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Making light work

Could light prove to be the ultimate weapon in the battle against deadly superbugs, asks Anthony King

A medical student working in Munich in 1900 made a chance discovery that may hold the key to fighting antibiotic resistance, one of the greatest challenges facing medicine today. Oscar Raab was studying the effects of dyes on the malarial protozoa when he realised that acridine solution is – in the presence of daylight – lethal to single-celled paramecia. It turns out that certain dyes, once illuminated, kill cells.

First used in extensive clinical trials in the 1980s, photodynamic therapy – or PDT – has harnessed the power of light to kill cancer cells in thousands of patients. PDT is also used to prevent the progression of age-related wet macular degeneration, a leading cause of blindness.

Researchers are increasingly pursuing PDT's potential against infectious diseases, with studies proving that the technique dispatches antibiotic resistant pathogens as quickly and efficiently as it kills their non-resistant brethren. PDT is a shining light of hope in the war against multi-antibiotic resistance superbugs, such as MRSA (methicillin-resistant *Staphylococcus aureus*).

PDT could also solve the riddle of how to maintain the effectiveness of antibiotics by avoiding overuse. The solution: to deploy PDT when it works best – against local infections in places in the body accessible to a light source – and save antibiotics for systemic infections.

Down to basics

When a photosensitiser is applied to an area of the body, in cream or ointment form for example, it is taken up by all cells non-specifically – but the bugs take it up within minutes whereas human cells take hours. Next, the photosensitiser molecules are excited by shining a specific wavelength of light on them. In their excited state, they transfer energy to any molecular oxygen present in the surrounding area, for example in tissues, generating reactive oxygen species (ROS) such as singlet oxygen and hydroxyl radicals. These ROS then damage and kill microbial cells.

Antibacterial use of PDT is probably 10 to 15 years behind its use in cancer, which kicked off in the 1980s, explains PDT expert Mark Wainwright from Liverpool John Moores University in the UK. He believes the success of antibiotics deflated early interest in PDT for infection control, at a time when MRSA was yet to rear its head. Today, in the era of the superbug, he says there are probably as many potential photosensitiser chemicals being looked at for infection treatment as there are new antibiotics under investigation.

The best photosensitisers for infection control are activated by red to far-red wavelengths of light, where light transmission through tissue is at its best. They tend to be organic molecules that are deeply coloured. The most popular candidate is the phenothiazinium salt methylene blue that has been used as a biological staining material for over 100 years. 'We know [methylene blue] is not toxic and we know the kind of doses people can withstand,' Wainwright explains.

Feeling blue

Another advocate of methylene blue is Michael Wilson from Eastman Dental Institute in University College London, UK. He is using this stain, and one of its derivatives toluidine blue, to target infections such as those found in wounds and burns, decaying teeth, and the tissues around the teeth (known as periodontitis). Patents from Wilson's lab have been licensed to a Canadian company – Ondine Biomedical – to treat periodontitis. More than 70,000 successful treatments have been carried out in Canada, he says.

Toluidine blue is not the only deriviative of methylene blue to find use as a photosensitiser. There is a whole family of derivatives with increasing methylation on the tricyclic molecular framework of the phenothiazinium dye. These include new methylene blue, methylmethylene blue and dimethylmethylene blue.

One of Wainwright's areas of interest is the potential use of PDT in the management of acne, normally treated with topical antibiotics. Trials have shown that a pentacyclic methylene blue derivative – that localises to the hair follicle, sebaceous gland and associated structures – could be a fruitful way to manage the condition.[1]

Think positive

In the 1990s, a fundamental difference in susceptibility to PDT was discovered between gram negative and gram positive bacteria. Gram positives often succumbed regardless of what the charge was on the photosensitiser molecule, but gram negative bacteria such as *Pseudomonas* and *Escherichia coli* were vulnerable only to cationic molecules (or by using alternative strategies to dissolve their protective barrier).

Cationic charges help because microbial cells have more anionic charges than mammalian cells, and therefore these positively-charged photosensitisers exhibit selectivity for microbes.

