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CNR NANOTEC
INSTITUTE OF NANOTECHNOLOGY

EDGE-TECH

LECCE 27 - 28 JUNE 2024

WORKSHOP

Emerging and disruptive next-generation technologies: materials, microfluidics and sensing for biomedicine

SCIENTIFIC COMMITTEE

- MariaSerenaChiriacò
- Antonio Turco
- Francesco Ferrara
- ElisabettaPrimiceri
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PROGRAM

27 - June - 2024

8:30 – 9:30 Registration

9:30 – 10:15 Introduction and Welcome

Dr. Alessandro Delli Noci (Department of economic development - Apulia Region); Dr. StefanoFabris (DSFTM – CNR); Prof. Alessandro Sannino (Experimental Medicine Department – UniSalento); Prof. Giuseppe Gigli (CNR NANOTEC – Tecnomed),

Materials for Biomedicine

Section co-Chairs: Antonio Turco, Viviana Vergaro

10:15 – 11:05 Prof. Maurizio Prato (LECTURE) – University of Trieste: *"Nanomaterials for the Repairof Spinal Cord Injury"*

11:05 - 11:25 Coffee Break

11:25 - 11:45 Dr. Francesca D'Elia - RWTH Aachen: "*Light-responsive polymers for neuro-inspired devices"*

11:45 - 12:05 Dr. Rossana Rauti – University of Urbino: *"Carbon-based nanomaterials interfacingwith neurons: impact on signalling and regrowth"*

12:05 - 12:25 Prof. Sonja Visentin – University of Torino: "*Mucosomes: innovative glycosylatedmucin based nanoparticles as multi drug delivery platform*"

12:25 – 12:45 Prof. Lucia Curri – University of Bari: "*Synthetic, bioinspired and natural nanovectors: versatile platforms for diagnostic and therapeutic applications"*

Technologies for sensing

Section co-Chairs: Francesco Ferrara, Elisabetta Primiceri

14:00 - 14:50 Dr. Raphael Pugin (LECTURE) – CSEM Neuchatel: *"Micro and nano fabrication technologies to the service of modern days medical challenges"*

14:50 - 15:10 Prof. Alejandro Criado Fernàndez – University of A Coruña: "*Tailored chemicaldesigns for graphene FETs as biosensors to detect large and small molecules*"

15:10 - 15:30 Dr. Stefano Bonaldo - University of Padova: *"Modeling and Multiphysics Simulationsof the Electrical Response of Electrochemical Sensors"*

15:30 - 15:50 Dr. Luca De Stefano - CNR ISASI Napoli: "Hard or soft? The next generation of optical transducers is…"

16:05 - 17:05 Coffee Break & poster session

28 – June - 2024

Devices for Microenvironment in Biomedicine

Section co-Chairs: Elisabetta Primiceri, Maria Serena Chiriacò

09:00 - 09:30 Introduction

09:30 -10:20 Prof. Jean-Luis Viovy (LECTURE) - Institut Curie-Paris: *"New microfluidic formats forthe growth and analysis of 3D cellular assemblies: droplets and gel-embedded microchannels"*

10:20 - 10:40 Prof. Marco Costantini – Polish Academy of Sciences: *"From muscle to tendon: engineering complex tissues and tissue interfaces using bioprinting"*

10:40 - 11:00 Dr. Adele De Ninno – CNR IFN, Roma: "Oncology meet Organs-on chips: dissecting cellular interactions in heterogeneous cancer ecosystems"

11:00 - 11:30 Coffee break

11:30 - 11:50 Prof. Marco Gaspari – University of Magna Graecia, Catanzaro: *"Biofluid proteomicsin prostate cancer"*

11:50 - 12:10 Dr. Valentina Marassi– University of Bologna "Seeking informativeness fromseparation science: the need for profilomics"

12:10 - 12:30 Dr. Christian Baumgartner – Graz University of Technology: *"Digital twins inbiomedical sciences: From the cell to the whole human"*

14:00 - 14:30 Best poster award and closing remarks14:30

- 15:30 Laboratory tour

Oral Contributions

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Abstract

Nanomaterials for the Repair of Spinal Cord Injury

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Abstract:
Spinal cord injury is a most devastating disease, as it causes a permanent loss of motor functions, causing enormous personal, social and economic problems. Neural regeneration has been shown to be a natural process; however, the regeneration mechanisms of the centralnervous system are generally ineffective in restoring appropriate function. Therefore, there is a tremendous social and medical pressure along with research interest to discover new therapeutic strategies for the effective repair of the spinal cord injury. Repairing spinal cordinjuries is far from simple, but new interdisciplinary research approaches through cutting- edge technologies and revolutionary concepts are raising hopes in promoting effective self- repair strategies. Cell- and biomolecule-based delivery strategies and therapeutic strategies based on novel tissue regeneration scaffolds have been developed in this direction. More recently, with a trend towards a combinatorial approach, regenerative/neural engineering therapies, prosthetics, neural engineering, rehabilitation engineering, bio-inspired robotics have been combined to develop advanced intelligent systems that promote spinal plasticity, regeneration and repair.
Nanomaterials are increasingly being used in this field, especially due to their size, which allows a particularly efficient control of their physical and chemical properties. In fact, connecting nanostructured materials to biological compartments is a crucial step in prosthetic applications, where the interfacing surfaces should provide minimal undesired perturbation to the target tissue. Ultimately, the (nano)material of choice has to be biocompatible and promote cellular growth and adhesion with minimal cytotoxicity or dis-regulation of, for example, cellular activity and proliferation.
In this context, carbon nanomaterials, including nanotubes and graphene, are particularly well suited for the design and construction of functional interfaces. This is mainly due to the extraordinary properties of these materials, which combine mechanical strength, thermal and electrical conductivity.
Our group has been involved in the organic functionalization of various types of nanocarbons, including carbon nanotubes, fullerenes and graphene. The organic functionalization offers the great advantage of producing soluble and easy-to-handle materials. As a consequence, since biocompatibility is expected to improve upon functionalization, many modified carbon nanomaterials may be useful in the field of nanomedicine.
In particular, we have recently shown that carbon nanotubes can act as active substrates for neuronal growth, a field that has given so far very exciting results. Nanotubes are not only compatible with neurons, but, especially, they play a very interesting role in interneuronal communication. Improved synaptic communication is just one example.
During this talk, we will discuss about the most recent attempts to regenerate the electrical connection in the lesioned spinal cord, with particular emphasis on the latest and most exciting

results obtained in our laboratories in this fast developing field.

Abstract

Light-responsive polymers for neuro-inspired devices

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Keywords: light-driven polymers, optoelectronic, neuromorphic

Abstract:
The development of novel electronic devices that interact with neurons is pivotal to understanding what drives neuronal behavior in order to address brain disorders and mirror efficient neuron-neuron communication mechanisms. So far, great efforts have been devotedto studying neuro-hybrid interfaces or neuromorphic platforms based on a wide range of inorganic and organic materials. Achieving perfect cell-chip coupling in these systems is stillchallenging, as they often do not exhibit the biocompatibility and mechanical compliance required for neuronal interfaces [1,2]. However, even when these conditions are met, most systems lack one of the main functionsof biological matter: dynamic adaptation to external stimuli. One way to achieve this is to use stimuli-responsive materials that change their properties in response to a specific trigger.Among them, light-responsive polymers represent a soft and biocompatible platform that canbe remotely tuned by applying or removing a light stimulus, with potential impact on next generation neuroelectronic interfaces [3].
Here we report the exploitation of polymers based on the light-sensitive azobenzene moleculeto realize two dynamic neuro-inspired devices. On the one hand, we engineered an azo- polymer to be re-shaped by green light illumination. This material shows cell instructive behavior, in fact cells cultured on azo-polymer patterns reorganize their cytoskeleton accordingly to the topographical change of the substrate underneath [4]. Furthermore, the re-shapable azo-polymer can be coated with a conductive polymeric layer functionalized witha supported lipid bilayer that adapts to the light-induced changes [5]. On the other hand, we cross-linked the azobenzene molecule to a conductive polymeric layer to develop organic photoelectrochemical transistors that exhibit short- and long-term plasticity as a function of light-stimulation [6].
All these results pave the way to real neuro-inspired devices capable of emulating physiological synaptic plasticity and learning abilities.

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Abstract

Carbon-based nanomaterials interfacing with neurons: impact on signalling and regrowth

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Keywords: neuronal network; carbon nanotubes; graphene; 3D-scaffolds; electrophysiology

Abstract:
Modern neuroscience increasingly relies on material science tools to engineer neuronal network models *in-vitro*. The synergy between engineering and technology with biology can lead to the development of materials and devices for the treatment and monitoring of pathological conditions as well as novel therapeutic strategies. In this framework, carbon- based materials, such as carbon nanotubes (CNTs) and graphene (GR), deserve particular attention, featuring dimensions and properties similar to neural machinery compartments; moreover, they have already been shown to govern *in-vitro* synapse formation, cell excitability, and synaptic processing [1,2]. These materials, composed by pure carbon withdifferent hybridization or structures, possess excellent mechanical strength, electrical and thermal conductivity, and optical properties [3]. Here we describe the development of carbon-based materials able to sustain neuronal survival and to promote neuronal process outgrowth. Moreover, their ability to boost the growth and activity of neuronal tissues, in 2D and 3D neuronal network models. The precise biophysical mechanisms of these special interactions are not completely understood, but the features and the remarkable applicationsof such materials, together with their ability to manipulate neural activity, still hold strong promises in manufacturing interfaces enriched by artificial cues that can improve neuronalperformance and guide tissue reconstruction.

