stimulation.

30 vol% graphene/collagen films exhibited optimal conductivity, low charge transfer resistance, and high charge storage capacity, which are ideal for implanted electroconductive biomaterials. The films demonstrated suitable biocompatibility with no significant loss in metabolic activity or proliferation with Schwann cells compared to collagen-glycosaminoglycan films. An NGC containing 30 vol% graphene homogeneously distributed throughout the scaffold was selected for further analysis due to its high reproducibility and graphene retainment. 3D collagen/graphene scaffolds exhibited excellent biocompatibility with Schwann cells and DRGs compared to the collagen-glycosaminoglycan control. In addition, when seeded on these electroconductive scaffolds, neuroprotective and regenerative genes, such as nerve growth factor and beta III tubulin, were upregulated in DRGs following applied electrical stimulation. These results demonstrated that an NGC with 30 vol% graphene homogeneously integrated throughout the core scaffold is a promising platform for peripheral nerve regeneration and may advance the treatment of severe peripheral nerve injury, providing much needed hope to patients.

Funding provided by the Fulbright Commission, RCSI, Taighde Éireann—Research Ireland, Advanced Materials and BioEngineering Research (AMBER) Centre, Integra LifeSciences.

Keywords: Biomaterial, Electroconductive, Nerve Guidance Conduit, Peripheral Nerve Injury, Graphene

Research Area: Biocompatible Materials, Scaffold Fabrication



HEMOSTATIC AND WOUND HEALING NON-ISOCYANATE POLYHYDROXYURETHANES (NIPHUS) DRESSINGS

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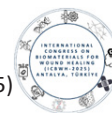
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The prevalence of chronic and non-healing wounds around the world is increasing, therefore, the use of biomaterials as wound dressings remains necessary. In this field, polyurethanes have gained importance due to their high biocompatible properties and versatility. However, only 0.1% of the polyurethanes come from renewable sources. Thus, the substitution of petrochemical biomaterials is crucial for sustainability, and polyhydroxyurethanes are a promising alternative due to their synthesis route. Based on this, polyhydroxyurethanes were obtained from soybean oil (epoxidation and carbonation) and two diamines: 1,4 butadiamine and 1,3-cyclohexanobis(methylamine). The NIPHUs obtained were evaluated for their antibacterial activity and *in vitro* biocompatibility with human dermal fibroblasts (HDFa) and human keratinocyte cells (HaCaT); and *in vivo* the hemostatic capability in mouse tail amputation model and skin irritation and wound healing assays. Results showed new formulations of NIPHUs with antibacterial activity against *Pseudomona aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, and cell lines viability above 70%. The amount of blood loss was significantly lower when the NIPHU 50/50 was applied than the wounds without any treatment. On day 14 all the wounds were closed.



The healed zone in animals treated with any NIPHUs was smaller and thin, while an elongated and thick scab was still observed in the healed skin of the control groups (wound without any treatment and wounds treated with a commercial patch). In correspondence with the macroscopic findings, histologically the healing zone (width of the skin) in each group was different, compared to normal skin. NIPHU 100/0 were approximately 1.07 ± 0.06 mm, NIPHU 67/33 0.94 ± 0.31 mm and NIPHU 50/50 0.27 ± 0.29 mm. For control treatments, the wound without any treatment, 0.70 ± 0.17 mm, and in the wounds treated with a commercial patch, 0.86 ± 0.36 mm. In all treatments the healing zone was epithelialized and below it, was occupied by residual granulation tissue. In NIPHUS 100/0, NIPHU 67/33 and controls no hypodermis was identified, but for the case of NIPHU 50/50, below this epithelium, several regions can be recognized, connective tissue with active fibroblasts was observed and had some young adipocytes, the healing edge had young pilosebaceous units, and the muscle layer is complete. In conclusion, NIPHU 50/50 accelerates the hemostasis phase and allows a remodeling phase on the wounds in 14 days, being a suitable dressing for wound healing without cytotoxic effects. Thus, NIPHU 50/50 is proposed as a candidate for wound dressings.

