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

Nanocoatings for Preventing Biofilm formation on Medical devices

Supervisor: Prof. Mamie Hui

Student: Poon Yeuk Lan, Nana (PhD student, Yr7)

Date: 14th December 2021

Outline

Introduction

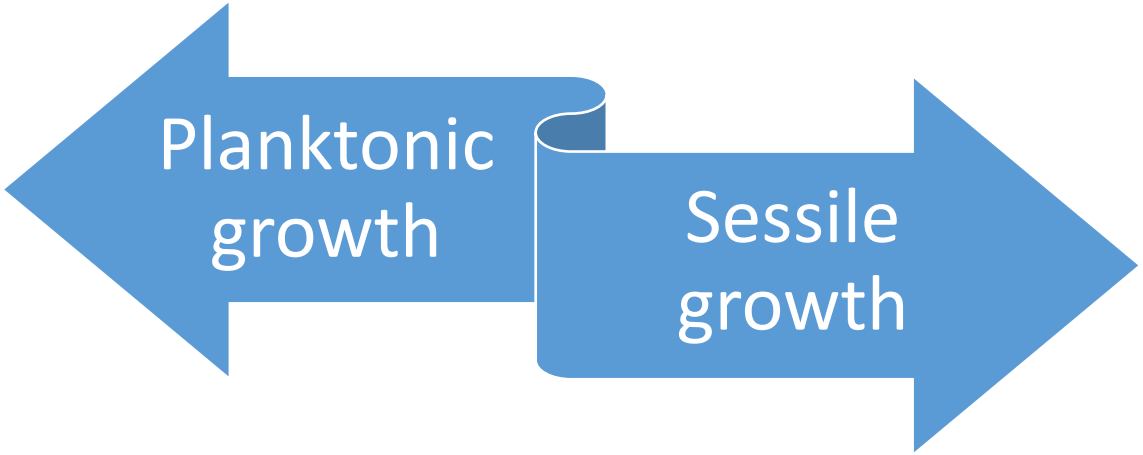
Nanocoatings

- Antifouling coatings
  - Hydrophilic polymers
  - Zwitterionic polymers
  - Superhydrophobic surface
- Antimicrobial coatings
  - Metal-based nanoparticles
  - Cationic polymers
- Limitations

Summary

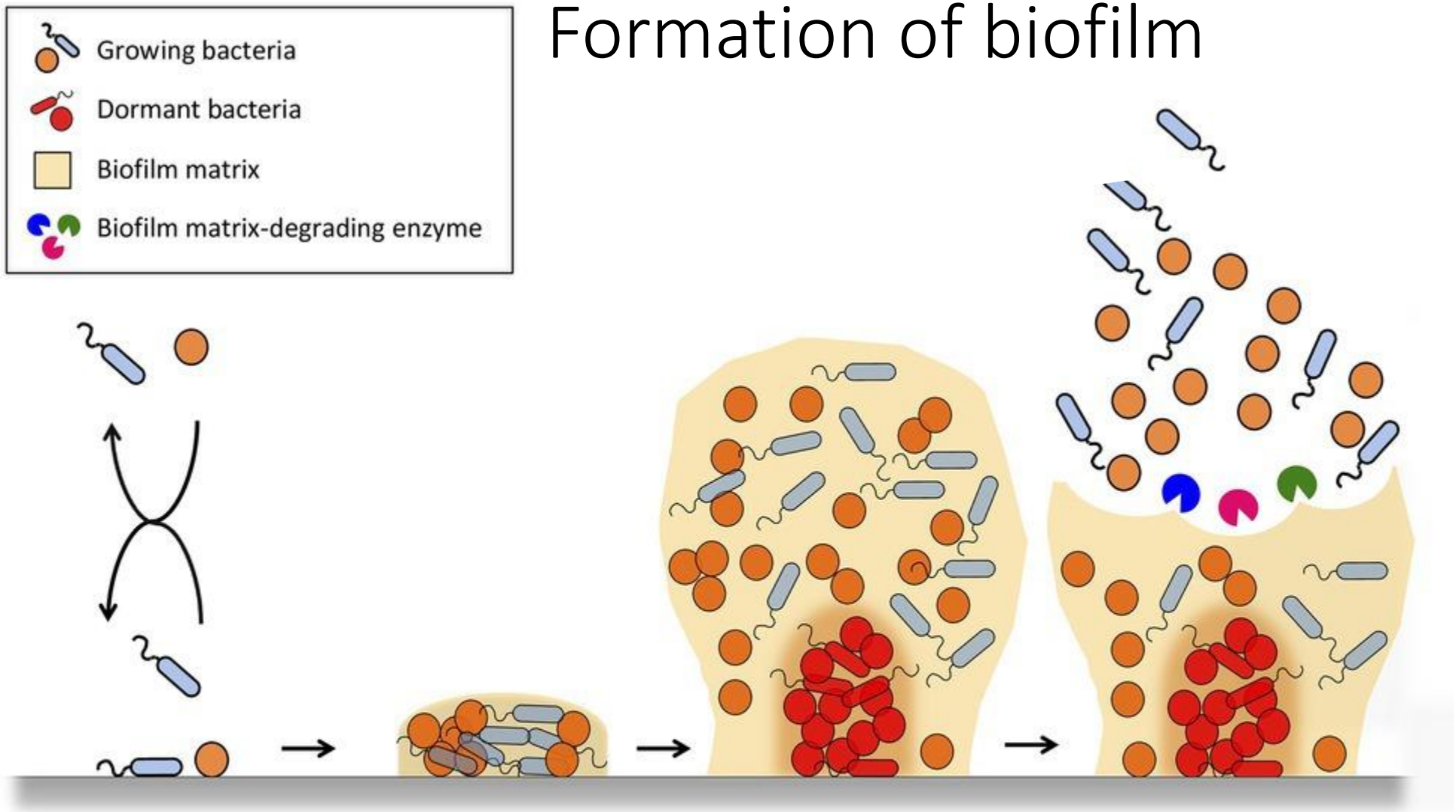
Introduction

Modes of growth of microorganisms



- Biofilm
  - “A structured community of bacterial cells enclosed in a self-produced polymeric matrix, adherent to a surface.” (Costerton et al., 1999)

Formation of biofilm



(Fig 1, Maali et al., 2020)

Introduction

- Clinically relevant, biofilm forming microorganisms
  - Gram positive bacteria
    - *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus viridans*
  - Gram negative bacteria
    - *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis,* and *Pseudomonas aeruginosa*
  - Fungi
    - *Candida* spp.
- Biofilms accounted for up 80% of microbial infections

(Khatoon et al., 2018)

Introduction

- Device-associated infections
  - Accounted for 25.6% of health care-associated infections (Magill et al., 2014)
  - Usually associated with microbial colonization on indwelling and prosthetic medical devices
  - Related to colonization of microorganisms on surface of implants
    - Implant as substrate for colonization and biofilm formation
    - Local immunosuppression in insertion site

Table 1. The magnitude of the problem of device-associated infections.

