
# SIFB-ALPE ADRIA Meeting on Photobiology

*with a Special Symposium on Photodermatology*

**20th-22nd June 2018**

# Program and Abstracts

## Palazzo Garzolini di Toppo Wassermann

*The Società Italiana di Fotobiologia (SIFB) is pleased to welcome you to the 2018 Annual Congress.*

*This year the Congress will be held in Udine, a small city located in Northeastern Italy, at the centre of the Friuli Venezia Giulia region between the Adriatic Sea and the Alps. This area together with two Austrian regions, Carinthia and Styria, all of Slovenia and the Veneto region is named ALPE-ADRIA. This is the reason for the name that has been given to the Congress.*

*The Società Italiana di Fotobiologia is grealy honored to have the opportunity to share the scientific and clinical results on Photobiology and Photodermatology with Experts from Italy and neighboring countries such as Austria and Slovenia.*

*The key objective of the Meeting is to provide an environment for both early career and senior investigators of different fields related to Photobiology to communicate their last results on relevant scientific topics and establish new interactions and collaborations.*

## Organization

***Organizing Committee***
Valentina Rapozzi (*Italy*), Giuseppe Stinco (*Italy*) Barbara Krammer (*Austria*)
Igor Frangež (*Slovenia*)

***Scientific Committee***
Giorgia Miolo, Enrico Caruso, Antonino Mazzaglia, Giovanni Romano, Francesco Milano, Marina Venturini, Valentina Rapozzi, Giuseppe Stinco

***Organizing Committee for the Photodermatology Symposium*:**
Giuseppe Stinco, Enrico Caruso, Valentina Rapozzi, Tiziana Arrighetti (SiDeMaST)

***The Local Conference Organizers***
Valentina Rapozzi, valentina.rapozzi@uniud.it
Giuseppe Stinco, giuseppe.stinco@uniud.it

***Official email address**:* info@sifb.it

***Patronage*:**

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hic sunt futura

***Sponsor Companies:***

***Scholarships for Student and Post-Docs***

The SIFB sponsored:

- 7 scholarships for students. These scholarships have handed out in the form of reimbursement for Conference Registration and Social Dinner.
- 2 postdoctoral fellows consisting of 300 euro.

***Acknowledgments:***

We aknowledge all the people who, to different extents, contributed to the realization of the Conference.

***Institutional representatives***

Roberto Pinton, Prorettore University of Udine

Franco Mallardi, Vice Direttore Department of Medicine, University of Udine

***Personnel from University of Udine:***

Anna Paulet, Ester Orlandi

***Slovenia Society of Photobiology***

Rebeka Strgar

***Conference hostess***: Iris Colombo, Camila Esposito, Francesca Pelosi

***Promo Turismo FVG***

# Location

The Conference is held at the University of Udine, at Palazzo Garzolini di Toppo Wassermann Aula T4-T9, Via Gemona 92.
The palace was built between 1705 and 1706 by Marzio Polcenigo. In 1901 the palace was bought by the Municipality of Udine to realize the boarding-school Toppo-Wassermann. During the first World War, part of building was used as a military hospital. In 2002 is granted in use at the University of Udine, which in the last years has worked for the recovery of the whole complex with particular attention to the interior fresco. The Palazzo Garzolini di Toppo Wassermann now hosts the Superior School of the University.
The building expresses the signs of the transition between Baroque and Rococo: it opens with a big porch that invites the spectacular staircase which ends with a dome and a narrow porch.

***The University***

The University of Udine was founded in 1978 as part the reconstruction plan of Friuli after the earthquake in 1976. Its aim was to provide the Friulian community with an independent centre for advanced training in cultural and scientific studies. The University currently has the following degree programmes: Agriculture, Biotechnologies, Economy, Engineering, Law, Modern Languages, Communication and Training, Humanities, Medicine and Surgery, Mathematics, Computer Science and Multimedia. The University is actively involved in student and staff exchange projects with universities within the eu and is currently engaged in close collaboration with several universities from Eastern Europe and other Non-EU countries. Moreover, the University participates in many research projects at the national and international level. Udine and its University are a point reference in a region which is historically known as a meeting place and the crossroads of different worlds and cultures. Geographically situated in the centre of the European Union, the University of Udine plays an active role in a close network of relations, committed to sharing its knowledge and ideas. Since its establishment, Udine University has pursued the policy of internalization, aimed at preparing students and forging relations and partnerships with universities and institutions in Europe and the rest of the world.

***Alpe-Adria*** is a bioregion in Central Europe, embracing all of Slovenia, the Austrian states of Carinthia and Styra, and the Italian regions of Friuli Venezia Giulia and Veneto. As of 2004, it is the subject of a proposal to create the world's first organic bioregion.

***The City***

Udine is a small city of 100,000 inhabitants; it has a bourgeois and refined heart, surrounded by typical small and dynamic rural villages whose architectures testify their rural origin. Udine is worldwide recognized for the number of its bookshops and this aspect unveils the nature of this people, also refreshing the ancient pleasure of being the third city, after Milan and London, to provide public lighting installations in the early 20$^{th}$ century. Udine is highly appreciated for its quality of life, it has a good bike sharing service which allows one to reach numerous sites, branches. Moreover, it offers all those opportunities typical bigger centers: traditional "osterie" (eateries), pubs, wine bars, cafes, restaurants, pizzerias, theaters, cinemas, discos, museums, and shopping malls among the biggest in Europe. A relaxing walk through the city center brings the visitors to the *Museo Diocesano* where one can appreciate the beauty of Giambattista Tiepolo frescos and, crossing one of the most beautiful venetian squares on terra firma "Piazza Libertà", to reach, in a refined context, a great variety of coffee bars, pastry shops, wine tasting shops, pizzerias and restaurants. Over the past several years, Udine has been host to several special national and international events: *Udin&Jazz*, refined and interesting jazz festival with international artists; *Vicino/Lontano* and the *Premio Tiziano Terzani*, which involves the world of international literature, for a debate on contemporaneity topics. The *Far East Fil Festival* is another important big event, hugely acknowledged and appreciated in its gender. *Friuli Doc* is the well-renowned as a wine-culinary regional kermesse that calls thousands of attendees. In the last years Udine hosted great pop and rock music shows with Madonna, Coldplay, Bruce Springsteen, U2, Bon Jovi, Pink Floyd and so on. The theatrical movement is very lovely and active with the main theatre *Giovanni da Udine* characterized of a traditional programme, and the more experimental *Teatro Contatto*, particularly dedicated to young people and students.

***The Territory***

Friuli Venezia Giulia, small land situated at the north-east of Italy, in the middle of the European landscape, in the past stage of wars and incessant changes of borders between Italy, Slovenia and Austria. In the land of floodings (south of Friuli) in 186 B.C. **Aquileia** was born as a military settlement, the second *latina* established by the roman empire to protect it from the invasions of the Gauls. It was the east gate of the empire Unesco heritage from 1998, had a past of richness due to prosperous trade, facilitated by water paths and a tremendous river harbor near the sea. Roman Aquileia, Christian Aquileia, in medieval age was elected the second capital city after Milan, and nowadays continues to disclose a fascinating history disclosed and written with the large contribution by the University of Udine with archeological digs and laboratories. Once upon a time, it was second only to Rome in matter of political influences, being for all medieval era the biggest diocese in Europe. In the cathedral, we can find a kaleidoscope of transformations occurred during the centuries; behind the cathedral we may find the cemetery where were buried the first soldiers died in the *Carso* area during the first World War; later one of those had been buried in the symbolic monumental grave of the *Milite Ignoto* at the Vittoriano in Rome.The ample friulian plain inspired a group of Venetian notables the realization of an idea; in 1593, during the Venetian dominion, born the perfect fortress for a strong defense from the refurbishment. Venzone had been one of the most demanding enterpreises in Venice history, symbol of strength and wisdom of the republic. The perimetral wall, strengthened by Napoleon Bonaparte who renamed the city **Palmanova** (Unesco heritage in 2017), remained to defend a military compound which had never been involved in any battle. Perfectly synthesizing the joint of both civil and military architectures, it won the most important challenge against the madness of nazists, whose projects were to completely destroy it. Nestled at the feet of the giulian alps, **Venzone**, the only "stone" of 4$^{th}$ century in all region, splendid example of architectonic and artistic refurbishment. Venzone was the staging post towards the north along the roman *via Julia Augusta*, rich and flourishing city during the Aquileian Patriarchate, so much to enforce itself with walls, castles and the moat, unfortunately suffered several invasions before the year 1000. It had been dominated by Napoleon Bonaparte first, then by Austrians, in 1866 entered in the Italian Reign. In 1348 was heavily stroken by an earthquake, and completely demolished by another in 1976. Rebuilt "*as was and where it was*" has in the dome the symbol of "*earthquake and rebirth*". Nowadays all the city is honoured as Italian national monument and last year elected the most beautiful village in Italy (*borgo dei borghi 2017*). A colony built by the Romans on the hills with an urban implant of "*cardo e decumano*" **Cividale del Friuli** became Unesco heritage in 2011. Favorite location of the longobardic dukedom, later became the abode of the aquileians patriarchs. Because of the heavy destructions suffered during the second World War, Cividale took the honor with the silver military medal. Nowadays Cividale shows itself as a small pearl around the green Natisone river; in the middle ages it was the capital city of the region with the name *Forum Julii*, giving later the name of the entire region Friuli. During the franks dominion it became *Civita Aaustriae*, city of the southern region of the reign, where Udine was named "*la terra grande*".

**Udine**, protectedby the alps, with the open spaces of the friulian plain and, towards south, the lagoon and the sea; "*terra grande*", dominated by the aquileian patriarchate first, the homeland of Friuli under the venetian dominion, is the center of the ideal geographic compass which allows the visitor to reach borders of a varied region in the range of one hour (from the mountains to the sea), a land defined in the nineteenth century by the poet Ippolito Nievo "*the summary of the Universe*".

**Trieste** is a Roman city approximately from 50 b.C. in it area conserved the ruins of the Foro and the Theater. After being repeatedly raided during the barbarian invasions and following the Frankish domination, the city, at the beginning of the XI century, began to revive. In 1382 the development of Trieste is restricted by the Republic of Venice, which ruled the Adriatic Sea. Hence, the town decides to embrace the Hapsburg influence. Under Maria Teresa of Austria'sreign (XVII century) the city establishes itself as an important commercial port, by taking the urban appearance that one admires today. In 1018 Trieste becomes a part of Italy, but after the Second World War, it is once more limited, this time by the Iron Curtain. Trieste, by being a city on the border, has become an important multicultural and multi-religious centre, which takes pride in its prominent university centre and cultural environment whilst searching for its role in today's Europe.

The **Miramare Castle** stands on the peak of a rock in the Gulf of Trieste. It was built by Archduke Ferdinand Maximilian of Hapsburg in the mid-nineteenth century as a residence for

himself and his wife, Charlotte of Belgium. From the castle, you can enjoy a magnificent view of the Gulf of Trieste and in its rooms you can admire many of the original furnishings. The castle is surrounded by a lush park consisting of hundreads of exotic plants.

# PROGRAM

## Wednesday June 20th, 2018

13.30-14.15 **Conference Registration**

14.15-14.40 **Opening Remarks:** Prof. Pinton ProRettore University of Udine, Prof. Franco Mallardi Vice Direttore Department of Medicine, University of Udine

*Chairs: Giorgia Miolo, Valentina Rapozzi*

14.40-15.10 ***Plenary Lecture***: **Alberto Diaspro** "Exploring space and time at the nanoscale in the realm of light" (*IIT Genoa, Italy*)

15.10-15.40 ***Plenary Lecture:*** **Andrea Lausi** and **Riccardo Mincigrucci** "Elettra: research at advanced light sources" (*Elettra – Sincrotrone Trieste, Italy*)

15.40-16.45 ***First Session:*** *Photosynthesis*

*Chair: Massimo Trotta*

15:40-16:10 **Maya Lambreva** "Insights on the interaction of carbon nanotubes and the photochemical reactions in microalgae" (*CNR, Rome, Italy*)

16:10-16:40 **Davide Vione** "The photochemistry of surface waters in the framework of climate change" (*University of Turin, Italy*)

16:40-16:55 **Livia Giotta** "Characterization of a photo-electrochemical transduction system based on photosynthetic reaction center proteins solubilized in the electrolyte medium" (*University of Salento, Italy*)

16.55-17.15 C*offee break*

17.15-18.40 ***Second Session:*** *Environmental Photobiology*

*Chair: Francesco Milano*

17.15-17.45 **Massimo Trotta** "Energy conversion in photosynthetic biohybrids systems" (*University of Bari, Italy*)

17.45-18.05 **Alberto Mezzetti** "Photoprotection mechanisms investigated by time resolved infrared spectroscopy: from isolated proteins to in vivo studies" (*University of Sorbonne de Paris, France*)

18.05-18.20 **Katja Malovrh Rebec** "Photobiological effects and parameters for non-image forming evaluation for different cases of inner built environment from the observers perspective" (*University of Ljubljana, Slovenia*)

18.20-18.40 **Giorgia Miolo** "PBL (Psoralens + Blue light): how blue light activates furocoumarin derivatives triggering tumor cell apoptosis" (*University of Padua, Italy*)

## Thursday June 21st, 2018

8.45-9.15 ***Plenary Lecture*** **Davide Zoccolan** "The rat as a model to study high-level visual functions" (SISSA, Trieste)

9.15-10.00 ***First Session***: *Photoreception, Circadian Rhythm*

*Chair: Giorgia Miolo*

9.15-9.45 ***Plenary Lecture*** **Sara Montagnese** "Circadian Rhythms" (*University of Padoa, Italy*)

9.45-10.00 **Manuela Liberi** "EPR- the technique extremely powerful for the identification and characterization of paramagnetic reaction intermediates, provides insights into the reaction mechanisms and kinetics of photochemical reactions" (*Bruker*)

10.00 -12.30 ***Second Session*****:** *Antimicrobial Photodynamic Therapy*

*Chairs: Enrico Caruso, Giovanni Romano*

10.00-10.15 **Paola Morici** "*In vitro* photodynamic antibacterial effect on *Helicobacter pylori* by a novel LED-based device" (*CNR, Pisa, Italy*)

10.15-10.30 **Antonella Battisti** "Exploiting endogenous bacterial photosensitizers for aPDT: investigation of porphyrin content in *Helicobacter pylori*" (*CNR, Pisa, Italy*)

10.30-10.50 *Coffee break*

10.50-11.10 **Kristjan Plaetzer** "Antimicrobial PDT based on natural substances: new horizons in fighting back resistant bacteria and fungi" (*University of Salzburg*, *Austria*)

11.10-11.25 **Lidjia Nemeth** "The Effect of Phototherapy of Major Salivary Glands on Caries Risk Factors" (*University Medical Centre Ljubljana*, *Slovenia*)

11.25-11.40 **Viviana Orlandi** "A promising combination of honey and blue light for antimicrobial applications" (*University of Insubria, Italy*)

11.40-11.55 **Roberto Zagami** "Exploring fabrics finished with poly(carboxylic acid)-cyclodextrin and photosensitizer as innovative tools in antimicrobial photodynamic therapy" (*CNR-ISMN c/o University of Messina, Italy).*