Keywords: Non-Isocyanate Polyhydroxyurethane, Biomaterial, Hemostatic, Healing Wound, Wound Dressing

Research Area: Biomaterials for Wound Healing, Biocompatibility and Safety of Biomaterials



DEVELOPMENT AND CHARACTERIZATION OF PLA/GELATIN-BASED BIOCOMPATIBLE NANOSPONGES ENRICHED WITH BIOACTIVE AGENTS

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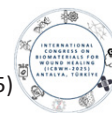
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Biomedical materials encompass a wide range of products, including biomedical devices such as biomaterials, biosensors, blood circulation tubes, hemodialysis systems, surgical sutures, plates, bone grafts, tendons, heart valves, lenses, dental materials, and artificial organs such as heart, kidney, liver, pancreas, lungs, skin, subcutaneous implants, particles, and wound dressings, as well as implantable materials. Depending on their structure, biomedical materials can be either natural or synthetic and are used to replace, treat, or support the proper function of tissues or organs. There is a wide variety of biomedical materials, including silicone hydrogels, biostable polyurethanes, polymer stabilization technologies, absorbable materials, hydrophilic and anti-biofouling coatings, dental materials, natural fibers, and single-use point-of-care products. The new generation of biomaterials is expected to focus on self-healing biomaterials for tissue engineering and regenerative medicine, dental biomaterials, natural substances for biomaterial synthesis, biocompatible coatings for bioactive devices, nano biosensors, and related innovations.

This study presents the development of a novel bioactive and biodegradable nanosponge system fabricated via electrospinning. The sponge was designed using polylactic acid (PLA) and bovine gelatin dissolved in HFIP, in formulations containing tranexamic acid (TXA), antimicrobial peptide LL-37, and coagulation factor IX, as well as control formulations. Nanofiber mats were produced at 20 kV and 2 mL/h, homog-



enized and lyophilized to yield porous nano sponge structures. Thermal crosslinking was applied for enhanced structural integrity.

All the samples were characterized by FTIR spectroscopy, wettability, antibacterial and biocompatibility tests. FTIR analysis confirmed the successful incorporation of TXA, contact angle measurements revealed a transition from hydrophobic to hydrophilic surface properties after TXA addition, indicating enhanced fluid interaction. TXA/LL-37/Factor IX-loaded extracts increased fibroblast proliferation by up to 162%, whereas placebo samples exhibited significantly lower values. This indicates not only biocompatibility but also a stimulatory microenvironment for cell proliferation. Antibacterial effect tests revealed that TXA/LL-37/Factor IX-loaded nano sponges inhibited *E. coli* and *S. aureus* viability. Confocal laser scanning microscopy analysis showed robust keratinocyte adhesion to TXA/LL-37/Factor IX-loaded surfaces, with intact cytoskeletons and nuclei. Z-stack images confirmed homogeneous and deep cell infiltration into the sponge matrix, supporting the material's suitability for wound contact. Overall, the multifunctional nano sponges demonstrate antimicrobial and biocompatible features, offering promising potential for wound healing applications.

Keywords: Biocompatibility, Electrospinning, Nanosponge, Tranexamic Acid, Confocal Laser Scanning Microscopy

Research Area: Biomaterials for Wound Healing, Advanced Drug Delivery Systems



ENHANCING BROWNING OF 3T3-L1 CELLS USING LIPOSOMAL NARINGENIN AND BERBERINE

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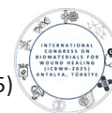
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Phytochemicals such as naringenin and berberine have attracted attention for their potential roles in metabolic modulation and tissue regeneration. However, their poor aqueous solubility, limited stability, and low bioavailability have posed significant challenges for clinical application. In this study, we aimed to address these limitations through a nanotechnology-based approach by developing liposomal formulations that could enhance the delivery, bioactivity, and sustained release of these compounds in a 3T3-L1 preadipocyte model. Liposomal carriers were prepared using the thin-film hydration method and characterized for particle size (berberine: 194.9 nm; naringenin: 151.9 nm), polydispersity index, zeta potential (berberine: -28.8 mV; naringenin: -24.4 mV), encapsulation efficiency (berberine: 87.8%; naringenin: 92.4%), and stability under 4°C storage. These formulations were designed to improve solubility, enhance cellular uptake, and support sustained release for integration into future wound healing biomaterials. Biological studies were conducted by applying both free and liposomal forms of naringenin and berberine during differentiation and maturation stages of 3T3-L1 adipogenesis. Browning markers (UCP1, PRDM16, PGC1- α , CIDEA) and adipogenic markers (PPAR γ , C/EBP β , FABP4) were quantified via RT-PCR and ELISA. Triglyceride accumulation was measured, and cytotoxicity was assessed using MTT assays. The results showed that liposomal berberine significantly increased UCP1 expression during differentiation ($p = 0.002$) and decreased intra-