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Mucosomes: innovative glycosylated mucin based nanoparticles as multi drug delivery platform

Sonia Visentin[1], Giuseppe Guagliano[1], Cosmin Stefan Butnarasu[3], Lorenzo Sardelli[1], Olga Valentino Garbero[1], Enrica Frasca1 Paola Petrini[2], Livia Visai[4,5], Elisa Restivo[4], Emanuela Peluso[4], Giuliana Banche[6], Narcisa Mandras[6], Lorenza Cavallo[6]

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Abstract:

Mucus is a complex barrier for pharmacological treatments and overcoming it is one of the major challenges faced during transmucosal drug delivery [1,2]. To tackle this issue, we introduce a novel class of glycosylated nanoparticles, named "mucosomes", which are based onthe most important protein constituting mucus, the mucins [3] Mucins are long polymeric glycosylated proteins composing the dense glycocalyx of mucosal epithelial cells or mucus layers covering the wet epithelia. In addition to protecting against shear stress and dehydration, mucins are also bioactive molecules towards microbes and mammalian cells [4]. Mucosomes were designed to improve drug absorption and residence time on the mucosal tissues. We have been able to synthesize mucosomes nanoparticles that are functionalized with glycans, and loaded with the desired drug in a single one-pot synthetic process. Usingthis method, we have been able to load a wide range of small, and macro, molecules with different physicochemical properties. Various *in vitro* models were used to test the mucoadhesive properties of mucosomes. *In vitro* and *in vivo* tests indicated that mucosomes did not induced adverse effects under the investigated conditions. We propose mucosomes asa ground-breaking nanosystem suitable for drug delivery. In particular, the ever-growing emergence of antimicrobial- resistant pathogens, demands innovative and transversal solutions. That is why mucosomes could be applied in several pathological contexts such as bacterial or fungal infections in mucus-related disorders. We will focus on testing mucosomes on pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and fungi like *Candida albicans* and *Candida auris*, determining how effectively mucosomes are able to deliver antimicrobial agents to inhibit or kill these pathogens. With their distinctive properties, mucosomes have the potential to revolutionise drug delivery, paving the way for the emerging field of mucin materials.

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Abstract

Synthetic, bioinspired and natural nanovectors: versatile platforms for diagnostic and therapeutic applications

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Keywords: Nanocarriers, Hybrid nanoplatforms, Exosomes

Abstract:
Recent nanotech advances have enabled the creation of therapeutic and diagnostic nanocarriers, offering potent solutions for diseases like cancer and neurological disorders. Lipid-based nanocarriers (e.g. micelles, liposomes, solid lipid nanoparticles) as well as polymeric nanoparticles represent the most commonly explored organic nanostructures for biomedical applications, being able to incorporate drugs or imaging agents and target them to the specific disease sites. Hybrid nanoplatforms, combining metals, semiconductors, carbon, silica, or oxide-based nanomaterials with nanocarriers, offer unparalleled targeting precision, drug delivery capabilities, and additional functionalities like photoactivity and magnetism, being all relevant in clinical field [1-3]. Natural vesicles such as exosomes and extracellular nanovesicles, are also employed as therapeutic and diagnostic delivery systemsdue to their superior stainability, safety, biocompatibility, bioavailability, and targeting abilities compared to artificial nanocarriers [4]. A broad platform that combines synthetic nanovectors such as mesoporous silica nanoparticles, lipid and polymer-based nanocarriers,and natural vesicles will be presented. This platform shows promise in developing precise nanoformulations with improved stability, biocompatibility, and encapsulation efficiency forthe diagnosis and treatment of diseases such as gastrointestinal cancers and neurological disorders. Finally, the use of luminescent, magnetic, and photoactive inorganic colloidal nanoparticles for multifunctional nanovector fabrication and showcases examples of naturalcell-derived exosomes in diagnosing gastrointestinal cancer will be proposed.

The work has been supported by Italian PON TITAN-Tumor Immunotherapy by Nanotechnology (ARS01_00906), the University of Bari Horizon Europe Seeds Project BIOMAD and Bilateral CNR-RFBR Project (2021-2023) and the PRIN 2022 PNRR NHYLODEA) Nanocrystalline Hydroxyapatite for the Local Delivery of Anticancer agents in the Treatment of Bone Tumors and Metastases ((code P2022RLFZB).

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Abstract

Micro and nano fabrication technologies to the service of modern days medical challenges.

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Abstract:
Since its inception CSEM has been at the forefront of microfabrication technologies for a broad range of applications, spanning from watch making, ultra-low power electronics, medical and life science, to environmental and renewable energy.
With its moder MEMS and Nano-scale operations, CSEM is pushing forward technologies and devices embedded in a variety of sensors and actuator in the bio-medical field.

Abstract

Tailored chemical designs for graphene FETs asbiosensors to detect large and small molecules

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Abstract:
Graphene field-effect transistors (GFETs) have emerged as a leading technology in biosensing due to their exceptional characteristics, which include high sensitivity, selectivity,low detection limits, and suitability for in vivo applications.[1] These advantages derive from graphene's outstanding carrier mobility and additional attributes such as biocompatibility, transparency, and flexibility. Consequently, GFETs can accurately and sensitively detect various biomolecules, including proteins, DNA, and small molecules, across diverse environments.
Various chemical approaches, such as covalent binding, non-covalent binding, and electrostatic adsorption, have been utilized to achieve necessary functionalization withdifferent receptors or biorecognition elements. However, determining the optimal immobilization strategy for the receptor presents a challenge, as not all graphene chemistry strategies readily lend themselves to transistor modification.
Through the optimal selection of the recognition element, precise control of graphene functionalization and refinement of device design, we have successfully developed a range of GFET microarrays capable of detecting small molecules such as neurotransmitters and air pollutants, as well as viral proteins, with remarkably low detection limits.[2–4] These achievements could pave the way for a new generation of analytical platforms utilizing innovative recognition elements and precisely tailored graphene modifications. Such platforms have the potential to identify an extensive range of biomarkers and pathogens evenprior to their isolation. Consequently, these capabilities offer vast potential in health and environmental monitoring, as well as in effectively addressing future pandemics.

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Abstract

Modeling and Multiphysics Simulations of the Electrical Response of Electrochemical Sensors

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Keywords: COMSOL Multiphysics simulation, cyclic voltammetry, screen-printedelectrode, electrochemical sensor, impedance spectroscopy.

Abstract:
This study introduces a novel multiphysics modeling technique using COMSOLMultiphysics® to simulate the electrochemical sensors' responses by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) [1,2]. The model accounts for key electrical and electrochemical physics at the electrodes and in the electrolyte, focusing on redox reactions at the metal/solution interface. It virtually links the device terminals to a potentiostat circuit model, enabling the application of bias and execution of CV readings, with the possibility to extend it for other voltametric analysis [1]. Calibration employs experimental measurements from screen-printed devices with 10 mM $[Fe(CN)_6]^{3-/4-}$ as a redox mediator in Phosphate Buffered Saline (PBS). Parameters such as double layer capacitance, redox diffusivity, electrolyte conductivity, and equilibrium potential are determined from these experiments. Additionally, the model is also calibrated with the addition of a self-assembled monolayer of 11-Mercaptoundecanoic acid (MUA) deposited on the working electrode between 1-100 µM [2]. After calibration, the model simulates CV responses at different scan rates and redox concentrations, closely matching experimental data with discrepancies in the CV simulated/experimental peaks under 50 mV [1,2]. The proposed model will allow the simulation of more complex electrode morphologies and electrochemical conditions, being useful to investigate the sensing system limitations, the non-uniformities, and to optimize the device layout and design.

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Abstract

Hard or soft? The next generation of optical transducersis…

Valeria Nocerino [1,2], Bruno Miranda [2], Ilaria Rea [2], Principia Dardano [2] and Luca De Stefano [2,*]

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Keywords: optical biosensors; porous silicon; hydrogels

Abstract

Optical (bio)sensors are based on materials that convert a physical, chemical, or biological interaction occurring on, or near, their surface into a change in the values of one of the features (amplitude, frequency, wavelength, phase, and so on) of the light impinging on them. Integrated optics, i.e. optical circuits on glass and silicon photonics, has been the main technology adopted in optical sensing until the advent of plasmonics and metamaterials [1]. Micro/nanomachining is still the most impactful industrial science on the large consuming electronics, and it is based onthe so-called hard materials, such as crystalline silicon and all the family of semiconductors (II-IV and III-V), that are nowadays exploited for all kind of optoelectronic as a well as photonic devices [2].

More recently, the diffusion of nanomaterials, that could be easily combined with other substances in bottom-up approaches, has strongly changed the development frame and opened new routes to applications until now forbidden to traditional hard materials. The soft matter, polymers, liquid crystals, macromolecules, has been added of optical features that well compete with traditional emitters, lightguides and detectors. Moreover, their mechanical properties, flexibility and weaving, can be used to design and fabricate smart wearables, innovative sensors and much more [3].

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Abstract

New microfluidic formats for the growth and analysis of 3D cellular assemblies: droplets and gel-embedded microchannels

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Keywords: Droplet microfluidics, cancer, cell spheroids, vascularization

Abstract:

3D in vitro biological systems are progressively replacing 2D systems to increase the physiological relevance of cellular studies. Microfluidics-based approaches can be powerful tools towards such biomimetic systems, but often require high-end complicated and expensive processes and equipments for microfabrication. We shall present here two new strategies for the formation and study of 3D cellular systems. The first involve the formation of spheroids, using highly confined droplets in a tube (or "plugs") [1] The platform then allows to implement multiple droplets merging events at programmable times. It was applied to screen cancer cells for drugs efficiency. The principle is to submit sequentially the spheroids to chemotherapy and to reagents for cytotoxicity screening. After a comprehensive study of tumorigenesis within the droplets, the system was validated for drug screening (IC50) with chemotherapies in cancer cell lines as well as cells from patientderivedxenografts (PDX). As compared to microtiter plates methods, this new system reduces the initial amout of cell up to 10 times and opens new avenues towards primary tumors drug screening approaches. A second platform, under development, combines microfluidicand textile technologies to prepare organ-on-chip systems. First proofs of concept to prepare vascular networks will be presented.

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Abstract

"From muscle to tendon: engineering complex tissuesand tissue interfaces using bioprinting"

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Keywords: Skeletal muscle, biofabrication, microfluidics

Abstract:
In the last ten years, biofabrication technologies have emerged as a promising tool to recapitulate in vitro the complexity of human tissues and organs. Here, we aim at demonstrating their potential to mimic the architecture and functionality of themyotendinous junction (MTJ), a region comprising skeletal muscle, tendon and theirinterdigitated interface. By using our wet-spinning rotary 3D bioprinting platform (3D- RoWS) equipped with microfluidic printing heads [1,2], we succeeded in developing structurally and functionally relevant MTJ-equivalents, including the transition zone wherewe observed the formation of finger-like projections of the multinucleated myotubes in thetenogenic compartment, epitomizing the MTJ signature architecture. Of note, we have alsodemonstrated by using high-resolution mass spectrometry-based proteomics with the integration of literature-derived signaling networks that: i) our 3D bioprinted biomimetic matrix (PEG-Fibrinogen) confers a less mitogenic microenvironment compared to standard2D cultures, favoring the formation of contractile-competent bundles of pericytes-derived myotubes in an anchoring-independent 3D state, and ii) our wet-spinning bioprintingmethod promotes an upregulation of muscle matrix structural protein besides increasing contractile machinery proteins with respect to other standard culture platforms [3]. Considering the quality of the obtained tissue models, the scalability potential and the operational simplicity of our platform, the superior capacity in controlling the cell differentiation processes, with future refining, our method seems promising for a possible translation into clinical practice.