Device	Estimated no. inserted in the United States per year	Rate of infection, %	Attributable mortality[a]
Bladder catheters[b]	>30,000,000	10–30	Low
Central venous catheters[b,c]	5,000,000	3–8	Moderate
Fracture fixation devices[b]	2,000,000	5–10	Low
Dental implants[d]	1,000,000	5–10	Low
Joint prostheses[b]	600,000	1–3	Low
Vascular grafts[b]	450,000	1–5	Moderate
Cardiac pacemakers[b,d]	300,000	1–7	Moderate
Mammary implants, in pairs[e]	130,000	1–2	Low
Mechanical heart valves[d]	85,000	1–3	High
Penile implants[b,d]	15,000	1–3	Low
Heart assist devices[d]	700	25–50	High

[a] Semiquantitative scale for attributable mortality: low, <5%; moderate, 5%–25%; high, >25%.
[b] Numbers estimated by analysis of market reports.
[c] Numbers estimated by review of the medical literature.
[d] Numbers estimated by personal communication with personnel from device manufacturing companies.
[e] Numbers estimated by review of data provided by medical associations.

(Table 1, Weinstein and Darouiche, 2001)

Introduction

- Current treatment of device-associated infection
  - High dose of antibiotics
    - Ineffective due to high tolerance and resistance to antibiotics of biofilm
    - Minimum biofilm inhibitory concentration (MBIC) usually higher than planktonic MIC
  - Last resort: Surgical replacement of the implanted devices
    - High cost and risk
    - High-chances of re-infection

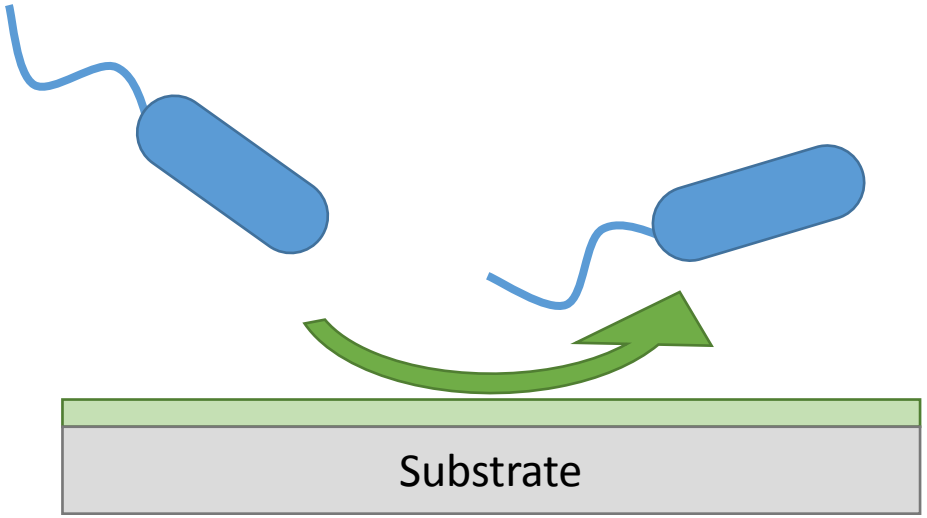
(Khatoon et al., 2018)

Introduction

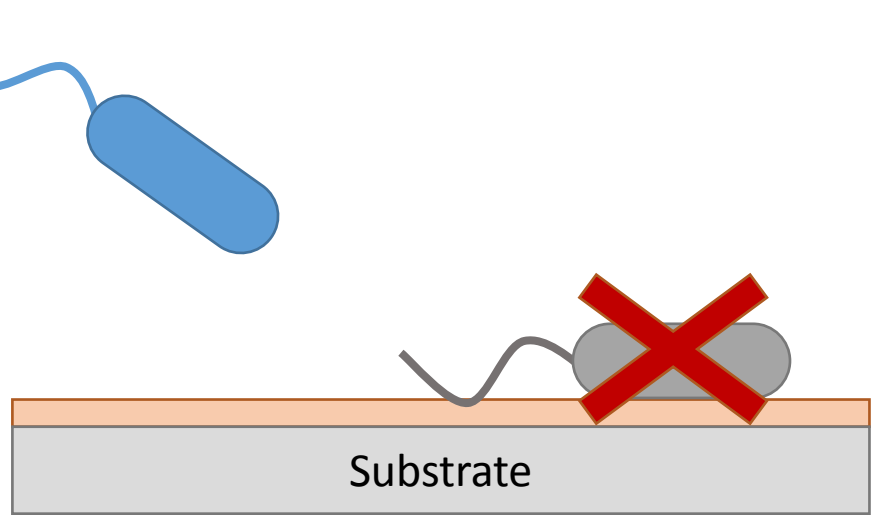
- Alternative approach:
  Prevention of biofilm formation on implants
- Nanocoating
  - Application of nanomaterial of which one dimension at a nanoscale (1-100 nm) onto a surface
  - Advantages of nanomaterial
    - Different properties compared to bulk counterpart
    - High surface area-to-volume ratio, thus high reactivity and capacity
    - Possibility for modifications

(Yousaf et al., 2015)

Strategies of nanocoatings to prevent biofilm formation



- Antifouling surface
  - Reduce and inhibit adhesion
  - Hydrophilic polymer
  - Zwitterionic polymer
  - Superhydrophobic surface