cellular triglyceride levels ($p < 0.05$). Liposomal naringenin significantly enhanced UCP1 expression during maturation ($p = 0.035$), although no reduction in triglyceride content was observed. At the protein level, liposomal naringenin increased PGC1- α concentration ($p = 0.015$). All liposomal formulations showed acceptable short-term biocompatibility (no significant cytotoxicity at 24 h), while prolonged exposure (48 h) at higher concentrations resulted in reduced cell viability ($p < 0.01$, dose-dependent). Liposomes retained structural integrity and demonstrated controlled release behavior, confirming formulation stability. This study supports liposomal encapsulation as a promising delivery platform for enhancing the metabolic effects of phytochemicals. Improved browning activity, coupled with controlled release and stability, highlights the potential of naringenin and berberine-loaded liposomes for future applications in obesity management and biomaterial-based wound healing systems. Further in vivo studies are warranted to confirm long-term therapeutic efficacy and systemic safety. This work was supported by TUBITAK 1001-The Scientific and Technological Research Projects Funding Program [project no. 220S74].

Keywords: Liposomal delivery, Browning, Berberine, Naringenin, Adipocyte

Research Area: Nanotechnology and Controlled Drug Delivery, Biomaterials for Wound Healing



DEVELOPMENT AND PRE-VALIDATION OF A NEW IN VITRO SKIN IRRITATION TEST KIT FOR SAFETY ASSESSMENT

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Skin irritation is one of the main side effects evaluated in the safety assessment of cosmetics, personal care products, and medical devices. With the ban on animal testing in the European Union and other regions, the demand for reliable, ethical, and reproducible alternative methods has increased significantly. There is no company in Türkiye that manufactures this test kit, which has examples around the world. The main objective of this study is to create a cost-effective, reproducible, and user-friendly test system based on reconstituted human epidermis (RhE) models, designed specifically for the cosmetics and medical device industries and compliant with OECD Test Guideline 439, and to present the pre-validation of this kit.

The test kit contains pre-prepared RhE tissues, application accessories, reagents for viability assessment (MTT test), and standardized protocols. During development, human keratinocytes were cultured in a 3D air-liquid interface to mimic the physiological structure of the epidermis. Histological sections were taken for RhE characterization, and the epidermal layers were identified using hematoxylin and eosin staining. Immunohistochemical staining for cytokeratin 10, pan-cytokeratin, and Ki67 was performed. Cell viability was assessed using a cell viability test following exposure to various chemicals in accordance with OECD Guideline 439, and results were interpreted based on a 50% cell viability threshold.

Histological analysis has shown that the model expresses the markers in question by confirming the integrity of the stratum corneum and multilayered keratinocyte structure. Preliminary validation results of the kit have shown high intra- and inter-batch reproducibility, with sensitivity and specificity values above 80%, in accordance with OECD acceptance criteria.



In conclusion, the developed in vitro skin irritation test kit offers a local, ethical, and practical solution for safety testing. Further validation and regulatory compliance studies are ongoing to support its widespread adoption and final regulatory acceptance in international markets.

Keywords: Biosafety, In Vitro Skin Irritation, Reconstructed Human Epidermis, OECD TG 439

Research Area: Biocompatibility and Safety of Biomaterials



COMPARATIVE EVALUATION OF CHEMICALLY AND GREEN SYNTHESIZED GOLD NANOPARTICLES: ANTIOXIDANT PROPERTIES AND WOUND-HEALING EFFECTS