11.55-12.10 **Gabrio Roncucci** "Vulnofast: from the bench to the bed side of the patients with infected wounds" (*Molteni Farmaceutici, Italy*)

12.10-12.25 **Clara Comuzzi** "New Polymeric materials with photo-induced antibacterial activity" (*University of Udine, Italy*)

12.30-14.00 *Lunch + Poster session*

14.00-16.30 ***Third Session:*** *Nanotechnology*

*Chair: Antonino Mazzaglia*

14:00-14:30 **Milo Malanga** "Synthesis and Applications of Photoresponsive Cyclodextrin Derivatives" (*CYCLOLAB, Budapest, Hungary*)

14:30-14:50 **Greta Varchi** "Keratin-based biomaterials for photodynamic antitumor and antimicrobial applications" (*ISOF-CNR Bologna, Italy*)

14:50-15:10 **Teresa Gianferrara** "New targeting photosensitizers for photodynamic therapy and imagining applications" (*University of Trieste, Italy*)

15:10-15:40 **Salvatore Sortino** "Photocontrollable NO Releasing Constructs and their Biomedical Applications" (*University of Catania, Italy*)

15:40-16:00 **Tatiana Da Ros** "Nanotechnology & Photobiology: The Role of Carbon Nanostructures" (*University of Trieste, Italy*)

16:00-16:15 **Antonino Mazzaglia** "Cyclodextrins nanoengineered with photosensitizers with PDT and aPDT potential" (*CNR-ISMN c/o University of Messina, Italy*)

16.15-16.30 **Ilse Manet** "Mesoporus silica particles for theranostic applications" (*CNR-Bologna, Italy*)

16.30-16.50 *Coffee break*

16.50-18:00 ***Fourth Session:*** *Photodynamic Therapy: Molecular pathways and therapeutic applications*

*Chairs Gianfranco Canti, Luigi Xodo*

16:50-17:20 **Barbara Krammer** "Mechanisms of hypericin-PDT" (*University of Salzburg, Austria*)

17.20-17.35 **Chiara Nardin** "*In vivo* activation of subcellular Ca2+ signals by PDT" (*University of Rome, Italy*)

17.35-17.50 **Francesca Moret** "Synergic cytotoxic activity of chemotherapy and PDT in drug-sensitive and -resistant cancer cells by co-delivery of docetaxel and disulphonate tetraphenyl chlorin in layer-by-layer nanoparticles" (*University of Padua, Italy*)

17.50-18.05 **Giovanni Romano** "Side effects of intragastric photodynamic therapy: an in vitro study on mucosa cells" (*University of Florence, Italy*)

18:05-19.15 **SIFB General Assembly**

20.00 **Cena Sociale "*Casa della Contadinanza*" - Castello di Udine**

Società Italiana di Dermatologia
(SIDeMaST)

# PHOTODERMATOLOGY SYMPOSIUM

## Friday, June 22nd, 2018

08.00-08.30 Registration

08-30-08.50 **LECTURE**

**Franz Trautinger** "Photoprotection needs of patients with acne?" *(Vienna, Austria)*

08.50-09.30 **1st SESSION**

Chairs*: Marco Dal Canton (Belluno, Italy), Nicola Zerbinati (Varese, Italy)*

08.50-09.10 **Antonina Agolzer** "Photoprotection - UV filters: properties, use and safety in sun products" (Udine, Italy)

09.10-09.30 **Marina Venturini** "Systemic photoprotection" *(Brescia, Italy)*

09.30-10.50 **2nd SESSION**

*Chairs: Franz Trautinger (Vienna, Austria), Giuseppe Stinco (Udine, Italy)*

09.30-09.50 **Mauro Salvatore Alesandro Alaibac** "Ultraviolet exposure and skin tumors" *(Padoa, Italy)*

09.50-10.10 **Marco Dal Canton** "Actinic Keratosis" *(Belluno, Italy)*

10.10-10.30 **Alberto Maria Bertoldi** "Treatment considerations in actinic keratosis" *(Venice, Italy*)

10.30-10.50 **Alessandro Gatti** "New and current preventive treatment options in actinic keratosis" *(Treviso, Italy)*

10.50-11.10 *Coffee break*

11.10-11.30 ***Plenary Lecture:*** Marina Venturini "Light sources for photodynamic therapy" *(Brescia, Italy)*

11.30-12.30 **3rd SESSION**

*Chairs: Alessandro Gatti (Treviso, Italy), Rapozzi Valentina (Udine, Italy)*

11.30-11.50 **Ana Benedičič** "Photodynamic Therapy in Praxis at Dermatology Department in General Teaching Hospital Celje" *(Celje, Slovenia)*

11.50-12.10 **Tanja Planinšek Ručigaj** "Our expirience with photodynamic therapy at Dermatovenereologic Clinic, University Medical Centre Ljubljana" *(Ljubljana, Slovenia)*

12.10-12.30 **Fabio Massimo Gavazzoni** "Photodynamic therapy: experience of Dermatovenereologic Unit in Bruneck Hospital" *(Brunico, Italy)*

12.30-13.30 **FOCUS ON PSORIASIS**

*Chairs: Giuseppe Stinco (Udine, Italy)***,** *Mauro Alaibac (Padoa, Italy)*

12.30-12.50 **Mariachiara Arisi** "Phototherapy of Psoriasis" *(Brescia, Italy)*

12.50-13.10 **Anna Chiara Fostini** "Italian guidelines on the systemic treatments of moderate-to-severe plaque psoriasis" *(Verona, Italy)*

13.10-13.30 **Cinzia Buligan** "What's new in the management of psoriasis?" *(Udine, Italy)*

13.30-14.30 *Lunch*

14.30-16.30 **FOCUS ON LASER**

*Chairs: Igor Frangež (Liubljana, Slovenia), Franz Trautinger (Vienna, Austria)*

14.30-14.50 **Leonardo Marini** "LASER and Incoherent Light Skin tissue interaction – Old and new concepts and their current clinical implications" *(Trieste, Italy*)

14.50-15.10 **Nicola Zerbinati** "Laser-assisted tattoo removal" *(Varese, Italy)*

15.10-15.30 **Daniela Cavalca** "How can the laser play a role in the treatment of infantile hemangioma?" *(Ome, Italy*)

15.30-15.50 **Matteo Tretti Clementoni** "Why to burn a burn. Laser treatment of scars and the laser assisted drug delivery" *(Milan, Italy)*

15.50-16.10 **Nicola Di Meo** "Blue diode laser: a new strategy for the management of lichen sclerosus et atrophicus" *(Trieste, Italy)*

16.10-16.30 **Cristina Zane** "Side effects of laser therapy" *(Brescia, Italy)*

16.30 -16.50 *Coffee break*

16.50-17.30 **4th SESSION**

*Chairs: Leonardo Marini (Trieste, Italy), Cristina Zane (Brescia, Italy)*

16.50-17.10 **Petra Bukovec** "Photobiomodulation with policromatic polarized light" *(Ljubljana, Slovenia)*

17.10-17.30 **Igor Frangež** "The effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients" *(Ljubljana, Slovenia)*

17.30-18.30 **5th SESSION**

*Chairs: Franz Trautinger (Vienna, Austria), Marina Venturini (Brescia, Italy)*

17.30-17.50 **Sara Trevisini** "The role of phototherapy in general dermatology: guidelines and real life practice" *(Trieste, Italy)*

17.50-18.10 **Enzo Errichetti** "Dermoscopy as a response predictor tool in psoriasis vulgaris and in vitiligo treated with narrowband ultraviolet B phototherapy" *(Udine, Italy)*

18.10-18.30 **Mariateresa Scaini** "Phototherapy of mycosis fungoides" *(Treviso, Italy)*

18.30-18.40 **Concluding Remarks – ECM test**

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# ABSTRACTS

# Liquid tunable microscopy towards 4D painting of the biological cell.

*Alberto Diaspro*[1,2,3]

[1] Department of Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy; [2] Department of Physics, University of Genoa, Italy; [3] Nikon Imaging Center, Genoa, Italy.

The possibility of integrating different light-matter interactions for producing optical microscopy images is the starting point for the design and realization of a brand new multiscale and multimodal optical microscope that has been recently named LIQUITOPY®, liquid tunable microscopy [1]. Optical fluorescence microscopy has been developed across a number of revolutions, namely: three-dimensional optical sectioning, confocal and two-photon laser scanning microscopy and F-methods (FRAP, FLIM, FCS, FRET). The current peak has been reached with the advent of super resolved fluorescence microscopy methods able to circumvent the classical diffraction limit [2, 3]. Most implementations "control" molecules between fluorescent ON- and OFF-states to obtain spatial super resolution. Such a control is mainly achieved by fluorescent molecule switching in two ways that became popular following the 2014 Nobel prize assignments, namely: "targeted" switching that confines the fluorescence emission to an area smaller than the diffraction of light – for example, STED [4] – and "stochastic" switching that implements single molecule localization exploiting the photo-switchable behavior of fluorescent molecules – for example, PALM or STORM [5]. Moreover, there is a trend towards label free methods like Mueller matrix and CIDS microscopy [6, 7]. Within this scenario, LIQUITOPY® is designed to integrate simultaneous acquisition of different contrast mechanisms taking a potential advantage from deep learning strategies and to foster a brand new way of forming images from a full multiscale and multimodal approach including expansion, light-sheet and image scanning microscopy.

[1] Diaspro A. et al 2018 *Biophysical Journal* 114 (3): 347a
[2] Diaspro A. 2014 *Il Nuovo Saggiatore* 30 (5-6): 45-51
[3] Hell S.W. et al. 2015 *Journal of Physics D: Applied Physics* 48 (44): 443001
[4] Vicidomini et al. 2018 *Nature Methods* 15(3): 190
[5] Deschout H. et al. 2014 *Nature methods* 11 (3): 253
[6] Mazumder N. et al. 2017 *Journal of Optics* 19 (2): 025301
[7] Diaspro A. et al. 1991 *IEEE Transactions on Biomedical Engineering* 38 (7):670-678

# Elettra and FERMI: research at advanced light sources.

*Andrea Lausi, Riccardo Mincigrucci*

[1] Elettra-Sincrotrone Trieste, Strada Statale 14, 34149 Basovizza (TS)

Elettra Sincrotrone Trieste is a multidisciplinary international research center of excellence, specialized in generating high quality synchrotron and free-electron laser light and applying it in materials and life sciences. The main assets of the research center are two advanced light sources, the electron storage ring Elettra and the free-electron laser (FEL) FERMI, c.
Continuously (H24) operated supplying light of the selected "colour" and quality to more than 30 experimental stations, these facilities enable the international community of researchers from academy and industry to characterize structure and function of matter with sensitivity down to molecular and atomic levels, to pattern and nanofabricate new structures and devices, and to develop new processes.
Advanced light sources offer unique tools to expand the boundaries of scientific investigations into new materials and living matter. As centers for fundamental and applied research, light sources play a key role in stimulating innovation and enhancing industrial competitiveness in fields such as agriculture, archaeology, biology, biomedicine, chemistry, cultural heritage studies, engineering, energy, environmental science, forensic science, geology, materials science, nanotechnology, new drugs, palaeontology and physics.

# Insights on the interaction of carbon nanotubes and the photochemical reactions in microalgae.

*Taras K. Antal[1], Silvia Orlanducci[2,3], Alena A. Volgusheva[1], Gianluca Fulgenzi[4], Andrea Margonelli[3], Giuseppina Rea[3], Maya D. Lambreva[3#]*

[1]Dep. of Biophysics, Faculty of Biology, Lomonosov Moscow State University, Russian Federation; [2]Dep. of Chemical Science and Technology, University of Rome Tor Vergata, Italy; [3]Institute of Crystallography, National Research Council of Italy, Monterotondo (RM), Italy; [4]Dep. of Molecular and Clinical Sciences, Marche Polytechnic University, Ancona, Italy

The integration of carbon nanotubes (CNTs) with plant machinery paved the way for various solutions to enhance plants vigor and their production efficiency [1,2]. Among others, the coupling of plant photosynthetic structures with nanomaterials has suggested the capability of single-walled CNTs (SWCNTs) to increase the efficiency of energy harnessing in the photosynthetic process [3]. This new knowledge may foster the exploitation of the nanotechnology tools to empower photosynthetic performance and production yields of commercially important microalgal species [4].
The research identified the physicochemical properties of SWCNTs enabling their entry into walled-cells of microalgae and developed experimental approaches to track their cell internalization. We tested the potential of the SWCNTs to improve algal fitness and photosynthetic performance by analysing the interaction of the carbon nanotubes and photochemical reactions in the model microorganism *Chlamydomonas reinhardtii*. The algal cultures were fed with stably dispersed SWCNTs and the effects of SWCNT-exposure on the function of Photosystem II and Photosystem I were studied under standard or stress-associated conditions.

[1] Wang et al. 2016. *Trends in Plant Science* 21: 699–712.
[2] Guatimosim et al. 2016. In: *Bioengineering Applications of Carbon Nanostructures*, A. Jorio (ed.), Springer Intern. Publ. Switzerland, pp. 17–29.
[3] Giraldo et al. 2014. *Nature Materials* 13: 400–408.
[4] Lambreva et al. 2015. *Photosynthesis Research* 125: 451–471.

# The photochemistry of surface waters in the framework of climate changes.

*Davide Vione*

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Sunlight illumination of surface waters induces several photochemical reactions involved in the transformation of dissolved compounds, inactivation of pathogens and biogeochemical cycles, via either direct photolysis (directly triggered by absorption of sunlight, if any) or indirect/sensitised transformation. In the latter case, sunlight is absorbed by naturally-occurring photosensitisers (e.g. chromophoric dissolved organic matter or CDOM, nitrate and nitrite) to produce reactive transient species that cause transformation reactions. The transients include, among others, the hydroxyl ($^{\bullet}OH$) and carbonate ($CO_3^{-\bullet}$) radicals, singlet oxygen ($^1O_2$) and CDOM triplet states ($^3CDOM^*$). Their occurrence in surface-water environments is linked to sunlight irradiance and to key water parameters such as chemistry and depth [1,2].
Depending on the photoreaction pathways that prevail for a certain compound, the environmental conditions can affect its fate in different surface-water environments [3]. The role of climate change on water chemistry and, as a consequence, on photochemical reactions is just starting to be investigated. The main difficulty is to disentangle climate effects from other disturbance factors (e.g. wastewater inputs) that may also operate on the long term [4]. Climate change has the potential to deeply alter the photochemistry of freshwaters, but its effects could be very different in boreal vs. temperate environments, where the respective main processes involve water chemistry (browning) versus a range of different phenomena (enhanced summer stratification of lakes, tree-line shifts, extended drought periods).

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# Characterization of a photo-electrochemical transduction system based on photosynthetic reaction center proteins solubilized in the electrolyte medium.