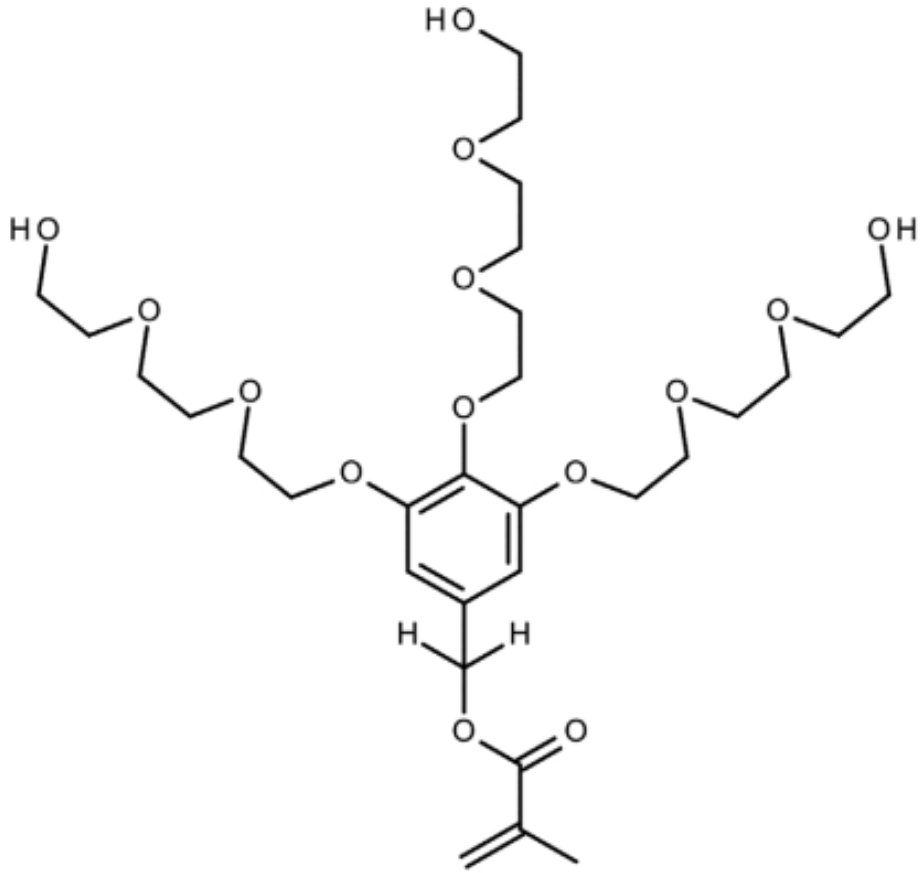


- Antimicrobial coating
  - Inhibit colonization
  - Metal-based nanoparticles
  - Cationic polymers

Antifouling strategy – Hydrophilic polymers

- Electrically neutral materials process polar ether, hydroxyl, or amide groups

- Mechanism: Surface hydration
  - Form a water layer on surface by hydrogen bonds with water molecules
  - Physical and energetic barrier
  - Preventing host-protein adsorption and bacterial adhesion

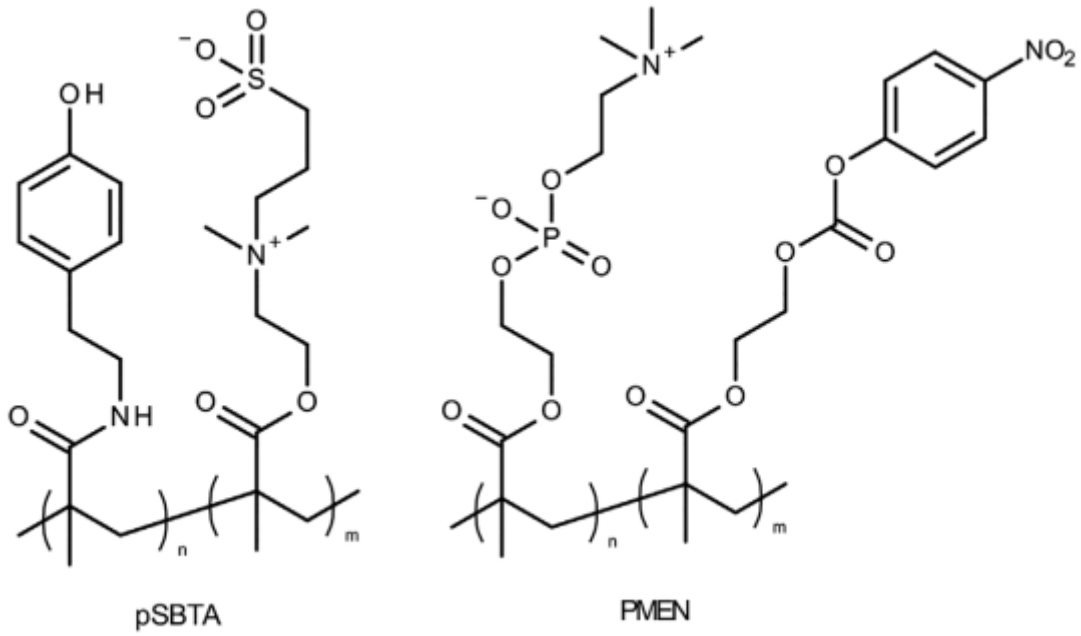


Dendronized PEG methacrylate (polyPEG)

(Fig. 5, Faustino et al., 2020)