'It is fair to say there are no microorganisms that cannot be killed by PDT. It is a relatively non-specific formation of reactive oxidant species which, by and large, will kill anything. The way to optimise is to target the dye to the species you want to kill,' explains Michael Hamblin from Harvard Medical School in Boston, US.

These 1990s studies were another thumbs up for the methylene blue family, that have a single cationic charge delocalised over their tricyclic structures. But that's not to say they can't be improved upon. 'Most of the clinical studies around the world have used methylene blue or

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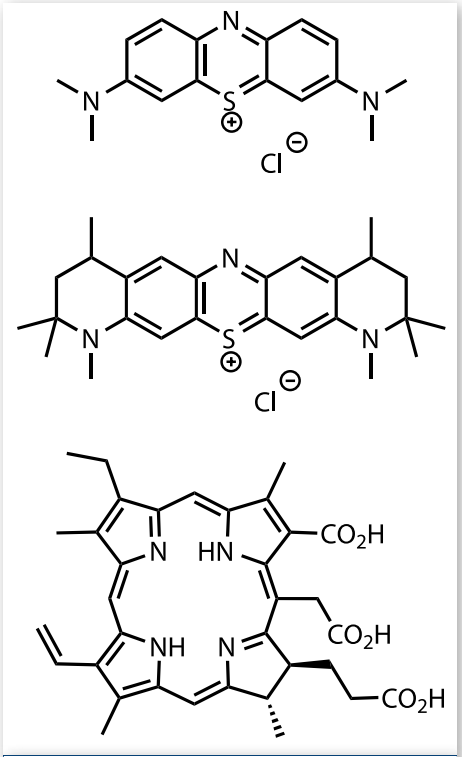


METHYLENE BLUE, A PENTACYCLIC DERIVATIVE AND CHLORIN E6

toluidine blue because they are approved for human use,' says Hamblin. 'But we believe it is relatively easy to get a photosensitiser that is 1000 times better.'

Hamblin's team has worked on a series of photosensitisers made from chlorin e6 and the polymer polyethylenimine. These cationic polymers can disrupt the bacterial permeability barriers – meaning they are even more effective.

They have also studied both bacteriochlorins and fullerenes with cationic substituents. 'The idea is you put a certain number of quaternary ammonium groups onto a standard photosensitiser [designed for use in cancer treatment] and it becomes an antimicrobial photosensitiser,' Hamblin explains.

Let there be light

Hamblin's team has also pioneered laboratory models for real time monitoring of PDT. His procedure uses genetically engineered bioluminescent bacteria, applied to wounds on the backs of mice. The bacteria are then allowed to multiply. Next, a photosensitiser is applied, followed by a light source. The number of infected bioluminescent cells is quantified before and after the PDT process.[2]

Hamblin was the first to investigate the effect of PDT on treating wounds infected by *Pseudomonas aeruginosa*. For this species of bacteria, he found that 90% of the PDT-treated mice survived, with around 98% of the bacteria killed.[3] '*Pseudomonas* is a very virulent pathogen that can kill a mouse with a few thousand cells in an infected wound or burn,' explains George Tegos, a biologist at the University of New Mexico, US, who has collaborated with Hamblin. 'By applying PDT with many different combinations [of sensitisers, mostly methylene blue derivatives], we were able to save the mice.'

'It's possible that PDT can be used in combination with existing antibiotics'

Burns are highly susceptible to infection, and large burns that occupy a high percentage of the body are often fatal owing to infectious complications. The microbial species responsible include the ubiquitous pathogens *P. aeruginosa* and *S. aureus*, yeast *Candida* and filamentous fungi.