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The photosynthetic reaction center (RC) from the purple non-sulfur bacterium *Rhodobacter sphaeroides* is a pigment-protein complex that exploits the free energy of visible photons to promote the transfer of electrons from ferrocytochrome $c_2$ to ubiquinone. A number of photoelectrochemical cells based on this biological photo-catalyst have been designed and characterized [1]. In this work we have explored in more detail the factors affecting the photocurrent generation in commercially available screen-printed electrochemical cells where RC proteins and suitable mediators are solubilised. We show that efficient generation of cathodic photocurrents occurs at an applied potential of 0.0 V *versus* quasi-ref Ag (the open circuit voltage of the system measured in the dark) in presence of ferrocenemethanol and decylubiquinone, which proved to guarantee high performances as electron donor and acceptor respectively. Moreover, a set of differential equations, describing reaction and diffusion processes, has been employed for modelling with high accuracy the chronoamperometry profiles recorded at variable RC concentrations. Through this model, the characteristic time course of the photocurrent has been explained in the light of the strict interconnection between the dynamical processes involved.

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# Energy conversion in photosynthetic biohybrids systems.

*S. la Gatta[1], M. Dell'Edera[1], F. Milano[2], R. Ragni[1], A. Agostiano[1,2], G.M. Farinola[1] R. Tangorra[1] and M. Trotta[2]*

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The complexity of the natural photosynthetic systems is difficult to reproduce in vitro; however, complexity is inherently associated to the efficiency of the living multienzyme character of photosynthesis any biomimetic attempts must cope with this stringent requirement. In this regard, we have designed and assembled efficient organic-biological hybrid systems formed by small to medium size organics molecules responsible of a given specific role and the photoenzyme responsible for energy transduction in photosynthetic organisms.
Applications to different fields will be presented to show drawbacks, limitations and potentials of such hybrid systems in energy conversion, along with some future developments.

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7) "*Garnishing" the photosynthetic bacterial reaction center for bioelectronics*. Alessandra Operamolla et al. (2015) *Journal of Materials Chemistry C. 3, 6471-6478* DOI: 10.1039/C5TC00775E
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10) *Synthetic Antenna Functioning As Light Harvester in the Whole Visible Region for Enhanced Hybrid Photosynthetic Reaction Centers (2016)* O. Hassan Omar, S.. Bioconj. Chemistry 27(7) 1614-1623.
11) *Highly oriented photosynthetic reaction centers generate a proton gradient in synthetic protocells* (2017) Emiliano Altamura et al. PNAS 114(15), 3837-3842

# Photoprotection mechanisms investigated by time-resolved infrared spectroscopy: from isolated proteins to *in vivo* studies.

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Time-res. FTIR difference spectroscopy (TR-FTIR-DS) provides details at the atomic level and kinetic information on the reaction mechanisms in photosynthesis (H+ transfer, e- transfer, cofactor displacement, conformational changes…) [1].
In a first study the photoactivation mechanism of the Orange Carotenoid Protein (OCP), a water soluble protein found in cyanobacteria implicated in photoprotection was studied. Whereas previous TR-FTIR-DS studies from our groups showed the molecular details of the conformational changes associated to the photoactivation of the protein [2], recent results on wild-type and mutants OCPs show that an intermediate state in the OCP photocycle can be trapped at low temperature, or observed by TR-FTIR-DS [3]. The use of specific mutants made it possible to assign specific IR bands to two different alpha-helices within OCP; on the other hand, time resolved studies showed that the disappearance of these two helices during the photocycle is not synchronous.
In a second study, we studied the photoprotective mechanism of diatoms *in vivo*. In this case in was possible, on a second time scale, to follow by TR-FTIR-DS the response of the microorganism to strong light, implying a xantophyll cycle. We could follow different chemical events (chemical epoxydation/de-epoxydation of carotenoids; plastoquinone pool reduction; localized pH changes; energy dissipation mechanisms; reorganization of the membrane structure) and calculate the kinetics for each of these processes [4].
The results will be discussed in the framework of the present knowledge of photoprotection mechanisms in cyanobacteria and diatoms, as well as in the framework of present and future capabilities of the TR-FTIR-DS technique applied to photobiology.

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SIFB-ALPE ADRIA Meeting on Photobiology
20th-22nd June 2018-Udine (Italy)

# Photobiological Effects and Parameters for Non-image forming evaluation for different cases of inner environment from the observers perspective.

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The congress contribution addresses possible ways to evaluate the photobiological effects of reflected light on observer. A real case scenario of office with different wall paintings and LED lighting fixtures has been evaluated. Assessments were done in terms of measuring spectral power distributions of light reflected from walls recorded in two different gaze directions: horizontal (wall facing) and vertical (table orientated). Both human reception of light; visual (image forming) and circadian effects (non-image forming effects) were calculated and compared based on measured reflected light spectra. Lighting fixture in experiment was tunable LED lamp, thus spectral power distribution of light source has been varied to simulate different scenarios.

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SIFB-ALPE ADRIA Meeting on Photobiology
20th-22nd June 2018-Udine (Italy)

# PBL (Psoralens + Blue light): how blue light activates furocoumarin derivatives triggering tumor cell apoptosis.

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Department of Pharmaceutical and Pharmacological Sciences, University of Padova

BACKGROUND. Furocoumarins are natural and synthetic compounds with high chemotherapeutic potency under UVA irradiation. To improve their activity and avoid severe side effects likely mainly related to the formation of interstrand crosslinks (XLs) with DNA pyrimidine bases, a variety of derivatives, hopefully monofunctionals, have been synthesized. Although angelicins, due to their angular geometry, do not generally form XLs, some of them, i.e. (TMA), can crosslink folded DNA upon UVA. The UVA photobiological effects of furocoumarins are mainly related to their capacity to photoreact with DNA. Furthermore, furocoumarins produce ROS that impair cellular functions through lipid peroxidation, oxidation of guanine and strand breaks in nucleic acids, oxidation of proteins and inactivation of enzymes.
To photoactivate 8- MOP and 4,6,4'-trimetylangelicin (TMA) towards human prostate (DU145 PCa) and bladder (T24) cancer cell lines, a new approach based on less toxic and more penetrating visible radiation (BL, 420 nm) is presented.
RESULTS. TMA and 8-MOP show high antiproliferative activity towards cancer cells, through induction of apoptosis. Besides ROS generation (less efficient under BL than UVA), the proapoptotic effect seems related to the activation of p38 and inhibition of p44/42 phosphorylation. Interestingly, the decrease of β nuclear-catenin is coupled with dropping of CD44-positive cells. The strong photocytotoxicity of TMA and 8-MOP can be related to the kind and number of DNA lesions. Under BL, no mutagenic crosslinks, no photocleavage nor photooxidative lesions are detected on isolated DNA by TMA treatment, but only MAs form. However, formation of XLs still remains for 8-MOP under BL but in a lower amount than UVA.
CONCLUSIONS. Overall, our results indicate that 8-MOP, and particularly TMA, can be efficiently activated by BL and may be considered good compounds for targeted phototherapy of prostate and bladder cancers and possibly for other solid tumors.

# The Rat as a Model to Study High-Level Visual Functions.

*Davide Zoccolan*

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The ability to recognize objects despite tremendous variation in their appearance (e.g., because of position or size changes) represents such a formidable computational feat that it is widely assumed to be unique to primates. Such an assumption has restricted the investigation of its neuronal underpinnings to primate studies, which allow only a limited range of experimental approaches. In recent years, the increasingly powerful array of optical and molecular tools that has become available in rodents has spurred a renewed interest for rodent models of visual functions. However, evidence of primate-like visual object processing in rodents is still limited and controversial.

In this seminar, I will present behavioral evidence showing that rats are capable of recognizing visual objects in spite of substantial variation in their appearance, i.e., in spite of changes in size, position, illumination, in-depth rotation and in-plane rotation. I will also show that rat object recognition relies on a shape-based, multi-featural processing strategy that makes close-to-optimal use of the discriminatory information afforded by the target objects across their various appearances.

Overall, these findings indicate that rats are able to process and efficiently use shape information, in a way that is largely tolerant to variation in object appearance, thus supporting spontaneous generalization of recognition to previously unseen views of learned objects. This suggests that their visual system may serve as a powerful model to study the neuronal substrates of invariant visual object recognition.

# Circadian Rhythms.

*Sara Montagnese*

Department of Medicine, University of Padova, Padova, Italy

The lecture will review:
- the basic concepts of circadian rhythmicity and circadian regulation [1];
- the definitions of chronotherapy and light therapy [2];
- the practical applications of the above, by way of a pertinent clinical example (i.e. circadian rhythmicity and chronotherapy in advanced liver disease) [3-5].

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# EPR- the technique extremely powerful for the identification and characterization of paramagnetic reaction intermediates, provides insights into the reaction mechanisms and kinetics of photochemical reactions.

*Manuela Liberi*

Bruker Italia Srl

Photochemistry provides us with one of the most generally useful methods of studying reactions of paramagnetic species (free radicals, radical pairs, excited states, and transition metals) by irradiating suitable molecules with light of a suitable wavelength. The studies of photochemical reactions involve elucidation of the primary process and of all secondary reactions to follow. The determination of the complete mechanism requires ideally identification of each elementary reaction and its intermediates together with quantitative assessment.
My presentation will show some examples on how the EPR, due to its quantitative and non-intrusive nature is extremely powerful for the identification and characterization of paramagnetic reaction intermediates, providing insights into the reaction mechanisms and kinetics of photochemical reactions
In particular, it is an unparalleled tool to detect free radicals in skin care products that are generated by UV exposure thereby damaging the skin via free radical reactions.
Screening of a product's efficacy and safety can be evaluated via stress testing: there is a need for highly efficient antioxidants that are resistant to environmental oxidative stress and remain active for a lasting time period, and EPR can be the answer.

# *In vitro* photodynamic antibacterial effect on *Helicobacter pylori* by a novel LED-based device.

*Paola Morici*[1#], *Antonella Battisti*[1], *Giuseppe Tortora*[2], *Giovanni Checcucci*[1], *Francesco Ghetti*[1], *Arianna Menciassi*[2] *and Antonella Sgarbossa*[1]

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The alarming antibiotic resistance in *Helicobacter pylori* is the main reason for conventional therapy failure of *H. pylori* infection, which is often associated with severe gastric diseases. In this context, antimicrobial PhotoDynamic Therapy (aPDT) represents a promising therapeutic approach. It requires a photosensitive dye and harmless light at appropriate wavelengths to kill several pathogens by producing cytotoxic reactive oxygen species. In the case of *H. pylori*, aPDT exploits photoactive porphyrins naturally produced and accumulated within the bacterium to induce its photokilling [1]. In the framework of the project "CapsuLight" [2], focused on the development of an ingestible LED-based robotic pill for the noninvasive intragastric treatment of *H. pylori* infection, the bactericidal effect of aPDT was evaluated on *H. pylori* strains by using a device with LEDs at various wavelengths. Photokilling assays were performed for different time points under two experimental conditions, aerophilic or microaerophilic atmosphere, since the latter is the most favorable for optimal growth of *H. pylori*, both *in vitro* and *in vivo*. The exposure to harmless levels of visible light through a novel LED-based device induced a marked bactericidal effect on the tested strains of *H. pylori* in both conditions. Hence, our findings suggest that aPDT could be a valid alternative therapy to conventional antibiotics for *H. pylori* infection.

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[2] The project: "CapsuLight - Design of an ingestible robotic pill based on LED sources for the treatment of gastrointestinal disorders" (CUP B52I14005760002) funded by Regione Toscana Bando FAS Salute 2014 (Italy)

# Exploiting endogenous bacterial photosensitizers for aPDT: investigation of porphyrin content in *Helicobacter pylori.*

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The increasing occurrence of antibiotic resistance is making conventional pharmacological treatments progressively less effective towards several pathogens [1]. Among these, *Helicobacter pylori* (Hp) represents a challenge since it infects about half of the human population [2], causing in some cases serious pathologies such as gastritis, ulcers, chronic inflammation and some forms of cancer. Antimicrobial PDT is a different therapeutic approach that can overcome the limits of conventional pharmacological treatments [3]: it requires the presence of $O_2$ and of a photosensitizing molecule able to induce photokilling upon irradiation of the microorganism with visible light. Hp spontaneously produces and accumulates pigments belonging to the family of porphyrins [4], naturally occurring macrocyclic molecules involved in several essential biological functions and able to take part in light-induced reactions within living organisms. In the context of a project aimed at the development of a novel miniaturized PDT device, these products were extracted from bacteria and characterized in terms of absorption, emission and fluorescence lifetime; the photophysical characterization of Hp porphyrins was also performed on planktonic and biofilm growing Hp cells [5]. HPLC-MS experiments allowed for the identification of the main porphyrinic components of the extracts [6] and for the observation of changes in the porphyrin mixture composition with aging of the bacterial cultures.

Framework project: "CapsuLight - Design of an ingestible robotic pill based on LED sources for the treatment of gastrointestinal disorders" (CUP B52I14005760002) financed by Regione Toscana Bando FAS Salute 2014 (Italy)

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# Antimicrobial PDT based on natural substances: new horizons in fighting back resistant bacteria and fungi.

*Michael Glück*[1], *Martina Hasenleitner*[1], *Christoph Hamminger*[1], *Markus Hörl*[1], *Kristjan Plaetzer*[1,#]

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Increasing resistance of microorganisms against antibiotics is one of the world's greatest health problems and necessitates the rapid and decisive development of new antimicrobial approaches. Photodynamic Inactivation allows for effective killing of bacteria and fungi [1] and the employment of natural photosensitizers ensures excellent biocompatibility of the treatment. Limitations of photoactive compounds derived from plants, *e.g.* low water-solubility and lack of positive charge, may be overcome by tailored formulations or synthesis of cationic derivates. In this study we discuss the photoantimicrobial properties of the natural photosensitizers hypericin (HYP), curcumin (CUR, approved as E100), chlorophyllin (CHL, E140) and cationic derivates of curcumin (SACUR) against bacteria and fungi. Furthermore, applications of these natural photoantimicrobials in human medicine, food decontamination and plant protection are presented. So, for example, 50 µM SACUR allows for effective photokilling (> 99.9%) of Gram- *Escherichia coli* in a porcine skin model. A formulation of 50 µM CUR with polyvinylpyrrolidone is applicable for decontamination of cucumbers and peppers from *Staphylococcus aureus*. The combination of 100 µM CHL and 5 mM ethylenediaminetetraacetic acid kills (> 7 $\log_{10}$ steps) the bacterial plant pathogen *Xanthomonas axonopodis* upon illumination. Photodynamic Inactivation based on natural photosensitizers is efficient, biocompatible and offers a wide spectrum of applications to improve life quality and safety of humans.

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# The Effect of Phototherapy of Major Salivary Glands on Caries Risk Factors.