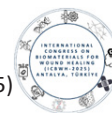
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Gold (Au) nanoparticles have gained considerable attention in biomedical research due to their excellent biocompatibility, inherent antioxidant properties, and potential to promote tissue regeneration in wound-healing applications. This study compares Au nanoparticles synthesized via a chemical reduction method using trisodium citrate with those produced through a green synthesis approach using a tetra-aqueous extract of *Cotinus coggygia*, a medicinal plant known for its wound-healing properties. Chemically and biogenically synthesized Au nanoparticles were characterized using advanced techniques including STEM, UV-Vis spectroscopy, XRD, FTIR, and zeta potential analysis to elucidate and confirm their physicochemical properties. Both types of nanoparticles were evaluated for their antioxidant activity and wound-healing potential. Antioxidant capacity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Here in, a synthetic antioxidant; ascorbic acid (Vitamin C) was used as positive control at the similar concentration ranges with Au NPs and both formulations exhibited notable free radical scavenging activity like those of the positive control. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cytotoxicity assays were performed on healthy L929 mouse fibroblast cells to evaluate the biocompatibility of the nanoparticles, and IC₅₀ concentrations were determined for both nanoparticle types. Subsequently, wound-healing experiments were conducted at these IC₅₀ concentrations to ensure physiological relevance and minimize cytotoxicity during biological evaluations. In vitro scratch assays demonstrated enhanced cell migration and proliferation in the presence of Au nanoparticles, indicating a positive effect on wound closure and tissue repair. Both chemically and green synthesized Au nanoparticles showed promising antioxidants and regenerative capabilities. However, green Au nanoparticles outperformed their chemically synthesized counterparts, likely due to the synergistic effects of plant-derived bioactive compounds present in the *Cotinus coggygia* extract. These phytochemicals, known for their intrinsic wound-healing activity, contributed significantly to enhanced cellular



responses and overall therapeutic performance. The findings underscore the potential advantages of incorporating plant extracts in the synthesis of gold nanoparticles, offering a green, cost-effective, and biocompatible strategy for developing multifunctional therapeutic agents. This comparative study highlights the superior efficacy of biogenic Au nanoparticles and supports their application in oxidative stress reduction and accelerated wound healing. Overall, plant-mediated gold nanoparticles demonstrate significant promise as eco-friendly nanomaterials for future biomedical and tissue engineering applications.

Keywords: Antioxidant, Green Synthesis, Gold Nanoparticles, Wound Healing

Research Area: Nanotechnology



PEGANUM HARMALA-MEDIATED ZINC OXIDE NANOPARTICLES WITH ANTIBIOFILM POTENTIAL AGAINST STAPHYLOCOCCUS AUREUS: IMPLICATIONS FOR WOUND HEALING APPLICATIONS

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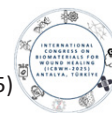
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S. aureus is one of the leading causative agents of wound infections, particularly in chronic and nonhealing wounds due to its ability to form biofilms and resist conventional treatments. In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized via a green method using *P. harmala* seed extract. This green synthesis approach offers an environmentally friendly and biocompatible alternative to conventional chemical methods. The synthesized nanoparticles were characterized using UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The UV-Vis absorption peak observed at 368 nm confirmed the optical properties of ZnO NPs. FTIR analysis revealed characteristic bands corresponding to Zn-O stretching vibrations and functional groups from phytochemicals present in the *P. harmala* extract, suggesting their role in reduction and stabilization. XRD patterns showed distinct peaks corresponding to the hexagonal wurtzite structure of ZnO, confirming the crystalline nature of the nanoparticles. SEM images demonstrated mostly spherical morphology with a size distribution ranging from 25 to 50 nm, while TEM analysis revealed an average particle diameter of 15.28 nm. The antibacterial and antibiofilm effects of these biosynthesized ZnO NPs against *S. aureus* were evaluated to provide supporting data for their potential use in infection control relevant to wound healing applications. Antibacterial activity was assessed by the broth microdilution method to determine the minimum inhibitory concentration (MIC), while biofilm formation was quantified by crystal violet staining in 96-well plates. The MIC value was determined to be 62.5 µg/mL, demonstrating notable antibacterial activity at low concentrations. Moreover, ZnO NPs reduced biofilm formation by up to 75% compared to the untreated control group ($p < 0.05$). These findings suggest that *P. harmala*-mediated ZnO NPs exhibit promising antibacterial and antibiofilm properties against *S. aureus*, supporting their potential application as alternative agents in the management of



wound-related infections. By targeting both planktonic and sessile bacterial forms, these nanoparticles may contribute to improved outcomes in wound healing through enhanced infection control.