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Abstract

Oncology meet Organs-on chips: dissecting cellular interactions in heterogeneous cancer ecosystems

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Keywords: organoids; tumor-on chips; immune system

Abstract:
In the age of immunotherapy and single-cell genomic profiling, cancer biology requires novelin vitro and computational tools for investigating the tumor-immune interface in a proper spatiotemporal context.
The tumor microenvironment is a complex tissue where cancer cells continuously interact and dynamically co-evolve with the other cellular components (immune, stromal, neuronal, and endothelial cells) and a chemical/physical landscape (i.e., the extracellular matrixconstituents, released soluble factors). In this context immune cells may play as friends or foes of malignant cells, thus strongly affecting both disease progression and response to therapy.
In the last decade significant advances in organoids and organs-on-chips (OOCs) technologies have enabled the construction of in vitro near-physiological three-dimensional microenvironments, allowing the possibility of reproducing the complexity and heterogeneity of in vivo tissues or organs, with the possibility to incorporate the personalizedcharacteristics of patients.
State-of-art of OOCs [1-4] and organoids models [5] for onco-immunology applications willbe presented as observation windows for understanding the role of immune contexture in cancer progression and resistance mechanisms, compatible with dynamic, multiparametric monitoring and visualization of cellular functions. The coupling of these strategies may provide a versatile and predictive preclinical model for precision medicine to accelerate the translation from lab to clinic.

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Abstract

Biofluid proteomics in prostate cancer

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Keywords: prostate cancer; machine learning; data-independent acquisition; protein glycosylation; liquid biopsy; proteomics

Abstract:

The molecular information contained in easily collectable biofluids such as blood and urinecould help in the early diagnosis of oncological malignancies. The dynamic complexity of the proteome and metabolome of these biofluids, though, represents a formidable challengefor current analytical methods, mainly based on mass spectrometry [1]. In this work, state of the art mass spectrometry analysis based on data-independent acquisition has been applied to proteomic profiling of urine and serum of patients suffering from either benign prostatic hyperplasia (BPH) or prostate cancer (PCa). Sample preparation and LC-MS methods have been designed to improve the dynamic range of detection of proteins, thus maximizing the quantification of low abundance proteins. The matrixes containing the quantified proteins in each patient (n=163 for serum and n=133 for urine samples) were integrated with clinical parameters such as prostate-specific antigen (PSA) level and glandsize, and the complete matrixes were analyzed by machine learning algorithms. In both sample sets, predictive models based on proteomic and clinical variables outperformed prostate-specific antigen in distinguish PCa from BPH [2,3]. Areas under the Receiver Operating Characteristic (ROC) curve of 0.93 and 0.78 were obtained for the serum-based predictor and the urine-based predictor, respectively.

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Abstract

Seeking informativeness from separation science: the need for profilomics

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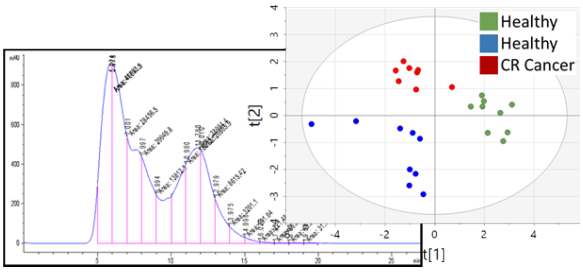
Keywords: liquid biopsy; FFF; profilomics; data-driven decisions

Abstract:
Isolating and characterizing liquid biopsy analytes from plasma and cell media is challengingdue to their low concentration, matrix complexity and heterogeneity, and nanoscale contaminants. Traditional isolation methods (ultracentrifugation, SEC, ultrafiltration, immunocapture) are slow, require large sample amount, offer limited-to-no characterization,and have low efficiency and purity, risking analyte integrity: methods that deliver reliable, quick, high-quality results in a shorter time are needed. Most of all, an isolation approach that also yields information is very desirable. In this context, I will present how a field flow fractionation (FFF) platform able to isolate intact EVs in less than 30minutes, can simultaneously provide multidimensional characterization through online detection, providing combined, holistic information on the entirety of the sample. These outputs are independent and supplemental to downstream molecular characterization and allow to map sample content in a univocal way. Using raw data as fingerprints with multivariate tools allows the development of models to predict sample characteristics and build libraries of profiles that can provide a signature of an individual or a clinical state. Elaboration of nativeseparation data offers the chance of integrating asyncronous, high-effort procedures with samples signature or *profilome*, reliably improving knowledge on cancer biology, diagnosisand prognosis.



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Abstract

Digital twins in biomedical sciences: From the cell to the whole human

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Abstract:

Digital twins are in-silico models that represent the virtual counterpart to real physical or biological systems and their interactions with each other at different levels of complexity andabstraction. These interactions open up numerous new application possibilities, especially inthe biomedical sciences. In this talk, three examples of digital twin approaches from our research will be presented, ranging from the level of a single cell, an organ to the whole human, and the advantages and challenges will be discussed.

The first functional digital twin of a cell in cancer electrophysiology will be demonstrated and discussed. It enables the investigation and evaluation of new research hypotheses on themodulation of the function of ion channels in the cell membrane, which are important for a better understanding of cancer development and progression as well as for the development of new drugs and the prediction of treatments.

At the organ level, an electromorphological model of the vestibular system of the inner ear will be demonstrated to answer clinical questions about vestibular dysfunction and its treatment by electrical stimulation. Using this model, a detailed analysis of intra- and extra- labyrinthine electrode configurations with different stimulation protocols and electrode designs can be performed on the patient-specific anatomy, providing a valuable tool for guiding vestibular implantations.

Finally, a digital model based on systems and control theory to predict the cumulative courseof fluid balance in intensive care patients is discussed. Since fluid balance is influenced by acomplex interplay of patient-, operation- and ICU-specific factors, the prediction of fluid balance is difficult and often inaccurate. This phenomenological model enables the estimation of cumulative fluid balance progression in a dynamically changing patient fluid balance system by simulating the response to current fluid management and could thus provide a useful digital tool for clinicians in daily intensive care.

Digital twins offer new computer-aided tools for diagnostic, prognostic and therapeutic questions in biomedicine, but their experimental and clinical validation remains a challenge.

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Poster Contributions

Abstract

Opto-electronic devices based on biological photoreceptor for quantum optics

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Keywords: Microbial Rhodopsin; Optoelectronics; Micro-detector; Artificial Membrane, Microfluidics; Photocurrent.

Abstract: In nature there are cellular photoreceptors, like those present in human retina that have single photon sensitivity. They convert the light energy into an electrical signal by a phototransduction process. It is the base of the rhodopsin function[1], [2]. Among this family, microbial Rhodopsins upon absorption of photons undergo a conformational change that leads to the opening of an internal channel through which ions can migrate. They are of particular interest in the optogenetic field[3], [4] but one of the innovative possibilities is to exploit these proteins for the generation of Opto-electronic devices based on biological photoreceptor.

In this work, Tara76 protein was selected and studied in an acellular system with the final aim to design a single-photon micro-detector with high precision and nanometric size. It has a measurable proton pumping activity and it is extremely resistant at different environmental conditions[5]. These characteristics make it appealing for its incorporation into a biocompatible and acellular device, enabling a substantial size reduction with respect to existing devices and could be implemented for the quantum technologies whilst also relevant to biomedical applications.

Tara76 was expressed by bacteria, extracted and, since it is a membrane protein, reconstituted in its functional conformation using Nanodiscs[6], [7]. Following, it was integrated into an artificial membrane[8], formed in a microfluidic device. The conversion of photons in electrical signals due to the light-mediated opening of ion channels was investigated.

Besides optoelectronics, this type of device could find important applications in the biomedical field, e.g. as components of the artificial retina with enhanced functionality.

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Abstract

Hybrid plasmonic Bound State in the Continuum entering the zeptomolar biodetection range

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Keywords: hybrid metasurfaces, bound states in the continuum, localized surface plasmon resonances, hybrid BICs, zeptomolar biosensing

Abstract: Optical Bound States in the Continuum, often referred to as embedded trapped modes, are peculiar localized states within the continuous spectrum of radiation characterized by potentially infinite quality factor and particularly narrow spectral linewidth [1]. Their lack of radiative losses have made them a promising tool in a variety of applications, especially biosensing. BIC-based dielectric solutions that use innovative structural architectures towards high sensitivity are emerging [2], even though they have not yet demonstrated detection below the femtomolar concentration levels of target molecules [3-7]. To enhance these performances, the hybridization with pure plasmonic modes has been considered [8,9], as these can confine light at a subwavelength scale leading to boosted light-matter interactions. However, to date BICs' exploitation in lossy media, such as plasmonic nanostructures, still remains a challenge. In this work, we reported the emergence of a hybrid photonic-plasmonic BIC state in a 2D system of silver-filled dimers, quasi-embedded in a high-index dielectric waveguide. The hybrid BIC onset relies on the bare modes' spectral and spatial overlap, and particularly on the plasmonic field's intensity confined in the nanogap between the nano disks. Thus, we selected the ideal coupling regime for which the nanostructures exhibit both high Q-factor values and strong near-field enhancement (associated with extremely small modal volume) by tailoring the hybridizing plasmonic/photonic fractions. We exploited this optical layout in a proof-of-concept experiment for the detection of TAR DNA-binding protein 43, which outperforms the sensitivity of current label-free biosensing platforms, reaching the zeptomolar range of concentration.

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Abstract

Aptamer-Enhanced Electrochemical Biosensor for the Detection of *Listeria monocytogenes*

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Keywords: Sensors-BioSensors, Aptamers, Polymers, Electrochemistry

Abstract:

Among the most recent scientific topics, electrochemical biosensors are becoming quite a hot topic in fields ranging from biomedical engineering to health-care and biological fields. Different biosensor have been investigated, but one category is under peculiar attention, the Aptasensors: these biosensors are based on Aptamers, bioreceptors which demonstrated very good sensing capabilities, such as high stability, good reproducibility, high sensitivity/specificity and good ease / cost-effectiveness [1]. thanks to this features, these species can be used for a wide range of applications. The aptamers of choice were selected based on the target of choice, which is *Listeria monocytogenes*. Different polymers (Polydopamine and its derivatives) were also investigated as an anchoring point for the aptamers. The polymers were tested at different concentrations by deposition on the electrode surfaces. The sensor was investigated step-by-step through Cyclic Voltammetry, Impedance Spectroscopy, Fourier-Transform Infra-Red spectroscopy, Raman Spectroscopy and XPS.

