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Sessions Summary

Light and human health

INVITED LECTURE

Light-responsive (nano)structure

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Luminescent molecules that can undergo self-assembly are of great interest for the development of new materials, sensors, biolabels…. However also systems that can disassembly or break on demand are of great importance in delivery active components or in diagnostics.
The talk will illustrate some of the recent results on the self-assembly of platinum complexes and their evolution in solution[1]. The different species that evolve from the initial assembly can be visualize thanks to their different photophysical properties and the control of the solvents determines the kinetics of their evolutions. The intense emission properties can be used for sensing application and as a tool to follow the formation of hybrid structures, virus-like capsules that can be realized using a unique approach to template virus proteins to reconstruct virus-like particles. We use luminescent Pt(II)-complex amphiphiles, able to form supramolecular structures in water solutions, that can act as templates of viruses capsid proteins. The platinum assemblies can have different morphologies and extremely high emission of which the color depends on the assembly. Interestingly we are able to change the size and shape of the particles even though we use the same natural proteins. The obtained virus-like particles can be visualized by their intense emission at room temperature, generated by the self-assembly of the Pt(II)-complexes inside the capside[2].
On the other hand, the stabilization of species in cage type structures can lead to their stabilization or even existence in solution, in a condition out of equilibrium. We recently demonstrated[3] that it is possible to entrap intermediate states of luminescent assemblies and prevent their thermodynamic evolution towards the equilibrium state. Furthermore, the use of nanocages able to break on demand allows the transport and release in cells of such species and therefore their dynamics can be observed in living cells. Finally, also the scaffold, the nanoparticles can be photoresponsive and the light leads to their destruction
with the release of desired active components[4].

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SHORT LECTURE

Blue LED light photobiomodulation in wound healing

Giada Magni[1], Martina Banchelli[1], Federica Cherchi[2], Paolo Matteini[1], Anna Maria Pugliese[2], Francesco S. Pavone[3,4] and Francesca Rossi[1]

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In the last two decades, two different effects induced by phototherapy were identified: the thermal effect and the ones mediated by the interaction of light with the endocellular photoacceptors [1]. This latter interaction was named PhotoBioModulation Therapy (PBMT) and induces many beneficial effects such as pain reduction, inflammation, and immunomodulation, leading to a promotion of wound healing and tissue regeneration [2]. To date, the mechanism of light is only partially known [3]. Still, it is clear that biological interaction - called primary reactions - stimulates secondary photoresponses at many cellular levels (metabolism, proliferation, increase of intracellular levels of Calcium, lymphocyte, macrophages and mastocytes activation and synthesis of growth factors and interleukins). Previously, we studied the role of a blue LED light (emission range 410-430 nm) in a superficial wound performed in a *in vivo* rodent model. Our results demonstrated that 20.6 J/cm^2 improves wound healing by modulating inflammatory response and activation of fibroblasts [4,5]. Recently, we showed that blue light modulates both cell metabolism and proliferation in a dose-dependent manner when irradiating fibroblasts isolated from keloid tissue and HaCaT cell line (Elabscience, Bologna, Italy) [6,7]. These findings suggest the role of blue light not only in the modulation of physiological healing but also its function in cutaneous fibrosis. Here, we reported the analyses about the effects of the blue LED light source (0.27 W/cm^2 power density) on HDFa cells (Human Dermal Fibroblasts, adult. Thermo Fisher Scientific, Milano, Italy) and in co-cultures of HDFa and HaCaT cells. Different light doses were applied, varying the treatment time: the effects on cell metabolism, proliferation and viability were evaluated by biochemical assays. Micro-Raman spectroscopy and patch-clamp recordings were used to explore the direct impact of the blue LED light on the Cytochrome C (Cyt C) oxidase and ionic membrane currents, respectively. Scratch test assay was performed on co-cultures to evaluate the involvement of blue LED light in wound healing. Our results demonstrate the potentials of the blue LED light: it can be used as PBMT, e.g. as an innovative and minimally-invasive approach in the management of wound healing, in association with current treatments.

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SHORT LECTURE

Nanoformulations of different photosensitizers in PDT

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Photodynamic therapy involves the concomitant action of three components, light with an appropriate wavelength, molecular oxygen, and a molecule able to absorb an electromagnetic radiation, called photosensitizer (PS) [1]. A fundamental aspect is the bioavailability of the PS that is directly related to some physico-chemical properties of the PS itself as it should feature a certain degree of lipophilicity to easily cross the cell membrane, however, at the same time, should be sufficiently water-soluble to navigate in the bloodstream [2,3]. Consequently, the use of a system for drug delivery becomes essential when photosensitizers with a high degree of lipophilicity are considered [4].
In this work, we present three different drug delivery systems, microemulsions, emulsions and liposomes capable of carrying two PSs belonging to the porphyrin family, the tetraphenyl porphyrin (TPP) and the 4-hydroxyphenyl porphyrin (THPP), characterized by a different degree of lipophilicity.
A series of microemulsions (ME) and emulsions (E) were prepared, among which the most stable formulations were chosen. The stability of these two vectors was monitored over time and under various temperature conditions. Liposomal formulations (L) were also identified and analyzed with the same criteria. The four formulations mentioned above (one ME, one E and two L) have been tested on SKOV3 tumor cell line. TPP is highly lipophilic, poorly soluble in those solvents suitable for the in vitro cell treatments; therefore, it is necessary to find a carrier thereby allowing the evaluation of its potential as photosensitizing agent. On the contrary, the more hydrophilic THPP can be formulated in a H_2O/DMSO mixture, and then the system chosen to convey the THPP can be compared with the dissolved porphyrin.
The results show that all the formulations have proved to be excellent carriers and that the liposomal formulation enhance the photodynamic efficacy of both porphyrins.

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INVITED LECTURE

Photopharmacology: new tools, considerations, and applications

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Current pharmacological treatments relay on using bioactive compounds that evoke a response by interacting with molecular targets in the human body. The selectivity of this interaction is crucial and the lack of it leads to the emergence of severe side-effects in the body and toxicity in the environment.

To solve this problem, drugs could be introduced whose activity could be reversibly or irreversibly turned on with light. The aim of this presentation is to describe the emerging concept of photopharmacology (Figure A),[1] which is currently being developed and applied to precisely control the activity of drugs using light. Light offers unparalleled advantages in regulation of bioactivity and as an input/output signal in medical (mostly optical/optoacoustic) imaging. Combination of those two paradigms along the principles of theranostics requires light-responsive tools that empower both therapy and imaging.

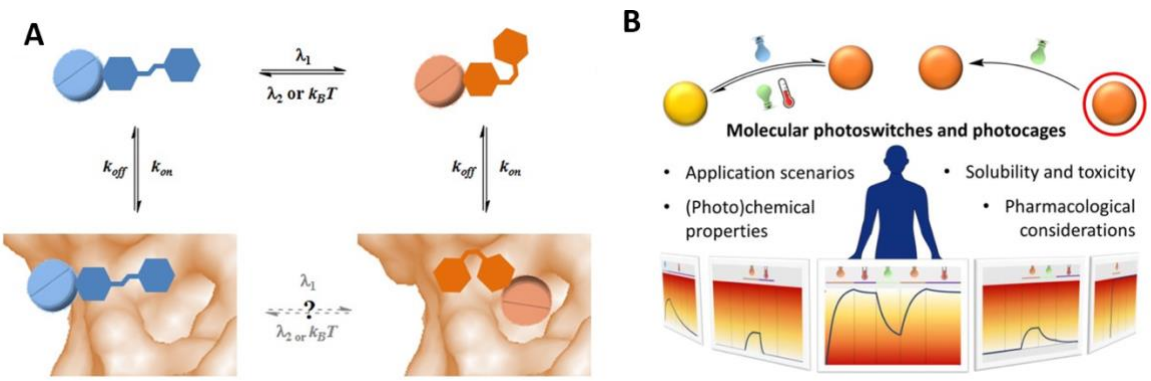

Figure. The principle of photopharmacology (A) and its key molecular tools (B)

The presentation will focus on our efforts towards bridging light and medicine, focusing first on new light-operated tools[2] (molecular photoswitches[3,4,5] and photocages[6], Figure B). Next, I will highlight the synergies between medical imaging and therapy, offered by light, through photoresponsive optical[7] and magnetic resonance[8,9] imaging agents. The examples of light-controlled bioactive molecules presented will include small molecules[10] and proteins[11]. Finally, using those examples, I will highlight the structural aspects[12] of photopharmacology.

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SHORT LECTURE

Innovative keratin-based bimodal nanoparticles for osteosarcoma treatment: a proof-of-concept study

Martella, E.,[1] *Dozza, B.,*[2] *Ferroni, C.,*[1] *Guerrini, A.,*[1] *Fini, M.,*[3] *Lucarelli, E.,*[3] *Columbaro, M.,*[3] *Serra, M.,*[3] *Martini, L.,*[3] *Donati, D.M. ,*[2,3] *Varchi, G.,*[1] and *Duchi, S.,* [1]

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Osteosarcoma (OS) affects children and young adults with an incidence of 0,2-3 cases/million per year in Europe [1]. Currently, the standard protocol for OS treatment is the administration of a cocktail of chemiotherapeutic drugs before and after the surgical resection to clear residual tumour cells and avoid lung metastasis. Despite this approach has increased patients' 5-years overall survival from 10% to 70%, no major breakthroughs have been made in the treatment of OS in the last 40 years and the onset of chemoresistance and key organs damage still represent major causes of chemotherapy failure in OS patients [2].

In the present work we propose a bimodal nano-platform that combines chemo- and photodynamic therapy (PDT) for increasing the efficacy of the pharmacological treatment. Paclitaxel (PTX) was selected as the chemotherapeutic drug due to its effectiveness against several cancers, and Chlorin e6 (Ce6) as the photosensitizer (PS) for PDT. To carry both drugs on the same formulation and increase the PTX solubility, high molecular weight and hydrosoluble keratin was chosen as carrier, and PTX-Ce6@ker nanoparticles were straigtforwadly prepared by a drug-induced aggregation method.

