Toluidine Blue-Mediated Photodynamic Effects on Staphylococcal Biofilms

Mrinalini Sharma, Livia Visai, Francesca Bragheri, Haria Cristiani, Pradeep Kumar Gupta, and Pietro Speziale

Antimicrobial Agents And Chemotherapy, 52(1): 299-305, 2008

Speaker: I-Lun Chiang

Adviser: Ching-Tsan Huang, PhD

Date: 2008.12.9

Biofilms are everywhere!

Biofilm on rock

Biofilm on pipe

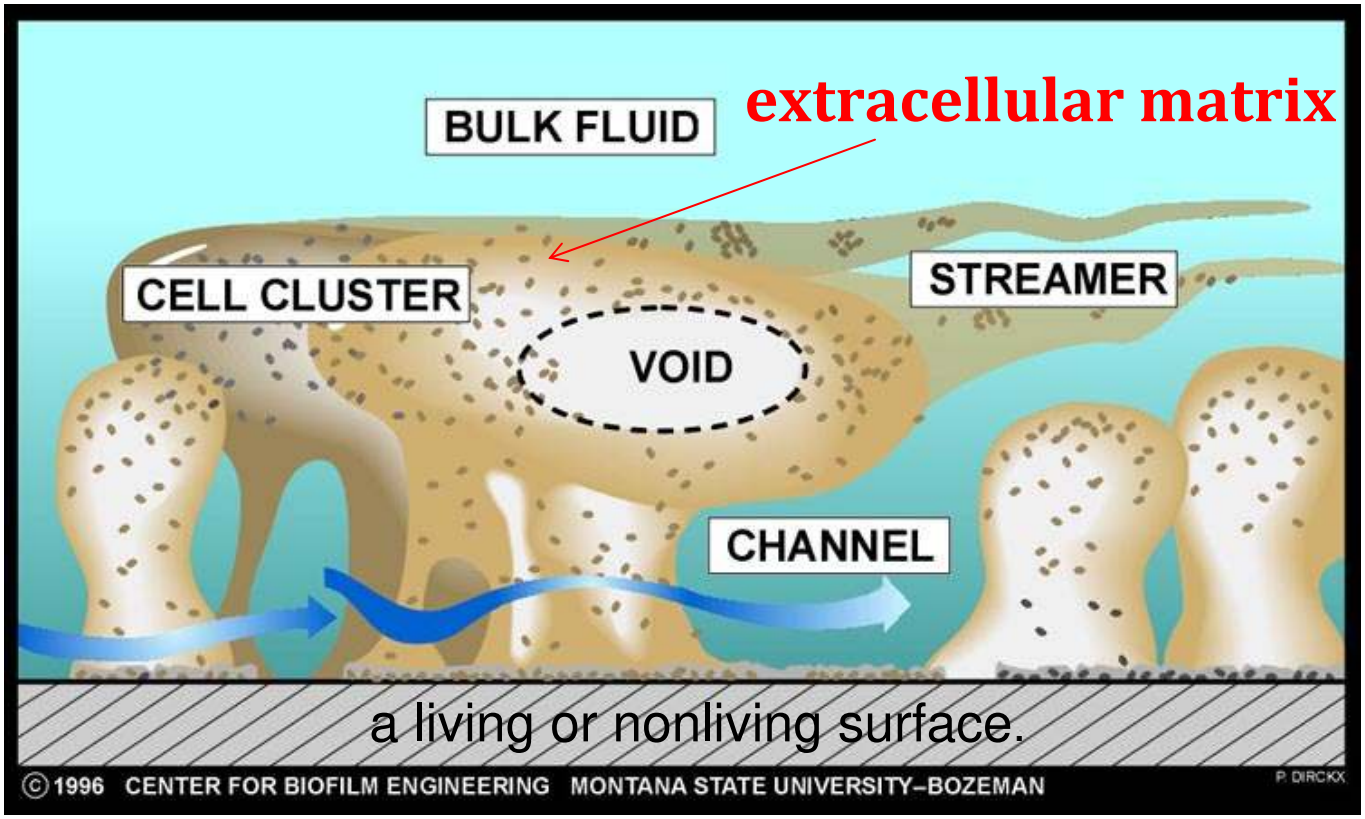
Biofilm in toilet

Had you ever slipped on a rock?

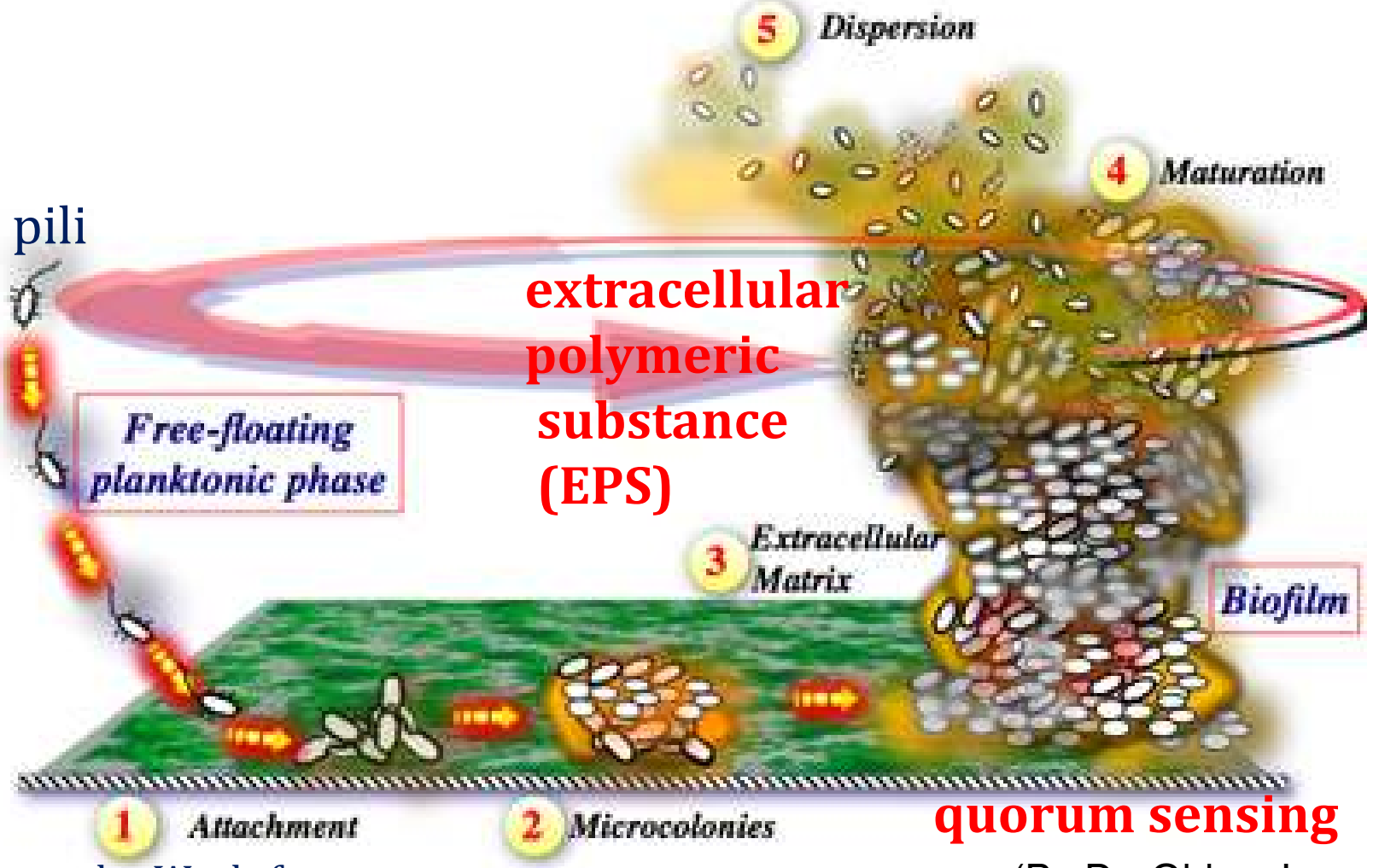
cleaned a clogged drain pipe or toilet?

What is Biofilm?

- a structured community of **microorganisms**
- Mushroom-like conceptual model

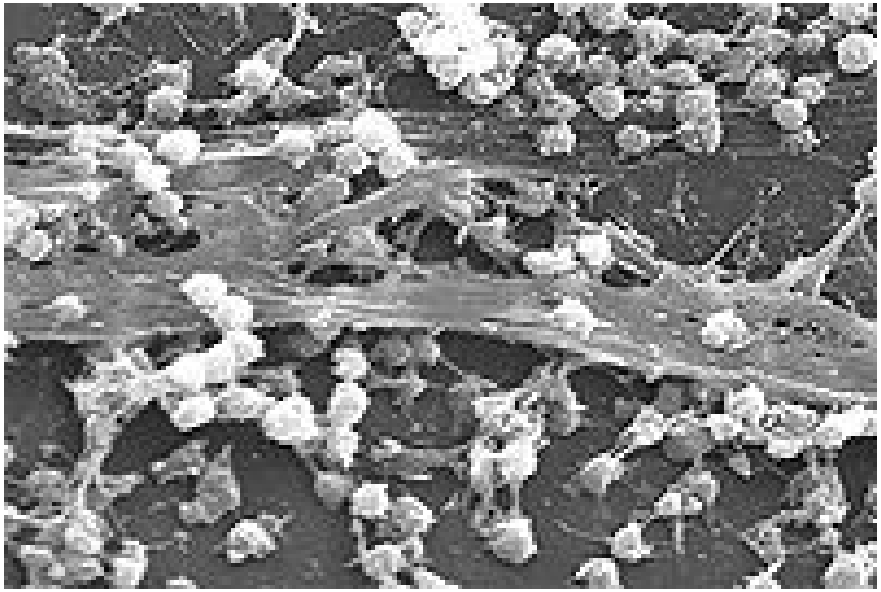


How do bacterial biofilms develop?

