NANOMED
EMJMDWORKSHOP

The 9th workshop NANOMED

NANO DRUG DELIVERY FOR IMMUNOTHERAPY

University of Pavia

22-24 July 2026

University of Pavia

University of Patras

Université Paris Cité

Université Angers

The 9th edition NANOMED workshop follows the previous editions that were held in Paris (July 2025), in Patras (July 2024), and in Angers (July 2023). The 2026 NANOMED workshop is organized by the Department of Drug Sciences of the University of Pavia.

The workshop "**Nano drug delivery for immunotherapy**" provides a road map for a successful development of nanobiotechnology products and serve as podium for master thesis dissertation of NANOMED students.

The workshop will take place in the historical town of Pavia from July 22nd to July 24th.

Scientific Committee

Prof. Bice Conti
Prof. Carla Caramella
Prof. Rossella Dorati
Prof. Ida Genta
Dr. Silvia Pisani
Prof. Silvia Rossi
Prof. Giuseppina Sandri
Prof. Marco Terreni
Prof. Livia Visai

Organizing Committee

Dr. Enrica Chiesa
Prof. Rossella Dorati
Prof. Sara Perteghella
Dr. Silvia Pisani
Dr. Marina S. Robescu
Dr. Marco Ruggeri
Prof. Milena Sorrenti

IMMUNOHub

GLOBEC

Ministero della Salute

The place

The Workshop will take place at the Department of Drug Sciences in the historical italian town of Pavia.

Venue Location:
Polo Didattico (Room Dompé)
Piazza Volontari del Soccorso
27100 Pavia (PV) ITALY

UNIVERSITÀ DI PAVIA
Department of Drug Sciences

Scientific Program

The workshop "Nano Drug Delivery for Immunotherapy" focuses on the latest advancements in biotechnology and nanomedicine. The program is designed to explore the synergy between engineered nanomaterials and biological systems to revolutionize therapeutic delivery and immune modulation. Special emphasis is placed on nanoimmunotherapy and nanovaccines, integrating cutting-edge research in organoids and regenerative medicine to enhance therapeutic efficacy.

July 22nd 2026	
2:00 - 2:30 pm	Workshop registration
2:30 - 2:45 pm	Institutional welcome
2:45 - 3:00 pm	Workshop opening introduction Prof. Bice Conti, Dept. of Drug Sciences, University of Pavia, Italy Prof. Karine Andrieux, Faculté de Santé, University Paris Cité, France
Session 1. Chairs: Proff. Silvia Pisani, Adriele Prina Mello	
3:00 - 3:30 pm	Dr. Marta Serafini, Dept. Medicine and Surgery, University of Milano Bicocca, Italy *CAR-T cell therapy development*
3:30 - 4:00 pm	Dr. Laura Locati, Medical Oncology, IRCCS Maugeri/University of Pavia, Italy *Clinical aspects of oncologic immunotherapy*
4:00 - 4:30 pm	Prof. Giovanni Tosi, Dept. of Life Sciences, University of Modena e Reggio Emilia, Italy *Nanomedicine and gene delivery: how to move from design to final products*
4:30 - 5.00 pm	**NANOMED Thesis Dissertation 1** Kiraz Beste - Development of Chitosan-Ulvan Nanoparticles for Drug Delivery Application
5:00 - 5:30 pm	Poster session
5:30 - 6:30 pm	**Welcome Happy Hour**

July 23rd 2026	
Session 2. Chairs: Proff. Bice Conti, Karine Andrieux	
9:30 - 10:00 am	Prof. Paolo Caliceti, Dept. of Drug Science, University of Padua, Italy *Immunosystem and immunization: the role of vaccines*
10:00 - 10:30 am	Dr. Luigi Calzolai, Joint Research Center (JRC) Ispra, Italy *Open Questions on LNP-RNA Structure-Activity Relationship*
10:30 -11:00 am	**Coffee Break**
11:00 - 11:30 am	Prof. Adriele Prina Mello, Dept. Of Clinical Medicine, Trinity College, Dublin, Ireland *The importance of preclinical testing: consideration on model relevance for testing innovation*
11:30 am - 12:00 pm	Prof. Rabah Gahoual, Faculty of Pharmacy, University Paris Cité, France *Title to be defined*
12:00 - 12:30 pm	**NANOMED Thesis Dissertation 2** Tahir Fiza - Development of Protein-Loaded Lipid Nanocapsules for Next-Generation Vaccines
12:30 - 1:00 pm	Poster Session
1:00 - 2:30 pm	**Light lunch**
Session 3. Chairs: Proff. Giuseppina Sandri, Alessio Malfanti	
2:30 - 3:00 pm	Dr. Ursa Lampreht, Dept of Experimental Oncology, Institute of Oncology, Lubjiana, Slovenia *Electroporation based application for the immunotherapy*
3:00 - 3:30 pm	Dr. Rahaf Mihyar, Dept of Nanomedicine and Theranostic, Aachem, Deutschland

	Tumor microenvironment biomarkers predict response to cancer (nano)therapy
3:30 - 4:00 pm	Prof. Guillaume Bastiat, Pharmacy Dept of the Faculty of Health, University of Angers, France *Tunable Paclitaxel-Loaded Lipid Nanoparticle Hydrogel for Intimal Hyperplasia Prevention*
4:00 - 4:30 pm	**Coffee Break**
Session 4. Chairs: Proff. Marco Terreni, Paolo Caliceti	
4:30 - 5:00 pm	Prof. Alessio Malfanti, Dept. of Drug Science, University of Padua, Italy *Exploring Hyaluronic Acid-Conjugates for Glioblastoma Local Chemo-Immunotherapy*
5:00 - 5:30 pm	Dr. Sara Tengattini, Dept. Drug Sciences, University of Pavia, Italy. *Chemically mannosylated elastin-like recombinamers as potential novel glyco-nanovaccines against tuberculosis*
5:30 - 6:00 pm	**NANOMED Thesis dissertation 3** Prieto Martinez Roxana - Exploring the Crosstalk Between Lipid Nanocapsules and the Gut Microbiota
July 24th 2026	
Session 5. Chairs: Proff. Milena Lillina Sorrenti, Guillaume Bastiat	
9:30 - 10:00 am	Prof. Mariangela Cavarelli, Université Paris-Saclay, Inserm, CEA, Center for Immunology of Viral, Auto-immune, Hematological and Bacterial diseases (IMVA-HB/IDMIT), France *Antibody-based antiviral immunotherapy: insights from non-human primate models*
10:00- 10:30 am	Prof. Letao Yang, School of Life Sciences and Technology, Tongji University, Shanghai, China *Engineering Insoluble Cues for Stem Cell Fate Control and Therapy*
10:30 - 11:00 am	Prof. Rongrong Zhu, School of Life Sciences and Technology, Tongji University, Shanghai, China

	Nanomaterials and Smart Manufacturing of Organoids for Advanced Regenerative Medicine
11:00 - 11:30 am	Dr. Isaac Godspower, CURAM research, Center for Medical Devices, Gallway, Ireland *Nitric Oxide nanoparticles for cancer therapy*
11:30 am - 12:00 pm	Conclusive Remarks
12:00 - 1:00 pm	Pedagogic Committee
1:00 - 1:30 pm	NANOMED Graduation

Poster List

P-1	Z. Abbasova	Design and development of dissolving microneedles for dermal vaccination
P-2	H. Almadawdi	Microparticle formation using water-in-water emulsions
P-3	J. Alnqeep	Development of enzymatic methods to evaluate the functional activities of beer samples and their melanoidin fractions
P-4	R. Araujo	Development of lipid nanoparticles for preeclampsia treatment
P-5	M. Belaribi	Study and characterization of novel cosmetic ingredients based on plant stem cell technologies
P-6	I. Bicerel	Development of liposomal formulations for the delivery of the antimicrobial peptide TMP
P-7	M. Biondi	Microwave-assisted technologies in organic synthesis and natural product extraction
P-8	L. Cao	Comparison of the particle size of two lipid nanoparticles before and after protein corona formation measured by TRPS
P-9	F. Carboni	Voltammetric detection of micro- and nanoplastics using an electroactive nanocarbon material based on a graphene oxide platform
P-10	E. Chavez-Ruiz	Organic nanocrystal vs. free drug ratio quantification by taylor dispersion analysis: an orthogonal validation
P-11	T. Fatima	New methodology to quantify lipid nanoparticle concentrations
P-12	V. Fracassi	Design and development of thermoresponsive hydrogels for the prevention of postoperative adhesion
P-13	J. Gandhi	Development of hybrid 3D-printed/nanoelectrospun scaffolds for esophageal tissue regeneration

P-14	O.D.A. Garcia	Evaluation of a plant-derived extract as a disruptor of tumor microenvironment crosstalk, survival, and invasion in 2D and 3D triple-negative breast cancer models
P-15	S. Giudici	Advanced 3D bioprinted hydrogels as in vitro platforms for breast cancer microenvironment modeling
P-16	N. Kainat	Ocrelizumab counteracts the upregulation of the PKCβ/HIF-1α/VEGF pathway in patients with relapsing multiple sclerosis
P-17	J. Keenaghan	Dextran-based electrospun nanofibers for biomedical application
P-18	M. Khalili	Study and characterization of peg-free oil-in-water nanoemulsion
P-19	I. Khan	Identification of formulation feasibility region of vancomycin loaded polymeric nanoparticles
P-20	E. Maffioli	Biomimetic electrospun composite scaffolds for cartilage tissue engineering
P-21	J.E.G Marroquin	Antimicrobial evaluation of chlorpromazine encapsulated in lipid-based formulations
P-22	M. Noumbissie	Stability of bacteriophage K-loaded liposomes with different lipid compositions during storage at 4 °C
P-23	G.I. Patat	4D Electrospun polyurethane scaffolds for tendon tissue engineering
P-24	N.Y. Patil	Semi-automated 3D segmentation of the rat inner ear using ITK-SNAP
P-25	M. Perucchini	Chitosan-coated fucoidan/poly-lysine nanogels for the prevention of oral dysbiosis-related respiratory infections
P-26	M. Podkidysheva	Fabrication and characterization of 4D PLA:PCL (70:30) scaffolds produced via direct ink writing (DIW)
P-27	Z. Rao	Thermal characterisation of eugenol-based nanostructured lipid carriers

P-28	P. Santagostini	Development of anisotropic core-shell electrospun nanofibers doped with lignin nanoparticles for quercetin delivery in volumetric muscle loss
P-29	I. Segato	Fast-dissolving prebiotic nanofibers loaded with oleic acid/β-CD inclusion complex for the treatment of bacterial vaginosis
P-30	G. Sensi	Formulation strategies for anti-eNAMPT nano and micro carriers
P-31	N. M. G. Silva	Secondary cell culture - seeding, cell passaging, and cell counting
P-32	M. G. Silvestri	Development and formulation of a highly soluble alpha-lipoic acid injectable solution
P-33	N. M. V. B. Tanaka	Exploring the *in vitro* efficacy of green-synthesized Prussian blue nanoparticles across different breast cancer models
P-34	F. Truddaiu	Design and characterization of amphiphilic cyclodextrin-based nanoemulsions
P-35	A. Zani	Microwave assisted synthesis of organic compounds