Keywords: Zinc Oxide Nanoparticles, Green Synthesis, *Peganum Harmala*, *Staphylococcus Aureus*, Biofilm Inhibition

Research Area: Infection Control and Antimicrobial Strategies



IN-VITRO EVALUATION OF BIOCOMPATIBILITY OF CBD ON KERATINOCYTE CELLS

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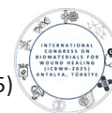
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The skin, as the largest organ in the human body, has important functions such as barrier function and thermoregulation. Slow-healing skin wounds create serious problems in healthcare systems and the economy. Cannabidiol (CBD) is a non-psychoactive compound obtained from the *Cannabis sativa* plant and has the potential to treat inflammation, pain and some skin diseases. The effects of CBD on wound healing have been examined in a limited number of studies. The aim of this study is to evaluate the potential effects of CBD on wound healing using two-dimensional (2D) and three-dimensional (3D) in vitro wound models. These models, which are preferred as alternative to animal experiments, were developed in line with the 3R (Replace, Reduce, Refine) principles defined in the European Union legislation and reflect cell-matrix and cell-cell interactions more realistically. Within the scope of the study, varying concentrations of CBD were applied to scratch wound models created on human fibroblast and keratinocyte cells. The cell viability, migration and wound closure rates were evaluated by microscopic imaging and quantitative analysis methods. The effect of CBD on cell proliferation was assessed using WST-1 assays at 24, 48, and 72 hours. The half-maximal inhibitory concentration (IC₅₀) was determined to be 12.5 µM. The wound healing potential of CBD was further evaluated using both inverted and confocal microscopy over a 7-day period. CBD treatment significantly enhanced wound closure in a 2D scratch assay, achieving 99.9% closure within two days. The wound closure rate in CBD-treated cells was 3.86 times higher than that of the control group. These findings highlight the wound healing-promoting properties of CBD and underscore the utility of advanced in vitro models for evaluating pharmacological agents. The authors acknowledge the support of the REGENEU project (no:101079123) funded by Horizon Europe.

Keywords: CBD, Wound Healing, Scratch Wound, 2D In Vitro Model, Endocannabinoid System

Research Area: Wound Healing Assessment and Imaging Techniques



PREPARATION OF STARCH-BASED AEROGELS VIA FREEZE-THAWING AS A POTENTIAL DRUG RELEASE SYSTEM

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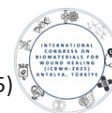
Aerogels are highly porous solid materials obtained by replacing the liquid phase in hydrogels with gas, while preserving the gel's internal network structure. Unlike conventional hydrogels, which are limited in mechanical strength despite their ability to retain moisture, aerogels exhibit superior properties such as high surface area, tunable porosity, low density, and excellent structural integrity. These features make them particularly attractive for biomedical applications, especially as drug delivery systems in wound healing. Starch is the most abundant and low-cost polysaccharide derived from plants through environmentally friendly mechanical methods. It can form physically cross-linked hydrogels, which is highly favorable for aerogel production. However, pure starch aerogels are generally weak and brittle, and their mechanical performance does not always satisfy the application. Therefore, composite formulations using synthetic polymers like polyvinyl alcohol (PVA) are preferred to enhance mechanical strength and structural integrity. In this study, starch-based aerogels were developed using PVA and corn starch through a freeze-thawing technique, and loaded with acetylsalicylic acid (ASA) to evaluate their potential as wound dressing materials. ASA was selected due to its well-established anti-inflammatory properties and its pro-angiogenic activity reported at low concentrations, both of which are known to support tissue regeneration processes. In addition to controlled release, the aerogels act as protective physical barriers and actively contribute to wound healing by creating a moist environment and facilitating cellular infiltration. The use of natural, biodegradable polymers such as starch and PVA provides excellent biocompatibility and eco-friendliness, while the freeze-thaw process offers a simple, solvent-free fabrication route. The synthesized aerogels were characterized by swelling and porosity tests, Fourier-transform infrared spectroscopy (FT-IR), and field emission-scanning electron microscopy (FE-SEM). The materials exhibited high water uptake and retained structural integrity during testing. FE-SEM images revealed a well-connected porous network favorable for moisture regulation, oxygen diffusion, and drug diffusion. Drug release profiles were evaluated in media of pH 4.0, 7.4, and 9.0, simulating different wound conditions. Overall, the developed ASA-loaded starch-



based aerogels demonstrate significant potential as responsive and customizable drug delivery systems for advanced wound care. The authors acknowledge the support of REGENEU project (no:101079123) funded by Horizon Europe.

