Sensitive to light

The University of Salzburg's Laboratory of Photodynamic Inactivation is exploring potential roles of photosensitizing agents in human health. With possible applications in areas as diverse as food decontamination and therapeutics, the lab is using a variety of photoactive compounds to generate reactive oxygen species which can kill key microbial pathogens or cancerous cells.

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Dr Plaetzer and the Laboratory of Photodynamic Inactivation team

UNIVERSITY of SALZBURG

Photodynamic inactivation (PDI) is a novel biophysical technique using light sensitive substances to disrupt biological activity. Following incubation with the photosensitizing agent, exposure to a visible light source causes the formation of reactive oxygen species (ROS) that kill the target cells by oxidative processes. This mechanism is both highly effective and extremely fast-acting, offering possible new strategies for dealing with multi-drug resistant pathogens – such as bacteria, yeasts and fungi – without affecting the host tissue. Due to the high proliferation rate of cancerous cells, this approach has also been approved by various national health institutions for applications in oncology, allowing malignant tumors to be specifically targeted using suitable photosensitizers.

Dr Kristjan Plaetzer, Principal Investigator in the Laboratory of Photodynamic Inactivation at the University of Salzburg's Division of Physics and Biophysics, explained: "Antimicrobial resistance is now a worldwide health problem, threatening our ability to effectively treat an ever-increasing range of infections caused by bacteria, viruses, fungi and other parasites. With very little progress in the development of new antibiotics, there is now a real need for alternative treatment strategies which can bypass the resistance mechanisms that have evolved in organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and resistant *Candida albicans*. The key advantage of PDI is that it has a non-specific mode of action – the resulting ROS disrupt cellular pathways in a random manner – and so pathogens cannot develop resistance mechanisms. Our research is focused on how we can translate this approach from the research bench – where it is already well characterized and understood – to environments that can benefit human health."

"Using *S. aureus*, *E. coli* and *C. albicans* as model organisms, we are looking at various applications for PDI technology, from food decontamination to chronic wound care. For example, in one recent study we used this approach to decontaminate porcine skin as a model of human tissue.[1] After infecting the model system with bacteria, we then treated the skin with a suitable photosensitizer and, after just 5-10 minutes, exposed it to a visible light source that is harmless to humans or the animal tissue. This proved extremely successful in killing the bacterial cells, but the porcine tissue was completely unaffected."

"We have used the same technique for the decontamination of plant materials. Pre-prepared salads are one of the most common sources of food contamination which affects human health, as these foodstuffs are rarely heated or cooked prior to consumption. Using photosensitive food additives which are already approved for other applications – such as the yellow food coloring curcumin (E100) – we have demonstrated that we can effectively decontaminate these foodstuffs with a quick and easy light treatment."

Kristjan continued: "Characterizing the uptake of the photosensitizing agents into cells is obviously crucial to ensure only the target is affected, and this is where our Spark™ 10M reader comes in. All photosensitive molecules are also fluorescent, so we can track the intracellular concentration over time using fluorescence measurements. The ability to incubate culture plates within the instrument's environmentally-controlled measurement chamber is a real advantage for this work, and we have even done some preliminary studies with the new Te-Cool™ cooling module. This allows us to simulate the conditions micro-organisms experience outside of the human body – for example on hospital equipment – helping us to investigate how PDI could be used for surface decontamination."

"The environmental regulation offered by the system is even more beneficial for studying eukaryotic tumor cells. These cells are affected by photosensitizers in a similar way to bacterial cells, and the Spark allows us to study the *in situ* formation of photoactive molecules over time – up to 72 hours – under stable, controlled conditions comparable to a CO_2 incubator.[2] The reader's integrated dispenser is an even greater benefit, as it uniquely offers continuous heating and stirring of reagent flasks prior to injection. This allows us to use the module for automated seeding of cells into assay plates, ensuring homogeneity and maintaining the media at a constant temperature to provide an even distribution of cells. This removes the need for staff to sit and manually pipette cells into plates. It also means that we can move from a 96-well to a 384-well plate format, which would be impossible to achieve manually, as the light sensitivity of the chemicals we use means that all pipetting and plate handling needs to be performed under very low light conditions. This has effectively quadrupled the throughput of our investigations – an experiment that previously took 12 days to perform four batches can now be achieved in a single three-day study – which is vital for multi-parameter studies. It also saves reagents, lowering costs while producing the same results in a shorter timeframe."

To view a webinar on the role of laboratory automation in complex translational research, go to www.tecan.com/spark10mwebinar

To find out more about Tecan's Spark 10M reader, visit www.tecan.com/spark

To learn more about the University of Salzburg's Laboratory of Photodynamic Inactivation, go to www.uni-salzburg.at/pdi

1) Tortik, N *et al.* A comparative study on the antibacterial photodynamic efficiency of a curcumin derivate and a formulation on a porcine skin model. *Photochemical & Photobiological Sciences*, 2015, DOI: 10.1039/C5PP00393H.

2) Kiesslich, T *et al.* Real-time analysis of endogenous protoporphyrin IX fluorescence from δ-aminolevulinic acid and its derivatives reveals distinct time- and dose-dependent characteristics *in vitro*. *J Biomed Opt*, 2014, **19**(8), 085007.