The Chinese University of Hong Kong, Faculty of Medicine, Department of Microbiology
Joint Graduate Student Seminar

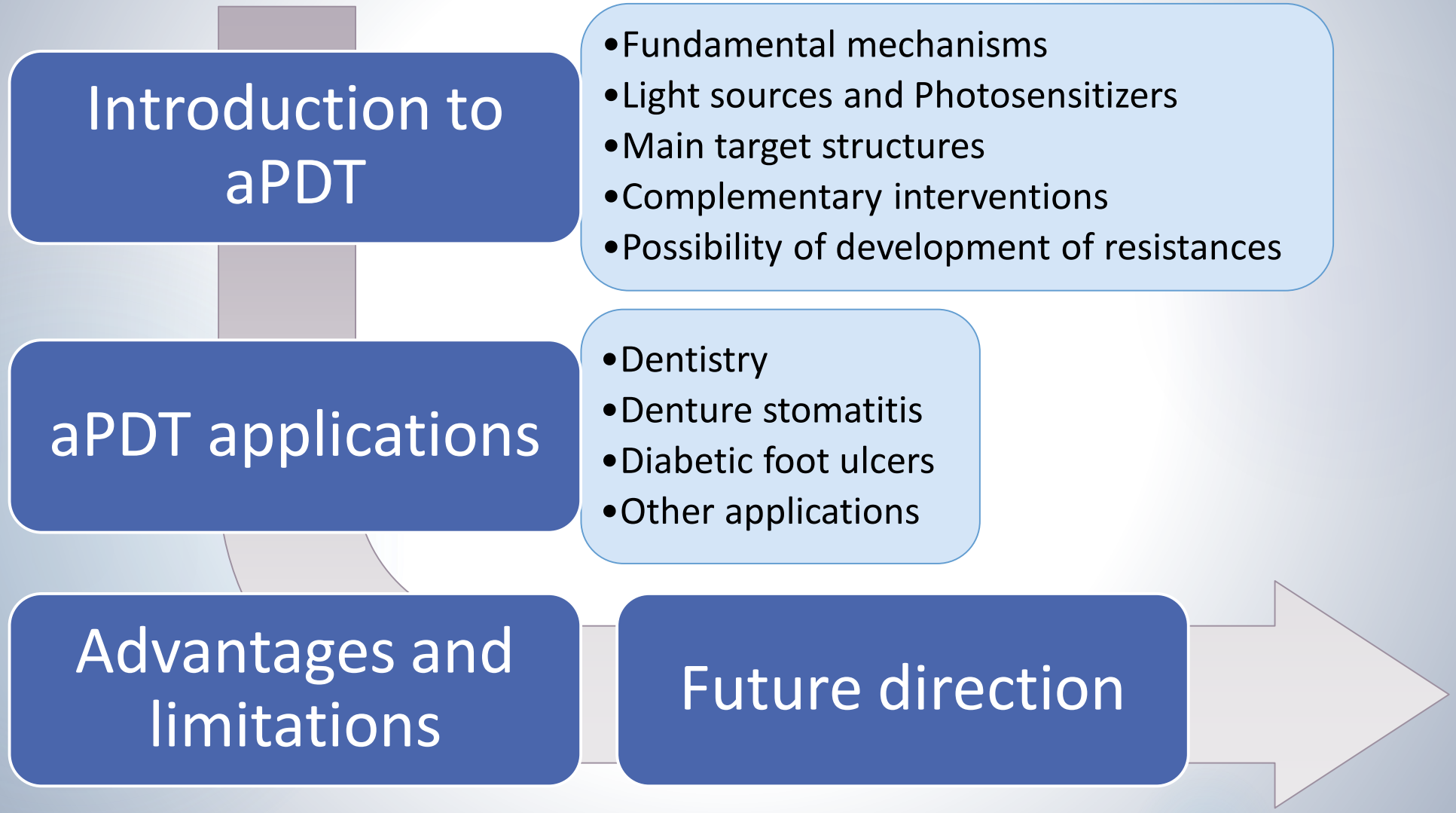
Cleansing light: Antimicrobial photodynamic therapy (aPDT)

Supervisor: Prof. Mamie Hui

Student: Poon Yeuk Lan, Nana (PhD student, Yr4)

Date: 14th December 2018

Outline


Introduction to aPDT
•Fundamental mechanisms
•Light sources and Photosensitizers
•Main target structures
•Complementary interventions
•Possibility of development of resistances
aPDT applications
•Dentistry
•Denture stomatitis
•Diabetic foot ulcers
•Other applications
Advantages and limitations
Future direction

Fundamental mechanisms of PDT

- 3 components

Photosensitizer (PS)