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Abstract

Electrochemical characterization of a new apta-sensor based on gold electrodes functionalized with poly-L-DOPA film

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Keywords: Electroanalysis; Aptamers; Biosensors; Electropolymers

Abstract: Food can be a source of chemical, physical or microbiological dangers, where the last mainly include pathogenic bacteria such as *Salmonella*, *Clostridium botulinum* and *Listeria monocytogenes*. The development of new biotechnological integrated and miniaturized devices, able to detect microorganisms responsible for food poisoning, give an interesting perspective in the application of biosensors in biomedicine sector. As catecholamines can be easily oxidized by electrode surface, the electrochemical modification of electrodes allows to build an anchor for other molecules. L-DOPA could be used to obtain a stronger film, more resilient than other polymerized catecholamines, although it has not yet been well characterized [1]. The present work is aimed at the development and characterization of a new type of biosensor for detection of pathogenic microorganism *Listeria monocytogenes*, exploiting the electrodeposition of a L-DOPA polymeric film on gold electrode surface in order to bind the suitable aptamer for *Listeria* surface protein. For this purpose, gold screen printed electrodes, appropriately modified with specific aptamers immobilized on poly-L-DOPA electropolymerized film, have been studied. The proposed biosensor is here characterized by using Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV).
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Abstract

A green strategy for the synthesis of a molecularly imprinted polymer based on a novel thiophene-derivative for tyrosine electrochemical sensing

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Keywords: green synthesis; thiophene-derivative; molecularly imprinted polymer; tyrosine; electrochemical sensing

Abstract:

Molecularly imprinted polymers (MIPs) are artificial receptors obtained from the polymerization of functional monomers around the target template, whose removal leaves complementary cavities allowing the selective target rebinding [1]. In this work, a molecularly imprinted polymer was produced *via* electropolymerization for tyrosine electrochemical detection. A novel thiophene-derivative, namely 2,2'-bis(2,2'-bithiophene-5-yl)-3,3'-bithianaphtene (BT_2-T_4), was chosen as functional monomer and the electrosynthesis was performed *via* cyclic voltammetry on screen-printed carbon electrodes (C-SPE) in the ionic liquid 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ((BMIM) TFSI). The sensor was employed for tyrosine amperometric detection, showing a linear response in the analytical concentration range 15 – 200 µM, with a detection limit of 1.04 µM, and good performances in terms of selectivity towards common tyrosine interfering molecules, as well as stability and reproducibility. The tyrosine electrochemical sensing was also carried out in human plasma, with a satisfactory recovery percentage. This work represents the first use of BT_2-T_4 as functional monomer for the synthesis of a molecularly imprinted polymer, while the use of a room temperature ionic liquid as solvent and low-volume electrochemical cells allowed the development of a sustainable strategy for the production of a tyrosine detection device that met the green chemistry guidelines.

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Abstract

Development of innovative MIP based sensors for liquid biopsy

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Keywords: biosensors, molecularly imprinted polymers, liquid biopsy

Abstract

The development of suitable, simple and practical diagnosis methods for cancer biomarkers detection is crucial in medical diagnostics. In the biosensor design, molecular recognition is a fundamental property of biological processes, and it is a powerful analytical tool in the form of antibody/antigen recognition. However, systems based on natural recognition elements have several drawbacks, including high cost and low stability. Molecularly imprinted polymers (MIPs) are synthetic receptors utilized as memetic antibody for selective molecular recognition which offer valuable opportunities for biosensing purposes providing templates able to non-covalently bind to antigens with the corresponding (imprinted) molecular morphology. In this study, the development and characterization of MIPs- based sensors for interleukins detection is reported as potential tool for liquid biopsy. MIPs have been integrated on different type of sensors: microelectrodes for electrochemical detection, porous silicon and BICs (bound states in continuum) based nanophotonic devices. The biomimetic surface have been obtained by electropolymerization of o-phenylenediamine (o-PD) or chemical synthesis of polydopamine (p-PD) on sensors' surface, in the presence of template molecules. MIP synthesis, template removal and target rebinding have been monitored by electrochemical or optical characterization and Atomic Force Microscopy (AFM). The MIP sensors performance has been tested in both buffer solution and spiked solutions demonstrating a high selectivity and LOD in the picomolar range.

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P07

Abstract

Preliminary studies on an impedimetric sensor based on molecularly imprinted polymeric nanoparticles for the detection of milk powder adulterants

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Keywords: melamine; molecularly imprinted polymer; electrochemical impedance spectroscopy; sensor.

Abstract: Proteins represent a crucial component of nutritional value in food. As a significant quality food factor, the illicit practice of adding adulterants to food, with the intention of enhancing the protein content, is frequently observed [1]. With this regard. melamine (2,4,6-triamino-1,3,5-triazine), a tasteless white monoclinic crystal of a triazine nitrogenous heterocyclic organic compound, contains high nitrogen content (approximately 66% nitrogen). When added to foodstuffs, particularly powdered milk, melamine does not exhibit acute toxicity. However, it readily adsorbs oxalic acid, tannic acid, calcium, and other substances in the urinary system, forming stones. Therefore, excessive ingestion of melamine can cause kidney failure and even death [2]. This issue has led researchers to develop methods for the detection of melamine in milk powder [3]. This study presents preliminary results regarding the development of an impedimetric sensor based on molecularly imprinted polymeric nanoparticles (nanoMIPs) for the determination of melamine protein in food. The sensor device comprised the chemically modified screen-printed commercial electrodes (SPPtE) which served as the anchoring point for the nanoMIPs. Melamine protein was subjected to testing in accordance with the WHO requirements for infant formula (1 mg/kg) [3]. In the presence of a redox probe, electrochemical impedance spectroscopy (EIS) was conducted, demonstrating the promising sensitivity characteristics of nanoMIPs at the ng/mL concentration range in comparison to negative controls.

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Abstract

Advancing Live Cell Imaging: Ratiometric Fluorescent Systems for Spatio-Temporal Mapping of Dissolved Oxygen in Cell Models

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Keywords: Optical ratiometric oxygen-sensors; core-shell silica microparticles; polymers blend; electrospun nanofibers; oxygen spatiotemporal tracking; cell metabolism; computational analysis.

Abstract: Fluorescence imaging allows non-invasive approach to visualize and quantify crucial physiological parameters such as pH and partial pressure of oxygen (PO_2)[1]. Here, we present two ratiometric fluorescent platforms designed for precise tracking of PO_2 levels in 2D and 3D cell models. These platforms utilize a tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) dichloride oxygen-sensitive probe along with rhodamine B isothiocyanate as reference dye. Notably, we designed and fabricated biocompatible core-shell silica-based microsensors and poly(ε-caprolactone)/poly(dimethyl)siloxane polymers-based electrospun nanofibers through simple, cost-effective, robust and scalable methods [2,3]. The morphology of the O_2-sensing particles and nanofibers were characterized using scanning electron microscopy and dynamic light scattering, while their physicochemical profiles were assessed via Fourier transform infrared spectroscopy, thermogravimetric analysis, andwater contact angle measurements. Next, we evaluated their sensing capabilities using spectrofluorimetry and confocal laser scanning microscopy, performing studies on photobleaching, reversibility, and calibration curves for different PO_2 levels. Additionally, we integrated the O_2-sensing particles into a pancreatic cancer model using alginate microgels. Utilizing customized computational methods for detailed data analysis, we generated 3D oxygen maps during live cell imaging, unveiling oxygen gradients within the tumor microenvironment recreated *in vitro* and highlighting a significant decrease in oxygen levels characteristic of solid tumors. Furthermore, the O_2-sensing nanofibers were assessed with three cancer cell lines (metastatic melanoma cell line SK-MEL2, breast cancer cell line MCF-7, and pancreatic ductal adenocarcinoma cell line Panc-1), demonstrating high biocompatibility, enhanced adhesiveness, and favorable cell growth.

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Abstract

Development of a Lateral Flow Assay Biosensor for miRNA-34a and miRNA-155 Detection Utilizing the Rolling Circle Amplification

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Keywords: Biosensor; Lateral flow immunoassay; MicroRNA detection

Abstract: MicroRNAs (miRNAs) are a class of small non-coding RNAs that play crucial roles in the regulation of gene expression and various biological processes, including cell differentiation, proliferation, and apoptosis. Aberrant expression levels of specific miRNAs have been implicated in the pathogenesis of numerous diseases, including cancer [1]. Detection and quantification of miRNAs hold great promise for early disease diagnosis, prognosis, and therapeutic monitoring. Among the various detection methods available, lateral flow immunoassay (LFIA) biosensors have emerged as powerful tools for rapid and point-of-care detection of biomolecules due to their simplicity, portability, and cost-effectiveness [2]. In our study, we present the development of a novel LFIA biosensor for the detection of two important oncogenic miRNAs, miRNA-34a and miRNA-155, using rolling circle amplification (RCA) as a signal amplification strategy. RCA is a robust isothermal nucleic acid amplification technique that generates long single-stranded DNA (ssDNA) products with tandem repeats of a DNA circle template [1]. The integration of RCA with LFIA offers several advantages, including enhanced sensitivity and specificity for target detection. In our biosensor design, the amplification step is done in the solution, gold nanoparticle-detection probe conjugates specific to miRNA-34a and miRNA-155 are immobilized onto the conjugate pad of the LFIA strip, and biotinylated DNA probes specific to miRNA-34a and miRNA-155 are immobilized onto the test lines of the LFIA strip [3]. Upon sample application, miRNA targets in the amplicon hybridize to the gold-labeled DNA probes, then captured by complementary DNA probes immobilized on the test lines of the LFIA strip [4]. This results in the formation of visible red bands at the test lines, indicating the presence of the target miRNAs. The current methodology is simple, sensitive, specific, and selective for miRNA-34a and miRNA-155 with the limits of detection (LOD) as low as 200pM.