The efficacy of PTX-Ce6@ker was tested on three different OS cell lines (MG63, SaOS-2, and U2-OS) and on a doxorubicin resistant cell line (SaOS-2/DX580), both in 2D and 3D *in vitro* models. To evaluate the contribution of the two components of the treatment, cell viability was measured 24 h after PTX-Ce6@Ker treatment (PTX cytotoxicity) and 24 h after light irradiation (PDT toxicity). Importantly, our results highlight that Ce6 cellular uptake is higher when loaded into keratin nanoparticles as compared to the free form, whereas PTX efficacy is well preserved, indicating a sustained release of the drug from NPs. All OS cell lines were significantly affected by both PTX and Ce6 action in an additive manner both in 2D and 3D systems [3]. Based on these very promising *in vitro* results, the nanoformulation was tested in an *in vivo* orthotopic OS model. Intra-tibia injections of SaoS-2 cells were performed in six-week-old male immunocompromised mice (BALB/c). Five weeks after cells injection, mice were treated with a paratibial injection of PTX-Ce6@ker and immediately after the injection, half of them, were exposed to light irradiation (LED source at 668±3 nm) for 15 min. The protocol was repeated the subsequent week and mice were euthanized one week after the end of treatment. Preliminary results show that the combination of chemotherapy and PDT induces a massive death of tumour cells. Furthermore, an important immune system response was detected inside the tumour mass and in all surrounding tissues.

Taken together these results support the efficacy of this innovative nanoformulation, prompting a deeper investigation of their clinical potential as a very promising approach to increase OS patient's life expectancy.

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SHORT LECTURE

Keratin nanoparticles and photodynamic therapy enhance the anticancer stem cells activity of salinomycin

Greta Avancini[1], Andrea Guerrini[2], Claudia Ferroni[2], Daniele Tedesco[2], Marco Ballestri[2], Marta Columbaro[3], Luca Menilli[1], Elena Reddi[1], Roberto Costa[1], Luigi Leanza[1], Greta Varchi[2] and Francesca Moret[1]

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The high rates of aggressiveness, drug resistance and relapse of breast cancer (BC) are mainly attributed to the inability of conventional therapies to equally eradicate bulk differentiated cells and cancer stem cells (CSCs). CSCs have the capability of self-renewal and differentiation into phenotypically diverse cancer cells [1], thus enormously increasing the complexity of the cell populations forming tuomor tissue. Breast CSCs plasticity plays a pivotal role in progression, metastasis, resistance and recurrence, thus negatively influencing disease prognosis. Therefore, in BC treatment, the use of drugs selective toward BCSCs combined with first-line chemotherapeutics or other therapeuitic modalities has remarkably grown in the last two decades, even if translation into clinical protocols remains a complex process. Interestingly, drug repurposing has unveiled the anti-CSCs potential of several compounds able to modulate tumour resistance by targeting intrinsic self-renewal pathways, TME and metabolism [2]. For instance, the antibiotic salinomycin (SAL), the most potent known-to-date anti-CSCs compound, besides acting as ionophore and inducing ferroptosis, also modulates ABC transporters expression and interferes with multiple signalling pathways, such as PI3K/Akt, Wnt/β-catenin, Hedgehog, and Notch [3]. Thus, we synthetized a novel keratin-based nanoformulation loaded with SAL, the photodynamic therapy (PDT) photosensitizer chlorin e6 (Ce6) and vitamin E acetate (SAL/Ce6@kVEs) to combine the anti-BCSCs potential of SAL with the widespread production of singlet oxygen and cell death mediated by Ce6 upon light irradiation. Highly reproducible SAL/Ce6@kVE nanoparticles were obtained in water by nanoprecipitation, using vitamin E acetate as aggregating agent, thus avoiding the use of toxic solvents and cross-linking agents to induce nanoparticle formation and stabilization. *In vitro* experiments on BC cell lines (MDA-MB-231, MCF-7) and CSC-enriched tridimensional mammospheres exposed to single or combined therapies showed that SAL/Ce6@kVEs determine synergistic cell killing, limit self- renewal capacity and decrease the stemness potential by eradication of BCSCs with a higher efficiency with respect to the delivery of the drugs without nanoparticles. *In vivo* experiments on zebrafish embryos confirmed the capacity of SAL nanoformulations to interfere with the Wnt/β-catenin signaling pathway, which is dysregulated in BC, thus identifying a target for further translation into pre-clinical models.

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INVIITED LECTURE

Photobiology in Medicine 2021: an open ongoing discussion”

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The study of Photomedicine and photobiology in Dermatology has known a gold period in the seventies and eighties because phototherapy and photochemotherapy were widely used for the treatment of psoriasis and other inflammatory disorders. However, their use is progressively declined in the past 20 years because of the progressive introduction of new effective “biologic” drugs. However, some interest for new light sources based on new technologies, like LED and excimer lamps, particularly in the UVA1 range remains although the number of companies producing lamps for phototherapy is smaller. Moreover, other light sources, like monochromators, are no more available for clinical research because of the lack of interest of companies to spend money for their certification as a medical device.
The lack of interest of companies to develop photosensitizers for anticancer PDT is a fact whereas some room remain for anti-microbial PDT because it can be approved as a disinfectant for external use and not as a drug for both humans and animals. Great interest but, at present, no clear future for the use of photosensitizers as an “enhancer” of the clinical response to new target therapies or new immunotherapies for cancer.
Photobiology plays a pivotal role in the development of new non-invasive diagnostic techniques and various approaches together with diagnostic systems driven by Artificial Intelligence will change medicine in the next few years. In addition, much work remains to be done to clarify the pathogenetic role of UV and visible radiation in the development of skin cancers. Finally, the development of sunscreens that are really protective for the human skin and safe for both humans and the environment are an urgent issue in medicine.

SHORT LECTURE

Safety and efficacy of UVA1 phototherapy for the treatment of extracutaneous GVDH

Maria Teresa Rossi[1], *Alessandra Gelmetti*[1], *Marina Venturini*[1], *PierGiacomo Calzavara-Pinton*[1].

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Graft-versus-host disease (GVHD) is a complex multiorgan disease, which mainly occurs in patients after allogeneic hematopoietic stem cell transplantation, rarely even after solid organs transplantation. Depending on the time of onset, acute and chronic GVHD are distinguished [1].

The skin is the most frequently affected organ. Clinical manifestations include palmoplantar erythema associated with a symmetrical, morbilliform, macular or maculopapular, sometimes hemorrhagic, exanthema with involvement of the face. Lesions might be painful, pruritic or asymptomatic. In most severe cases, widespread epidermolysis and erosive involvement of the adjacent mucosae might be observed. Mucosal involvement, particularly oral, genital, conjunctival and intestinal, is not infrequent even in milder cases, leading to a significantly impaired quality of life [1].

UVA1 phototherapy is a safe and effective option for the treatment of cutaneous GVHD [2,3].

Among all the patients undergoing UVA1 phototherapy at our Center, those affected by cutaneous GVHD in association with mucosal and/or systemic involvement, were selected. A retrospective analysis based on clinical and laboratory data showed the potential therapeutic action of UVA1 phototherapy beyond the site of application, being effective for treating both the cutaneous and extracutaneous manifestations of the disease.

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SHORT LECTURE

A multiphoton intravital microscopy study of skin photodamage induced by femptosecond pulsed laser radiation

Viola Donati[1,*], Chiara Peres[2,*], Chiara Nardin[2], Ferdinando Scavizzi[2], Marcello Raspa[2], Mario Bortolozzi[1] and Fabio Mammano[1,2] (*equal contribution)

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BACKGROUND: The mammalian skin, the body's largest single organ, is a highly organized tissue that forms an essential barrier against dehydration, pathogens, light and mechanical injury. Damage triggers perturbations of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) that spread from cell to cell (known as intercellular Ca^{2+} waves) in different epithelia, including epidermis. Ca^{2+} waves are considered a fundamental mechanism for coordinating multicellular responses, however the mechanisms underlying their propagation in the damaged epidermis are incompletely understood.
AIM OF THE PROJECT: To dissect the molecular components contributing to Ca^{2+} wave propagation in a murine model of epidermal photodamage.
METHODS: To trigger Ca^{2+} waves, we used intense and focused pulsed laser radiation and targted a single keratinocyte of the epidermal basal layer in the earlobe skin of live anesthetized mice. To track photodamage-evoked Ca^{2+} waves, we performed intravital multiphoton microscopy in transgenic mice with ubiquitous expression of the sensitive and selective Ca^{2+} biosensor GCaMP6s. To dissect the molecular components contributing to Ca^{2+} wave propagation, we performed *in vivo* pharmacological interference experiments by intradermal microinjection of different drugs.
EXPERIMENTAL RESULTS: The major effects of drugs that interfere with degradation of extracellular ATP or P2 purinoceptors suggest that Ca^{2+} waves in the photodamaged epidermis are primarily due to release of ATP from the target cell, whose plasma membrane integrity was compromised by laser irradiation. The limited effect of the Cx43 selective inhibitor TAT-Gap19 suggest ATP-dependent ATP release though connexin hemichannels (HCs) plays a minor role, affecting Ca^{2+} wave propagation only at larger distances, where the concentration of ATP released from the photodamaged cell was reduced by the combined effect of passive diffusion and hydrolysis due to the action of ectonucleotidases. The ineffectiveness of probenecid suggests pannexin channels have no role. As GCaMP6s signals in bystander keratinocytes were augmented by exposure to the Ca^{2+} chelator EGTA in the extracellular medium, the corresponding transient increments of the $[Ca^{2+}]_c$ should be ascribed primarily to Ca^{2+} release from the ER, downstream of ATP binding to P2Y purinoceptors, with Ca^{2+} entry through plasma membrane channels playing a comparatively negligible role. The effect of thapsigargin (a well-known inhibitor of SERCA pumps) and CBX (a recently recognized inhibitor of Ca^{2+} release through IP_3 receptors) support this conclusion.
CONCLUSIONS: The one presented here is an experimental model for accidental skin injury that may also shed light on the widespread medical practice of laser skin resurfacing, used to treat a range of pathologies from photodamage and acne scars to hidradenitis suppurativa and posttraumatic scarring from basal cell carcinoma excision. The results of our experiments support the notion that Ca^{2+} waves reflect chiefly the sequential activation of bystander keratinocytes by the ATP released through the compromised plasma membrane of the cell hit by laser radiation. We attributed the observed increments of the $[Ca^{2+}]_c$ chiefly to signal transduction through purinergic P2Y receptors. Several studies have highlighted fundamental roles of P2Y receptors during inflammatory and infectious diseases, and the initial phase of wound healing involves acute inflammation. In addition, hyaluronan is a major component of the extracellular matrix and its synthesis is rapidly upregulated after tissue wounding via P2Y receptor activation. It is tempting to speculate that response coordination after injury in the epidermis occurs via propagation of the ATP-dependent intercellular Ca^{2+} waves described in this work.
ACKNOWLEDGEMENTS: Supported by the University of Padova (Grant No. BIRD187130 to FM) and Fondazione Telethon (Grant No. GGP19148 to FM).