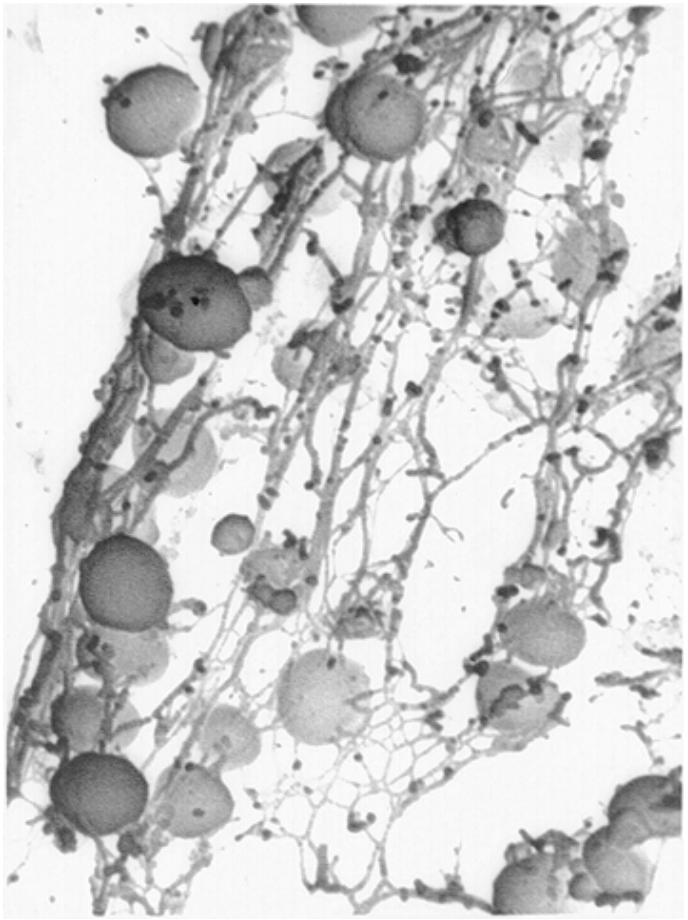


(By Dr. Ghigo Jean-Marc)

Staphylococcal biofilms



Staphylococcus aureus biofilm
- Gram (+) bacteria



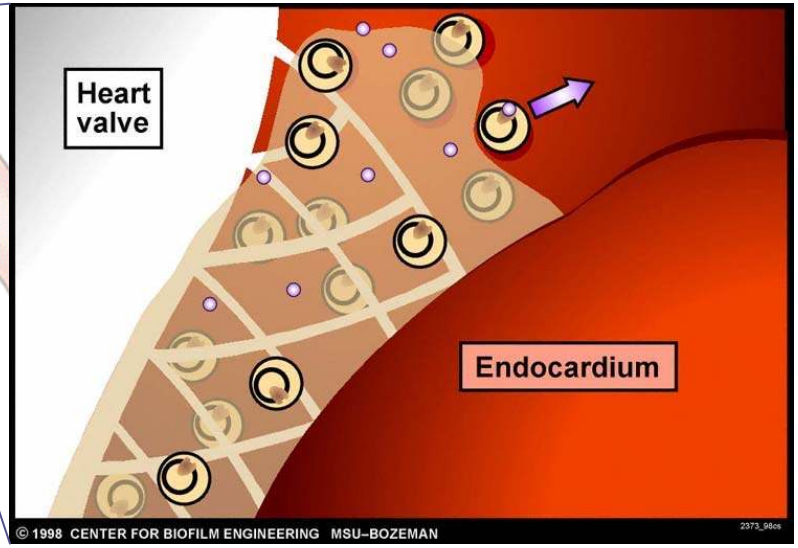
Staphylococcus epidermidis biofilm
- Gram (+) bacteria

Common Sites of Staphyloccocal Biofilm Infections

Catheter Infection

peridontal disease

Mechanical heart valves



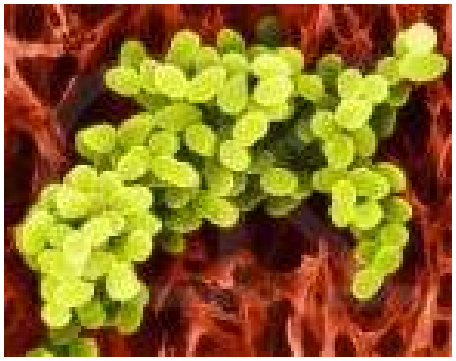
Artificial hip implant

Wound infection

Nature Reviews | Microbiology

Staphylococcus aureus

— **invade the wound after surgical procedure**



S. aureus

Penicillin (1940s)

2 years

Penicillin-resistant *S. aureus*

Methicilin (1960)

1 year

Methicillin-resistant *S. aureus* (MRSA)

Vancomycin

Vancomycin-intermediate *S. aureus* (VISA)

Vancomycin-resistant *S. aureus* (VRSA)

? ?

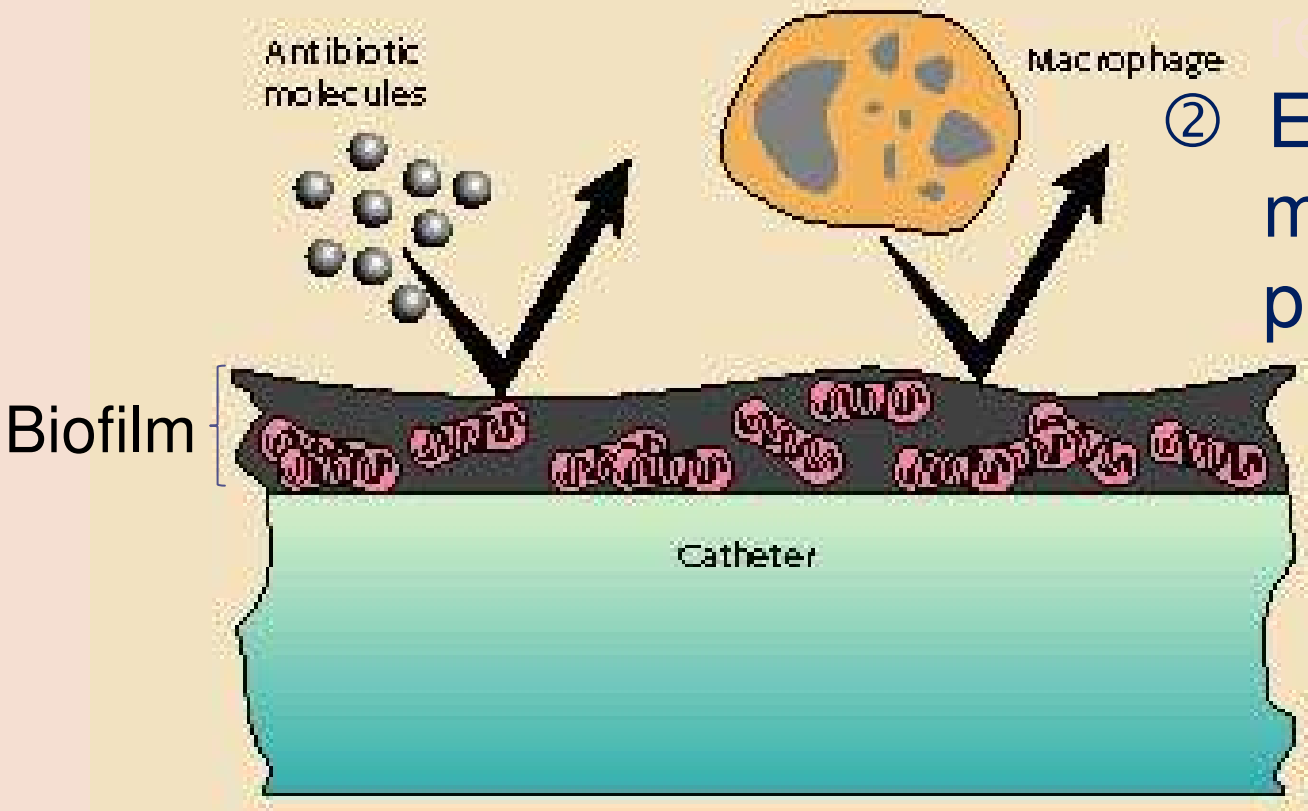
The discovery of new antibiotics might not catch up with the appearance of antibiotics resistance.

Extracellular pulymeric substance (EPS) protect from phagocytosis

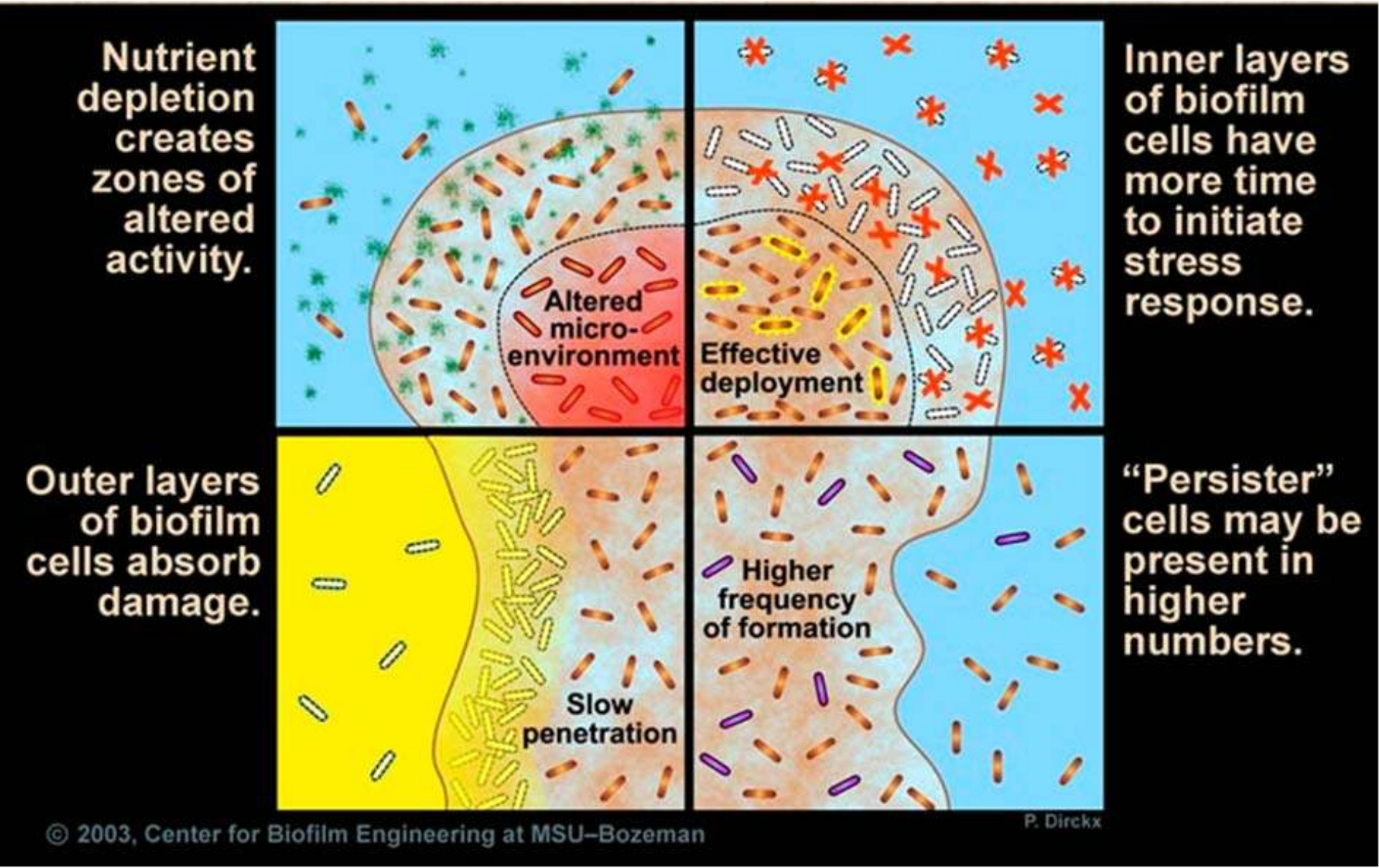
① Antibiotics resistance

resistant to phagocytosis

② Extracellular matrix protection



Biofilm resistance mechanisms



Strategies of Biofilm Control-

1. Prevention is better than cure
2. Removing or Killing

Traditional ways

- ✓Stop growth - by antimicrobial agents
- ✓Block attachment - by changing surface material
- ✓Promote detachment - by surfactants
- ✓Mechanical removal - by ultrasonic device
- ✓Kill - by biocide, chemical agents