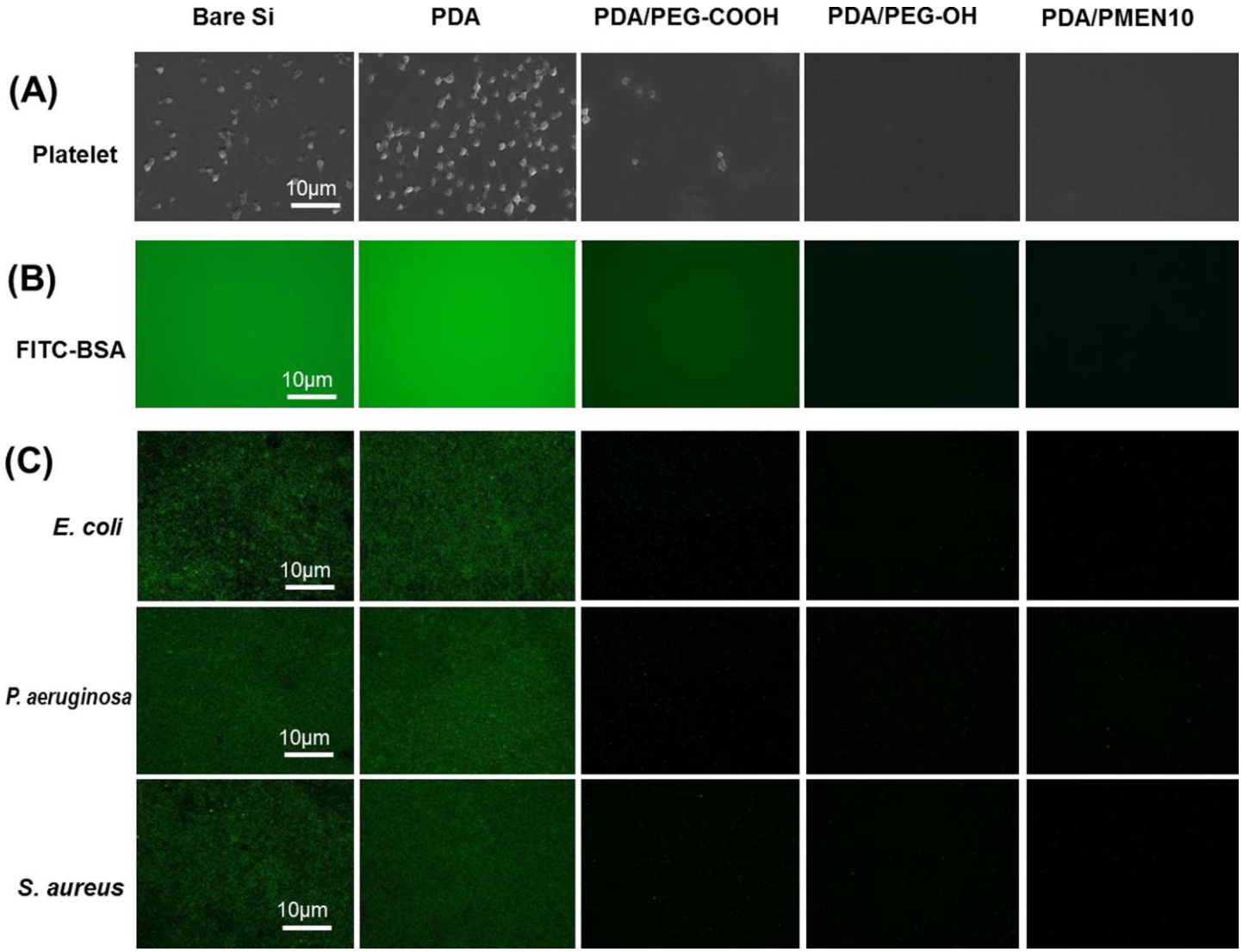
Antifouling strategy – Zwitterionic polymers

- Polymers with an identical number of negatively and positively charged groups
- Form hydration layer by ionic interactions
- High biocompatibility
- High stability against oxidation



(Fig. 5, Faustino et al., 2020)

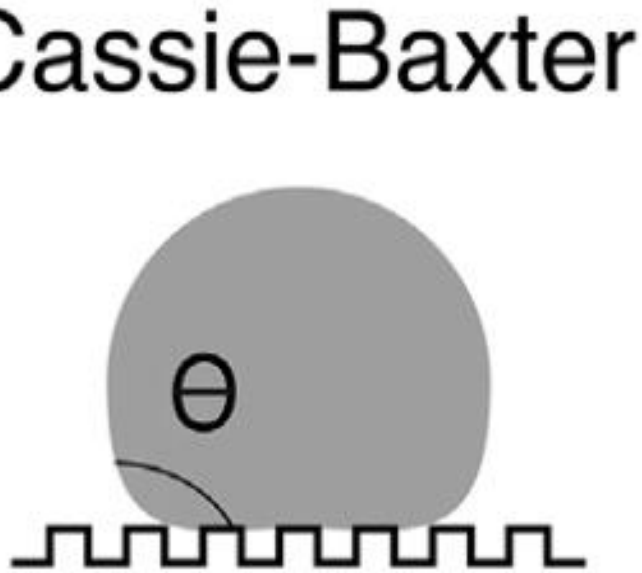
- Xing et al., 2017
  - PEG and PMEN10 with polydopamine (PDA) intermediate layer
  - *in vitro* incubation of coated silicon wafers with platelet, BSA, bacterial suspensions
  - Both PEG and PMEN10 reduced fouling and bacterial cell adhesion and BSA adsorption



(Fig. 8, Xing et al., 2017)

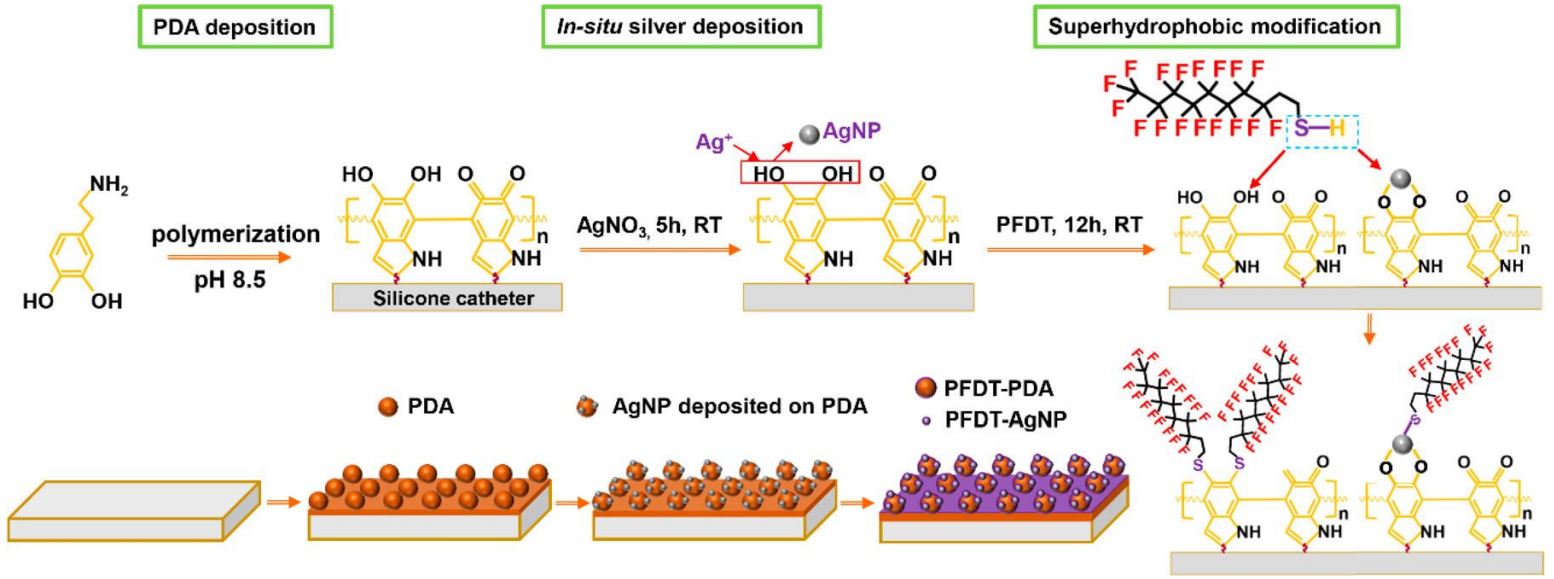
Antifouling strategy – Superhydrophobic surface