- *per* se non-toxic

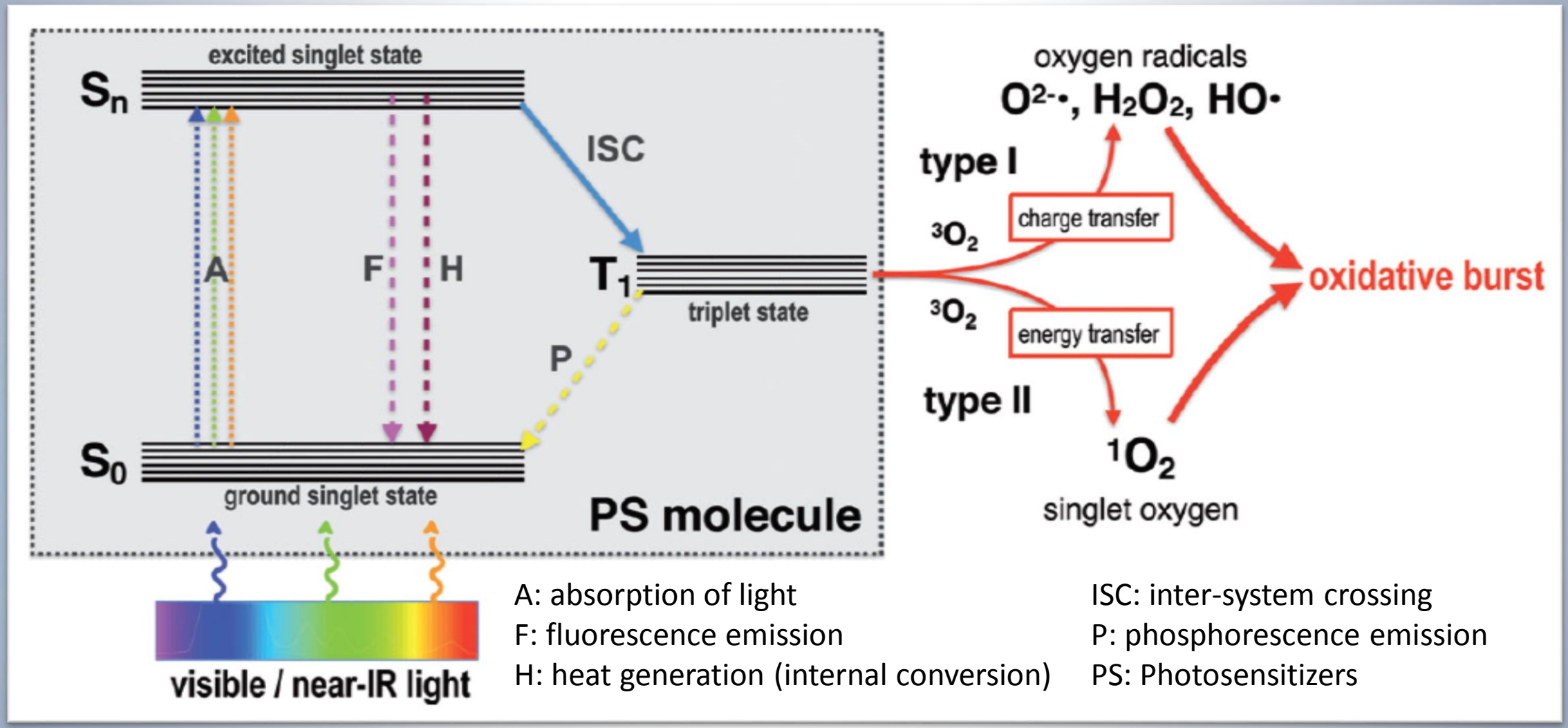
Visible light

- Of specific wavelength that excite PS
- Sources: Lasers, Light-emitting diodes (LEDs), and Halogen lamps

Molecular oxygen (O_2)

(Cieplik et al., 2018)

Fundamental mechanisms of PDT



A: absorption of light
F: fluorescence emission
H: heat generation (internal conversion)

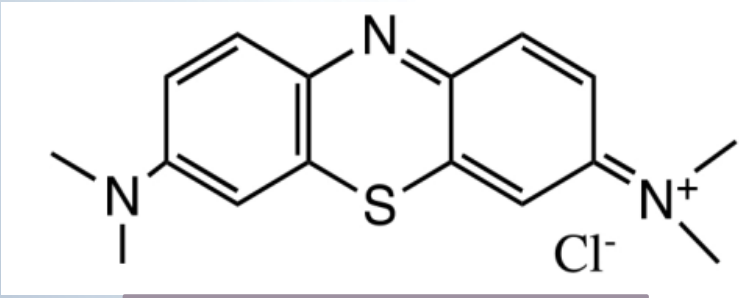
ISC: inter-system crossing
P: phosphorescence emission
PS: Photosensitizers