But some ways cannot be carried out in hospitalized patients…

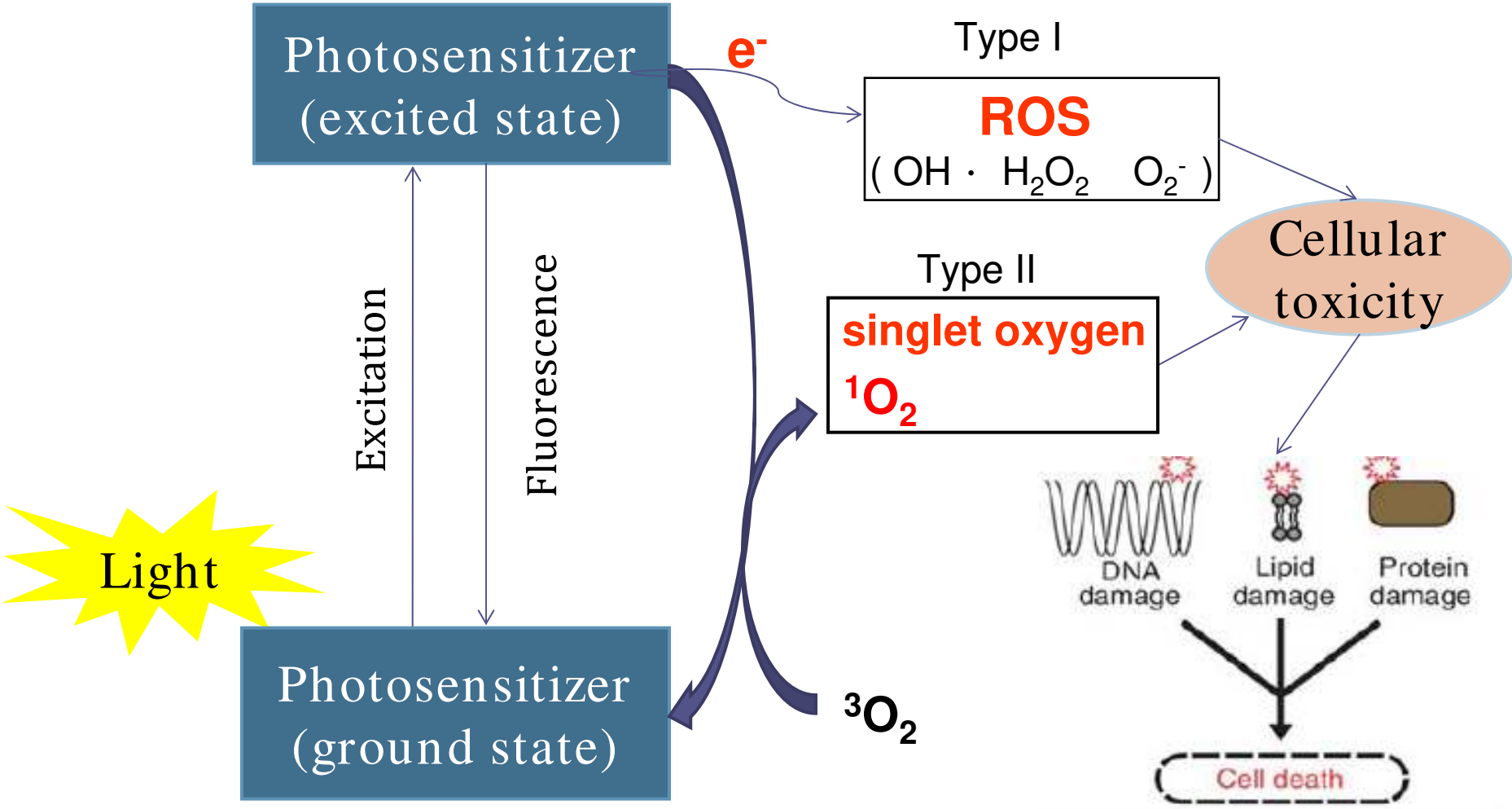
Besides traditional ways…

- Is there any **alternative way** to inhibit the bacteria growth in biofilms?

There is one way…

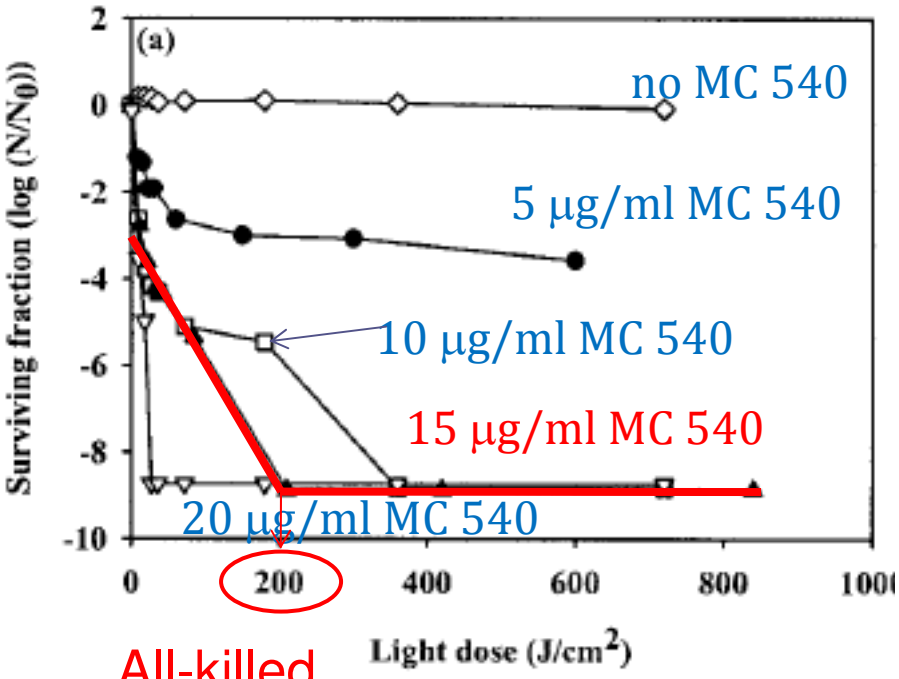
Photodynamic Therapy (PDT)

Three factors of PDT: Light , PS , Oxygen



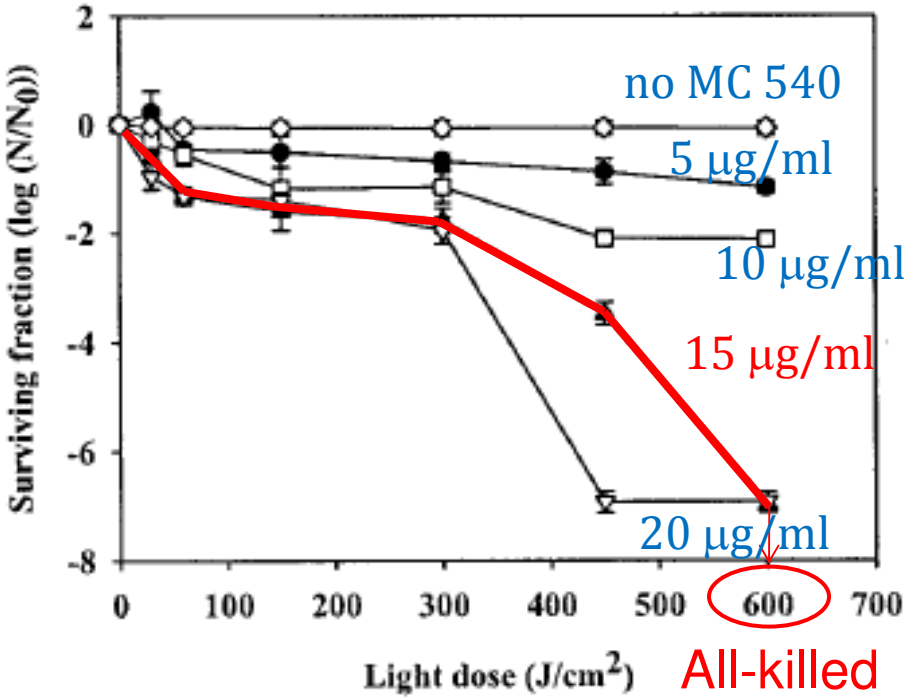
The difference of PDT effect between planktonic cells and biofilms

S. aureus planktonic cells



All-killed light dose

S. aureus biofilms



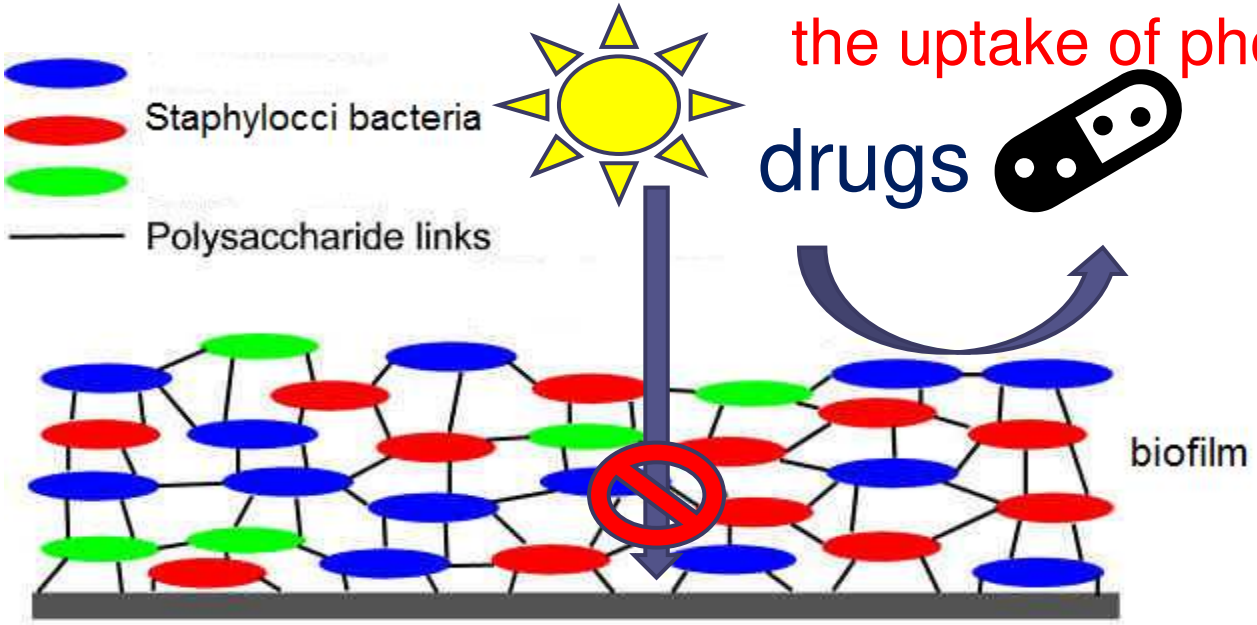
All-killed light dose

Photosensitizer: Merocyanine 540 (MC540)