- Ultra low water adhesion
  - Lotus leaf effect
  - Self-cleaning property
- Hydrophobic surface + micropatterning
- Cassie-Baxter state
  - Water contact angle $\theta > 90^{\circ}$
  - Reduced adhesion force



(Fig. 3, Faustino et al., 2020)

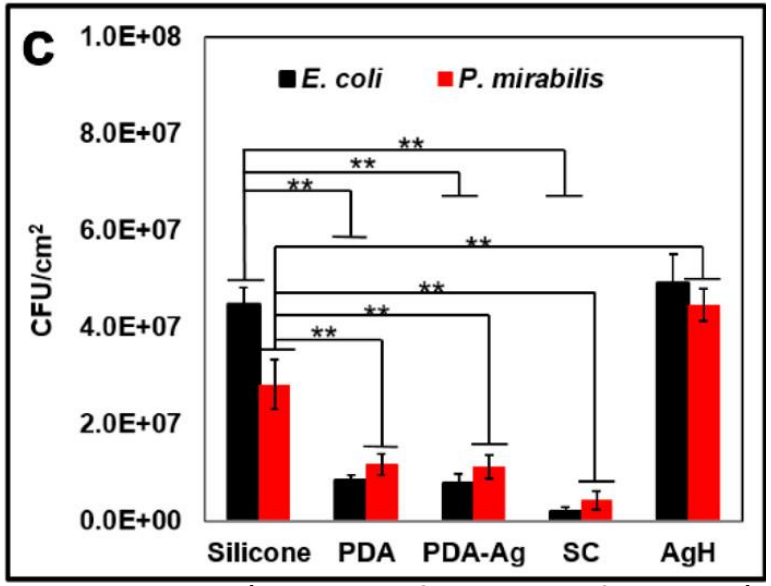
- Zhang et al., 2020



(Scheme 1, Zhang et al., 2020)

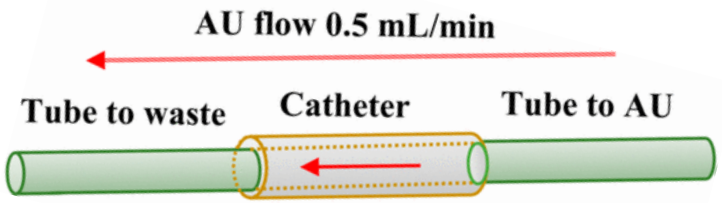
- Superhydrophobic coating on silicon catheter
  - Deposition of PDA and silver nanoparticles (AgNPs)
  - Hydrophobic modification with 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol (PFDT)
  - Water contact angle: 154.7°
- Compared with all silicon or silver-alloy-hydrogel-coated catheters

- Biofilm adhesion assay
  - Incubation of 2 cm of coated catheter with tryptic soy broth with *E. coli* or *P. mirabilis* for 2 days



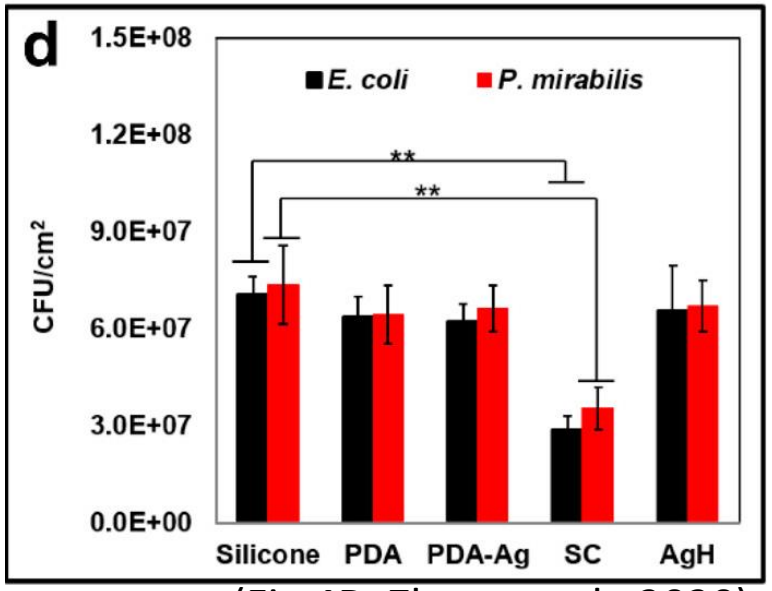
(Fig 4C, Zhang et al., 2020)

- Dynamic flow model



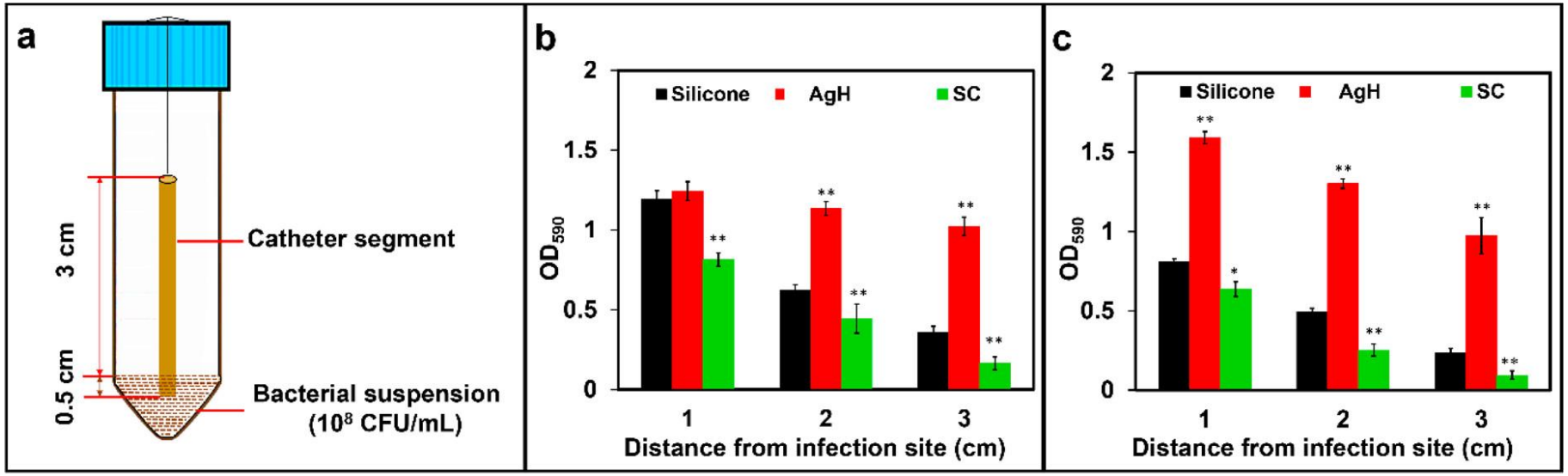
(Scheme 2, Zhang et al., 2020)