DESIGN AND DEVELOPMENT OF DISSOLVING MICRONEEDLES FOR DERMAL VACCINATION

Zhala Abbasova, Barbara Vigani, Caterina Valentino, Marco Ruggeri, Giuseppina Sandri, Silvia Rossi
Department of Drug Sciences, University of Pavia, Italy

zhala.abbasova01@universitadipavia.it

Dissolving microneedles (dMNs) represent a promising platform for dermal vaccination, enabling minimally invasive antigen delivery across the stratum corneum barrier. Natural polysaccharides are particularly attractive materials due to their biocompatibility, biodegradability, and water solubility. Aim of the work was to design and characterize dMNs based on chondroitin sulfate (CS) combined with dextran (DEX) or pullulan (PULL).
Different polymeric solutions were prepared and characterized in terms of rheological behavior. dMNs were prepared from these solutions by a centrifugation-assisted micro-molding approach. Their morphology was evaluated by scanning electron microscopy, while the mechanical performance by compression and Parafilm® insertion tests using a texture analyzer. A multilayer hydrogel model was developed to simulate the different layers of the skin and evaluate MN dissolution over time.
Rheological studies demonstrated that all polymeric solutions exhibited shear-thinning behavior, which is advantageous for mold filling during manufacturing. PULL-containing solutions showed higher viscosity values than DEX-containing ones, suggesting stronger intermolecular interactions and enhanced structural stability. All polymeric solutions were able to form needle structures. Among them, four formulations presented the most regular and well-defined geometry and were therefore selected for further characterization. dMNs obtained from solutions containing 20% w/w CS and 8 or 10% w/w PULL showed the highest mechanical robustness. Parafilm® insertion test indicated that dMNs obtained from the solution containing 20% w/w CS and 10% w/w DEX provided the best penetration performance. Dissolution studies demonstrated gradual hydration and erosion of all dMNs. DEX-based formulations dissolved faster than PULL-containing ones (20-30 vs 40 min).
In conclusion, polymer composition strongly influenced dMN performance. The dMNs obtained from the solution containing 20% w/w CS and 10% w/w DEX showed the most balanced overall performance, combining suitable morphology, mechanical properties, insertion capability, and dissolution behavior. This formulation represents a promising candidate for further development as a platform for dermal vaccination.

MICROPARTICLE FORMATION USING WATER-IN-WATER EMULSIONS

Hasan Almadawdi[1], Asso. Prof. Dr. Emilie Roger[2], Eng. Olivier Thomas[3]
[1]Faculty of Health, University of Angers, France
[2]Pharmaceutical Technology Department, Faculty of Health, University of Angers, France
[3]Medical Education Department, Faculty of Health, University of Angers, France

hasan.almadawdi@univ-anger.fr

Water-in-water emulsions, also known as aqueous two-phase systems, are promising platforms for pharmaceutical applications because they enable the encapsulation of sensitive biomolecules without using organic solvents. However, their stabilization remains challenging due to the low interfacial tension between the aqueous phases. The objectives of this study were to construct three phase diagrams of water-in-water emulsions prepared from dextran, dextran sulfate, and polyethylene oxide (PEO), and to identify suitable direct and inverse emulsion formulations for microparticle production.

A total of 87 water-in-water emulsion formulations were prepared using three dextran/dextran sulfate ratios (25/75, 50/50, and 75/25) with varying polymer concentrations, and their phase behavior was evaluated by fluorescence microscopy. Three phase diagrams were constructed to classify direct emulsions, inverse emulsions, bicontinuous phases, and single-phase systems. Direct emulsions, in which dextran/dextran sulfate droplets were dispersed in a continuous polyethylene oxide (PEO) phase, were the predominant and most stable structures, particularly at PEO concentrations of 10% w/w or higher, with the 25/75 ratio providing the broadest stability region. Based on the phase diagrams, one stable direct emulsion (point 14) and one inverse emulsion (point 21) were selected as templates for microparticle production using polyethyleneimine-induced gelation in acetic acid. Microparticles were successfully produced from the direct emulsion and confirmed by fluorescence microscopy, whereas the inverse emulsion failed to generate microparticles. These results demonstrate that stable direct emulsions provide a more reliable template for microparticle formation than inverse emulsions under the conditions investigated.

This study allowed the identification of the phase behavior of dextran/dextran sulfate–PEO water-in-water emulsions through phase diagram analysis and demonstrated their potential for microparticle production. Further work is needed to optimize droplet size homogeneity and enable microparticle production from inverse emulsions for future pharmaceutical applications.

DEVELOPMENT OF ENZYMATIC METHODS TO EVALUATE THE FUNCTIONAL ACTIVITIES OF BEER SAMPLES AND THEIR MELANOIDIN FRACTIONS

Julia Alnqeep[1], Martina Piluso[1] and Adele Papetti[1]
[1]Department of Drug Sciences, University of Pavia, Italy

juliamohammedn.alnqeep01@universitadipavia.it

Beer is a chemically fermented beverage containing a wide range of bioactive compounds, including polyphenols, peptides, and Maillard reaction products. Among these, melanoidins are high-molecular-weight compounds formed during thermal processing that have attracted increasing attention due to their potential health-promoting properties. This study aimed to isolate beer melanoidin fractions and evaluate their inhibitory activity against the carbohydrate-hydrolysing enzymes a-amylase and a-glucosidase. Melanoidin fractions were isolated from three selected beer samples (A, B, and C) using ultrafiltration system equipped with a 10 kDa molecular weight cut-off membrane, allowing the separation of high- and low-molecular-weight constituents. The beer samples and melanoidin fractions were evaluated using in vitro spectrophotometric enzyme inhibition assays. Results showed that the beer samples exhibited weak a-glucosidase inhibitory activity. The highest inhibition was observed for sample A (7.10 ± 0.78%), while samples B and D showed no inhibition at 1 mg/mL. The melanoidin filtrate fractions inhibited a-glucosidase by 8.24 ± 0.49%, 5.28 ± 0.37%, and 11.01 ± 0.23% for samples A, B, and D, respectively. Similarly, the melanoidin retentate fractions exhibited inhibition of 9.25 ± 0.14%, 4.10 ± 0.15%, and 6.90 ± 0.97%, respectively, at 1 mg/mL. In contrast, the reference inhibitor acarbose showed dose-dependent activity, reaching 80.74 ± 0.52% inhibition at 1 mg/mL. For a-amylase, samples A and D exhibited inhibition effects of 6.22 ± 0.64% and 4.14 ± 0.47%, respectively, at 1 mg/mL, whereas sample B and filtrate and retentate showed no detectable activity. By comparison, acarbose demonstrated strong, dose-dependent inhibition, reaching 89.44 ± 0.05% at 100 µg/mL. Overall, the isolated beer melanoidin fractions exhibited limited inhibitory activity against both enzymes under the tested conditions. Future studies will expand the enzymatic screening to additional biological targets, including elastase, tyrosinase, and acetylcholinesterase. The isolated fractions will also be chemically characterized to clarify the relationship between melanoidin structure and biological activity.

DEVELOPMENT OF LIPID NANOPARTICLES FOR PREECLAMPSIA TREATMENT

Rita Araújo[1], Benjamin Le Rolland[1], Karine Andrieux[1]
[1]Université Paris Cité, CNRS, INSERM, UTCBS, 4 avenue de l'observatoire, F-75006, Paris, France

rita.araujo@etu.u-paris.fr

Pre-eclampsia is a pregnancy associated hypertensive disorder characterized by an imbalance of angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) secreted in excess by the placenta. Elevated blood sFlt-1 levels contribute to maternal hypertension and endothelial dysfunction associated with the disease.
This work explores formulation of lipid based nanocarriers, including lipoplexes and Ionizable Lipid nanoparticles (LNPs), for drug delivery of small interfering RNA (siRNA) targeting sFlt-1 in a placental model based on human primary cytotrophoblasts. Liposomes were produced by microfluidic manufacturing and complexed with siRNA to create lipoplexes. Then they were characterized in terms of size, polydispersity index (PDI) and zeta potential. The impact of the addition of alginate, an anionic polymer, in the zeta potential of the lipoplexes was studied. No significant changes in particle size were observed after 24 h of storage, revealing stability of the formulations under storage at 4°C for 24h.
Lipid Nanoparticles were formulated using Bip20 (a novel lipid) and a commercial ionizable lipid (MC3). Results indicate that the use of the ionizable lipid Bip20 renders smaller particles than lipoplexes, with a negative zeta potential.
Ribogreen assay results showcased encapsulation efficiency above 95% across all formulations. MTT assay showed that cell viability is dependent on the concentration of siRNA complexed to lipoplexes.
These findings support the potential of the developed lipid-based nanocarriers as a promising platform for siRNA delivery in preeclampsia.
Future work will focus on evaluating sFlt-1 gene silencing efficacy, nanoparticle stability, and biological performance in a pre-eclampsia model.

STUDY AND CHARACTERIZATION OF NOVEL COSMETIC INGREDIENTS BASED ON PLANT STEM CELL TECHNOLOGIES

Meriam Belaribi, Vitiana Cerone, Paola Perugini
Department of Drug Sciences, University of Pavia, Italy

meriam.belaribi01@universitadipavia.it

In the modern cosmetic and nanomedicine sectors, the pursuit of innovative, sustainable, and highly effective active ingredients is crucial for developing next-generation anti-aging treatments. This study evaluates the feasibility of two novel plant stem cell-derived ingredients developed by ACTV Biotechnology for the preparation of cosmetic products. The primary objective was to investigate the physicochemical stability, structural morphology, and biological properties of these multi-active systems to validate their regenerative and protective potential.
A multi-methodological approach was employed to characterize the formulations, Multiple light scattering (MLS) technique was utilized to monitor kinetic instability phenomena and phase separation over time. While MLS technique provided initial particle diameter estimations, Dynamic Light Scattering (DLS) was subsequently implemented to achieve high-precision size measurements. To check structural morphology, optical microscopy and Fluorescence microscopy were used to visually confirm the structural integrity, distribution, and morphology of the ingredients within the complex formulation matrix. Furthermore, the free radical scavenging capacity of the plant stem cell products was also quantified via a spectrophotometric DPPH assay.