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Dental caries is one of the most prevalent chronic diseases worldwide. Main factors influencing caries lesions formation are: previous caries experience, salivary risk factors such as amount of oral cariogenic bacteria, salivary flow and buffering capacity, oral hygiene habits, diet, and fluoride supplements. Phototherapy is a treatment with different modalities of light, such as laser, light emitting diode (LED), halogen lights or other light sources. The therapeutic light absorbed by chromophores stimulates tissue regeneration, reduces inflammation, and controls pain.
The aim of our study was to elucidate how phototherapy of salivary glands with polychromatic light or LED therapeutic light affects salivary risk factors in patients with high caries risk. The hypothesis was that polarized polychromatic non-coherent or LED light phototherapy reduces caries risk in high caries risk patients.
In prospective randomised case-control study 40 patients with high caries risk according to Cariogram [1] were assigned into four groups. In experimental groups the patients were irradiated with polarized polychromatic non-coherent light (Bioptron AG, Switzerland) wavelengths 480–3400 nm, average power density 40 mW/cm$^2$, with continuous LED light (Ortholumm, Votan d.o.o., Slovenia), wavelengths 625, 660 and 850 nm, average power density 16 mW/cm$^2$, and with same LED light in pulsed mode. For control group non-therapeutic low power visible light was used. In all patients light was administered extraorally bilaterally on the parotid and submandibular glands for 10 minutes and intraorally on the sublingual glands for 5 minutes, 3 times a week, for 4 weeks in a row. Every patient's caries risk was assessed according to Cariogram at the start and the end of therapy. Salivary caries risk factors were determined from saliva samples before the therapy, after two weeks, at the end of treatment and 4 weeks after the end of therapy. The study was approved by Republic of Slovenia National Medical Ethics committee (Nr. 0120-539/2016-2 KME 40/11/16).
As a result of the study at the end of the treatment, in group, irradiated with polarised polychromatic light (N = 9), and in group, irradiated with continuous LED light (N = 8), *Streptococcus mutans* and *Lactobacillus* colony forming unit/mL (CFU/mL) counts were reduced and salivary buffering capacity was increased (one-way repeated measures ANOVA, Dunnett's test, $p < 0.05$). At the end of the treatment, in group, irradiated with LED light in pulsed mode (N = 7), *Streptococcus mutans* CFU/mL counts were reduced, unstimulated salivary flow and buffering capacity were increased (one-way repeated measures ANOVA, Dunnett's test, $p < 0.05$). In all three experimental groups caries risk at the end of the phototherapy was lower (Wilcoxon test, $p < 0.05$). In placebo control group (N = 12) there were no statistically significant differences between parameters during and after the therapy.
The conclusions are that phototherapy can reduce the amount of cariogenic bacteria in saliva and improve some salivary risk factors thus diminishing the chances for new caries lesion formation. The results from our study confirm the hypothesis that phototherapy with polarized polychromatic non-cocherent light or LED light reduces caries risk factors in patients with high caries risk.

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# A promising combination of honey and blue light for antimicrobial applications.

*Viviana T. Orlandi[#], Eleonora Martegani, Francesco Franzi, Fabrizio Bolognese*

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The antimicrobial activity of honey has been known for millennia, as ancient Egyptians and Greeks used honey to treat infections. The antimicrobial power of honey seems to be ascribable to several factors such as osmotic stress induced by high concentrations of fructose and glucose, acidic pH, oxidative stress, methylglyoxal and bee-derived defensing-1. However, honey have complex chemical compositions that vary depending on the floral or the honeydew (HD) source, the climate and the harvesting conditions.
Since antibiotics and disinfectant are often unable to fight infections caused by multidrug resistant pathogens, new and/or alternative antimicrobial approaches are needed. In this study, the effect of visible light irradiation on Italian honey antimicrobial power has been evaluated. The opportunistic pathogen *Pseudomonas aeruginosa* PAO1 has been chosen as model microorganism. *In vitro* experiments pointed out that the antimicrobial activity of fir- and forest honeydew honeys was enhanced upon irradiation by a broad-spectrum visible light. The observed enhancement was energy-dose and honey-concentration dependent. In particular, the blue LED source (460 nm) increased significantly the antimicrobial power of HD honeys. The combination of blue light/honey was also efficient in inhibiting the biofilm formation.
The quality improvement of honey upon irradiation by blue light seems very promising. Further investigations will be aimed at evaluating if honey chemical compounds, such as riboflavin and gallic acid, could play the role of endogenous photosensitizers.

# Exploring fabrics finished with poly(carboxylic acid)-cyclodextrin and photosensitizer as innovative tools in antimicrobial photodynamic therapy.

*Roberto Zagami[1,#], Maria A. Castriciano[1], Maria P. Casaletto[2], Bernard Martel[3], Mariachiara Trapani[1], Andrea Romeo[1,4,5], Salvatore Guglielmino[4], Luigi Monsù Scolaro[1,4,5], Antonino Mazzaglia[1].*

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Antimicrobial photodynamic therapy (aPDT) is a well-known alternative way to treat local infection caused by different microorganisms such as Gram (+) and Gram (-) bacteria, viruses, fungi and protozoa. In this communication, we present a polypropylene (PP) fabric finished with citrate-hydroxypropyl-βCD polymer (PP-CD) entrapping an anionic porphyrin (TPPS) as photosensitizer-eluting scaffold (PP-CD/TPPS) for aPDT. The concept is based on host−guest complexation of porphyrin in the cavities of CDs immobilized on the PP fibers, followed by its sustained and controlled delivery in release medium and simultaneous photoinactivation of microorganisms. Morphology of fabric was characterized by optical (OM) and scanning electron microscopies (SEM). X-ray photoelectron spectroscopy (XPS) and FT-IR revealed the surface chemical composition and the distribution map of the molecular components on the fabric, respectively. Release kinetics of TPPS in physiological conditions pointed out the role of the CD cavity to control the TPPS elution. PP-CD/TPPS fabric-treated *S. aureus* cells were photokilled of 99.98%. Moreover, low adhesion of *S. aureus* cells on textile was established. Conversely, no photodamage of fabric-treated *P. aeruginosa* cells was observed, together with their satisfying adhesion. In this direction studies to entrap in PP-CD other photosensitisers for dejection of Gram-negative bacteria are in due course.

# Vulnofast®: from the bench to the bed side of patients with infected wounds.

*Gabrio Roncucci*

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Photodynamic Therapy (PDT) is a strategy combining the administration of visible light and a substance called photosensitizer, molecule capable of capturing and transforming the provided light energy into Reactive Oxygen Species (ROS). PDT has been used for decades to treat skin pre-cancerous and cancerous lesions. More recently PDT has been considered due to its antimicrobial potential, yet, in most instances, the clinical data published reported the experiences with older, not specifically designed photosensitizers. During the last few years, a new series of phthalocyanine photosensitizers capable of absorbing light in the red region of the visible spectrum where tissues are more transparent have been synthesized and developed.
The studies performed on a number of candidates in a large set of *in-vitro* and *in-vivo* pharmacological studies have shown a great promise due to their very broad antimicrobial spectrum activity, including wild types and clinical bacterial strains, resistant bacteria , fungi such as yeasts and dermatophytes, protozoa and biofilms. Such photosensitizers binds selectively, due to the idro/lipophilic nature and electrical charges of the compound, to the bacterial cell membrane or cell wall, and when illuminated causes a production of singlet oxygen and other ROS within the bacterial cell, leading to the immediate destruction of the organism. Besides being highly selective thus sparing the normal tissue cells, it has also been shown not to be affected by the previous resistance to antibiotics nor to induce resistance to microorganism due to a multitarget physical type of inactivation.
This promising approach was studied in a randomized, placebo controlled trial study with a single application of the previously identified lead photosensitizer compound RLP068, and light in infected DFUs showing a very potent antibacterial activity causing a rapid reduction of bacterial load of the lesion.
Actually the commercially available CE marked formulation of RLP068 named Vulnofast® plus was used to treat a set of patients with infected DFUs, with repeated applications, to assess the healing potential.

# New polymeric materials with photo-induced antibacterial activity.

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Infection diseases are one of the main mortality causes worldwide and the first death reasons in undeveloped areas. Nowadays the growing threat of resistant bacterial strain prompts also developed countries for the adoption of new strategies to control and minimize the spreading of pathogens. Contamination of surfaces together with inappropriate disinfection routines has been recognized as critical in the transmission of pathogens to humans and environment. So, the development of self-disinfecting materials or coatings able to guarantee constantly sanitized surfaces looks like a promising strategy to limit the risk of exposure to pathogens. Several materials exploiting photodynamic inactivation (PDI) of bacteria have been recently synthetized either by conjugating the photosensitizer (PS) to an electro-polymerizing unit or by forming a conductive film bearing a negatively charged moiety devoted to the ionic interaction with a positively charged PS [1]. In this study we synthetize a photoactive material using an imprinting technique based on the electro-polymerization of a monomer capable of coordinating, via non-covalent interactions, the photosensitizer. 5-phenyl-dipyrromethane and 5-(4-pyridyl)-dipyrromethane were chosen as electroactive monomers and 20-(4-carboxyphenyl)-2,13-dimethyl-3,12-diethyl-(22p) pentaphyrin (PCox) was selected as photoactive molecule. Our results showed that the new material was able to reduce a $10^8$ CFU/ml solution of S. Aureus of 4log in 90min using a multi led blue lamp at a radiance of 40 W/m$^2$.

[1] Spagnul, Cinzia et al. 2015. "Immobilized photosensitizers for antimicrobial application." *J. of Photochem. Photobiol. B: Biology* 150: 11–30.

# Synthesis and Applications of Photoresponsive Cyclodextrin Derivatives.

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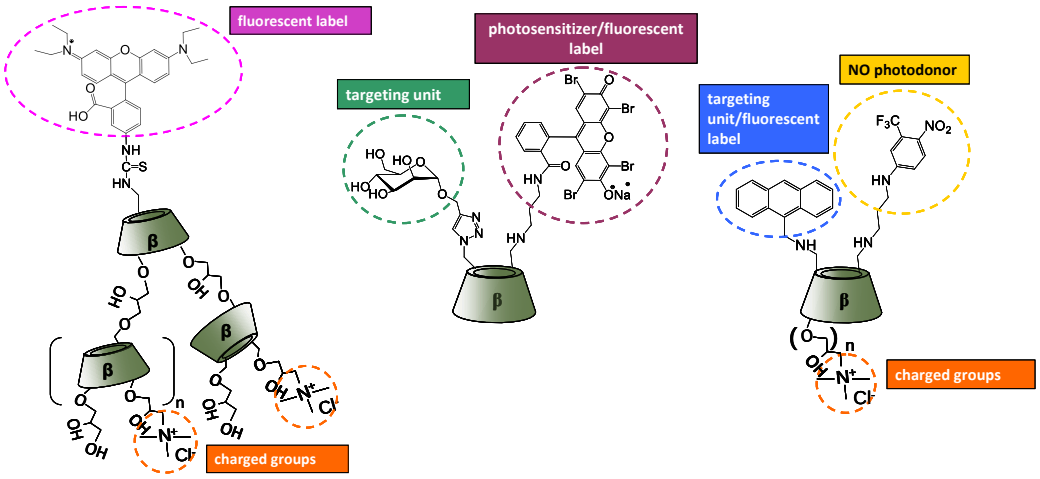
Cyclodextrins (CDs) are cyclic oligosaccharides able to form inclusion complexes with drugs. This phenomenon can be utilized both to visualize the guest molecules and for their biological-targeted delivery. Effective detection of the carrier and/or of the encapsulated cargo in biological media can be achieved by fluorescent labeling of the CD moiety. In our ongoing research a wide variety of fluorescent dyes were covalently attached to various CD scaffolds through thioureido[1], amido[2] or amino linkages. Our works resulted in a library of fluorescently labeled CDs including positively and negatively charged CD monomers and various cross-linked CD-polymers[1], having multiple CD cavities in their molecule. Several examples for the preparation of photoactive CD-based system will be presented. Photodynamic therapy (PDT) can be used to fight against multi-drug resistance (MDR) in infectious diseases and in cancer thanks to the selective release of cytotoxic species as singlet oxygen and nitric oxide. In order to obtain multifunctional systems with both photoreleasing and targeting properties, the CD scaffolds were simultaneously modified with photoactivable prodrugs, such as Eosin Y or orto-trifluoro-nitroaniline and targeting units such as mannoside or anthracene for targeting bacterial lectin or DNA, respectively. The anticancer and antibacterial activity of these multifunctional systems will be shown.

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[2] Benkovics, G. et al. 2017. “Novel β-cyclodextrin–eosin conjugates.” *Beilstein J. Org. Chem.* (13

# In vitro efficacy of keratin based bimodal nanoparticles: chemotherapy and photodynamic therapy to address drug toxicity and chemoresistance.

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Chemoresistance and damage of key organs are the major causes of chemotherapy failure in patients affected osteosarcoma (OS) [1]. To decrease the cytotoxicity and increase the efficacy of farmacological treatment in OS, we propose a bimodal nano-platform that combine chemo and photodynamic therapy (PDT) [2].
Bimodal keratin nanoparticles (PTX-Ce6@Ker), functionalized with Paclitaxel (PTX) and the photosensitizer Chlorin-e6 (Ce6), were synthetized by drug-induced aggregation method [3] with the aim of increasing cell death in OS cell lines including chemoresistant one where PTX alone is less effective. The efficacy of PTX-Ce6@Ker was tested on MG63, SaOS-2, U2-OS and doxorubicin resistant SaOS-2/$^{DX580}$ cells using viability assays in 2D and 3D systems. To evaluate the contribution of the two treatment therapies, cell viability was measured 24 h after PTX-Ce6@Ker treatment in the dark (PTX cytotoxicity) and 24 h after photoirradiation at near-infrared wavelength (Ce6 photo-toxicity).
Results highlight that our nanoformulation enhance PTX intrnzalization as compared to PTX alone, while generally preserving PTX efficacy. Most importantly, all OS lines were significantly affected by both PTX and Ce6 action in an additive manner, confirming that the combination of the cytostatic blockage due to PTX and the oxidative damage induced by ROS, have a far superior efficacy as compared to PTX alone. If proven to be effective in vivo, this approach would significantly enhance the efficacy of farmacological tratment in based treatments increasing the life expectancy of OS patients.

[1] Miller, Cram, Lynch, Buckwalter 2013 “Risk factors for metastatic disease at presentation with osteosarcoma: an analysis of the SEER database.” *The Journal of Bone and Joint Surgery. American Volume*, *95*(13): e89.
[2] Duchi, Sotgiu, Lucarelli, Ballestri, Dozza, Santi, Varchi, et al. 2013 “Mesenchymal stem cells as delivery vehicle of porphyrin loaded nanoparticles: Effective photoinduced in vitro killing of osteosarcoma.” *Journal of Controlled Release*, *168*(2): 225–237.
[3] Varchi, Aluigi, Sotgiu, Guerrini, Ballestri Italian patent application N. 102017000067430

# New Targeting Photosensitizers for Photodynamic Therapy and Imaging Applications.

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Targeting peptides are very promising molecules to increase tumor selectivity of anticancer drugs. [1] Herein, we report the synthesis of three complexes, which might act at the same time as photosensitizers (PSs) for cancer photodynamic therapy (PDT), as delivery systems of Re(I)/$^{99m}$Tc(I) fragments into tumor cells as well as targeting agents for prostate cancer cells. To this aim, we designed a structure containing a PS such as a water soluble +3-charged tris-methylpyridinium porphyrin or a fulleropyrrolidine derivative, a [1,4,7]-triazacyclononane (TACN) chelator suitable for coordination, and a bombesin BN[7-14] as a targeting peptide. We could successfully prepare molecules bearing tumor-targeting PSs and which are suitable for imaging applications. Overall, this work paves the way for the synthesis of theranostic complexes although the yields will need to be seriously improved and the synthetic process optimized.