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Abstract

SILVER NANOCLUSTERS AS INNOVATIVE BUILDING BLOCK IN NANOTECHNOLOGICAL-BASED THERAPEUTIC SOLUTIONS FOR EPIDERMOLYSIS BULLOSA

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Abstract

Epidermolysis bullosa (EB) represents a heterogeneous group of genetic disorders characterized by skin and in some cases mucosal fragility which led to the development of blisters and/or erosions after minimal trauma, if not appropriately treated it leads to secondary infections. EB can produce painful wounds and erosions in the skin, eyes, and mucosal tissues. Patients affected by EB require the adoption of appropriate lifestyles for preserving the affected tissues from being seriously compromised as for example, by secondary infections. Indeed, the appearance of blisters is careful monitored, and a meticulous protection of the skin has to be employed to prevent their appearance. This scenario becomes even more complicated with patients affected by severe forms of EB such as in the case of recessive dystrophic epidermolysis bullosa (RDEB), the classic form of the condition that is associated with a high risk of extracutaneous complications. For this reason, gene therapy is currently being explored as innovative therapeutic approach for RDEB treatment. Nanotechnology can offer valid tools in gene therapy of RDEB thanks to the employment of polymeric nanoparticles functionalized with inorganic nanostructures able to perform gene

delivery and inhibition of microbial growth. Consequently, sub-nanometer-sized silver nanoclusters (AgNCs) with potential antimicrobial properties were designed by employing a protein-mediate reduction. Hence, bovine serum albumin (BSA) was used as protein model for synthesizing of AgNCs. The cage effect of the protein stabilizes the clusters as demonstrated by morphological characterization through transmission electron microscopy (TEM). Their photophysical properties were deeply assessed, thus receiving important information about their size, their encapsulation in the protein structure and their interaction in biological milieu. Different pathogen and opportunistic yeasts and bacteria strains were used to test the ability of AgNCs to inhibit and/or affect growth of microorganisms. In particular, the nanoclusters showed a fungistatic effect when administered at a final concentration ranging between 0.075-0.3 mM for the yeast pathogen species *Cryptococcus neoformans* and *Candida parapsilosis.* In the case of *C. albicans* the inhibiting efficacy was observed at a final concentration ranging between 0.3-0.6 mM. A complete growth inhibition (bactericidal effect) was reported at the concentration of 0.6 mM, whereas a growth reduction corresponded at the concentration of 0.3 mM for the pathogen *Staphylococcus aureus* strain. These microorganisms were tested because of their ability to colonize skin lesions and infections. Our results demonstrate that this AgNCs produced by using BSA as reductive and capping agent can represent promising nanotechnology-based building blocks for the fabrication of hybrid nanovectors for gene therapy of EB, able to efficiently control secondary infections.

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Keywords: EB; gene therapy; secondary infections; AgNCs

Abstract

Slpi delivery to intestinal macrophages by non viral nanovectors for the prevention of inflammation in ulcerative colitis

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Keywords: Nanovectors; gene delivery; colitis ulcerous

Abstract:

Secretory leukoprotease inhibitor (Slpi) is a serine protease inhibitor secreted by several cells including dendritic cells (DCs), neutrophils and macrophage. The anti-inflammatory effectof several nutritional derived bioactive compounds, including quercetin, requires Slpi upregulation. Slpi-positive DCs were founf in the intestinal lamina propria suggesting a role as checkpoint for supporting intestinal homeostasis and prevent chronic inflammatory syndromes.

Targeting Slpi of intestinal resident macrophages could represent an in important issue in the development of innovative adjuvants able to promote disease remission in patient with inflammatory bowel disease such as ulcerative colitis.

In this frame, we are working on the development of innovative nanoformulations able to promote the delivery of Slpi to intestinal resident macrophages in order to sustain disease remission in Ulcerative Colitis (UC) in combination with nutritional regimes enriched in polyphenols. We designed and characterized non-viral nanovectors for Slpi pDNA delivery by using FDA approved polymers in order to assess their physical chemical properties. Then, nanovectors were biologically evaluated *in vitro* by using bone marrow derived murine macrophages and *ex vivo* by using intestinal 3D culture, organoids from WT and the UC model Winnie mice. Results showed that the efficiency of these nanovectors are influenced by the bio-nanointeractions occurring between macrophages and nanostructured materils. The best nanoformulation will be used to enriched chow for WT and Winnie mice to evaluate UC remission and dysbiosis.

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Non-viral Nanovectors for Gene Delivery: design and production through a combinatorial approach

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Keywords: gene delivery, non-viral nanovectors, combinatorial synthesis,

Abstract: Nowadays, immune cell engineering rely on the employment of viral vectors, with serious safety issues and high production costs. To overcome these limitations, nanotechnological approaches represented by non-viral nanovectors (NV) can pave the way towards safe and low-cost solutions for cell engineering. [1].

The development of efficient non-viral nanovectors requires in-depth studies of their interactions at the cellular level in order to obtain appropriate design principles. In particular the identification of the correlation of factors influencing the behavior of NPs in terms of physico-chemical properties and biological responses could be a successful approach to produce efficient delivery systems through a combinatorial approach. In this frame, the use of Box-Behnken Design and Response Surface Methodology represent an efficient and economical method compared with other conventional combinatorial synthesis methods [3]. In this study, the combinatorial synthesis of polymeric NPs for gene delivery employs Box-Behnken based on 3 factors and 3 levels, thus enabling the design of appropriate experiments. The analyzed factors were represented by the ratio of amine groups (N) of the cationic polymer to plasmid phosphate groups (P), the concentration of the nanostructurant polymer polycaprolactone, and the helper polymer polyvinyl alcohol. Responses evaluated were encapsulation percentage, average size, surface charge, polydispersity index (PdI), cellular uptake, cell viability and transfection efficiency .

The optimized NPs are found to have a hydrodynamic radius of about 230 nm, a PdI of 0.20, encapsulation efficiency of up to 40%, high cellular uptake and excellent biocompatibility to immune cells. The key question we are addressing by these combinatorial approach concerns the importance of parameters that influence the structure of NPs on cellular uptake,release of encapsulated genetic material, and biological activity. Further characterization studies are in progress in order to obtain structural correlations with transfection response.

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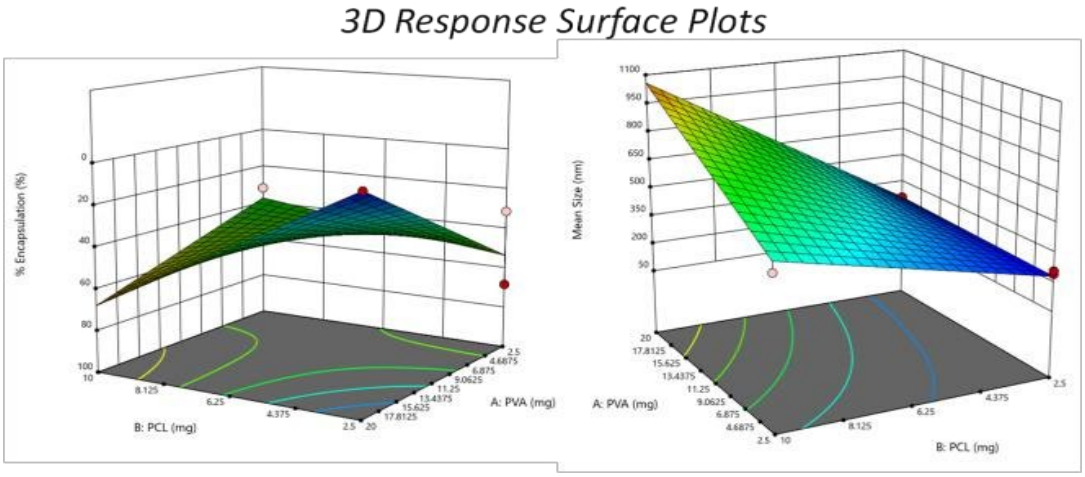

Figure 1: Three-dimensional response surface plots showing the effects of independent variables on particle size, loading efficiency for NPs belonging to group N/P 2.

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Abstract

Biomimetic nanovectors for advanced drug and gene delivery

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Keywords: precision medicine, nanovectors, nanomaterials, biomimetics nanoparticles, gene delivery, drug delivery, targeting

Abstract: Nowadays, the mainstream therapeutic approaches to treat cancers are represented by chemotherapy, radiotherapy and surgery [1]. However, setbacks are still related to their adverse toxic effects in different tissues and organs due to off-target accumulation and drug resistance [2]. Nanocarriers represent a promising approach to overcome these limits, thus representing a powerful tool for precision therapy. Smart nanovectors could be designed to achieve gene/drug delivery with enhanced targeting abilities against malignant cells, as well as to properly engineer immune cells to fight against cancer [3]. However, biological macro- and microbarriers such as tumor heterogeneity, stromal environment, osmotic pressure, and clearance have to be considered [4]. In this frame, we focused on the development of cell-membrane coated nanoparticles (NPs) by exploiting their ability to mimic both biological and functional features of the native cells, thus ensuring to nanoparticles self-recognition, immune elusion and long-time circulation *in vivo* [5,6]. To this aim, we already demonstrated the ability of glioblastoma membrane-coated NPs to achieve homotypic targeting, thus obtaining specificity and high internalization rate in glioblastoma cells (source cells). Instead, heterotypic, autologous targeting between both red blood cells (RBCs) and peripheral blood mononuclear cells (PBMCs) membrane vesicles to T lymphocytes was investigated in this work. First results showed that T cells displayed a preferential uptake of PBMC-derived vesicles. Studies are ongoing in order to identify the cell population belonging to PBMCs able to preferentially interact with these cells. By this way, important information for the design of biologically active membrane coated NPs will be obtained for the design of a biocompatible and selective cell membrane coated nanovector for engineering T cells that could be employed for targeted cancer therapy.

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Abstract

CD19 CAR-T cells generation using nanovectors for the delivery of minicircle DNA

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Keywords: gene delivery, nanocarriers, immunotherapy, T cells.

Abstract:

Among innovative therapeutic strategies for the treatment of cancer, CAR-T (Chimeric Antigen Receptor T-cells) therapy represents one of the most promising frontiers of modern oncology. Up to now, *ex-vivo* gene delivery to T lymphocytes involved the use of viral vectors, that have several drawbacks for clinical use, including safety concern, complex manufacturing and high cost [1]. Therefore, a safer, easier and cheaper alternative is strictly needed. In this context, minicircle DNA (mcDNA), a small non-integrating episomal vector, represents an advanced genetic tool option [2]. Here, we generated mcDNA encoding CD19-CAR, three times smaller than conventional plasmid, due to the lack of bacterial backbone. The short size of this plasmid induced higher transfection of T cell line, compared to standard-size plasmids. However, conventional non-viral transfection methods were not effective in primary T cells, raising the necessity to find suitable nanocarriers for gene delivery. In this frame, different factors need to be considered, as the activation status of T lymphocytes and the interaction of T cells with different kinds of nanoparticles (NPs) and their components. Till now, different nanomaterials have been tested on activated T cells evaluating the morphological changes through flow cytometry. Results highlight that severalNPs induce dimensional and internal complexity changes, thus resulting cytotoxic, while single constituent of NPs do not entail any morphological changes. This evidence reveals therole of nanostructuration in conferring biocompatibility/cytotoxicity properties to nanoformulations. The combination of NPs with short-size plasmid will offer not only high transfection efficacy of T cells, but also a manufacturing advantage and safeness compared to other transfection methods.