DEVELOPMENT OF LIPOSOMAL FORMULATIONS FOR THE DELIVERY OF THE ANTIMICROBIAL PEPTIDE TMP

İrem Biçerel[1], Eirini Mallikopoulou[1], Athanasios Skouras[1], Spyridon Mourtas[2]
[1] Department of Pharmacy, School of Health Sciences, University of Patras, 26504 Patras, Greece
[2] Department of Chemistry, University of Patras, 26504 Patras, Greece

Antibiotic resistance in bacteria has been rising in recent years due to its overuse, leaving current treatments ineffective. Antimicrobial peptides (AMPs) are promising alternatives owing to their activity against drug-resistant bacteria, however, their clinical application is limited by poor in vivo stability, enzymatic degradation, cytotoxicity, and low bioavailability. Liposomal drug delivery systems may overcome these limitations by protecting AMPs and improving their therapeutic performance. This study aimed to investigate the encapsulation of the synthetic antimicrobial peptide TMP into liposomes and evaluate how lipid composition influences physicochemical characteristics, stability, and antibacterial activity. Liposomes composed of phosphatidylcholine/cholesterol (PC/Chol, 2:1 and 1:1) and phosphatidylcholine/phosphatidylglycerol/cholesterol (PC/PG/Chol, 8:2:10) were prepared by thin-film hydration followed by probe sonication and purified by ultracentrifugation. All formulations produced nanoparticles with mean diameters of approximately 109 nm and low polydispersity (PDI < 0.30), remaining physically stable over four weeks. Loading capacities of approximately 40% were obtained for both PC/Chol (2:1) and PC/PG/Chol (8:2:10). High-performance liquid chromatography demonstrated significantly greater peptide retention in PC/PG/Chol than in PC/Chol, suggesting that incorporation of the negatively charged phospholipid phosphatidylglycerol enhances peptide-lipid interactions. While free TMP exhibited potent antibacterial activity (MIC 12.455 µg/mL), liposomal encapsulation reduced antimicrobial efficacy. Among the formulations tested, only PC/Chol (1:1) retained measurable antibacterial activity (MIC 56.535 µg/mL). These findings indicate that lipid composition strongly influences peptide incorporation and antimicrobial performance. Further optimization of liposomal formulations is required to preserve the antimicrobial activity of encapsulated TMP while maintaining favorable physicochemical properties.

MICROWAVE-ASSISTED TECHNOLOGIES IN ORGANIC SYNTHESIS AND NATURAL PRODUCT EXTRACTION

Marta Biondi, Giacomo Rossino
Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia PV, Italy

marta.biondi02@universitadipavia.it

Microwave-Assisted Organic Synthesis (MAOS) and microwave-assisted solvent extraction (MASE) have emerged as invaluable strategies in modern chemical research. By leveraging direct dielectric heating, these techniques provide rapid, volumetric energy transfer, significantly reducing processing times and enhancing efficiency compared to conventional thermal methods. The primary focus of this internship project was to master specialized microwave instrumentation, specifically the CEM Discovery and CEM Mars systems, across diverse synthetic and extractive applications.

Regarding the utilization of MASE for synthetic protocols, a microwave-assisted SN2 nucleophilic substitution was optimized and evaluated against traditional patent procedures. Specifically, the N-alkylation of a secondary amine with an alkyl halide substrate was subjected to systematic modifications of temperature, time and irradiation cycles with the aim of accelerating reaction kinetics and maximizing conversion. Reaction progress was monitored via Thin-Layer Chromatography (TLC), and the target intermediate was isolated through liquid-liquid extraction. Additionally, preliminary training encompassed transition-metal-catalyzed transformations, specifically a palladium-catalyzed Heck coupling. Both reactions were necessary for the synthesis of multitarget directed ligands as potential anticancer agents.

Utilizing the CEM Mars system for plant-based extractions, microwave technology was applied to natural product chemistry to isolate bioactive compounds from Arthrospira platensis (Spirulina algae). The resulting extracts were analytically characterized using both TLC and High-Performance Liquid Chromatography (HPLC) to ensure reproducible profiling. The extracted matrices are scheduled to be subjected to in vitro biological assays to evaluate their cytotoxicity against specific thyroid cancer cell lines. Overall, these findings highlight the potential of microwave technology as a powerful, versatile tool in medicinal chemistry, streamlining both the synthesis of intermediate targets and the extraction of promising natural anticancer agents.

COMPARISON OF THE PARTICLE SIZE OF TWO LIPID NANOPARTICLES BEFORE AND AFTER PROTEIN CORONA FORMATION MEASURED BY TRPS

Li Cao, Maria Cristina Bonferoni, Laura Catenacci, Sara Perteghella, Rachele Rossi, Milena Sorrenti
Department of Drug Science, University of Pavia, Italy

li.cao01@universitadipavia.it

Nucleic acid nanoparticles (NPs), once in the bloodstream, rapidly interact with plasma proteins, forming of a protein corona that can alter their physicochemical properties, compromise structural stability, and accelerate clearance by the immune system. Assessing the structural stability of NPs in biological fluids remains a critical challenge for the successful systemic nucleic acid delivery. In this study, two lipid nanoparticle (LNP) formulations, both unloaded and mRNA-loaded, were developed and compared to investigate particle size changes associated with human plasma incubation. LNPs based on either the cationic lipid DDA (dimethyldioctadecylammonium bromide - DDA-LNPs) or the ionizable lipid ALC-0315 (ALC-LNPs), were prepared using a microfluidic system, both with and without a model mRNA payload. The LNPs obtained were dialyzed in phosphate buffer solution (pH 7.4), and subsequently incubated in human plasma for 1 hour . Particle size distributions were characterized using Tunable Resistive Pulse Sensing (TRPS). This technique provides for an accurate assessment of the particle distribution, since it analyses each particle passing through a nanopore. Preliminary results demonstrated that plasma incubation induced no significant size changes in either DDA- or ALC-LNPs. Overall, ALC-LNPs showed a slightly lower mean diameter compared to the cationic counterpart, achieving in any case acceptable size values (≤ 200 nm). These findings indicate that both NP formulations maintained their structural integrity and colloidal stability under physiologically relevant conditions. Overall, the data suggest that both DDA- and ALC-LNPs exhibit robust stability during mRNA loading and human plasma proteins exposure, supporting their potential use as vehicles for systemic nucleic acid delivery.

VOLTAMMETRIC DETECTION OF MICRO- AND NANOPLASTICS USING AN ELECTROACTIVE NANOCARBON MATERIAL BASED ON A GRAPHENE OXIDE PLATFORM

Francesco Carboni[1], Alessandro Carvani[2], Alessandra Bonanni[2]
[1]Department of Drug Sciences, University of Pavia, Italy
[2]Department of Analytical Chemistry, University of Pavia, Italy

francesco.carboni02@universitadipavia.it

To address the critical need for rapid detection of pervasive micro- and nanoplastic (MNP) pollution, this study introduces a novel "signal-off" electrochemical sensor based on the controlled suppression of the intrinsic redox activity of graphene oxide. Driven by strong hydrophobic and π-π interactions, the spontaneous adsorption of these non-electroactive contaminants onto the graphene matrix hinders the intrinsic electroactivity of the latter, yielding a current decrease utilized for label-free quantification.
This work aims to carry out the detection of polystyrene MNPs through the reduction of the intrinsic electrochemical signal of colloidal graphene once it has been modified with a layer of MNPs; the signal is measured through a differential pulse voltammetry (DPV) analysis, which measures the current difference between two potential pulses applied at regular intervals to minimize capacitive background noise and enhance the sensitivity of redox peak detection.
For the experiments, five glassy carbon working electrodes have been first modified with colloidal graphene layer. The voltametric signal from the graphene layer was then recorded in a phosphate buffer solution, using a platinum counter-electrode and a silver/silver chloride reference electrode. Subsequently, a second layer of micro- and nanoplastics (100 nm, 300 nm, and 1100 nm) was applied to form a double-layer surface, allowing for the measurement of the suppressed graphene signal and the calculation of the signal difference and ratio (0.6 – 0.8) caused by the plastics.
Moving forward, this study can be transitioned from environmental monitoring into a high-throughput screening tool within pharmaceutical sciences to detect trace of nanoplastic leachables from polymer-based drug packaging and intravenous delivery systems. Furthermore, by exploiting the same suppression mechanics, the sensor can be adapted to investigate whether nanoplastics bind to, transport, and alter the bioavailability of hydrophobic drug molecules in biological systems.

ORGANIC NANOCRYSTAL VS. FREE DRUG RATIO QUANTIFICATION BY TAYLOR DISPERSION ANALYSIS: AN ORTHOGONAL VALIDATION

Emiliano Chavez-Ruiz[1], Cédric Sarazin[2], Nathalie Mignet[1], Rabah Gahoual[1], Yohann Corvis[1]
[1]Unit of Chemical and Biological Techniques for Health (UTCBS), Faculty of Pharmacy, Université Paris Cité, 75 006 Paris, France
[2]Nanoscale Metrix SAS, 53 route de Lavaur, 31 240 L'Union, France

emilianochavezofficial@gmail.com

Over 90% of new chemical entities are poorly water-soluble. Organic Drug nanocrystals (NCs), carrier-free systems composed of ~99% APIs, address this by increasing the surface area-to-volume ratio, which enhances dissolution and apparent solubility. However, NC formulations dynamically coexist with the free active pharmaceutical ingredient in a concentration-dependent equilibrium, a complexity that hinders in vitro-in vivo correlations and limits clinical translation.
This study aimed to develop an ultraviolet-visible (UV-Vis) spectroscopy methodology to quantify the NC-to-free drug ratio, serving as an orthogonal validation for Taylor Sizer®, a new characterization tool based in Taylor Dispersion Analysis (TDA) in a fisetin-based model.
Fisetin NCs were synthesized using a solvent-antisolvent method and characterized across a wide concentration range (0.812–2000 µg/mL) via TDA, UV-Vis, fluorescence, dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR).
Absorbance and fluorescence were proportional to concentration, though a quenching effect was noted at higher concentrations for fluorescence, FTIR spectra matched the reported previously for fisetin NC in the literature.
The transition limit from nanocrystals to free drug was determined when the sample absorbance equaled or dropped below that of pure fisetin; this was confirmed by the DLS derived count rate, establishing the NC persistence threshold at 3.25 µg/mL. By calculating the free fraction using the characteristic absorbance at 360 nm, the obtained values closely matched the primary TDA measurements. Furthermore, TDA data successfully predicted sample absorbance values, achieving a Pearson correlation of 0.999 and a 95% accuracy with differences below 0.22 a.u., as validated by Bland-Altman analysis.
This innovative orthogonal methodology successfully quantifies free versus nanocrystal drug ratios, confirming cross-method predictability and establishing TDA as a robust tool to advance the study and development of organic nanocrystal medicines.

NEW METHODOLOGY TO QUANTIFY LIPID NANOPARTICLE CONCENTRATIONS

Toseef Fatima[1], Talah Sagheer[2], Emili Roger [2], Guillaume Bastiat[1]
[1]UNIV Angers, INSERM 1066, CNRS 6021, MINT, Angers, France
[2]UNIV Angers, INSERM 1066, CNRS 6021, MINT, Angers, France

toseeffatima26@gmail.com

Lipid nanoparticles (LNPs) are versatile drug delivery systems that require accurate characterization for formulation development. While Nanoparticle Tracking Analysis (NTA) directly measures particle concentration, its high cost limits accessibility in many laboratories. This study investigated the potential of rheological viscosity measurements as an indirect method for estimating LNP concentration. LNP formulations with varying lipid concentrations, particle sizes, and dispersing media (Milli-Q water and PBS) were prepared using the phase inversion zone (PIZ) method and characterized using a Malvern Zetasizer Ultra (MADLS), NTA, and a rotational rheometer.