SHORT LECTURE

Squaraines as photosensitizers: a structure-function analysis by means of *in vitro* cytotoxicity test and Calcium signaling assessment

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Photodynamic therapy (PDT) is an emerging, minimally invasive therapeutic modality approved by the U.S Food and Drug Administration for the treatment of several conditions including oncological applications [1]. The approach is based on light-induced photosensitizers (PS) activation which, in turn, promotes the intersystem crossing and triplet state that easily reacts with molecular oxygen, thus generating free radicals and singlet oxygen species with a final increase in apoptosis, necrosis, and autophagy processes and tumor mass reduction [1]. In this context, the development of new PS for the PTD is of great interest to increase the treatment efficacy minimizing side effects. Among numerous PS already proposed, NIR polymethine dyes (PMDs) such as cyanines (CY) and squaraines (SQ) proved to be successful singlet oxygen ($^1O_2$) generators and promising candidates as PS in PDT applications [2,3]. In the present work, we investigated the structure-activity relationship of some SQ based on the indolenine ring. In particular, we compared the phototoxic activity of unsubstituted SQ, carboxyl (COOH)- and bromine (Br)-substituted SQ, as well as SQ in which the oxygen atoms of the squaryl ring were replaced with sulfur atoms (S-SQ). Indeed, the presence of heavy atoms such as Br and the replacement of oxygen with sulfur are predicted to shift the emission to the far NIR region and increase the quantum yield of singlet oxygen [4], thus improving the overall effect in PDT. As expected, Br-SQ and S-SQ demonstrate a higher phototoxicity on MCF-7 cells compared to the COOH-SQ dye, which showed no effect. However, surprisingly, our data reveal that also the unsubstituted SQ has a strong phototoxic activity. In addition, going deeper into the molecular mechanisms underlying SQ phototoxicity, we analyzed PS-mediated intracellular signaling by correlating the phototoxic effect of Br and S-SQ with cytoplasmic and mitochondrial Ca^{2+} signals and ROS generation. Our results shed new light on the structure-function relationship of SQ and the intracellular pathways involved in their exploitable photo-activity in PDT, demonstrating a critical role for intracellular Ca^{2+} signals in ROS activation and cell death.

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SHORT LECTURE

The Effect of Substitutions on Cyanine dyes on their Photodynamic Activity

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Over the recent years, extensive efforts have been devoted to the development of near-infrared (NIR) dyes for biological applications, especially for photodynamic therapy (PDT).
In this context, polymethine dyes can be considered as innovative photosensitizers (PS) due to the easy and low-cost synthesis along with remarkable absorption property in the far-red NIR region, perfectly matching the biological tissues' transparency window (600-900 nm) [1]. In particular, NIR polymethine cyanines (CY) are well suited for this purpose and have been extensively studied for many biomedical applications [2], thanks to their high molar absorption coefficients, remarkable brightness, fluorescence and photostability, especially in organic media [3]. However, despite their excellent photodynamic activity, their chemical instability and self-aggregation properties when in contact with biological media still limit their effective clinical application.
To overcome these drawbacks, the incorporation of these dyes in nanoparticles (NPs) is extremely important to prevent the formation of dye aggregates in aqueous environment.
The present contribution deals with the design and synthesis of a new series of CY polymethine dyes based on indolenine ring and with different substitution groups synthetized by microwave irradiation and photophysically characterized by UV-Vis and fluorescence spectroscopy, with the aim to highlight a structure-activity relationship.
Afterwards, to assess the suitability of these dyes to be used in PDT, the Reactive Oxygen Species (ROS) production ability of the CY samples has been evaluated by using the 1,3-diphenylisobenzofuran (DPBF) probe and compared with standards PS well-known in literature (i.e Methylene Blue and Bengal Rose).
In addition, the *in vitro* biological assessments were examined by exposing the MCF-7 cells to increasing concentration of each PSs to select the maximum non-cytotoxic concentration suitable for performing the *in vitro* PDT. The potential photocytotoxicity of each cyanine dyes was evaluated by performing an *in vitro* photodynamic treatment, showing low cytotoxicity in dark, but promoting phototoxic effect upon irradiation.

Finally, these dyes have been also encapsulated into Solid Lipid Nanoparticles (SLNs) and Human Serum Albumin (HSA) NPs to increase their solubility and improve their photochemical properties.

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SHORT LECTURE

RKIP as a potential target to improve photodynamic therapy in PC3 prostate cancer cells

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Raf- kinase inhibitor protein (RKIP) is involved in various cellular processes through interaction with several signaling pathways such as mitogen activated protein kinase (MAPK), nuclear factor kappa light chain enhancer of activated B cell (NF-kB), G protein-coupled receptors (GPCR), and glycogen synthase kinase 3Beta (GSK 3B). RKIP negatively affects tumor survival and proliferation and acts as a metastasis suppressor. In addition, overexpression of RKIP is reported to abrogate tumor chemo/immune/radio resistance and support cancer host immunosurveillance.
In this work, we aimed to investigate the role of RKIP expression on the sensitivity of PC3 prostate cancer cells to photodynamic therapy (PDT).
Our previous studies demonstrated the involvement of a specific NF-kB/YY1/Snail/RKIP loop in PDT tumor cell response. The expression of the pro-survival NF-kB gene is closely associated with that of the pro-apoptotic RKIP gene. A low dose of PDT stimulates survival and proliferation of PC3 prostate cancer cells, as highlighted by an increase in pro-survival NF-kB, YY1, and Snail genes and low expression of the pro-apoptotic RKIP gene. An optimal dose of PDT determines tumor growth arrest with a strong increase in RKIP expression and consequently inhibition of pro-survival genes.
Another molecular pathway associated with PDT-induced photooxidative stress is Nrf2.
Nrf2 is a transcription factor that upregulates the antioxidant cellular system to ensure cell survival.
Here, we evaluate the role of RKIP in modulating Nrf2 and NF-kB signaling pathways in determining the fate of PDT tumor response and propose the RKIP gene as a potential therapeutic target.

SHORT LECTURE

Photodynamic effects of positive-charged diaryl porphyrins on multicellular tumor spheroids

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Photodynamic therapy (PDT) of cancer is a non-invasive clinical option in which reactive oxygen species are formed only when three harmless components, a photosensitizer (PS), light and molecular oxygen are present at the same time, leading to cell death [1]. Most of the PSs have been tested on monolayer cells, but differences between 2D cells and solid tumors significantly limit the value of *in vitro* PDT studies, whereas the use of 3D spheroid might be more suitable for drug development and preclinical drug testing for PDT [2].
In a previous work we have shown that two positive-charged diaryl porphyrins (**2** and **4**) were more potent than the corresponding neutral molecules (**1** and **3**) on a panel of 2D-cultured cancer cell lines [3]. In the present study the photodynamic effects of these molecules have been evaluated on HCT116 and MCF7 spheroids. Induction of apoptotic and necrotic cell death, and generation of reactive oxygen species (ROS) have been also evaluated, along with accumulation and localization of PSs into spheroids.
Our findings indicate that **2** and **4** retained their phototoxic effects also in 3D spheroids; furthermore, they were more potent than **1** and **3** and as potent as Foscan (m-THPC), the most successful PS approved for clinical PDT of cancer, used as reference. Although further aspects of their mechanisms of action need to be addressed, our results strongly suggest a potential *in vivo* photodynamic application of **2** and **4**, considering that spheroids represent a more realistic indicator of *in vivo* therapeutic efficacy than 2D cell lines.

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AWARD LECTURE

Photodynamic Therapy and *ras* genes: Effect of Bifunctional Alkyl-Modified Porphyrins on G-quadruplex RNA Structures

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The *ras* genes are frequently mutated in human cancer, in particular mutations in *KRAS* are implicated in the development and progression of pancreatic ductal adenocarcinoma (PDAC). In this study we propose a new targeted therapy to inhibit *KRAS* in PDAC cells. Our strategy stems from four observations. First, PDAC cells are strongly dependent on *KRAS*, as this oncogene reprograms the metabolism to produce the biomass required to fuel a high rate of proliferation. Second, *KRAS* mRNA contains a 5'-untraslated region (5'-UTR), that is characterized by a high amount of guanines (45%), harboring three G-quadruplex motifs that fold into G-quadruplex RNA structures stabilized by two tetrads of guanines (RG4s) [1]. Third, cationic phorphyrins (CPs) are known to strongly bind to RG4s, by end-stacking over the G-tetrads [2,3]. Fourth, photoactivated CPs bound to RG4s generate reactive oxigen species (ROS) and singlet oxygen ($^1O_2$) that degrade mRNA and induce apoptosis [4,5]. On these grouds we reasoned that an interesting approach against PDAC would be to target RG4s with CPs that, upon photoactivation, will breakdown mRNA and suppress *KRAS* in PDAC cells. To increase the cellular uptake, we designed CPs with an alkyl chain of more than 12 carbons and investigated their capacity to bind to and suppress *KRAS* mRNA. The suppression of *KRAS* induced apoptosis and a strong cell growth arrest. We also investigated a different delivery strategy for the designed CPs. We fixed CP-decorated porphyrins, through their alkyl chain, on the lipid bilayer of POPC liposomes. We found that CP-decorated liposomes are efficiently transported in PDAC cells by endocytosis. These nanoparticles efficiently arrest cell growth and colony formation of PDAC cells. Finally, considering that the anticancer activity of CPs is mediated by ROS, we investigated if the suppression of Nrf2, which controls the cell response to oxidatice stress, in Panc-1 cell is a proper strategy to boost the bioactivity of CPs. To sum up, our data show that alkyl modified porphyrins, delivered as free molecules or bound to liposomes, are efficient photosensitizers to treat PDAC. These molecules act by suppressing KRAS and inducing apoptosis in pancreatic cancer cells.