(Lin, H. Y. et al. 2004)

Extracellur matrix in biofilms

Hindrance of the penetration of light and the uptake of photosensitizer



polysaccharide intercellular adhesion (PIA)

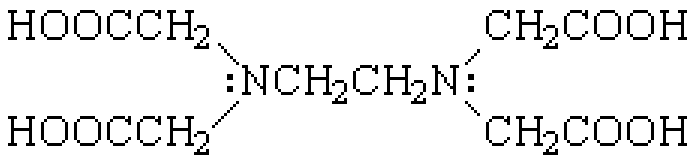
extracellular polymeric substance (EPS)

How to solve this problem?

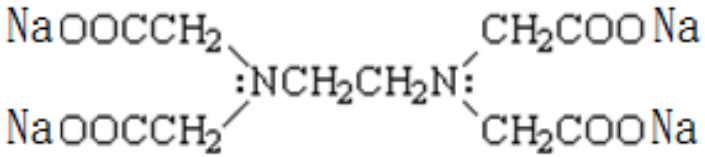
Increase the permeability

- **Metal chelators**
  - EDTA and tetrasodium EDTA (TEDTA)

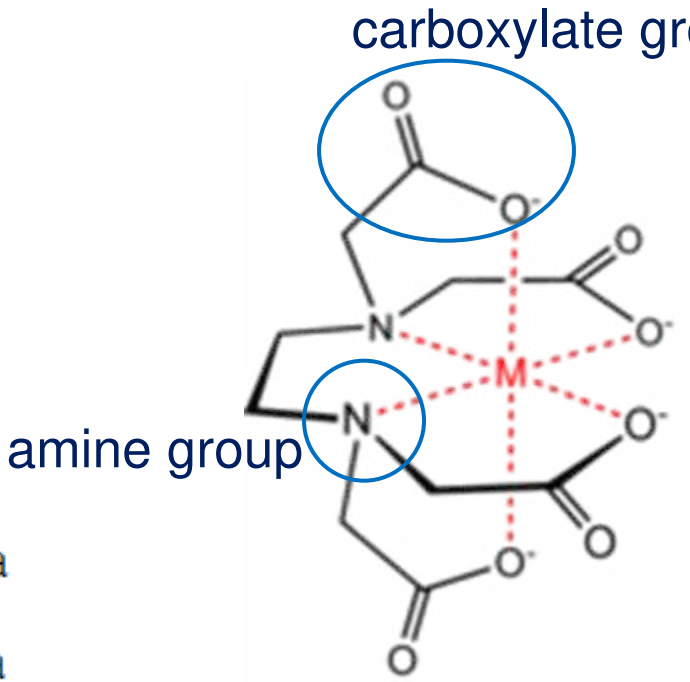
EDTA



TEDTA



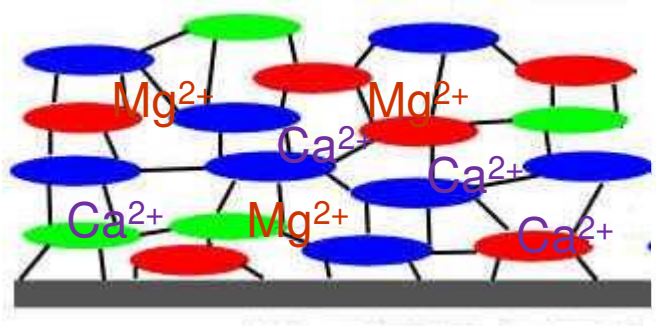
(salt form of EDTA, better solubility)

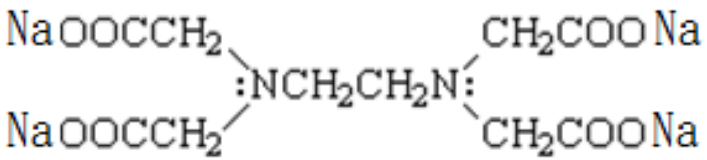


EDTA

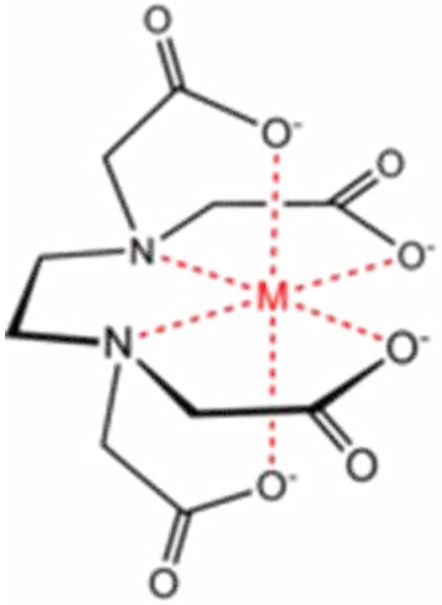
How Metal Chelators dispersing the structure of a biofilm?

- The sequestration of divalent cations, such as Ca^{2+} and Mg^{2+}, are important for maintaing the integrity of EPS in the biofilm.





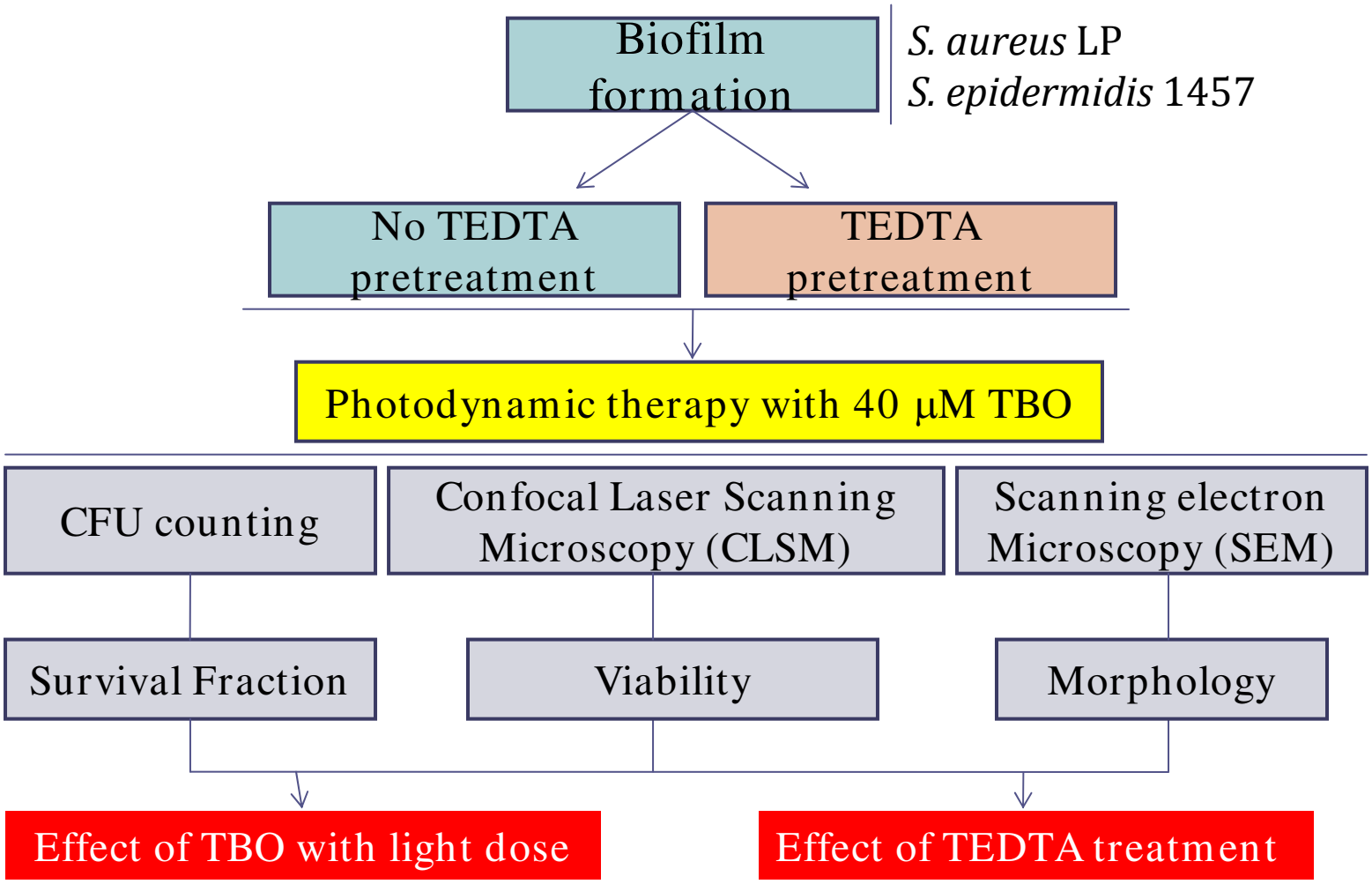
TEDTA



The purpose of this study

1. To examine the photodynamic effects of TBO on the **viability** and **structure** of staphylococcal biofilms.
2. To investigate the effect of TEDTA pretreatment on the efficacy of the photodynamic inactivation of staphylococcal biofilms.