The findings elaborated LNP concentration and suspension viscosity were positively correlated, whereas particle size did not significantly affect viscosity within the size range under investigation. Double-blind formulations were used to design and verify a viscosity based model that demonstrated a potential predictive capability. MADLS analysis verified consistent particle sizes in all formulations, showing that there was no fusion or aggregation of nanoparticles. Additionally, a significant linear correlation between the MADLS measured particle concentration and lipid concentration was observed. In comparative study between Malvern zetasizer Ultra and NTA Particle size measurements showed similar results, however particle concentration measurements showed significant discrepancies, perhaps because NTA analysis requires a large sample dilution.

Overall, this work shows that viscosity measurements have the potential to be a quick and useful method for determining LNP concentration. This methodology may offer a straightforward and effective approach for LNP particle concentration measurements when combined with MADLS characterization; however, additional validation across a wider range of formulations is necessary.

Keywords: Lipid nanoparticles, Viscosity, Multi-Angle Dynamic Light Scattering (MADLS), Nanoparticle Tracking Analysis (NTA), Rheology, Particle concentration.

DESIGN AND DEVELOPMENT OF THERMORESPONSIVE HYDROGELS FOR THE PREVENTION OF POSTOPERATIVE ADHESION

Veronica Fracassi, Marco Ruggeri, Barbara Vigani, Silvia Rossi, Giuseppina Sandri
Department of Drug Sciences, University of Pavia, Italy

veronica.fracassi01@universitadipavia.it

Thermoresponsive hydrogels represent a promising strategy for the prevention of postoperative adhesions, as they can be administered in a minimally invasive manner undergo an *in situ* sol-gel transition under physiological conditions.
In this work, hydrogels based on methylcellulose (MC) and hyaluronic acid (HA) were designed and developed to identify formulations suitable for postoperative adhesion prevention.
Rheological analyses were performed using a Modular Compact Rotational Rheometer.
A Design of Experiments (DoE) approach was employed to optimize the hydrogel formulation by investigating the effect of three variables: methylcellulose molecular weight, methylcellulose concentration, and hyaluronic acid concentration. Eight formulations were prepared according to the experimental design. As a response variable, the relative viscosity increase between room and physiological temperature ($\Delta\eta/\eta$) was used. The results revealed that MC molecular weight and concentration were the most significant factors affecting the response, whereas the concentration of HA showed a limited influence. In particular, formulations containing low-molecular-weight MC at 8% w/w exhibited the highest $\Delta\eta/\eta$ values, regardless of the hyaluronic acid concentration.
Based on these findings, the selected formulations were subsequently subjected to further rheological characterization, including viscosity, strain sweep, frequency sweep, temperature ramp analyses. Rheological studies confirmed the thermoresponsive nature of the selected hydrogels. In particular, all formulations showed a marked increase in viscosity at 37 °C compared with 25 °C, together with a decrease in the loss factor (tan δ), indicating a progressively more elastic behavior at physiological temperature. Temperature ramp analyses further demonstrated a sol-gel transition, with the crossover between the storage modulus (G') and the loss modulus (G'') occurring at temperatures close to physiological conditions.

DEVELOPMENT OF HYBRID 3D-PRINTED / NANOELECTROSPUN SCAFFOLDS FOR ESOPHAGEAL TISSUE REGENERATION

Jimil Gandhi[1], Silvia Pisani[1], Aleksandra Evangelista[1], Luigi Ruccolo[2], Bice Conti[1]
[1]Department of Drug Sciences, University of Pavia, Italy
[2]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

jimilsgandhi@gmail.com

The present study aimed to develop hybrid 3D-printed and nanoelectrospun scaffolds for esophageal tissue regeneration. A Polylactic acid/polycaprolactone copolymer (70:30) was used to fabricate the 3D-printed (8%w/v), electrospun (15%w/v), and hybrid scaffolds (composed of a 3D-printed support coated with electrospun fibers). Triamcinolone acetonide (TA) was loaded into the electrospun fibers at 1 %w/w (relative to polymer). TA is widely used for the postoperative treatment of esophageal scarring. The scaffolds were characterized in terms of morphology (scanning electron microscopy), surface wettability (contact angle measurement), mechanical properties (tensile testing), and interfacial adhesion strength (peel testing). For hybrid scaffolds, the electrospun side exhibited a fiber diameter of 430 ± 10 nm, and the surface porosity was approximately 39% and 24% for the 3D-printed and electrospun sides, respectively. The contact angle (for phosphate buffer saline) decreased from around 122□ to 111□ over 10 minutes, indicating increased wettability of the drug-loaded hybrid scaffold. The hybrid and electrospun scaffolds showed significantly higher contact angles than 3D-printed scaffolds, consistent with their porosity differences. Loading of the hydrophobic drug caused an increase in the values; however, the difference was statistically insignificant. The 3D-printed scaffolds displayed excessive stiffness for soft tissue applications, whereas the hybrid and electrospun scaffolds showed Young's modulus values around 4 MPa, which are closer to those of the human esophagus. The hybrid scaffold, evaluated by T-peel testing, demonstrated adhesive (interfacial) failure mode with complete separation of the individual layers. The average peel resistance was 0.83 ± 0.09 N/100mm and drug loading did not significantly affect it. The multiscale material characterization performed in the present study is crucial for the development of bioengineered scaffolds. Future work necessitates drug quantification, assessment of release behavior, and biological evaluation.

EVALUATION OF A PLANT-DERIVED EXTRACT AS A DISRUPTOR OF TUMOR MICROENVIRONMENT CROSSTALK, SURVIVAL, AND INVASION IN 2D AND 3D TRIPLE-NEGATIVE BREAST CANCER MODELS

Oscar Dario Angel Garcia[1], Veronica Giugnetti[1], Erica Buoso[1]
[1]Department of Drug Sciences, University of Pavia, Italy

oscardario.angelgarcia01@universitadipavia.it

Triple-Negative Breast Cancer (TNBC) is a particularly aggressive breast cancer subtype accounting for 15% to 25% of all diagnoses, characterized by extreme molecular heterogeneity, high metastatic propensity, and a lack of targeted therapies due to the absence of key biomarkers (ER, PR, HER2). The dynamic crosstalk between TNBC cells and the tumor microenvironment (TME) establishes a co-signaling network promoting progression, while the scaffolding protein RACK1 coordinates critical pathways governing cell survival and invasion.
To investigate the efficacy of the plant-derived extract A1 on TNBC aggressiveness and modulating TME, mesenchymal-stem-like MDA-MB-231 and SUM159PT cell lines were treated with different concentrations of A1 and evaluated with in vitro models:
● 2D model: Wound healing assays were employed to assess migration, with crystal violet staining for overall proliferation, alongside an MTT assay for cell viability. Western blot analysis monitored the corresponding expression profiles of RACK1. To model TME communication, Transwell assays measured THP-1 monocyte recruitment toward tumor-derived conditioned media.
● 3D model: An invasion assay was used to assess spatial invasiveness, by embedding spheroids into a biomimetic Matrigel matrix, and treating them with A1 to track morphological growth up to 72 hours.
Our findings indicate that A1 reduced viability in a dose-dependent manner and severely impaired 2D cell migration and invasion. This correlated with a marked down-regulation of RACK1 levels. Furthermore, a significantly reduced monocyte migration index was observed due to an altered tumor secretory profile. Remarkably, in the 3D invasion model, A1 treatment effectively suppressed spheroid growth and significantly reduced invasive branching protrusions, inhibiting tumor dissemination.
These results demonstrate that A1 severely impairs TNBC aggressiveness and disrupts the TME crosstalk, holding strong potential as a novel adjuvant therapeutic strategy. However, both in vitro and in vivo studies are needed to validate its efficacy and clinical application.

ADVANCED 3D BIOPRINTED HYDROGELS AS *IN VITRO* PLATFORMS FOR BREAST CANCER MICROENVIRONMENT MODELING

Steven Giudici[1], Giovanni Lo Bello[1], Amirhossein Elahi[1], Mariella Rosalia[2], Livia Visai[1,3], Nora Bloise[1,3]

[1]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), Unità di Ricerca (UdR) INSTM, Operative Unit (OU) of the Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research (Centro 3R), University of Pavia, 27100 Pavia, Italy
[2]Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy
[3]UOR6 Nanotechnology Lab., Department of Prevention and Rehabilitation in Occupational Medicine and Specialty Medicine, Istituti Clinici Scientifici Maugeri IRCCS,27100 Pavia, Italy

steven.giudici01@universitadipavia.it

Breast cancer is one of the leading causes of cancer-related mortality among women worldwide. This reflects the pressing need for advanced *in vitro* models that accurately reproduce the complexity of the tumour microenvironment. Conventional two-dimensional (2D) cell cultures and animal models often fail to replicate human tumours accurately, while three-dimensional (3D) bioprinting enables the creation of biosimilar tissue constructs with customizable architecture and microenvironmental properties. The aim of this study is the development and characterization of bio-printable alginate-gelatine hydrogels, which can reproduce breast tumour tissue and to evaluate their suitability as 3D platforms for breast cancer microenvironment modelling. At the beginning of the project, four formulations were prepared, followed by structural and mechanical characterization. Subsequently, biological behaviour was evaluated using triple-negative breast cancer cell lines encapsulated in both hydrogels and 3D bio-printed constructs. Among the tested formulations, an optimal hydrogel composition was identified that most closely mimicked breast tumour tissue, exhibiting the most suitable combination of stiffness, printability, and cytocompatibility. Cell viability assays demonstrated persistent survival of different cell lines throughout the culture period, while metabolic activity progressively increased over time, indicating cell growth and adaptation within the 3D matrix. A homogeneous distribution of viable cells with minimal cell death after prolonged culture was demonstrated using confocal laser scanning microscopy. In addition, gene expression analyses were performed after 7 days underlining the preservation of markers associated with proliferation, cell adhesion, and extracellular matrix remodelling. These highlight active cell-matrix interactions and cell-specific responses within the engineered microenvironment. These findings demonstrate that the selected formulation provides a supportive and physiologically relevant microenvironment for long-term culture of triple-negative breast cancer cells. This 3D bio-printed platform represents a promising tool for investigating tumour biology, disease progression, and therapeutic responses, with potential applications in preclinical drug screening and personalized medicine.