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AWARD LECTURE

Folate-decorated amphiphilic cyclodextrins as cell-targeted nanophototherapeutics

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Nowadays, active targeting of nanotherapeutics is a challenging issue. Here, we propose a rational design of a ternary nanoassembly (SAP) composed of nonionic amphiphilic β-cyclodextrins (amphiphilic CD) [1-2] incorporating pheophorbide (Pheo) as a phototherapeutic and an adamantanyl-folic acid conjugate (Ada-FA) to target tumor cells overexpressing α-folate receptor (FR-α(+)). Dynamic light scattering and ζ-potential pointed out the presence of nanoassemblies bearing a negative surface charge (ζ = −51 mV). Morphology of SAP was investigated by atomic force microscopy and microphotoluminescence, indicating the presence of highly emissive near-spherical assemblies of about 280 nm in size. Complementary spectroscopic techniques such as ROESY-NMR, UV/vis and steady-state fluorescence revealed that the folic acid protrudes out of amphiphilic CD rims, prone for recognition with FR-α. Pheo was strongly loaded in the nanoassembly mostly in monomeric form, thus generating singlet oxygen ($^1O_2$) and consequently showing phototherapeutic action. SAP remained stable until 2 weeks in aqueous solutions. Stability studies in biologically relevant media pointed out the ability of SAP to interact with serum proteins by means of the oligoethylenglycole fringe, without destabilization. Release experiments demonstrated the sustained release of Pheo from SAP in environments mimicking physiological conditions (~20% within 1 week), plausibly suggesting low Pheo leaking and high integrity of the assembly within 24 h, time spent on average to reach the target sites. Cellular uptake of SAP was confirmed by confocal microscopy, pointing out that SAP was internalized into the tumoral cells expressing FR-α more efficiently than SP. SAP showed improved phototoxicity in human breast MCF-7 cancer cells FR-α(+) (IC_{50} = 270 nM) with respect to human prostate carcinoma PC3 cells (IC50 = 700 nM) that express a low level of that receptor (FR-α(−)). Finally, an improved phototoxicity in FR-α(+) MCF-7 cells (IC_{50} = 270 nM) was assessed after treatment with SAP vs SP (IC_{50} = 600 nM) which was designed without Ada-FA as a targeting unit.

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SHORT LECTURE

Potentiating connexin hemichannel-mediated bystander effects improves photodynamic therapy outcome in a syngeneic melanoma mouse model

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Cutaneous melanomas are the most aggressive skin cancer types [1]. Despite its uncomparable qualities, the application of photodynamic therapy (PDT) to the treatment of pigmented melanomas is limited by several factors, including melanin antioxidant properties [2] and attenuation of light penetration due to pigment absorbance [3]. To find a strategy to improve therapy efficacy also in deeper layers of tumor mass, we investigated the propagation of bystander effects induced by PDT with the photosensitizer Aluminum Phthalocyanine Chloride (AlClPc) in a syngeneic murine melanoma model [4].

In multiphoton intravital microscopy experiments using genetically encoded fluorescent biosensors expressed by melanoma cells *in vivo*, we showed that intercellular calcium (Ca^{2+}) waves propagated from irradiated to non-irradiated cells promoting cytosolic Ca^{2+} rise and intracellular Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria, paralleled by rapid activation of apoptotic pathways. The propagation of bystander processes was mediated by paracrine signaling due to ATP release from connexin (Cx) hemichannels (HCs) and was amplified in the presence of EGTA, that chelates extracellular Ca^{2+}, increasing HC opening probability. Thus, we reasoned that combination treatment with HC openers could potentiate bystander cell killing via enhanced Ca^{2+} signaling. To verify this hypothesis, prior to irradiation we intratumorally administrated S-Nitrosoglutathione (GSNO), an endogenous nitric oxide (NO) donor that biases the HCs towards the open state in our melanoma model. Combination treatment with GSNO greatly improved the efficacy of AlClPc-mediated PDT, leading to 86 % reduction of post-irradiation tumor mass.

These findings strongly indicate that HCs expressed by tumor cells are candidate targets for enhanced PDT and potential application of HC activators as PDT adjuvants for the treatment of melanoma and possibly other cancer types should be further investigated in clinical settings.

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INVITED LECTURE

Chronophotopharmacology: controlling the circadian clock with light

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Inspired by the crucial role of circadian clock disruption in disease development,[1] during the last decade chemical biology studied how to adjust cellular clocks using small molecule modifiers.[2] However, these modifiers face a big drawback when in vivo application is needed: due to the similarity in cellular regulation of clocks, besides curing the disrupted biological rhythm, they would affect all the others, healthy rhythms. To overcome this problem, a potential strategy would be regulation of a compound`s bioactivity with light, which can be delivered precisely in space and time.[3]

Here, new photo-responsive modulators (cages and photoswitches) were successfully designed, developed, and applied to modulate circadian rhythm in a light-inducible manner. The control was achieved by interfering with the proteins of the core clock transcription-translation negative feedback loop or by regulation of the protein kinase activity involved in posttranslational modifications. As a result, we have applied photo-modulation of the circadian rhythm in cells, *ex vivo* and a living organism, and for the first time enabled the restoration of the phase shift under jet leg experimental conditions.

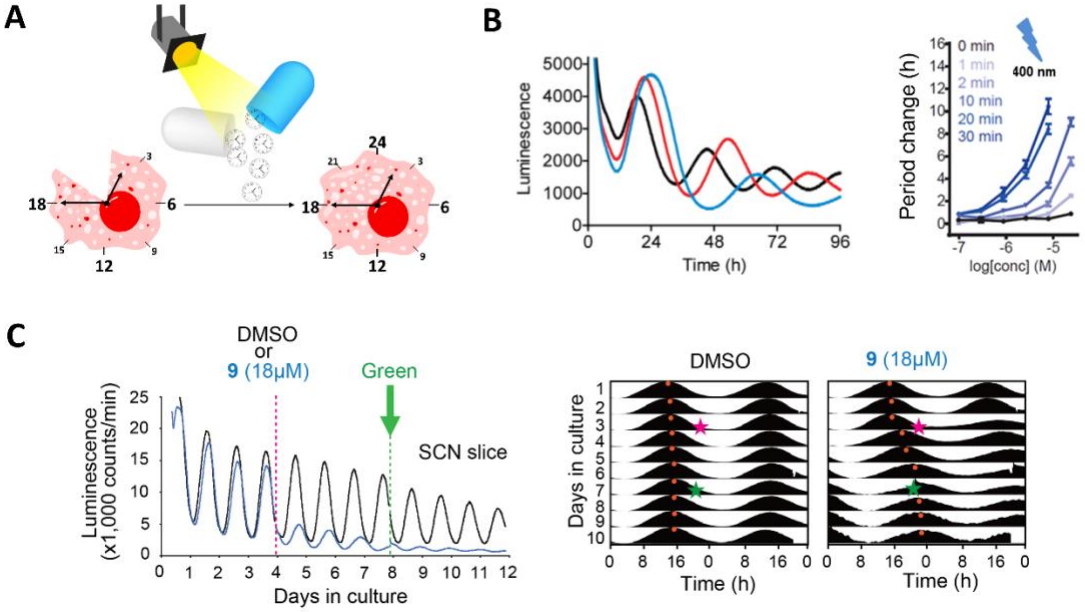

Figure 1. A) The illustration of chronophotopharmacology; B) Irradiation-time dependent circadian period change in cells; C) Reversible modulation of the circadian rhythm in tissue (suprachiasmatic nucleus or master clock) using visible light.

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INVITED LECTURE

Novel phthalocyanines as potential light-activated insecticides

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Oxidative radicals generated by light-excitation of dyes or photosensitizers have long been examined as biocides against insect pests [1-2]. We have begun to screen some novel phthalocyanines (PCs) in comparison to other dyes for light activation to produce singlet oxygen-initiated cytotoxic activities against insect cells in vitro and insect pests of medical and agricultural importance [3]. The results illustrated activities of structurally different PCs, porphyrins and halogenated fluoresceins, e.g. Rose Bengal and Cyanosine, consistent with the in vitro findings of the mosquito larvicidal activities of the PCs in vivo [4]. Promising outcomes were noted against plant sap-sucking whiteflies and aphids – vectors for transmitting viral diseases of plants. Halogenated fluorescein-fed whiteflies were killed, but only after exposure to dim light. Army worms –a significant leave-chewing pest [5] were light-sensitive when fed with PC-containing diets. Initial work also showed PC-mediated killing of newly emerged cockroach nymphs, omnivorous insect of nuisance worldwide. Unresponsiveness of adult roach points to the necessity of further investigation. In summary, the preliminary results obtained clearly demonstrate the potential advantages and effectiveness of PC as a new approach for consideration to develop light-activated insecticides.

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Singlet oxygen-initiated inactivation of Leishmania for safe and effective delivery of vaccines against infectious and malignant diseases

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The trypanosomatid protozoa include Leishmania species, which cause miner skin infection [1]. Patients cured of such cutaneous disease invariably develop life-long immunity to re-infection, pointing to the potential use of Leishmania as vaccines and vaccine carriers [2]. This is made possible by inactivation of Leishmania initiated with singlet oxygen, rendering them completely non-viable for safe application, while preserving their immunological properties. Photosensitizers of different properties are used to load separate cellular compartments of Leishmania in vitro for light exposure to generate cytotoxic singlet oxygen. Leishmania accumulates UV-excitable uroporphyrin I cytosolically when genetically engineered to express the 2nd and 3rd enzymes in the heme biosynthetic pathway followed by exposure to delta-aminolevulinate – the product of the 1st enzyme in this pathway [3]. This outcome is expected from the genetic defects of Leishmania in missing first five of the 8 enzymes in the pathway for heme biosynthesis [4]. Uroporphyrinogen I produced in the transfectants is thus auto-oxidized into uroporphyrin I in the absence of the downstream decarboxylase. The

uroporphyrinogenic Leishmania are further loaded in their endosomes with red light sensitive phthalocyanines, which are made cationic by chemical engineering for endocytic uptake [5]. The Leishmania rendered non-viable by installation of this dual mechanism of inactivation delivers vaccines to activate immune T cells in vitro [5], protect laboratory animals against leishmaniasis [6-8] and prevent the emergence of tumors in animal models [9]. Current plan of work is to carry out in vitro vaccination with inactivated Leishmania expressing cancer vaccines in the direction of DC-based immunotherapy of human lung cancer.

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Optical and spectroscopic methods applied to biology, medicine and biosafety

INVITED LECTURE

Organic optoelectronic components in highly integrated systems for plasmonics sensing in food security/quality

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The increase of contaminants levels in food has led to negative human health effects from exposure to toxic substances. The interactions between environment and food supply chain that mainly occur at primary production level can cause serious both short- and long-term detrimental effects on human health. Moreover, quality parameters directly affect both the industrial process and the final nutritional and organoleptic properties of the finished products and need to be assessed routinely to increase process monitoring efficiency of the food supply chain and, in turn, competitiveness of the European food processing industry.

The smart integration of multiple devices in a single functional unit is boosting the advent of compact optical sensors for cost-effective and on-site analysis. Considering plasmonic sensors, the strict constraints on the detection scheme are hampering the deployment of this extremely powerful sensing technology.

The MOLOKO project aims at the manufacturing and implementation of miniaturized organic photonic sensor for plasmonics-based screening of safety and quality parameters for sustainable milk and dairy industry. In particular, the multiplexed and (semi)quantitative detection is expected to be of up to 10 analytes among which food safety parameters e.g. antibiotics (i.e. penicillin, cephalsporin) and toxins (i.e. enterotoxins) and food quality parameters e.g. lactoferrin and caseins.