For Hamblin's mouse model, third degree burns covering around 5% of the body surface area were induced and then infected with bioluminescent *S. aureus*. Using a cationic porphyrin photosensitiser – both topically and injected under the burn – more than 98% of the bacteria were eradicated after a dose of light.[4]

Extra level of complexity

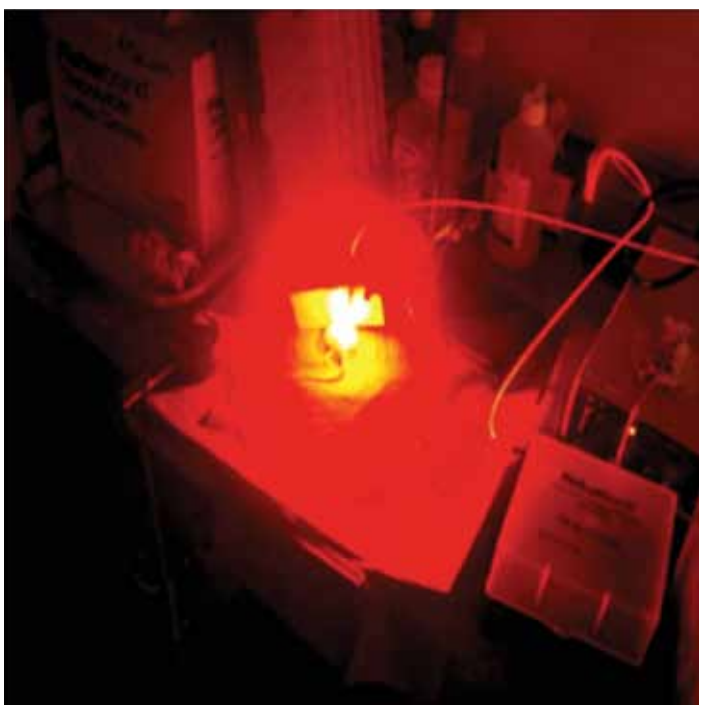
Though he describes it as a little speculative, Hamblin notes that some investigators are looking at PDT for latent tuberculosis, hidden in granulomas in the lungs. Extreme drug resistant TB is almost incurable, he says. 'The theory goes that you inject photosensitiser into the granuloma, probably by putting a needle through the chest wall. Then you put a fibre through the chest wall using the same track and deliver light.' This concept was developed for a benzoporphyrin molecule.

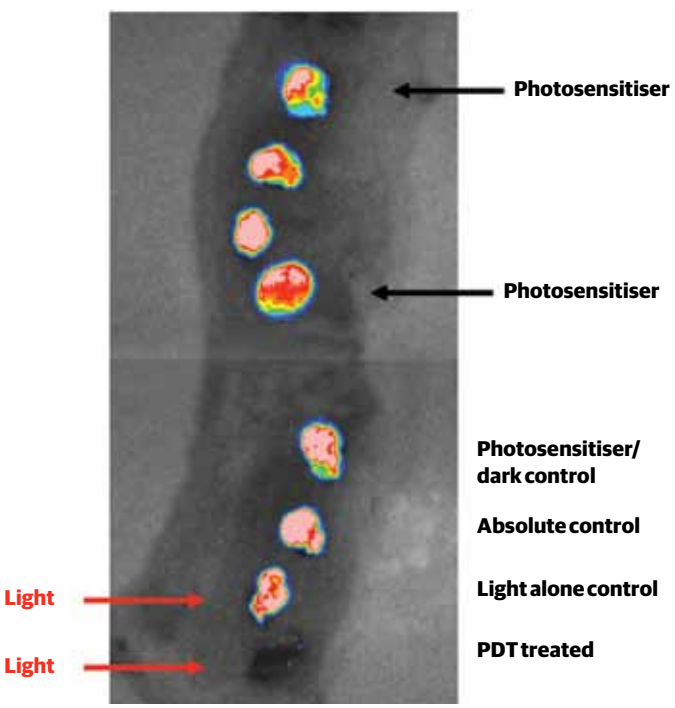
Tegos says he reads around 25 to 30 papers each year describing new photosensitiser drugs. 'People have an active interest in the chemistry of these molecules,' he concludes. His own lab is using methylene blue derivatives, porphyrins and cationic fullerenes.