  - Inoculated artificial urine (AU) pumped through catheter for 7 days



(Fig 4D, Zhang et al., 2020)

- Bacterial migration assay



E. coli *P. mirabilis*

(Fig 6, Zhang et al., 2020)

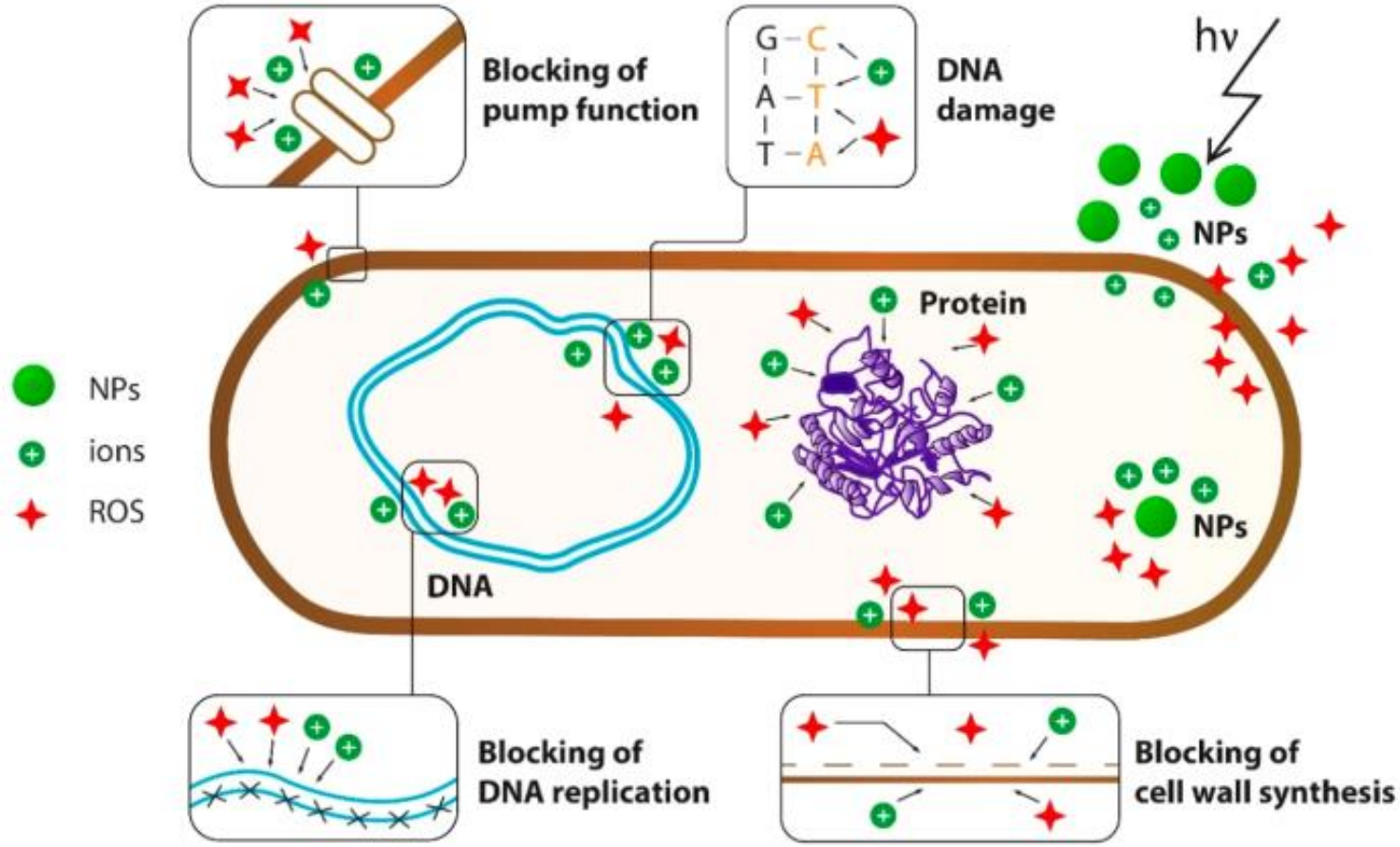
- Encrustation assay
  - In vitro bladder model with artificial urine
  - Time to blockage delayed from 41.3 ± 1.7 to 101.4 ± 6.9 hours

Antimicrobial strategy – Metal-based NPs

- Widely studied metal-based nanoparticles (NPs)
  - Gold (Au), and silver (Ag)
  - Magnesium oxide (MgO), copper oxide (CuO), titanium dioxide (TiO_2), and zinc oxide (ZnO)
- Metal NPs outperform microscale counterparts

Antimicrobial strategy – Metal-based NPs

- Possible mechanisms of action



(Fig 2, Shkodenko et al., 2020)