(Figure: Cieplik et al., 2018)

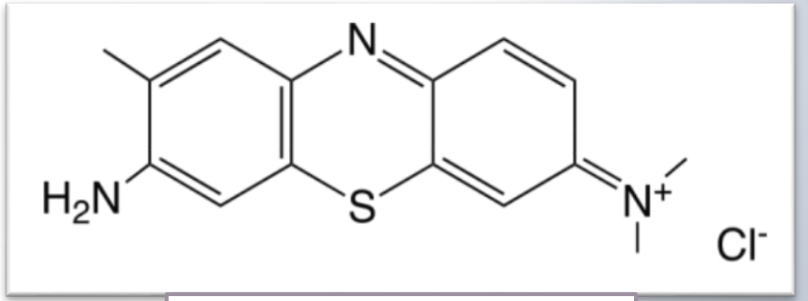
Photosensitizers of aPDT

Phenothiazinium derivatives

- Three-ring π-system with auxochromic side groups
- Mainly Type I mechanism
- Absorption in red spectrum (600-680nm)
- Two PS were clinically approved for aPDT:



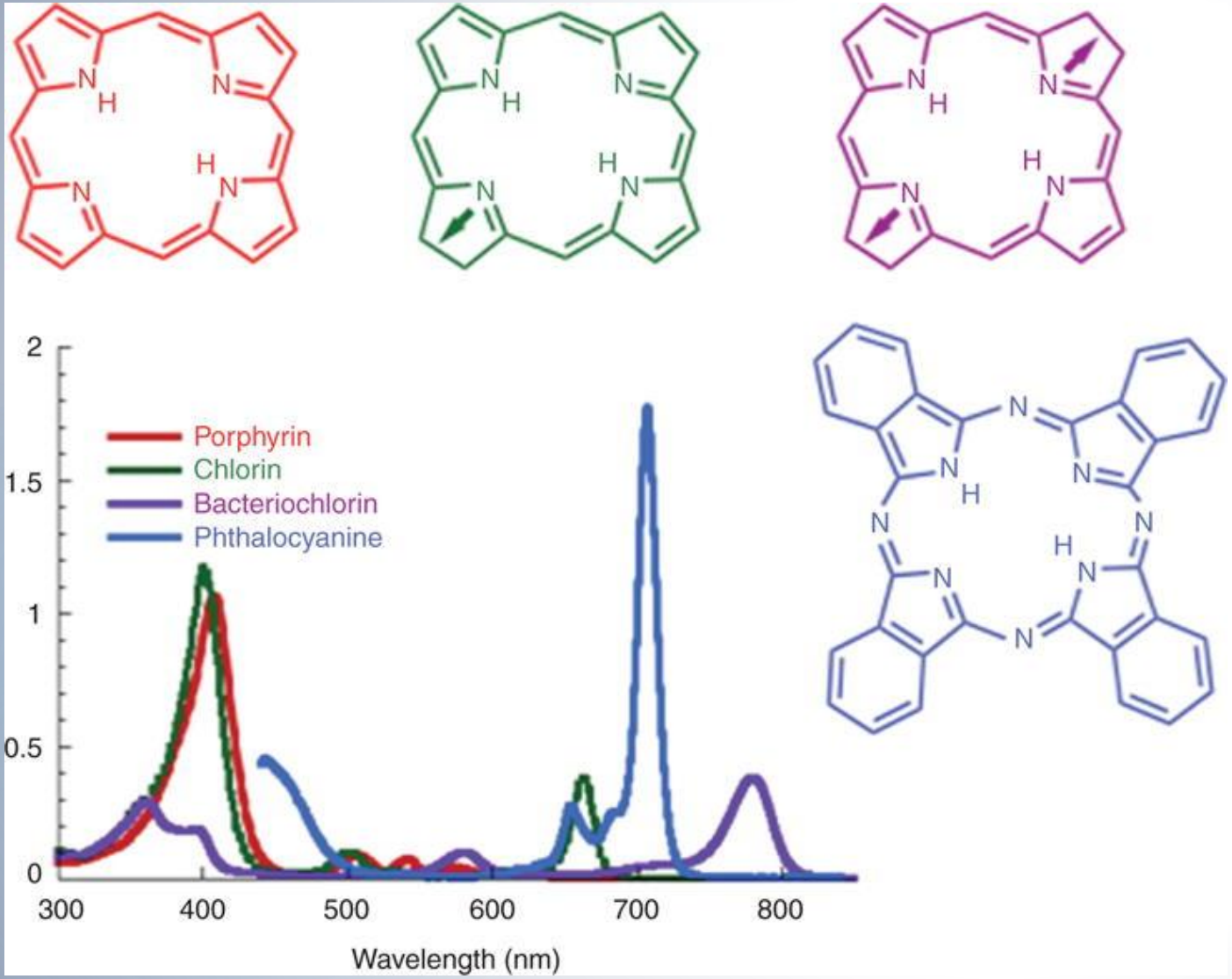
Methylene Blue



Toluidine Blue

(Cieplik et al., 2018; Hu et al., 2018)