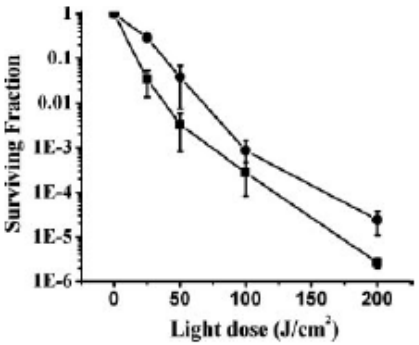
The framework of this study



Materials and Methods

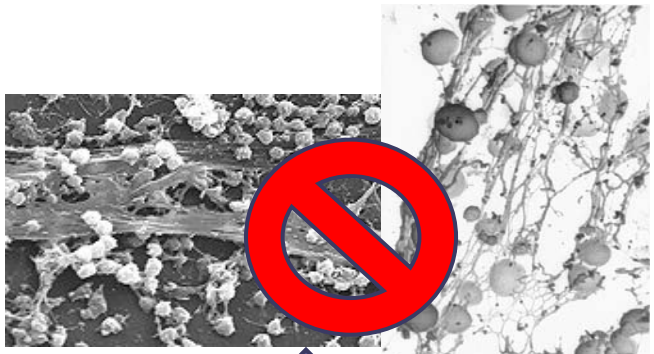
CFU count (TSA)

Survival Fraction



Stapylococcal biofilms

S. aureus LP/ *S. epidermidis* 1457



+TEDTA

(1 h

640 nm diode laser

CLSM images

ROS

Tissue O_2

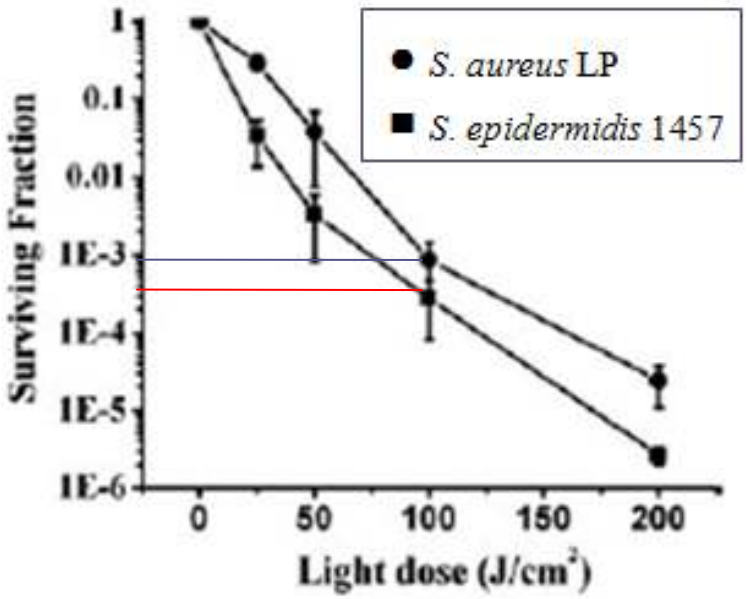
SEM images

r (TBO)

e: 30 min

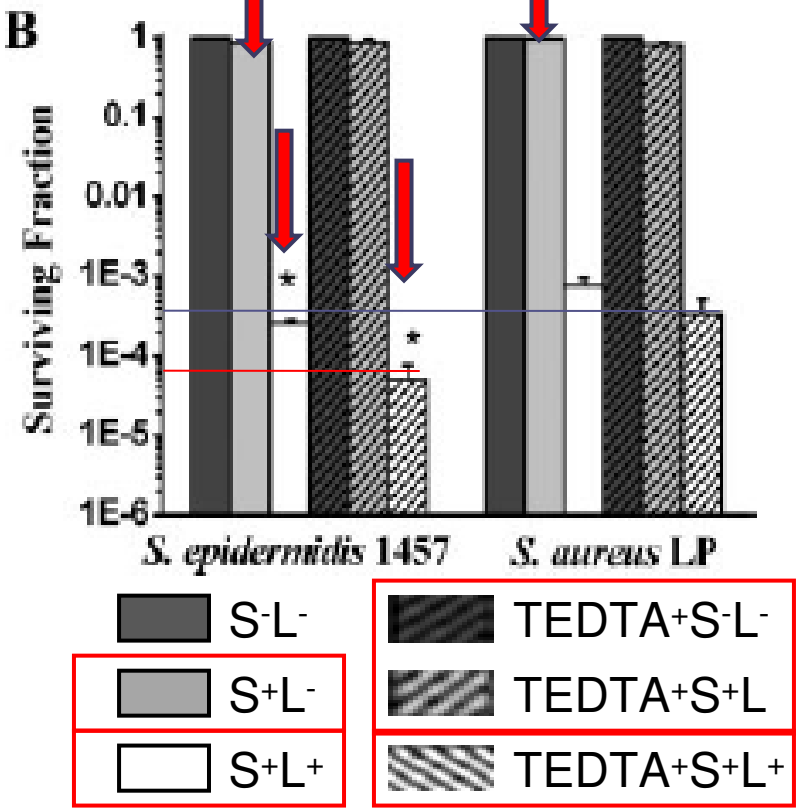
Results

No TEDTA pretreatment



TEDTA pretreatment

TBO itself do no harm staphylococcal biofilms



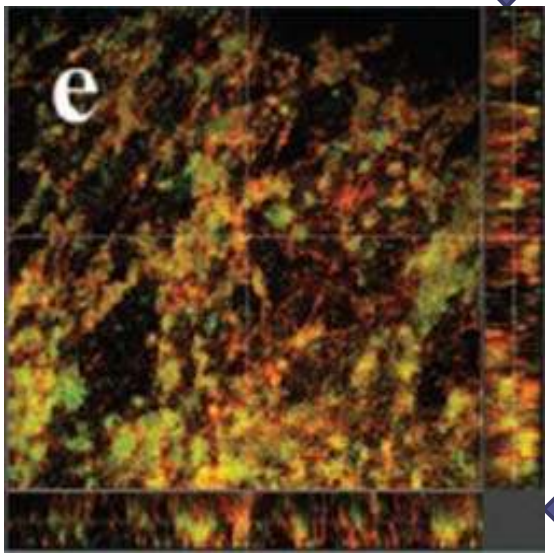
$$\text{Survival fraction} = \frac{\text{CFU of } (S^+ L^+)}{\text{CFU of } (S^+ L^-)}$$

$$\text{or} \quad \frac{\text{CFU of } (TEDTA^+ S^+ L^+)}{\text{CFU of } (TEDTA^+ S^- L^-)}$$

S:sensitizer (TBO:40 μM)
L: light dose (100 J/cm^2)
TEDTA: 20 mM

Confocal laser scanning microscopy (CLSM)

- Use Live/Dead BacLight Bacterial Viability Kits
  -Two fluorescent nucleic acid stains:
  SYTO9 : green(live)
  Propidium iodide(PI): red(dead)



Cross section

Cross section

CLSM image

SEM images

green(live)
red(dead)