Keywords: Aerogel, Drug Release, Freeze-Thawing, Starch, Wound Healing

Research Area: Biomaterials for Wound Healing, Scaffold Fabrication Techniques for Wound Healing



COMPARISON OF PHB PRODUCTION EFFICIENCIES OF CEREIBACTER SPHAEROIDES AND CUPRIAVIDUS NECATOR USING FOUR DIFFERENT CARBON SOURCES

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The increasing demand for safer and more sustainable healthcare solutions has driven the development of advanced biomaterials, particularly in the field of wound healing. Traditional synthetic materials used in medical applications often present challenges such as limited biocompatibility, non-biodegradability, and adverse immune responses. As a result, there is growing interest in biodegradable and non-toxic biomaterials that can support tissue regeneration while minimizing environmental impact. Microbially derived biopolymers, such as polyhydroxybutyrate (PHB), have emerged as promising alternatives due to their biodegradability, biocompatibility, and structural similarity to native tissues. These materials offer multifunctional properties ideal for wound healing applications, including support for cell proliferation, oxygen permeability, and gradual degradation into non-toxic byproducts. Their natural origin and tunable characteristics make them suitable candidates for use in wound dressings, tissue scaffolds, and bioactive coatings aimed at accelerating tissue repair and reducing complications. *Cereibacter sphaeroides*, a photosynthetic purple non-sulfur bacterium, is capable of accumulating PHB up to 60–70% of its dry cell weight under nitrogen-limited conditions. *Cupriavidus necator*, a versatile and robust model organism, can store PHB up to 90% of its dry weight using various carbon sources. This study aimed to compare the PHB production efficiencies of *C. sphaeroides* O.U.001 (DSM 5864) and *C. necator* H16 (DSM 428) using four different carbon sources: acetic acid, glucose, fructose, and sucrose. Glucose, fructose, and sucrose were tested at varying concentrations between 10–50 g/L. Each condition was replicated in triplicate, and bacterial growth was monitored through pH and optical density (OD) measurements over time. Results showed that *C. necator* did not grow in any sucrose concentration, while it demonstrated significant growth in glucose (optimal at 50 g/L), fructose (optimal at 20 g/L), and acetate (optimal at 70 mM). In contrast, *C. sphaeroides* grew only at 1 g/L fructose and showed optimal growth at 10 g/L glucose and sucrose, with 70 mM acetate also supporting growth. The findings of this study provide critical insights into selecting suitable carbon sources and microbial strains for efficient PHB

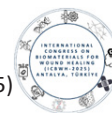


production. These results contribute to the optimization of sustainable bioplastic production processes by guiding substrate–organism compatibility.

Keywords: Biopolymers, *Cereibacter sphaeroides*, *Cupriavidus necator*, poly-hydroxybutyrate (PHB)

Research Area: Bioactive and Natural Biomaterials, Production, Extraction and Purification of Natural Biomaterials

Acknowledgements: This study was supported by the Scientific Research Projects Coordination Unit of Necmettin Erbakan University (Project No: 24YL15006) and the European Union Horizon Europe REGENEU project (Project No: 101079123).



NATURAL CAROTENOID EXTRACTION FROM *CEREIBACTER SPHAEROIDES* O.U.001 CULTIVATED UNDER CARBON DIOXIDE FIXATION CONDITIONS

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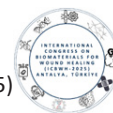
Cereibacter sphaeroides, a photosynthetic purple non-sulfur bacterium, can fix CO₂ via the Calvin cycle and convert it into high-value compounds such as carotenoids. These lipophilic pigments are located in the intracytoplasmic membranes and act as potent antioxidants by scavenging free radicals. In particular, spheroidene and spheroidenone produced by *C. sphaeroides* show potential for functionalization of biomaterials used in wound healing. In this study, carotenoids were extracted from *C. sphaeroides* O.U.001 grown under CO₂ fixation conditions, and their potential in wound healing applications was investigated. Customized media based on Biebl & Pfennig (B&P) and acetate were designed, supplemented with sodium thiosulfate (5 mM) as an electron donor. Control and experimental groups were prepared using various carbon (malic acid or acetic acid) and nitrogen (sodium glutamate) sources, with or without potassium bicarbonate (30 mM) and CO₂ gas. Bacterial growth was monitored via optical density and pH measurements. The highest growth was observed in the first experimental groups of both media, indicating enhanced CO₂ fixation through bicarbonate utilization. Carotenoid extraction yielded 0.558 mg/L and 0.54 mg/L for B&P and acetate groups, respectively. The isolated carotenoids were characterized by NMR, FTIR, and UV-Vis-NIR spectroscopy. NMR signals appeared in the ranges of 3.5–4.0, 5.11–6.62, and 6.93–7.5 ppm; FTIR absorption bands were detected at 3026–3023, 2853, 1735–1733, 1556–1540, and 1070–1060 cm⁻¹; UV-Vis-NIR scanning revealed characteristic absorbance peaks between 400–900 nm. The findings confirm that *C. sphaeroides* performs effective CO₂ fixation and produces valuable carotenoids under bicarbonate-supplemented conditions.