OCRELIZUMAB COUNTERACTS THE UPREGULATION OF THE PKCβ/HIF-1α/VEGF PATHWAY IN PATIENTS WITH RELAPSING MULTIPLE SCLEROSIS

Naseer Kainat[1], Campagnoli Lucrezia Irene Maria[1], Pascale Alessia[1]
[1]Department of Drug Sciences, University of Pavia, Italy

kainat.naseer01@universitadipavia.it

Ocrelizumab (OCR) is a humanized anti-CD20 monoclonal antibody approved for Relapsing Multiple Sclerosis (RMS), a complex demyelinating and neurodegenerative disease of the central nervous system (CNS). While its mechanism of action mainly involves B-cell depletion, growing evidence suggests that CD20 binding also exerts notable effects on intracellular signalling cascades, including the Protein Kinase C (PKC) pathway. Specifically, the PKCβ isoform regulates Vascular Endothelial Growth Factor (VEGF) expression through the hypoxia-inducible factor 1 (HIF-1), a protein consisting of a constitutively expressed β subunit and an oxygen-sensitive active α subunit, whose expression is increased in hypoxic conditions. VEGF is an angiogenic factor implicated in critical physiological processes; however, a deleterious role has been reported in the early stages of RMS, where it acts as a pro-inflammatory factor mediating CNS injury and blood-brain barrier disruption.
In this study, we explored the effects of OCR on this novel intracellular signaling pathway by assessing PCKβ, HIF-1α, and VEGF protein expression in peripheral blood mononuclear cells (PBMCs) from RMS patients at baseline (T0) and after 12 months (T12) of treatment. PBMCs from RMS patients and age- and sex-matched healthy controls (HC) were used to evaluate the relative protein contents by Western blotting (WB) at both time points. At baseline (T0), WB analysis showed significantly higher expression levels of PKCβ, HIF-1α, and VEGF in MS patients compared to the HC group. After 12 months of OCR administration, a significant reduction in these protein levels was found, reaching values comparable to those of HC. Our study demonstrates that PCKβ, HIF-1α, and VEGF protein content is elevated in MS patients compared to HC, and that OCR treatment counteracts this increase. Given the detrimental role played by VEGF in early MS, this effect may represent an additional beneficial anti-inflammatory mechanism exerted by OCR.

DEXTRAN-BASED ELECTROSPUN NANOFIBERS FOR BIOMEDICAL APPLICATION

Julian Keenaghan, Marco Ruggeri, Barbara Vigani, Silvia Rossi, Giuseppina Sandri
Department of Drug Sciences, University of Pavia, Italy

julian.keenaghan01@universitadipavia.it

Electrospun nanofibrous scaffolds have attracted considerable attention for biomedical applications due to their ability to mimic the architecture of the extracellular matrix and support tissue repair.
This work focused on the development and characterization of two dextran-based electrospun scaffolds for potential regenerative applications.
A preliminary formulation study was carried out to optimize the dextran concentration for electrospinning. Different polymer concentrations were investigated, and the resulting fiber morphology was evaluated by scanning electron microscopy (SEM). The optimal dextran concentration was selected based on the production of homogeneous, bead-free nanofibers.
Once found the suitable dextran concentration, citric acid was incorporated, as crosslinking agent into the polymeric blend and different thermal conditions were evaluated to identify the optimal crosslinking treatment.
Subsequently, two distinct scaffold formulations were developed by incorporating norfloxacin together with either chitosan oligosaccharide or chondroitin sulfate as functional components to enhance cell proliferation and support tissue regeneration.
The obtained scaffolds were characterized in terms of morphology, fiber diameters, swelling behavior, degradation profile and mechanical properties.
SEM analyses confirmed the preservation of the fibrous architecture after crosslinking and hydration, while both formulations exhibited mean fiber diameters of approximately 800 nm. Mechanical characterization was performed by tensile testing to determine the maximum force at break, elongation at break, and Young's modulus of the electrospun scaffolds.
Overall, the developed electrospun scaffolds exhibited suitable physicochemical and mechanical properties, highlighting their potential for tissue engineering and regenerative medicine applications.

STUDY AND CHARACTERIZATION OF PEG-FREE OIL-IN-WATER NANOEMULSION

Maedeh Khalili, Francesca Birolini, Paola Perugini
Department Drug Sciences, University of Pavia

maedeh.khalili01@universitadipavia.it

The growing demand for safer and more sustainable cosmetic products has increased interest in PEG-free delivery systems and natural antioxidant actives. This work aimed to develop and characterize a PEG-free oil-in-water nanoemulsion for topical delivery of a hydro-glycolic Plantago lanceolata L. extract.
The optimised formulation was based on 5% medium-chain triglyceride oil phase, 10% PGPR/Polyglyceryl-6 Laurate surfactant blend and 0.25% xanthan gum and was processed by probe ultrasonication.
The free radical scavenging capacity of the extract as well and of nanoemulsions were quantified through spectrophotometric DPPH assay. The extract showed marked phenolic content and antioxidant activity, while the extract-loaded nanoemulsion preserved radical scavenging activity, showing 43.27% DPPH inhibition and 80.4% activity retention after 48 days of storage.
Multiple light scattering (MLS) technique was utilized to monitor the physical stability and evolution of the formulations during storage, with particular attention to the role of xanthan gum in influencing the kinetic behaviour of the system.
Dynamic Light Scattering (DLS) was used to determine droplet size, polydispersity index and ζ-potential. The formulations showed droplet sizes in the nanometric range (approximately 48–60 nm), confirming the formation of a nanometric system.
Overall, the developed PEG-free nanoemulsion represents a promising cosmetic platform for the topical delivery of P. lanceolata antioxidant extract.

IDENTIFICATION OF FORMULATION FEASIBILITY REGION OF VANCOMYCIN LOADED POLYMERIC NANOPARTICLES

Imad Khan[1], Abdourahmane Nidaye[2], Emilie Roger-Talibart[1]
MINT Laboratory, University of Angers, France

khanimad145@gmail.com

Oral delivery of peptide therapeutics remains challenging due to enzymatic degradation, poor gastrointestinal permeability, and low bioavailability. Vancomycin, a glycopeptide antibiotic used to treat severe Gram-positive bacterial infections, exhibits poor intestinal absorption following oral administration, highlighting the need for alternative delivery strategies. This study aimed to develop polymeric nanoparticles for oral vancomycin delivery, map the feasible formulation space using ternary phase diagram analysis, evaluate nanoparticle physicochemical properties, and compare the formulation region with that of nisin.

Vancomycin-loaded polymeric nanoparticles were prepared by polyelectrolyte complexation using varying proportions of Polymer 1, Polymer 2 and Vancomycin. A ternary phase diagram was constructed to define the feasible formulation region based on nanoparticle formation, particle size, and polydispersity index. Formulations that met the criteria of size and PDI were further characterized for encapsulation efficiency and drug loading capacity using ultra-performance liquid chromatography. Stable nanoparticles were obtained predominantly at intermediate Polymer 2 concentrations over a broad range of Polymer 1 compositions, whereas formulations containing ≤10% Polymer 1 resulted in precipitation and those with >60% were unstable. Particle size ranged from 162 to 422 nm, with formulation F8 exhibiting the highest encapsulation efficiency (>50%) and formulation F14 achieving the highest drug loading capacity (45%). Comparison with a previously established nisin formulation demonstrated distinct feasibility regions, indicating that nanoparticle formation is dependent on peptide characteristics and that formulation strategies are not directly transferable between peptides.

The identified formulation region provides solid foundation for subsequent Design of Experiments based optimization and further evaluation of these nanoparticles as a potential oral delivery system for vancomycin.

BIOMIMETIC ELECTROSPUN COMPOSITE SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

Elisabetta Maffioli, Marco Ruggeri, Carmela Tommasino, Barbara Vigani, Silvia Rossi, Giuseppina Sandri
Department of Drug Sciences, University of Pavia, Italy

elisabetta.maffioli01@universitadipavia.it

Articular cartilage has a limited capacity for self-regeneration, and untreated defects often progress to degenerative joint disease. Tissue engineering represents a promising strategy to support cartilage repair by mimicking the extracellular matrix while providing mechanical stability. The aim of this study was to develop and characterize electrospun nanofibrous scaffolds based on thermoplastic polyurethane, chitosan oligosaccharide, and collagen for cartilage tissue engineering applications.

Electrospun scaffolds containing increasing concentrations of chitosan oligosaccharide (MW ≤5 kDa, HMC–Chitoceuticals) were based on medical-grade thermoplastic polyurethane (Pathway™, Lubrizol Advanced Materials) and manufactured under optimized electrospinning conditions (15 kV, 0.397 mL·h^{-1} flow rate, 20 cm needle-to-collector distance, 25 °C, 25% RH). The scaffolds were subsequently coated with type I collagen (Advanced BioMatrix, 3 mg/mL, 2 min dip coating) to enhance cell attachment and provide a biomimetic microenvironment for chondrocytes. Scaffolds morphology, surface topography, nanomechanical and mechanical properties, collagen coating, wettability, degradation, and antioxidant activity were evaluated using scanning electron microscopy, atomic force microscopy, dynamic mechanical analysis, tensile testing, Fourier-transform infrared spectroscopy, bicinchoninic acid assay, contact angle measurements, in vitro degradation, and DPPH radical scavenging assay. All scaffolds consisted of randomly oriented nanofibers with uniform morphology and mean diameters of approximately 400 nm. The materials exhibited high flexibility and elastic moduli mimicking those of native cartilage while maintaining structural and mechanical integrity for up to 12 weeks. Increasing chitosan oligosaccharide content enhanced scaffold hydrophilicity, facilitating uniform collagen coating and creating an extracellular matrix-like surface suitable for cell adhesion. The presence of chitosan oligosaccharide also conferred antioxidant activity.

Overall, the developed composite scaffolds combine favorable mechanical performance, long-term stability, enhanced surface properties, and antioxidant capacity, making them promising candidates for cartilage regeneration. Future studies will investigate their biocompatibility and chondrogenic potential using mesenchymal stem cells and chondrocytes, as well as the antimicrobial properties imparted by chitosan oligosaccharide.

ANTIMICROBIAL EVALUATION OF CHLORPROMAZINE ENCAPSULATED IN LIPID-BASED FORMULATIONS

Jorge Eduardo Galván Marroquín[1], Eirini Mallikopoulou[1], Athanasios Skouras[1], Sophia Antimisiaris[1]

[1]Department of Pharmacy, School of Health Sciences, University of Patras, 26504 Patras, Greece

jorge.gama@exatec.tec.mx

Multidrug-resistant bacterial infections represent one of the major challenges facing modern healthcare. Drug repurposing and lipid-based drug delivery nanosystems are some strategies explored for solving this issue. Already approved medicines have well-established safety profiles, while nanocarriers can protect the drug and enhance its therapeutic efficacy. This study aimed to encapsulate chlorpromazine, a first-generation antipsychotic which has shown antimicrobial effects, into DOPS and PC/DPPG/Cholesterol liposomes, and DOPS cochleates, followed by evaluation of the physicochemical properties of these lipid-based formulations as well as their antibiofilm activity against *S. epidermidis* (ATCC 35984) mature biofilms. Physicochemical characterization showed that DOPS liposomes exhibited the smallest particle size, lowest polydispersity index and highest encapsulation efficiency. Despite these differences, all formulations remained physicochemically stable throughout a one-month evaluation. Regarding the antimicrobial assessment, the MTT and crystal violet assays showed that all treatments reduced bacterial metabolic activity and total biofilm biomass to similar extents. Loaded liposomes demonstrated a better antibiofilm activity than empty vehicles. Nonetheless, their activity (~25% biofilm reduction) was not significantly different from that of free chlorpromazine (~16% biofilm reduction). For cochleates, both empty and loaded samples showed a significant reduction of the biofilm, by approximately 50% and 90%, respectively. Empty cochleates displayed intrinsic antibiofilm activity, suggesting that the carrier itself contributes to biofilm disruption. Although liposomal encapsulation did not improve the antibiofilm activity of chlorpromazine under the evaluated conditions, additional studies on drug stability, degradation and cytotoxicity are needed to determine other potential advantages of encapsulation. In contrast, the marked intrinsic activity of cochleates highlights these structures as promising candidates for the development of alternative strategies against the multidrug-resistant crisis. However, the specific mechanism underlying their antimicrobial activity and their interactions with bacterial cells remain to be elucidated.