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Induction of immunogenic cell death in cancer cells by using polymeric nanocarriers

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Keywords: immunogenic cell death; natural compounds; nanoparticles.

Abstract:
Nowadays, cancer immunogenicity is considered a more important predictor of cancer progression than tumor stage or pathological grade, and numerous efforts are done to improve the efficacy of canonical immunotherapies for the treatment of cold tumors, characterized from lack of tumor infiltrating immune cells and defective antigen presentation machinery [1]. In this scenario, immunogenic cell death (ICD) is a regulated cell death mechanism in which several tumor-associated antigens,neoantigens and Damage Associated Molecular Patterns (DAMPs) are released to recruit and activate immune cells in the tumor microenvironment [2]. The induction of an appropriate ICD in cancer cells could represent a promising strategy for treating cold tumors. Different compounds were identified and validated as ICD inducer until now, but our attention is primarily focused on natural compounds as polyphenols, cardiac glycosides and terpenoids, thus exploiting their wide availability of sources and relatively low cytotoxicity. However, the poor bioavailability and the lack of tumor-targeting abilities of these compounds impacts their application in cancer research, especially in preclinical studies [3]. In this context, the use of nanomaterials represents a promising strategy for the selective and controlled delivery of natural compounds, while preserving their physic- chemical properties [4]. For our issue, we produced polymeric nanocarriers to attempt a controlled delivery of digoxin in different cancer cell lines in order to exploit its anticancer and immunogenic properties. Drug-loaded nanocarrier are constituted mainly by polycaprolactone, a FDA approved polymer, their physical-chemical properties were deeply assessed as well as their biological properties as ICD inducers.

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Abstract

Liposomal nanoformulations targeting NLRP3 inflammasome for fatty liver disease treatment

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Keywords: liposomes; luminescent carbon dots; NLRP3 inflammasome

Abstract: The chronic inflammation plays a pivotal role in the progression of Non-Alcoholic Fatty Liver Disease (NAFLD) towards hepatocellular carcinoma. The NLRP3 inflammasome mediates the interaction between the inflammatory microenvironment and immune cells whose dysregulated activation promotes immunosuppression, cancer, and metastases. Targeting macrophages by inhibiting NLRP3 inflammasome activation holds therapeutic promises for avoiding NAFLD progression. MCC950, a NLRP3 inhibitor, faces limitations such as short half-life and inadequate targeting, which could be addressed through nanocarrier encapsulation. Liposomes (LPs), versatile and biocompatible nanovectors, allows to deliver various compounds or nanoparticles (NPs)[1]. Carbon Dots (CDs), luminescent NPs with easy surface functionalization and high biocompatibility, have emerged as imaging tools for monitoring biological processes. Here, polyethyleneglycol (PEG)-LPs (co-)loaded with MCC950, and luminescent CDs[2] were prepared to obtain optically traceable nanovectors for selectively inhibiting the NLRP3 activation in macrophages. The reformulation of MCC950 in LPs emerges as a promising strategy to substantially reduce the required drug doses. These specifically engineered LP-based systems offer the advantage of achieving comparable therapeutic efficacy with lower drug concentrations compared to the free form. This innovative approach holds considerable promise for enhancing the therapeutic potential of MCC950 in the management of inflammatory-driven diseases.

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Abstract

Synthesis and characterization of polymeric nanoparticles for quercetin delivery

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Keywords: quercetin; polymeric nanoparticles; neurodegenerative disorders

Abstract:

Quercetin (QC) is a polyphenolic compound, a secondary metabolite of plants, with antioxidant and anti-inflammatory properties found to be important in the treatment of neurodegenerative disorders [1, 2]. Here we have synthesized nanoparticles (NPs) using polymaleic anhydride-1-octadecene (PMAO) loaded with quercetin. The aim of this work was to obtain biocompatible nanosystems capable of protecting the active compound up to the site of release and the possibility of targeting the tissues of interest. For this purpose, the NPs were functionalized with L-DOPA that can also elicit a therapeutic effect against Parkinson disease once converted into dopamine [3]. The characterization was performed through dynamic light scattering (DLS) and transmission electron microscopy (TEM) analysis revealing an average size of 200 nm and 100 nm, respectively. The encapsulation efficacy (EE%) was studied through and indirect measurement at UV-Vis spectrophotometer. The release assay, performed using a phosphate buffer at different pH, has showed a higher quercetin release at pH 4.5 (intracellular endosomal environment) than pH 7.4 (physiological extracellular environment). Moreover, antioxidant tests, as TEAC and DPPH, have revealed a better scavenging activity of free QC and NPs loaded with QC in respect to plain NPs. A great biocompatibility was assessed through MTT assay. The antioxidant effect was also analyzed in C6 gliobastoma cells through DCFH-DA assay. Furthermore, the cellular uptake at different time points was studied by encapsulating Rhodamine 101 inside the NPs as fluorescent tracer showing, under a fluorescent microscope, a great internalization of L-DOPA-functionalized NPs. Multivariate analysis of lipidomics data was also performed.

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Abstract

Development of core-shell nanoparticles for CT/MRI dual-modal imaging

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Keywords: Nanomaterials; Multimodal imaging; Brain diseases

Abstract:

In recent years, inorganic nanomaterials have gained a major role in medical imaging techniques thanks to their adjustable compositional and physicochemical characteristics, allowing detailed cellular and molecular characterization. Metal-based nanoparticles (NPs) have been particularly investigated as contrast agents (CAs) in MRI, with improved relaxivity and biocompatibility. Their surfaces can be functionalized with biomolecules or polymers to enhance pharmacokinetics and biodistribution, thereby increasing diagnostic efficiency. Various synthetic approaches have been proposed for preparing inorganic nanoparticles with different compositions, sizes, and surface functionalization, which can be tailored according to their application, such as diagnosing solid cancers and imaging neurodegenerative diseases. These conditions are often diagnosed late; thus, early detection with non-invasive methods is crucial for timely treatment before irreversible neural damage occurs [1,2].

The aim of this work is to develop inorganic nanoparticles as contrast agents for brain imaging. The goal is to create new tools to better understand and image neuropathologies at an early stage using nano-approaches combined with imaging techniques like MR and X-ray phase contrast tomography (XPCT). This involves studying these diseases at a nano-sized level to recognize specific cell populations without tissue manipulation, to provide an accurate diagnosis, crucial for improving treatment outcomes [1]. FePt alloy nanoparticles show great potential as dual-modality contrast agents for CT and MRI, combining the superparamagnetic properties and stability of the Fe domain with the high X-ray absorption coefficient of Pt. Controlling nanoparticle size is essential, aiming to keep them small enough to cross biological barriers like the blood-brain barrier. As well as identifying molecules of special interest to functionalize the surface with to ensure passage of larger NPs and specific targeting [3].

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Abstract

Fluorescent Probes for Mucins: Polymethine Dyes in early Cancer Detection

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Keywords: mucin; polymethine dyes; cancer detection

Abstract:

Mucins, a family of long polymeric glycoproteins, play critical roles in various biological processes and are linked to mucus-related disorders [1]. Over recent years, mucins have gained recognition as biomarkers of adverse prognosis, making them attractive therapeutic targets. Research has explored the roles of mucins in several cancers, including gastric, pancreatic, colon and rectal, breast, and ovarian cancers. Early diagnosis of these cancers is pivotal for improving patient outcomes, treatment efficacy, and overall healthcare. Consequently, the identification and detection of specific and sensitive biomarkers are of paramount importance. Fluorometric detection mediated by fluorescent probes represents a promising strategy for early diagnosis. Among these probes, polymethine dyes have garnered significant attention due to their sharp and intense absorption and emission in the near-infrared (NIR) region [2]. Here, we present a novel spectrophotometric method for the detection of mucins in biological samples, offering a promising approach for early cancer diagnosis.

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Abstract

CRISPR/Cas9-mediated genome editing in T-cells using non-viral nanovectors

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Keywords: Nanovectors; genome editing; T-cells

Abstract: Immunotherapy using Chimeric Antigen Receptor (CAR)-T cells is one of the most exciting recent developments in cancer treatment [1]. The therapy involves genetic modifications of a patient's T-cells to improve immune activation against cancer cells [2]. The process requires the use of viral vectors for efficient and stable DNA editing of T cells with relevant side effects related to unsafety procedures, potential for integration into the host genome, long-term effects in terms of mutagenesis and carcinogenesis [3]. This is leading to the necessity of using alternative delivery vectors for genome engineering of T-cells. To this end, we are developing biocompatible and biodegradable nanovectors, to prevent the side effects of viral carriers, coupled with the CRISPR/Cas9 technology for modifying precisely the genome of T-cells. In particular, we have chosen one of relevant gene involved in escape of immunosurveillance as *PDCD1* (encoding PD-1), an inhibitory receptor that, through the binding to its ligand, PD-L1, promotes self-tolerance [4]. Turning off PD-1 genetically means inducing autoimmunity, so is more appropriate to endogenously tune the binding affinity of PD-1 with PD-L1. Here, we show that new synthetized polymeric CRIPSR/Cas9-nanovectors are able to target *PDCD1* at genomic and protein level. We are aiming to introduce site-specific modifications of *PDCD1* which lead to tune the affinity forits ligands. This may pave the way for new therapeutic avenues offering highly innovative and promising technology for immunotherapy of cancer at genomic level.