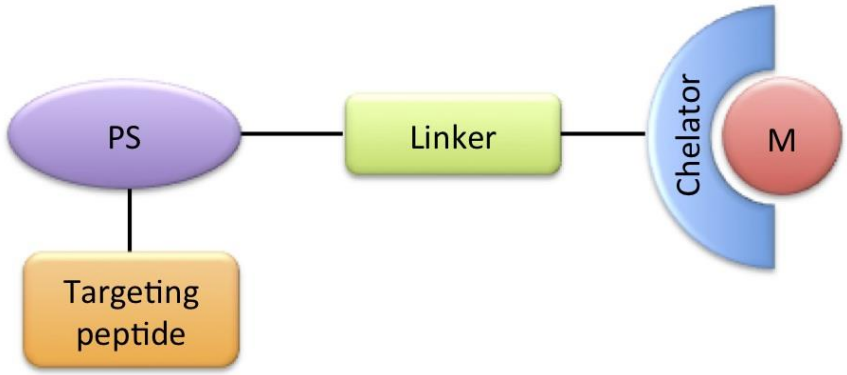


Figure 1. Structure of peptide-PS conjugate

[1] a) Majumdar S., Siahaan T. J. 2012. "Peptide-mediated targeted drug delivery."*Med. Res. Rev.* 32(3): 637–658. b) Jamous M., Haberkorn U., Mier W. 2013 "Synthesis of peptide radiopharmaceuticals for the therapy and diagnosis of tumor diseases." *Molecules* 18(3): 3379–3409. c) Böhme D., Beck-Sickinger A. G. 2015. "Drug delivery and release systems for targeted tumor therapy." *J. Pept. Sci.*, 21(3): 186–200.

# Photocontrollable NO Releasing Nanoconstructs and their Biomedical Applications.

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The multiple role nitric oxide (NO) plays in a number of physiological and pathophysiological processes has stimulated a massive interest in the development of new strategies and methods for generating NO in a controlled fashion, with the exciting prospect to tackle important diseases. In this context, the therapeutic outcome of any NO-based drug is strictly dictated by three main parameters: i) concentration, ii) delivery site and iii) dosage. In some cases, this creates a complex role for the molecule in opposing beneficial and deleterious events. This dichotomy has made the development of new strategies and methods for generating NO with precise spatiotemporal control a *hot topic* in the burgeoning field of nanomedicine [1]. Light represents a minimally invasive "micosyringe" to provide a highly localized "burst" of NO in biological systems with a superb spatiotemporal control through the aid of suitable NO photoprecursors. In fact, photoexcitation permits not only to confine site of action of NO at the illuminated area with high precision but also to define its dosage with great accuracy by tuning the light intensity and/or duration. These unique features make the NO photoreleasing compounds a powerful therapeutic arsenal much more appealing than those based on either thermal, enzymatic or pH stimuli.
In this contribution, an overview of the most significant examples of NO photodelivering molecular constructs, including molecular hybrids, nanoparticles, nanosheets and thin films, developed in our laboratories over the last years is presented, emphasizing the logical design and the potential applications in vasodilatation, anticancer and antibacterial therapy.

[1] Sortino, Salvatore 2016 Ed."Light-responsive nanostructured systems for applications in nanomedicine" *Topics in Curr. Chem*. 370.

SIFB-ALPE ADRIA Meeting on Photobiology
20th-22nd June 2018-Udine (Italy)

# Nanotechnology & Photobiology: The Role of Carbon Nanostructures.

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Many fields have been strongly influenced by the advent of nanotechnology, and, among them, photobiology.
Carbon nanostructures (CNSs) are a rather heterogeneous family of materials differing in shape and size but presenting some peculiarities, which allow their use in many biological areas. Fullerenes, nanotubes, nanoonions, nanohorns, nanodots, graphene, graphene oxide, carbon and graphene quantum dots, nanodiamonds are the most important and representative members of this family. Herein we will present their main characteristics and the most interesting results related to their use in PDT for cancer and antimicrobial therapy, thanks to their intrinsic characteristics, as for fullerene, or as vectors for photosensitizers. [1]

[1] Goodarzi, Saba; Da Ros, Tatiana; Conde, Joao; Sefat, Farshid; Mozafari, Masoud. 2017. “Fullerene: Biomedical engineers get to revisit an old friend.” *Materials Today*, 20 (8): 460-480. Karunya, Albert, Hsin-Yun, Hsu 2016. “Carbon-Based Materials for Photo-Triggered Theranostic Applications.” *Molecules* 21 (11): 1585.

# Cyclodextrins nanoengineered with photosensitisers with PDT effectiveness.

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Cyclodextrins (CDs) offers, either as molecule or in self-assembled form, functional constructs to bind PS guests by non-covalent interactions. In the rent past, we reported the design of different supramolecular assemblies of amphiphilic CDs with high efficacy for application in PDT and aPDT [1]. Actually, the design of novel nanophototherapeutics for PDT generally complies with efficient uptake of PS within tissues, cell membranes and/or intracellular components with production and diffusion of singlet oxygen in the neighboring areas to the sites of action. Here we report a brief overview on the most recent outcomes on nanoconstructs (NC) based on self assembly of CD building block complexing different PSs of undoubted interest for PDT such as Zn(II)-phthalocyanine, Bodipy and Pheophorbide A [2-3]. The choice of CD molecule can determine the amount of entrapped PS within of NC, modulating the *in vitro* PDT activity, thus allowing spatial-temporal control of PS release *in* upcoming in *vivo* applications.

[1] Scala, A., Piperno, A.., Grassi, G-., Monsù Scolaro, L., Mazzaglia A. 2017. In Nano- and Microscale Drug Delivery Systems Design and Fabrication (Alexandru Mihai Grumezescu editor). Elsevier, p. 229-241.
[2] Zagami, R., Sortino, G., Caruso, E., Malacarne, M. C. ; Banfi, S. ; Patanè, S. ; Monsù Scolaro, L. and Mazzaglia, A. 2018. (submitted).
[3] Zagami,R., Rapozzi, V. ; Sortino, G. ; Piperno, A. ; Scala, A. ; Xodo, L. ; Monsù Scolaro, L. and Mazzaglia, A. 2017. 17th Congress of ESP, Pisa , 4-8 September 2017.

# Cyclodextrin- and silica-based carrier systems for theranostic applications.

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Infectious diseases due to multidrug resistant bacteria are becoming a global health concern. The EU has published her Action Plan in 2011 against the rising threats from antimicrobial resistance. Developing new antibiotics is mandatory but takes years. A short-term alternative is the development of novel strategies to deliver existing drugs in an optimized way. In this frame, the use of antibiotic nanocarriers is one of the most promising options. The objective of our research in the frame of the ITN project Cyclonhit is to take full advantage of nanotechnology to efficiently encapsulate antibiotics in nanocarriers to kill both intracellular and extracellular bacteria. We focused on two drugs, Ethionamide (ETH), a second line drug in the treatment of tuberculosis, and Clofazimine, a drug used in the case of leprosy. Both have severe side effects due to their low therapeutic index and suffer from insolubility in water hampering their administration. We addressed this problem by loading ETH in polymeric β-cyclodextrin (β CyD)-based carriers and CLZ in fluorescent mesoporous silica particles as well as sulfobutyl β CyD oligomeric carriers. CLZ was successfully loaded in three types of mesoporous silica particles exhibiting intrinsic fluorescence with long fluorescence lifetimes up to 10 ns.[1] The system has very appealing properties for theranostic applications. For both drugs highly improved solubility in water has been obtained with the β CyD-based polymeric carriers.[2-3] The CLZ/ sulfobutyl β CyD carrier system has very interesting IC50 values in the nanomolar range against MDR *S. Epidermidis*.

[1] I. Manet et al. 2017. *J. Mater. Chem. B,* 5 (17): 3201–3211.
[2] I. Manet et al. 2017. *Int. J. Pharm,* 531 (2): 568–576.
[2] I. Manet et al. 2017. *Int. J. Pharm,* 531 (2): 577-587.

# Mechanisms of photodynamic therapy with hypericin.

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Hypericin (Hyp) serves as a powerful natural photosensitizer (PS) for photodynamic therapy (PDT) especially of tumors. After selective accumulation in tumor tissue and vessels, and (selective) activation by visible light, the PS generates reactive oxygen species, which can be used to destroy the tumor. Actually different molecular biological mechanisms may lead to a variety of cellular endpoints ranging from "biostimulation" (increased proliferation rate) over repair of the damage resulting in cell recovery to autophagy, apoptosis and necrosis. The processes are supported by changes of the tumor microenvironment and vessels, and by activation of immune reactions.

The different modes of cellular responses depend mainly on the PDT-protocol, photosensitizer localisation, cellular damage protection and the available intracellular energy. Hyp accumulates mainly in the endoplasmatic reticulum, Golgi apparatus and lysosomes; in some cases also in mitochondria.

One important challenge of PDT is supplementing local tumor treatment with systemic anti-tumor immunity to eliminate tumor cells inaccessible for PDT, circulating tumor cells and metastases. Hyp-PDT is known to trigger immunogenic cell death (ICD) in tumor cells leading to exposure or release of damage-associated molecular patterns (DAMPs). DAMPs act as effective danger signals and comprise *i.a.* calreticulin, high-mobility group box 1 protein, heat shock proteins 70/90, and adenosine triphosphate. ICD has been successfully used as anticancer vaccine in mice experiments. In accordance with the vaccine concept, we could demonstrate in a previous study that low-dose Hyp-PDT combined with a short drug-light interval not only achieved a complete elimination of CT26 colon carcinoma of BALB/c mice, but additionally prevented tumor recurrence upon re-challenge. Vessel damage and hypoxia seemed to be involved in ICD of tumor cells followed by systemic immunity.

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# *In vivo* activation of subcellular Ca 2+ signals by PDT.

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Photodynamic therapy (PDT) is a valid treatment for superficial neoplasms. A better understanding of intracellular pathways activated by PDT is critical to improve treatment for different types of tumors. In particular, the molecular mechanism by which PDT induces apoptosis or necrosis depends on several factors, such as cellular context and photosensitizer agent. Here, we performed live cell imaging experiments on the mouse melanoma cell line B16-F10. Using selective fluorescent biosensors, we found that PDT promotes activation of caspase-9 and caspase-3 within seconds of photosensitizer activation. To unravel the underlying apoptotic mechanisms, we used B16-F10 cells expressing a novel generation of genetically-encoded fluorescent $Ca^{2+}$ probes targeted to endoplasmic reticulum or mitochondria (G-CEPIA1er, R-CEPIA1er, CEPIA2mt, LAR-GECO1.2). Experiment performed *in vitro* , as well as *in vivo* , in the dorsal skinfold chamber surgically implanted on the tumor-bearing mouse back, indicate that PDT-induced stress response involves $Ca^{2+}$ release from endoplasmic reticulum and its transfer to juxtaposed mitochondria. Supported by the Italian Ministry of Health, Project Code RF-2011-02348435 (P.I. FDV).*

# Synergic cytotoxic activity of chemotherapy and PDT in drug-sensitive and -resistant cancer cells by co-delivery of docetaxel and disulphonate tetraphenyl chlorin in layer-by-layer nanoparticles.

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The use of biodegradable polymer-based multifunctional nanoparticles (NPs), able to encapsulate drugs with different solubility properties and at fixed concentration ratios, represents an interesting opportunity to co-deliver chemotherapeutic and photosensitizing agents using a single nanovehicle to perform combination therapy. Thus, we investigated on the capability of hyaluronic acid (HA) targeted polymeric layer-by-layer NPs co-loaded with the chemodrug docetaxel (DTX) and the photosensitizer (PS) meso-tetraphenyl chlorin disulphonate (TPCS2a) to induce synergistic cell photo-killing of DTX -sensitive (MDA-MB231 triple negative breast cancer cells, HeLa cancer cervix cells) and DTX-resistant cells (HeLa-R) over-expressing HA receptors (CD44). Therefore, applying the Chou and Talalay method developed to study synergism in combination therapy by the calculation of the Combination Index (CI) with the software Compusyn, we observed the highest extent of synergism when MDA-MB231 and HeLa cells were treated with the combination of DTX and TPCS2a co-loaded in the same NP (DTX/TPCS2a NPs) with respect to the combination of the drugs delivered in the respective standard solvents or in separate HA-targeted NPs. The DTX/TPCS2a NP formulation turned out to be the most efficient in inducing cell death also in DTX-resistant HeLa-R line, as outlined also by the calculated Drug Reduction Index (DRI) for the chemotherapeutic which indicates the possibility of reducing the DTX dose more than 100 times if delivered by DTX/TPCS2a NPs.

# Side effects of intragastric photodynamic therapy: an *in vitro* study on mucosa cells.

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In the framework of antibacterial PDT, our group has recently developed a prototype of ingestible luminous capsule [1]. In view of its use in clinical trials, we have evaluated the possible side effects on the healthy stomach tissue caused by illumination, due either to an intrinsic cell sensitivity to illumination or to a sensitization following porphyrin release in the tissue by *H. pylori*. To this aim, we performed a phototoxicity study on the AGS cell line, commonly used as a model for gastric mucosa, with a 400nm LED light source by varying the light power, irradiation time and PPIX concentration.
First we observed that PPIX itself becomes toxic for AGS cells only for incubations at concentration > 10μM. Irradiation doses were tested in the range 0.6 to 13 J/cm$^2$. The results in terms of photo-treatment dose-effect curves allow the conclusion that a photo-treatment comparable with that of the luminous capsule (~4mW/cm$^2$ for ~30 minutes) may cause a cell damage only for PPIX concentration >200nM, much higher than the one estimated to be released in the surrounding tissues by *H. pylori* [2]. Out analyses were completed by a study of the light-induced oxidative stress and preliminary enzymatic activity. The prosecution of our study plans the quantitative determination of the amount of porphyrins present into the bacteria, released in its culture medium and internalized into AGS cells after incubation with known porphyrin concentration, by LC-MS techniques.
These results will hopefully open the way to a more complex *in vitro* model such as an *H. pylori*/gastric cell co-culture, to better understand their interaction and the possible exchange of photosentizers from prokaryotic to eukaryotic cells.

[1] G. Tortora et al. IEEE/ASME TRANSACTIONS ON MECHATRONICS, 21 (2016) 1935-1942
[2] M.R Hamblin et al. , Antimicrob. Agents Chemother. 49 (2005) 2822-2827.

# POSTERS

# P1

## Photodynamic activity of novel diaryl porphyrins against cancer cell lines.

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Photodynamic therapy (PDT) of cancer uses photosensitizers (PS), a light source and oxygen to generates highly reactive oxygen species (ROS), which exert a cytotoxic action on tumor cells. Recently, has been shown that mixed non-symmetrical diaryl porphyrins, with two different pendants, are more efficient for PDT than symmetrical diaryl porphyrins.
In the present study we investigate the in vitro photodynamic effects of diaryl porphyrins with a pentafluoro-phenyl and a bromo-alkyl pendant (MCM21, MCM42) and with a pentafluoro-phenyl and a cationic pyridine pendant (MCM26, MCM46) in a panel of cancer cell lines.
The results of the cytotoxicity studies indicate that molecules with the cationic pendant are more potent in vitro than those with apolar pendant and as potent as m-THPC (Foscan), a powerful PS already approved for clinical use in PDT. To elucidate some aspects in the mechanism of PS-induced phototoxicity, induction of apoptotic, autophagic and necrotic cell death, and generation of reactive oxygen species (ROS) were evaluated. The effect of the PSs on the migratory activity of the cells was also assessed.
While all the compounds tested induced apoptosis as the major type of cell death, necrosis and autophagy were also observed to varying degrees in a cell-specific fashion. None of the four PSs induced any significant increase in intracellular ROS levels. The scratch wound healing assay indicate an anti-migratory effect; interestingly, Foscan was totally devoid of this activity.
In conclusion, the data obtained from this work support a greater efficiency of diaryl porphyrins with a positive charge in inducing cell death, compared to those with the bromo-alkyl pendant; most importantly, some of these novel compounds exhibit features that might make them superior to the clinically approved PS Foscan.