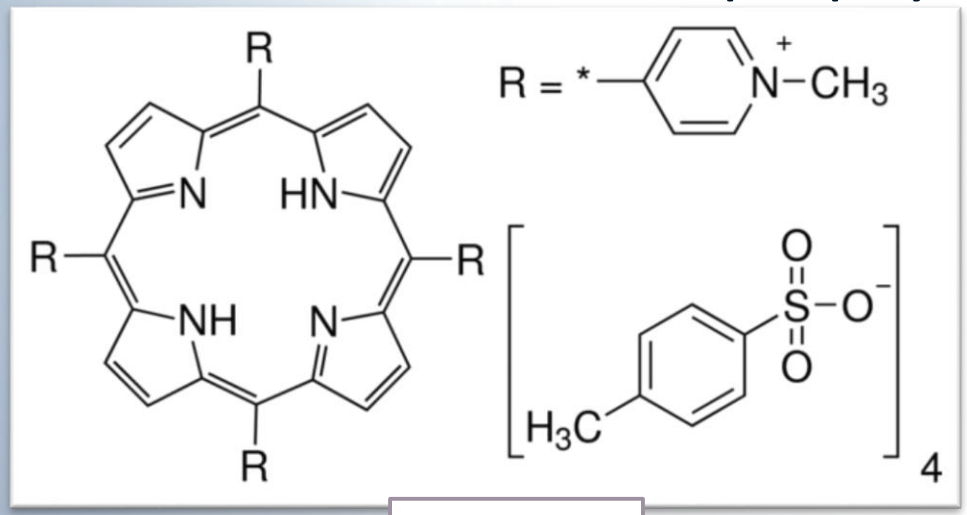
Porphyrins, chlorins, and phthalocyanines



- Mainly Type II mechanism
- Absorption:
  - Porphyrins & chlorins: Around 405 nm
  - Phthalocyanines: Around 700 nm

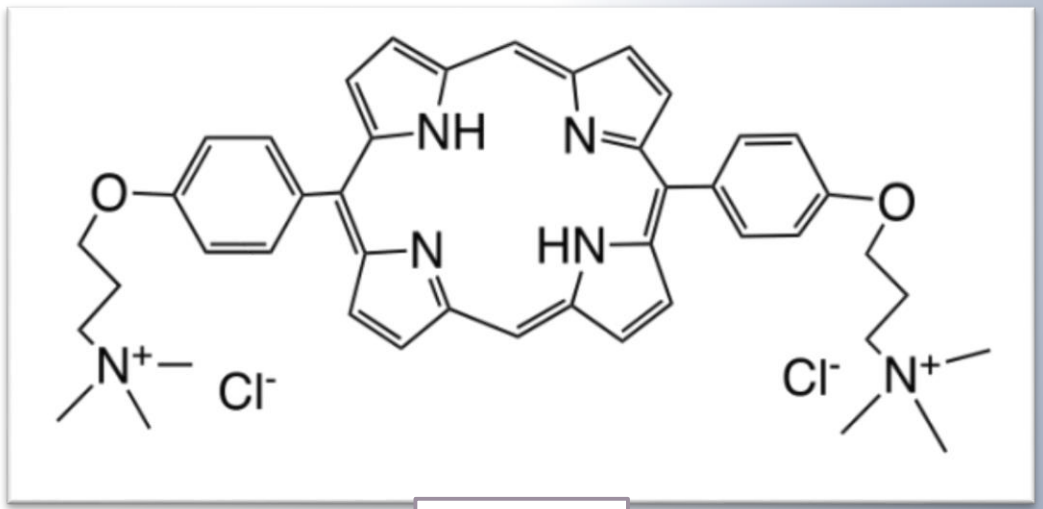
(Kashef et al., 2017)

- Porphyrins
  - Endogenous produced by bacteria
    - *Helicobacter pylori* (Hamblin et al., 2005)
    - *Aggregatibacter actinomycetemcomitans* (Cieplik et al., 2014)
    - *Prevotella* and *Porphyromonas* species (Pummer et al., 2017)
  - Chemical modified porphyrins