CLSM images

S. epidermidis

Experiment conditions

TEDTA(-)
Dark

TEDTA(-)
TBO(40 μM)
100 J/cm²

TEDTA(-)
TBO(40 μM)
200 J/cm²

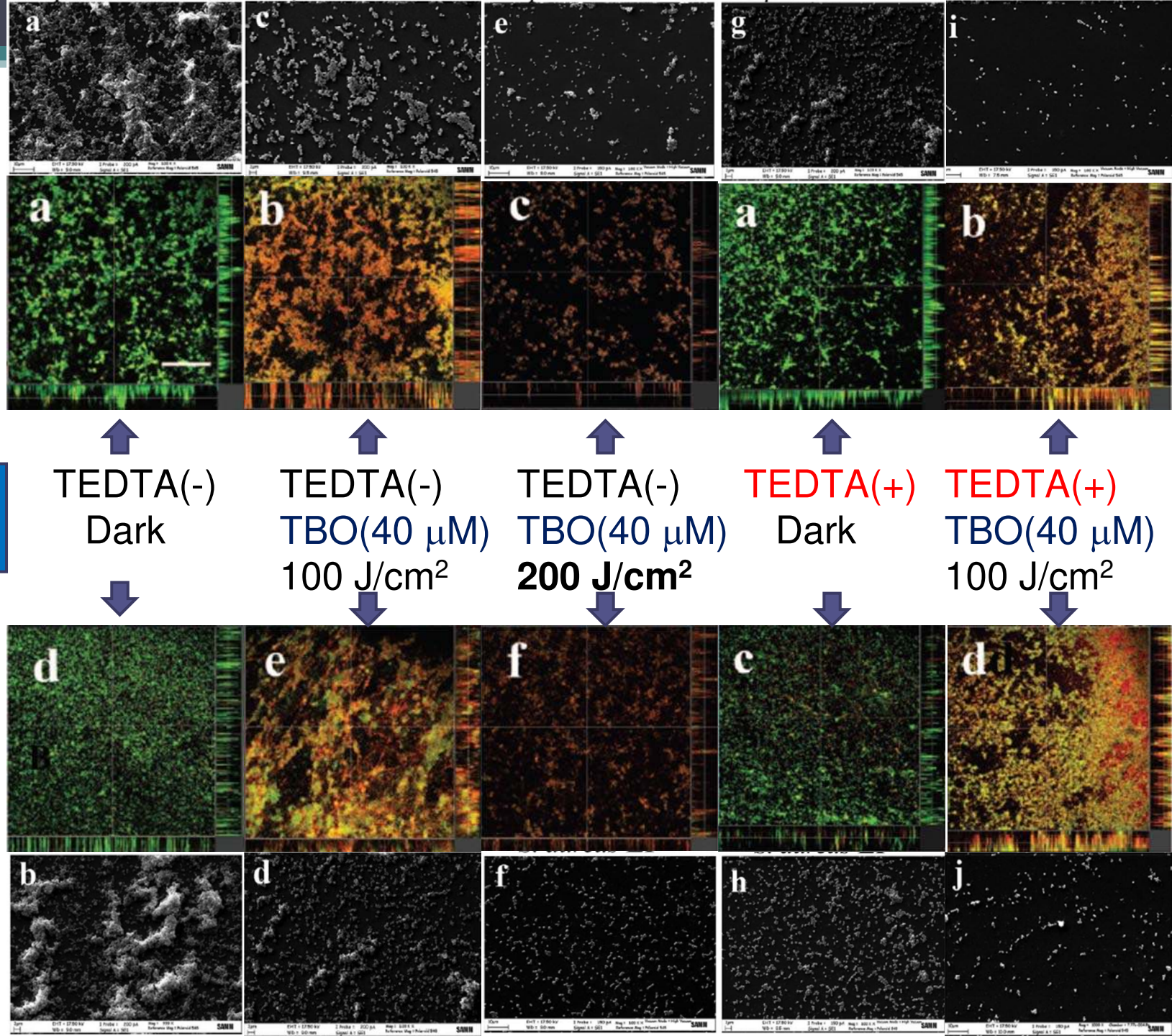
TEDTA(+)
Dark

TEDTA(+)
TBO(40 μM)
100 J/cm²

S. aureus

CLSM images

SEM images



Antibiotics resistance

Staphylococcal biofilms

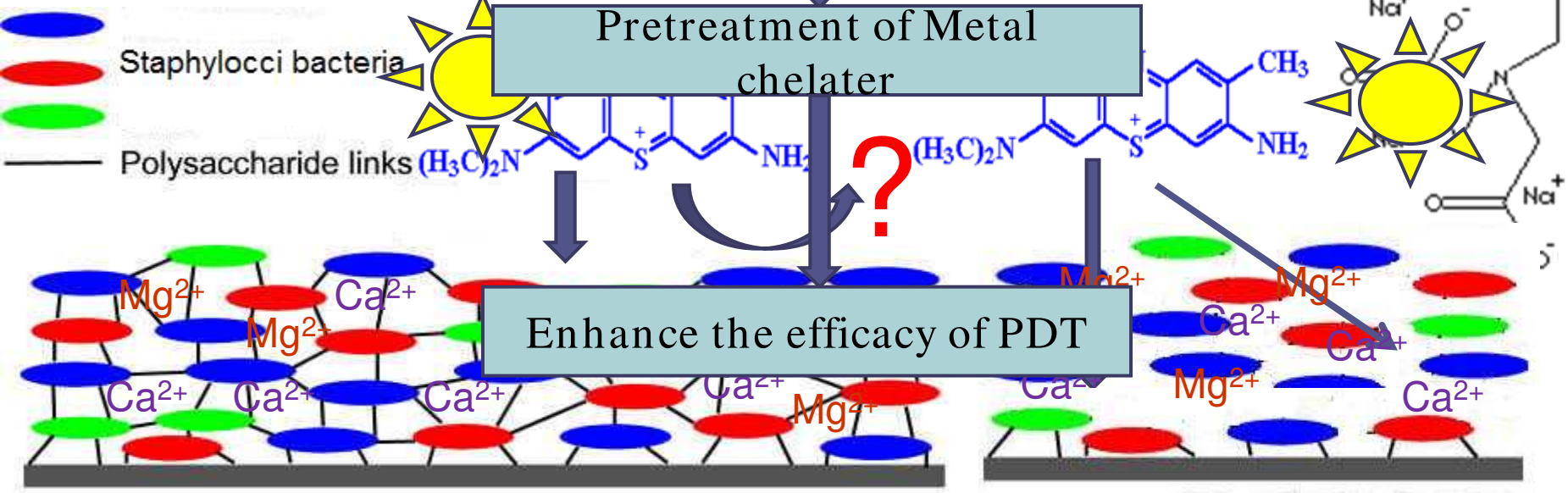
Resistant to Phagocytosis

Photodynamic therapy

Hindrance of penetration

Pretreatment of Metal chelater

Enhance the efficacy of PDT



Photodynamic inactivation

Effect of TBO with light dose

The main cause of cell death

Photodynamic inactivation

Effect of TEDTA treatment

Disperse biofilm structure

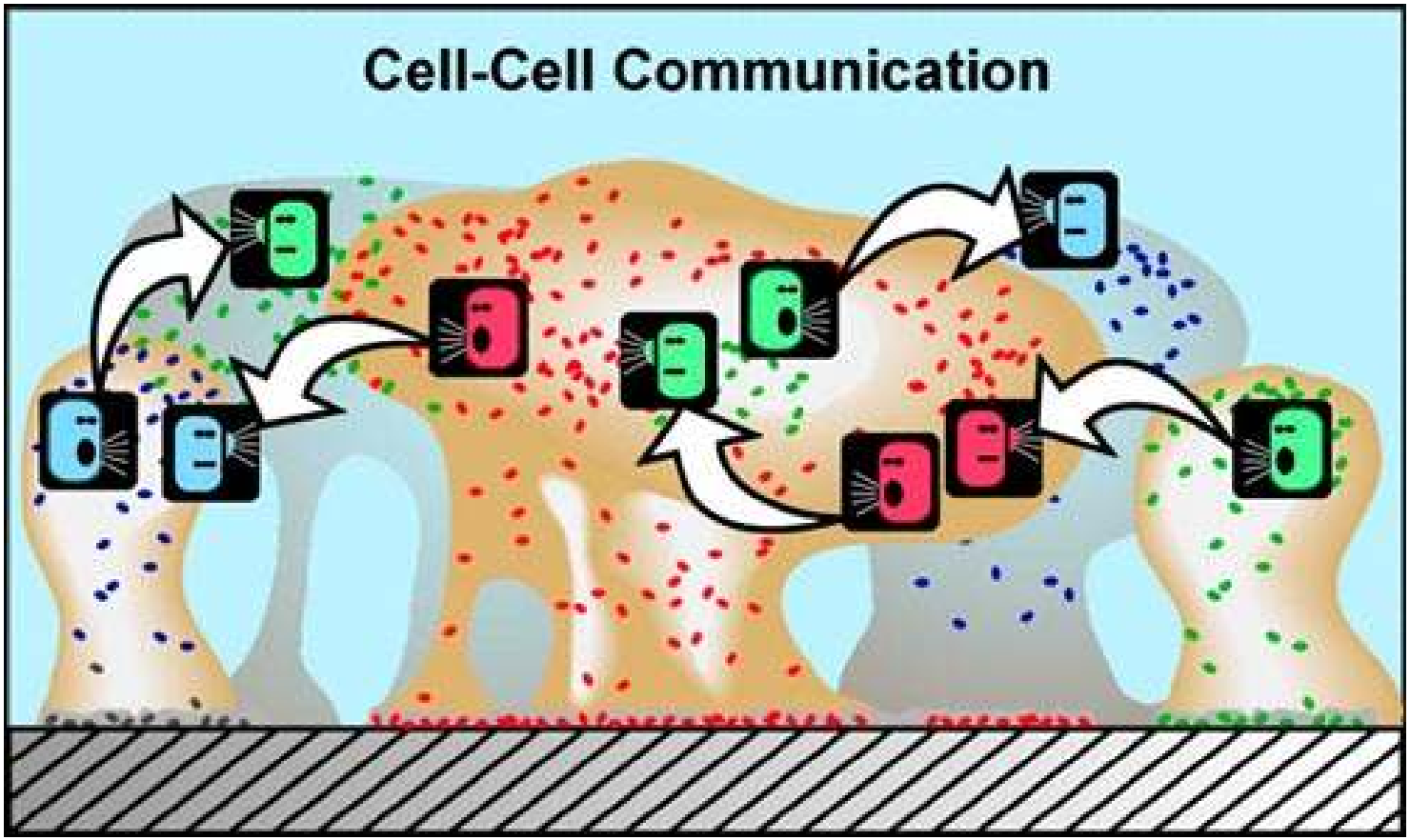
The End

Thanks for your attention!!

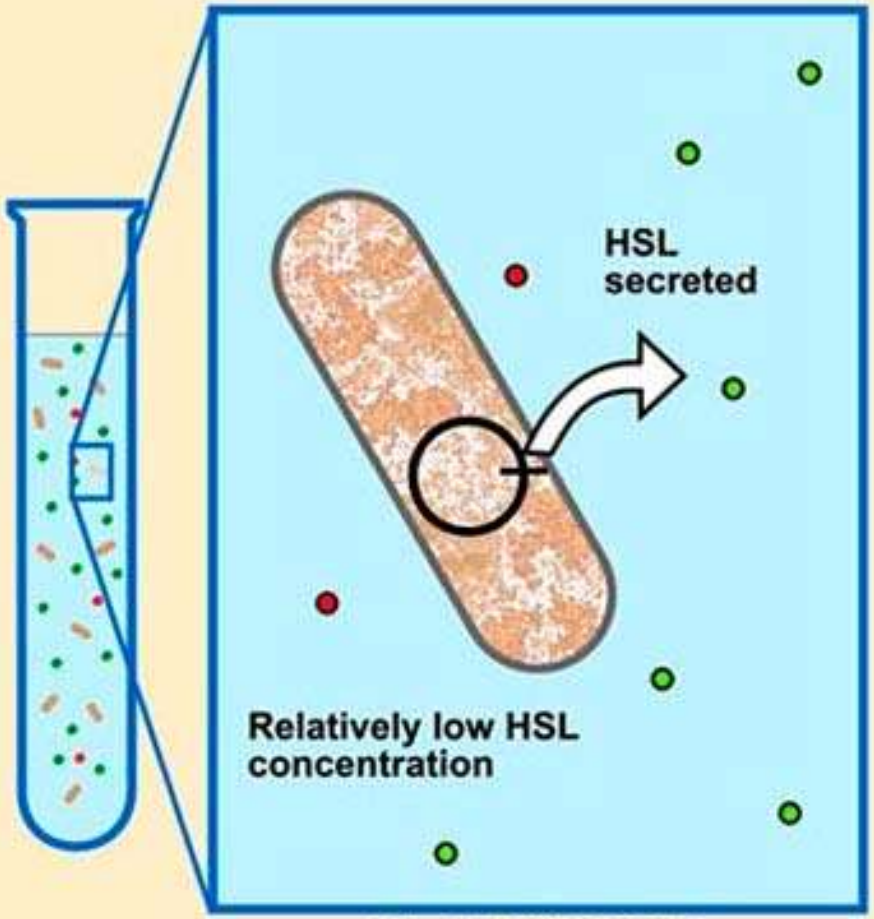
Special thanks to Prof. Huang !

Cell-cell communication

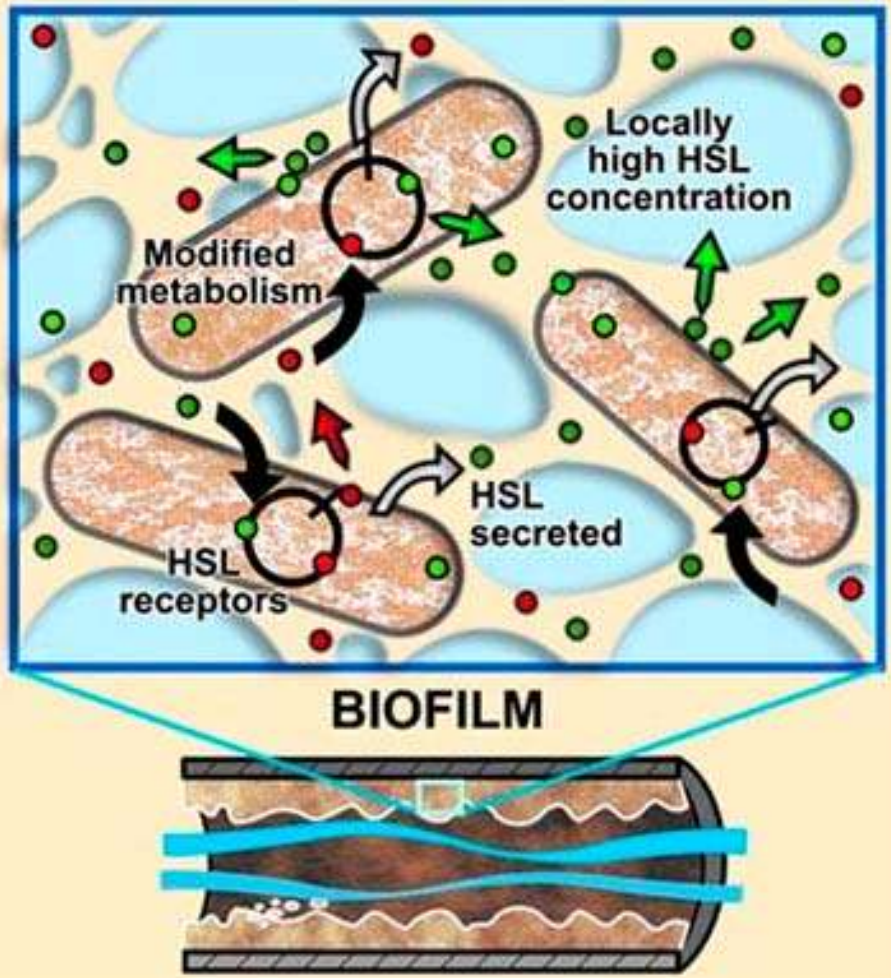
- Cells in a biofilm "talk" to each other via quorum sensing.