Keywords: *Cereibacter Sphaeroides*, Carbon Dioxide Fixation, Carotenoids

Research Area: Bioactive and Natural Biomaterials, Production, Extraction and Purification of Natural Biomaterials



Acknowledgements: This study was supported by the Scientific and Technological Research Council of Turkey-2209-A project and the European Union Horizon Europe REGENEU project (Project No: 101079123).



OPTIMIZING POLYHYDROXYBUTYRATE BIOSYNTHESIS IN *CEREIBACTER SPHAEROIDES* AND *RHODOPSEUDOMONAS* *PALUSTRIS* VIA GROWTH-INDUCTION APPROACH

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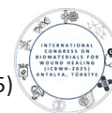
Petroleum-derived plastics are commonly used in everyday life, yet their resistance to degradation poses major environmental concerns. As a result, there is growing interest in biodegradable alternatives such as polyhydroxybutyrate (PHB)-a renewable, biocompatible thermoplastic produced by various microorganisms. In bacterial systems, PHB accumulation typically occurs when cells are exposed to an excess of carbon sources under conditions of nutrient limitation. This study focused on enhancing PHB synthesis in two purple non-sulfur bacterial strains: *Cereibacter sphaeroides* O.U. 001 (DSM5864) and *Rhodopseudomonas palustris* 7850 (DSM 127). A two-phase cultivation method was applied. Initially, cultures were propagated in a molasses-enriched medium to promote biomass growth. In the subsequent phase, cells were shifted to a nitrogen-deficient medium containing 70 mM acetate to trigger PHB biosynthesis. During the growth phase, *C. sphaeroides* and *R. palustris* reached high optical densities, accompanied by moderately alkaline pH levels. After transfer to the acetate-based production medium, optical densities slightly declined for both strains, while pH levels increased further, indicating a shift to more alkaline conditions. The formation of intracellular PHB granules was visualized using Sudan Black B and Nile Red staining via light and confocal microscopy. Upon acetate supplementation, PHB content increased by approximately 5.6% in *C. sphaeroides* and by about 93.1% in *R. palustris*. Structural verification of PHB was conducted using ¹H-NMR and FTIR spectroscopy. These results indicate that sequential cultivation-beginning with biomass enrichment in molasses followed by induction in acetate medium-effectively enhances PHB accumulation in both bacterial strains.

Keywords: Polyhydroxybutyrate (PHB), Purple Non-Sulfur Bacteria (PNSB), Two-Stage Fermentation

Research Area: Biomaterials for Wound Healing, Bioactive and Natural Biomaterials, Production, Extraction and Purification of Natural Biomaterials



Acknowledgements: This study was supported by Necmettin Erbakan University BAP (23YL15005) and the EU Horizon Europe REGENEU project (101079123).



BIOCONVERSION OF ACETIC ACID TO PHB BY *CEREIBACTER SPHAEROIDES* AND *RHODOPSEUDOMONAS PALUSTRIS*: YIELD OPTIMIZATION AND STRUCTURAL CHARACTERIZATION