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P21

Concentrated growth factor-permeated implants meliorate osteointegration

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Keywords: CGF; osteogenic differentiation; regenerative medicine

Abstract: Concentrated growth factors (CGF) is an autologous blood-derived biomaterial, the latest generation of platelet derivatives, produced by centrifugation of the whole blood sample [1]. Many studies reported the ability of CGF to induce osteogenic differentiation, indeed it contains growth factors, platelets, white blood cells and stem cells that play an important role in the processes of tissue regeneration and differentiation. During the last decade the need of implant services in edentulous patients was dramatically increased. In this study, the structural and biological characterization of CGF was carried out and its role in the process of osteointegration of CGF-permeated dental implants has been evaluated. The CGF structural characterization was carried out by SEM analysis, whereas the molecular characterization was performed by ELISA to measure growth factors and matrix metalloproteinases (MMPs) release. CGF primary cells were isolated and their osteogenic differentiation was evaluated through matrix mineralization by alizarin red staining and through mRNA quantification of osteogenic differentiation markers by Real-Time PCR [2]. Likewise, the effects of CGF-permeated implants on human BMSC was evaluated. We found that CGF has a complex inner structure capable of influencing the release of growth factors and cells. These cells had the capability to differentiate into osteoblasts. The CGF-permeated implants showed a kinetic of biomolecules release similar to CGF, this could be due to the growth factors released by CGF primary cells. We demonstrated the ability of CGF-permeated implant to induce osteogenic differentiation in vitro. Finally, data obtained from surgical interventions showed that CGF-permeated implants improved osseointegration respect to control implants (without CGF). These data highlight new interesting perspectives in the use of CGF in regenerative medicine and, particularly, in the dental implantology field [3].

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Abstract

Osteogenic differentiation of CGF primary cells can be stimulated by Hydroxyapatite-silicon scaffold

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Keywords: CGF; osteogenic differentiation; hydroxyapatite–silicon scaffold

Abstract: The combination of scaffolding materials and stem cell technologies is widely used in tissue regeneration. Concentrated Growth Factor (CGF) is an autologous and biocompatible product derived from blood, rich in growth factors and able to stimulate the osteogenic differentiation of bone marrow stem cells [1]. It contains, also, multipotent stem cells, found capable to differentiate into osteoblasts when stimulated [2]. In this study, we explored the use of CGF together with a hydroxyapatite and silicon (HA-Si) scaffold, a material of great interest for bone reconstructive surgery [3]. The aim was to assess the ability of HA-Si scaffolds to induce osteogenic differentiation in CGF primary cells. We evaluated the viability of CGF primary cells on HA-Si scaffolds using MTT assay and conducted structural characterization through SEM analysis. Matrix mineralization was assessed using Alizarin red staining. The expression of osteogenic differentiation markers was measured through mRNA quantification by real-time PCR. Our findings revealed that HA-Si scaffolds are non-cytotoxic for CGF primary cells, supporting their growth and proliferation. Additionally, the HA-Si scaffold promotes the expression of osteogenic markers, reduces the levels of stemness markers, and facilitates mineralized matrix formation. In conclusion, HA-Si scaffolds appear to be a promising biomaterial support for CGF in tissue regeneration applications.

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Abstract

Light-mediated Processes to Selectively Biofunctionalize 2PP 3D Microstructures

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Keywords: biofunctionalization; two-photon laser lithography; light-induced click reaction; selective protein binding

Abstract:

The extracellular matrix (ECM) plays a role in influencing cell behavior. In recent years it has become evident that cell biological functions are also closely linked to the three-dimensional (3D) spatial distribution of ECM proteins [1,2], and the geometric and mechanical constraints of their microenvironment. These parameters can hardly be assessed under classical 2D culture conditions [1]. Using the two-photon polymerization (2PP) prototyping technique, static and dynamic 3D structures can be microfabricated, which can be selectively functionalized with bioactive molecules (e.g., ECM proteins, oligonucleotides, etc.) [2,3]. In turn, these enable cells anchorage [4], thus mimicking the physiological extracellular microenvironment [5]. In this work, we focused on the biofunctionalization of microstructures using widely used 2PP photoresists. The obtained structures were selectively functionalized by exploiting a light-induced reaction, allowing for the covalent binding of maleimide compounds, which in turn can be decorated with biologically active streptavidin. The patterning of 3D structures for protein immobilization has already been the subject of various complex approaches, that also require chemical modification of the resist using photo-reactive molecules, such as photoenols [2,4].

Therefore, we simplify currently available approaches by extending the light-induced activation for protein binding properties on 3D structures to user-grade 2PP photoresists without introducing chemical modifications in the used materials.

This work is carried out in cooperation with Dip. di Ingegneria civile, informatica e tecnologie aeronautiche, Università Roma Tre.

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Abstract

Improving Wound Healing assay: achieving High Reproducibility through 3D-Printed Inserts and Automated Cell Analysis

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Keywords: Cell migration, wound healing, 3D insert, biocompatibility, customizable

Abstract:
Massive cell migration and proliferation mechanisms take place in many fundamental physiological processes from embryonic development, to tissue restore and angiogenesis. Damages due to external factors may induce a "wound". Through the wound healing assay, it is possible to observe and investigate the proliferation, migration and behaviour of cells during the "repopulation" of an injury (1). This process is exploited more in general to verify the invasiveness of a cell line or drug effect limiting metastatic processes.
Usually, the wound healing assay is carried out in mechanical way by a tip or a micropipette, to obtain a cell-free region over a cell monolayer. The alternative to the "*scratch-wound healing assay" is the "electrical-wound healing"* in which the electrical field application removes cells from a specific area of the monolayer. *I*n both cases, cell death, debris accumulation and lack of reproducibility take place as well as difficulty in defining the exact area (2). Moreover, also the image analysis of results can be challenging,despite it is usually performed with open-source programs such as ImageJ. These toolsneed manual tuning of various parameters, are time-consuming and suffer from limited high-throughput image analysis (3).
To overcome these drawbacks going in parallel toward scratch performance and standardized image analysis, customized 3D-inserts have been developed with a 3D Digital Light Processing (DLP) printer (4) allow a high reproducibility of the assay. We chose a rectangular and round shape of resin inserts. Three cell lines were used to set the test: prostate cancer (PC3), neuroblastoma (SH-Sy5y) and oral cancer (OECM-1), also in presence of TGFβ molecule, a drug able to induce proliferation and migration of cells. In addition, the MATLAB software has been developed to automatically calculate the cell- free area healing, thus overcoming the user-dependent limits of manual border design, especially when the edges became irregular and difficult to be perceived by the human eye. The aim of MATLAB tool is to realize in one script an algorithm that, collecting a high number of images from different experiments can automatically elaborate all images, detectedges, calculate areas and save all results in one folder. The algorithm is designed to be as much as possible user-friendly, leading the worker to enter only the source and the destination folder.
To conclude, here we would like to show how, thanks to the use of 3D printers and

biocompatible resins we are able to realize an ad-hoc device for biological testing. Furthermore, we developed a software that contributes to a growing trend that aims to quickly, accurately and quantitatively analyse high quantities of biological data in automated manner.

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Abstract

Rapid tooling and quality control of micro injection moulding for biomedical microfluidics applications

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Keywords: micro injection moulding; microfluidic devices; rapid tooling, digital twin.

Abstract: Lab-On-Chips and Organ-On-Chips are revolutionising medical practice by offering researchers sustainable, non-invasive, and straightforward methods to study diseases. These devices, produced using MEMS technologies, are typically complex and expensive, which can inhibit fabrication speed and customisation. In this context, micro injection moulding (µIM) seems to be a disruptive technology for the industrialisation of these chips ensuring their transition from the lab to the real world [1]. However, reproducing the micro/nano features required by microfluidic devices on mould inserts is very challenging. Currently, researchers are focusing their efforts on studying materials and micro-manufacturing technologies more suitable for prototyping and mass-producing these chips using µIM [2]. Additionally, methods and tools for continuous monitoring of the micro-production process are also being developed with the final aim of improving reliability [3]. Today more than ever, the creation of a highly reconfigurable technological platform for the large-scale production of microfluidic devices is crucial. The interdisciplinary and long-established collaboration among CNR-STIIMA (Bari), CNR-NANOTEC (Lecce), STMicroelectronics, University of Salento, and University of Brescia is giving a concrete contribution in this direction. High TRL microfluidic devices have been tested with rapid prototyping approach for micro injection moulding and quality control systems have been implemented to validate the process chain.

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Pericytes-Assisted Vascular Lumen Organization in a Novel Dynamic Human Blood-Brain Barrier-on-Chip Model

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Keywords: Blood-brain barrier; Organ-on-chip; Pulsating Flow.

Abstract: Organ-on-Chips (OoCs) are pivotal in neurovascular research, particularly for modeling the blood-brain barrier (BBB), due to their ability to replicate the complex architecture and dynamics of the blood flow in the BBB [1]. This study introduces an innovative microfluidic device that enhances BBB modeling by incorporating human endothelial cells (ECs), pericytes, and astrocytes. The study provides critical insights into the alignment and organization of actin filaments in brain ECs under flow and the role of pericytes and astrocytes in vascular lumen stability. The microfluidic device features both "open" and "enclosed" versions, catering to static and dynamic flow conditions. Findings reveal that the presence of pericytes is essential for the structural organization of the endothelial lumen and its stability under dynamic conditions. Permeability assays and TEER measurements indicate that the model's permeability values closely match those observed in vivo. This human BBB-on-chip model offers a robust platform for studying the interactions among neurovascular components and has significant potential for drug screening and therapeutic research for central nervous system diseases.

(A paper of this work is under review on Advanced Healthcare Materials)

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Abstract

Unlocking the AhR Therapeutic Potential for Cystic Fibrosis with an Integrated Mucosal Platform for Drug Screening

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Keywords: mucus; permeability; AhR

Abstract: This study explores the potential of bacteria-derived metabolites (BDM) as innovative therapies for cystic fibrosis (CF), a severe genetic disease characterized by abnormal mucus production in the lungs, leading to inflammation and infections. Despite existing treatments, new therapies are needed. BDM have shown promise due to their antimicrobial and immunomodulatory properties, with some capable of modulating the Aryl hydrocarbon Receptor (AhR), which is vital for immune regulation in fighting infections [1]. The research aims to evaluate the permeability of nine AhR-targeting molecules through CF mucus using an in vitro system called PermeaPad, designed to mimic the biological barrier of the cellular membrane and measure passive paracellular diffusion. In the study, a CF-mucus model [2] was applied to a PermeaPad phospholipid membrane, and nine AhR-binding molecules were tested for their ability to permeate through this setup. Results revealed that while 90% of the compounds could pass through the phospholipid layer alone, the presence of CF mucus significantly hindered their permeability. Specifically, phenolic derivatives and quorum sensing molecules showed reduced efficiency in reaching the cytoplasmic target via passive diffusion. The study highlights the pathological impact of mucus and suggests that targeting this barrier could be a viable strategy in improving CF treatment outcomes.

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Abstract

Multi-chamber organ-on-a-chip device for analyzing interactions between human iPSC-derived motor neurons and Schwann cells in physiology and ALS pathology.