Quorum Sensing



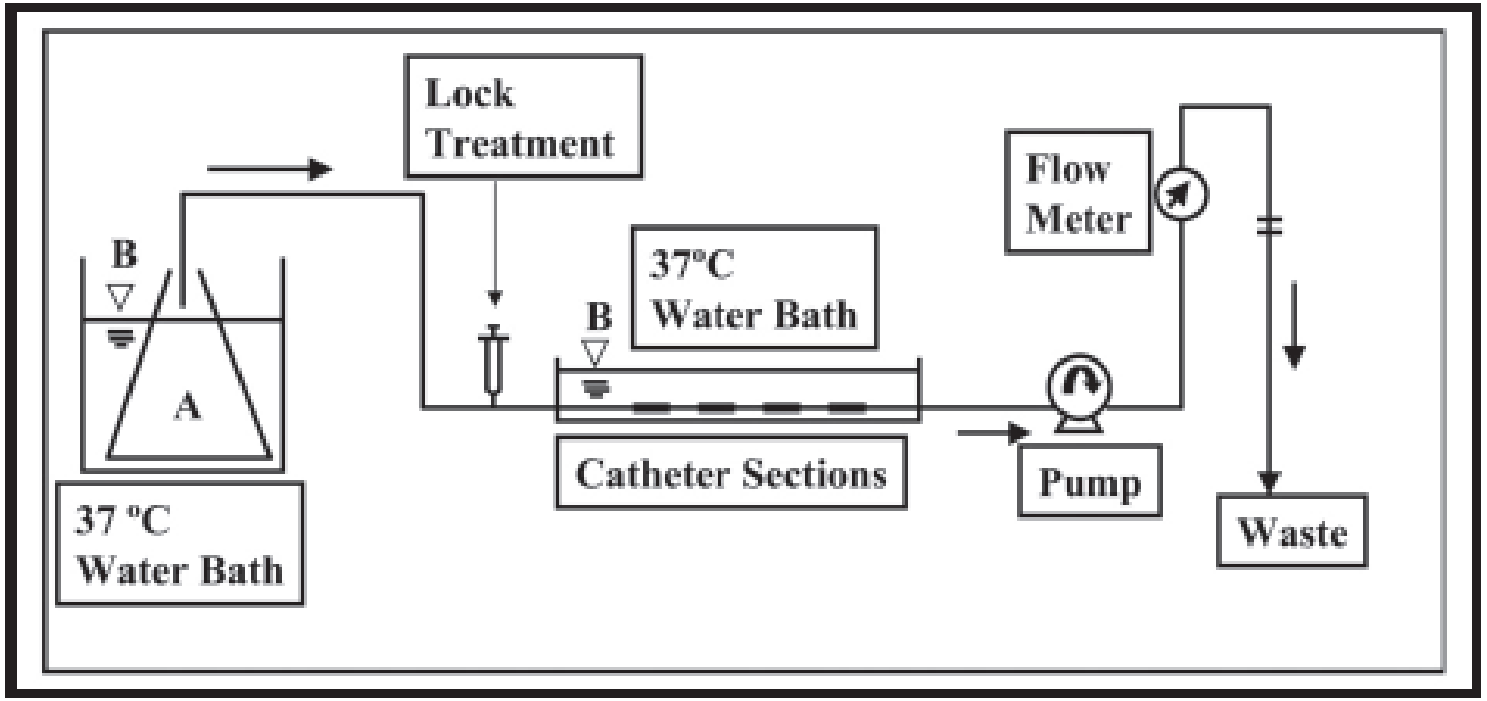
PLANKTONIC



BIOFILM

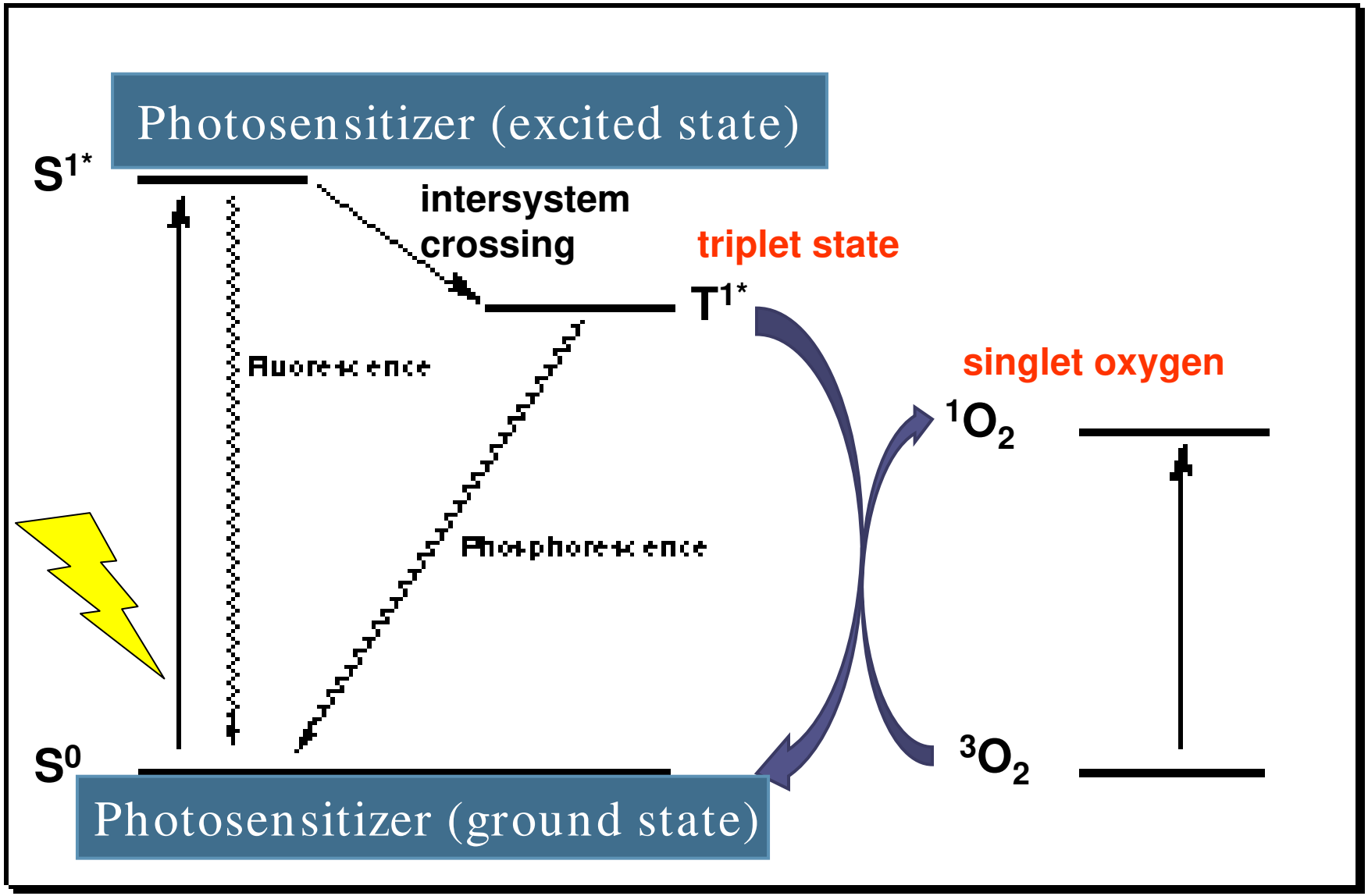
Metal Chelator-Tetrasodium EDTA

- TEDTA is a novel central venous catheter lock solution against biofilm.



(Infection Control and Hospital Epidemiology Vol.26 N0.6)

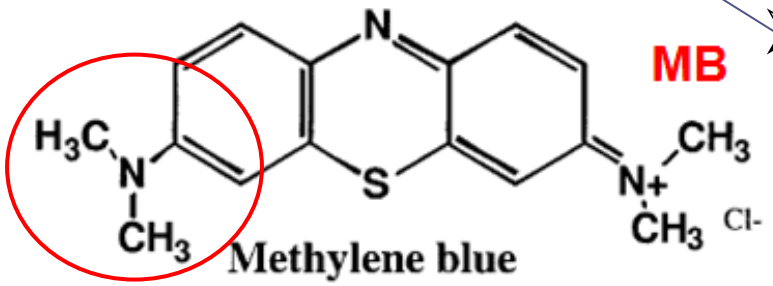
Photodynamic therapy (PDT)



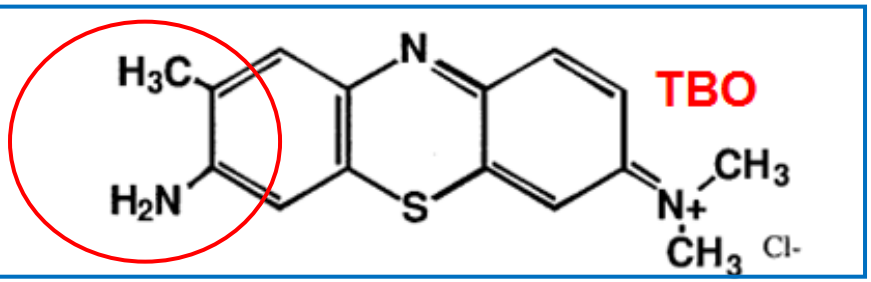
Photosensitizers used in PDT

Amphiphilic , cationic

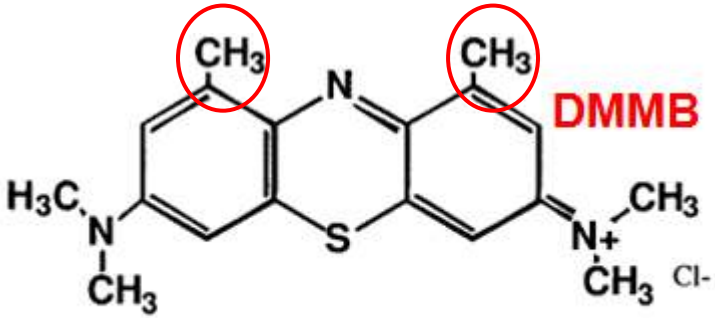
- Hydrophobic– helps penetration in cellular membrane
- Hydrophilic – helps diffusion.