The rapid and reusable final prototypal sensor is based on an opto-microfluidic detection scheme and designed according to milk production and distribution end-users. These challenging objectives are achieved by the unprecedented integration within the same device platform of multiple key-enabling technologies as organic photonics, nanoplasmonics, immunoassay diagnostics and microfluidic system.

In this contribution, we report on the latest results obtained in the project with particular attention to (i) the scheme of monolithic integration of the different nanostructured device components resulting in a sensor size as low as 0.1 cm^3, (ii) the proof of concept of the innovative detection scheme in lab environment reporting dose-response curves for analytes of interest and (iii) two specific application scenarios for screening milk at the different levels of the value chain (i.e. cow, farm and plant levels).

This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 780839.

SHORT LECTURE

Brillouin and Raman micro-Spectroscopy: a new tool for the chemo-mechanical characterization of human bone and cartilage in physiological and pathological conditions

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Brillouin and Raman micro-Spectroscopy (BRamS) is a scattering technique allowing the simultaneous analysis of both the sample mechanical and chemical properties with a micrometric resolution. It is contact-less, not-destructive, and label-free, thus it is suitable for the imaging of biological tissues *ex vivo* and *in vivo* [1]. Since its first use, it has been gaining more and more attention into the characterization of cells, microbial biofilms, tissue phantoms [2], and biological tissues involved in pathological processes, such as Barrett's esophagus, Alzheimer's disease and corneal keratoconus [3]. Human bone and cartilage tissues are characterized by a stringent structure-function relation. In particulat, bone exhibits a hierarchical architecture with several different levels of organization, while the bearing-like structure of the articular cartilage is specifically designed to allocate mechanical stresses throughout the joint interface, preventing bone friction [4,5]. Several diseases, such as Osteoarthritis, originate from the failure of these tissues to maintain the correct arrangement of their constituents already at the microscale, and thus resulting in an impairment of the whole-tissue performance. Here, we present the BRamS chemo-mechanical characterization of the human femoral head and diaphysis [6] and the first results obtained into the application of this technique to the diagnosis of orthopedic disease, i.e the hip joint Osteoarthritis.

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Anti-microbial PDT

INVITED LECTURE

Antimicrobial photidynamic inactivation of microorganisms

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The worldwide and continuous increase in antibiotic resistance requires alternative strategies to combat bacteria. A promising complement to the conventional use of antibiotics is the principle of antimicrobial photodynamic inactivation. In this procedure, a per se non-toxic dyes, so-called photosensitizers, are excited with visible light in the presence of oxygen. Thereby it produces reactive oxygen species that irreversibly cause an oxidative damage to biological molecules, which enables the non-specific killing of bacteria, including multi-resistant pathogens. In this talk the current knowledge about antimicrobial photodynamic inactivation will be discussed.

SHORT LECTURE

Antimicrobial Photodynamic Action of Photosentising Nanoassemblies based on Cyclodextrins

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New broad-spectrum antimicrobial strategies, alternative to antibiotics and able to efficiently inactivate pathogens without inducing resistance, is one of the main objectives in public health. Antimicrobial photodynamic therapy (aPDT), based on the light-induced production of reactive oxygen species from photosensitizers (PS), is attracting growing interest to infection treatment, also including biofilm distruction. Due to limited photostability of free PS, delivery systems are highly required to decrease PS photodegradation, thus improving the therapeutic efficacy and to reduce collateral effect on unaffected tissues [1]. In this study, we propose two photosensitizing nanosystems based on the same cationic porphyrin 5,10,15,20-tetrakis (N-methyl- 4-pyridyl)-21H,23H-porphyrin (TMPyP) assembled with two different cyclodextrins (CD), namely CD nanosponge (CDNS) [2] and the trade sulfobutylether-beta-cyclodextrin (CAPTISOL®) [3]. Complementary spectroscopic techniques such as UV-Vis, fluorescence emsission as well Dynamic Light Scattering (DLS) and ζ-potential pointed out the complexation between β PS and CDs. Nanoassemblies with photodynamic features exhibited photoantimicrobial activity on Gram-negative and Gram-positive bacteria, in addition to generally sustained release properties and a higher photostability. Moreover, preliminary results of TMPyP/CAPTISOL® and CAPTISOL® alone against biofilms from *P. aeruginosa* reveal that i) CAPTISOL® alone inhibits biofilm formation (regardless of light exposure) and ii) TMPyP/CAPTISOL® nanoassembly significantly increases biofilm degradation respect to TMPyP alone. Altogether *in vitro* photo-antibactericidal studies elucidated the aPDT efficacy of our photosensitizing nanosystems based on CD.

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SHORT LECTURE

Aerosol-based antimicrobial photoinactivation in the lungs: an action spectrum study

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Chronic lung infections are important clinical challenges, being often associated with biofilm-forming and multidrug-resistant bacteria. In this framework, the European project "Light4Lungs" aims at synthesizing and testing an inhalable light source to control lung infections by antimicrobial photoinactivation (aPDI), addressing endogenous photosensitizers only (porphyrins) which induces cell death by the production of cytotoxic reactive oxygen species (ROS). This work defines and calculates the photo-killing action spectrum for lung aPDI in the exemplary case of biofilm-associated infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* in patients affected by cystic fibrosis. This was obtained by applying a semi-theoretical modelling with Monte Carlo simulations to the infected lung regions, according to a simple optical theory [1] and previously published methodology related to stomach infections [2]. The variability of *in vivo* pathologic conditions [3] was accounted for by a careful analysis of the variation in the concentration of oxygen, bacteria and relative endogenous porphyrins, besides the presence of other relevant absorbers/diffusers inside the biofilm/mucous layer. The obtained semi-theoretical action spectrum is peaked at 394 nm and mostly follows porphyrins extinction coefficient behavior. To confront these results with an experimental validation, we permorfed *in vitro* experiments using preformed biofilms grown on a standardized static model (MBEC Assay). Reference and clinical strains from cystic fibrosis were tested (for a total of four strains). Biofilms were irradiated with representative wavelengths, considering LED sources centred in the violet region (415nm) and in the green (525nm). Light at 415nm showed a dose-dependent antimicrobial effect, with reduction of at least 2 log CFU at 60 J/cm^2 observed with three tested strains (including the *P. aeruginosa* and *S. aureus* clinical isolates from cystic fibrosis). Light at 525 nm showed a scant or no effect.
The obtained results can offer important indications for the synthesis of the aerosolized light source and definition of its most effective emission spectrum, suggesting also a flexible platform for dosimetry calculation to be considered in further applications.

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SHORT LECTURE

Novel BODIPY derivatives possessing aliphatic tertiary and quaternary amine groups as efficient anticancer and antibacterial photosensitizers

Weronika Porolnik[1], Malgorzata Kucinska[2], Jolanta Dlugaszewska[3], Marek Murias[2], Jadwiga Mielcarek[1], Jaroslaw Piskorz[1]

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Cancers and microbial infections are recently two severe and urgent global health problems. Thus, developing new approaches for the treatment of both these medical challenges is currently of great interest. Photodynamic therapy (PDT) has been successfully applied for the treatment of localized cancers and other premalignant or non-malignant dermal lesions, as well as a perspective modality to treat microbial-induced pathologies. PDT involves using a photoactive compound, which after irradiation with light of appropriate wavelength generates toxic oxygen species, such as singlet oxygen or free radicals. These species can damage various cellular components leading to the death of targeted cells [1].

Boron dipyrromethene (BODIPY) derivatives are organic chromophores possessing many attractive properties, such as strong absorption, high fluorescence quantum yields, and good stability. Moreover, their optical features can be easily modified by structural changes. Bromine or iodine substitution in BODIPYs core drastically increases singlet oxygen generation, making them promising candidates for PDT [2]. Also, BODIPY with positive charge shows increased solubility and optimal activity on both Gram-positive and Gram-negative bacteria, as well as other pathogens such as fungi and protozoa [3].

A series of novel BODIPY derivatives possessing dimethylaminopropoxyphenyl substituents and their cationic derivatives was synthesized and characterized using mass spectrometry, UV-Vis spectrophotometry, and various NMR techniques. Photochemical studies, including the absorption and emission properties, as well as the ability to singlet oxygen generation assessment, were performed. It was found that the introduction of iodine atoms at 2 and 6 positions of the BODIPY core caused an about 30 nm bathochromic shift of the absorption band and significantly increased values of the singlet oxygen generation quantum yields. *In vitro* photodynamic antimicrobial activity studies were performed on Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, as well as on human androgen-sensitive prostate adenocarcinoma cell line (LNCaP). Also, the impact of the presence of quaternary ammonium cation and iodine atoms, in the structure of BODIPYs, on physicochemical and photochemical properties, as well as photodynamic activity, was assessed. BODIPY derivative possessing both a positive charge and iodine atoms revealed the highest activity towards all studied cells [4].

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SHORT LECTURE

Antimicrobial photodynamic treatment of *Candida albicans*

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Candida albicans is a yeast living in many natural environments and commensal of various animals. In humans, it can be found as part of the skin flora or in gastrointestinal, respiratory, and genitourinary tracts. *C. albicans* is considered an opportunistic pathogen because, even if it is harmless for the host, sometimes causes vaginal and oral infections, may enter the bloodstream leading to deep-tissue infections leading to a mortality rate of almost 40 % [1]. The selection of *C. albicans* strains resistant to common antifungal agents (azoles, echinocandins, polyenes) is an important issue in clinical environment. Furthermore, the tolerance to antifungals is favored by biofilm formation on tissues or devices surfaces. The production of extracellular polymeric substance (EPS) represents a physical barrier to antimicrobial compounds. In particular, β1,3 glucan is responsible for azole, echinocandins and polyene sequestration, while eDNA prevents the activity of polyenes and echinocandins [2]. In this scenario, light-based techniques could represent anti-fungal tools in medical care both in environmental sanitization and clinical devices or in host treatment.

In this study, we compared the photoactivity of two-family compounds of photosensitizers, diarylporphyrins and borondipyrromethens (BODIPYs), in yeast cultures. A rich panel of neutral and cationic compounds belonging to both classes was investigated. The model strain *C. albicans* ATCC 14053 showed to be more sensitive to PSs in dark conditions than Gram-positive and Gram-negative bacteria. The tested cationic diarylporphyrins activated by light at 410 nm were able to kill suspended cells and inhibit the biofilm formation both of *C. albicans* type strain and clinical strains. Among BODIPYs, different neutral compounds and one monocationic, under irradiation at 520 nm, showed to be efficient in controlling the growth of suspended cultures of *C. albicans*. A good rate of photoinhibition of biofilm was obtained under low fluence rate (30 J/cm^2). Confocal microscopy analysis showed that the photodynamic treatment with active PSs belonging both to porphyrins and BODIPYs caused the loss of eukaryotic compartmentalization, in accordance with their cytoplasmic uptake.