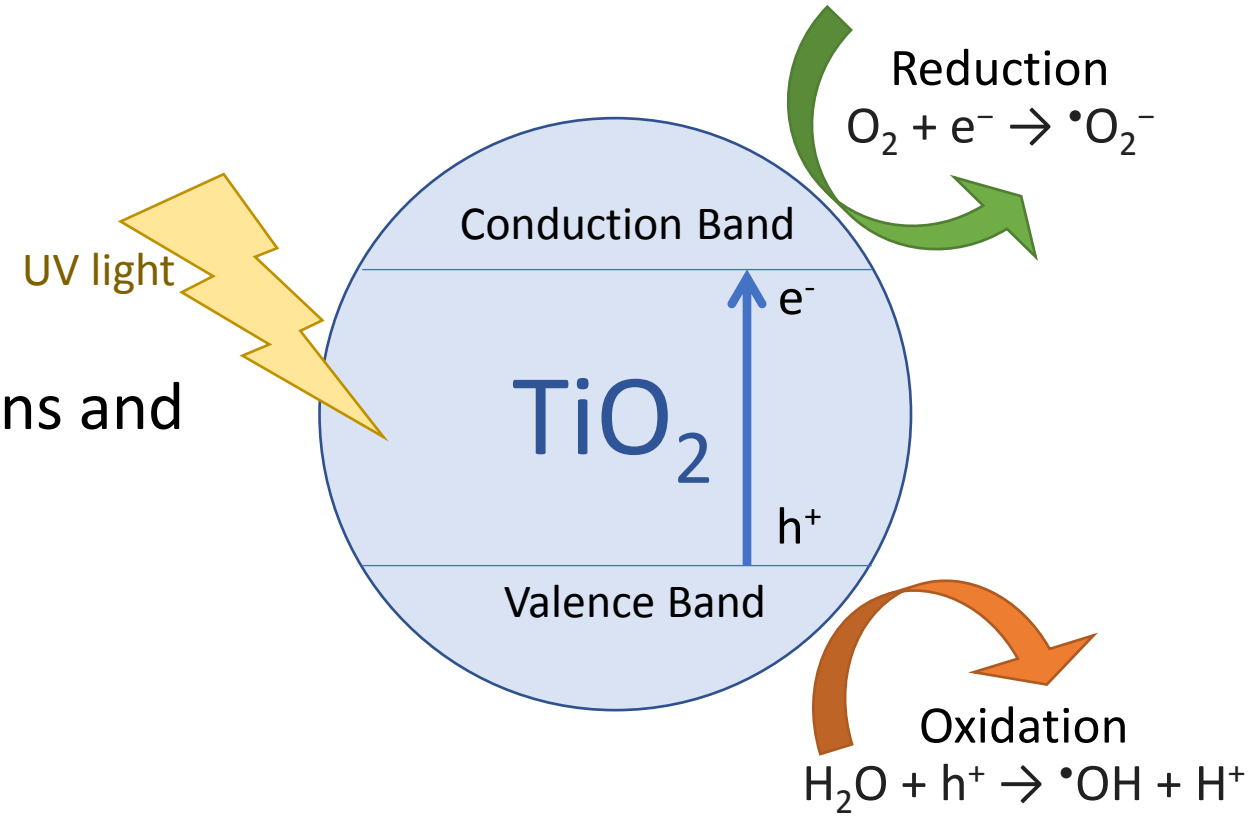
Antimicrobial strategy – Metal-based NPs

- Zinc oxide (ZnO)
  - Low toxicities in mammalian cells
  - More effective at inhibiting biofilm formation and growth of *E. faecalis*, *S aureus*, *S. epidermidis*, *B. subtilis*, and *E. coli*
  - Ineffective to *P. aeruginosa* and *Proteus* due to resistance

(Ramasamy and Lee, 2016)

Antimicrobial strategy – Metal-based NPs

- Titanium dioxide (TiO_2)
  - Inert, non-toxic
  - Photocatalyst
    - Produces superoxide anions and hydroxyl radicals



- Biofilm inhibition on MRSA, *Streptococcus mitis*, *P. aeruginosa*, and *Candida albicans*

(Shkodenko et al., 2020)

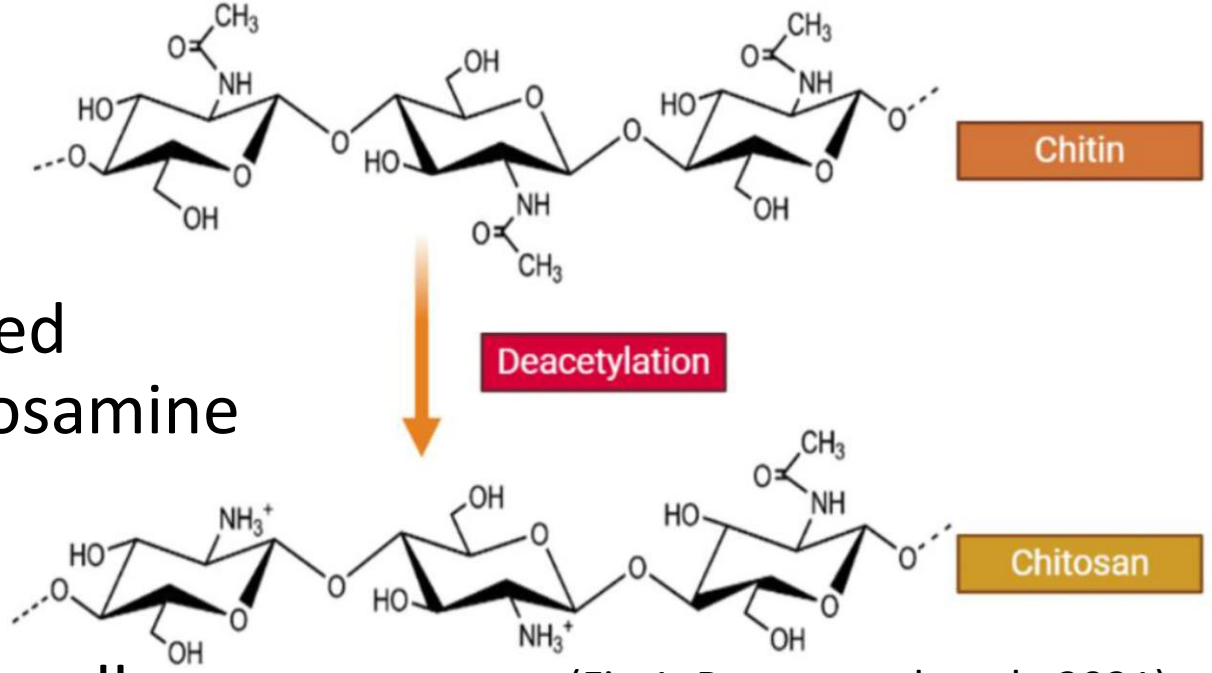
Antimicrobial strategy – Cationic polymers