# P2

## Synthesis and photodynamic activity of a poly-methylmethacrylate nanoparticle loaded with a covalently bonded BODIPY photosensitizer.

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Photodynamic therapy (PDT) is a therapeutic option for the treatment of several solid tumors. PDT uses the combination of a photosensitizer (PS), light at an appropriate wavelength and molecular oxygen ($O_2$), to produce the reactive oxygen species (ROS). These last are particularly toxic and induce cell death through oxidative processes. Among the commonly known PS drawbacks, the main limits is their poor solubility in water because of most PS are characterized by a hydrophobic organic structure.
The purpose of this work was the synthesis of one nanoparticle functionalized with a PS belonging to the BODIPY family and the evaluation of its photodynamic activity.
The synthesis of the functionalized nanoparticle (NP) required the preparation of a BODIPY bearing an aminomethylmethacrylate. This monomer was then used to obtain NP through a copolymerization process with meta methyl methacrylate and 2-(dimethy-octyl) ammonium ethyl-methacrylate bromide.
Photodynamic activity was evaluated, on 3 cell lines (HCT116, SKOV3 and MCF-7), through the MTT assay, following the treatment with increasing PS concentrations, incubation for 24h, and exposure for 2h to a green LED light radiation. The results showed that the NP was a slightly less effective than the corresponding BODIPY in free form on HCT116 and SKOV3 instead slightly more efficient on MCF-7.
In order to better understand the mechanism of action of the NP, the percentage of apoptotic cells, the production of ROS and $^1O_2$ and, finally, the cellular uptake were then determined.

# P3

## A photoproduct of ketamine was identified in hair samples irradiated with artificial light in a solar simulator.

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Knowledge of the effect of sun light on drug content in hair can be helpful to the forensic toxicologist, in particular when investigating on drug concentrations above or below pre-determined cut-offs. In this study authentic positive hair samples were selected that had previously tested positive for ketamine. Washed hair were exposed to 765 W/m$^2$ (310-800 nm spectrum of irradiance) of artificial sun light for 48 hours in a solar simulator. This dose corresponds to about 2 months of exposure to natural sun light. Same samples were kept in the dark as controls. Hair drugs were extracted as [1] and analyzed by LC-HRMS. The percentage of photodegradation was calculated for ketamine and its metabolite norketamine. In parallel, photodegradation processes of both standard molecules dissolved in aqueous and organic solutions were studied. In hair samples, exposure to artificial sun light induced an appreciable decrease of drug concentrations. When more drugs were present in the same hair sample (e.g. MDMA and ketamine) the degradation yields were compound dependent. A degradation product induced by irradiation of ketamine in aqueous and methanol solutions was identified; this was also present in a true positive hair sample after irradiation. Thus, when decisional cut-offs are applied to hair analysis, photodegradation must be taken into account since sun light may produce false negative results. Moreover, new markers could be investigated as evidence of illicit drug use.

[1] D. Favretto, S. Vogliardi, G. Stocchero, A. Nalesso, M. Tucci, S.D. Ferrara. High performance liquid chromatography-high resolution mass spectrometry and micropulverized extraction for the quantification of amphetamines, cocaine, opioids, benzodiazepines, antidepressants and hallucinogens in 2.5mg hair samples. J. Chromatogr. A 2011, 1218, 6583

# P4

## Focus on acquired resistance mechanisms after oxidative damage induced by photodynamic therapy in prostate cancer cells.

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Emerging evidence indicates that Photodynamic therapy (PDT) can lead to acquired resistance in cancer. This particular event seems to depend on the structure, dose and subcellular localization of photosensitizer and by the light-fluence employed. The new challenge of the basic researchers is to deeply investigate the molecular mechanisms involved in this phenomenon in order to find new strategies to prevent it. Our previous studies confirmed the crucial role of nitric oxide (NO) together with singlet oxygen ( $^1O_2$ ) and reactive oxygen species (ROS) in determining the efficacy of PDT on tumor growth. We have found that repeated low dose Pheophorbide *a* (Pb*a*)-PDT treatments in prostate cancer cells determined a low "chronic" iNOS/NO level that stimulating the NF-kB/YY1/RKIP loop resulted in tumor progression with the development of a more aggressive and resistant cell subpopulation [1].
Considering that (i) Nuclear factor E2-related factor2 (Nrf2) is involved in the regulation of antioxidant proteins that protect cells against oxidative stress and (ii) Nrf2 has a dual role in cancer development and progression [2], our study is now focused on the relationship between Nrf2 and iNOS/NO in PDT acquired resistance.

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[2.] Leinonen, HM, Kansanen E, Polonen P, Heinaniemi M, Levonen AL. 2014. "Role of the Keap1-Nrf2 pathway in cancer." *Adv Cancer Res* 122: 281-320.

# P5

## Captisol/porphyrin nanocomplexes with potential in PAT.

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Cyclodextrins (CDs) improve the bioavalaibility and release properties of entrapped PS, thus their use is advantageous to fabricate efficient biocompatible nanoformulations for photodynamic therapy of cancer (PDT) [1] and photodynamic antimicrobial therapy (PAT) [2]. Aim of this work was to design a novel photosensitizing nanocomplex based on CAPTISOL® (sulphobutylether-beta-cyclodextrin, SBE-beta-CD) with the tetracationic water soluble meso-tetrakis(N-methylpyridinium- 4-yl)porphine (TMpyP), as nanosytems for PS controlled release in PAT [2]. Nanocomplexes were prepared in aqueous media and characterized with complementary techniques such as UV-Vis, fluorescence spectroscopy, thus elucidating complex stoichiometry, stability constant and photophysical properties of entrapped porphryin. Furthermore size and ζ-potential were measured by DLS. Finally, photoantimicrobial activity of the CAPTISOL®/TMpyP complex *vs* free porhyrin were investigated against Gram-negative *Pseudomonas aeruginosa* ATCC 27853, by showing the suitability of the proposed systems in photokilling bacterial cells.

[1] Conte, C., Scala, A., Siracusano, G., Leone N., Patanè, S., Ungaro, F., Miro A., Sciortino M.T., Quaglia F., Mazzaglia, A. (2014) RSC Adv., 4, 43903.
[2] Castriciano, M.A., Zagami, R., Casaletto M. P., Martel. B., Trapani, M., Romeo, A., Villari, V., Sciortino, M. T., Grasso, L., Gugliemino, S., Monsù Scolaro, L., Mazzaglia A. (2017) Bioamacromolecules, 18, 1134.

Financial support from Ligand (San Diego, CA, US) is gratefully acknowledged

# P6

## Photodynamic Inactivation of microorganisms using an approved LED-based light source.

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If not tackled immediately, antibiotic resistance will kill 300 million people by 2050 [1]. Therefore, the rapid development of new antimicrobial approaches is urged by health agencies such as the WHO. To bring Photodynamic Inactivation (PDI) into clinical practice approved light sources with sufficient power and a homogenous spatial intensity distribution are mandatory. Here we test the application of two LED-based lamps (Repuls7PDI-blue/Repuls7PDI-red) by Repuls Lichtmedizintechnik, for a PDI against Gram+ *Staphylococcus aureus*, Gram- *Escherichia coli* and the yeast *Candida albicans*. As photosensitizers, methylene blue (MB) and a natural compound, chlorophyllin (CHL, approved as E140) were used.
Methylene blue was photoactivated with red light (635 nm, 25.6 J/cm²) and allowed for a reduction of the number of viable *E. coli* (10 µM MB) by more than 6 $\log_{10}$ steps. Using the same parameters, more than 99.99999% of *S. aureus* (20 µM MB) or 99.99% of *C. albicans* (50 µM MB) were killed. Blue light activation (410 nm, 6.6 J/cm²) of the natural and therefore biocompatible photosensitizer CHL resulted in a 7 $\log_{10}$ reduction of *S. aureus* (5 µM) and a more than 6 $\log_{10}$ killing of *C. albicans* (50 µM). However, CHL-based PDI failed to inactivate *E. coli* without addition of cell wall permeabilizers.
In conclusion, the Repuls7PDI LED light sources are ideally suited for PDI. Due to approvals as medical devices in combination with an easy-to-use concept, high power and homogenous light distribution, these lamps will likely be accepted by practitioners.

[1] King, Anthony, "Antibiotic Resistance Will Kill 300 Million People by 2050" Scientific American, 2014

**P7**

# Fighting back mold: Photodynamic Inactivation of *Aspergillus niger*.

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The fungus *Aspergillus niger* is one of the species responsible for mold in buildings and may cause health problems for humans. A treatment with chlorine compounds is effective but problematic due to possible carcinogenic effects. Here the principle for the application of Photodynamic Inactivation (PDI) to kill *A. niger* is proven. In order to enable a safe and harmless treatment the natural photosensitizers curcumin and chlorophyllin were used. *A. niger* was grown in liquid suspension to a colony diameter of approximately 5 mm. After illumination using an LED array (435 nm, 15.8 J/cm²) the colonies were transferred onto agar plates and their growth was measured for up to 48 h. Antifungal PDI using 20 µM curcumin induced a significant growth reduction (0.6 cm² *versus* 8.1 cm² colony area of double negative control) irrespective of the dimethyl sulfoxide content (0.5 *versus* 10%). Above a concentration of 50 µM all hyphae were killed. Both natural photosensitizers therefore prove applicable as photofungizides on surfaces. Further experiments using ambient light to activate the photoactive compound are ongoing.

# P8

## Save the crop: Photodynamic Inactivation against phytopathogenic fungi.

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Phytopathogens are responsible for plant illnesses and cause partial or total loss of crop worldwide. Estimates of direct crop failures caused by pathogens, animals and weeds range between 20% and 40% of global agricultural productivity [1]. To combat phytopathogens the use of fungicides and pesticides is mandatory in industrialised fruit plantages, which induces drug resistance and accumulation of the substances in the human food chain. Photodynamic Inactivation (PDI) is a powerful approach to kill microorganisms [2]. We here proof PDI being effective against fungal phytopathogens if based on formulations of NaChlorophyllin (CHL, E140). For the antifungal PDI of *Alternaria solani* and *Botrytis cinerea* mycelia were grown in liquid medium for 24 hours (*A. solani*) or 48 h (*B. cinerea*). Small spheres of the mycelia (average diameter 2 mm) were incubated for 100 min with CHL or a formulation of CHL with $Na_2EDTA$. Samples were illuminated with 395 nm (fluence 106.6 J cm-2) and the radial growth of mycelial patches after 7 days on agar medium was measured. The treatment of fungal mycelia with CHL did not show any antifungal effect. Upon addition of 5 mM $Na_2EDTA$ the phototreatment turned effective. It was most effective at 100µM CHL, where *A. solani* and *B. cinerea* were almost completely inactivated. PDI is a new and powerful approach to combat plant pathogens. Usage of natural photosensitizers like CHL in a formulation with $Na_2EDTA$ might help to avoid effects to the host plant or the food chain.

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[2] Wainwright, Mark; Maisch, Tim; Nonell, Santi; Plaetzer, Kristjan; Almeida, Adelaide; Tegos, George P. and Hamblin, Michael R. 2016. "Photoantimicrobials – are we afraid of the light?". *Lancet Infectious Deseases*. 17(2).

**P9**

# Combating environmental pathogens: Photodynamic Inactivation of *Erwinia amylovora* and *Xanthomonas axonopodis* by the natural photosensitizer Chlorophyllin.

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Due to bad practices, misuse of antibiotics or lack of a better alternative treatment, bacterial plant pathogens cause severe financial losses in agriculture. [1] In this study, Photodynamic Inactivation is applied against bacterial plant pathogens as first proof-of-principle. The natural photosensitizer Na-Chlorophyllin (CHL, approved as food additive E140) was used to combat the Gram (-) bacterial strains *E. amylovora* (causing fire blight in apple) and *X. axonopodis* (causing citrus canker). To ensure plant health during PDI-treatment, a plant compatibility assay using *Fragaria vesca* in BBCH-stage 14 [2] was established. Photoactivated CHL (100 µM @ 395 nm, 26.6 J/cm²) reduced the number of colony forming units (CFU) of *X. axonopodis* by one $\log_{10}$ step. 100 µM CHL in combination with 5 mM ethylenediamine tetraacetic acid ($Na_2EDTA$) was able to photokill (>7 $\log_{10}$ steps) *X. axonopodis* and inhibited the number of *E. amylovora* by 4 $\log_{10}$ steps. CHL had no adverse effects on soft growing *F. vesca* plants inside the greenhouse, but the addition of $Na_2EDTA$ produced minor leaf damage. These adverse effects of $Na_2EDTA$ were not observed on plants that were grown outside. We conclude that PDI based on CHL is applicable in plant protection against bacterial pathogens and might minimize negative effects to the environment due to the excellent biocompatibility of the photoactive compound.

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[2] Meier, Uwe, Graf, H. Hack, H. Hess, M. Kennel, W. Klose, R. Mappes, D. Seipp, D. Stauss, R. Streif, J. and Van den Boom, T. 2001. "Growth stages of mono-and dicotyledonous plants." Federal Biological Research Centre for Agriculture and Forestry 62-63

# P10

## Response of *Pseudomonas aeruginosa katA* mutant to photo-oxidative stress.

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*Pseudomonas aeruginosa* is a well-known human opportunistic pathogen, causing severe nosocomial infections. Since *P. aeruginosa* is often difficult to eradicate, new therapeutic strategies to combine with antibiotics are necessary. Among these, the antimicrobial Photodynamic Therapy (aPDT) seems to be a promising one. The aPDT combines the use of photosensitizers (PSs), visible light and oxygen to induce oxidative stress in bacterial cells. Recently, the antimicrobial Phototherapy (aPT), based on antimicrobial power of visible light, has emerged as new approach. In particular, blue light seems to be efficient against different microbial species.
Some authors hypothesized the involvement of detoxifying enzymes in bacterial defense against photo-oxidative stress. Hence, the attention was focused on the putative role of *P. aeruginosa* PAO1 catalase A (KatA) in response to photo-oxidative stress induced by aPDT and/or aPT. At this aim the genetic approach was chosen. *P. aeruginosa katA*- mutant was more sensitive than wild-type strain PAO1 to wide-spectrum light and blue LED (464 nm) treatments. The complementation of KatA, in *katA*- mutant, restored the light response of wild-type PAO1. Upon Toluidine Blue O treatment and irradiation by visible light (halogen lamp or LED), *katA*- mutant was significantly more sensitive than PAO1 strain.
In conclusion, it seems that in P. aeruginosa PAO1 KatA is involved in response to photo-oxidative stress induced by irradiation (aPT) and photodynamic inactivation (aPDT) TBO-mediated.