This study supports the potential of photodynamic therapy as antifungal treatment. In particular, monocationic diaryl-porphyrins and BODIPYs showed to be optimal photosensitizers under studied settings. The presence of one positive charge on PSs periphery favored the binding to fungal cell without eliciting an intrinsic toxic effect.

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Applied Photobiology and Biophysics

INVITED LECTURE

Nanomaterial Engineering and Bioengineering of Living Photovoltaics for Enhanced Performance

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Living photovoltaics are microbial technologies that convert solar energy into electricity using live cells. Photosynthetic microbes, in particular, contain innate light-harvesting metabolisms that are conducive to realizing such technologies. Despite their advantages, these microbes have insulating membranes that limit extracllular electron transfer, resulting in poor device efficiencies.

This presentation focuses on inter-disciplinary approaches to overcoming this barrier with cyanobacteria using complementary bioengineering and materials engineering techniques. We discuss recent advancements in synthetic biology that allows us to increase extracted photocurrent through the expression of foreign heme proteins inside cyanobacteria [1]. Further improvements in device performances can also be achieved through materials engineering of the electrode surface to facilitate charge transfer from the microbe [2]. Finally, we discuss the development of nanobionic cells that show augmented capabilities through the internalization of nanoparticles. Together, these endeavors represent an emerging movement that aims to blur the interface between living and non-living materials, both metaphorically and literally, to improve the performances of bioelectric microbial devices.

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SHORT LECTURE

Electro-optical interaction of carbon nanotubes with photosynthetic assembles

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The development of various biohybrid natural or artificial systems for promoting the solar energy conversion is of high priority in contemporary energy research. The fusion of highly dynamic and adaptive photosynthetic structures with easily manipulated inorganic material at nanoscale level might pave the way of new opportunities to improve plant photosynthetic features. It was suggested that part of the energy absorbed by single-walled Carbon NanoTubes (CNTs) may be transferred to photosynthetic reactions and increase photosynthetic activity *in vitro* and *in situ* [1]. This experimental clue promoted the possibility to exploit the high light-capturing efficiency and broad absorbance of CNTs to enhance plant light harvesting capacity. The actual mechanism of this phenomenon is still unclear and the limited number of studies dealing with the CNT interplay with PhotoSynthetic Complexes (PSCs) provide controversial indications including both energy/charge transfer forward and from the nanotubes.

Here we aimed to gain insights into the electro-optical interactions of CNTs with light-dependent photosynthetic reactions using isolated PSCs and supramolecular assemblies with different level of complexity such as thylakoid membranes, Photosystem II (PSII)-enriched membrane fragments and light-harvesting complexes of PSII. The energy and electron fluxes in the biohybrid (PSCs/CNTs) systems were analysed by steady-state chlorophyll fluorescence and time-resolved fluorescence spectroscopy. The possible processes involved in the energy excitation decay in the photosynthetic structures in the studded model systems will be discussed.

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SHORT LECTURE

Mussel-inspired purple bacteria biohybrid photoanodes.

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Purple bacteria are anoxygenic microorganisms with one of the most versatile metabolisms among all bacteria, as they use sunlight to oxidize a broad variety of organic compounds in addition to heterotrophic and photoautotropic alternative metabolisms. This versatility led to an increasing interest in interfacing these organisms with abiotic electrodes to convert solar energy into electrical (and/or chemical) energy while providing the enzymatic machinery for self-repair and replication of the biocatalysts [1, 2] However, various challenges remain prior to implement the technology in the field, such as enhancing photoexcited electron transfer from the biocatalyst to the electrode and maximizing stability of the biohybrid architecture.

In this context, a mussel-inspired approach to achieve a firmly anchored layer of purple bacteria on the electrode with included exogenous redox mediators is reported. Polydopamine (PDA), containing both catechol and amine groups, is a polymer preseting similar characteristic to the adhesive plaque of mussel byssus, and it has been recently utilized to encapsulate isolated photosynthetic apparatus [3]. Here, the biohybrid photoanode was obtained by a one-step immobilization of intact purple bacteria, PDA, and quinone-based redox mediators. Electrochemical and spectroscopic evidence for the obtained biohybrid photoanodes will be discussed together with future research possibilities.

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SHORT LECTURE

Photosynthetic bacteria in the synthesis of dopamine-based polymers

Maria Varsalona[1,2], *Rossella Labarile*[1,2], *Danilo Vona*[1], *Gabriella Buscemi*[1,2], *Matteo Grattieri*[1,2], *Gianluca Maria Farinola*[1], *Massimo Trotta*[2]

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The self-polymerization of dopamine in a aerobic and anaerobic environment is investigated in presence and in absence of growing purple non sulfur photosynthetic bacterial strain *Rhodobacter sphaeroides* (*R. sphaeroides*) R-26.
Dopamine (DA) is a neurotransmitter that self-polymerizes into polydopamine (PDA), a synthetic analogue of melanin [1] of great interest in organic and bioinorganic electronics.
To study how the photosynthetic bacterium affects polymerization, dopamine solutions were initially studied in bacterial culture medium exposed to light under aerobic and anaerobic conditions confirming that oxygen acts as a catalyst for the self-polymerization of DA.
In a second set of experiments, dopamine DA monomers were added to the biological feeding medium along with a bacterial inoculum [2, 3] and exposed to light under anaerobic conditions as previously reported [4, 5] to assess the role of the bacterial cells on the PDA formation.
Spectroscopic characterization was used to show that, in absence of oxygen, *R. sphaeroides* acts as a catalyst for the PDA formation.

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SHORT LECTURE

Electric response of purple non-sulfur bacterial cells coated with polydopamine

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Bioelectronics promises fundamental advances in the sustainable production of energy thanks to the possibility to exploit living and metabolically active microorganisms through the optimization of the interface of poorly conductive phototrophs with electronic components [1]. The *in vivo* coating of the membrane of single metabolically active cells of the purple non sulfur photosynthetic bacterium *Rhodobacter sphaeroides* (*R. sphaeroides*) R26 with a melanin-like material [2, 3] was achieved.
The oxidative conditions employed for the polymerization are mild and biocompatible and were obtained by adaptive feeding technique of DA monomer into the culture medium. The PDA artificial conductive coating assembles around the cells, acts as soft functional matrix allowing the photosynthetic organisms [4] to thrive using light and ensuring the satisfactory electronic communication with the conductive surfaces, namely glassy carbon electrodes.
Electrochemical characterization was performed to investigate the electronic behavior of these biohybrids, unveiling that the polymer layer on the bacterial cells does not hinder the diffusion of the mediator and its capability to react at the electrode surface. The effects of dopamine concentration on the light-induced photoresponse of the biohybrid systems will be discussed and compared to bare bacteria.

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SHORT LECTURE

Embedding photosynthetic Reaction Center into polydopamine/ethylenediamine nanoparticles for efficient photocurrent generation.

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The reaction center (RC) from *Rhodobacter sphaeroides* is a transmembrain photoenzyme that behaves as a highly efficent photoconvertor, able to perform light transduction into charge separated states through electron cascade processes. The RC can be exploited as photoactive element to produce photocurrents and, by engineering bioelectronic devices, to gain an eco-friendly and scalability technology using sunlight as primary green energy source [1-2]. One of the main problems regarding the efficient implementation of the photoenzyme into optoelectronic devices is the electronic communication between the biological and electronic domains. Trying to solve this problem many approaches have been tested, and one of particular interest involves the incapsulation in soft organic materials [3].
With this aim, photoactive bio-hybrid soft nanoparticles were assembled by embedding the reaction center in polydopamine (PDA) aggregates treated with ethylenediamine (EDA). The PDA:EDA@RC nanoparticles obtained were investigated for photocurrent generation in photoelectrochemical cells, capable to convert sunlight into electrical energy.
By engineer PDA aggregates with EDA, the structural and functional integrity of the photosynthetic protein is retained, and the nanoparticles exhibit higher water dispersity and improved light collection ability than PDA@RC precursors. Consequently, higher photocurrents were obtained in the engineered nanoparticles as compared to pure and dark PDA. The optimized hybrid aggregates confined photoenzyme and produce charge separated states with a yield comparable to the pristine enzyme in solution, overcoming the limitation of photoactive system encapsulated in bare PDA, which is the polymer low light transmission ability, yet retaining the adhesive properties of the starting material. Photocurrents generated in RC embedded in the PDA:EDA environment almost doubled with respect to the PDA@RC.
These engineered bio-hybrid composites represent an interesting example of effective functional nanostructures for sunlight photoconversion based on a biological component addressed in a tunable bio-compatible polymer composite, also showing the potentialities of fine chemical tailoring of polydopamine bio-interfaces [4-5].

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SHORT LECTURE

Biohybrids from diatoms microalgae as new tools for photonics and electronics

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Diatoms microalgae are photosynthetic microorganisms capable of coupling the photosynthetic energy with the production of mesoporous biomineralized silica shells, called frustules. These shells are promising source of silica-based materials which exhibit high surface area, transparency, and mechanical resistance. Their easy aptitude to be chemically functionalized paves the way to the use of silica shells for applications in photonics, sensing, optoelectronics and biomedicine [1]. In this context we present a plethora of chemical or *in vivo* decorations for directly giving specific properties to silica from diatoms microalgae.[2] We recently demonstrated the *in vivo* incorporation of organometallic emitters, with Iridium, Rhutenium and Aluminum metal cores, into *Phaeodactylum tricornutum* diatom specie.[3] We let diatom cells bearing luminescent complexes self-populate and propagate on transparent conductive ITO glasses, successfully aiming to produce simple devices in principle suitable for producing photocurrent under light. Processing are totally green, and diatoms act as self-adherent and auto-propagating and living supports for photoactive materials. Furthermore we considered the *in vivo* incorporation of a positively charged Iridium Complex into *Coscinodiscus spp.* and *Thalassiosira weissflogii* diatoms frustules, in order to produce respectively new silica-based luminescent micro-materials and phosphorescent nanoparticles for biomedicine and drug delivery.[4] Moreover, organic dyes can be chosen for improving biomass and oxygen response of living microalgae thanks to their specific spectroscopic interactions with the photosynthetic apparata.[5]

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Light -responsive materials

INVITED LECTURE

Polymeric TiO_2-biomaterials with photocatalytic activities for drug degradation in aqueous environment