(Picture: Sigma-Aldrich)

TMPyP



XF-73

(Picture: Hu et al., 2018)

- Chlorins
  - Mainly cationic derivatives of chlorin-*e*6
  - e.g. Photodithazine® produced by VETA-GRAND Company
- Phthalocyanines
  - Hydrophobic and uncharged → Low solubility
  - Solubilization could be accomplished by
    - Nano-emulsion
    - Modification of structure

Other PS classes

Classes	Examples	Absorption range	Mechanism
Xanthene	Eosin Y, Erythrosine, Rose Bengal	≈ 480 to 550 nm	Type II
Fullerenes	C60	UV and visible range	Type I or II*
Cationic water-soluble Phenalenone derivatives	SAPYR	≈ 320 to 430 nm	Type II
Riboflavin	--	≈ 350 to 470 nm	Type II
Curcumin	--	≈ 300 to 500 nm	Type I

*depending on modifications and solvents

(Cieplik et al., 2018)

Examples of microorganisms inhibited by aPDT

Classes	Microorganisms	Reviewed by
Viruses	Vesicular stomatitis virus, Herpes simplex virus, Suid herpes virus, Hepatitis C virus, Human immunodeficiency virus, Adenovirus, Dengue virus etc.	Costa et al., 2012
Fungi	*Candida* spp., *Malassezia* app., *Aspergillus fumigatus*	Lyon et al., 2011
Bacteria	*Escherichia coli*, *Staphylococcus aureus* (including MSSA and MRSA), *Pseudomonas aeruginosa*, *Enterococcus faecalis* etc.	Liu et al., 2015

Putative main target structures of aPDT

- Properties of oxidative damage in aPDT
  - Non-selective, wide range of targets
  - Located in close vicinity to the PS molecules
- Targets depend on the locations of PS molecules

Location of PS	Possible targets	Consequences
Inside biofilm	Extracellular polymeric substances (EPSs), lipids	Disruption of biofilm
Binds to cell walls	Cell wall, cytoplasmic membrane	↑Membrane permeability, ↑PS uptake
Inside the cell	Cytoplasmic proteins and DNA	Inhibition of metabolism and cell growth

(Cieplik et al., 2018)

Putative main target structures of aPDT

- Reported location of PS
  - Phenothiazinium classes: Intracellular
    - Addition of efflux pump inhibitors enhanced inactivation efficacy of MB and TBO (Tegos et al. 2008)
  - TMPyP (Porphyrin derivative): Extracellular
    - No uptake in *Escherichia coli* (Preuß et al. 2013)
    - Minor accumulation in cytoplasm of *Staphylococcus aureus* (Gollmer et al. 2017)

Complementary interventions to potentiate aPDT efficacy

- Addition of inorganic salts, e.g. potassium iodide
  - Increase killing by up to 6 $\log_{10}$ (Hamblin, 2017)
- Addition of efflux pump inhibitors (Tegos et al., 2008; Kishen et al., 2010)
- Addition of saponins
  - Increase cell permeability and thus facilitate penetration of PS
  - Increased susceptibility to photodynamic inactivation of *Candida albicans* (Coleman et al., 2010)
- Conjugation of PS with chitosan
  - Increased uptake of PS in biofilm and increased biofilm disruption (Shrestha and Kishen, 2012)

Possibility of development of resistances to aPDT

- Oxidative damage of aPDT is non-selective and multi-targeted
- Studies had shown incapability of bacteria to develop resistance after continual exposure to aPDT
  (Giuliani et al., 2010; Lauro et al., 2002; Pedigo et al., 2009; Tavares et al., 2010)
- Highly improbable for microbes to develop resistances and tolerance against aPDT (Kashef and Hamblin, 2017; Maisch, 2015; Wainwright et al., 2017)
- More evidences are required

aPDT applications

- Dentistry
- Denture stomatitis
- Diabetic foot ulcers
- Other applications

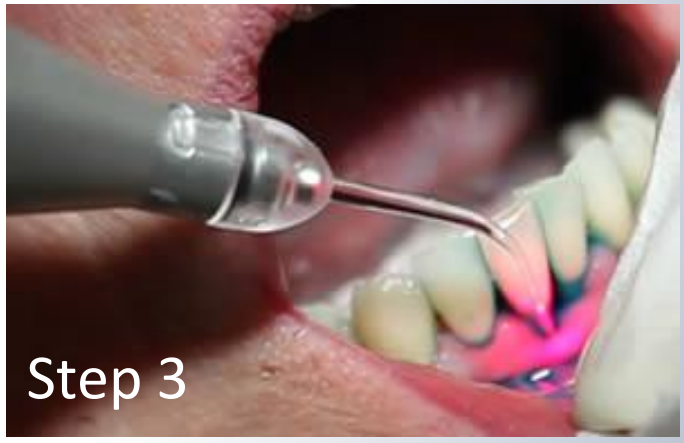
aPDT application in dentistry

- Two approved aPDT systems: Periowave™ & HELBO®
- PS: Methylene blue
- Light source: Laser (650-675nm)
- Used for disinfection in
  - Periodontitis
  - Gingivitis
  - Endodontics
  - Peri-implantitis disease