# P11

## Advancement of the reasearch project CAPSULIGHT for the photokilling of *Helicobacter pylori* by endogenous pigments.

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The research project «CAPSULIGHT: Design of an ingestible robotic pill based on LED sources for the treatment of gastrointestinal disorders», funded by Regione Toscana[1], is aimed at the development of a light capsule suitable for ingestion with which to eradicate infections of *Helicobacter pylori*. This gram-negative microaerophilic bacterium colonizes the mucus layer of stomach and duodenum of up to 50% of the world population, with infection rates nearing 90% in some countries. Since the removal of *H. pylori* is a complex process, usually attained by means of cocktails of drugs (e.g., bismuth, metronidazole, tetracycline, and a proton pump inhibitor, or clarithromycin, amoxicillin and a proton pump inhibitor), the antibiotic treatments reach eradication rates of only about 80%, sometimes with heavy side effects for the patients; moreover *H. pylori* strains are beginning to show antibiotic resistance and patients are found to be unresponsive to treatment. A possible alternative to the antibiotic treatment is to take advantage of the presence of endogenous porphyrins of *H. pylori*, irradiating the mucus layer of the stomach with light produced by LEDs inserted in an ingestible capsule, a device thought to be less invasive than endoscopy. In the framework of this project, we are testing the effect of light of different wavelength and intensity in order to identify the best irradiation conditions for bacterial eradication.

[1] Project CAPSULIGHT: "Design of an ingestible robotic pill based on LED sources for the treatment of gastrointestinal disorders" supported by Regione Toscana (Italy) Bando FAS Salute 2014 (grant number CUP B52I14005760002).

# P12

## Possible light-antibiotic synergy in the photo-treatment of *Helicobacter pylori* strains.

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In the framework of antibacterial PDT, stomach infections caused by *Helicobacter pylori* (*H. pylori)* are among the most studied. Different illumination schemes have been proposed, all characterized by the absence of external photosensitizer, due to endogenous production of photoactive porphyrins by *H. pylori* itself. As *H. pylori*-associated infections are among the most studied in the search for antibiotic-resistance solutions, in this work we have investigated the possible synergies due to the contemporary use of both antibiotics and therapeutic light. To this aim, we performed a phototoxicity study on three different *H. pylori strains* (ATTC 700392, 43504, 49503), sensible to doxycycline. Cultures have been grown on solid medium containing doxycycline at sub-MIC concentration, then irradiated for 10, 20, 30 minutes with a 400nm-peaked light source (4.8 $mWcm^{-2}$). Vitality has been evaluated by post-treatment CFU counting. Controls corresponded to irradiated-only samples were prepared for comparison. A synergistic effect was noticed when both antibiotic and light treatments were performed, indicating an enhancement of the photokilling efficacy due to possible bacteria photosensitization by doxycycline. Further studies will be necessary to clarify all the possible mechanisms at the basis of the observed effect.

SIFB-ALPE ADRIA Meeting on Photobiology
20th-22nd June 2018-Udine (Italy)

# ABSTRACTS

# Symposium on PHOTODERMATOLOGY

# Photoprotection needs of patients with acne.

*Franz Trautinger*

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Due to its high prevalence and its preference to mainly afflict the face of adolescents acne is surrounded by a number of myths and beliefs passed on among affected persons, their friends and family. These lay conceptions most commonly relate to the role of diet, local skin care, squeezing of offending lesions and the effects of climate, season and solar radiation. Due to its predilection for sun-exposed body sites interactions of radiation from the sun (and occasionally also from sunlamps) with acne itself and with the topical and systemic medications used for its treatment are inevitable. Knowledge of the pertinent evidence on these interactions is important for proper counselling and to ensure compliance and satisfactory treatment outcomes. The belief of improvement of acne through sunrays may have encouraged sun-seeking behavior among patients and has led in the past to trials of UV-phototherapy. However, neither was it possible to convincingly demonstrate a beneficial effect of UV nor could a positive (seasonal) effect of sunlight on the severity of acne ever be confirmed. Consequently UV-phototherapy has almost completely lost its place in our armamentarium against acne and sun-exposure should not be encouraged. Although the influence of UV-radiation on the pathophysiology of acne is insufficiently understood there are two major reasons in addition to the general prophylactic benefit to promote photoprotection to acne patients: First, acne in darker skin is likely to result in postinflammatory hyperpigmentation, which may be long lasting and cosmetically even more disturbing than the causative condition, and second the potential photosensitization from topically and systemically applied acne medication. Recommendations for photoprotection in acne follow the same rules as in the general population with regular reassurance for adjustments according to acne severity, treatment, season, and occasional travel.

# Photoprotection-UV filters: properties, use and safety in sun products.

*Antonina Agolzer*

MD Dermatologist, UDINE

Sunscreen application is a common strategy used to prevent the damage induced by UV radiations. In the last 30 years a variety of protective agents against UV exposure have been developed: physical filters, chemical filters and organic filters. Physical filters are the safest: they scatter and reflect UV rays, but are difficult to apply on the skin. Chemical filters are capable of absorbing these rays, they are cosmetically pleasant, but recent studies suggest a toxicological nature for some of these agents. Organic filters are the newest advance in sun products . They are easy to apply and safer than UV filtering/scattering filters. Since 2003 the COLIPA Index (Europe) has regulated sun product availability on the market and suggests a classification of sunscreen protection into low, middle, high and very high protection. In the USA, the FDA establishes the classification and the safety of sun products, which are considered topical medical drugs. There are many cosmetical formulations for all requirements: cream, fluid, spray, stick for small areas (e.g. lips, eyelids, scars). In the last two decades dermocosmetical research has developed the correct tools for all kinds of patients and demands (children, pregnant women, patients with skin tumors and other dermatological problems and diseases).

# Systemic photoprotection.

*Marina Venturini*

Department of Dermatology, University of Brescia, Brescia, Italy

As experimental, clinical and epidemiological evidences indicate that both sunburns and high cumulative lifetime exposure together with progressive aging of the population are the most important causes of cutaneous tumors. However, sunscreens use proved only partially effective because they cannot afford a uniform protection against the entire spectrum of ultraviolet wavebands of the solar spectrum and because in daily use they are applied at a largely lower dose than needed and they are unevenly distributed on the skin surface. A promising alternative strategy is the use of natural non-toxic botanicals called nutriceuticals. After oral administration, they distribute uniformly on the skin surface. Here, they have not a filtering activity against ultraviolet radiation but they have a beneficial activity by contrasting deleterious cell damages at a molecular level. Most nutriceuticals marketed against solar UV effects have antioxidant activity because they can prevent and repair cell damages mediated by UV generated reactive oxidative species. In addition, a few of them, such as the extract of polypodium leucotomos, have demonstrated activities against UV related anaerobic DNA damages. In addition, other nutriceuticals, such as nicotinamyde and sulphoraphane, and (pro-)vitamins A, E, D and F have beneficial effects that are due to the modulation of molecular pathways with an overall increase of the natural reparative mechanisms. Cocoa beans are rich in polyphenols which have a well known and high antioxidant capacity. Studies in vivo and in vitro have shown how oral assumption or topical application of cocoa extracts carry out a photoprotective effect by reducing UV sensibility expressed as cutaneous erythema induced by the irradiation with a solar simulator.

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# Ultraviolet exposure and skin tumors.

*Mauro Alaibac*

Unit of Dermatology, University of Padua, Italy

Skin cancer is the most common type of cancer in fair-skinned populations in many parts of the world and recent trends show an increase in incidence of both melanoma and nonmelanoma skin cancers. The initiation and propagation pathways of melanoma and nonmelanoma skin cancers differ but have some elements in common. In particular, ultraviolet radiation (UVR) is considered the major etiologic agent in the development of skin cancers and several epidemiological data strongly support this view, as UVR promotes DNA damage and genetic mutations, which subsequently lead to skin cancer. This presentation aims to provide an overview of the different subtypes of skin cancer and their relationship with UVR.

# Aktinic Keratosis.

*Marco Dal Canton*

Dermatologist - Belluno

Medicine has significantly improved the care and prevention of primary melanoma, refining our diagnostic accuracy, training and teaching physicians, improving information and awareness in the population. NMSC, particularly epithelial tumors, have remained in background, because of a lower mortality rate and of a traditional underestimation of the consequences and costs of epithelial tumors in respect to melanoma. NMSC, particularly epithelial tumors, display a steep prevalence curve from the '60s, likely related to a better diagnostic accuracy and documentation, and a slightly lowered age at onset, mainly of SCC, particularly when localized on the limbs. The higher attention for the SCC in the recent guidelines, as the 8$^{th}$ AJCC revision (2018), induces a more aware and a proactive consideration also to its precursor, the actinic keratosis (AK). The recent consensus papers describe AKs as true non-invasive squamous cell carcinomas, redirecting the previous concept of epidermal dysplasia and pre-cancerosis, albeit there is an agreement in not intimidating patients affected by AKs sharing this concept, due to the very low likelihood of evolution to SCC. Our instruments to read the clinical frame, to correlate the clinical features with hystologic degree of invasion and to intercept the transformation to truly invasive carcinoma are under scrutiny and likely to be the new edge in upcoming research. In the clinical practice the main challenge is the update of the clinical classification of the patient affected by AKs, and possibly the true nature and definition of skin photoaging. The crucial point at the moment is the demarcation of the field of cancerization, upon which should depend the choice between a focal or a field directed therapeutic tactic. This bare borderline influences directly our therapeutic choices, among a wide array of options available today, and physicians are requested to balance the related economic implications, the effects on the patient compliance, and on their sustainability. The latter are today one important emerging issues in all health care systems worldwide.

# Treatment considerations in actinic keratosis.

*AM. Bertoldi*

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Actinic keratosis (AK) is a frequent health condition caused by chronic exposure to ultraviolet radiation; several treatments are available to deal with that. Chronic exposure to UV radiation has a fundamental role in the pathogenesis of AK, as reflected by the term 'actinic', and the synonym 'solar keratosis'. UVB radiation can cause direct DNA damage with the formation of cyclobutane pyrimidine dimers and pyrimidine-pyrimidone 6,4- photoproducts. As a result of DNA mutations, the function of tumour suppressor proteins such as p53 can be suppressed, leading to a clonal expansion of keratinocytes into an AK. A dysregulation of the p53 pathway seems to play the most important role in the development of AK lesions, as well as in the further development of SCC. Absorption of UVA radiation by skin chromophores generates reactive oxygen species, which oxydize guanine residues on the DNA; these oxidative products are mutagenic. The AK are considered pre-malignant and they can become squamous cell carcinoma. Reliable data on the progression rates of single AK lesions are few, so that the actual risk of progression of single AK lesions to invasive SCC remains unclear. For AK, the choice of a lesion-directed treatment aims at the physical destruction or removal of atypical keratinocytes that constitute a singular AK lesion. These treatments earmark the clinically manifest AK lesions. Also field-directed treatment options for AK aim at the destruction, removal or remission of atypical keratinocytes. Here, therapy of latent, subclinical areas of atypical keratinocytes within a field of chronic sun damaged skin and not only a reduction in manifest areas of AK is intended. Topical therapies allow the treatment of a whole area of affected skin and currently include diclofenac sodium gel, 5-fluorouracil cream, 5-fluorouracil and acetylsalicylic acid solution, imiquimod cream, and ingenol mebutate gel. Retinoids is a systemic therapy that have been assessed for its potential role in suppression or treatment of multiple AK. Photodynamic therapy combines a dedicated light source of appropriate wavelengths with the application of a photosensitizing cream to produce apoptosis and necrosis of the target tissue. Photosensitizing agents include 5-aminolaevulinic acid (5-ALA) and the methyl ester of 5-ALA, 5-MAL. BF-200ALA was recently used, showing increased stability and penetration. Daylight PDT involves the application of MAL to the skin without occlusion and subsequent exposure to ambient daylight. A high-SPF sunscreen without mineral filters is applied 15 min before the photosensitizing cream. Cryosurgery, it is a long-established treatment for AKs requiring a cryospray (or cotton wool and orange sticks) and a supply of liquid nitrogen. The most recent scientific acquisitions have been explained. The favourite treatment for keratosis is:

A. Ingenol mebutate

B. Solaraze 3% gel

C. Cryotherapy

D. The treatment has to be for each single person and based on the case history of the patient (number and type of lesions) and on the reaction to previous treatments.

# New and Current Preventive Treatment Options in Actinic Keratosis.

*Alessandro Gatti*

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Actinic keratosis (AK) is characterised by proliferation of atypical epidermal keratinocytes in sun damaged skin, especially in fairy skin population. It is considered a precursor or an initial form of squamous cell carcinoma (SCC) and so it represents a public health problem.
Long term exposure to sun is the main risk factor for AK and SCC; this correlation is not so clear for basal cell carcinoma (BCC) and melanoma (MM).
MM and BCC are more correlated with intermittent and intense short-term sun exposure and in contrast chronic less intense sun exposure has not been found to be a risk factor for melanoma; in fact outdoor workers generally has not an higher risk for MM than general population.
The aim of preventive treatments of AK is avoiding SCC in high risk population.
The first milestone is sun protection measures in general population, to reduce global lifetime UV irradiation of the skin with particular attention in fairy skin population and in patients treated for AKs or SCC, that are at higher risk.
Field treatments for AK seem to be more effective to prevent the appearance of new AKs than lesion directed treatment; field treatments are directed against clinical and subclinical lesions in field of cancerization while lesion directed treatments are directed only towards clinical evident lesions. Treatment choice is important for multiple AKs in sun damaged skin; it's better to use one of the field treatment available. Lesion directed treatments appear to be adequate only for isolated lesions.
Oral nicotinamide in some studies has demonstrated activity in AK treatment and prevention and it is a promising alternative option.

# Light sources for photodynamic therapy.

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A photodynamic effect is produced through the light activation of a photosensitizer. At the beginning of the modern photodynamic era in the second half of the last century, the treatment goal was to activate sensitizers that were accumulated in the tumor tissue of internal organs. Therefore, lasers were the ideal light source because the sensitizers of the second and following generations had narrow peak of absorption and high quantum yields and because laser light could be easily delivered with an optical fiber. However, if you treat an easily accessible external tumor, like skin tumors, with a sensitizer like protoporphyrin IX (that is produced intracellularly after application of a cream containing aminolevulinate or methylaminolevulinate) with many activation peaks, you have several effective alternatives: white light from tungsten lamps, monochromatic light from lasers, selected wavebands from light emitting diodes (LED), intense pulsed light (IPL) or filtered metal-halide lamp (MHL) and broadband visible natural light from the sun. LED red light with a peak around 630 nm is the preferred light source from the ALA or MAL photodynamic therapy (PDT) of basal cell carcinoma and Bowen's disease because of its deep skin penetration. It is also used for the treatment of multiple actinic keratoses. Alternatively, exposure to visible content of the sunlight are increasingly used for the treatment of multiple actinic keratoses that are, by definition, confined in the epidermis. Indeed, daylight PDT proved equally effective but much more tolerated and with fewer adverse effects than conventional PDT with red LED light.