STABILITY OF BACTERIOPHAGE K-LOADED LIPOSOMES WITH DIFFERENT LIPID COMPOSITIONS DURING STORAGE AT 4°C

Malissa Noumbissie[1]; Eirini Mallikopoulou[1]; Sophia G. Antimisiaris[2]

[1]Department of Pharmacy, School of Health Science, University of Patras, 26504, Patras, Greece.
[2]Institute of Chemical Engineering & Sciences FORTH/ICES, Patras, Greece.

noumbissiemalissa17@gmail.com

The emergence of antimicrobial resistance has renewed interest in bacteriophage therapy as a promising alternative to conventional antibiotics. However, the therapeutic application of bacteriophages is limited by their susceptibility to environmental degradation during storage, highlighting the need for effective delivery systems. This study aimed to evaluate the influence of lipid composition on the physicochemical and biological stability of bacteriophage K-loaded liposomal formulations during storage at 4 °C. Three liposomal formulations with different lipid compositions, including a conventional formulation, a cationic formulation containing stearylamine, and a polyethylene glycol (PEG)-modified formulation, were prepared using the thin-film hydration method. Following membrane extrusion and purification, the formulations were characterized by particle size, polydispersity index, zeta potential, lipid concentration, encapsulation efficiency, and bacteriophage titer. Stability was assessed weekly over six weeks using Dynamic Light Scattering for physicochemical characterization and the Small Drop Agar Assay for bacteriophage titration. The cationic formulation achieved the highest encapsulation efficiency (100%), compared with 83.8% for the PEGylated formulation and 62.5% for the conventional formulation. Liposomal encapsulation significantly improved bacteriophage preservation compared with the free phage throughout storage. While the cationic formulation exhibited the greatest biological stability by maintaining the highest phage titer, the PEGylated formulation demonstrated superior physicochemical stability, maintaining a consistent particle size distribution, low polydispersity index, and stable zeta potential over the six-week study period. These findings demonstrate that lipid composition plays a critical role in the performance of bacteriophage-loaded liposomes. The incorporation of stearylamine enhances bacteriophage encapsulation and retention, whereas PEGylation improves long-term physicochemical stability, supporting the potential of tailored liposomal formulations for future bacteriophage-based therapeutic applications.

4D ELECTROSPUN POLYURETHANE SCAFFOLDS FOR TENDON TISSUE ENGINEERING

Gaston Ignacio Patat, Marco Ruggeri, Carmela Tommasino, Barbara Vigani, Silvia Rossi, Giuseppina Sandri
Department of Drug Sciences, University of Pavia, Italy

gastonignacio.patat01@universitadipavia.it

Electrospun fibrous scaffolds represent a promising strategy for tendon tissue engineering due to their ability to mimic the native extracellular matrix while providing suitable mechanical and biological properties. Thermoplastic polyurethane (TPU) is particularly attractive for biomedical applications because of its elasticity, mechanical stability, and availability of medical-grade formulations. In this study, aligned electrospun TPU scaffolds doped with magnetite nanoparticles (Fe_3O_4-MNPs) with different particle sizes were developed to obtain stimuli-responsive (4D) scaffolds for tendon tissue engineering. TPU solutions containing 0.1% and 0.02% concentrations and types of Fe_3O_4-MNPs were electrospun under optimized conditions to produce aligned fibrous scaffolds, which were characterized for morphology, nanoparticle distribution, physicochemical properties, wettability, mechanical behavior, and degradation. SEM confirmed the successful production of aligned fibers with diameters of approximately 500–600 nm. TEM and SEM-EDX analysis demonstrated the homogeneous incorporation and distribution of nanoparticles without affecting fiber alignment, while FTIR confirmed that nanoparticle incorporation did not alter the chemical structure of TPU. The scaffolds exhibited high stability during degradation, retaining more than 95% of their initial weight after twelve weeks, and their wettability and mechanical properties were influenced by nanoparticle formulation and loading. These findings demonstrate that aligned TPU-based electrospun scaffolds incorporating magnetite nanoparticles can be successfully fabricated and tuned to achieve desirable physicochemical and mechanical properties. Their magnetic responsiveness makes them promising candidates for the development of 4D scaffolds capable of providing electromagnetic stimulation to support tendon regeneration and guide cell proliferation and alignment.

SEMI-AUTOMATED 3D SEGMENTATION OF THE RAT INNER EAR USING ITK-SNAP

Nikita Yogesh Patil[1], Elisa Vivado[2], Roberto Pizzala[2], Paola Perin[3]
[1]Department of Drug Sciences, University of Pavia, Italy
[2]Department of Molecular Medicine, University of Pavia, Italy
[3]Department of Brain and Behavioral Sciences, University of Pavia, Italy

nikitayogesh.patil01@universitadipavia.it

Micro-computed tomography (micro-CT) is a valuable tool to visualize small and highly complex anatomical structures that are otherwise difficult to resolve from classical two-dimensional slides. The rat temporal bone is one such region where three-dimensional reconstruction can greatly improve anatomical understanding. The aim of this project was to learn and apply image-based segmentation techniques to generate a 3D reconstruction of the rat inner ear from micro-CT data.
High-resolution (6.43 µm) micro-CT scans of the rat temporal bone were first pre-processed in FIJI to adjust contrast and define a region-of-interest (ROI). The dataset was then imported into ITK-SNAP for semi-automated segmentation. Fluid-filled cavities corresponding to the rat inner ear were traced slice by slice using seed points ("bubbles") and active contour tool ("Snake segmentation"), followed by user-guided boundary refinement. The resulting segmentation was rendered into a three-dimensional model that accurately reflected the convoluted structures of the rat bony labyrinth.
This work shows that user-refined semi-automated segmentation provides a sufficiently quick and reliable approach for visualizing small and complex anatomical regions. The resulting 3D reconstruction supports clear anatomical interpretation and highlights the usefulness of image-based workflows in biomedical and nanomedicine research.

CHITOSAN-COATED FUCOIDAN/POLY-LYSINE NANOGELS FOR THE PREVENTION OF ORAL DYSBIOSIS-RELATED RESPIRATORY INFECTIONS

Mariasofia Perucchini[1,2], Barbara Vigani[1], Caterina Valentino[1], Marco Ruggeri[1], Giampiero Pietrocola[3], Giuseppina Sandri[1], Silvia Rossi[1]
[1]Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia, Italy
[2]PhD National Program in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, 27100, Italy
[3]Department of Molecular Medicine, University of Pavia, Via Taramelli 3/b, 27100, Pavia, Italy

mariasofia.perucchini01@universitadipavia.it

The oral cavity hosts a complex microbial ecosystem essential for both oral and systemic health. Disruptions of this balance, known as oral dysbiosis, has been associated with local diseases and increased susceptibility to respiratory infections. Current preventive strategies (e.g. antiseptic mouthwashes and probiotic-based products) are limited by low specificity and short residence time within the oral cavity. This study aimed to develop chitosan-coated fucoidan/-poly-L-lysine nanogels (Cs-coated FD/Poly-L NGs) as a mucoadhesive oral spray designed to prevent dysbiosis-related respiratory complications.
Nanogels (NGs) were prepared via ionotropic gelation by mixing FD and Poly-L solutions (0.25–2% w/v and 0.025–0.2% w/v) at different v/v ratios (1:1, 2:1, 3:1). The 1:1 and 2:1 FD/Poly-L ratios yielded the highest particle concentrations with a hydrodynamic diameter (HD) around 200 nm and negative zeta potential values (-20 mV), as determined by NTA and ELS. The most promising formulation, selected for the smallest HD and the highest particle concentration, was coated with Cs solutions (0.25–1% w/v) to obtain mucoadhesive NGs (CsNGs). Cs coating shifted the zeta potential to positive values (+20 mV) and increased the HD to 300–400 nm. Spherical NG morphology was confirmed using SEM and TEM. Turbidimetric assay demonstrated NG interactions with mucins, particularly at Cs concentrations of 0.35% and 0.5% w/v. Suitability for oral spray administration was supported by viscosity, spray pattern, surface tension, and contact angle analyses. Finally, CsNGs were proved to be fully cytocompatible with Normal Human Dermal Fibroblasts through Alamar Blue assays and showed antimicrobial properties against S. aureus and P. aeruginosa.
In conclusion, mucoadhesive Cs-coated FD/Poly-L NGs were successfully developed. Their mucoadhesive properties may prolong residence time on the oral mucosa, while their sprayability ensures uniform distribution throughout the oral cavity. Furthermore, their antibacterial activity supports NG potential as a promising formulation for preventing dysbiosis-related complication.

FABRICATION AND CHARACTERIZATION OF 4D PLA:PCL (70:30) SCAFFOLDS PRODUCED VIA DIRECT INK WRITING (DIW)

Mariia Podkidysheva[1], Luigi Ruccolo[2], Silvia Pisani[1], Bice Conti[1]
[1]Department of Drug Sciences, University of Pavia, Italy
[2]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

mariia.podkidysheva01@universitadipavia.it

Biodegradable 4D porous scaffolds have emerged as promising platforms for tissue engineering (TE) and localized drug delivery. Unlike conventional three-dimensional constructs, 4D scaffolds are designed to undergo a controlled change in shape or function in response to external stimuli, enabling dynamic interactions with the surrounding biological environment. Combined with their ability to provide temporary mechanical support and progressively degrade after implantation, these features make them attractive candidates for a broad range of regenerative medicine applications requiring minimally invasive deployment and site-specific therapeutic delivery. Among these materials, blends of offer a promising combination of mechanical performance and controlled biodegradation.
Therefore, the aims of this study were to manufacture poly-llactic acid and polycaprolactone (PLA/PCL 70:30) scaffolds by using DIW, investigate shape-memory effect and evaluate the degradation properties under physiological and accelerated conditions by Gel Permeation Chromatography. Furthermore, the structural and morphological changes of the scaffolds after 35 days of exposure to PBS (pH 7.4) and PBS/H2O2 (pH 6.5) media were characterized by Scanning Electron Microscopy (SEM).
As a result, the developed scaffolds demonstrated excellent shape-memory properties with a high shape fixity ratio (~88.5%) and shape recovery ratio (~95.5%). Degradation studies revealed a faster polymer breakdown in physiological PBS compared to accelerated PBS/H_2O_2 conditions, as evidenced by a greater reduction in molecular weight, higher mass loss, and a significant increase in porosity (from 27.85% to 49.65% after 35 days). SEM analysis confirmed progressive surface erosion while the overall scaffold architecture remained preserved.
Future investigations should extend the degradation studies to longer time periods and employ experimental models that more closely reproduce the physiological conditions encountered in vivo. In particular, dynamic testing environments capable of simulating fluid exchange and mechanical stimuli would provide a more realistic assessment of scaffold integrity, shape-memory functionality, and degradation behavior throughout the implantation period. Such studies would contribute to a better understanding of the long-term performance of these systems and support their translation toward a broad range of tissue engineering and drug delivery applications, including minimally invasive regenerative approaches for congenital defects such as spina bifida.