(Picture: Periowave™)

aPDT application in dentistry

- Brief procedures for periodontitis:
  1. Scaling and root planning
  2. Applying PS into root pocket
  3. Illumination
- Outcome reported in clinical trials:
  Reduction of bacterial load,
  pocket depth and bleeding on probing



(Pictures: Periowave™)

aPDT application on denture stomatitis

- Clinical trial by Mima et al. (2012)
  - PS: PHOTOGEM® (hematoporphyrin derivative)
  - Light source: LED light at 455nm
- Results

Treatment	aPDT (3 sessions weekly for 15 days)	Nystatin (4 times daily for 15 days)
No. of patients	20	20
Clinical success* rates	45%	53%

*absent or lower degree of inflammation at the end of treatment

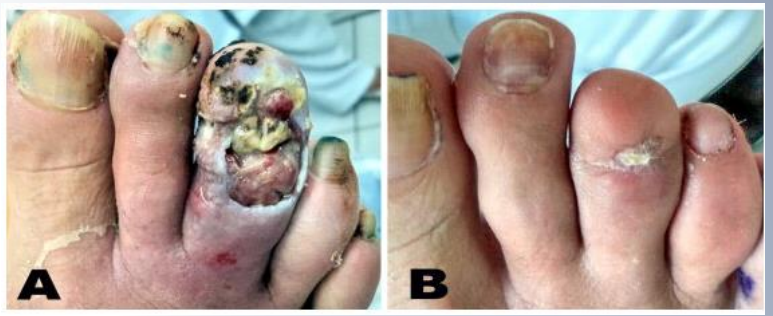
- Conclusion
  - aPDT was as effective as topical nystatin

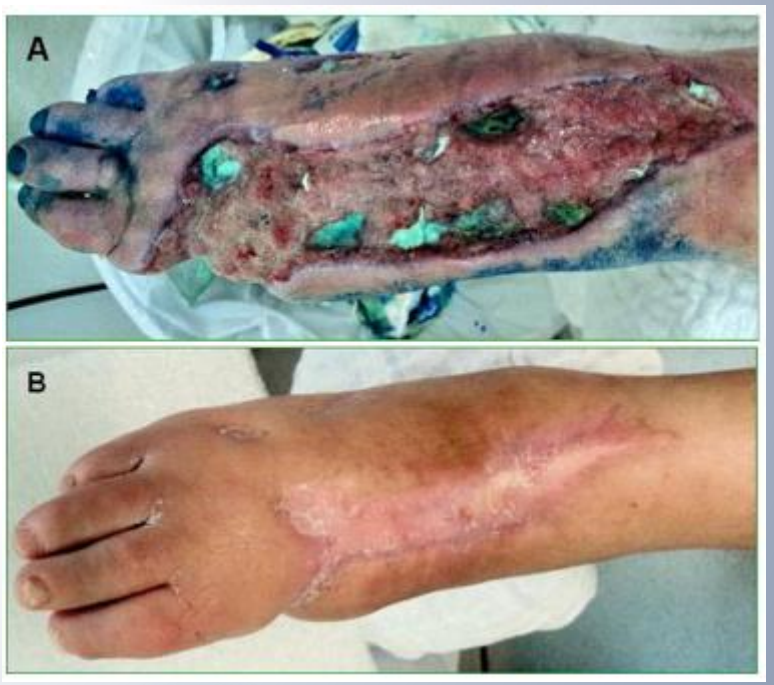
aPDT application on diabetic foot ulcers

- Clinical trial by Tardivo et al. (2014)
  - PS: 1:1 solution of Methylene blue and O-toluidine blue
  - Light sources:
    1. LED (640nm) for whole infected tissue
    2. Halogen light source (400nm to 725nm) with optical fibers for foot bones through fistula
  - Study design:
    - All patients presented with ulcers of Wagner Grade 3 (deep ulcer with abscess or osteomyelitis)
    - 16 patients in control group (Systemic antibiotics + debridement)
      18 patients in treatment group (Systemic antibiotics + aPDT)
    - aPDT treatment was performed twice a week

aPDT application on diabetic foot ulcers

- Clinical trial by Tardivo et al. (2014)
  - Outcome
    - Control group: All patients resulted in amputation
    - Treatment group
      - 17 out of 18 patients were considered cured (Wagner Grade 0, intact skin)
      - Accelerated healing of the fistulas and tissue reconstruction
      - 2 cured patients were previously infected by multi-resistant *Pseudomonas aeruginosa* and carbapenemase-producing *Klebsiella pneumoniae*