# Photodynamic Therapy in Practice at Department of Dermatology in General and Teaching Hospital Celje.

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Photodynamic therapy (PDT) involves the activation of a photosensitizing drug by visible light to produce reactive oxygen species within target cells, resulting in their destruction. In Dermatological indications, PDT is usually performed by topical application 5-aminolaevulinic acid (5-ALA) or its ester, methyl aminolaevulinate (MAL). Within target cells they are converted into photoactivatable porphyrins, especially protoporphyrin IX. After incubation period, visible light of an appropriate wavelength activates the photosensitizer, promoting the photodynamic reaction. Before PDT it is possible to use UV induced skin surface fluorescence by which even incipient lesions can be detected. Topical PDT can be used as lesion or as area/field-therapy. It is approved for treatment of actinic keratosis (AK) and certain non-melanoma skin cancers (NMSC), with superiority of cosmetic outcome over conventional therapies. Recurrence rates are typically equivalent to existing therapies, although inferior to surgery in nodular basal cell carcinoma [1]. Conventional topical PDT with red and green spectrum of light has been in regular use at the Department of Dermatology in Celje General and Teaching Hospital since 2010 and day-light PDT (dPDT) for AK since 2015. As a photosensitizing drug we use a 20% formulation of 5-ALA, prepared by our pharmacy, and registered nanoemulsion of ALA (for AK only). In the period of 2010-2016 PDT was used in 509 treatments, with a male predominance. The average age of the patients was over 70-years, as expected from official indicating diagnoses. In most cases topical PDT was used for field directed treatment (for non-hyperkeratotic AK and actinic cheilitis), followed by lesion treatments for basal cell carcinoma of superficial spreading type (sBCC) and for Bowen's disease (BD) as a form of "in situ" squamous cell carcinoma of the skin. Following the recommended protocols (including curettage and repetition scheme for sBCC and BD) the treatments were, as expected, successful in immunocompetent patients. Side effects as tingling discomfort or pain during illumination phase (pronounced at classic treatments for big surfaces of AK and least at dPDT) were treated with different ways of pain control, e.g. air cooling, oral analgesics therapy and, in some cases, infiltrative anesthesia of regional nerves.

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# Our Experience with Photodynamic Therapy.

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From 7th March 2013 to 16th February 2018, at the Dermatovenereology Clinic, University Medical Center Ljubljana, Slovenia, at 76 patients (5 women, 71 men), an average of 82.3 years old, 2.42 photodynamic therapy (from 1-11) of actinic keratoses was perfomed. They have actinic keratoses an average of 5.6 years before the photodynamic therapy, and before that, 3.42 x (from 1 to 16) were performed cryotherapy. These patients also had 3 x diagnosed Mb. Bowen, 53 x basal cell carcinoma, 4 x keratoacanthoma, 7 x melanoma and 18 x squamous cell carcinoma. Previously were treated 5 x with Ingenol Mebutate gel and 29x with Imiquimod Cream too.

# Photodynamic Therapy: Experience of Dermatovenereologic Unit in Brunek Hospital.

*Fabio Massimo Gavazzoni*

Dirigente Medico SABES Comprensorio Sanitario Brunico (Italia)

Topical photodynamic therapy (PDT) is a widely approved therapy for many skin diseases, such as actinic keratoses, superficial and thin basal cell carcinomas, in-situ squamous cell carcinomas. During the last years PDT has been used to treat other cutaneous disorders, such as acne, viral warts, skin rejuvenation, psoriasis and localized scleroderma. Here we report the experience of our Dermatovenereologic Unit in Bruneck Hospital.

# Phototherapy of Psoriasis.

*Mariachiara Arisi*

Department of Dermatology, University of Brescia, Brescia, Italy

In the past several decades, phototherapy has been widely used to treat psoriasis using a variety of light with different mechanisms of action. Because each light source has specific therapeutic and adverse effects, it is important to adequately choose the sources and parameters in management of psoriasis with different pathogenic sites, severities, and duration of the disorder. Various phototherapeutic approaches have been used either in different combinations or as monotherapy and phothotherapy has remained a mainstay option for patients with moderate-to-severe psoriasis resistant to topical treatments due to its efficacy, cost-effectiveness, and relative lack of side effects, in particular a lack of systemic immunosuppression seen with traditional and biologic systemic therapies..This presentation aims at providing most updated clinic information about how to select UV sources and individual therapeutic regimens.

# Italian guidelines on the systemic treatments of moderate-to-severe plaque psoriasis.

*Anna Chiara Fostini, Paolo Gisondi*

Section of Dermatology and Venereology, Department of Medicine, University of Verona, Verona, Italy.

Psoriasis is one of the most common dermatologic diseases. It is a chronic inflammatory disease of the skin that affects about 2-3% of the population and it is characterized by erythematous and scaly plaques, mainly localized on elbows, knees, and scalp. In 70% of cases it occurs in mild or moderate forms, but one in three patients is affected by a widespread and severe disease. Available therapies include topical, systemic and phototherapy treatments. In recent years, biological drugs have also been introduced. The Italian guidelines contains indications on the screening and monitoring of various treatments, also in relation to some subpopulations of patients such as children and the elderly, pregnant women and carriers of chronic infections such as viral hepatitis and latent tuberculosis. The opportunity to have national guidelines also derives from the fact that the reimbursement criteria of the drugs vary among European countries.

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# What's new in the management of psoriasis?

*Cinzia Buligan*

Clinica Dermatologica, Dipartimento di Area Medica, Università degli Studi di Udine.

During the presentation the latest news on the treatment of psoriasis will be illustrated, including topical and systemic therapies recently introduced on the market or during marketing in Italy.

# Laser and Incoherent Light skin tissue interaction – Old and new concepts and their current clinical implications.

*Leonardo Marini*

SDC The Skin Doctors' Center Trieste

Technical achievements in the field of Dermatological LASER and Incoherent Light Source systems are simply amazing. The choice of treatment parameters is incredibly vast allowing a wide range of laser and incoherent light tissue interactions variably applying the theories of selective photo-thermolysis and its expanded version. Pulse modulation, fractional delivery, nano- and sub-nanosecond laser sources, controlled stability of IPL spectral bands, and sequential combination treatments contribute to improve clinical results, reduce complications and side effects, and expand treatment indications. Low level lasers and LED sources are also currently used and we are just beginning to better understand their local and systemic tissue effects. Choosing among this enormous range of possibilities is not easy and requires specific knowledge of light-tissue interaction to take maximum advantage of modern technologies.

# How can the laser play a role in the treatment of infantile hemangioma?

*Daniela Cavalca*

Rome, Italy

Infantile hemangiomas are the most frequent vascular tumors in infancy in Caucasian newborn (4-10%). The superficial component of the hemangioma is sometimes already evident at birth or in the following few days; on the contrary the subcutaneous component grows later. The proliferative phase may not always last until 5 months of age but quite often beyond this time, until one year of age and then it is followed by a slow regressive phase from 2 to 10 years of age. Although 85-90% of all IH undergo spontaneous involution (completely or partially), a small number can cause disfigurements, functional impairments, life threatening or present ulceration not responding to local treatment and pain. Actually in these cases the treatment with oral non selective β-blocker propranolol is the first line option. It is known that the sooner propranolol is started, the greater its efficacy (usually from the 2$^{nd}$ month of age) although the involution may not be complete.
There may be cases where propranolol therapy should be stopped because of the side effects and cases in which the therapy is not effective. Relapse, not always longer responding to propranolol, may occur after therapy discontinuation. Furthermore, we shall not forget that regression, spontaneous or after therapy, is rarely accompanied by a restitution ad integrum of the tegument: the dystrophic and dysmorphic results can be equally disabling.
Finally, we shall remember that the non complicated IH not falling within the indications for propranolol therapy, may still be a psycho-social problem for the patient.
In all these cases the laser sources (Dye, Nd-YAG, Alexandrite, $CO_2$, Erbium, Thullium) can play a key role with the aim of improving not only the quality of the final result but also the little patient's quality life.

# Why to burn a burn. Laser treatment of scars and the laser assisted drug delivery.

*Matteo Tretti Clementoni, MD*

Laserplast – Milano – Italy

Advances in burn treatment has dramatically increased survival of severely burned patients. The increase of survival of burn patients has resulted in an increased demand to treat the resulting scars. In addition to impairing self-esteem and body image, these scars severely limit functional recovery, compromise activities of daily living, and prevent return to work. Restoration of form and function of these scars remains anyway a challenge, but emerging treatments utilising laser can now help to improve them. The aim of the talk will be to present a new algorithm of treatment of severe burn scars combining different kind of laser and several drugs. Lasers determine a collagen remodelling and therefore a clinical improvement. The combination of lasers and drugs, through the so-called laser assisted drug delivery, has a synergistic effect and leads to better outcomes.

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# Blue diode laser: a new strategy for the management of lichen sclerosus et atrophicus.

*Di Meo Nicola*

Dermatology and Venereology Departement, University of Trieste

Lichen sclerosus represents a frustrating pathology for the patient and for the dermatologist who has to perform the treatment.
We will discuss about a new therapeutic aid by LASER for the management and treatment of patients affected by this pathology. The blue diode laser delivering wavelengths similar to those of UVA-1 could be a useful tool in the management of these patients.

# Side effects of laser therapy.

*Cristina Zane*

ASST-Spedali Civili di Brescia, Università degli Studi di Brescia

The demand for the treatment of many dermatological disorders using laser or intense pulsed light (IPL) technology has been rising for years. Although such procedures used to be performed almost exclusively by physicians, due to their commercial potential and apparent simplicity, a multitude of wellness facilities, cosmetology institutes are also now offering them. Most treatments are performed by trained laypersons without any medical supervision. In addition, the expectations of patients are often raised. Yet dermatologists are seeing growing numbers of patients with complications following such treatment. Typical side effects include alterations of pigmentation (hypo/hyperpigmentation depending on laser/IPL setting, skin type, and preinterventional or post-interventional sun exposure), crusts, blistering, burning, hypertrophic scarring/keloids, pruritus, localized herpes virus infections, folliculitis, color changes (with removal of permanent make-up), as well as paradoxical hair growth (especially with IPL technology). The biggest problems are the treatment of pigmented lesions of uncertain benign/malignant nature without prior diagnosis or histological controls, which often leads to the appearance of an atypical post-operative recurrent nevus or pseudomelanoma. Conversely, amelanotic melanomas may be allowed to progress without detection and may even metastasize.

# Photobiomodulation with polychromatic polarized light.

*Petra Bukovec, Igor Frangež*

Clinical department for surgical infections, University Medical Centre Ljubljana

Due to the aging population with many vascular and endocrine (diabetes) comorbidities, the incidence of chronic wounds has increased lately. As such, they represent an exceptional burden on the entire health system, health care and economy itself. At the same time, they still represent the challenge to specialists, who are involved in its the prevention and treatment [1]. Treatment of chronic wound is difficult and expensive, that is why new noninvasive treatment methods are being sought. As a successful method of treatment, a photobiomodulation is also considered. Photobiomodulation is using a precisely determined light regime for regulation of cellular processes. Among the various phototherapeutic possibilities, the most promising is use of polychromatic polarized light, which the light similar to a part of the electromagnetic spectrum produced by the sun but without UV radiation [2,3]. It has many positive effects due to its polarization and polychromaticity. Due to polarization, light waves move in parallel planes and create a narrow, concentrated beam, compared to conventional light, in which the light waves swing in all directions. Furthermore it contains a wide spectrum of wavelengths and colors (480-3,400 nm), which enables it to stimulate a number of light receptors on the skin. Unlike laser light, which has only one wavelength - monochromatic. Such characteristics enables light to penetrate into the skin and underlying tissue easier and thus accelerate various biological processes [3]. Photons trigger complex processes that lead to favorable clinical effects such as analgesia, regeneration, inflammation modulation, edema reduction, vasodilatation and consequently improved microcirculation [4]. The success of chronic wound healing with photobiomodulation has already been demonstrated in the past [5].

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[3] Reddy M, Gill SS, Kalkar SR, Wu W, Anderson PJ, Rochon PA. Treatment of pressure ulcers: a systematic review. JAMA. 2008;300:2647–62.
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[5] Hawkins D, Heidi A., 2007. “Phototherapy – a treatment modality for wound healing and pain relief.” African Journal of Biomedical Research 2007; 10: 99-109.

# The effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients.

*Igor Frangež, Helena Ban Frangež*

University of Ljubljana

**Purpose**: Chronic wounds, especially in diabetic patients, represent a challenging health issue. Since standard treatment protocols often do not provide satisfactory results, additional treatment methods—like phototherapy using low-level light therapy—are being investigated. The aim of our study was to evaluate the effect of phototherapy with light-emitting diodes on chronic wound treatment in diabetic and non-diabetic patients. Since a sufficient blood supply is mandatory for wound healing, the evaluation of microcirculation in the healthy skin at a wound's edge was the main outcome measure.

**Methods**: Forty non-diabetic patients and 39 diabetics with lower-limb chronic wounds who were referred to the University Medical Center Ljubljana between October 2012 and June 2014 were randomized to the treated and control groups. The treated group received phototherapy with LED 2.4 J/cm$^2$ (wavelengths 625 nm, 660 nm, 850 nm) three times a week for 8 weeks, and the control group received phototherapy with broadband 580-900 nm and power density 0.72 J/cm$^2$. Microcirculation was measured using Laser Doppler.

**Results**: A significant increase in blood flow was noted in the treated group of diabetic and non-diabetic patients ($p=0.040$ and $p=0.033$), while there was no difference in the control groups. Additional Falanga wound bed score evaluation showed a significant improvement in both treated groups as compared to the control group.

**Conclusion**: According to our results, phototherapy with LED was shown to be an effective additional treatment method for chronic wounds in diabetic and non-diabetic patients.

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# Phototherapy.

*Sara Trevisini*

Azienda Ospedaliera Universitaria, Trieste

Phototherapy represents the use of ultraviolet radiation for the treatment of several skin diseases such as psoriasis, atopic dermatitis, mycosis fungoides, vitiligo and pruritic disorders. Currently phototherapy encompasses irradiation with broadband UVB, narrowban UVB, excimer laser, UVA1, UVA plus psoralen or alone and extracorporeal photopheresis. Proper selection of the phototherapy modality for different disorderds is necessary for therapeutic success and right dosimetry is required to avoid acute side effects such as sunburn reactions and burning. The major long term side effect is represented by the risk of carcinogenesis.

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# Dermoscopy as a response predictor tool in psoriasis vulgaris and in vitiligo treated with narrowband ultraviolet B phototherapy.

*Enzo Errichetti*

University of Udine, Italy

Dermoscopy is a noninvasive method that allows to appreciate findings which are hardly visible to the naked eye through an in vivo magnification. Consequently, such a technique is more and more used to assist the noninvasive diagnosis of many skin conditions. Besides diagnostic purposes, dermoscopic examination may also have a potential role in identifying possible response predictors to treatments of several dermatoses. In the presentation, I will show the results of two prospective studies aiming to find positive and/or negative response predictor factors of psoriasis vulgaris and vitiligo to narrowband UVB phototherapy.

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