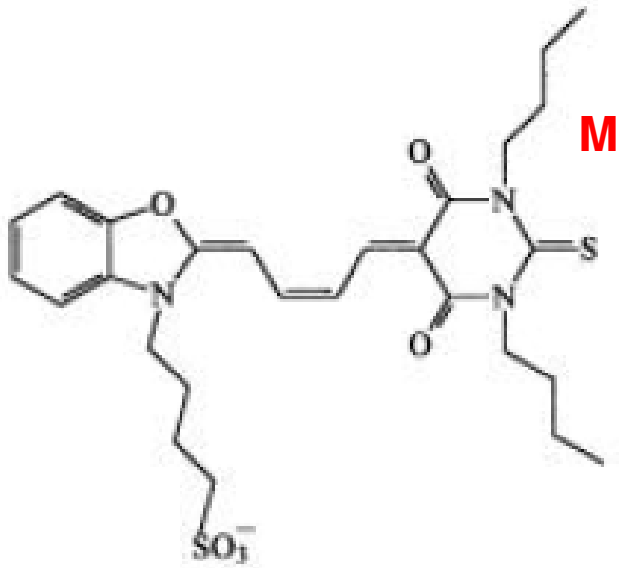
Methylene blue



Toluidine blue O



1,9-Dimethyl-methylene blue

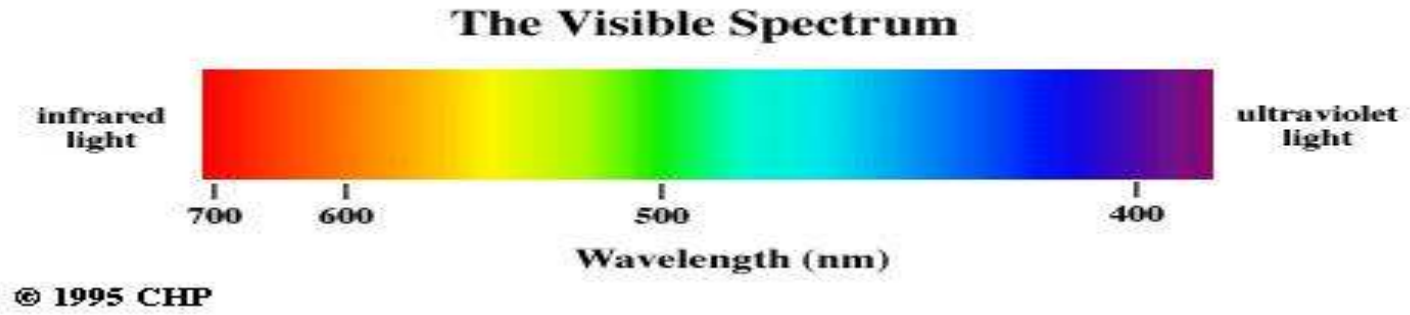


Merocyanine 540

Lipophilic , anionic

Wavelength Absorption of Photosensitizer

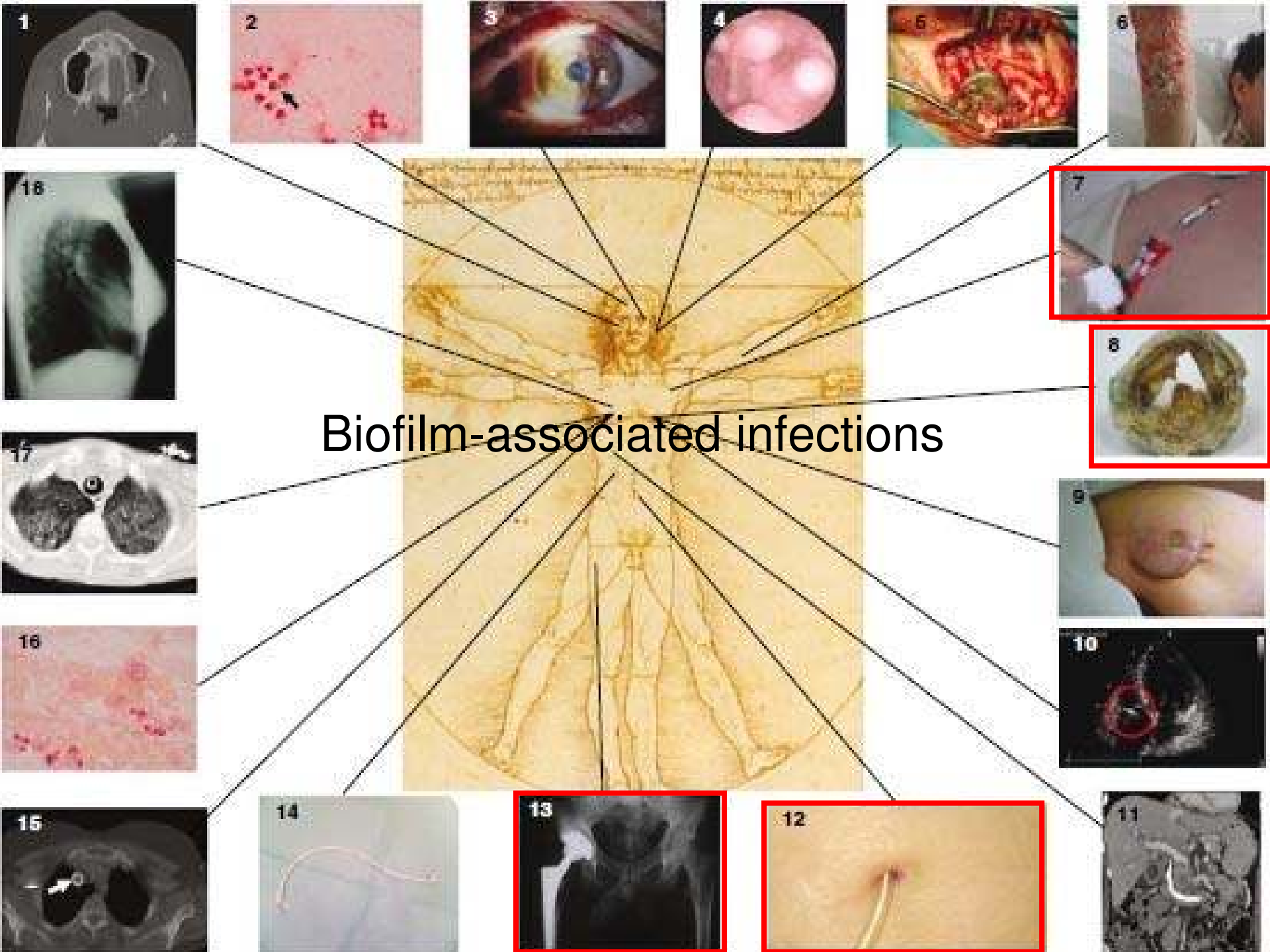
- Best if absorb at longer wavelengths
- Longer wavelength emissions can penetrate tissue farther.
- Decreases probability of damage to surrounding tissue.
- Must have enough energy to excite O_2



Light source of PDT

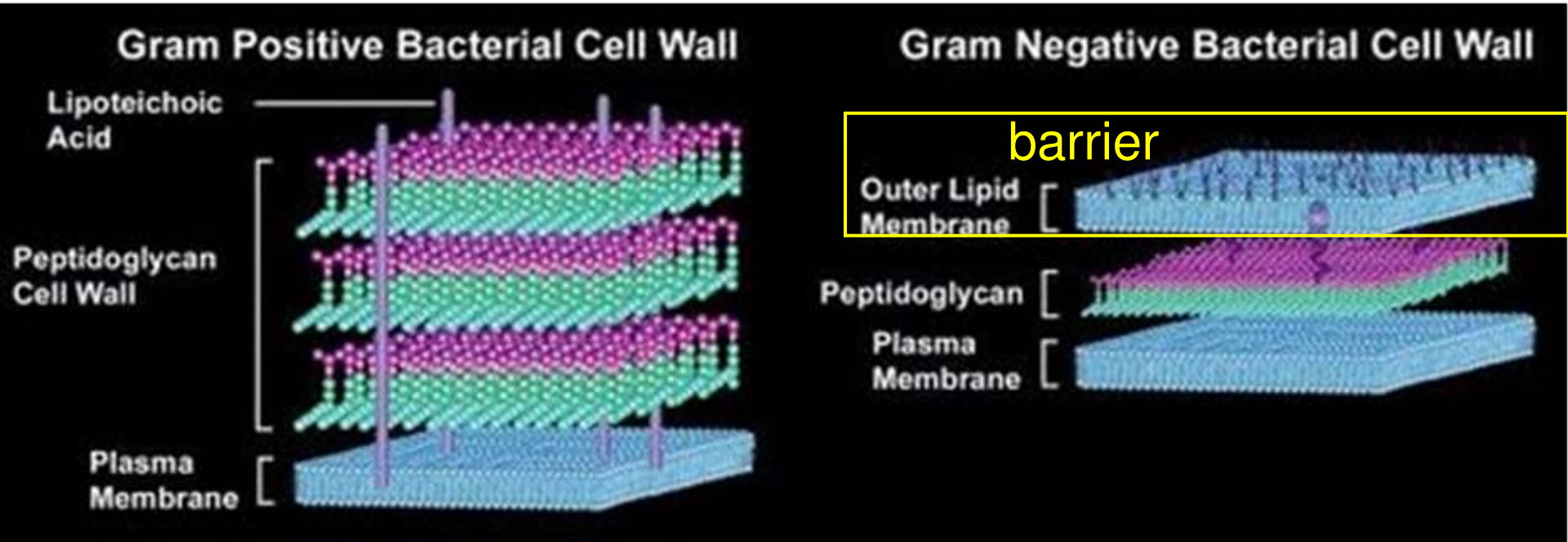
- LED (Light-emitting diode):
  -coherent
  -long useful lifetime
  -high photoelectric conversion efficiency

- Laser
  -noncoherent
  -specific wavelength → high intensity
  -low divergent effect


Biofilm-associated infections

PDT has better effect on Gram(+) bacteria due to the cell wall structure

- Thick peptidoglycan cell wall integrated by several lipoteichoic acid →forms many holes
- Photosensitizers can pass through Gram positive bacterial cell wall easier.



S. aureus v.s. *S. epidermidis*

	Staphylococcus aureus	*Staphylococcal epidermidis*
Culture medium	TSB (Tryptic soy broth)	TSB (Tryptic soy broth)
Gram-staining	Gram(+)	Gram(+)
Biochemical characteristics	catalase-positive coagulase-positive	catalase-positive coagulase-negative
habitat	facultative anaerobe	facultative anaerobe
color	golden-yellow	white