A. Pre-treatment
B. Post-treatment

Other possible applications

- Summarized in the review by Hu et al. (2018)

Preclinical animal studies	Clinical trials and patient studies
• Burn injury • Osteomyelitis • Nasal infection • Superficial fungal skin infection • Otitis media	• Nasal Decontamination • *H. pylori* infection

Advantages of aPDT over antibiotic treatment

- Broad spectrum
  - Effective against bacteria, fungi, and viruses
  - Effective against antimicrobial resistant microorganisms
- Unlikely for microorganisms to develop resistance against aPDT
- Light-activated action
  - Immediate reduction of pathogen load
  - Reactive oxygen species production ceases without light
- Less adverse effects
  - Less damage to host cells and tissue
  - No reported allergy
  - Less disruption to microbiome

Limitations of aPDT

- Limited to localized infections at easily accessible parts
  - Oxygen level
  - Illumination
- Lower efficacy when used against biofilm
  - EPS hinder the diffusion of PS

Future directions

- Understanding of the mechanism of action of different classes of PS
  - To apply complementary interventions to increase efficacy
  - To predict potential for development of resistances
- *In vitro* testing on biofilm, preferably on clinical specimens
- Translation into clinical practice
  - Dose and regimen
  - Cost

(Cieplik et al., 2018; Hu et al., 2018)

Take home messages

- Principle of antimicrobial photodynamic therapy (aPDT)
  1. Photosensitizers are excited by light
  2. Reactive oxygen species (ROS) are generated
  3. ROS cause oxidative damage and kill nearby microorganisms
- aPDT is broad-spectrum and
  effective even against antimicrobial resistant microorganisms
- aPDT was already being applied in dentistry for years
- Future applications of aPDT include tropical and oral infection,
  nasal decontamination, and *H. pylori* infection

Q&A

References

- Alves, F., Alonso, G.C., Carmello, J.C., Mima, E.G. de O., Bagnato, V.S., and Pavarina, A.C. (2018). Antimicrobial Photodynamic Therapy mediated by Photodithazine® in the treatment of denture stomatitis: A case report. Photodiagnosis Photodyn. Ther. *21*, 168–171.
- Cieplik, F., Späth, A., Leibl, C., Gollmer, A., Regensburger, J., Tabenski, L., Hiller, K.-A., Maisch, T., and Schmalz, G. (2014). Blue light kills Aggregatibacter actinomycetemcomitans due to its endogenous photosensitizers. Clin. Oral Investig. *18*, 1763–1769.
- Cieplik, F., Deng, D., Crielaard, W., Buchalla, W., Hellwig, E., Al-Ahmad, A., and Maisch, T. (2018). Antimicrobial photodynamic therapy – what we know and what we don't. Crit. Rev. Microbiol. *44*, 571–589.
- Coleman, J.J., Okoli, I., Tegos, G.P., Holson, E.B., Wagner, F.F., Hamblin, M.R., and Mylonakis, E. (2010). Characterization of plant-derived saponin natural products against Candida albicans. ACS Chem. Biol. *5*, 321–332.
- Costa, L., Faustino, M.A.F., Neves, M.G.P.M.S., Cunha, Â., and Almeida, A. (2012). Photodynamic Inactivation of Mammalian Viruses and Bacteriophages. Viruses *4*, 1034–1074.
- Giuliani, F., Martinelli, M., Cocchi, A., Arbia, D., Fantetti, L., and Roncucci, G. (2010). In vitro resistance selection studies of RLP068/Cl, a new Zn(II) phthalocyanine suitable for antimicrobial photodynamic therapy. Antimicrob. Agents Chemother. *54*, 637–642.
- Gollmer, A., Felgentraeger, A., Maisch, T., and Flors, C. (2017). Real-time imaging of photodynamic action in bacteria. J. Biophotonics *10*, 264–270.
- Hamblin, M.R. (2017). Potentiation of antimicrobial photodynamic inactivation by inorganic salts. Expert Rev. Anti Infect. Ther. *15*, 1059–1069.