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Polyhydroxyalkanoates (PHAs) are a family of biologically synthesized polyesters produced by a variety of microorganisms as carbon and energy storage materials. These biodegradable polymers, particularly polyhydroxybutyrate (PHB), offer attractive physicochemical and mechanical properties that make them suitable for biomedical and industrial applications. Among the different PHA types, PHB is a short-chain-length polymer (C3-C5) accumulated intracellularly by numerous gram-positive and gram-negative bacteria, archaea, and some eukaryotic microbes, especially under nutrient-limited conditions in the presence of excess carbon. The biosynthesis of PHB involves a two-step process: initial bacterial growth in nutrient-rich medium followed by PHB accumulation in a carbon-rich, nutrient-limited medium. Purple non-sulfur bacteria (PNSB) such as *Cereibacter sphaeroides* and *Rhodopseudomonas palustris* are recognized for their ability to utilize various carbon sources including organic acids like acetate for PHB accumulation. This study aimed to optimize the production parameters for PHB biosynthesis using *C. sphaeroides* and *R. palustris* under aerobic conditions. The bacterial strains were cultivated at 30°C and 150 rpm in media supplemented with varying concentrations of acetic acid (10–100 mM). The optimal acetate concentration for maximum biomass and PHB yield was determined to be 70 mM. After a 48-hour incubation period, solvent extraction was employed to recover intracellular PHB, a method known for its efficiency and high-purity yield. *C. sphaeroides* and *R. palustris* both produced measurable amounts of cell dry weight and PHB in the acetate-based medium. The results confirmed that both strains efficiently assimilated acetic acid and converted it into PHB granules, with *R. palustris* showing a relatively higher PHB yield and content compared to *C. sphaeroides*. Structural characterization via NMR and FTIR confirmed the purity and identity of the extracted PHB. The results highlight the importance of selecting appropriate carbon sources and growth conditions to enhance PHB production. The study also supports the use of simple, cost-effective carbon sources such as acetic acid for PHB biosynthesis. However, further optimization strategies including medium design and the use of industrial or agricultural waste substrates could significantly improve PHB

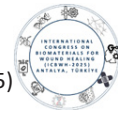


yield and reduce production costs. Thus, the integration of waste valorization into PHB production systems may offer sustainable solutions for large-scale biopolymer synthesis, promoting a circular bioeconomy.

Keywords: Biopolymer, Polyhydroxybutyrate (PHB), Purple Non-Sulfur Bacteria (PNSB)

Research Area: Biomaterials for Wound Healing, Bioactive and Natural Biomaterials, Production, Extraction and Purification of Natural Biomaterials

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BIOCOMPATIBLE AND ANTIMICROBIAL PHB-BASED NANOFIBROUS DRESSINGS FOR WOUND HEALING APPLICATION

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Effective skin regeneration is critical for the treatment of burns and open wounds. In this context, tissue engineering plays a vital role by offering innovative strategies to restore skin structure and function. Central to these strategies is the use of biocompatible and biodegradable scaffolding materials that support cell growth and tissue repair. Among such materials, biopolymers are particularly promising due to their beneficial characteristics, including bioactivity, biodegradability, resorbability, and compatibility with living tissues. This study focuses on the development of a nanofibrous wound dressing composed of polyhydroxybutyrate (PHB) and marine-derived collagen, further enhanced with the antimicrobial peptide LL37. The composite material was fabricated through electrospinning, a technique that produces fibrous mats at the nanoscale. PHB was sourced both from *Cereibacter sphaeroides* through bacterial extraction and from a commercial supplier. The PHB and collagen were dissolved in hexafluoroisopropanol (HFIP), and solutions were formulated with varying bacterial PHB contents. Electrospinning was performed under optimized parameters to generate uniform nanofibers. The structural and thermal properties of the fibers were analyzed using Field Emission Scanning Electron Microscopy (FESEM), Thermogravimetric Analysis (TGA), X-Ray Diffraction (XRD), and optical tensiometry. The antibacterial potential of the materials was tested against *Staphylococcus aureus* and *Escherichia coli*. Biodegradation behavior was examined in DMEM medium, while cytotoxicity was evaluated using the L929 fibroblast cell line. The wound healing efficacy of the nanofibers was further tested using HS2 keratinocytes, with cell morphology assessed via



inverted microscopy. The findings demonstrated that the fabricated nanofibers were non-toxic, supported cell attachment, and were suitable for biological applications. Additionally, the incorporation of LL37 significantly improved antibacterial efficacy and enhanced the in vitro wound healing response. These results suggest that the PHB/Collagen/LL37 nanofiber dressing holds strong potential for advanced wound care applications.

Keywords: Antibacterial Peptide, Biofiber, Collagen, LL37, PHB

Research Area: Biomaterials for Wound Healing, Bioactive and Natural Biomaterials

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