THERMAL CHARACTERISATION OF EUGENOL-BASED NANOSTRUCTURED LIPID CARRIERS

Zalkifel Rao, Maria Cristina Bonferoni, Laura Catenacci, Sara Perteghella, Rachele Rossi, Milena Sorrenti
Department of Drug Sciences, University of Pavia, Italy

zalkifel.rao01@universitadipavia.it

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are lipid-based Nano carriers that can offer a promising platform to enhance the bioavailability and controlled release of encapsulated actives. In this study, eugenol was introduced into an NLC formulation as an "active" excipient, acting as a structural liquid lipid component and a bioactive agent. To overcome the aqueous instability and high volatility of bioactive adjuvants such as essential oils, the liquid Nano carrier suspensions were converted into dry powders via freeze-drying. Because the structural integrity of lipid carriers can be compromised during lyophilisation, this research investigated the efficacy of two different cryoprotectants, trehalose and hydroxypropyl-beta-cyclodextrin (HP-β-CD), evaluating how their positional placement, within the particle matrix (IN), in the external aqueous phase (OUT), or in both compartments simultaneously (IN+OUT) influences lipid matrix crystallinity. SLN based on the same solid lipid phase were prepared as a reference.
This study aimed to characterise the solid-state properties of lyophilized samples by means of thermal analysis, in particular using differential scanning calorimetry (DSC) to analyse the samples in terms of crystalline or amorphous nature related to the excipient used and its placement in the matrix. This preliminary characterization was performed using a single heating cycle from −30 °C to 400 °C at 10 K/min under nitrogen.
DSC analyses revealed that NLC formulations exhibited significantly lower lipid crystallinity indices compared to SLN controls, consistent with eugenol disrupting lipid crystal packing. The cryoprotectant placement critically determined the post-lyophilisation physical state and lipid matrix ordering; dual IN+OUT arrangement offering superior stabilization; future work will assess long-term storage stability.
Eugenol-based NLCs can be rationally engineered as multifunctional systems by integrating structural and bioactive functionalities within a single platform. The integration of eugenol, combined with the optimized HP-β-CD lyophilisation strategy, resulted in a robust solid-state Nano carrier system.

DEVELOPMENT OF ANISOTROPIC CORE-SHELL ELECTROSPUN NANOFIBERS DOPED WITH LIGNIN NANOPARTICLES FOR QUERCETIN DELIVERY IN VOLUMETRIC MUSCLE LOSS

Paolo Santagostini[1], Caterina Valentino[1], Barbara Vigani[1], Marco Ruggeri[1], Giuseppina Sandri[1], Silvia Rossi[1]
[1]Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia, Italy

paolo.santagostini01@universitadipavia.it

Volumetric Muscle Loss (VML) is a severe condition caused by traumatic or surgical loss of skeletal muscle tissue. This irreversible injury is characterized by prolonged oxidative stress and inflammatory responses, leading to functional loss and disability. This project aims to develop polycaprolactone/hydrolyzed collagen (PCL/HC) anisotropic core–shell nanofibers (ACS-NFs) loaded with lignin nanoparticles for quercetin delivery in skeletal muscle tissue. PCL was selected for its mechanical resistance and biodegradability, while HC as a bioactive component capable of rapid dissolution. Lignin was chosen as a natural polymer able to sustain the controlled release of quercetin, an antioxidant polyphenol.
PCL/HC solutions in acetic acid were prepared at different concentrations. Surface tension and conductivity were evaluated. Subsequently, these solutions were subjected to electrospinning process using a static collector plate. Homogeneous and smooth randomly-oriented NFs (R-NFs) were successfully obtained from PCL/HC blends, whereas bead-free nanofibers could not be produced from PCL alone. Aligned NFs (ANFs) were then obtained by using a rotating collector. Both R-NFs and A-NFs were characterized, in dry and hydrated state, for their: i) morphology by means of SEM; ii) topography by means of AFM; iii) static and dynamic mechanical properties by means of texture analyzer and DMA.
Thereafter, to obtain blank lignin nanoparticles (L-NPs) lignin was dissolved at different concentrations in 80:20 acetone:water as organic phase and mixed with 0.5 M aqueous acetic acid in the microfluidics device. The total flow rate was kept constant at 3 mL/min. Different flow rate ratios ranging from 1:1 to 8:1 were tested. L-NPs were characterized for their: i) particle size and ζ potential by means of DLS and ELS; ii) concentration by means of NTA; iii) morphology by means of TEM. Similarly, quercetin will be co-dissolved with lignin to obtain quercetin loaded L-NPs.
Finally, ACS-NFs will be fabricated through coaxial electrospinning by incorporating the most promising QL-NPs into the fiber core. *In vitro* assays on C2C12 myoblasts will be performed.

FAST-DISSOLVING PREBIOTIC NANOFIBERS LOADED WITH OLEIC ACID/B-CD INCLUSION COMPLEX FOR THE TREATMENT OF BACTERIAL VAGINOSIS

Irene Segato[1,2], Barbara Vigani[1], Caterina Valentino[1], Chiara Milanese[3], Giorgia Piccinini[2,4], Fabio Longo[4], Marco Ruggeri[1], Giuseppina Sandri[1], Silvia Rossi[1]
[1]Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia, Italy; [2]PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, 27100, Italy
[3]University of Pavia, Department of Chemistry, Via Taramelli 12, 271004Department of Public Health and Pediatrics, University of Torino, Turin, Italy
[4]Department of Public Health and Pediatrics, University of Torino, Turin, Italy

irene.segato01@universitadipavia.it

Bacterial vaginosis (BV) is a recurrent dysbiosis characterized by Lactobacillus spp. depletion and anaerobic bacterial overgrowth, requiring strategies that restore microbial balance while limiting pathogen proliferation. In this context, oleic acid (OA), a plant-derived fatty acid, is a promising broad-spectrum antibacterial agent, although its clinical use is limited by poor water solubility. This study aimed to improve OA solubility through β-cyclodextrin (β-CD) inclusion complexation and incorporate the resulting OA/β-CD inclusion complex (IC) into fast-dissolving dextran/pectin (DEX/PEC) electrospun nanofibers as a potential local delivery system for BV treatment.
The IC was prepared via co-precipitation and characterized by SEM, FTIR, XRPD, and DSC, using a physical mixture as reference. Its antibacterial activity was evaluated against Gardnerella spp. and several Lactobacillus strains.
Different solutions containing DEX (25–30% w/w) and PEC (1.0% w/w) were prepared in PBS 1X, assessed for their rheological properties, conductivity, and surface tension, and subsequently electrospun. Process parameters, including applied voltage and flow rates, were optimized to ensure proper fiber formation. The obtained nanofibers were analyzed for morphology and diameter through SEM and ImageJ software, respectively. Physicochemical analyses confirmed IC formation. Antibacterial assays demonstrated IC selective activity, with MIC values of 125–250 µg/mL against Gardnerella spp. and dysbiosis-related Lactobacillus strains, compared to >2000 µg/mL against beneficial L. gasseri. Among the tested polymeric solutions, the one containing 30% DEX,1% PEC, 5% IC and 0.3% (w/w) Tween-80 produced uniform nanofibers with a mean diameter of 0.552 ± 0.071 µm. Moreover, the nanofibers were biocompatible with normal human dermal fibroblasts at all tested doses. Ongoing studies will evaluate fiber mechanical properties and their prebiotic potential. Overall, this approach provides a feasible strategy to enhance OA processability and selective antibacterial performance within a biocompatible fast-dissolving nanofibrous matrix.

FORMULATION STRATEGIES FOR ANTI-eNAMPT NANO AND MICRO CARRIERS

Gaia Sensi, Bice Conti, Silvia Pisani, Cristina Travelli, Ida Genta, Rossella Dorati
Department of Drug Sciences, University of Pavia, Italy

gaia.sensi01@universitadipavia.it

Poly(lactic-co-glycolic acid) (PLGA) micro and nanoparticles are biodegradable and biocompatible drug delivery systems widely investigated for the controlled delivery of therapeutic proteins. Their ability to protect biologics from degradation and provide sustained release makes them attractive carriers for antibody-based therapies targeting extracellulr nicotinamide phosphoribosyltransferase (eNAMPT), a protein implicated in kidney cancer progression. The aim of this study was to develop and optimize PLGA submicron nano- and microcarriers for anti-eNAMPT antibody encapsulation, preventing cellular uptake and enabling controlled extracellular release for approximately 360 hours. This strategy is intended to reduce injection frequency and enhance therapeutic activity against eNAMPT-overexpressing kidney tumors. PLGA particles were prepared by nanoprecipitation and double emulsification. A Design of Experiments (DOE) approach was applied to nanoprecipitation by varying PLGA concentration (10, 15, 20, and 40 mg/mL) and needle diameter (14 and 15 G) to identify conditions yielding larger particles suitable for the intended application. Bovine serum albumin (BSA) was used as a model protein to evaluate encapsulation feasibility. Nanoprecipitation generated PLGA particles, with particle size positively correlated with PLGA concentration, whereas needle diameter had a limited effect. However, all formulations showed high polydispersity and failed to encapsulate BSA, indicating poor suitability for BSA loading. Double emulsification was investigated using magnetic stirring and probe sonication. Formulations prepared by probe sonication with a Sonics Materials VC-505 Ultrasonic Processor exhibited encapsulation efficiency 58.87% and drug content 0.04%, higher than those obtained by magnetic stirring, and particle size in the range 20-0.5µm, although particle heterogeneity remained a challenge.
Overall, double emulsification, particularly when combined with probe sonication, was more suitable than nanoprecipitation for encapsulating BSA in PLGA carriers. Future perspectives include anti-eNAMPT antibody loading and formulation optimization to achieve sustained anti-eNAMPT release in extracellular environment.

Funding: the project is funded by Immunohub grant from Italian Ministry of Health.