References

- Hamblin, M.R., Viveiros, J., Yang, C., Ahmadi, A., Ganz, R.A., and Tolkoff, M.J. (2005). Helicobacter pylori Accumulates Photoactive Porphyrins and Is Killed by Visible Light. Antimicrob. Agents Chemother. *49*, 2822–2827.
- Hu, X., Huang, Y.-Y., Wang, Y., Wang, X., and Hamblin, M.R. (2018). Antimicrobial Photodynamic Therapy to Control Clinically Relevant Biofilm Infections. Front. Microbiol. *9*.
- Kashef, N., and Hamblin, M.R. (2017). Can microbial cells develop resistance to oxidative stress in antimicrobial photodynamic inactivation? Drug Resist. Updat. *31*, 31–42.
- Kashef, N., Huang, Y.-Y., and Hamblin, M.R. (2017). Advances in antimicrobial photodynamic inactivation at the nanoscale. Nanophotonics *6*, 853–879.
- Kishen, A., Upadya, M., Tegos, G.P., and Hamblin, M.R. (2010). Efflux Pump Inhibitor Potentiates Antimicrobial Photodynamic Inactivation of Enterococcus faecalis Biofilm. Photochem. Photobiol. *86*, 1343–1349.
- Lauro, F.M., Pretto, P., Covolo, L., Jori, G., and Bertoloni, G. (2002). Photoinactivation of bacterial strains involved in periodontal diseases sensitized by porphycene–polylysine conjugates. Photochem. Photobiol. Sci. *1*, 468–470.
- Liu, Y., Qin, R., Zaat, S.A.J., Breukink, E., and Heger, M. (2015). Antibacterial photodynamic therapy: overview of a promising approach to fight antibiotic-resistant bacterial infections. J. Clin. Transl. Res. *1*, 140–167.
- Lyon, J.P., Moreira, L.M., Moraes, P.C.G. de, Santos, F.V. dos, and Resende, M.A. de (2011). Photodynamic therapy for pathogenic fungi. Mycoses *54*, e265–e271.
- Maisch, T. (2015). Resistance in antimicrobial photodynamic inactivation of bacteria. Photochem. Photobiol. Sci. *14*, 1518–1526.
- Mima, E.G., Vergani, C.E., Machado, A.L., Massucato, E.M.S., Colombo, A.L., Bagnato, V.S., and Pavarina, A.C. (2012). Comparison of Photodynamic Therapy versus conventional antifungal therapy for the treatment of denture stomatitis: a randomized clinical trial. Clin. Microbiol. Infect. *18*, E380–E388.

References

- Pedigo, L.A., Gibbs, A.J., Scott, R.J., and Street, C.N. (2009). Absence of bacterial resistance following repeat exposure to photodynamic therapy. In Photodynamic Therapy: Back to the Future, (International Society for Optics and Photonics), p. 73803H.
- Preuß, A., Zeugner, L., Hackbarth, S., Faustino, M. a. F., Neves, M.G.P.M.S., Cavaleiro, J. a. S., and Roeder, B. (2013). Photoinactivation of Escherichia coli (SURE2) without intracellular uptake of the photosensitizer. J. Appl. Microbiol. *114*, 36–43.
- Pummer, A., Knüttel, H., Hiller, K.-A., Buchalla, W., Cieplik, F., and Maisch, T. (2017). Antimicrobial efficacy of irradiation with visible light on oral bacteria in vitro: a systematic review. Future Med. Chem. *9*, 1557–1574.
- Shrestha, A., and Kishen, A. (2012). Polycationic Chitosan-Conjugated Photosensitizer for Antibacterial Photodynamic Therapy†. Photochem. Photobiol. *88*, 577–583.
- Tardivo, J.P., Adami, F., Correa, J.A., Pinhal, M.A.S., and Baptista, M.S. (2014). A clinical trial testing the efficacy of PDT in preventing amputation in diabetic patients. Photodiagnosis Photodyn. Ther. *11*, 342–350.
- Tavares, A., Carvalho, C.M.B., Faustino, M.A., Neves, M.G.P.M.S., Tomé, J.P.C., Tomé, A.C., Cavaleiro, J.A.S., Cunha, Â., Gomes, N.C.M., Alves, E., et al. (2010). Antimicrobial Photodynamic Therapy: Study of Bacterial Recovery Viability and Potential Development of Resistance after Treatment. Mar. Drugs *8*, 91–105.
- Tegos, G.P., Masago, K., Aziz, F., Higginbotham, A., Stermitz, F.R., and Hamblin, M.R. (2008). Inhibitors of Bacterial Multidrug Efflux Pumps Potentiate Antimicrobial Photoinactivation. Antimicrob. Agents Chemother. *52*, 3202–3209.
- Wainwright, M., Maisch, T., Nonell, S., Plaetzer, K., Almeida, A., Tegos, G.P., and Hamblin, M.R. (2017). Photoantimicrobials—are we afraid of the light? Lancet Infect. Dis. *17*, e49–e55.