Tegos's lab has also tested a combination of cationic fullerenes and a fluoroquinone antibiotic. 'We used a suboptimal dose of the antibiotic with the PDT and this worked much better than the antibiotic alone, so there is a possibility that PDT can be used for therapeutic purposes in combination with antibiotics,' says Tegos.

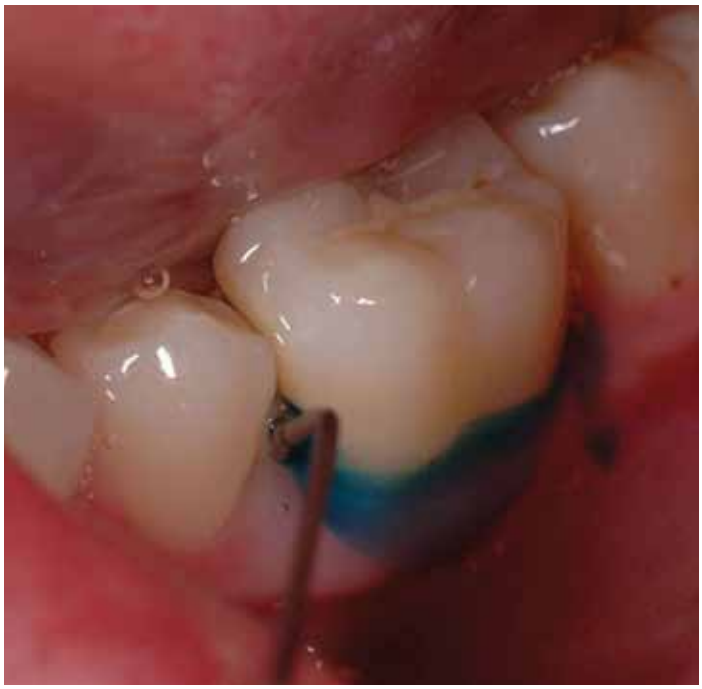
Most of the PDT for infection control work done so far has been based on topical application – where the photosensitiser is applied exactly where you want it – or injection of the chemicals into the right spot in the body. But a recently reported colon PDT photosensitiser is swallowed. The photosensitiser is protected from the stomach's acidity by a polymer system that is designed to dissolve away in the higher pH environment of the colon.[5]

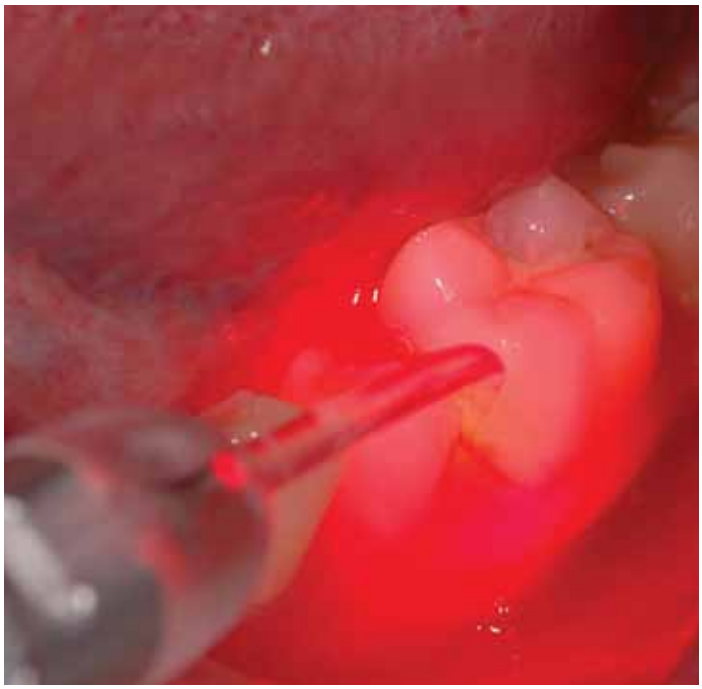
Ryan Donnelly, part of the research team at Queen's University Belfast, UK, explains: 'The idea here would be that you could administer your photosensitiser orally in a tablet but it would only be released in the colon where it is needed. And then the *Clostridium difficile* infection could be treated by using a suitable flexible endoscope to apply the light.' The research is still at the laboratory stage, he notes, and will require significant extra funding to translate into clinical use.