SECONDARY CELL CULTURE - SEEDING, CELL PASSAGING, AND CELL COUNTING

Nallahandige Malmi Gayashi Silva[1], Lavatelli Francesca[2]
[1]Department of Drug Sciences, Università di Pavia, Italy
[2]Department of Molecular Medicine, Università di Pavia, Italy

nallahandigema.silva01@universitadipavia.it

In vitro cell culture techniques are essential for studying cellular behavior, ranging from primary cultures obtained directly from tissues via tissue dissociation to secondary cultures derived from subculturing.
The objective of this work was to follow a protocol for the routine cell cultivation, seeding, and passaging of secondary cells to ensure high cell viability and homogeneity for reproducible studies.
To maintain sterile environment, all procedures were performed inside a laminar air flow cabinet after sanitizing hands and working surfaces with 70% ethanol.
First, we removed the cryovial from liquid nitrogen and liquified the frozen cells rapidly at 37°C to limit cryoprotectant toxicity. Using sterile serological pipettes, the cells were seeded into culture flasks containing high-glucose Dulbecco's Modified Eagle Medium with appropriate supplements. Flasks were incubated at 37°C with slightly loosened caps to enhance gas exchange. Once the cells reached high confluence, the monolayer was washed with phosphate-buffered saline to remove serum-derived trypsin inhibitors. We then added a trypsin-EDTA solution for five minutes to detach the cells from the flask through enzymatic dissociation; cells were counted utilizing a Bürker chamber to check the cell density. Trypsin was neutralized by adding serum-containing medium, and the suspension was centrifuged at 1000 g for five minutes. At this point, for cell passaging, the pellet was resuspended into fresh medium and cells were distributed into new flasks; instead, for long-term storage, cells were resuspended in freezing medium (50% serum, 40% basal medium, and 10% dimethyl sulfoxide), and cooled gradually to -80°C, before storing them into liquid nitrogen.
Following these disciplined cell culture and aseptic procedures keeps the cells healthy, prevents cellular stress, and eliminates microbial contamination. Properly executing this baseline seeding and passaging steps reduces experimental variability, giving us reliable data for future applications using these secondary cell lines.

DEVELOPMENT AND FORMULATION OF A HIGHLY SOLUBLE ALPHA-LIPOIC ACID INJECTABLE SOLUTION

Maria Grazia Silvestri[1], Tommaso Pelagatti[2]
[1]University of Pavia, master's degree in Industrial nanobiotechnologies for pharmaceuticals
[2] Farmacia Tili, Erba (CO)

mariagrazia.silvestri02@universitadipavia.it

Alpha-lipoic acid (ALA) is a potent antioxidant with widespread therapeutic potential across various clinical fields. However, its clinical efficacy and formulation feasibility are severely constrained by its poor aqueous solubility (0.01 g to 0.87 g per 100 mL), which limits its development into stable, high-concentration injectable forms. Therefore, it was necessary to devise a strategy to overcome these hydrophilic limitations and develop a biocompatible injectable solution.
To enhance solubility, ALA was reacted with sodium hydroxide (NaOH) in a precise 1:1 molar ratio in an aqueous medium. This neutralization reaction targets the carboxylic acid group of ALA (pKa approx 4.7), converting the lipophilic free acid into its highly water-soluble sodium salt, sodium thioctate. During the process, it is clearly observable that the solution transitions from a turbid yellow—indicating that the substance is undissolved—to a clear yellow, signifying the complete dissolution of alpha-lipoic acid. Given the extreme alkalinity of the resulting primary mixture, the solution was carefully buffered and titrated using hydrochloric acid (HCl) to achieve a physiological pH of 7.4, ensuring safety and tolerability for intravenous or intramuscular administration.
The salt-formation approach demonstrated a remarkable increase in aqueous solubility. While raw ALA remains practically insoluble in pure water, the newly formulated sodium thioctate solution achieved a maximum solubility threshold of up to 34 g per 100 g of water. The resulting injectable solution remained clear, fully neutralized, and physically stable at physiological pH.
Utilizing a 1:1 molar neutralization with NaOH successfully bypasses the inherent solubility barriers of alpha-lipoic acid. By transforming the compound into sodium thioctate and adjusting it to physiological pH, this method yields a highly concentrated, versatile injectable solution suitable for diverse biomedical and therapeutic applications.

EXPLORING THE IN VITRO EFFICACY OF GREEN-SYNTHESIZED PRUSSIAN BLUE NANOPARTICLES ACROSS DIFFERENT BREAST CANCER MODELS

Nadia Mayumi Vilela Bartnick Tanaka[1], Marialaura Giannaccari[1], Laura Ghidoni[2], Eleonora Galleano[3], Paolo Minzioni[3], Giacomo Dacarro[2], Livia Visai[1,4] and Nora Bloise[1,4]
[1]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), Unità di Ricerca (UdR) INSTM, Operative Unit (OU) of the Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research (Centro 3R), University of Pavia, 27100 Pavia, Italy; [2]Department of Chemistry, University of Pavia, Viale Taramelli, 12, 27100 Pavia, Italy; [3]Department of Electrical, Computer and Biomedical Engineering, University of Pavia, 27100 Pavia, Italy; [4]UOR6 Nanotechnology Laboratory, Department of Prevention and Rehabilitation in Occupational Medicine and Specialty Medicine, Istituti Clinici Scientifici Maugeri IRCCS, Via Maugeri 4, 27100 Pavia, Italy

nadiamayumi.vilelabartnick01@universitadipavia.it

Nanoparticle-based cancer therapy represents an area of emerging interest for research focused on overcoming the challenges of traditional therapies, increasing efficiency for the treatment of oncological patients. In this field, Prussian Blue Nanoparticles (PBNPs) can be used as potential breast cancer therapeutic applications, due to their high porosity, large surface area, and photothermal potential application.
This project focused on biocompatible PBNPs, synthetized with citrus-derived pectin as a sustainable and reducing agent, instead of conventional Polyvinylpyrrolidone (PVP), loaded with Doxorubicin (Dox), and the evaluation of their therapeutical efficacy across different breast cancer models.
A comprehensive physicochemical characterization of PBNPs through Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Ultraviolet–visible Spectroscopy (UV-Vis) confirmed the formation of stable nanocarriers.
Moreover, nanoparticles exhibited good Dox encapsulation efficiency together with a favorable drug release.
Then, the effect of both empty and Dox-loaded PBNPs was evaluated on SKBR-3 and MCF-7, two different breast cancer cell lines. Cells viability was analyzed by assay Cell Counting Kit-8 (CCK-8) that demonstrated a marked decrease in cell viability. TEM imaging confirmed effective nanoparticle uptake and cytosolic localization.
Finally, Confocal Laser Scanning Microscopy (CLSM) revealed pronounced cytoskeletal rearrangements in cells treated with Dox-loaded PBNPs, supporting the quantitative evidence of their anticancer activity.
Overall, these results demonstrate the potential of pectin-stabilized PBNPs as biocompatible and effective platforms for therapeutic drug delivery. Future studies will explore the synergistic effect between drug-loaded PBNPs and photothermal therapy, with expected results of laser-induced hyperthermia, which increases the chemotherapeutic release and effect.

DESIGN AND CHARACTERIZATION OF AMPHIPHILIC CYCLODEXTRIN-BASED NANOEMULSIONS

Filippo Truddaiu, Laura Catenacci, Sara Perteghella, Rachele Rossi, Milena Sorrenti, Maria Cristina Bonferoni
Department of Drug Sciences, University of Pavia, Italy

filippo.truddaiu01@universitadipavia.it

Cyclodextrins (CDs) are useful excipients featuring a hydrophobic inner cavity and a hydrophilic outer surface that enables the formation of inclusion complexes to improve the solubility and stability of poorly water-soluble compounds.
This study aims to design and characterize nanoemulsions based on two amphiphilic γ-CD derivatives (ACDs), CDγ170 and CDγ171, loaded with α-(+)-pinene in a lipophilic phase (Labrafac™ lipophile or olive oil) evaluating their self-assembly behavior, based on the HLB balance. The resulting nanoemulsions are intended as precursors for future topical formulations enabling controlled α-(+)-pinene release.
Nanoemulsions were prepared by self-assembling in aqueous phase, after prior solubilization of the components in suitable organic solvents and subsequent solvent evaporation. After an initial exploratory phase at low concentrations, the work was then extended to a formulation scale-up, and the stabilization ability of ACDs was tested for two total oily phase concentrations, 8 mg/ml and 24 mg/ml.
The resulting dispersions were characterized by dynamic light scattering and zeta potential measurements by a DLS apparatus (Litesizer 500, Anton Paar, Rivoli, TO, Italy). A dimensional stability study of the nanoemulsions was also performed at 4 °C or −20 °C for up to 30 days. Dispersions with the lower concentration and stored at 4 °C proved to be the most stable, maintaining hydrodynamic diameters below 300 nm, with limited time-dependent changes. Increasing concentration led, for both ACDs, to a slight increase in particle size and a slight broadening of size distribution. The results obtained under freezing conditions indicate aggregation/coalescence and the loss of the original nanostructure.
Overall, these findings highlight that these ACDs effectively stabilize α-(+)-pinene nanoemulsions. Storage under freezing conditions requires further investigation regarding the potential use of cryoprotectants.

MICROWAVE ASSISTED SYNTHESIS OF ORGANIC COMPOUNDS

Alice Zani[1], Giacomo Rossino[1]
[1]Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100, Pavia PV, Italy

alice.zani01@universitadipavia.it

Microwave-assisted organic synthesis has become a valuable approach for performing chemical transformations under rapid and controlled heating conditions, often enabling efficient reaction optimization and reduced processing times. In this work, small- and medium-scale organic reactions were carried out using a monomodal microwave reactor (CEM Discovery®) under temperature-controlled irradiation conditions.
The aim of this internship project was to perform selected organic reactions under microwave irradiation and to purify and characterize the resulting products.
A palladium-catalyzed microwave-assisted Heck coupling between 2-bromonaphthalene and ethyl crotonate was performed under microwave irradiation, yielding the desired tri-substituted E alkene as main product, and a mixture of isomers as byproducts. These were subjected to microwave-assisted isomerization using catalytic iodine in isopropanol to promote conversion toward the thermodynamically favored E isomer, thereby reducing waste. To evaluate the applicability of microwave heating to a different reaction class, an N-alkylation between tert-butyl 4-(phenylamino)piperidine-1-carboxylate and ethyl 2-bromoacetate was also investigated in preliminary experiments, affording the corresponding functionalized piperidine derivative.
Reaction progress was monitored by thin-layer chromatography (TLC), and products were isolated via liquid–liquid extraction using a separatory funnel, followed by purification using flash column chromatography. The purified products were characterized by proton nuclear magnetic resonance spectroscopy (NMR), confirming their structures. All investigated transformations were successfully accomplished under microwave irradiation, demonstrating good compatibility of the method with different reaction types and substrates.
The study highlights the potential of microwave irradiation as a useful tool in synthetic and medicinal chemistry, particularly for rapid and efficient screening and optimization of reaction conditions, and for the acceleration of reactions traditionally performed under conventional heating conditions.