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Keywords: neurodegeneration; Stem Cells; neurological disorders

Abstract:

Background. The peripheral nervous system (PNS) plays anearly role in amyotrophic lateral sclerosis (ALS) pathogenesis. Axon degeneration, peripheral axon loss, and nerve terminal destruction are among the first events in the disease process preceding motor neuron (MN) degeneration and the onset of symptoms.In this context, Schwann cells (SC) have recently been investigated as either promoting or protecting factors in the pathogenesis of ALS. However, to date, the specific contribution of Schwann cells to ALS remains to be elucidated. In this work, we used a customizable tri-chamber microfluidic device that allows physical separation between MN soma, axons and SCs to study the interactions occurring between MNs and SCs derived from both control and ALS patients and to assess the non-cell-autonomous effect of SCs in ALS.

Materials &Methods. MNs and SCs were differentiated from healthy control (HC) and ALS (carrying the TDP-43 p.A382T mutation) iPSCs using a combination of small molecules that regulate multiple signaling pathways. Soft-lithography and PDMS replica molding were used to create a three- chamber microfluidic device suitable for culturing up to three cell populations in a fluidically independent circuit. Immunofluorescence, Western blot, and RT-PCR assays were used to characterize MNs and SCs and to investigate the interactions between MNs and SCs.

Results. We demonstrated that: i) SCs differentiated from HC iPSCs specifically enhanced the axonal migration of iPSC-derived MNs from HC; ii) SCs differentiated from ALS iPSCs (TDP-43 A382T) failed to promote the axon outgrowth of either HC or ALS-derived MNs.

Conclusions. In the present study, we used a custom-designed microfluidic device to investigate the interaction between human iPSC-derived MNs and SCs. Our findings highlight the importance of SCs as essential neuronal partners for axonal migrationin physiology and ALS pathology.

Development of Autologous Organ-on-Chip Models for Precision Medicine in Rheumatoid Arthritis

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Keywords: Organ-on-chip, Hydrogel, Rheumatoid Arthritis, hFLS, 3D Printing, DLP, Precision Medicine, Mechanical Actuator

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disorder affecting joints and leading to severe disability [1,2]. The complexity of RA pathophysiology demands advanced *in vitro* models that can accurately recapitulate the human joint environment to facilitate precision medicine approaches [3].

Our research introduces autologous organ-on-chip (OoC) systems using patient-derived human fibroblast-like synoviocytes (hFLS) encapsulated in two innovative hydrogels: GelMA and a chitosan-Matrigel composite. These were cultured, assessed for viability, and formed a three-dimensional (3D) network over time. We engineered an OoC platform with digital light processing (DLP) 3D printing and micro milling, allowing precise microfluidic structures and dynamic culture. Then, we developed and integrated a pneumatic actuator to provide mechanical stimulation, mimicking joint movement.

Encapsulated hFLS within the OoC platform exhibited sustained viability and network complexity over a week-long culture under dynamic flow conditions, suggesting an effective *in vitro* environment.

The pneumatic actuator delivered a mechanical stimulation and preliminary data indicated that stimulation contributed to a more physiological-like response compared to static culture conditions.

The firtst results obtained demonstrated the system's potential for pathophysiology studies and drug screening applications.

Collaborative efforts within the framework of a European project (Flamin-GO, Grant Agreement number: 953121), aims to integrate additional joint tissues into a larger joint-on-chip system for comprehensive RA studies, driving the development of this advanced OoC system and fostering translational research and precision medicine in RA.

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Abstract

Development of OoC platforms for studying GBM in vitro through a combined experimental-computational approach

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Keywords: GBM; Organ-on-Chip; Computational model; Microfluidics

Abstract:

Glioblastoma multiforme (GBM) is the most common and most deadly form of primary brain cancer in humans. The current standard of care is marginally effective, and patients have a median survival time of approximately 12-15 months after diagnosis [1, 2]. Most used disease models are 2D cell cultures (on classical petri dishes) and animal models, which however, show significant molecular and pathological differences from human GBM [3, 4]. Therefore, there is an urgent need of bioengineered 3D *in vitro* GBM models that can recapitulate *in vivo* features. Hydrogels are increasingly used as biomaterials for cell biology [3]. In this study a chitosan-based hydrogel has been used as a biomimetic material for U87 cells encapsulation because of its excellent biocompatibility and similarity to glycosaminoglycans structure [5]. Since hydrogel-based 3D cultures are usually in static conditions neglecting the fluid shear, here we developed a GBM-on-chip, using a straightforward approach, represented by 3D printing technology (Digital Light Processing) [6]. Moreover, in order to optimize the chip design and to correctly set the operational parameters, such as flow rate, a computational model of the chip, which couples laminar flow with mass transport physics, has been developed and validated by experimental results. The in silico model allowed to study the velocity and shear stress that cells undergo, along with oxygen diffusion and consumption within the chip. Computational tools are useful to explore the full design space for specific design parameters [7] and to collect data into a coherent theoretical framework [8]. This GBM-on-chip could open new landscapes for a better understanding of cancer, personalized medicine, and drug screening.

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Abstract

On-chip devices for flow-driven release of extra-cellular vesicles and their characterization as diagnostic biomarkers

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Keywords: Extracellular Vesicles (EVs); Lab-On-Chip (LoC); microfluidics

Abstract: Precision and personalized medicine advancements, allow to tailor the clinical approach, depending on the makeup of patients' DNA and expression issues. Liquid biopsy and the chance to use selected biomarkers from biological fluids, could strongly contribute to the improvement of an increasingly patient-oriented method, avoiding invasive assays and tissue biopsies. Extracellular Vesicles (EVs) act both as a snapshot of the cells from which they originate and as depository of important information, facilitating direct extracellular transfer of proteins, lipids, and miRNAs/mRNAs/DNAs. For all these reasons, EVs are on the rise for the possibility to be considered as powerful biomarkers. In this study, we realized a Lab-On-Chip (LoC) device starting from fluid dynamic simulations and using microfabrication techniques (micro-milling and 3D printing) to realize EV samples characterization from different cell lines in response to dynamic conditions and mechanical stimuli.

Molded plastic substrates assembled on a glass slide create a microfluidic chamber where Oral Squamous Carcinoma (OECM-1), neuroblastoma (SH-SY5Y) and microglial (CHME-5) cell lines were seeded reaching 50% of confluence and exposed to a controlled culture medium flow for different time-points. A complete medium replacement in the dynamic condition was obtained setting the flow medium rate, avoiding shear stress. The large- and small-EVs released from cells in dynamic and static conditions were isolated by differential ultracentrifugation and the different EV populations were characterized using high-resolution flow cytometry, western blot assay and transmission electron microscopy (TEM).

Moreover, in addition to our in-flow device for EVs release, our purpose is to build a benchtop-ready device able to identify arrays of biomarkers associated to the size and to different functions of EVs subclasses, customizable according to clinical needs. The aim of this platform is to integrate the function of microfluidic sorting and electrochemical characterization of EVs in order to identify a panel of EVs related biomarkers on a single lab-on-chip. A key point will be the effort in removing all the highly impacting processing procedures to operate in the most native conditions possible, reducing time and cost, and preserving EV morphology and properties.

Abstract

Towards human liver-on-chip models for investigating metabolic diseases

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Keywords: 3D liver cell culture; liver-on-chip; metabolic disease; drug screening platform.

Abstract:

High-throughput and physiologically relevant *in vitro* models are increasingly required to improve the predictive value of toxicity testing as valid alternatives to animal experiments. In this scenario, Organs-on-Chip (OoC) may fill this gap, allowing scientists to develop miniaturized and complex human disease models holding high pathophysiological relevance for drug screening [1]. Here we present our liver-on-chip platform designed to mimic the onset of metabolic dysfunction-associated fatty liver disease (MAFLD) status, with the future aim to establish a platform for drug screening. Besides, we show the further evolution of such systems by including hepatic organoids, based on co-culture of parenchymal cells (PCs) with non-parenchymal cells (NPCs), for increasing the complexity and predictivity of our OoC platform.

Microfluidic devices were fabricated in polydimethylsiloxane (PDMS) through a two-layer soft-lithographic process according to a previously validated layout [2,3]. HepG2 cells loaded and cultured in a 3D fashion in the chip under microfluidic perfusion. For hepatic steatosis induction, cells were supplemented with long-chain free fatty acids (FFAs), namely oleic acid (OA) and palmitic acid (PA) (2:1 molar ratio). Our preliminary results suggested that the co-culture of PCs with NPCs better mimic the *in vivo* microenvironment and that our MAFLD model may represent a suitable platform for recapitulating *in vitro* the chronicity of the disease. Altogether, integration of PC and NPC-derived hepatic organoids into microfluidic devices will enable a deeper investigation of chronic liver diseases mechanisms. In a future perspective, these cutting-edge technologies may carry future potential for hepatic disease modelling and drug screening in the fields of personalized medicine and *in vitro* toxicology.

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Abstract

Development of an *in vitro* intestinal barrier model as a platform for precision medicine

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Keywords: Gut-on-chip; iPSCs; precision medicine

Gut on chip (GoC) models represent a tool to study intestine pathophysiology [1]. In recent years GoC have been integrated with systems to recapitulate the biomechanical stimuli typical of the intestine, such as shear stress, cyclic strain and oxygen gradients [2]. Here, we present the development of a dynamic in vitro intestinal barrier model achieved by the culture of a human epithelial colorectal adenocarcinoma cell line (Caco2). The device is composed of an upper and a lower fluidic chamber separated by a thin porous flexible membrane with circular pores. The two chambers of the chip were individually prepared by casting PDMS (Sylgard 184, Dow Corning) on a mold obtained by 3D printing (DLP, digital light printing). The porous membrane was produced by spinning a thin layer of PDMS onto a photolithographically obtained master, consisting of an array of circular pillars. Chip assembly was performed by oxygen plasma bonding. Dynamic culture conditions were achieved through a perfusion system (Ibidi Pump System). Perfusion parameters, such as flow rate, pressure and shear stress, were optimized in order to achieve a confluent intestinal barrier with tightly interconnected cells. The continuous monolayer of epithelial cells was verified by confocal microscopy imaging. As a further implementation of the platform for precision medicine applications, human induced plutipotent stem cell (hiPSCs)-derived intestinal epithelial cells will be used as a more relevant cell model, and the device will be integrated with electrodes for the real-time monitoring of the barrier formation through transepithelial resistance (TEER) measurement.

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