Wounds harbour bacteria and are ideal candidates for PDT. Here, only the combination of light and photosensitiser works; neither is effective alone





Big pharma says no thanks

And here he has hit the nail on the head: one of the big issues facing this field at the moment is funding. Big pharma, in particular, has not yet been persuaded to invest.

Instead, small and medium enterprises have taken up the PDT baton. One such company is the Leeds, UK-based Photopharmica. This firm is currently developing a toluidine blue derivative for treating infections in leg ulcers. In October 2011, the company announced positive results from a Phase IIb clinical trial. Photopharmica also has ongoing trials for treatment of acute surgical wounds, acne and fungal infections.

Meanwhile, Canadian firm Ondine Biomedical is developing a PDT to eradicate bacterial pathogens from the nasal passages. The company's photosensitiser formulation is gently applied to the nasal openings prior to illumination. Those of us colonised with MRSA tend to have large quantities of this bacteria in our noses. The idea is to hit the MRSA in their stronghold prior to surgery; by knocking the resistant bacteria back for a few days before and after surgery, it is hoped to reduce the risk of deadly MRSA infection.

Tegos reckons big pharma has had difficulty swallowing the idea of a pill plus something else, the latter being the need to deliver light to wherever the photosensitiser has been applied. Hamblin is similarly downbeat on the prospect of big pharma riding in, all guns (or lamps) blazing. They have shown no interest in PDT for the last 50 years, whether for antimicrobial or cancer treatment, he says.

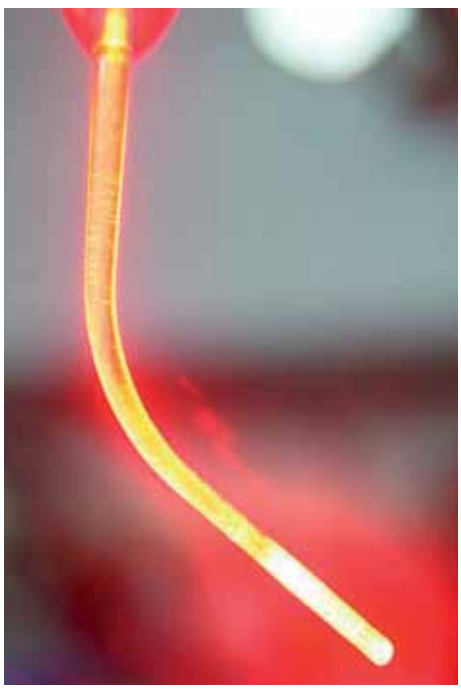
'They would much rather just make a drug, because they are used to doing that. And medical device companies would like to make a device and know nothing about drugs. So drug-device combinations kind of confuse people,' Hamblin explains.

'Big pharma has shown no interest in PDT for the last 50 years. They would rather just make a drug'

RSC president David Phillips has worked in the PDT arena for over 25 years. He was involved in the spin-out of the company Photobiotics from Imperial College London. Photobiotics focuses on using PDT for cancer treatment, and uses monoclonal antibody fragments attached to sensitisers to improve targeting in the body. The company is also currently seeking commercial funding for trials.

Phillips says he is hopeful that 'at last the UK government is recognising that PDT has a role in [medicine] and so there is some emphasis on it'. Another challenge, he notes, is persuading the community of medics that PDT is a viable option. This is a frequent refrain in the PDT community. Medics want to stick to the tried and tested pill, but PDT is making itself heard nonetheless. The more clinical applications get approved, the stronger its case. PDT could be stocked in your local pharmacy yet.

Anthony King is a science writer based in Dublin, Ireland



Ondine Biomedical is developing PDT methods for treating periodontitis and tooth decay

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