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The increasing pollution, due to a wide range of micropollutants, particularly in the aquatic ecosystem, has become a serious issue for the harmful effects on the environment and human health. Pharmaceuticals and in particular antibiotics (up to 95%) can be discharged into the sewers in unaltered state and with conventional wastewater treatment only incomplete removal can be achieved [1]. One of the most known effects on the environment is the development of resistant pathogenic bacteria. Advanced oxidation processes (AOPs), based on the *in-situ* generation of highly reactive species, are a promising route for the nonselective oxidative degradation of a wide variety of organic and inorganic water pollutants into harmless end-products. Heterogeneous photocatalysis, a photoinduced reaction, is an effective and environmentally friendly AOP that uses semiconductors such as TiO_2, to increase the production of reactive species, without the use of potentially hazardous oxidants, to decompose and completely mineralize organic pollutants. TiO_2 mediated photocatalysis is one the most used AOPs to degrade a wide range of water recalcitrant contaminants; indeed, due to its photo and chemical stability, nontoxicity and high photoactivity, nanocrystalline TiO_2 has been employed for many applications such as photocatalytic drug degradation where particle size and surface area are the key factors in heterogeneous catalysis. The main drawback of the TiO_2 nanoparticles, limiting the widespread technical application of heterogeneous photocatalysis in water treatment, is the difficulty of the photocatalyst separation from water and its regeneration after use. To solve this problem, researchers attempt to immobilize TiO_2 nanoparticles on some active support such as activated carbon, glass, zeolites, polymer films or natural biopolymers [2]. More recently, 3D printing has been proposed as a new method for immobilizing TiO_2 nanoparticles in biopolymer-based scaffolds [3,4]. As advantages, 3D printing allows to obtain identical objects, defined in terms of designed geometries that can be arbitrarily set during the design phase with high surface areas.
Here, the preparation and use of biomaterials as support for the photocatalytic activity of TiO_2 on drug degradation in aqueous environment will be discussed.

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INVITED LECTURE

Novel bioinspired UV-filters for safer and ecosustanaible sunscreen formulations

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The sunlight is essential for our well-being, it is responsible for stimulate vitamins the immune system, furthermore, thanks to the production of serotonin (knows as the molecule of happiness), the sun helps to fight depression, but whether you decide to expose yourself to sunlight for an aesthetic or healthy reason, today we know that it is important to protect yourself.

With the increasing awareness of the risks associated with exposure to the sun, ever greater amounts of organic and mineral filters included in the commercial sunscreen formulations are found among the pollutants of coastal waters, with effects on phytoplankton and consequent important ecological impact. Therefore, among the initiatives related to the European "Green Deal" it is envisaged that all the harmful materials and chemicals that are threatening the environment must be replaced within 2050 with eco-sustainable products.

With these evidences, the availability of new technologies offering efficacious protective barriers against skin damage and cancer, by absorbing harmful UVA and UVB rays, and respectful for the environment is becoming an increasingly important issue. To reach the target this research was aimed to investigate, develop and validate new physical UV filters and offer important innovations to the sector of sunscreen.

Commercial products are typically containing titanium dioxide (TiO2) or zinc oxide (ZnO) [1], that are able to reflect, scatter and absorb UV radiation, thus preventing sunlight-related skin photodamage. However, TiO2 is well known to generate reactive oxygen species (ROS) under photoexcitation, in fact, it has to be chemically modified when used in sunscreens [2]. Calcium phosphates, especially hydroxyapatite (HA), have been deeply investigated for several applications in biomedical field and nanomedicine due to their high biomimicry and biocompatibility. An interesting feature of the biomimetic HA is to incorporate some foreign ions in its lattice, in fact, in the past years we developed a number of protocols for the incorporation of different kind of ions (e.g. Mg, Sr, CO_3, and Fe) and the achievement of multifunctional products for regenerative- and nano-medicine [3]. In cosmetic field, unmodified HA was also involved, however, it does not absorb in the UV range, so our research was focused into modify its structure and design a new biomimetic HA-based formulation with improved UV filtration behavior.

By exploiting a nature inspired biomineralization process, hybrid particles were successfully obtained composed of alginate as polymeric template on which are growth poorly crystalline Ti-doped HA (alg-TiHA) and Ti- and Fe-doped HA (alg-FeTiHA). During the mineralization process the mineral phase nanocrystals are nucleated on alginate chains blocking the particles' growth and allowing the formation of a hybrid material where the nanophase is merged and stably bonded with the alginate matrix. The composition is suitable to be dispersed in sunscreen cream without damaging skin for the penetration of nanoparticles, moreover, the low crystallinity and the hybrid nature make these particles fully degradable in aqueous medium and thus an ecosustainable filter respecting the marine environment.

Deep investigations were performed to evaluate the particles' morphology (ESEM), chemical properties (XRD, FTIR, TGA) and interaction with UV-VIS radiations (adsorption spectra and photodegradation potential) and their safety. Results demonstrate that the sample with alg-TiHA reflect in the range of UVA and UVB, however, the sample with alg-FeTiHA showed a good absorption only in UVB. Although the worst properties of alg-FeTiHA in terms of reflectance index, its combination with alg-TiHA is important because the presence of Fe ions provide for a brown color range avoiding the whitening effect typical of highly protective sunscreen. Furthermore, both samples do not show photocatalytic effect thus avoiding the formation of free radicals and reactive species under irradiation cause of skin disease. Are ongoing the production of a complete sunscreen formulation for the demonstration of its filtration efficiency, stability and safety in vitro and in vivo by means of standard validation protocols.

The technology has already been patented [4] and the preliminary data collected suggest that it can be useful for an effective protection from UV radiation and with a strong potentiality to make innovation in sunscreens field, at the same time allow to meet the standard requirements imposed by current regulations. In fact, these

particles are fully biodegradable in sea water, and its degradation products are not harmful, moreover they are devoid of photocatalytic potential so that, if after their use they end in coastal waters, they will not be responsible to damage marine ecosystems.

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Poster presentations

Surface-enhanced Raman spectroscopy (SERS) for sensitive determination of flavonoids in plant extracts

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Preliminary data on the use of surface-enhanced Raman spectroscopy (SERS) as highly sensitive, non-destructive and cheap analytical technique for the rapid determination of flavonoids in biological extract are presented. Apigenin, luteolin, kaempferol, quercetin and their glycosylated forms were analysed using SERS substrates based on spotted silver nanowires [1]. Our results show structure-related SERS profiles especially originating from the catechol moiety and the possibility to identify characteristic peaks of flavonoids down to micromolar concentration with high reproducibility.

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Camouflaging $TPCS_{2a}$-loaded PLGA nanoparticles with mesenchymal stem cell membranes for targeted photodynamic therapy

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Among clinically approved cancer therapies, photodynamic therapy (PDT) represents an alternative treatment, based on the administration of a photosensitizer (PS) that, after its accumulation in cancer cells, could be activated through an appropriate wavelength of visible light. The PS irradiation stimulates the production of reactive oxygen species (ROS), especially singlet oxygen that can cause local oxidative stress, cell death and the activation of the immunological and anti-inflammatory response in treated tissues [1]. Since most of the PSs present a high hydrophobicity and tend to aggregate in aqueous solution, the idea of entrapping *meso*-tetraphenyl chlorin disulfonate ($TPCS_{2a}$) into a polymeric nanoparticle (NP) could be a strategy to enhance its delivery efficiency, bioavailability and phototoxicity [2]. Furthermore, it is well known that NPs blood circulation time is reduced by the rapid uptake by reticuloendothelial system, thus preventing NPs to reach their targets. Hence, the coating of NPs with mesenchymal stem cell-derived plasma membranes (mMSC) could both prolong circulation time and enhance cancer cells targeting due to the tumor-homing capacity related to their enrichment in chemokines receptors [3].
$TPCS_{2a}$-loaded NPs were prepared using a NanoAssemblr® Benchtop instrument and purified using Amicon® Ultra4 centrifugal filters unit. Previously isolated mMSC stained with DiO were mixed with $TPCS_{2a}$-loaded nanoparticles and ultrasonicated to produce the biomimetic nanosystem (mMSC-$TPCS_{2a}$-NPs). Size distribution, zeta potential and morphology of NPs were measured by DLS and TEM. The amount of $TPCS_{2a}$

entrapped in the nanoparticles was determined using UV-Vis spectrophotometer. *In vitro* phototoxicity was measured by MTS assay on breast cancer MDA-MB-231 and MCF-7 cells treated with increasing concentrations of PS delivered free or in NPs and irradiated with red light (total fluence 1 J/cm^2).
$TPCS_{2a}$-loaded NPs showed a spherical and homogenous shape with a mean diameter around 130 nm and an encapsulation efficiency of 88%. TEM images revealed that mMSC-$TPCS_{2a}$-NPs have a core-shell structure indicating the successful coating of NPs with membranes. Moreover, *in vitro* phototoxicity experiments in MDA-MB-231 and MCF-7 cells revealed a PS concentration-dependent and comparable viability reduction with $TPCS_{2a}$ and $TPCS_{2a}$-loaded NP formulations.
Our results demonstrate the efficient encapsulation of $TPCS_{2a}$ into the polymeric nanosystem, avoiding PS aggregation and ensuring the production of singlet oxygen upon light irradiation. Indeed, the *in vitro* cancer cells killing efficiency of $TPCS_{2a}$ loaded in NPs was comparable with that measured for $TPCS_{2a}$ delivered in the standard solvent.

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Red light-activatable peroxynitrite generator for cancer treatment

Menilli L.[1], Parisi C.[2], Failla M.[2], Fraix A.[2], Moret F.[1], Reddi E.[1], Rolando B.[3], Spyrakis F.[3], Lazzarato L.[3], Fruttero R.[3], Gasco A.[3], Sortino S.[1]

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The use of reactive oxygen species (ROS) and reactive nitrogen species (RNS) generators as "unconventional" therapeutics with precise spatiotemporal control by using light stimuli may open entirely new horizons in innovative therapeutic modalities for cancer. Among ROS and RNS, peroxynitrite ($ONOO^-$) plays a dominant role for its potent oxidizing power and cytotoxic action.[1] Therefore, we synthetized an unprecedent red-light activable molecular hybrid (BPT-NO) based on a benzophenothiazine derivative, functioning as light-harvesting antenna, joined to an N-nitroso appendage able to trigger the release of nitric oxide ($^{\bullet}NO$) and simultaneously produces superoxide anions ($O_2^{\bullet-}$) after irradiation with red light. The diffusion-controlled reaction between these two radical species generates $ONOO^-$. The red fluorescence of the BPT-NO hybrid was exploited to follows it intracellular uptake and localization in different cancer cell lines (breast adenocarcinoma MDA-MB-231 and cervix carcinoma HeLa cells). BPT-NO was well tolerated by cells in the dark but induced remarkable cell mortality when irradiated with very low red-light doses (1 J cm^{-2}). This $ONOO^-$ generator activatable by highly biocompatible and tissue penetrating single photon red light can open intriguing prospects in cancer treatment research, where precise and spatiotemporally controlled concentrations of $ONOO^-$ are required.