- Net positive charge
- Cationic groups on side chain or polymer backbone
  - Cationic centres including ammonium ions, sulfonium ions, phosphonium ions
- Proposed mechanism
  1. Adsorption and penetration of the cationic polymers into microbial cell well
  2. Reaction with cell membrane (lipid and protein components)
  3. Membrane disassembly
  4. Leakage of intracellular material
  5. Degradation of proteins and nucleic acids

(Francolini et al., 2017)

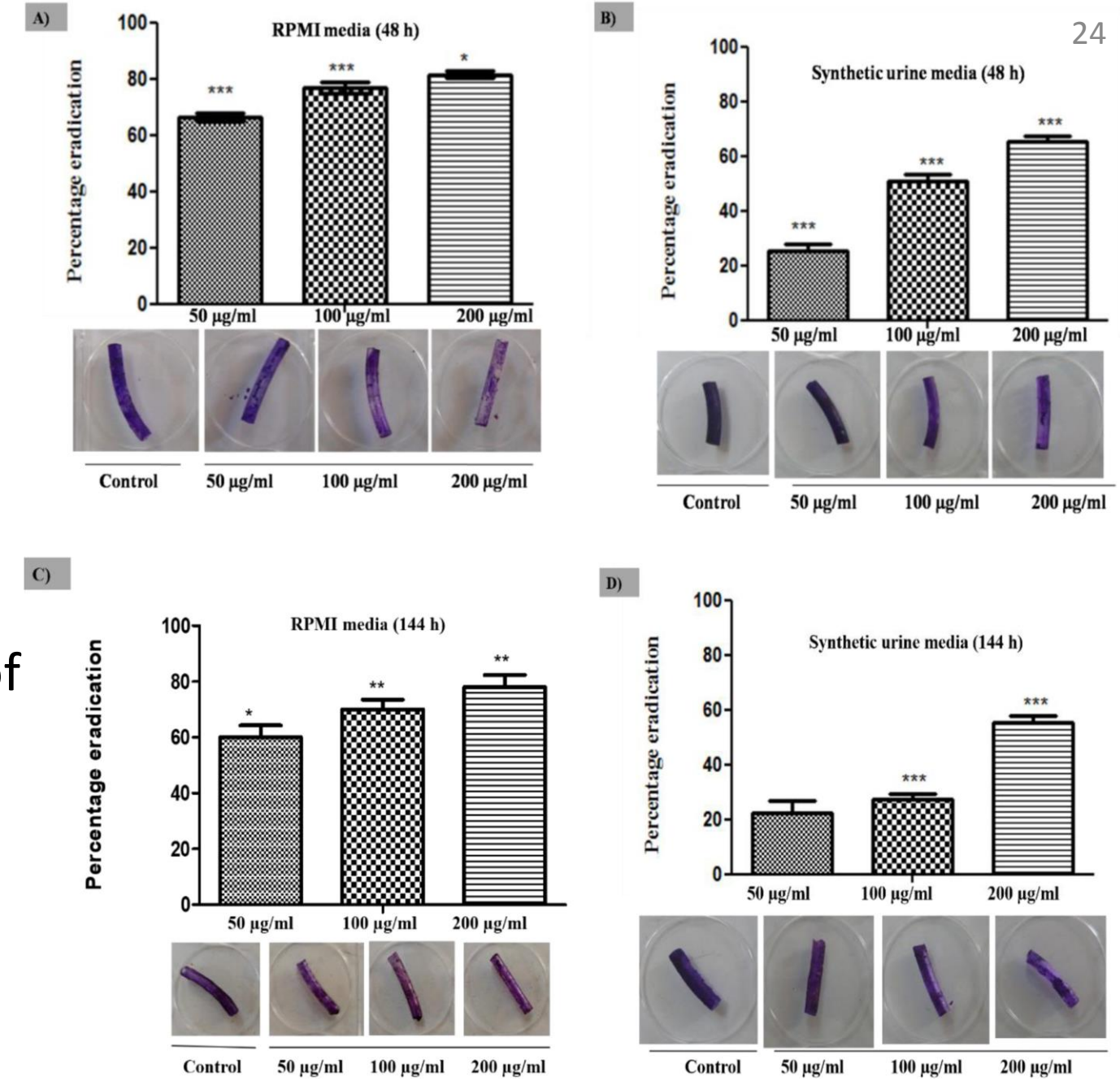
Antimicrobial strategy – Cationic polymers

- Chitosan
  - Derived from natural polymer
  - Composed of randomly distributed N-acetylglucosamine and D-glucosamine
  - Low toxicity towards mammalian cells
  - Antibacterial activity against Gram + and Gram - bacteria



(Fig 1, Boroumand et al., 2021)

- Rubini et al., 2021
  - In-house extracted chitosan, coated onto silicon catheter
  - Incubated in *S. epidermidis* and *C. albicans* co-culture
  - Dose-dependent reduction of biofilm formation



(Fig 2, Rubini et al., 2021)

Chitosan

- Nanocomposite with metal-based NP
  - Wang et al., 2012
    - Chitosan-Ag/PVP nanocomposite, coated on PET film
    - Eliminated 100% of *S. aureus* and *E.coli* in 10 ml of suspension (10^5 CFU/ml) in 5 min
    - Retained antimicrobial activity after submerging in PBS for 35 days
    - Reduced adhesion of bacteria
  - Pandiselvi and Thambidurai, 2015
    - Chitosan-ZnO/polyaniline nanocomposite
    - Reduced biofilm formation of *S. aureus* (97%), *P. aeruginosa* (95%), and *C. albicans* on coated glass slide

Limitations of nanocoatings

- Antifouling coatings
  - Not biostatic or biocidal
  - Can be overwhelmed by high concentration of microbes
- Antimicrobials coatings
  - Different antimicrobial spectra
  - Active ingredients depletes gradually
  - Accumulation of debris of dead microbes
- Future trend – Integrated strategies
  - Combination of antimicrobial NPs
  - Antifouling + antimicrobial coatings
  - Nanotopography

Take-home messages

- Colonization and biofilm formation of microbes on medical devices can cause device-associated infections
- Biofilms on medical devices are difficult to eradicate by antibiotics
- Nanocoatings can be used to prevent microbial colonization and biofilm formation on medical devices
- Antifouling nanocoatings repel protein and microbial adhesion
- Antimicrobial nanocoatings inhibit microbial colonization
- The future trend of development is multifunctional coatings with integrated strategies

Q & A

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