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Photodynamic therapy in macrophages infected with *Leishmania*

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Cutaneous Leishmaniasis is one of the manifestations caused by infection with the protozoan of the genus Leishmania. It is a neglected disease, with little pharmacological development for its treatment. Currently, the treatment available is systemic and highly toxic[1,2]. Photodynamic therapy combines a photosensitive component, light and oxygen, to trigger the formation of reactive oxygen species and consequently cell death[3]. Thus, this study aimed to compare the effect of photodynamic therapy with porphyrin and methylene blue, on macrophages infected with *Leishmania major* and *Leishmania braziliensis*, on their viability and mitochondrial activity. The macrophages were grown in DMEM, maintained at 37 ° C and for the infection process, 10: 1 protozoa were added and kept in an overnight greenhouse. The concentrations of 500, 250, 125 and 62.5µg/ml were tested for both photosensitizers. After 1 hour of interaction, it was irradiated with Biotable (660 nm, 25mW/cm² and 10J/cm²). The same groups kept in the dark were also tested to evaluate cytotoxicity. Viability was assessed by the exclusion test with Trypan blue, a vital dye that only penetrates cells with damaged membrane. Mitochondrial activity was assessed by the MTT test (3- (4,5-Dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide), a salt that is converted by active mitochondria. Toxicity was observed in the dark for infected cells, at concentrations greater than 250µg/ml in methylene blue and reduction in mitochondrial activity, while porphyrin showed low cytotoxicity and little change in mitochondrial activity in the dark. After PDT with both PS tested, no viable cells were observed, and cell activity decreased, indicating severe damage to the cell membrane, culminating in cell death. The results of porphyrin, which showed less toxicity in the dark than methylene blue, are important, but both compounds are promising for use in the treatment of skin lesions of leishmaniasis.

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Photostability studies on Nivolumab in formulation and in diluted saline and glucose solutions for parenteral infusion

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We investigated the impact of some stress factors, *i.e.*, shaking, vibrations and light exposure on Nivolumab, a fully human immunoglobulin G4 anti-PD-1 monoclonal antibody approved for multiple advanced tumours, including melanoma [1], trying to mimic the real life of this protein drug once it has been released from the pharma industry and shipped to the hospital pharmacy.

A critical exogenous stressing factor is indoor and outdoor light exposure to which a drug product could be exposed during transport but particularly within the hospital and during the hours of administration to the patient [2].

Chromatographic, electrophoretic, and spectrophotometric analyses were performed on the intact product and after dilution in sterile saline and glucose solutions to compare the structure of Nivolumab before and after the stressor steps. Nivolumab demonstrated a general instability to light and particularly in the diluted glucose solution, suggesting the role of glucose degradation products in the formation of aggregates and photooxidation products of the mAb.

In conclusion, upon exposure to light, this product may undergo a change in structure, chemistry and likely functional properties that could have a potential impact on the safety and efficacy of this very active mAb in the anticancer therapy.

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Novel dipyrrole and tetrapyrrole-based photosensitizers with various biphenylmethyl substituents of potential application in PDT

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Numerous compounds are consisting of pyrrolic rings linked together to form various cyclic and acyclic structures. Porphyrinoids are a large group of aromatic macrocycles containing four pyrrolic rings. In porphyrazines (Pzs) structure, pyrrole units are linked together with azide groups instead of methine bridges found in the naturally occurring porphyrins. In recent years, porphyrinoids have been researched for medical applications, especially as agents for photodynamic therapy (PDT). PDT is a novel, alternative, anticancer treatment, which has also been used to cure cardiovascular, dermatological, and ophthalmic diseases, as well as different microbial infections [1,2]. Boron dipyrromethene (BODIPY) derivatives are a new class of photosensitizers, consisting of two pyrrole units connected with methine bridge, forming a tricyclic complex with boron as the central atom. BODIPY dyes exhibit a number of properties, making them excellent candidates for photodynamic therapy, including high absorption coefficients, good stability, and chemical versatility, allowing easy modification of their photophysical properties such as absorption wavelength, solubility, and singlet oxygen generation efficiency. Although there are no BODIPY-based photosensitizers currently approved for clinical use, it is believed that this situation will change [3].

Novel sulfanyl porphyrazines possessing substituted biphenylmethyl groups were synthesized. In the first step, alkylation reactions of dimercaptomaleonitrile disodium salt with appropriate bromides led to maleonitrile derivatives possessing biphenylmethylsulfanyl moieties with fluoro, cyano, or methylcarboxylate substituents. These derivatives were subjected to macrocyclization reactions using Linstead conditions to give novel porphyrazines. Also, BODIPY derivatives with biphenylmethyl groups were obtained. Firstly, 1,3,5,7-tetramethyl-8-(-4- hydroxyphenyl)-4,4'-difluoroboradiazaindacene was synthesized from 4-hydroxybenzaldehyde and 2,4- dimethylpyrrole. Subsequent alkylation reactions with appropriate biphenylmethyl bromides gave novel BODIPY analogs bearing biphenylmethyl groups with fluorine, methylcarboxylate, and cyano substituents. Finally, obtained BODIPY analogs were iodinated with the mixture of I_2 and HIO_3. Novel compounds were characterized using mass spectrometry, UV-Vis spectrophotometry, and various NMR techniques. Obtained dipyrrole and tetrapyrrole derivatives were subjected to photochemical studies to evaluate their spectral properties, photostability, and ability to generate singlet oxygen, which is considered a crucial cytotoxic agent in photodynamic therapy.

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Comparison on photodynamic activity among pyridylporphyrins alkylated with four different alkyl chain

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Photodynamic therapy (PDT) is a minimally invasive treatment that uses the combination of a photosensitizing agent (PS) and light to selectively target solid tumors, as well as several nonneoplastic proliferating cell diseases. Following systemic administration, PSs are activated by localized irradiation with visible light; in the presence of adequate concentrations of molecular oxygen, this results in reactive oxygen species (ROS) formation and subsequent tissue demage [1].

The family of porphyrins and their derivatives encompass the majority of photosensitizers and studies concerning the synthesis and activity of new porphyrin derivatives, aiming the improvement of chemical-physical or activity characteristics, are abundant in the scientific literature [2,3].

In this study, two series of tetra cationic alkyl pyridyl porphyrins, including five derivatives, were synthesized, differing for the presence or absence of the zinc atom in the tetrapyrrole core; the five derivatives are characterized by a different length of the alkyl chain (from one to twelve carbon atoms). The compounds were fully chemically characterized, their light stability and singlet oxygen production determined. To determine a possible relationship between the photodynamic activity and the length of the alkyl chain, the effects on cell viability, ROS production, apoptosis and necrosis induction were also evaluated on a panel of tumor cell lines, following PSs activation.

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Inhalable aerosol light source for controlling drug-resistant bacterial lung infections (Light4Lungs project)

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Antimicrobial resistance (AMR) is an increasingly serious threat that can compromise the achievement of the Sustainable Development Goals (SDGs), affecting health and food security, poverty and economic growth [1]. Light4Lungs is a European project whose purpose is to develop and introduce a novel therapeutic scheme for the treatment of multidrug-resistant lung bacterial infections, with notable applications in cystic fibrosis. In that case, the leading cause of morbidity and mortality is due to *Pseudomonas aeruginosa* infection. Other pathogens, such as methicillin-resistant *Staphylococcus areus*, also play a key role in cystic fibrosis and hospital-acquired chronic and acute lung infections.
The underlying concept of the Light4Lungs project is the use of an inhalable light-emitting aerosol containing long-decay phosphorescent particles to perform lung antimicrobial inactivation. The aerosol particles will be excited prior to inhalation and will deliver therapeutic light in all the infected lung regions. The treatment will rely on the presence and activation of endogenous bacterial porphyrins, that are natural photosensitising molecules [2], selectively killing the lung-infecting bacteria [3]. In this scheme, the aerosol will mediate light-delivery, being not necessarily associated to a delivery of its constituent particles into the infected tissue.
The development stages of the project include: (i) the design, synthesis, characterization, and testing of the light-emitting material; (ii) its aerosolization and excitation; (iii) *in vitro* and *in vivo* biocompatibility and efficacy tests, together with the definition of the treatment parameters, considering the representative species of *P. aeruginosa* and *S. aureus*.

In conclusion, Light4Lungs addresses a clear and radical vision in the treatment of recalcitrant respiratory tract infections, with exemplary applications in Cystic Fibrosis. This will be enabled by a new non-intrusive light-emitting technology that challenges current paradigms in anti-infective treatments, photodynamic therapy, materials science and lighting technology.
Future and potential applications are in the treatment of other pulmonary diseases, such as fungal infections and lung cancer, possibly extended to other internal organs, having a profound impact on the fields of materials, photonics and healthcare.

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Do photosynthetic bacteria dream of heavy metals?

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Purple non sulphur (PNS) photosynthetic bacteria have a high affinity toward metals, being prone to bioaccumulate metals and – in some case – lower their toxicity by catalising the change of their redox states. The poster will present the most recent advancement on the topic of bioremediation of sites polluted with heavy metals by the use of PNS [1-12].

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Raman detection of polychlorinated biphenyls in human skin.

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Polychlorinated biphenyls (PCBs) are aromatic compounds including a family of 209 congeners that, owing to their lipophilic properties, tend to accumulate in lipidic tissue and have the potential to induce inflammatory and/or tumoral skin diseases.

Few previous studies investigated the association between PCBs plasma levels and risk of cutaneous melanoma with conflicting results; however, the potential distribution and storage of these compounds in cutaneous layers has not yet been proven.

Here we investigated the *in-vitro* uptake of 3,3',4, 4'tetrachlorobiphenyl (PCB n°77) in healthy human skin specimens prepared from histological sectioning protocols in the absence of any dyes, by means of µ-Raman spectroscopy.

The skin specimens were soaked in 1 mL of PCB n°77 aqueous solution at different concentration (from 1mg/mL down to 50 ng/mL) and time (from 2h to 5 days) ranges. The analysis of PCB adsorption and its distribution across the skin layers (from stratum corneum to subcutaneous tissue) was evaluated by acquiring µ-Raman spectroscopy in backscattering configuration, using a 632.81 nm He-Ne laser excitation, without using any labels or SERS reporters. The Raman analysis clearly revealed that PCB n°77 accumulates preferentially in the adipocytes of subcutaneous fat.

This study shows the importance and the usefulness of µ-Raman spectroscopy for the study of biological tissues, paving the way to the development of new methods for the detection of persistent organic pollutants dispersed in human skin.

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